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Response of eleven stored product pest species to modified atmospheres with high carbon dioxide concentrations

J. Riudavets[1,*], C. Castañé[1], O. Alomar[1], M.J. Pons[1], R. Gabarra[1]

Abstract

Modified atmospheres with high carbon dioxide concentrations have been used as an alternative treatment to methyl bromide for the control of stored product pests. In this study, two different methods were chosen to apply carbon dioxide (CO_2): modified atmosphere packaging and high pressure treatments. The aim of our research was to compare the mortality of a range of stored product pests when treated with the two methods in order to identify which species were the most tolerant and at what stages of their development. The coleoptera *Lasioderma serricorne*, *Cryptolestes ferrugineus*, *Oryzaephilus surinamensis, Tribolium confusum*, *Rhyzopertha dominica*, *Sitophilus oryzae* and *Acanthoscelides obtectus*, the lepidoptera *Ephestia kuehniella* and *Plodia interpunctella*, the psocid *Liposcelis bostrychophila*, and the mite *Tyrophagus putrescentiae* were the species tested. Eggs, larvae, pupae and adults were exposed to two sub-lethal dosages of each method (packaging: 50 % and 90 % CO_2 for 4 days, and high pressure: 15 and 20 atm of pressure for 15 minutes) and the resulting mortality was recorded. For modified atmosphere packaging, pupae of coleoptera and lepidoptera were the least sensitive stages and could therefore be used to further fine-tune optimal dosages for controlling all their developmental stages. For high pressure treatments, the least sensitive stages were *L. serricorne*, among coleoptera with external feeding habits, and preimaginal developmental stages of *S. oryzae* and *A. obtectus* among internal feeders. These may therefore be the best stages for studying the efficacy of CO_2 treatments. The egg of *L. bostrychophila* was the least sensitive stage and was therefore recommended for testing both methods of CO_2 application. Determining pest species and the developmental stages that infest a particular food commodity could facilitate selection of a best method of application for modified atmospheres and most effective dosage

Key words: Modified atmospheres, carbon dioxide, packaging, high pressure, stored product pests.

Introduction

Modified atmosphere treatments are safe and environmentally friendly ways of controlling insects and mites that affect a large number of raw and manufactured products. In several developed countries, they have been adopted as feasible alternative treatments since the use of methyl bromide was phased out in 2005. Modified atmospheres have been used for many years and have been tested in the laboratory and under industrial conditions for the control of various different insect and mite species (Fleurat-Lessard, 1990; Adler et al., 2000; Navarro, 2006). According to the Navarro (2006) classification,

[1] Departament de Protecció Vegetal. IRTA Cabrils. Ctra. Cabrils Km 2. E-08348 Cabrils (Barcelona). Spain.

* Corresponding autor: Fax: +34937533954. e-mail address: jordi.riudavets@irta.es

they can be applied at atmospheric pressure and under vacuum or high pressure conditions. In general, when seeking a high degree of control efficacy, high pressure treatments are much faster than vacuum or atmospheric pressure applications. However, the application of high pressure treatments also calls for high pressure equipment, which represents an important capital investment. Most studies of high carbon dioxide (CO_2) modified atmospheres have focused on the control of pests affecting raw or semi-processed food products as an alternative to the use of conventional chemical fumigants and insecticides. However, this technology can also be applied to final products during the packaging process in order to prevent the development of pests that are present after the manufacturing process.

Most pest species affecting stored products are cosmopolitan. A good number of them are polyphagous, infesting cereals, cereal by-products, dried fruits, dried herbs and spices, semi-processed food materials and manufactured final products. Other species only infest specific food products, such as legumes. Among the pest species present in the north east of Spain, the coleoptera *Lasioderma serricorne* (F.) is the most important on dried herbs and spices, but it also infests many semi-processed and manufactured food products of plant origin (Riudavets et al., 2002a). For dried fruits, the external feeding coleoptera *Cryptolestes ferrugineus* (Stephens), *Oryzaephilus surinamensis* (L.) and *Tribolium confusum* Jacquelin du Val, and the lepidoptera *Ephestia kuehniella* Zeller and *Plodia interpunctella* (Hübner) are the most abundant pest species. They are also the most problematic pests in cereal by-products and many other manufactured food products. The key pests affecting stored whole grain cereals are the internal feeding Coleoptera *Rhyzopertha dominica* (F.) and *Sitophilus oryzae* (L.). The psocid *Liposcelis bostrychophila* Badonnel is traditionally considered a polyphagous secondary pest, although it is now also often present in stored cereals and cereal by-products. The main pest affecting dried beans is the internal feeding coleoptera *Acanthoscelides obtectus* (Say). The mite *Tyrophagus putrescentiae* (Schrank) is the most important pest affecting a wide range of food products stored under high humidity conditions.

