EFFICACY OF CONTROLLED ATMOSPHERES ON TEN STRAINS OF THE GRANARY WEEVIL *SITOPHILUS GRANARIUS* (L.) FROM DIFFERENT PLACES OF ORIGIN

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ABSTRACT

The present study aims to compare the efficacy of controlled atmospheres (CA) against laboratory and newly collected field strains of the granary weevil from different places of origin (F, GB, CAN, USA, AUS and FRG). The following three gas mixtures, each resembling the gas composition in a silo bin after initiation of a commonly used CA fumigation method, were tested under laboratory conditions at 20°C / 75% r.h.:

Gas 1: 99% N_2 1% O_2 (resembling a purge with pure N_2)
Gas 2: 80% N_2 1% O_2 19% CO_2 (resembling inert atm. generator)
Gas 3: 4% N_2 1% O_2 95% CO_2 (resembling a purge with pure CO_2)

Five developmental stages of defined age and adult weevils of each strain were fumigated in 2.3 l Drechsel flasks for periods from one day to 8 weeks. Pupae and late larvae proved to be the most tolerant stages, the longest LT_{99} was 29.7 days. Against these stages the gas mixture containing a high carbon dioxide content (gas 3) was not in all strains significantly more effective than the other two gas mixtures (gas 1 and gas 2). Results indicate that different time spans required for egg to adult-development have an effect on the relative tolerance of the strains. This relative tolerance of one strain compared to the others is not inherited from one developmental stage to the next. There is some evidence for a correlation between the efficacy of CO_2 and the degree of respiratory activity of developmental stages. None of the tested strains showed any sign of developing resistance against low oxygen atmospheres. The degree of tolerance did not correlate with medium body size or weight of adult weevils.

INTRODUCTION

If one compares the efficacy of controlled atmosphere (CA) fumigations on stored-product insects, one is often confronted with widely diverging or even contradictory results. On one hand, this may be caused by the use of different methods (rearing conditions, temperature, r.h., age of developing stages, gas composition, fumigation methods, etc.). On the other hand, genetic differences within a species of test insects could also play a role. The granary weevil, *Sitophilus granarius* (L.) (Col., Curculionidae), is one of the stored-product insects, most tolerant to a variety of control measures. Therefore, this species is often chosen for assessments of the efficacy of control methods.

Experimentally, it is possible to induce an increased tolerance of *Sitophilus* species against atmospheres containing high amounts of carbon dioxide. Bond and Buckland (1979) exposed adult granary weevils to an atmosphere containing 42% CO_2 in air (25°C / approx. 100% r.h.) until the mortality reached 60% or more. After selecting 7 successive generations of adult weevils, the authors achieved a 3.3 fold increase in tolerance to the gas mixture. Navarro et al. (1985) selected ten consecutive generations of adult rice weevils *Sitophilus oryzae* (L.) (at 26°C / 100% r.h.) and found that an atmosphere containing 75% CO_2 in air produced a tolerance factor of 3.34.
Thus, it could be possible that in laboratory cultures high population densities, resulting in locally low oxygen concentrations and high CO_2 contents, also cause a selective pressure on the weevils for the development of increased tolerance against controlled atmospheres (Jay, personal comm.). Another cause for different levels of tolerance could be the genetic differences developing in geographically separated populations (genetic drift).
To study the influence of genetic predisposition on the response towards CA fumigations, ten laboratory and field strains of the granary weevil *S. granarius* from France, the United Kingdom, the United States of America, Canada, Australia and the Federal Republic of Germany were compared in fumigation experiments. At the same time, the efficacy of three different controlled atmospheres commonly used in practice was tested.

