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Susceptibility of *Plodia interpunctella* (Hbn.) and *Lasioderma serricorne* (F.) to nitrogen protective pressurized atmosphere

Abstract - Susceptibility of *P. interpunctella* and *L. serricorne* in airtight package containing coffee has been evaluated in presence of protective atmosphere with pressurized nitrogen and carbon dioxide (0.2-0.4 bar).
In each jar, previously filled with coffee (250 g) 2 cylindrical containers (Ø 3.7 cm; h 5.5 cm) in plastic rigid transparent material were placed centrally, closed by a lid in soft plastic, with a rectangular opening, protected by a wire net to permit the content to be aired; in each container containing artificial substrate and coffee individuals of the same stage and species were placed.
On the whole *P. interpunctella* individuals show a longer survival on artificial diet. II-III and IV instar larvae of *P. interpunctella* are the least sensitive stage, as 6 days are required to obtain 100% mortality.
L. serricorne resulted to be very susceptible to nitrogen and carbon dioxide atmosphere; 100% mortality occurs after 24 hours from the packaging for both diets. Eggs are the most resistant stage as no egg hatching is observed after a stay of 7 and 8 days in nitrogen atmosphere respectively on coffee and artificial diet.

Riassunto - *Suscettibilità di* Plodia interpunctella *(Hbn.) e* Lasioderma serricorne *(F.) in presenza di atmosfera protettiva pressurrizzata.*

In questa ricerca è stata valutata la sensibilità di *P. interpunctella* e *L. serricorne* in imballi ermetici contenenti caffè in presenza di atmosfera protettiva con azoto e anidride carbonica pressurizzata (0,2-0,4 bar).
In ogni recipiente precedentemente riempito di caffè (250 g) sono stati posti in posizione centrale due contenitori di forma cilindrica (Ø 3,7 cm; h 5,5 cm) in materiale plastico rigido trasparente, chiusi da un coperchio in plastica morbida con apertura rettangolare protetta da una rete metallica, per garantire l'areazione del contenuto; in ognuno dei contenitori contenente rispettivamente substrato artificiale e caffè sono stati posti individui del medesimo stadio e specie.
Gli individui di *P. interpunctella* mostrano una maggiore sopravvivenza su dieta artificiale. Larve di II-III età e larve mature di questo lepidottero risultano essere lo stadio meno sensibile; sono necessari infatti 6 giorni per ottenere il 100% di mortalità su dieta artificiale. Gli adulti di *L. serricorne* risultano molto suscettibili all'atmosfera di azoto e anidride carbonica; 100% di mortalità si verifica dopo 24 ore dal confezionamento per entrambe le diete. Le uova di questo coleottero

sono lo stadio più resistente, in quanto non si osserva alcuna schiusura dopo permanenza di 7 e 8 giorni rispettivamente su caffè e su dieta artificiale.

Key words: *Plodia interpunctella, Lasioderma serricorne,* nitrogen, and carbon dioxide protective pressurized atmosphere

INTRODUCTION

The controlled atmosphere can be obtained by using 2 inert gases, nitrogen and /or carbon dioxide which show different problems: high concentration of nitrogen, by reducing oxygen concentration to very low values, have an effect mainly stifling on the forms of life there present, while the absorption of carbon dioxide causes a direct toxic action.

Modifications of atmosphere cause in fact pathologic effects on all the aerobic organisms, limit the danger of micotoxins by moulds (Wilson & Jay, 1975) and prevent from oxidation (Jay, 1984; Lu Qianyu, 1984).

The use of carbon dioxide had a greater diffusion , as it can also occur when the rooms are not perfectly airtight; moreover it requires, compared to nitrogen, fewer concentration and shorter time of application.

The use of nitrogen is more successful for packaged products where gas concentration can be kept by using material impermeable to gas.

Time required to control pests, using carbon dioxide and/or nitrogen depends on temperature; which parameter can affect the insects respiration and therefore the efficacy of the treatment (Bailey & Banks, 1980; Reichmuth, 1986).

Jay (1984) shows that, in a test with carbon dioxide, significant increase of mortality of all the species of the insects considered occurs increasing temperature from 16 to 32°C.

Pests control by fumigation with this gas, at atmospheric pressure, at 20-25°C temperatures, can be reached on the average after 10 days (Annis, 1987), while in an atmosphere rich in carbon dioxide (90%), at 14.7 bar, 100% mortality of adults of *Rhyzopertha dominica* (F.) *Sitophilus zeamais* Motsch., *Tribolium castaneum* (Herbest) and *Lasioderma serricorne* (F.) is obtained after 6 minutes of treatment .

