Evaluation of the attractiveness of an organic litter compared to breeding substrate

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Abstract
In a pet shop warehouse, stored food pest insects can develop in various preserved animal feeds (dog’s pasta, puffed rice, kibble). However, there is another commodity that is rarely considered, such as the organic litter which is composed of bran, flours and other residues of the screening of corn that may result attractive to the same pest insects. The purpose of this laboratory test was to evaluate the attractiveness of organic litters on Plodia interpunctella, Tribolium confusum, Oryzaephilus surinamensis in comparison with breeding substrate. The results confirmed that the test insects were attracted by the breeding substrate rather than by the organic litter.

Keywords: stored food pest insects, pet food, organic litter, Plodia interpunctella, Tribolium confusum, Oryzaephilus surinamensis.

1. Introduction
The stored food insects can attack several food products, the importance in stored food losses are estimated about 16% (World Bank et al., 2011). Food industries pest are also involved in losses in pet food industries. Roessli et al. (2003) reported that it is possible to record up to thirty insect species belonging to 20 families in four pet stores chain during February to August 2001. The most common and abundant species were Plodia interpunctella (Hübner), Oryzaephilus mercator (Fauvel), Tribolium castaneum (Herbst) and Sitophilus spp.

In the pest food stores this attack by stored pest food can cause damage to various preserved animal feeds (dog’s pasta, puffed rice, kibble). However, there is another commodity that is rarely considered, such as the organic litter which is composed of bran, flours and other residues of the screening of corn that may result attractive to the same pest insects. In July 2017, a package of dog’s pasta found infested by O. surinamensis by LEA (Laboratory of applied entomology Agroblu). The origin of infestation was investigated, and it was discovered it had developed from a pallet of ecological litter for pets. Thanks to these events, the aims of the present work is to investigate the attractiveness of organic litter applied to O. surinamensis, P. interpunctella and T. confusum in comparison with a balanced diet, typical of breeding. This test was combined to a development evaluation test of the same species on such substrate.

2. Materials and Methods

2.1 Insects
The insects used in the test was provided by the Agroblu Laboratory of Applied Entomology (LEAA: via Isonzo 20, Rozzano- Milano – Italy) where are reared at 26 ± 2 °C 70% RH and photoperiod light darkness 16L:8D. The test organisms used were typical insects infesting food industries and also collected in pet stores. For the test were employed larvae II instar of Plodia interpunctella (Hübner), adult stage of Tribolium confusum (Jaqcquelin du Val) and Oryzaephilus surinamensis (Linnaeus).

Table 1 reported the species and stage used for the test.

<table>
<thead>
<tr>
<th>Insect</th>
<th>Stage</th>
<th>Quantity</th>
<th>Substrate TNT</th>
<th>Substrate T</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. interpunctella</td>
<td>II instar larvae</td>
<td>20</td>
<td>Honey, glycerin, white flour, semolina, yellow flour, oatmeal, sesame, bran</td>
<td>Organic litter</td>
</tr>
<tr>
<td>T. confusum</td>
<td>Adult</td>
<td>20</td>
<td>Semolino, brewer’s yeast, bran</td>
<td>Organic litter</td>
</tr>
<tr>
<td>O. surinamensis</td>
<td>Adult</td>
<td>20</td>
<td>Honey, glycerin, white flour, semolina, yellow flour, oatmeal, sesame, bran</td>
<td>Organic litter</td>
</tr>
</tbody>
</table>
2.2 Substrate

The test was conducted to compare the attractiveness of a commercial organic litter and a balanced diet normally used for breeding. *P. interpunctella* and *O. surinamensis* were reared on a diet composed as follow honey 15%, glycerin 5%, white flour 20%, semolina 20%, yellow flour 15%, oatmeal 5%, sesame 15%, bran 5%.

*T. confusum* diet was composed 70% semolina, 29% bran and 1% brewer’s yeast.

The composition of the organic litter, vegetable granules, obtained by extracting and drying the fibrous part of the corn’s ear, was reported in table 2.

**Tab. 2** Composition of organic litter.

<table>
<thead>
<tr>
<th>Component</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw ashes</td>
<td>1 – 2%</td>
</tr>
<tr>
<td>Raw protein</td>
<td>0.5 – 1.5%</td>
</tr>
<tr>
<td>Raw lipids</td>
<td>0.1 – 1%</td>
</tr>
<tr>
<td>Raw fiber</td>
<td>33 – 40%</td>
</tr>
<tr>
<td>Extraction inazotati</td>
<td>50 – 60%</td>
</tr>
<tr>
<td>Humidity</td>
<td>4 – 10%</td>
</tr>
</tbody>
</table>

2.3 Y-olfactometer

To test the preference between the two substrates, a plexyglas Y-olfactometer was used. The arms of the structure were 10 cm long and 3 cm wide. At the end of the arms a plastic panel (3x3 cm) was fixed with a 0.5 cm wide hole to allow the air flow created thanks to an extractor fan. To allow the assessment, the Y-olfactometer was fixeded on a rectangular plexiglass panel. On the top of the structure, another rectangular plexiglass panel was placed, featuring a rubber gasket to avoid insects escape.

