Can environmental dust from silo area allow the development of stored product insects?

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Abstract

Dust derived from food processing can accumulate in places difficult to reach, where stored-product pests could thrive. The purpose of this work was to verify the development of *Plodia interpunctella*, *Ephestia kuehniella*, and *Tribolium castaneum* in dust collected on pipes and beams (15 m and 7.5 m) in a silo area of a pasta industry. Proximate analyses showed a higher metal content in the dust collected at the two different heights than semolina, including the presence of chrome, cobalt, arsenic, and lead. Particle size distribution analysis showed that in the two samples of dust the highest percentage was constituted by particle sizes smaller than 106 μm. The tests were carried out by using two quantities 4 g or 0.15 g of dust (corresponding to 3 mm and 0.1 mm), at controlled conditions. Fifty larvae, 0-24 hours old, of each species, were used for each dust, semolina, and thickness test. The number of emerged adults was assessed daily. *T. castaneum* developed on all the tested substrates, despite the high content of metals and the small particle size in the environmental dust. A significant interaction between diet and thickness of the layer was observed, but thickness had a stronger influence than diet. Moreover, light filth analysis detected a large number of fragments of *Tribolium* sp. in dust collected at a different height. Dust was unsuitable for the development of moths; only two *E. kuehniella* adults emerged from 3-mm-deep dust collected at 15 m, and development lasted more than 90 d.

1. Introduction

Cereals and their byproducts are susceptible to attack of several species of stored product insects (Keskin and Ozkaya, 2013). Food processing industries offer optimal conditions for the development of such pests, including shelter, high temperature and relative humidity, the absence of predators and parasitoids, and plenty of food. Moreover, dust derived from food processing can accumulate in places difficult to reach, inside and under machinery, on electric cables and cabinets, and on windowsills where insects can develop unobserved (Dal Monte, 1984; Trematerra et al., 1984; Rotundo et al., 1995; Trematerra and Süss, 2006; Phillips and Throne, 2010).

Flying insects can thrive on uncollected dust and contaminate food on production lines. The presence of insects or their fragments in processed food can damage company reputations; therefore, taking targeted action to avoid insect contamination is of primary importance. This action includes checking raw materials, modifying the environment to make it less favorable for pest establishment, and applying integrated pest management strategies. Dust that accumulates in the upper areas of the food plants is particularly difficult to reach and remove, and regular monitoring cannot be carried out easily. Consequently, cleaning of high areas such as beams, pipes, and windowsills cannot be included in routine cleaning for several reasons; instead, these operations must be performed by specialized cleaning companies, and they are expensive and, therefore, occur rarely. It must be considered that, in dust derived from food processing, stored-product pests such as darkling beetles and pyralid moths can settle, as they find food and suitable conditions for development, and make them permanent hotbeds of infestation in the plant. On the other hand, dust, deposited as time goes by, can be enriched with particles of various origin that are derived from the surrounding environment. In particular, they may be enriched with metals that could affect the development of stored-product pests (Perron et al., 1966; Baker et al., 1976; Davis and Boczek, 1987).

The purpose of this work was to observe the development of *Plodia interpunctella* (Hübner) (Indian Meal Moth), *Ephestia kuehniella* Zeller (Mediterranean Flour Moth), and *Tribolium castaneum* (Herbst) (Red Flour Beetle) on dust collected at different heights in the silo area of a pasta plant. The aim was, first, to verify whether flour dust, produced during processing and enriched with airborne dust, is suitable for the development of the above-mentioned insects and secondly, to establish the amount of dust that can be tolerated to improve cleaning operations.

2. Materials and methods

2.1. Collection and analysis of flour dust and semolina

Samples of flour dust were collected in the North of Italy, from a silo area of a pasta plant belonging to a company that produces more than 30,000 tons per year of traditional and organic pasta. Pyrethrum (Piretro Safe H, Copyr S.p.A.) was used 2-3 times a year, during spring and summer to control flying adults.

Dust was collected with a flat brush and a dustpan, during a rare comprehensive cleaning operation, by pipelines (7.5 m height) and beams (15 m heights) surfaces and placed in plastic bags. The amount of dust collected from pipelines and beams was respectively 942 g and 791 g. Dust samples were sieved with 20 mesh to separate any larger concrete particles. Samples of semolina, 500 g for each of the four silos, were also collected during charging operation; the samples were mixed before tests. The particle size distribution of dust and semolina was measured by sieve analysis, using six sieves of 20, 45, 70, 100, 125, and 140 US mesh, on a sample of 100 g (Table 1). In dust collected at 7.5 m and at 15 m, the highest percentage of particles was smaller than 106 μm, 62.69% and 79.10%, respectively. In semolina, the highest percentage of particles (49.70%) ranged from 212 to 354 μm. The semolina was ground to obtain a particle size distribution similar to the collected flour dust. Semolina and ground semolina were used as controls. All samples were kept at -18°C for 15 days before the tests, to eliminate any possible prior infestation.

