EFFECT OF IRRADIATION AND EXPOSURE TO NITROGEN ON MORTALITY OF ADULTS OF *TRIBOLIUM CONFUSUM* J. Du V.

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Abstract

For insect control purposes, the effect of irradiation can be improved by applying it in combination with other methods thus limiting the dose and hence preserving the sensory qualities of some foods like fresh fruits. In addition , combined treatment will kill more rapidly the adult stage, thereby avoiding the risk that a shipment will be rejected because of the presence of live insects.

The effect of irradiation with long exposure to inert gas has not been reported previously. This study presents preliminary results of combined irradiation plus nitogen gas treatment on *Tribolium confusum*.

Irradiation alone from 120 to 1000 Gy killed 100 % of *T. confusum* adults in 12-15 days. At 60 Gy, 10 % insects were still alive after 28 days and at 40 Gy no mortality was observed. Mortality of adults *T. confusum* observed 10 days after exposure to nitrogen increased with the exposure time; 100% mortality was attained after 17 hours' exposure.

When insects were exposed to nitrogen before or after irradiation synergistic effects were observed. The greatest efficiency was obtained when insects were irradiated at 600 Gy after 9 h exposure to nitrogen. When insects were exposed to nitrogen during irradiation, a 30-min exposure to nitrogen prevented mortality at doses of < 60 Gy. Exposure for 9 h to nitrogen prior to irradiation at 600 Gy was more efficient than irradiation followed by exposure to nitrogen.

Introduction

In food preservation, irradiation is often combined with other chemical or physical treatments in order to enhance the quality of the products and sometimes to reduce costs. For insect control, irradiation can also be improved by integration with other methods for two main objectives:

1. To limit the dose applied in order to preserve the sensory qualities of some foods such as fresh fruits, or for economical reasons.

2. To kill rapidly the adult stage: when irradiation alone is applied at doses high enough to control the population, larval stages are killed and adults are sterilized but they can live several

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days or even weeks and hence damage the food. Moreover the presence of living adults during routine inspection may bring about rejection of the infested product.

Different treatments can be combined with irradiation, such as: variation of temperature, microwaves and infra-red waves, fumigation, mechanical control and exposure to inert gases (Tilton and Brower, 1985).

The protection of stored products by controlled atmospheres has been used since antiquity. Oxygen deprivation for several hours causes the death of larval and adult stages of stored product insects (Carlson, 1968 a and b; AliNiazee, 1971; Le Torc'h, 1983; Spratt, 1984).

The combined effect of irradiation and long exposures to inert gases has yet not been studied. Most of the studies deal with irradiation under short exposure periods of about half an hour in an inert gas (Clark and Herr, 1955; Tilton *et al.*, 1965; Griffiths and Ducoff, 1973; Tilton *et al.*, 1985). They generally are intended to sterilize insects without decreasing their competitiveness, because the radioprotecting effect produced by the absence of oxygen is greater in somatic tissues than in reproductive ones (Baumhover, 1963; Langley and Maly, 1971; Hooper, 1971; Lachance and Richard, 1974; Ashraf *et al.*, 1975; Ohinata *et al.*, 1977; Economopoulos, 1977; Earle *et al.*, *1979;* Chang and Lee, *1984)* .

From a technical point of view it seems that the combination of irradiation with long exposures to an inert gas can be interesting for several reasons: inert gases do not leave toxic residues, they can protect the treated food, and the efficacy of the two treatments on the different stages complement each other since the adult stage is more radioresistant and more sensitive to anoxia than the larval stages.

Our purpose was to study the combined effects of exposures to nitrogen during varying time and irradiation doses on *Tribolium confusum* J. du V. adults. We intended more specifically to study whether the combined treatments have synergistic effects, and whether the order of application of each treatment relative to the other is important.

Materials and Methods

One week old *T. confusum* adults were obtained from our laboratory culture initiated in 1981. Insects were reared in darkness at 28°±1°C and 65±5% relative humidity (r.h.), on whole flour and yeast (19:1). Groups of 50 insects were placed in Petri dishes with 10 g of food.