As to nitrogen, concentration of oxygen not higher than 1% are generally advised to control pests, but some species don't survive at oxygen concentration lower than 3% (Reichmuth, 1987); Shejbal (1979) suggests treatments with at least 99.7% of nitrogen, observing that lower concentration cannot prevent development of species which lay eggs inside the kernels. All the stages of *Lasioderma serricorne* (F.) (Williams *et al.*, 1980) die on stored maize in small silos, in presence of 100% nitrogen after 10 weeks of storage.

According to Marzke *et al.* (1970) mixtures of nitrogen and oxygen can be used to control *P. interpunctella*, if the oxygen concentration is lower than 1%; in fact the eggs of this species cannot hatch after 3 days of exposure to atmosphere with 98.3%

of nitrogen, while a 7 day exposure are required when the concentration decreases to 96.4%.

The insects become differently susceptible to mixtures of carbon dioxide and nitrogen also in relation to the species and to the development stage. Jay & Pearman (1972) observe that in modified atmosphere (CO_2 38.5%; N_2 49.0%, O_2 12.5%) *Tribolium castaneum* results more susceptible to the treatment compared to *T. confusum* J. Du Val. Adults, for instance, are generally more susceptible compared to larvae and pupae (Lingren & Vincent, 1970).

In this research the susceptibility of *P. interpunctella* and *L. serricorne* in an atmosphere enriched of CO_2, and with a low content of oxygen, at a pressure higher than the atmospheric one, has been valued.

MATERIALS AND METHODS

Tests were carried out by placing *Plodia interpunctella* and *Lasioderma serricorne* inside jars containing roasted coffee; this vegetal, if it wasn't properly aired after roasting, produces carbon dioxide.

As coffee isn't a complete nourishment for these insects, mortality which is recorded can be due not only to the treatment but also to food shortages; consequently tests were carried out also on specific artificial diet, formed by pellets [(1)] in the case of *L. serricorne* and by a mixture of ingredients, in equal parts [(2)], for *P. interpunctella.* The humidity percentage of ground coffee is 1.0±0.1, while that one of the artificial substrate used for *P. interpunctella* is 10.8±0.7.

In each jar, previously filled with coffee (250 g) 2 cylindrical containers (Ø 3.7 cm; h 5.5 cm) in plastic rigid transparent material were placed centrally, closed by a lid in soft plastic, with a rectangular opening, protected by a wire net to permit the content to be aired; in each container containing artificial substrate and coffee individuals of the same stage and species were placed.

Afterwards the jars were packaged in atmosphere enriched with CO_2 and low content of oxygen at a pressure higher than the atmospheric one (0.2-0.4 bar).

As to *P. interpunctella* eggs, II-III, and IV instar larvae and cocoons were undergone to the treatment while adults were not considered as they are the most vulnerable stage.

100 eggs, 24-48 hour old, were controlled to the stereo-microscope (10-15 enlargements), putting aside the subjects with malformations; 10 individuals, respectively for II-III, and IV instars and cocoons were also used.

As to *L. serricorne* only adults were counted (10 individuals for each repetition) as the juvenile stages mostly develop themselves inside the substrate of rearing. The biological material was consequently prepared according to the following methods:

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(1) Ingredients: maize flour, wheat flour, bran and germ of wheat.

(2) Ingredients: bran, wheat flour, germ of wheat, maize flour and ground hazel nuts.

100 adults were placed to lay eggs on the food substrate and after 10 days, time required for oviposition, they were removed.

Larvae and pupae were obtained at different intervals respectively after 30-40 and 45-55 days from adults' removal (26±1°C; 65±5% R.H.)

For each test 4 replications were carried out for any species and stage. The containers were kept in an environment at 21°C and 65±5% R.H.; 4 untreated individuals for each stage and species were put at the same temperature conditions. The coffee packaging was afterwards opened at fixed intervals (1,2,3...n. days), registering oxygen and carbon dioxide concentration; the values were reported in Table 1. As time passes, also a steady increase of the pressure from 0.2 to 0.4 bar occurs, with a decrease of oxygen content; in fact coffee releases carbon dioxide, which progressively increases from 14.6% of the first day of the test till 40.4% recorded after 7 days.

