



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

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The purpose of this laboratory test was 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.

### MATERIALS AND METHODS

The insects used in the test were provided by the Agroblu Laboratory of Applied Entomology (LEAA, via Isonzo 20, Rozzano- Milano - Italy) where they are reared at 26 ± 2 °C 70% RH and photoperiod light darkness 16L:8D.

72 h laid eggs of *Plodia interpunctella* (Hübner), newborn larvae of Tribolium confusum (Jaqcquelin du Val) and Oryzaephilus surinamensis (Linnaeus) were used for the test.

The substrates with the test species were put into small plastic container 6 cm diameter and 6,5 cm high. Each container was covered with a plastic twist cap provided with a small hole 1cm diameter, filled with a special filter to avoid the escape of insects and allow the air exchange.

	Stage	Substrate TNT	Substrate T
P. interpunctella	Eggs 72 h laid	Honey, glycerin, white flour, semolina, yellow flour, oatmeal, sesame, bran	Organic litter
T. confusum	Larvae Semolino, brewer's yeast, bran Organ		Organic litter
O. surinamensis	Adults	Honey, glycerin, white flour, semolina, yellow flour, oatmeal, sesame, bran	Organic litter

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Organic litter components	Range
Raw ashes	1-2%
Raw protein	0,5 – 1,5%
Raw lipidis	0,1 – 1%
Raw fiber	33 – 40%
Extraction inazotati	50 - 60%
Humidity	4 – 10%.









### **Application method**

For the test with P. interpunctella, 8 groups of 50 vital eggs were sorted each in one plastic container four of which were filled with the balanced diet (TNT) and the other four have been filled with organic litter (T) to give the possibility to the newborn larve to feed.

For O. surinamensis, 8 groups with 10 adults were sorted in 8 plastic containers 4 with balanced diet (TNT) and 4 with test substrate. For T. confusum 10 new born larvae per group were arranged as above.

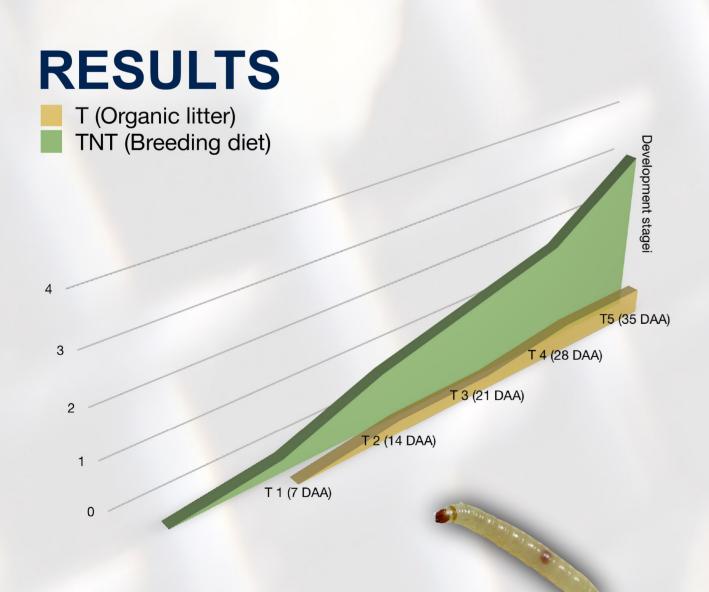
### Stage achieved (at least one individual)