MATERIAL AND METHODS

Rearing Procedures

Rearing methods of the strains at their places of origin are described in table I. As indicated, eight strains came from laboratory cultures (-L) while two strains had been collected from infested grain stores (field strains:-f). Upon arrival, all the strains were reared on the same kind of wheat (German soft wheat, moisture content 14 ± 0.2%) at 25 ± 1°C. About 2500 young weevils per strain were placed on approx. 3000 wheat grains (142.2 g) for three days. As found in preliminary experiments (Adler and Reichmuth, 1988 and 1989), an infestation rate of 70-90% is thereby achieved. This procedure was repeated weekly to provide developmental stages of defined age. Because the fumigation started one day after separating the weevils from the grain that had been offered for oviposition, experiments were carried out with the following age groups:

Stage 1: eggs, 1-4 days after oviposition
Stage 2: 1^{st} and 2^{nd} larval stage, 8-11 days after oviposition
Stage 3: 2^{nd} and 3^{rd} larval stage, 15-18 days after oviposition
Stage 4: 4^{th} larval stage, 22-25 days after oviposition
Stage 5: prepupae and pupae, 29-32 days after oviposition
Weevils: adults, 1-3 weeks after emergence.

The same weevils were used for oviposition for a maximum period of five weeks to avoid decreasing oviposition rates due to aging females or injuries caused by sieving off the weevils.

Gas mixtures

In commercial CA fumigations of storage facilities, it is technically difficult and too expensive to expell the oxygen completely. Because oxygen contents of 1% do not negatively affect CA treatments and are easier to maintain in practice, the following gas mixtures were chosen:

Gas 1: 99% N_2 1% O_2
Gas 2: 80% N_2 1% O_2 19% CO_2
Gas 3: 4% N_2 1% O_2 95% CO_2

These gases were mixed manometrically from pure components using a SETARAM gas mixing apparatus (Rampé à gaz) and a vacuum pump. After 24 hours, the oxygen concentration was determined with a SERVOMEX Oxygen Analyser 570 A, which measures the paramagnetic properties of oxygen.
Gas 1 represents the gas composition in a silo bin after a purge with pure nitrogen until the amount of oxygen present is reduced to 1%. Gas 2 represents the use of an Exothermic Inert Gas Generator. Usually, fuel burning generators can produce a concentration of 11-12% CO_2 (Fleurat Lessard and Le Torc'h, 1986). But because an earlier study had shown that the addition of 10% CO_2 to an atmosphere containing N_2 and 2% O_2 cannot significantly enhance its toxic effects on the granary weevil (Adler and Reichmuth, 1988), a somewhat "idealized" gas mixture with a higher CO_2-content was used in this study. Gas 3 stands for a CO_2-fumigation with the ratio of N_2:O_2 remaining 4:1 (as in air).

Table I Culture conditions of the tested strains at their place of origin

culture strain	place of origin	culture temp.°C	grain m.c.%	indiv. /jar	ovip.. time	grain variety	grain (g)/jar	culture since
F-L	*INRA, Po nt de la Maye, France	25	14	500	14 d.	soft	1000	1968
GB-L1	*Min. of Agric., Slough Lab. UK	25	13	160	21 d.	Engl. hard	320	1952
GB-L2	*Min. of Agric., Slough Lab. UK	25	13	160	21 d.	Engl. hard	300	1979
CN-L1	*Agric. Canada, London,Ont.,CAN	25	14	200	2 d.	Fred. winter	580	1948
CN-L2	*Agric. Canada London,Ont.,CAN	25	14	200	2 d.	Fred. winter	580	1979
USA-L	*USDA, Savannah GA., USA	27	13	1000	3 d.	soft r. winter	380	1978
AUS-L	*CSIRO, Div.Ent. Canberra C.,AUS	25	11.5	1000	70 d.	Corella soft	400	1974
AUS-f	*CSIRO, Div.Ent. Canberra C.,AUS	28	12.5	200	42-56 d.	Corella soft	1000	1986/87
D-L	Fed. Biol. Res. C., Berlin, FRG	25	14	2500	3 d.	German soft	142	1952
D-f	Fed. Biol. Res. C., Berlin, FRG	25	14	2500	3 d.	German soft	142	1988

-L :laboratory strain -f: field strain

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In the above mentioned laboratories the strains are named as follows:
F-L: Bx L.I.D. / GB-L2: MeBr-resistant (originally from Canada) / CN-L2: CO_2-selected strain / AUS-L: SG 4 / AUS-f: GSG 66 (PH_3-resistant)(for others no special name given).