A lot of work has been published on modified atmospheres and the effects of different CO_2 treatments and dosages on key pests (Navarro, 1978; Donahaye et al., 1994; White et al., 1995; Banks and Annis, 2000). Some of these studies have compared the responses of a few key-pests to the same set of treatments. Even so, these studies have seldom included a wide range of different species or developmental stages or represented a major pest complex for a wide range of food products. The objective of this study was therefore to compare the toxicity of high CO_2 modified atmospheres for the 11 cited pests and their respective development stages. An additional objective of this study was to identify insect species and/or developmental stages that are difficult to kill and could be used as models for further determining critical methods and dosages. We therefore compared the effect of the toxicity of two different modified atmosphere treatments (modified atmosphere packaging and CO_2 at high pressure) on eggs, larvae, pupae and adults of these pest species. Based on previous experience (Riudavets et al., 2002b), we selected 50 and 90 % CO_2 for 4 days for modified atmosphere packaging, and 15 and 20 atm for 15 minutes for the high pressure treatment, expecting that this should show clear mortality patterns but not kill all stages of the different pest species tested.

Material and methods

The species tested were four external feeding coleoptera (*L. serricorne, C. ferrugineus, O. surinamensis, T. confusum*), three internal feeding coleoptera (*R. dominica, S. oryzae* and *A. obtectus*), two lepidopterans (*E. kuehniella* and *P. interpunctella*), a psocid (*L. bostrychophila*), and a mite (*T. putrescentiae*). Individuals of these species were reared on standard diets in a climatic chamber at 25 ± 1 ºC, 70 ± 10 % r.h., and with a photoperiod of 16:8 L:D. Eggs, larvae, pupae and adults of each species were evaluated separately, although in the case of *T. putrescentiae*, all stages were tested together, while for *S. oryzae* and *A. obtectus*, larvae and pupae were tested together. Ventilated plastic cages were prepared containing a minimum of 50 individuals per cage and 20 g of standard food diet.

Two different methods were tested: (a) modified atmosphere packaging (MAP) at atmospheric pressure, and (b) CO_2 at high pressure. To apply MAP, one ventilated cage was placed inside a plastic bag (Cryovac BB4L, of 300 x 210 mm) that was then filled with the desired atmosphere, using a vacuum packaging machine (Multivac A 300/16). Modified atmospheres were previously prepared using a gas mixer (Witt KM 100-3M/MEM). Two different modified atmospheres were prepared: with 50 % and 90 % CO_2, 3 % residual oxygen, and 47 % and 7 % balances of nitrogen, respectively. A gas analyzer (Abiss model TOM 12) was used to verify the concentrations of carbon dioxide and oxygen inside the plastic bags, which were kept in a climatic chamber for 4 days. The bags were subsequently opened to release the modified atmosphere and the cages were removed from the bags.

To apply CO_2 at high pressure, the cages were placed in a high pressure chamber (Carburos Metálicos S.A.) with a volume of 2.2 m^3 connected to a CO_2 tank. Gas was introduced into the chamber until the desired pressure was achieved. Two different pressures were tested: 15 and 20 atm. Once the target pressure was reached, it was maintained for 15 minutes. The process of decompression took 30 seconds.

For both methods, cages were kept in the climatic chamber for up to seven weeks after treatment to allow individuals of the different developmental stages to develop until adulthood. When adults were treated, cages were left for 24 hours. The number of living adults in each cage was counted and the percentage of mortality was calculated using the initial number of individuals placed in each cage. Fifteen repetitions were prepared for each developmental stage and species. Sets of control cages were used to determine the percentage of natural mortality. The observed mortality data were corrected for control mortality using Abbott's formula. The resulting average mortalities were then classified according to toxicity categories developed for laboratory testing of the residual effects of pesticides on insects (Hassan et al., 1987): 1 = harmless (< 30 % mortality); 2 = slightly harmful (30 – < 80 % mortality); 3 = moderately harmful (80 - < 99 % mortality); 4 = harmful (= > 99 % mortality).

Results and discussion

In tables 1, 2, 3 and 4 we present toxicity categories for all tested species and stages. Category 4 (equal to or greater than 99 % mortality) is highlighted.

