Study on the Influence of Temperature on the Mortality of *Tribolium confusum* J. du Val. Exposed to Carbon Dioxide or Nitrogen

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In the pest control of stored products like grain and flour, treatment with gases like CO_2 or nitrogen plays an increasing role, as this type of treatment leaves no residues in the food product and is less questionable than treatment with ionizing radiation. An important pest of stored grain is the beetle *Tribolium confusum*. *T. confusum* pupae, young and aged adults were exposed to pure nitrogen, CO_2 or air at different temperatures between 38 and 46 °C. In nitrogen as well as in CO_2 pupae were the most resistant. Nitrogen efficacy on adult mortality was much higher than CO_2 which was higher than air. For pupae CO_2 was more efficient than nitrogen or air in the range 38–45 °C. The temperature dependence of the sensitivity to both gases was noticeable in every stage, particularly below a given specific temperature. It was concluded that the combination controlled-atmosphere-temperature could be a valuable time saving and non aggressive technique for the stored-foods insects control.

Introduction

Physical methods of insect control of stored-foods are developing now since they do not leave toxic residues and do not lead to resistance phenomena. Various physical methods are available at the present time [1]. Their efficiency is often improved when several methods are combined [2, 3].

Irradiation is one of the available physical methods that has proved very useful in grain preservation [4] and quarantine treatments [5]. Its combination to other techniques like temperature [6] and exposure to nitrogen [7, 8] has given good results. However the preservation by irradiation of stored-foods has not been authorized in certain countries.

Various thermal treatments (fluidized bed, high frequency) are applied in grain preservation, processed food [9] and quarantine disinfestation of fruits [10].

Controlled-atmospheres are used in grain preservation as well as in quarantine control. They usually are performed in air tight containers during a period of several days. Since the storage bins or silos are never completely gas tight, renewal of the gas is always necessary. It appears possible to reduce the time of treatment and hence to avoid the replacement of the gas if the effect of the *controlled-atmosphere* addition is accelerated by temperature, as has been noticed by several workers [11]. Our present aim is to study this possibility in the temperature range of 35 to 50 °C. This zone lies between the temperature scale usually used in *controlled-atmosphere* treatments (from 5 to 35 °C) and the one used in thermal treatments (45 to 65 °C). The present study is carried out with *T. confusum* pupae and adults exposed to CO_2 or nitrogen, during various times.

Abbreviations: Probit 9 mortality, 99,9968% mortality; LT9, letal time to reach probit 9 mortality; CAT, *controlled-atmosphere-temperature*.

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Materials and Methods

T. confusum pupae and adults were derived from our laboratory colony. Insects were reared at 28 ± 2 °C and 65 ± 5% rh on whole wheat flour and yeast (19:1). The different treatments were assigned to 3 groups of 50 insects. The different stages studied were pupae 1 to 7 days old, young adults less than 1 day old and adults 2 to 3 months old.

Each group of 50 insects was placed in a 100 ml glass jar in which the gas was introduced until the oxygen concentration became less than 0.3% (Fig. 1). The jar was then partly submerged in a laboratory bath which was maintained at a given temperature ± 0.2 °C. After the exposure the in-

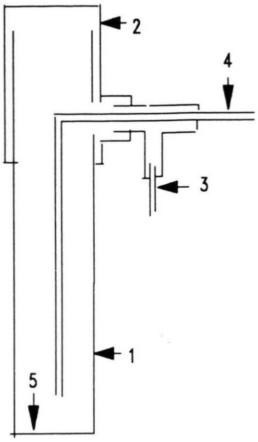

Fig. 1. Schematic diagram of the gas exposure setup. 1: Exposure glass jar. 2: glass stopper. 3: gas tube to the oxygen analyser. 4: gas inlet through a capilary tube. Tube 4 is removed when the oxygen content reaches 0,3%, then the stopper 2 is rotated on the vessel 1 in order to close the system. 5: group of 50 insects.

sects were transferred with wheat flour to petri dishes (dia 5 cm).

Mortality was recorded in adults that did not move 5 days after the treatment. In the experiments with pupae the pupae which did not emerge and the adults which did not live more than 5 days were recorded as dead. Control insects were used in all experiments. The letal time required to provide probit 9 (99.9968%) mortality (LT9) was calculated by a computer-programmed probit analysis according to "Voyons" [12] in which the mortality average of the three groups of insects was fitted to the integral of a Gaussian distribution. The 95% confidence limits of LT9 was obtained from the product of the LT9 standard deviation and the t-value of Student [13].