Fumigation methods

While the weevils were reared at 25 ± 1°C, the experiments were carried out at 20 ± 1°C because this temperature better resembles post harvest conditions in Central European grain stores. Earlier investigations had shown that a temperature drop from 25°C to 20°C does not cause increased mortality among adult granary weevils and developmental stages.

For the fumigation, each of the developmental stages was put into fine wire-mesh cages (50 mm long, 14 mm diam.). For each stage, exposure time and gas mixture, 70 complete kernels of the infested wheat were counted and placed into one cage. 50 young adult weevils (1-3 weeks after hatch) were placed into slightly larger wire-mesh cages (80 mm long, 14 mm diam.) together with approximately 3 g of wheat. All cages were closed with soft rubber stoppers. In fumigation experiments, the developmental stages and weevils of three different strains were fumigated together in 2.3 l Drechsel flasks. A different flask was used for each of the exposure times (1 day, 3 days, 1, 2, 3, 4, 5, 6 and 8 weeks) and gas mixtures, respectively. The gas was introduced into the flask through a glass tube with an opening close to the bottom, driving out the air through an outlet at the top of the flask. Drechsel flasks flushed with the same gas mixture were connected by PVC-tubing (i. diam. 8 mm, wall 2 mm). The gas had a temperature of 20°C and an r.h. of 75% that was adjusted by a saturated NaCl-solution (Winston and Bates, 1960). A row of flasks was fumigated for approximately 20 min. (flow rate 1000 ± 50 ml/min.) until the oxygen concentration at the gas outlet of the last flask was 1%. Because a continuous gas flow with low flow rates is difficult to install and even an airflow can cause considerable mortality among insects (Navarro, 1975), the flasks were flushed only once. After purging, the flasks were separated and kept closed for the desired time span. For comparison, untreated stages and weevils were kept under similar conditions in flasks which were just closed by a cotton cloth and a rubber band, thus allowing the continous exchange of air. In order to provide the same moisture conditions as in treated flasks, the air in the experimental chamber was kept at 75 ± 5% r.h. All experiments were carried out in at least three replicates.

After exposure, the flasks were opened and the oxygen concentration in the fumigated flasks was determined. All samples (infested grain and weevils) were transferred into glass petri dishes (diam. 60 mm) and kept at 25 ± 1°C / 75 ± 5% r.h. for at least 10 weeks. Fumigated eggs (stage 1) were kept for at least 12 weeks because in preliminary studies, weevils had been found hatching from stage 1-infested grain over 10 weeks after the end of the fumigation experiment. Hatch and weevil mortality were checked weekly. The per cent mortality of the developmental stages was determined by comparison with the numbers of weevils hatching in untreated samples. The per cent mortality of adult weevils was calculated by direct comparison of the numbers of survivors in treated and untreated samples. To avoid bias by natural mortality among weevils, Abbott correction (Abbott, 1925) was applied where necessary. LT_{99}-values were calculated by Probit analysis (Finney, 1971) using a special program on the personal computer (Noack and Reichmuth, 1978).

Weight and size determination of adult weevils

The medium weight was determined by measuring the weight of 1000 live adult weevils per strain in three replicates. The medium size was calculated by placing individuals (killed by cooling) under a microscope (20x magnif.) and by determining the length from the tip of the rostrum to the distal end of the abdomen. 25 male and 25 female weevils per strain were measured.

RESULTS AND DISCUSSION

When the flasks were opened after fumigation, the oxigen content was found to be reduced to 0.8% after 1 day, to 0.4% after 3 days and to 0.1% or less after 1 week. The oxygen concentration dropped more slowly in bottles fumigated with gas 3 reaching 0.1% or less after 2-3 weeks. Upon opening, often a reduced pressure was found in flasks fumigated with gas 2 or gas 3, which impaired the correct measurement of the oxygen content. This could have been caused by carbon dioxide being dissolved in the water fraction of the grain.