Proximate analyses were performed on 50 g the dust and semolina samples to determine the nutritional value (2 replicates). Different methods were used, fiber content was analyzed according to Prosky et al. (1988); carbohydrates were determined with Rocklin and Pohl (1983) method; Association Of Analytical Communities and American Association for Clinical Chemistry methods were performed to measure proteins (AOAC 34.01.05 n.925.31), fats (AOAC 31.04.02 n. 963.15), moisture (AACC 44-15.02), ashes (AACC 08-01.01). The results of analysis are summarized in Table 2. Semolina has the highest moisture content (11.4%), followed by dust collected at 7.5 m (8.6%) and dust collected at 15 m (7.8%). Insoluble and soluble fiber content in dust collected at the two different heights was twice the one observed in semolina. Similar amounts of proteins, fats, and sugars were present in all three substrates. Ash content was higher in dust collected at 15 m (2.4%) and lower in dust collected at 7.5 m (1.7%). The lowest ash content was observed in semolina (0.8%). To determine elements of interest (Al, Cr, Fe, Co, Ni, Cu, Zn, As, Sr, Mo, Ag, Cd, Tl, and Pb), 0.25 g of each of the dust and semolina samples were digested by a microwave digestor system (Multiwave-Eco Anton Paar GmbH, Graz, Austria) in Teflon tubes filled with 10 mL of 65% HNO_3 by applying a one-step temperature ramp (at 210°C in 10 min, kept for 10 min). After being cooled for 20 min, the mineralized samples were transferred to polypropylene test tubes and diluted at a ratio of 1:40 with MILLI-Q water. Then, the concentration of elements was measured by ICP-MS (BRUKER Aurora-M90 ICP-MS). An aliquot of a 2 mgL^{-1} of an internal standard solution (72Ge, 89Y, 159Tb) was added to both samples and by calibration curve to give a final concentration of 20 mgL^{-1}. Typical polyatomic analysis interferences were removed by using CRI (Collision-Reaction-Interface) with an H_2 flow of 75 mL min^{-1} flow through a skimmer cone. Metal content (Table 3) was higher in dust collected at 15 m, and it gradually decreased in dust collected at 7.5 m, and in semolina. In particular, the amount of aluminum in dust collected at 15 m was twice the one observed in dust collected at 7.5 m, and iron was three times higher. Furthermore, both dust collected at two different height contained chrome, cobalt, arsenic, and lead, and all these metals were absent in semolina.

2.2. Light filth analysis

Light filth analysis was performed to detect insects, insect fragments, and rodent hair, in semolina and in dust collected at 7.5 m and 15 m (Anonymous, 2007). For each sample, 3 replicates, each 50 g, were analyzed. Firstly HCl solution (970 ml H_2O, 30 ml HCl) was added and autoclaved 30 minutes at 121 °C; the sample was sieved on No. 230 sieve with hot tap water to remove all original liquid and fine material. Sieve retainings were diluted with water and mineral oil and magnetically stirred, then transferred to the percolator. Oil - H_2O interface was drained and the contents transferred to filter paper and examined under the stereomicroscope. Insects and fragments were identified by comparison with photos and drawings (Domenichini, 1997) and counted at 30x magnification.

2.3. Insect rearing

Laboratory cultures of *Tribolium castaneum*, *Ephestia kuehniella,* and *Plodia interpunctella* kept in a rearing room at 26±1°C and 70±5% r.h., with a photoperiod of 16:8 (L:D) were used for these experiments. *T. castaneum* was reared on wheatmeal added with 5% brewer's yeast. The Indian meal moth and the Mediterranean flour moth were reared on an artificial diet consisting of bran (60 g), corn meal (65 g), wheat meal (55 g), glycerol (85 g), wheat germ (17 g), honey (67 g) and brewer's yeast (14 g).

2.4. Tests on dust from pasta plant

Tests were carried out in glass Petri dishes (diameter 50 mm) using dust samples collected in the pasta plant at 7.5 m and 15 m height, and with semolina and ground semolina as a control. For each dust and semolina, two quantities were tested. In each Petri dish 4 g (thickness: 3 mm) or 0.15 g (thickness: ~0.1 mm), were distributed evenly by gently shaking. The different samples of dust or semolina were used to evaluate the influence of dust thickness on insect development.