- 0 No development
- 1 Newborn larvae
- 2 Mature larvae
- 3 Pupae

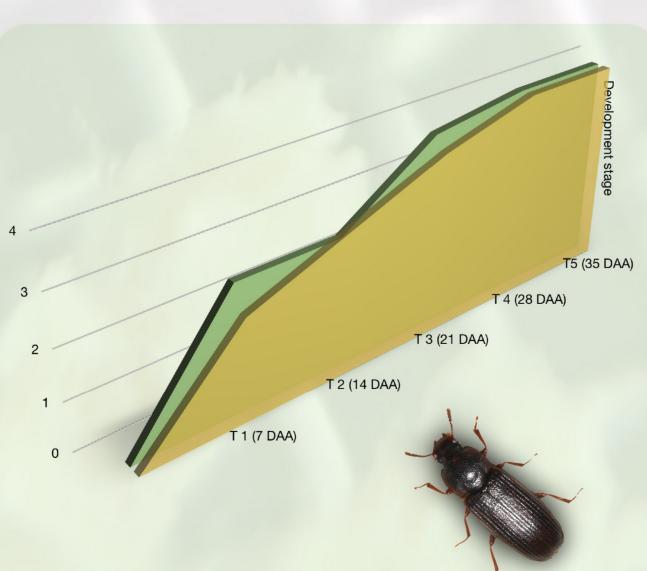
4 Adults

### **Evaluation method**

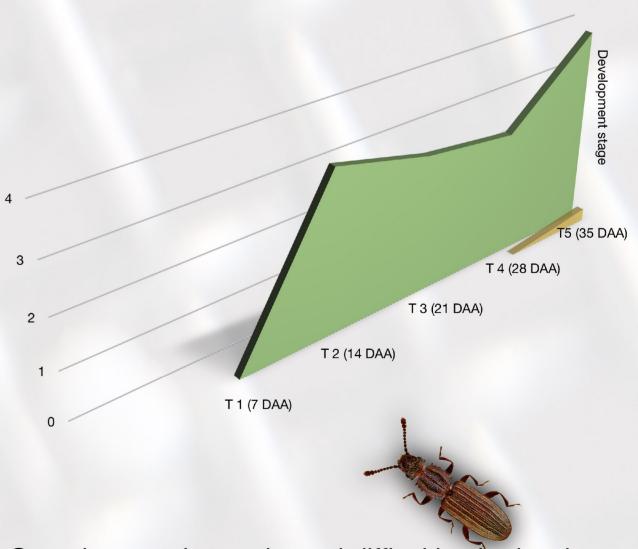
After the start of the test, the experimental units were checked every seven days and the development stage of the test species at the time of the assessment was noted. In according to the scale shown in table 3 the qualitative data was converted in number for statistical analysis.



P. interpunctella was not able to develop on organic litter, indeed young larvae failed to develop in any replicates.



T. confusum showed the ability to complete the life cycle on organic litter successfully, with no significant difference in comparison to the development on the breeding substrate.



O. surinamensis too showed difficulties in develop on organic litter and only one adult emerged by one replicates in all the test

### CONCLUSION

This study showed that *Tribolium confusum* was able to complete successfully his development on organic litter. These data are preliminary and require further investigations on the possible development on organic litter by other stored food pest in addition to adjustments to the experimental protocol





BREEDING

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

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The purpose of this laboratory test was to evaluate the attractiveness of organic litters on larvae of Plodia interpunctella and adults of Tribolium confusum and Oryzaephilus surinamensis in comparison with breeding substrate.

Organic litter components	Range
Raw ashes	1 – 2%
Raw protein	0,5 – 1,5%
Raw lipidis	0,1 – 1%
Raw fiber	33 – 40%
Extraction inazotati	50 – 60%
Humidity	4 – 10%.

	Stage	Substrate TNT	Substrate T
P. interpunctella	II instar larvae	Honey, glycerin, white flour, semolina, yellow flour, oatmeal, sesame, bran	Organic litter
T. confusum	Adult	Semolino, brewer's yeast, bran	Organic litter
O. surinamensis	Adult	Honey, glycerin, white flour, semolina, yellow flour, oatmeal, sesame, bran	Organic litter

### **MATERIALS AND METHODS**

The insects used in the test were 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 olfactometers were built in plexiglas and the air outputs connected by rubber pipes to a centralized air extractor so to ensure the airflow to be uniform from the two source arms.

#### **Application**

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.

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.