The treatment produced a "knockdown" effect which made difficult the determination of mortality. Hence 3 classes were differentiated: dead insects showing black spots on the cuticle, lethargic and weak insects (knockdown), and insects able to move toward the edge of the screen.

Irradiation was applied in a 60 Co irradiator delivering 4.5 Gy/min for doses $\leqslant$ 60 Gy and in a 137 Cs irradiator delivering 60 Gy/min for doses > 60 Gy. The doses applied ranged from 20 to 1000 Gy.

Exposure to nitrogen was done before, after, or during irradiation.

For the former 2 treatments 6 Petri dishes were inserted into chambers that were flushed with nitrogen and then closed by electropneumatic valves when the oxygen content was < 0.1% as assessed by an oxygen analyser connected to the chamber. Exposure times were 3, 6, 9, 12, 15 and 17 h.

When irradiation was done during exposure to nitrogen, insects were exposed with the diet in an Erlenmeyer flask tightly closed by a rubber stopper through which two syringe needles allowed to introduce nitrogen and to measure the oxygen content. The needles were removed when 0.1% oxygen was attained. Exposure times were 1/2 and 9 h.

Results and Discussion

Effect of irradiation alone.

From 120 to 1000 Gy no mortality was observed until the 8th day and 100% mortality was attained between 12 and 14 days after irradiation (Figure 1). When a dose of 60 Gy was applied, mortality began on the 9th day, total mortality was not reached within the time scale of experiments, and 28 days were needed to reach 90% mortality (Figure 1). At 40 Gy no mortality was observed. It can be noted that above 60 Gy insect response was extremely homogenous while under this dose the effects of irradiation produced heterogenous insect response.

Counting knockdown and dead insects together resulted in a pseudo mortality curve which preceded the true one by about one day. After 12 days post treatment, no more "knockdown" insects were observed.

Effects of Exposure to Nitrogen Alone

Total mortality was attained on the first, 5th and 6th day following 17, 15 and 12 h exposure respectively. For 9 h exposure, only 90 % mortality was reached after 6 days and for 6 h exposure mortality fell to 23 %. No mortality was observed after 3 h exposure.

When 9 h exposure was applied nearly all the insects were unable to move the following day, but about 10 % had recovered by the 5th day. This ability to recover activity was not observed in irradiated insects as described previously.

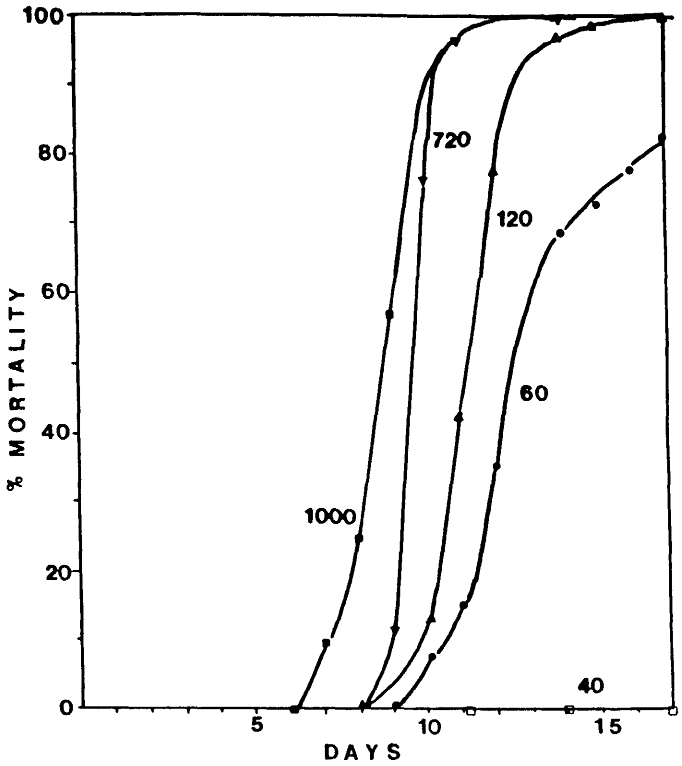

FIGURE 1. EFFECT OF IRRADIATION ON THE MORTALITY OF *T. CONFUSUM* ADULTS. NUMBERS INDICATE THE DOSES IN GY.