Eggs of *T. castaneum*, *E. kuehniella,* and *P. interpunctella* were collected separately in Petri dishes and observed daily to collect first instar larvae, 0-24 hours old, for the experiments. Fifty replicates, with a single larva for dust collected at 7.5 m and 15 m, semolina and ground semolina in the two different quantities, were carried out. We chose to carry out tests with a single larva to avoid cannibalism (Savoldelli, 2006).

Glass Petri dishes were placed in an incubator at 26.0±0.1°C, 45±5% r.h. with a photoperiod of 16:8 (L:D). Tests were monitored daily for a period of four months, and the number of emerged adults was recorded.

2.5. Statistical analysis

Data on the number of fragments identified from light filth analysis in flour dust and semolina were analyzed with one-way ANOVAs and LSD test ($\alpha = 0.05$) was performed; data on the development period of insects were submitted to one-way ANOVAs and LSD tests; the influence of quantity of dust and semolina on development period was analyzed by Student T-tests. Two-factor ANOVA was performed to verify the interaction between diet and thickness of the layer on development period (SPSS Statistics 22, SPSS Inc., IBM, Chicago, IL, USA).

3. Results

3.1. Light filth analysis

The highest number of identified (One-way ANOVA: $F_{2, 6}$=182.248), unidentified (One-way ANOVA: $F_{2, 6}$=272.981), and total (One-way ANOVA: $F_{2, 6}$=182.248) insect fragments was detected by light filth analysis in dust collected at 15 m (55.0±8.39, 328.0±15.13, 383.3±23.33, respectively), significantly different from dust collected at 7.5 m (19.7±1.76, 121.3±7.80, 140.7±7.13, respectively), and semolina (1.3±0.33, 5.0±2.08, 6.3±2.40, respectively) (Fig. 1). Identified fragments represent 14% of the total fragments in dust collected at the two heights.

The majority of identified fragments, in dust collected at the two heights, belonged to *Tribolium* spp. In dust collected at 15 m, several fragments of *Sitophilus* spp., and few of *R.*

dominica and Lepidoptera (femur and tibia) were also identified. The identified fragments in semolina were mandibles of *Tribolium* spp. and *Sitophilus* spp., and tibia of *R. dominica* (Fig. 3). In particular, in dust collected at 15 m different parts of larva and adults of *Tribolium* spp. were identified. In the case of *Sitophilus* spp., only adult body fragments were detected, most of them were mandibles (Fig. 3). In dust collected at 7.5 m no fragments of *Sitophilus* spp. were identified, while *Tribolium* spp. fragments belonged to body parts of larva and adult (Fig. 4).

3.2. Development of insects on dust collected at 7.5 m and 15 m, and semolina

The results of tests carried out on dust showed that *T. castaneum* developed on all the tested substrates (Table 4). The development period on thin layers was significantly shorter (43.7±0.49 days) on dust collected at 15 m height than on the other substrates (One-way ANOVA: $F_{3, 154}$=9.370). For 3 mm layers, the longest development period was observed on sifted semolina (One-way ANOVA: $F_{3, 178}$=12.323). The development period observed on different layers of the same substrate was significantly shorter on the 3 mm layer (dust collected at 15 m: t (85) = 13.273, p = 0.000; dust collected at 7.5 m: t (80) = 14.161, p = 0.000; semolina: t (83) = 12.495, p = 0.000; sifted semolina: t (84) = 11.392, p = 0.000). A two-factor ANOVA showed significant interaction between diet and thickness of the layer ($F_{3, 332}$=4.29), but thickness had a stronger influence than diet.

The 0.1 mm layers of dust collected at 7.5 m and 15 m heights were unsuitable for the development of *Ephestia kuehniella*, and only two adults emerged from the 3 mm layers of dust collected at 15 m, but their development was extremely slow (91.5±7.50 days). Fewer adults and longer development periods were observed in 0.1 mm layers of semolina (29 adults and 59.4±1.33 days) and sifted semolina (10 adults and 80.4±2.62 days) than in 3 mm layers of the same substrates: 45 and 46 adults; 48.1±0.46 and 60.7±0.51 days, respectively. The development period on ground semolina was significantly longer than on semolina, both on 0.1 mm layers (t (37) = -7.658) and on 3 mm layers (t (89) = -18.313).

