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STORED PRODUCT PESTS

Semifield study on the effect of membrane-based nitrogen production for the control of the eggs of *Sitophilus oryzae* (L.) and *Tribolium confusum* J. du V.

D.P. Locatelli,[1] P. Girgenti,[1] L. Moncini,[2] L. Limonta[1]

[1]Department of Food, Environmental, and Nutritional Sciences, University of Milan; [2]Centro Ricerche CRISBA-ISIS "Leopoldo di Lorena", Grosseto, Italy

Abstract

In this paper, the effect of a highly purified N_2 (98.5%) controlled atmosphere-generated in situ by a membrane nitrogen separator on mixed age eggs of *Sitophilus oryzae* and *Tribolium confusum* was evaluated. Trials were carried out in an airtight stainless silo filled with wheat, in which a nitrogen atmosphere was maintained for durations lasting from one to several days. The mean temperature recorded during the treatment varied from 22 to 24 °C. After the treatment, the eggs were transferred to a jar with rearing medium and kept at 27±1°C and 70±5% R.H. The jars were checked every two days to record adult emergence. Eighty percent mortality was observed in *T. confusum* and *S. oryzae* with two and four days of treatment, respectively. One hundred percent mortality was obtained after five days of treatment in the case of *T. confusum* and six days for *S. oryzae*.

Correspondence: Lidia Limonta, Department of Food, Environmental, and Nutritional Sciences (DeFENS), Università degli Studi di Milano, via G. Celoria 2, 20133 Milano, Italy.
E-mail: lidia.limonta@unimi.it

Key words: Rice weevil, Confused flour beetle, nitrogen, controlled atmosphere.

Contributions: the authors contributed equally.

Conflict of interest: the authors declare no conflict of interest.

Funding: This work was funded by Fondazione Ente Cassa di Risparmio di Firenze; Eurosider S.a.s. by Ottavio Milli & C. provided the nitrogen separation system.

Received for publication: 27 July 2020.
Accepted for publication: 23 October 2020.

Journal of Entomological and Acarological Research 2020; 52:9278
doi:10.4081/jear.2020.9278

Introduction

Controlled atmospheres are increasingly considered an alternative to insecticides in the control of stored grain pests (Carvalho *et al*., 2012, 2019; Navarro *et al*., 2012; Aulicky *et al*., 2017; Njoroge *et al*., 2019; Vassilakos *et al*., 2019). Nitrogen- and carbon-dioxide-controlled atmospheres require air-tight storage systems in order to prevent gas leakage and guarantee the constant concentrations to achieve an effective result. Nitrogen is more volatile and more air-tight demanding than carbon dioxide, and it is mainly employed in packaging (Riudavets *et al*., 2009) because it prevents the development of pests while the strong packaging prevents pest entrance. It was also demonstrated that the use of a N_2 controlled atmosphere (CA) can prevent mold (Moncini *et al*., 2020a) and preserve the nutritional value of stored wheat (Moncini *et al*., 2020b).

Premises that are not perfectly air-tight hardly keep oxygen concentration below 1% and increase the time required to kill pests. One hundred percent mortality was reached with 2% oxygen after 20 days (Navarro *et al*., 2012), and after ten days with oxygen at concentrations of 1% or less (Aulicky *et al*., 2017). Temperature influences the length of the treatment: high temperatures shorten the required CA treatment (Donahaye *et al*., 1996).

In this study, the effect of a purified N_2 atmosphere (98.5±0.5%) generated in situ by a membrane nitrogen separator in airtight silos was tested on mixed age eggs of two of the most widespread pests of cereal grains, *Sitophilus oryzae* and *Tribolium confusum*.

Materials and Methods

Mass rearing and egg collection

Sitophilus oryzae and *Tribolium confusum* were reared in the Department of Food, Environmental, and Nutritional Sciences (DeFENS) in a climatic chamber (27±1°C, 70±5% UR).

Wheat seeds were used as rearing media for *S. oryzae*, while a mixture of wheat flour (70%), bran (20%), and wheat germ (10%) was used for *T. confusum*. A total of 300 g of rearing medium and 200 adults were placed in glass jars (height, 19 cm; Ø, 10 cm; volume, 1.5 L) and the lid with a hole was closed with wired net (18 mesh). Adults were transferred monthly to another jar and the rearing medium was renewed.