![Fig. 1 Scheme of the plexyglass Y-olfactometer setup for the test](image)

2.4 Test System

The Y-olfactometer was set as follow:

- Principal arm: starter point of insect;
- Arm 1: TNT (breeding substrate);
- Arm 2: T (organic litter);
The position of the two alternative substrate was randomized in the replicates. (figure 1).

2.5 Replicates
The test was replicated 20 times.

2.6 Test site
The test was conducted in the Peet Grady Room of LEAA at 26 ± 2 °C 70% RH and photoperiod light darkness 16L:8D.

2.7 Application method

2.7.1 Coleoptera
For *O. surinamensis* and *T. confusum* 50 adult insects were sampled from the breeding and placed in a Petri dish for 72 hours. After this period a single insect was placed in the principal arm of the olfactometer to choose between two alternative substrates.

2.7.2 Lepidoptera
For *P. interpunctella*, 50 larvae at the II instar, high trophic activity development stage, were collected and placed in a petri dish for one hour. The larvae were placed in the principal arms individually to choose between two alternative substrates.

2.8 Evaluation method

2.8.1 Coleoptera
To ensure accurate assessments, the insects were observed up to their choice for 5 minutes, as choices adopted after 3 minutes do not statistically differ from choices taken within the first 3 minute (Wakefield et al. 2004).

2.8.2 Lepidoptera
For *Lepidoptera* the assessment lasted longer because the choice took place in max 25 minutes. The choice of substrate and the time of choice was recorder and compared with t-test.

3. Results
The results showed that all the species taken into consideration, preferred to head towards the substrate typically used for breeding (table 3).

**Tab. 3** Percentage of choice between T and TNT.

<table>
<thead>
<tr>
<th>Test insect</th>
<th>T-Organic litter</th>
<th>TNT-Breeding substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Plodia interpunctella</em></td>
<td>15%</td>
<td>85%</td>
</tr>
<tr>
<td><em>Tribolium confusum</em></td>
<td>30%</td>
<td>70%</td>
</tr>
<tr>
<td><em>Oryzaephilus surinamensis</em></td>
<td>20%</td>
<td>80%</td>
</tr>
</tbody>
</table>

The observed mean values were significantly different for \( p<0.05 \) (t-test) for all the species and stages considered (table 4).

**Tab. 4** Mean of insects that have chosen the test substrate (organic litter). Means followed by "*" are significantly different \( p<0.05 \) than the response to the control (t-test).

<table>
<thead>
<tr>
<th>Test insect</th>
<th>T-Organic litter</th>
<th>TNT-Breeding substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Plodia interpunctella</em></td>
<td>0,15*</td>
<td>0,85</td>
</tr>
<tr>
<td><em>Tribolium confusum</em></td>
<td>0,30*</td>
<td>0,70</td>
</tr>
<tr>
<td><em>Oryzaephilus surinamensis</em></td>
<td>0,20*</td>
<td>0,80</td>
</tr>
</tbody>
</table>
The mean time of choice of the two alternatives was recorded and compared with t-test. *Plodia interpunctella* larvae took more time than Coleoptera to choose, the mean time of choice of the preferred substrate for Coleoptera was respectively 3.21 minutes for *T. confusum* and 1.63 minutes for *O. surinamensis*, only the mean choice time of *T. confusum* between organic litter and breeding substrates was statistically significant for p<0.05 (t-test) (table 5).

**Tab. 5** Mean time used by insects in chosen the test substrate (organic litter). Means followed by “*” are significantly different (p<0.05) than the response to the control (t-test).

<table>
<thead>
<tr>
<th>Test insect</th>
<th>Choice time (min)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T-Organic litter</td>
<td>TNT-Breeding substrate</td>
</tr>
<tr>
<td><em>Plodia interpunctella</em></td>
<td>11.67</td>
<td>12.76</td>
</tr>
<tr>
<td><em>Tribolium confusum</em></td>
<td>5.00*</td>
<td>3.21</td>
</tr>
<tr>
<td><em>Oryzaephilus surinamensis</em></td>
<td>1.75</td>
<td>1.63</td>
</tr>
</tbody>
</table>

4. Discussion

The results obtained show that the test insects in front of a choice between a balanced diet substrate and a commercial litter, prefer the first substrate. This result complete and integrate the information available in literature (Phillips *et al.*, 1994, Tsuji, 2000, Mowery *et al.*, 2002). These data are preliminary and require further investigations on the possible attractiveness of organic litter compared to other commodities stored in pet food shops by other stored food pest or its attractiveness in interaction with other volatile components.

References


Evaluation of the difference in the development of stored insect pests on organic litter

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Abstract

On July 2017 in a warehouse of pet food shop in Italy an infestation of *Oryzaephilus surinamensis* was found on a pallet of organic litter, near an infested pallet of dog’s pasta. In order to investigate the origin of the infestation, and to support the risk assessment by the pest control operator, one test was conducted at Agroblu Laboratory of Applied Entomology (LEAA) to observe the feasibility of development of *O. surinamensis*, *Plodia interpunctella* and *Tribolium confusum*, in a substrate of 2.5 g of organic litter and to compare it to a balanced diet substrate.