No adults of *Plodia interpunctella* were observed in either dust collected at 7.5 m and 15 m. All the larvae reared on a 3 mm layer of semolina completed development in a significantly shorter period (49.5±0.58 days) than those reared on a 0.1mm layer (56.5±0.86) (t (91) =6.984). Only 7 adults emerged from 3 mm layer of ground semolina and their development period was longer (73.9±0.55 days) than in the one in the same layer of semolina.

4. Discussion

This research reported that dust collected at two different heights (7.5 m and 15 m) from a silo area in a pasta plant were suitable as a rearing substrate for *Tribolium castaneum*, but not for *Ephestia kuehniella* and *Plodia interpunctella*.

The proximate analysis identified similar amounts of proteins, lipids, and carbohydrates in dust and semolina, but different metal content. In semolina, low metal content was detected. Metal content increased with the dust collection height. The metals detected in this study can originate from production equipment as nickel and molybdenum are components of steel, and aluminum and iron are components of steel and cement. Chrome, cobalt, arsenic and lead were absent in semolina but were detected in dust samples. Arsenic is used in the production of ceramic and electronic components and lead is used in the coatings of electric wires. Studies on the influence of metals on insect development were carried out but they focused on insect nutritional requirements. Potassium, phosphate, and magnesium are reported to be essential for all insects; but they require little calcium, sodium, and chlorine, and the quantities present in food are generally sufficient (Trager, 1953; Medici and Taylor, 1966; 1967; Kruk et al., 1983). Zinc, copper, manganese, and iron are important cofactors in enzymatic reactions in *Tribolium confusum* (Kruk et al., 1983).

Research on the toxic effects of mineral salts on stored product insects is limited to only a few elements. Some authors proved that the addition of tricalcium phosphate to the diet prevented the development of the different stored-product insects, including *T. castaneum* (Baker et al., 1976; Davis and Boczek, 1987). Perron et al. (1966) demonstrated that aluminum, arsenic, cobalt, and molybdenum are toxic to *P. interpunctella*. All these elements are present in dust collected in the pasta plant and they prevented the development of the Indian meal moth. On the contrary, *T. castaneum* development was seemingly unaffected by the high metal content in the dust because adults were raised successfully in both 0.1 and 3 mm layers. These results were also confirmed by light filth analysis which detected a high number of *Tribolium* spp. legs, wings, and larval exuviae in dust collected at both 7.5 and 15 m. As whole insects were absent, the presence of only insect fragments suggests a previous infestation.

Additionally, the particle size of semolina did not prevent the development of red flour beetles, although their development was significantly shorter, with 3 mm layer of all the substrates tested. The results of this work suggest that a rapid increase of *T. castaneum* infestation can occur in the presence of accumulated dust because it is able to develop even when only a small, thin layer of accumulated dust is present. In fact, one larva of *T. castaneum* can develop on 0.1-mm-deep dust, corresponding to 150 mg spread on a surface of 19.6 cm^2.

As to the moths, only 4% of *E. kuehniella* eggs reared in 3 mm of dust, collected at 15 m height, developed into adults and their development required a period of time twice as long as their development on semolina. The absence of *P. interpunctella* and the poor, lengthy development of *E. kuehniella* on dust can depend on various factors. We previously mentioned that the content of metals in dust can negatively affect moth development. The high numbers of *Tribolium* spp. fragments recorded in filth analysis suggests a previous infestation, with the consequent accumulation of quinones, which are produced by *Tribolium* spp. adults, in the dust. It is well known that a high population density of *Tribolium* spp. causes the production of quinones that act as anti-aggregation pheromones, chemical defenses, and bacteriostatic agents (Duehl et al., 2011;

Senthilkumar et al., 2012; Trematerra et al., 2015). Yezerki et al. (2007) also suggested that quinones can act as an "antipredatory defense against rats that cohabitate grain storage with flour beetles". In previous studies, it was observed that substrates infested by *E. kuehniella* were attractive to *T. confusum* (Athanassiou et al., 2006), but the effect of substrates previously infested by *Tribolium* on the development of *E. kuehniella* or *P. interpunctella* is unknown.

The small dust particle size is another factor that could influence moth development. This study found a longer development period on sifted semolina and few adults of *P. interpunctella* emerged. Almost half (49.7%) of the particle size in semolina is between 212 μm and 354 μm; dust, collected at 7.5 and 15 m height, consists of particle sizes less than 106 μm (79.10% and 62.69%, respectively). Locatelli et al. (2008) observed that the particle size of soft wheat flours influences both the adult emergence percentage and the mean development time of *E. kuehniella*.