Results

With pupae the LT9 decreased more quickly with temperature in air than in nitrogen or CO_2 (Fig. 2). The probit mortality was reached in less than 60 min at 45 °C in both gases whereas in air for the same LT9 a temperature of 50 °C was needed. Nitrogen and CO_2 had nearly the same effect below the LT9 level of 100 min whereas nitrogen was more efficient than CO_2 above this level.

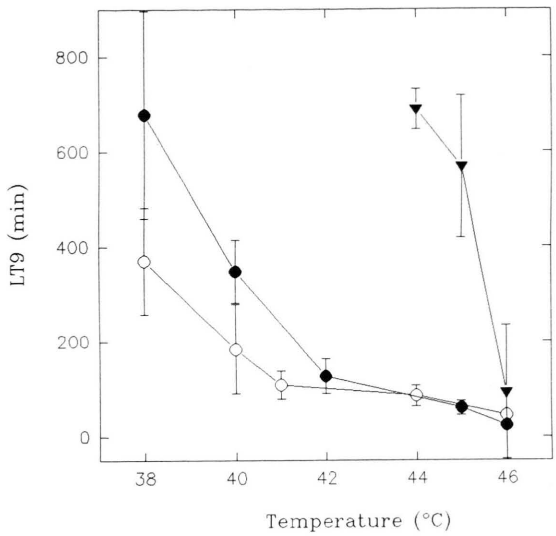

Fig. 2. Dependence of the probit 9 lethal time in *T. confusum* pupae, exposed to nitrogen (●), CO_2 (○) and air (▼), on temperature. Bars represent +/−95% confidence limits.

For old adults the dependence of LT9 on temperature shows that the slope suddenly changes at 30 °C if the gas is nitrogen (Fig. 3). In CO_2 and in air this change was not as steep as before and occurred respectively at 38 and 45 °C. For these three

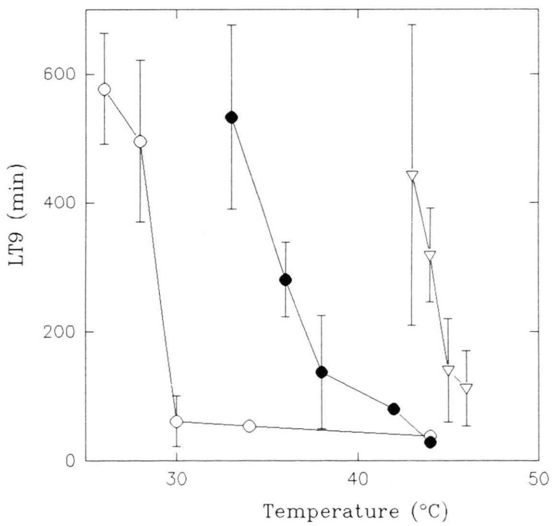

Fig. 3. Dependence of the probit 9 lethal time in *T. confusum* adults exposed to nitrogen (○) CO_2 (●) and air (▽), on temperature. Bars represent +/−95% confidence limits.

specific temperatures in nitrogen, CO_2 and air the corresponding LT9 were respectively 60, 120 and 120 min.

Young and old adults had the same thermosensitivity in CO_2 and in air (Fig. 4). By contrast the thermosensitivity of these two stages was quite different in nitrogen. At 38 °C the LT9 in young adults was 5-fold greater than in the old ones.

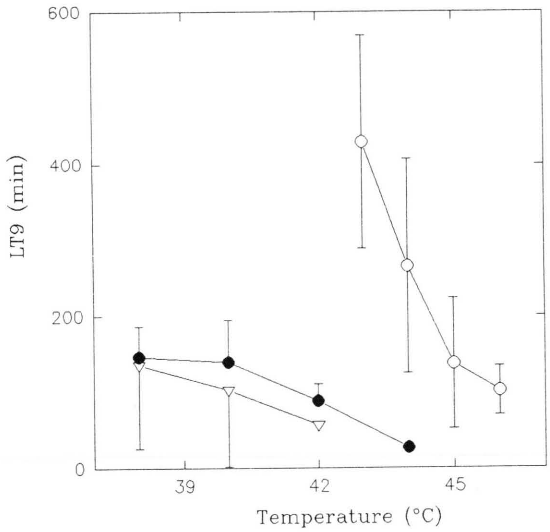

Fig. 4. Dependence of the probit 9 lethal time in *T. confusum* young adults exposed to nitrogen (▽), CO_2 (●) and air (○), on temperature. Bars represent +/−95% confidence limits.