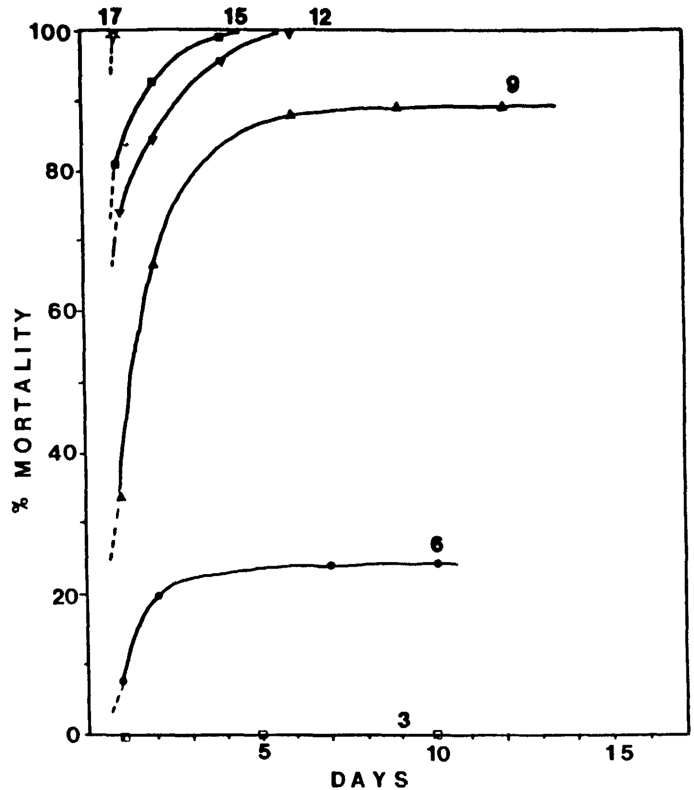

FIGURE 2. MORTALITY OF *T. CONFUSUM* ADULTS FOLLOWING DIFFERENT EXPOSURE TIMES (HOURS) TO NITROGEN.

In some experiments the 9 h exposure was fractionated in 6 and 3 h with various intervals of exposure to air between exposures to nitrogen. No recovery occurred after 1 h in air. There was a partial recovery after 3 h exposure to air which was not improved by a longer period of 6 h. From the relationship between mortality counts 10 days after exposure and time of exposure to nitrogen it was shown that the effect of 9 h of intermittent exposure to nitrogen with a 3 h exposure to air after 6 h in nitrogen is equivalent to about 7.5 h continuous exposure to nitrogen.

Effect of irradiation and nitrogen exposure combined successively

In this study irradiation at doses ranging from 240 to 600 Gy was applied before or after 6 or 9 h exposure to nitrogen.

Synergistic effect was determined from the area between the mortality curve of the combined treatments and the one obtained from the two separate treatments. Maximum efficiency was attributed to treatments that killed all insects in one or two days.

When 6 h exposure was applied before irradiation synergism was greater at 600 Gy than at 240 Gy (Figure 3). For 600 Gy synergism was lower for 9 h exposure than for 6 h.

The order of application of each treatment had no effect on mortality for 6 h exposure. However for 9 h, post-irradiation exposure was more efficient than pre-irradiation exposure (Figure 4).

Effect of irradiation during nitrogen exposure

In this study irradiation at doses ranging from 60 to 600 Gy was applied during 1/2 or 9 h exposure to nitrogen.

Irradiation under 1/2 h nitrogen exposure had a protective effect on mortality for doses up to 120 Gy. Above this level, total mortality was only delayed by 1 to 5 days. Early irradiation at 600 Gy had less effect than late irradiation during exposure to nitrogen for 9 h (Figure 4).