Also, *P. interpunctella* and *E. kuehniella* larvae developed faster in the 3 mm layer of semolina. The number of emerged adults of *P. interpunctella* was high on both layers of semolina but for *E. kuehniella*, the number of emerged adults was higher in the 3 mm layer. The larvae of the latter species are bigger than *P. interpunctella* larvae; therefore, the quantity of food in the 0.1 mm layer was probably insufficient for *E. kuehniella* development. We observed that the 0.1 mm layer of semolina did not cover mature *E. kuehniella* larval. Moreover, the adults were smaller than the ones that emerged when reared on a standard diet. In this study, *E. kuehniella* development time was longer than the one reported by Bhavanam et al. (2012) on an artificial diet consisting of wholemeal wheat flour, maize meal, brewer's yeast and glycerine. The nutritional composition of semolina is poorer than the diet used by Bhavanam et al. (2012). *E. kuehniella* has fewer nutritional needs compared to other pyralids because it can also develop on refined flours (Jacob and Cox, 1977; Stein and Parra, 1987). It was observed that *E. kuehniella* needs a diet rich in carbohydrates (Fraenkel and Blewett, 1943). In any case, a diet with flours deprived of protein fractions (gliadins, albumins, and globulins) retards the development of the insect and increases mortality in pupae (Nawrot et al., 1985).

The results obtained in this research show that *T. castaneum* was able to develop on dust collected at different heights of a local silo, despite the high content of metals. The development also occurred with a small amount of dust (0.15 g; thickness: ~0.1 mm). Given the results, it is impossible to determine a quantity of dust that can be tolerated because even small amounts of dust can enable the development of some insects. Therefore in the case of *T. castaneum*, the infestation would not be prevented by a routine cleaning but it would be necessary to carry out a targeted cleaning to eliminate debris in areas difficult to reach, such as high windowsills, pipes, and electrical conduits, in order to eradicate any outbreak,

This paper emphasizes the importance to monitor carefully insect species and suggests targeted actions to eliminate the infestation, according to integrated pest management strategy.

5. References

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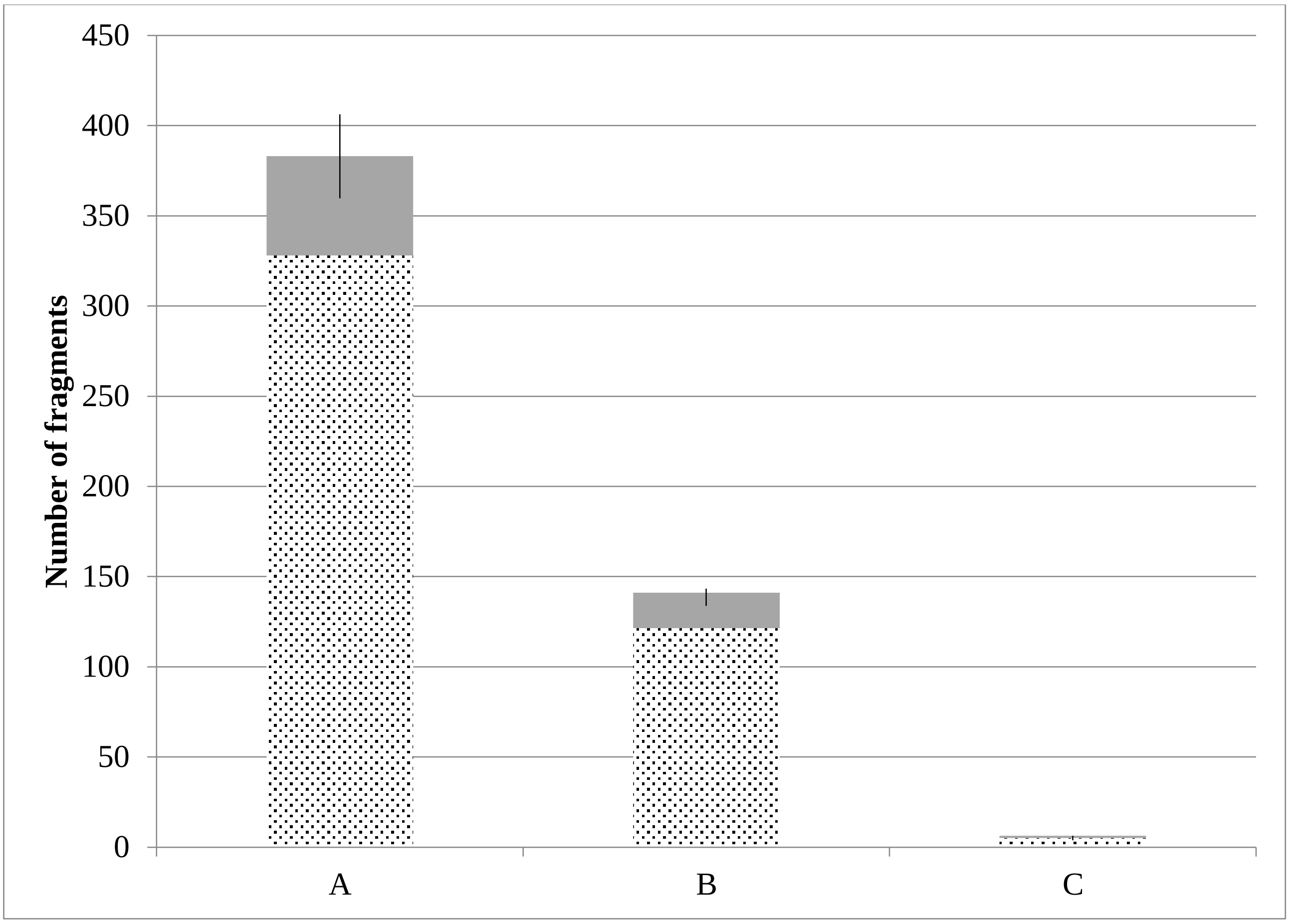