Discussion

Thermal effect alone

Among the insects occurring in stored foods *Rhizoperta dominica* is the most heat tolerant species and is therefore often used as a reference model in practical investigations [14]. For *T. confusum* fewer bibliographical data are available. The LT9 values given by Lai and Ducoff [15] in the temperature interval of 44–46 °C were greater than ours (Fig. 5) partly because the experimental conditions were different. In fact the insects were given food during the treatment and the assigned temperature was reached with a certain delay. At 50 and 55 °C Knipling and Sullivan [16] indicated that complete mortality occurred in 50 and 15 min respectively. According to Faria Estacio (cited by Sokoloff, [17]) 15 min at 51 °C were fatal to all the stages of *T. confusum*.

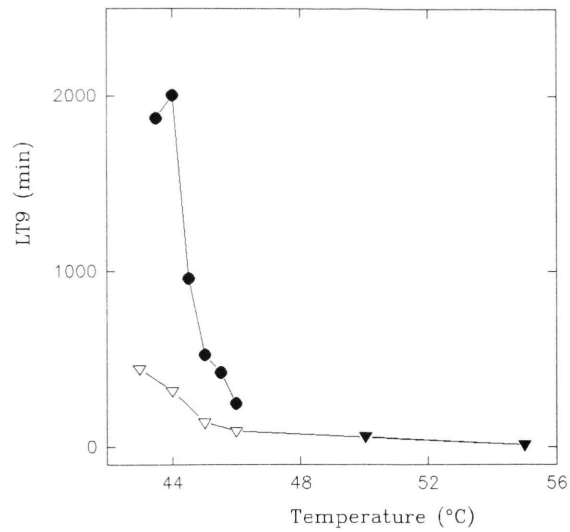

Fig. 5. Dependence of the probit 9 lethal time in *T. confusum* adults exposed to air on temperature according to Lai and Ducoff [15], (●) and Knipling and Sullivan [16] (▽) and to our results (▼).

Gas exposure

Usually *controlled-atmosphere* studies deal with different species exposed to gas mixtures containing various ratios of CO_2, nitrogen and oxygen. A detailed review was given by Annis [18].

In *T. castaneum* the efficacy of nitrogen exposure is highly dependent on the oxygen concentration which ought to be as low as possible. For instance Navarro [19] stated that the 95% lethal time at 26 °C increased from 1,5 to 4,5 days if the oxygen content increased from 0 to 1%. It seems that the sensitivities of *T. confusum* and *T. castaneum* to gas exposure are similar [20].

By contrast the effect of CO_2 is not influenced by low contents of oxygen as shown by Aliniazee [21]. For various oxygen concentrations below 10% at 26.7 °C, the 100% mortality in *T. confusum* was reached after 36 h.

The description of the efficacy of nitrogen compared to CO_2 in *T. castaneum* adults differs from one author to the other. Zakladnoy (cited by Bailey and Banks [20]) stated that CO_2 was more rapidly toxic than nitrogen (from 20 to 35 °C). According to Press and Harein [22] and Verma and Wahdi [23] pure nitrogen was more efficient than CO_2. Our results were in accordance with the latter statement in the temperature range 26–46 °C.

The sensitivity to CO_2 of the different stages varies. Aliniazee [21] noticed that the 100% mortality at 26.7 °C was reached in respectively 60, 42, 60 and 18 h in the eggs, larvae, pupae and adults. At 38 °C we observed correspondingly a 3-fold greater sensitivity of adults compared to pupae.