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Trials were carried out with wheat kernels infested with eggs of *S. oryzae* and with eggs of *T. confusum*. To obtain seeds with eggs, 200 adults of *S. oryzae* at 3 to 6 days old and 200 g of wheat seeds were put in PS jars (height, 6 cm; Ø, 11 cm; volume, 500 ml). After 5 days, adults were separated from seeds with a sieve (10 mesh).

Adults of *T. confusum* were sifted from the rearing medium with a 20 mesh sieve. Groups of 200 adults were put in PS jars (height, 6 cm; Ø, 11 cm; volume, 500 ml) with 70 g of wheat flour sieved with a 45 mesh sieve. After 3 days, adults were separated using a 20 mesh sieve and eggs were separated from wheat flour with a 45 mesh sieve.

Silos

Trials were carried out in a prototype of laboratory-scale silos (Moncini *et al*., 2020b) in the CRISBA-ISIS Research Center "Leopoldo II di Lorena" (Grosseto, Italy). The stainless-steel silos (60 L) were airtight and can maintain a controlled atmosphere with N_2 gas.

The N_2 controlled atmosphere was generated using a membrane nitrogen separator. The system separated N_2 from atmospheric air with membranes that fractionated N_2 by selective permeation. N_2 remained under slight overpressure (0.07 bar) and was maintained automatically at a pre-set percentage (98.5±0.5% v/v N_2 controlled atmosphere).

The silos were filled with 40 kg (corresponding to 32 L) of organic wheat cultivar Bologna, which was chilled for 24 h before the trials. Experiments were performed in six silos: three silos were kept airtight and three silos had the lid partially open to simulate traditional storage (control: CNTRL). Nitrogen was insufflated for different lengths of time, from 1 to 6 days.

Trials set up

The eggs were put in a PP jar (height, 7 cm; Ø, 5.5 cm; V, 125 ml) and the lid with a hole was closed with wired net. In the case of *S. oryzae*, 40 g of wheat seeds with eggs were put in the jar, while in the case of *T. confusum*, 100 eggs with 45 g of grounded wheat and 5 g wheat flour were added.

One jar for each species were placed in the silos in the middle of the cereal mass. The environmental temperature was measured with a data logger (Cryopak Europe mod. Mini TH, 76150 Maromme - France).

After the treatment, the insects were transferred to a glass jar with rearing media at 27±1°C and 70±5% R.H. Emerged adults were counted three days a week until adults were not observed for at least another week.

Statistical analysis

Data were submitted to one-way ANOVA, LSD test and Student's t-test (α=0.05) (IBM SPSS Statistics 26). Mortality (%) was corrected using Abbott's formula (Abbott, 1925).

Results

Table 1 shows the mean number of adults of *Sitophilus oryzae* that survived in the treatment with N_2 and in the control. Comparing the number of adults recorded in the 1-day treatment, there was no significant difference between the N_2 treatment and the control (Student's t-test, $t(4) = 0.189$ n.s.). No significant differences were observed in the number of adults in the 4 and 5 days N_2 treatment (ANOVA LSD post hoc). To prevent the survival of eggs, 6 days of N_2 treatment was required.

In the case of *Tribolium confusum* eggs, the number of adults from eggs of 1-day N_2 treatment was not significantly different from the control (Student's t-test $t(4) = 2.294$ n.s.) (Table 2). The treatments of two and three days obtained less than 20% survival, while survival was less than 5% for the 4-day treatment. At five days of N_2 treatment, there was no egg survival.

Figure 1 shows the mean temperature detected during the trials and the corrected percentage egg mortality for *S. oryzae* and *T. confusum*. The mean temperature was fairly constant, varying from 22 to 24 °C. Two days of N_2 controlled atmospheres obtained 80% mortality of *T. confusum* eggs and 25% mortality of *S. oryzae* eggs. The four-days treatment achieved 100% egg mortality in *T. confusum* and 85% in *S. oryzae*. For the latter species, a six-days treatment was necessary to obtain 100% egg mortality.

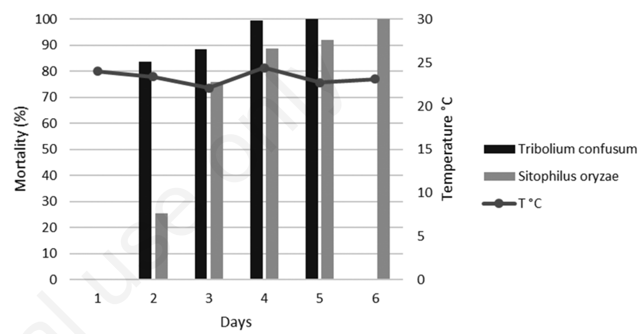

Figure 1. Mean temperature (°C) registered during the nitrogen treatments and mortality (%) of *Sitophilus oryzae* and *Tribolium confusum* eggs.