### AIR IN ORGANIC LITTER

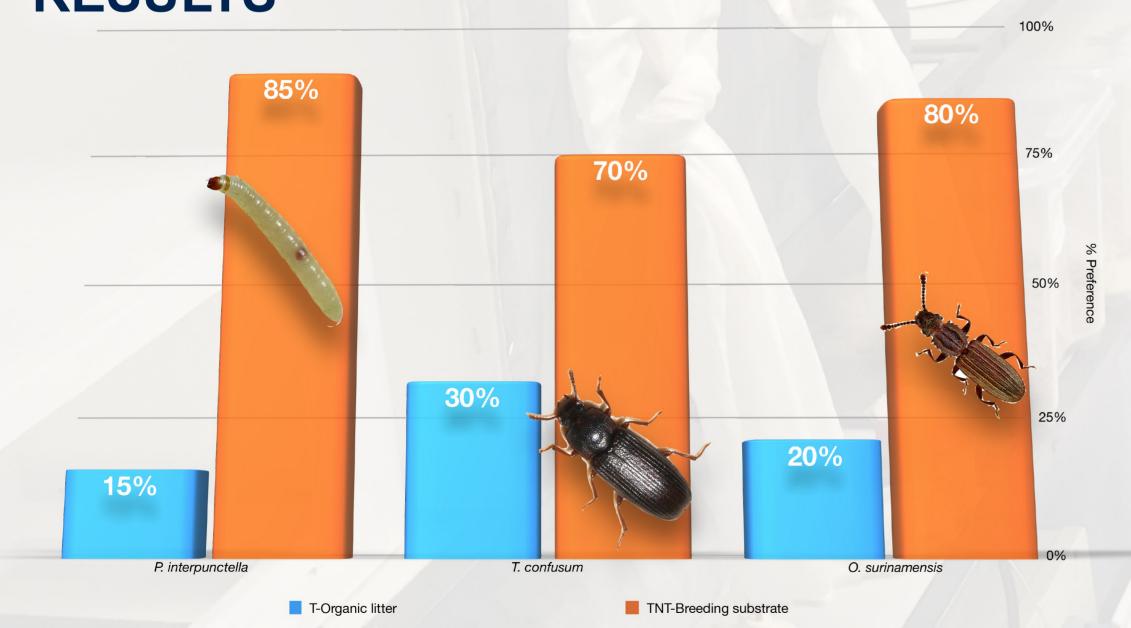
### Evaluation

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).

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.

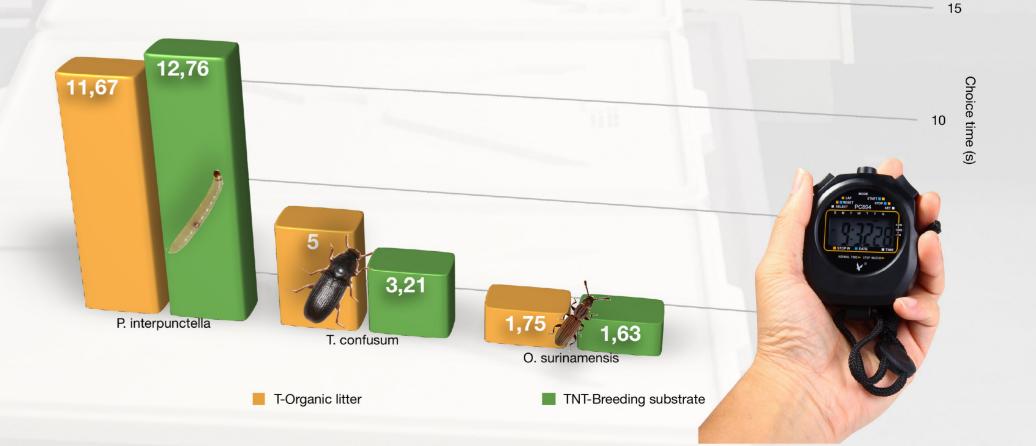
The **mean time of choice** of the two alternatives was also recorded and compared with t-test.

### **RESULTS**



The results obtained show that the test insects in front of a choice between a balanced diet substrate and a commercial litter, prefer the balanced substrate.

*P. interpunctella* larvae took more time than Coleoptera to choose. Only the mean choice time of *T. confusum* between organic litter and breeding substrates was statistically significant for p < 0.05 (t-test)



### CONCLUSION

These results 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.





# Biocidal efficacy of nitrogen (anoxic atmosphere) applied in operational condition to stored hazelnuts against pest insects at different stages of development.

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The purpose of this field-test was to providing data support to avoid phosphine in the process of stocking fresh hazelnuts by verifying that the biocidal effect of the exposure to 99,9% concentration of N2 for 21 days at 15-18°C temperature is sufficient to ensure total control on five common pests of stored food, through evaluation on alive insects at different stages of development.

### MATERIALS AND METHODS

**INSECTS** - The insects used in the test were 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.

**SUBSTRATES** - Special biotests containing eggs, larvae and adult insects were prepared with non-infested substrate normally used for breeding.