Conclusions

The best results using a combination of irradiation and anoxia were obtained when doses above 240 Gy were associated with exposure times in nitrogen which for nitrogen alone resulted in high mortality (9 h). Post exposure irradiation was better than irradiation before or during exposure to nitrogen. The advantage of the treatment relative to irradiation alone was to shorten the time needed to achieve total adult mortality. Compared to anoxic treatment alone 100 % adult mortality was obtained within about half the time otherwise required. This advantage is expected to be still much greater for the mortality of larvae since more than two days in nitrogen (Le Torc'h, 1983), and about 100 Gy would be necessary to kill them (Hoedaya *et al.*, 1972).

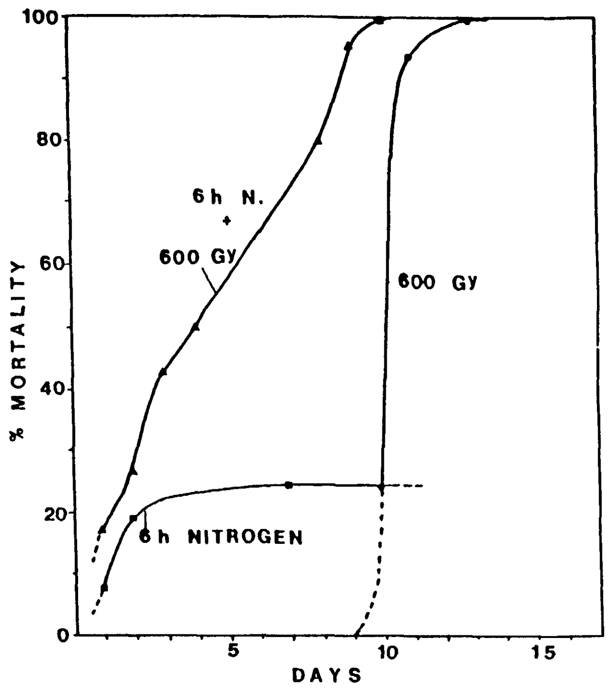

FIGURE 3 SYNERGISTIC EFFECT OF IRRADIATION AT 600 GY FOLLOWING 6 HOURS EXPOSURE TO NITROGEN.

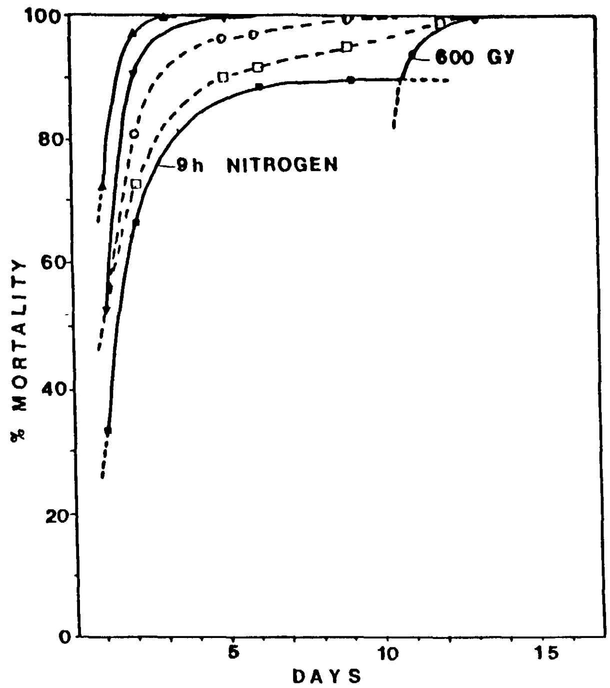

FIGURE 4 SYNERGISTIC EFFECT AND EFFICIENCY OF IRRADIATION AT 600 GY AND 9 H EXPOSURE IN NITROGEN. IRRADIATION AFTER EXPOSURE (▲) ; IRRADIATION BEFORE EXPOSURE (▼) ; LATE IRRADIATION DURING EXPOSURE (○) ; EARLY IRRADIATION DURING EXPOSURE (□)

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