Fig. 1 - Mean number (±S.E.) of insect fragments obtained from 50 g of dust samples collected at 15 m (A) and 7.5 m (B) and of semolina from silos (C) (dots: unidentified fragments; gray: identified fragments).

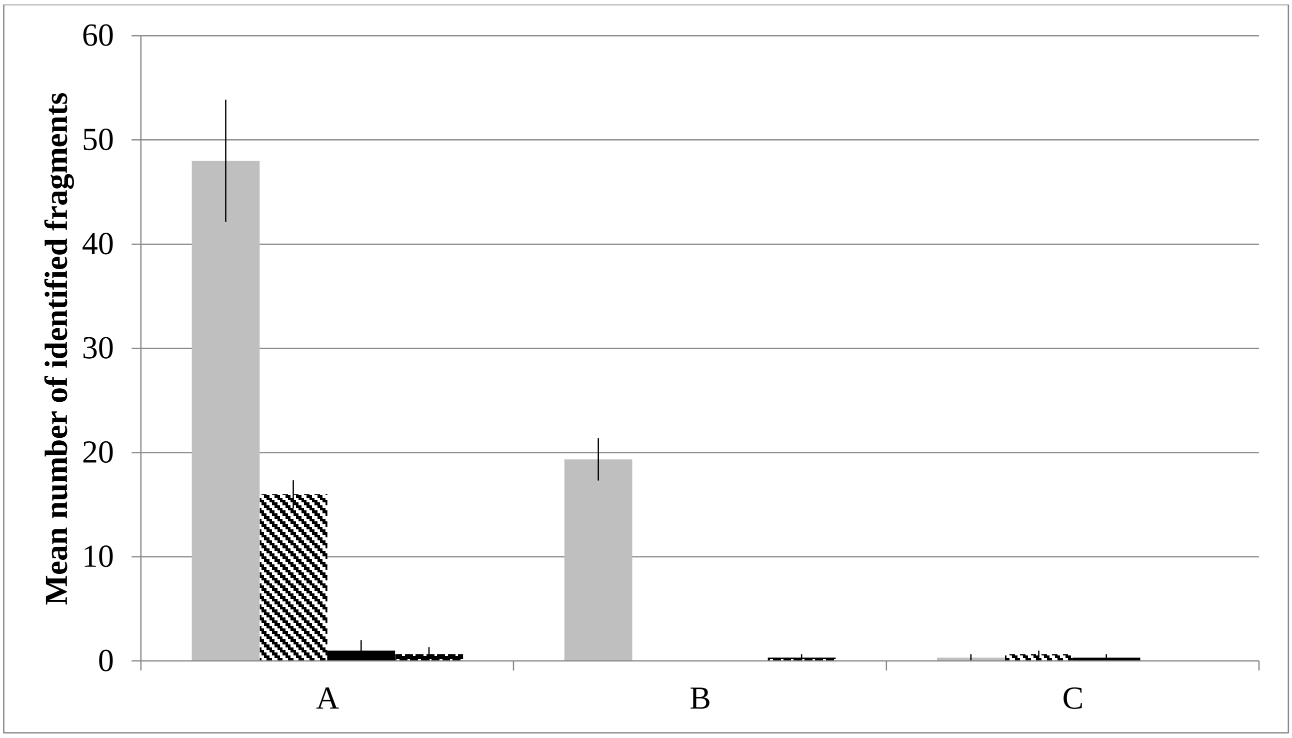

Fig. 2 - Mean number (±S.E.) of identified fragments of *Tribolium* spp. (gray), *Sitophilus* spp. (diagonal line), *Rhyzopertha dominica* (black), and Lepidoptera (dots) obtained from 50 g of dust samples collected at 15 m (A) and 7.5 m (B) and of semolina from silos (C).