	Contraction of the state of the
P. interpunctella	Eggs and Larvae
C. cautela	Eggs and Larvae
C. cephalonica	Eggs
T. confusum	Mix population
O. surinamensis	Mix population



**TEST SITE** - A logistic center in northern Italy, with climatic cell. The cell had a controlled atmosphere permanent plant, capable of extracting Oxygen and pumping Nitrogen to reach a 99,9% saturation at a chosen range of temperature (15-18°C). **Such range of temperature was chosen to achieve a minimum pest development condition.** This cell had 752 pallets capacity, sorted in 9 lines of 8 units, each replicated by 4 vertical levels (figure 1). The total dimension of the cell is 27,8 m length, 15,14 m width and 8,60 m height.

**TEST SYSTEM** - The biotests were inserted from the top opening, 30-35 cm deep, in 58 fresh shelled hazelnuts big bags sorted in a specific position to ensure homogenous distribution in the cell at all 4 levels.



**APPLICATION** - The test started when the  $N_2$  saturation reached 99,9%. After **21 days** of complete and constant  $N_2$  saturation, the desaturation process started. During the whole period of the test, the temperature and % saturation by  $N_2$  were constantly monitored and kept.

**EVALUATION** - The evaluation of the biotests was performed at LEAA by the laboratory staff, through visual assessment, count and record of alive and dead individuals, within 5 days after the extraction. The assessment was based on the observation of alive individuals, the

biotests recorded as "positive" at the first alive individual observed.



### **RESULTS**

P. interpunctella larvae
C. cephalonica eggs

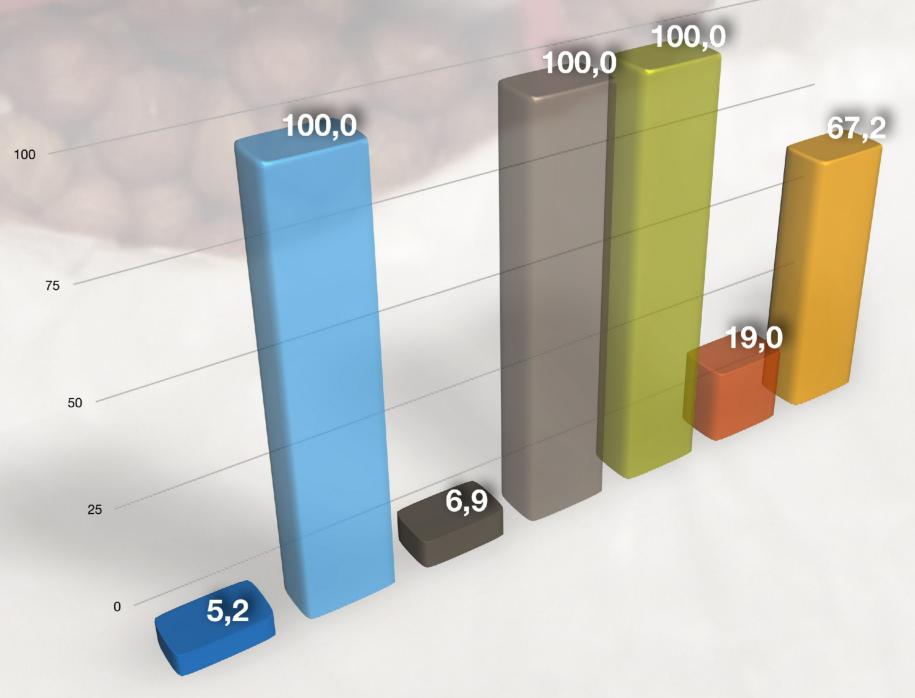
P. interpunctella eggs
T confusum mix pop.

C. cautella larvae
O. surinamensis mix pop. mixed

C. cautella eggs

CONTROL ON **LEPIDOPTERA**: the results elaborated with Abbott's formula showed that the treatment achieved total mortality on eggs of all Lepidoptera species tested, while on larvae of *P. interpunctella* and *C. cautella* performed very low control, respectively 5,2 and 6,9 percent.

CONTROL ON **COLEOPTERA**: the effect observed was 19% and 67% mortality respectively on *Tribolium confusum* and *Oryzaephilus surinamentis* 



### CONCLUSION

The test highlighted that an exposure to N2 saturation at temperatures of 15-18 °C for 21 days was **not sufficient** for a total control on mobile stages of all pests tested, except for eggs of Lepidoptera species.

This tests also points out the importance of using biotests when an innovative stored food pest control method is tested.