Temperature and exposure to the gases

Different authors noticed that temperature increased the efficiency of gas exposure in different species. Navarro and Calderon [24] have given the temperature dependence between 15 and 30 °C for *Sitophilus granarius, Rhizopertha dominica* and *T. castaneum*. When *Sitophilus granarius* was exposed to an atmosphere containing less than 1.3% oxygen [20] 2 weeks were needed to reach 99.5% mortality at 29.4 °C and 3 weeks at 23.9 °C. According to Zakladnoy (cited by Bailey and Banks [20]) the temperature had a great influence on the effect of pure nitrogen but a weaker one in the case of pure CO_2. To the contrary Aliniazee [21] found an appreciable effect of temperature on CO_2 exposure in *T. confusum* and *T. castaneum*.

The practical advantage of the *controlled-atmosphere-temperature* (CAT) treatment did not escape to other authors. With their experiments on *S. oryzae* treated between 15 and 35 °C Banks *et al.* [11] showed the importance of temperature in the control of insects in stored food products by the *controlled-atmospheres*. For quarantine purposes Whiting *et al.* [25] studied the combined effect of CO_2 and temperature treatment on an apple parasite *Epiphyas postvittana*. Newton [26] showed the efficacy of fumigation with an atmosphere containing 60% CO_2 heated to 35 °C in a plastic "bubble". Recently Soderstrom *et al.* [27] studied the effects of high temperature combined with CO_2 enriched atmospheres or reduced oxygen atmospheres.

Our results confirm the literature data on the temperature dependant efficacy of the *controlled-atmosphere* technique in the range 35–45 °C. In Fig. 6 our observations on the CO_2 exposure of *T. confusum* adults in the range 15–45 °C are compared to those of other workers.

The dependence of LT9 on temperature (Fig. 2 and 3) exhibits generally two slopes. This shows that for any insect stage, exposed to a given atmosphere a specific temperature value exists which

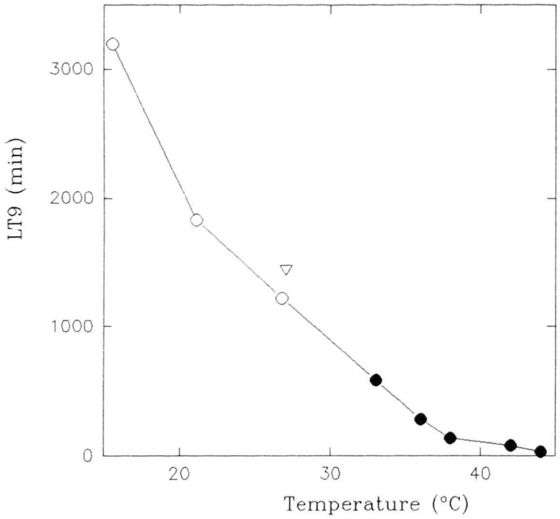

Fig. 6. Dependence of the probit 9 lethal time in *T. confusum* adults exposed to CO_2 on temperature according to Aliniazee [21] (○), Jay and Cuff [29] (▽) and our present studies (●).

corresponds to the optimum efficacy. As the temperature increases above this value the gain in time on the LT9 increases more slowly. Thus for pupae exposed to nitrogen this threshold value is 120 min at 42 °C. For practical purposes this limit should be determined for different species, stages and gases in order to choose the most efficient treatment. To give an example three efficient treatments against pupae (the more resistant stage) would be nitrogen–41 °C–275 min, nitrogen–42 °C–120 min, nitrogen–43 °C–100 min (Fig. 2). This result clearly shows that the second treatment is the best one as it saves time and heating.

Moreover, it was observed in our study that old adults exhibited in comparison to pupae and young adults an exceptionally high sensitivity to nitrogen (Fig. 3) which has apparently never been seen before. This particularity is probably a physiological characteristic of this species since we did not observe this high sensitivity to nitrogen in *Sitophilus granarius* adults subjected to the same treatment. At 42 °C 60 min was needed to get 100% mortality.

In conclusion one could ask wether the CAT would be useful in practice. At first view this method seems to set too many technological difficulties and to be too expensive to favour any further development even if it appears to be a time saving and non aggressive technique. Nevertheless the usefulness of this combined treatment has been illustrated by the technique of Newton [25] using heated circulating CO_2 in a plastic bubble. Hence, it appears that this study could be profitably extended to other species like the thermoresistant *Rhizopertha dominica* and also combined with other technologies as for instance the combination of *controlled-atmosphere* and plastic package (as used by New and Rees, [28]) together with high frequency or microwave treatment.

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