Comparison of strains

LT_{99}-values are presented in figure 1. As may be noted, the two German strains (D-L and D-f), the Australian field strain (AUS-f) and the first Canadian lab strain (CN-L1) were most tolerant among the tested strains. The British lab strain (GB-L1) was most sensitive to all tested gas mixtures. Another outstanding fact is that the eggs of this strain were found to be more tolerant to gas 3 than the pupae (stage 5), while in all other strains and gas mixtures the pupae were more tolerant than the eggs.

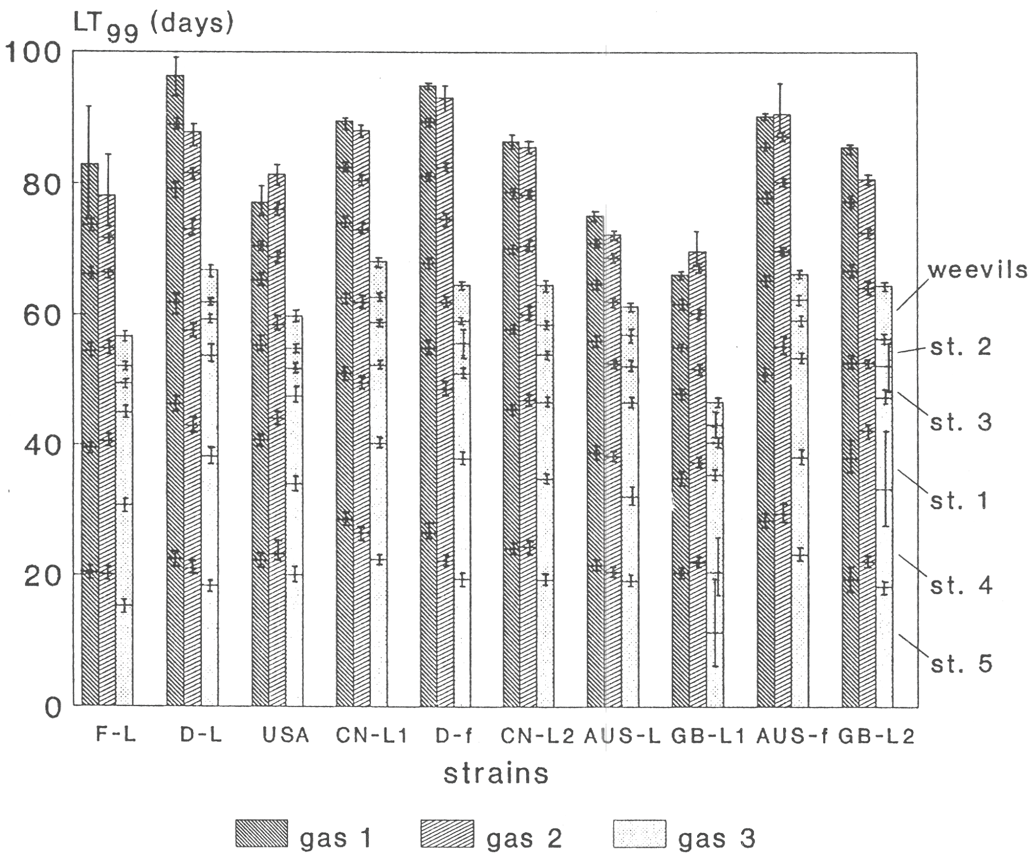

Figure 1 Comparison of strains

The Canadian lab strain 2 (CN-L2), formerly selected for CO_2-tolerance, seems to have lost its aquired tolerance during the timespan of non-selection. No increased tolerance to CO_2 was found in this strain, in comparison to the others.
Most important from the aspect of pest control is the comparison of the most tolerant stages (stage 4 and 5). That is why these stages were placed at the bottom of each column (fig. 1). Among these stages the differences between strains are the greatest (see also standard deviations in tab. III). The LT_{99}-values differ up to 13.7 days (stage 4, gas 1). However, only a few stages were found to be significantly more tolerant to a certain gas mixture than the rest. Among the most tolerant stages (stage 4 and 5), stage 4 of D-f is significantly more tolerant against gas 1 and stage 5 