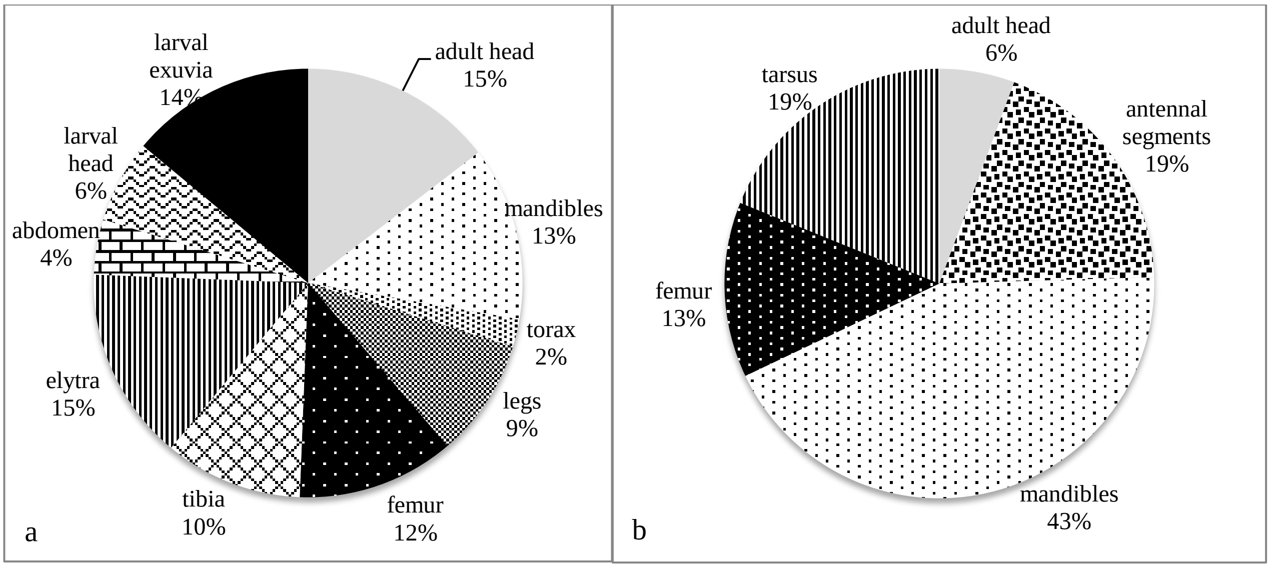

Fig. 3 – Percentage distribution of identified fragments of *Tribolium* spp. (a) and *Sitophilus* spp. (b) in dust collected at 15 m.

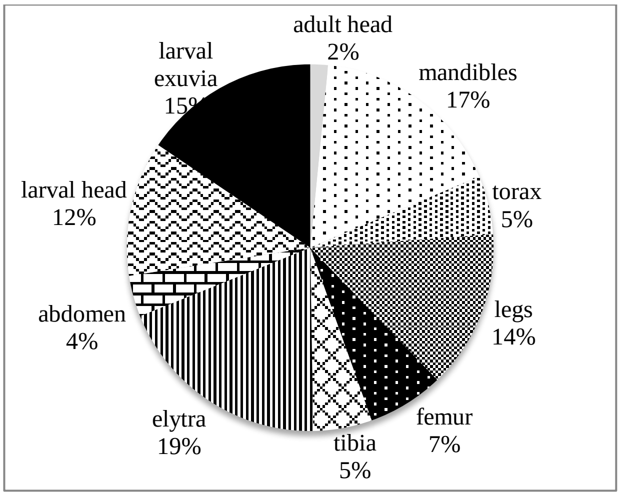

Fig. 4 - Percentage distribution of identified fragments of *Tribolium* spp. in dust collected at 7.5 m.

Table 1- Percentage distribution of particle size (µm) of dust collected at 15 m (A) and 7.5 m (B) and of semolina (C) and ground semolina (C-ground) from silos.