Modified atmosphere packaging. Results of applying this method can be seen in Tables 1 and 2. When all the tested species were exposed to 50 % CO_2 for four days, only two (*E. kuehniella* and *L. bostrychophila*) had mortality rates higher than 99 % for all of the stages tested. It is worth noting that, except for *R. dominica*, this treatment was harmful for all coleopteran adults, which indicated that it was sensitive to the application of CO_2. The beetle *R. dominica* and the mite *T. putrescentiae* showed a low level of sensitivity to this treatment. Increasing the CO_2 level to 90 % increased mortality for the lepidoptera and coleoptera tested. All stages of both lepidoptera (*E. kuehniella* and *P. interpunctella*) and of two stages of coleoptera (*O. surinamensis* and *A. obtectus*) had mortality rates of above 99 %. All adult coleoptera and lepidoptera, and the psocid also died under these conditions. However, pupae of five coleoptera species demonstrated low levels of sensitivity to CO_2. *T. putrescentiae* also showed a low level of sensitivity to this dosage.

Table 1. Toxicity of 50 % CO_2, 3 % O_2 and 47 % N_2 modified atmosphere packaging for 4 days with eleven stored product pests. Toxicity class: 1 = harmless (< 30 % mortality); 2 = slightly harmful (30 – < 80 % mortality); 3 = moderately harmful (80 - < 99 % mortality); 4 = harmful (= > 99 % mortality).

	Eggs	Larvae	Pupae	Adults
COLEOPTERA external feeders				
Lasioderma serricorne	3	2	3	4
Cryptolestes ferrugineus	4	4	3	4
Oryzaephilus surinamensis	4	4	3	4
Tribolium confusum	3	3	3	4
COLEOPTERA internal feeders				
Rhyzopertha dominica	3	3	3	3
Sitophilus oryzae	3	2	4	
Acanthoscelides obtectus	NT	NT	NT	
LEPIDOPTERA				
Ephestia kuehniella	4	4	4	NT
Plodia interpunctella	4	4	3	NT
PSOCOPTERA				
Liposcelis bostrychophila	4	4	-	4
ACARI				
Tyrophagus putrescentiae			3	

Table 2. Toxicity of 90 % CO_2, 3 % O_2 and 7 % N_2 modified atmosphere packaging for 4 days with eleven stored product pests. Toxicity class: 1 = harmless (< 30 % mortality); 2 = slightly harmful (30 – < 80 % mortality); 3 = moderately harmful (80 - < 99 % mortality); 4 = harmful (= > 99 % mortality).

	Eggs	Larvae	Pupae	Adults
COLEOPTERA external feeders				
Lasioderma serricorne	4	4	3	4
Cryptolestes ferrugineus	4	4	3	4
Oryzaephilus surinamensis	4	4	4	4
Tribolium confusum	3	4	3	4
COLEOPTERA internal feeders				
Rhyzopertha dominica	4	4	3	4
Sitophilus oryzae	4	3	4	
Acanthoscelides obtectus	4	4	4	
LEPIDOPTERA				
Ephestia kuehniella	4	4	4	NT
Plodia interpunctella	4	4	4	NT
PSOCOPTERA				
Liposcelis bostrychophila	3	4	-	4
ACARI				
Tyrophagus putrescentiae			3	

Table 3. Toxicity of carbon dioxide 15 atm pressure treatments for 15 minutes with eleven stored product pests. Toxicity class: 1 = harmless (< 30 % mortality); 2 = slightly harmful (30 – < 80 % mortality); 3 = moderately harmful (80 - < 99 % mortality); 4 = harmful (= > 99 % mortality).

	Eggs	Larvae	Pupae	Adults
COLEOPTERA external feeders				
Lasioderma serricorne	1	3	2	2
Cryptolestes ferrugineus	2	2	1	4
Oryzaephilus surinamensis	2	2	4	2
Tribolium confusum	2	NT	NT	NT
COLEOPTERA internal feeders				
Rhyzopertha dominica	4	4	3	1
Sitophilus oryzae	2	3	2	
Acanthoscelides obtectus	2	1	1	
LEPIDOPTERA				
Ephestia kuehniella	3	4	3	NT
Plodia interpunctella	4	NT	NT	NT
PSOCOPTERA				
Liposcelis bostrychophila	3	4	-	4
ACARI				
Tyrophagus putrescentiae		4		

Table 4. Toxicity of carbon dioxide 20 atm pressure treatments for 15 minutes with eleven stored product pests. Toxicity class: 1 = harmless (< 30 % mortality); 2 = slightly harmful (30 – < 80 % mortality); 3 = moderately harmful (80 - < 99 % mortality); 4 = harmful (= > 99 % mortality).