of AUS-f is significantly more tolerant against gas 2 with LT_{99}-values of 28.4 and 29.7 days, respectively. Stage 4 of the GB-L strain is significantly less tolerant to gas 1 and gas 3. All other LT_{99}-values of stage 4 and stage 5 were overlapping in their confidential zones.
Figure 2 shows the LT_{99}-values for gas 1 as a comparison of stages and it is obvious that different stages of a given strain vary considerably in their relative tolerance. Clearly, the degree of relative tolerance is not inherited from one developmental stage to another. This elucidates that while the height of the column of two strains in figure 1 may be equal, there could be significant differences in the degree of tolerance if stages are compared separately.

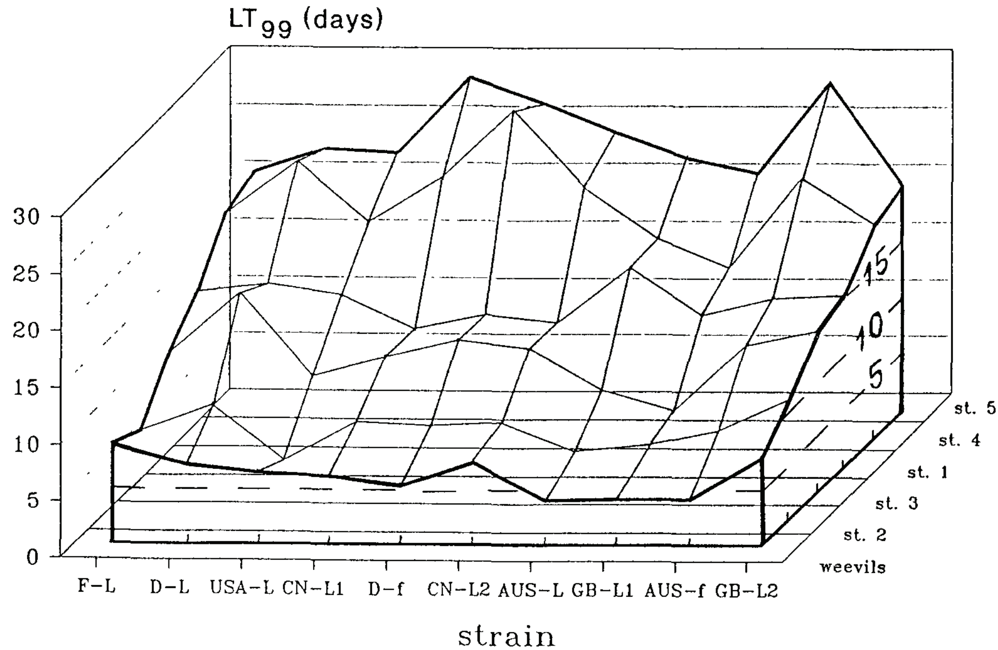

Figure 2 Comparison of stages, gas 1

Mean weight and size of adult weevils are listed in table II. Among the tested strains, there is no correlation between the mean weight or size and the degree of tolerance to the three gas mixtures.
As the results indicate, there are some differences in the degree of tolerance, but none of the tested strains shows any signs of developing resistance against low oxygen atmospheres.
As noted in earlier studies, all strains showed a marked retardation in egg-to-adult-development that was caused by all tested gas mixtures. It took up to 11 weeks at 25°C until adults developed out of fumigated eggs.