µm	Particle size distribution (%)			
	A	**B**	**C**	**C-ground**
<106	79.10	62.69	16.24	79.00
106–124	3.83	3.08	2.92	4.00
125–149	2.40	4.21	5.74	2.00
150–211	4.66	5.75	7.13	5.00
212–354	7.99	16.32	49.70	8.00
355–850	2.02	7.95	18.27	2.00
>850	0.00	0.00	0.00	0.00

Table 2—Results (%) of proximate analysis of dust collected at 15 m (A) and 7.5 m (B) and of semolina from silos (S.E.: standard error; CV: coefficient of variation).

Substrate	**A**		**B**		**C**	
	Mean ±S.E.	CV%	**Mean**±S.E.	CV%	**Mean**±S.D.	CV%
Moisture	7.8±0.08b	0.9	8.6±0.02b	0.3	11.4±0.20a	3.8
Ash	2.4±0.02a	1.0	1.7±0.00b	0.1	0.8±0.02c	3.8
Protein*	11.0±0.02b	0.3	11.0±0.03b	0.4	11.3±0.10a	1.3
Fat	1.3±0.04	4.6	1.4±0.03	3.5	1.4±0.01	0.6
Insoluble fiber	4.2±0.27a	8.9	4.2±0.06a	2.2	2.0±0.08b	5.8
Soluble fiber	2.2±0.16a	10.5	2.2±0.17a	10.9	1.0±0.08b	11.9
Glucose	0.3±0.01a	3.9	0.2±0.02a	14.8	0.1±0.01b	28.1
Fructose	0.3±0.01a	6.9	0.3±0.01a	6.9	0.1±0.00b	9.3
Saccharose	1.2±0.01a	1.5	1.2±0.01a	1.5	0.8±0.07b	12.2
Maltose	1.6±0.09	7.7	1.6±0.09	7.7	1.2±0.09	10.6
Starch**	67.7±0.24b	0.5	67.5±0.23b	0.0	70.0±0.00a	0.0

*A conversion factor of 5.70 was used for protein.

** Estimated by difference.

The means followed by different letters in the same line are significantly different (LSD, $P < 0.05$).

Table 3 – Mean (±SE) metal content (μg g^{-1}) of dust collected at 15 m (A) and 7.5 m (B) and of semolina from silos (C)*.

	Metal content (μg g^{-1})		
	A	**B**	**C**
Na	156.3±1.60a	121.7±2.20b	15.7±1.27c
Mg	1397.0±15.81b	1557.6±19.17a	446.4±5.64c
Al	216.8±3.93a	99.4±4.05b	1.8±0.17c
P	2.4±0.02b	2.6±0.04a	1.8±0.02c
K	3485.4±34.01b	3754.1±43.52a	2646.9±32.60c
Ca	969.9±6.38a	685.7±38.27b	267.7±3.69c
Cr	19.2±0.67a	14.2±0.66b	0.0±0.00c
Mn	24.1±0.25a	17.4±0.27b	9.3±0.14c
Fe	1579.7±96.62a	481.8±28.4b	9.3±0.16c
Co	0.4±0.02a	0.2±0.01b	0.0±0.00c
Ni	10.4±0.79a	7.8±0.15b	0.2±0.01c
Cu	27.0±1.22a	11.4±0.23b	3.4±0.14c
Zn	196.0±13.26a	123.9±5.74b	15.5±0.51c
As	0.2±0.01a	0.1±0.00b	0.0±0.00c
Sr	3.3±0.03a	2.4±0.03b	0.9±0.85c
Mo	1.5±0.03a	1.2±0.02b	0.8±0.04c
Pb	3.4±0.15a	1.9±0.05b	0.0±0.00c

The means followed by different letters in the same line are significantly different (LSD, $P < 0.05$).

*Ag, Cd, Tl were absent.

Table 4. Number of emerged adults of *Tribolium castaneum* (Herbst) and mean development period (dd ± S.E.) (from newly hatched larva to adult) in the different substrates (A: dust collected at 15 m height; B: dust collected at 7.5 m; C: semolina from silos; C-ground: semolina with particle size similar to dust A and B) and layers (0.1 mm and 3 mm).

Substrate	0.1 mm		3 mm	
	N	**Mean±S.E.(dd)**	**N**	**Mean±S.E.(dd)**
A	40	43.7±0.49a	47	36.3±0.30a
B	38	46.8±0.59b	44	36.6±0.43a
C	40	47.7±0.89b	45	36.6±0.27a
C-ground	40	48.6±0.74b	46	39.1±0.44b

The means followed by different letters in the same column are significantly different (LSD, $P < 0.05$).