	Eggs	Larvae	Pupae	Adults
COLEOPTERA external feeders				
Lasioderma serricorne	1	3	3	2
Cryptolestes ferrugineus	4	4	3	4
Oryzaephilus surinamensis	4	4	4	4
Tribolium confusum	4	4	3	4
COLEOPTERA internal feeders				
Rhyzopertha dominica	4	4	4	4
Sitophilus oryzae	2	3	4	
Acanthoscelides obtectus	2	2	4	
LEPIDOPTERA				
Ephestia kuehniella	4	4	4	NT
Plodia interpunctella	4	4	4	NT
PSOCOPTERA				
Liposcelis bostrychophila	2	4	-	4
ACARI				
Tyrophagus putrescentiae		4		

CO_2 at high pressure. Results for this method can be seen in Tables 3 and 4. When the species tested were exposed to 15 atmospheres of pressure for 15 minutes, the mite *T. putrescentiae* was the only species killed. None of the individuals tested of this species survived to adulthood. Eggs and larvae of *R. dominica* were also very sensitive to this treatment. *Plodia interpunctella* was sensitive too, but since we only tested their eggs, it is difficult to draw other than limited conclusions. When pressure was increased to 20 atmospheres of CO_2 applied for 15 minutes, all stages of the lepidoptera, the coleopteran *O. surinamensis*, and the mite had mortality rates of equal to or above 99 %. Adults of all coleoptera and of the psocid were also easily killed. On the other hand, coleopteran pupae and eggs of both the coleoptera *S. oryzae* and *A. obtectus* and of the psocid *L. bostrychophila* showed no sensitivity to this high pressure treatment. None of the stages of *L. serricorne* was sensitive to this treatment.

Although it is often accepted that internal feeders on whole grains could be more difficult to kill than external feeders, due to the protection that the grain may offer the insect, this did not seem to be the case in our study. For both the modified atmosphere packaging and high pressure treatments, the sensitivity of the coleoptera tested was apparently unrelated to with their feeding habits. In other words, coleopteran pests that affect whole grain crops (*R. dominica*, *S. oryzae*) and legumes (*A. obtectus*) and have internal larval stages, o coleopteran pests for cereal by-products and other processed food products and have external larval stages (i.e. *L. serricorne*). In our study, both groups of coleopterans exhibited comparable sensitivities under each of the four test conditions (Tables 1, 2, 3, 4).

In our studies, some insect species and/or developmental stages showed different levels of sensitivity to the modified atmosphere packaging and high pressure treatments. They could therefore be used as models to further fine-tune the selection of optimal dosages for controlling all the developmental stages of most species.

Among pests affecting cereal by-products, dried fruits, and other manufactured food products, the pupae of coleoptera (the most tolerant of which are *L. serricorne*, *C. ferrugineus* and *T. confusum*) could be used to determine effective dosages of control in modified atmosphere packaging. However, when moths are the only target pest, the pupae of *P. interpunctella* could be selected as the best stage to test for determining effective dosages. On the other hand, for high pressure treatments, the eggs of *L. serricorne* could be used to determine effective control dosages for coleoptera, while the eggs and pupae of the moth *E. kuehniella* could provide better models than those of *P. interpunctella*. As with results obtained with other fumigants (Ho and Winks, 1995; Leong and Ho, 1995), the eggs of *L. bostrychophila* were the least sensitive stage and therefore the ideal model stage for studying the two methods.

Pests affecting grain cereals include the pupae of *R. dominica* and the larvae/pupae of *S. oryzae*, which could be used to determine the most effective dosages for control in modified atmospheres treatments. For high pressure treatments, the eggs or larvae/pupae of *S. oryzae* seem to offer the best options. For testing the main pest species that affect legumes, *A. obtectus*, the eggs and larvae/pupae were the most tolerant stages treated at high pressure. No differences were observed between stages when this species was treated under modified atmosphere packaging conditions at the dosages tested.

In the case of the results obtained with the mite *T. putrescentiae*, it is important to stress that the levels of mortality observed were greatly determined by the control method chosen. The mortality rates for all stages of this pest were above 99 % for high pressure treatments, even at 15 atm for 15 minutes, a dosage at which all other species were tolerant. For modified atmosphere packaging mortality rates were as low as for tolerant stages of other species tested. Due to the methodology applied, it was not possible to determine the most sensitive stage for this species.

From our results, it can be concluded that mortality responses varied according to the treatment method applied, developmental stage and species tested. It is therefore important to determine the candidate pest species and the developmental stages that could infest a food commodity to select the best method of application and most effective dosage with which to obtain the most effective level of control.

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