Table II Mean weight and size of adult weevils

	F-L	D-L	USA-L	CN-L1	D-f	CN-L2	AUS-L	GB-L1	AUS-f	GB-L2
weight of 1000 indiv. (g) *	2.62 ±.03	4.29 ±.13	3.33 ±.02	3.87 ±.04	3.35 ±.01	4.19 ±.07	3.05 ±.03	3.51 ±.04	2.53 ±.02	4.66 ±.03
mean size (mm) **	3.77 ±.20	4.89 ±.26	4.22 ±.24	4.36 ±.29	4.33 ±.24	4.33 ±.35	4.56 ±.26	4.29 ±.36	4.24 ±.31	4.41 ±.24

* : weight of 1000 live adult weevils, 3 replicates per strain

**: mean values from 25 male and 25 female weevils per strain, sizes of both sexes were combined, because the standard deviations were greater than the differences due to sexual dimorphism.

Comparison of gases

To compare the gas mixtures an average LT_{99}-value was calculated combining the values of all strains stage by stage (tab. III).
Gas 1 and gas 2 are quite similar in their efficacy against the stages of the granary weevil. From the mean values per developmental stage, the following order of increasing tolerance against gas 1 and gas 2 can be deduced: weevils / stage 2 / stage 3 / stage 1 / stage 4 / stage 5.
The order of increasing tolerance against gas 3 differs slightly in that the positions of stage 2 larvae and adult weevils are exchanged. (This aspect will be discussed later on).
It should be considered, however, that these orders of increasing tolerance are not found in all the strains. For example, in eight out of ten strains stage 5 (pupae) was most tolerant to all tested gas mixtures. But in two strains (D-L and D-f), stage 4 (late larvae) was more tolerant against gas 1 and gas 2 (D-L also against gas 3) than stage 5. In total, 73% of all LT_{99}-values were found on the positions described by the above given order.
The deviations from this order were at least partially caused by different time spans required for development: Even within one strain of granary weevils, egg-to-adult-development may vary by up to three weeks at 25°C. Therefore, intraspecific differences in the medium developmental times of different strains would not be surprising. As calculated from the maximum hatch in untreated samples exposed to 20°C for 1 day or 3 days, the two German strains (D-L and D-f) took only about 5.5 weeks on average for development from oviposition to adult emergence while the French (F-L) strain needed 5.8 weeks, the two Canadian strains needed 5.9 weeks and all other strains required 6 weeks or more on average until a maximum of weevils hatched. Thus, stage 4-infested grain of the two German strains could already

have contained a certain number of pupae. This assumption was confirmed when stage 4-infested grain of the D-L-strain was examined for other purposes. As a result, stage 5-pupae of the two German strains were closer to completion of their development and the hatching weevils may have been killed within the grain. This could explain the higher tolerance of stage 4 in these two strains.
Another evidence for this hypothesis is that in the F-L strain, the LT_{99}-values of stage 4 and stage 5 are close to eachother, as well (stage 4-values are smaller than stage 5-values in gas 1-, greater in gas 2- and equal in gas 3-fumigations). Moreover, the two strains that take the longest time span for development (AUS-L with 6.8 weeks, GB-L1 with 6.7 weeks) are in this experimental setting the most succeptible strains, as well. In order to avoid uncomparable results caused by variations in larval development it is important to gather data from all developmental age groups in future

Table III Comparison of gases

age group	LT_{99} in days gas 1	gas 2	gas 3
stage 1	14.5 ± 1.9	13.8 ± 2.4	14.0 ± 1.3
stage 2	8.1 ± 1.6	7.5 ± 1.0	3.4 ± 0.8
stage 3	12.2 ± 2.7	11.6 ± 1.7	5.4 ± 1.0
stage 4	21.0 ± 3.7	21.7 ± 2.9	15.4 ± 3.0
stage 5	23.9 ± 3.5	23.5 ± 2.8	18.8 ± 3.4
weevils	6.6 ± 1.6	5.9 ± 2.4	4.9 ± 1.4