After the packaging was opened, the number of II-III and IV instar of *P. interpunctella* and of adults of *L. serricorne* survived to the treatment, was counted. The eggs of the two species and the larvae and the pupae of *L. serricorne* cannot be directly counted as they cannot be separated from the substrate. These individuals, at the end of the test, were put in a thermostatic cell at 26±1°C and 65±5% R.H. and the emerged adults were counted.

II-III instar larvae and emerged adults were respectively counted in order to evaluate the survival of *P. interpunctella* eggs and cocoons.

The test was carried out also by placing bio essay in jars at environmental pressure and with coffee previously scattered on a tray exposed to the air and daily mixed for a month, in order to help carbon dioxide dispersion, naturally produced by the vegetable.

The jars were kept for 10 days in a thermostatic cell at 65±5% R.H. and 21±1°C.

RESULTS

In Tables 2-5 the average number of survived adults recorded on coffee and artificial diet at different intervals of preservation in nitrogen and carbon dioxide atmosphere at the pressure of 0.2-0.4 bar was listed.

Eggs resulted to be the least vulnerable stage for *P. interpunctella*; in fact 4 days were required to obtain 100% mortality on coffee, while the same result was obtained for cocoons after 24 hours on coffee and after 48 hours on artificial diet (Table 2).

On the whole *P. interpunctella* individuals show a longer survival on artificial diet (Table 3).

L. serricorne resulted to be very susceptible to nitrogen and carbon dioxide atmosphere; 100% mortality occurs after 24 hours from the packaging for both diets. On the contrary eggs are the most resistant stage as no egg hatching is observed after a stay of 7 and 8 days in nitrogen atmosphere respectively on coffee and artificial diet (Tables 4-5).

A high survival of individuals is observed in the tests carried out at atmospheric pressure, in jars with coffee previously aired (Table 6)

Table 1 - Percentage of CO_2 and O_2 and pressure varying from 0.2 to 0.4 bar registered in coffee packages after 1-7 days from test starting.

Day	%CO_2	%O_2
1	14.6±0.5	0.7±0.2
2	16.9±0.3	0.5±0.2
3	25.2±0.3	0.5±0.2
4	28.9±0.3	0.3±0.2
5	38.0±0v5	0.3±0.2
6	38.0±0.5	0.4±0v2
7	40.4±0.2	0.6±0.2

Table 2 - Average number (Dev.st ±) of eggs, II-III, IV larvae and cocoons of Plodia interpunctella *(Hbn.) survived on artificial diet after staying for different period inside a jar with not aired coffee in modified atmosphere (0.3-0.7% O_2;14.6-40.4% CO_2; 0.2-0.4 bar).*

Days	Eggs	II-III instar larvae	IV instar larvae	Cocoons
1	1.5±1.3	1.8±1.0	4.0±2.2	0.2±0.5
2	0.8±1.0	1.3±1.5	3.3±1.7	0
3	0.5±1.0	0.5±1.0	1.0±0.8	-
4	0.5±1.0	1.3±1.3	0.8±1.0	-
5	0	0.5±0.6	0.3±0.5	-
6	-	0	0	-
Control*	80.3±4.6	9.5±0.6	9.5±0.6	9.7±0.5

** Average number (Dev.st+) of individuals of* Plodia interpunctella *survived on artificial diet observed after a 6 day staying in thermostate (26±1°C; 65±5% R.H.).*

Table 3 - Average number (Dev.st ±) of eggs, II-III, IV larvae and cocoons of Plodia interpunctella *(Hbn.) survived on coffeet after staying for different period inside a jar with not aired coffee in modified atmosphere (0.3-0.7% O_2;14.6-40.4% CO_2; 0.2-0.4 bar).*

Days	Eggs	II-III instar larvae	IV instar larvae	Cocoons
1	0.5±1,0	0.3±0.5	1.8±1.3	0.7±0.9
2	0.3±0,5	0.3±0.5	1.0±1.2	0
3	0.3±0,5	0	0	-
4	0	-	-	-
Control*	76.2±4.4	-	9.5±0.6	9.5±0.6

** Average number (Dev.st+) of individuals of* Plodia interpunctella *survived on artificial diet observed after a 4 day staying in thermostate (26±1°C; 65±5% R.H.).*

Table 4 - Average number (Dev.st±) of eggs, larvae, pupae and adults of Lasioderma serricorne *(F.) survived on artificial diet after staying for different period inside a jar with not aired coffee in modified atmosphere* (0.3-0.7% O_2;14.6-40.4% CO_2; 0.2-0.4 bar).