Table 1. Number of adults of *Sitophilus oryzae* (mean ± S.E.) that emerged after the nitrogen controlled atmosphere (CA) treatment (Treated) and in the control (CNTRL).

Treatment days	Treated	CNTRL
1	182.0±1.15[a]	182.3±1.33[b]
2	121.0±2.65[b]	162.3±4.63[c]
3	49.0±3.51[c]	203.0±2.89[a]
4	22.3±1.20[d]	200.3±0.88[a]
5	16.7±0.33[d]	209.7±4.98[a]
6	0.0±0.00[e]	185.7±7.69[b]

One-way ANOVA: treated $F_{5, 12} = 1374.969$; $P<0.005$; untreated $F_{5, 12} = 15.526$, $P < 0.005$.

Table 2. Adults of *Tribolium confusum* (mean ± S.E.) emerged after the nitrogen CA treatment (Treated) and in the control (CNTRL).

Treatment days	Treated	CNTRL
1	74.3±1.33[a]	71.0±0.58[c]
2	14.3±2.33[b]	88.7±0.88[a]
3	10.3±3.18[b]	88.7±1.76[a]
4	0.3±0.33[c]	80.0±2.52[b]
5	0.0±0.00[c]	80.0±5.13[b]

One-way ANOVA: treated, $F_{4, 10} = 276.904$, $P<0.005$; control, $F_{4, 10} = 7.364$, $P<0.005$.

Conclusions

To obtain 100% mortality with a 98.5% nitrogen atmosphere, six days were needed in the case of the eggs of *Sitophilus oryzae*, which are laid inside the kernel, while in the case of *Tribolium confusum* eggs, five days sufficed, as these eggs are laid outside the kernel. After 48 h, the mortality of *S. oryzae* was 25%, while Dal Monte and Pasqui (1979), who developed a method to obtain free eggs of *S. granarius* and *S. oryzae* and exposed the eggs to pure nitrogen for 48 hours, obtained 60% mortality and a delay in the embrional development of the remaining eggs, which hatched after 11 days.

In our trials, we tested eggs of mixed ages as they are considered inactive stages and are less sensitive to modified and controlled atmospheres (Hoback and Stanley, 2001; Navarro, 2006; Wong-Corral *et al*., 2013; Tutuncu and Emekci, 2019), although Mbata *et al*. (2004) observed a different vacuum susceptibility according to the egg developmental period. Shortly after egg-laying, the soft corium releases water and oxygen, and before hatching a high respiratory activity was observed; therefore, young and old eggs seem to be more sensitive.

The temperature registered during our trials with a nitrogen-controlled atmosphere varied from 22 to 24°C. Donahaye *et al*. (1996) used modified atmospheres at 26, 30, and 35°C, and observed a decrease of the time required to obtain 99% mortality of all the stages of *T. castaneum* with an increase of temperature. Modified atmospheres with 1% O_2 and 26 °C required 6.8 days to obtain 99% mortality, while at 35°C this time decreased to 44 hours. In concrete silos, the time required to obtain 100% mortality varies according to the species and temperature. In tests with N_2 and O_2 from 0.1 to 0.9%, to obtain 100% mortality of adults of *Oryzaephilus surinamensis*, *T. confus*um, and *Rhyzopertha dominica*, at 26°C and 22°C were required 18.7 and 23.8 days, respectively (Navarro *et al*., 2012).

In field trials with an O_2 concentration from 1 to 2.2%, Fleurat Lessard and Le Torch (1987) observed a different mortality at the top and bottom of the silo. One hundred percent mortality in *S. granarius* was obtained in 21 days at the top of the silo, while in the lower part 99.5% mortality was reached after 28 days. The size of cereal storage silos makes it difficult to maintain uniform gas concentrations, especially considering that nitrogen is an extremely volatile gas.

The positive outcomes achieved in our trials were largely due to the size and airtightness of the stainless-steel silo, and to the constant concentration of N_2, provided by a membrane nitrogen system under slight overpressure (0.07 bar) and maintained automatically at the pre-set concentration.

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