All-strains-average and standard deviations

In fumigations with gas 3, young larvae (stage 2) were the most sensitive stage. Their medium LT_{99}-value was less than half of those produced in fumigations with the other two gas mixtures. Young larvae are known to have the highest rate of oxygen consumption of all developmental stages (Bailey, 1967). Thus, the toxic action of carbon dioxide could be correlated to the respiration rate. This interpretation is backed up by several other findings:
1. Also stage 3 and weevils, stages with a high oxygen consumption, were found to have significantly shorter LT_{99}-values after fumigations with gas 3.
2. Moreover, the LT_{99}-values of eggs, late larvae and pupae (stage 1, 4 and 5) are not much smaller than those produced by gas 1 and the standart deviations of these values are overlapping (tab. III).
3. In fumigation experiments with eggs of *Plodia interpunctella* (Hübner) and *Ephestia elutella* (Hübner) Reichmuth (1986) found that the same three gas mixtures produce almoust identical LT_{95}-values. Also his studies on the efficacy of gas 1 and gas 3 on the different developmental stages of *S. granarius* (Reichmuth, 1990) produced results comparable to the findings of the present survey.
An explanation for the correlation of CO_2-efficacy and respiration rate could be that stages with a high respiratory activity have the need to excrete

their exessive carbon dioxide. Usually, excretion by diffusion is no problem because of the high CO_2 gradient. But in an artificial atmosphere with 95% CO_2 stages with a high respiratory activity rapidly accumulate CO_2 before the process of dissimilation breakes down due to the lack of oxygen. This impaired excretion of CO_2 could speed up the toxic action of the surrounding CO_2-atmosphere.

The exposure times required for complete control of the tested granary weevil strains are only a few days shorter when CO_2 (gas 3) is used in comparison to a purge with N_2 (gas 1). The addition of 19% CO_2 to a nitrogen atmosphere (gas 2) does not increase the efficacy of the gas mixture. Therefore, in future other factors like worker safety, logistics, biochemical reactivity, environmental aspects and the price per volume may gain importance in the choice of the fumigant.

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LES EFFETS DES ATMOSPHERES MODIFIEES SUR DIX SOUCHES GEOGRAPHIQUEE DU CHARANÇON DES GRENIERS, *SITOPHILUS GRANARIUS* (L.)

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RESUME

L'étude vise à comparer l'efficacité des atmosphères modifiées (CA) vis-à-vis du charançon du grains, sur des souches de laboratoire et d'autres collectées dans différents pays (F, GB, CAN, USA, AUS et RFA). Les trois mélanges gazeux suivants, chacun ayant une composition similaire à celle du gaz des cellules des silos après mise en route d'une méthode de fumigation en atmosphère modifiée classique, ont été étudiés en laboratoire à 20° C et 75 % HR.

gaz 1 : 99 % N_2 1 % O_2 (rappelant la purge avec N_2)
gaz 2 : 80 % N_2 1 % O_2 19 % CO_2 (rapp. génération atm. inerte)
gaz 3 : 4 % N_2 1 % O_2 95 % CO_2 (rappelant la purge avec CO_2)

Cinq stades de développement des charançons de chaque souche géographique, d'âge défini et les adultes, ont été soumis à des expositions aux gaz dans des récipients en verre de 2 l de capacité pendant une période allant de 1 jour à 8 semaines. Le pourcentage de mortalité a été calculé par comparaison avec les témoins non traités et les valeurs de TL50 et TL99 ont été obtenues par analyse des probits de mortalité. Les nymphes et les dernières larves se sont montrées les plus tolérantes. Les mélanges gazeux contenant du dioxyde de carbone (gaz 2 et 3) n'ont pas été plus efficaces que l'azote (gaz 1). Certaines souches étaient plus tolérantes que d'autres mais aucun signe de résistance n'a été décelé, le plus long TL99 étant de 30 jours. Le degré de tolérance des différentes souches n'était pas fonction de la taille corporelle moyenne ou des différences de poids.