Days	Eggs	Larvae	Pupae	Adults
1	4.0±1.4	2.3±1.0	1.3±0.5	0.5±0.6
2	2.5±1.3	2.0±0.8	0.5±0.6	0
3	1.5±1.3	1.5±1.3	1.5±1.3	-
4	0.8±1.0	0.8±1.0	0.8±1.0	-
5	0.3±0.5	0	0	-
6	0.5±0.6	-	-	-
7	0.3±0.5	-	-	-
8	0	-	-	-
Control*	23.2±2.6	16.8±1.6	22.4±3.4	10

* *Average number (Dev.st±) of individuals of* Lasioderma serricorne *survived on artificial diet observed after a 8 day staying in thermostate (26±1°C; 65±5% R.H.).*

Table 5 - Average number (Dev.st ±) of eggs, larvae, pupae and adults of Lasioderma serricorne *(F.) survived on coffeet after staying for different period inside a jar with not aired coffee in modified atmosphere (0.3-0.7% O_2;14.6-40.4% CO_2; 0.2-0.4 bar).*

Days	Eggs	Larvae	Pupae	Adults
1	2,8±1,4	3.0±0.8	0.5±1.0	0.7±0.9
2	3.0±0.8	1.8±1.3	0.5±0.6	
3	1.8±1.3	1.0±1.2	0.3±0.5	-
4	0.5±0.6	0.3±0.5	0.5±0.6	-
5	0.5±0.6	0	0	-
6	0.3±0.5	-	-	-
7	0	-	-	-
Control*	23.6±2.9	14.8±2.4	21.2±3.8	9.7±0.5

* *Average number (Dev.st±) of individuals of* Lasioderma serricorne *survived on artificial diet observed after a 7 day staying in thermostate (26±1°C; 65±5% R.H.).*

CONCLUSIONS

Time required to control *Plodia interpunctella* and *Lasioderma serricorne* in the different stages increases in presence of a substrate which favours the species development. II-III and IV instar larvae of *P. interpunctella* and *L. serricorne* eggs are the least susceptible stages.

Table 6 - Average number (Dev.st ±) of individuals in the different stages of Plodia interpunctella *(Hbn.)* and Lasioderma serricorne *(F.) on artificial diet and coffee after a 10 day staying inside a jar with coffee previously aired for a month.*

Plodia interpunctella				
Substrate	**Eggs**	**II-III instar larvae**	**IV instar larvae**	**Coocons**
Artificial diet	78.7±4.1	8.7±0.5	8.7±0.5	9.7±0.5
Coffee	81.7±2.2	7.5±0.6	8.0±0.8	9.7±0.5
Lasioderma serricorne				
Substrate	**Eggs**	**Larvae**	**Pupae**	**Adults**
Artificial diet	20.7±1.7	16.5±2.1	20.5±1.9	9.5±0.6
Coffee	19.5±1.3	15.3±1.5	20.2±0.9	9.7±0.5

Jay *et al.* (1990) point out that 4 days are enough to obtain 100% mortality of *L. serricorne* eggs exposed to atmosphere 99% of nitrogen at 32°C an 55-60% R.H., while 3 days are enough for adults. Soderstrom *et al.* (1986) observe that, at a nitrogen concentration of 99% (at 21°C and 60% R.H.) 70 hours are required to obtain 95% mortality of *P. interpunctella* cocoons. Soderstrom and Brandl (1983) discover 100% mortality of this insect after 48 hours of exposure in modified atmosphere (O2 0.5%; $CO_2$12-14%; N_2 85%) at 27°C and 50% R.H. Caliboso *et al.* (1994) observed a significant reduction of treatment period (time) in presence of carbon dioxide at high pressures (5-10-20-30 kg/cm^2) compared to that one obtained with carbon dioxide at standard pressure.

The results of this test, carried out at a pressure higher than the atmospheric one, due to the presence of carbon dioxide, normally produced by coffee, show a decrease of treatment time to control these insects.

Also a light pressure inside the packages (0.2-0.4 bar) helps to reduce time required to obtain the control of the population.

Roasted coffee, ground and stored in airtight packages in presence of protective atmosphere with nitrogen and pressurised carbon dioxide, don't permit the survival of *P. interpunctella* and *L. serricorne* in all the developmental stages, considering also the average technical days of presence in the market (at least 10 days from after the production).

The presence of alive insects can be casually verified in the case of jars previously opened by the consumer and not properly closed.

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