ECOLOGICAL ASPECTS OF CONTROL OF A STORED PRODUCT INSECT BY OZONATION

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INTRODUCTION: Microclimatic changes are effective in reducing insect populations; however, unexpected results such as increases of mite infestations, have occurred (1). Aeration to control insect infestation has met with limited success due to heavy infestation (2) and with almost complete success (3).

Ozone as a potential agent to control insect pests in stored products was chosen because of its oxidative ability to kill pathogenic bacteria (4, 5), viruses (6, 7, 8), fungi (9, 10). One mechanism of damage is associated with a membrane effect in bacteria (11), fungi (10), green plants (12), and mammalian cells (13, 14). Insect toxicity to ozone should prove interesting because of their tracheolar respiratory system rather than the complex alveolar system of humans.

Also, ozone is known for its radiomimetic effects: mutagenicity in bacteria (11); chromosome breakage in mice and chickens (15); chromatid breakage in human tissue culture cells (16); and mitotic inhibition in oysters (17). A documented compilation of comparable effects of ozone and ionizing radiation in living organisms includes these major areas: general, behavioral, congenital, physiological, chemoprotection, biochemical and genetic effects (18). Molecular events at the cellular level due to ozone, oxygen, and radiation were examined on the basis of interactions and the fate of free radicals in biological systems (19).

Ozone, like ionizing radiation, for control of insects might function as a killing agent and/or a sterilizing agent.

This report concerns the effects of ozone on specific life cycle stages and on population performance of two species of economically important insect pests of stored products, namely, wild type strains of the flour beetles, *Tribolium confusum* and *T. castaneum*.

MATERIALS AND METHODS: Wild type strains of the flour beetles, *Tribolium confusum* duVal and *T. castaneum* Herbst, were cultured on standard food at 30±2°C and 65-75 percent relative humidity.

An Orec Model 03Vi-0 ozonator (Ozone Research and Equipment Corporation, 3840 North 40th Avenue, Phoenix, Arizona 85019, U.S.A.) operated at 39 percent power setting, 46 volts, 0.50 amps with clean dry air flow at 0.33 liter per minute produced 450 ppm of ozone. A boric acid buffered potassium iodide absorbent and

UV photometry were used to determine the ozone concentration (20). All exposures were at room temperature in a vented hood.

Life cycle stages of both species as 15 and 20 day old larvae; 23 day old white unpigmented pupae; 28 day old pigmented pupae; and, stock adults were exposed to ozone for 1/4, 1/2, 3/4 hour and each hour and half-hour thereafter up to 7 hours. Five samples of 20 individuals per stage were observed for lethality and sterility one month after adult eclosion.

Single-species populations were begun with 50 stock adults and 3 g food. Monthly three replicates were given: no exposure-census-new food (control); air-census-new food; ozone-census-new food; or census-new food-ozone. Hereafter these groups will be referred to as: control, A-C-F, O-C-F, and C-F-O, respectively. Air or ozone was given for 5 hours. At each census numbers of living and dead larvae, pupae and adults were counted. Dead forms were discarded. Large larvae, pupae, and adults were restrained in a 0.0278 inch U.S. Standard Sieve. In the flour, larvae which were visible to the naked eye were counted and returned to the other live forms.

RESULTS AND DISCUSSION: Ozone effects on specific life cycle stages. A differential lethal response to ozone on a species basis for a given life cycle stage indicated that *T. castaneum* was consistently more ozone sensitive than *T. confusum* at the stages tested except as 15 day old larvae at which time the sensitivities were comparable (Figure 1). The 100 percent lethality induced by ozone for these species indicated that 20 day old larvae, white or unpigmented pupae, and adults of *T. castaneum* were more sensitive than those of *T. confusum* (Table I). For both

Table I. Hours exposure to 450 ppm of ozone which induced 100 percent lethality at specific life cycle stages of wild-type strains of the flour beetles, *Tribolium confusum* and *T. castaneum*.

HOURS OF OZONE EXPOSURE FOR 100 PERCENT LETHALITY

LIFE CYCLE STAGE	*T. CONFUSUM*	*T. CASTANEUM*
15 DAY LARVAE	3 1/2	3 1/2
20 DAY LARVAE	6 1/2	5 1/2
WHITE PUPAE	5 1/2	3 1/2
PIGMENTED PUPAE	6	6
ADULTS	6 1/2	5

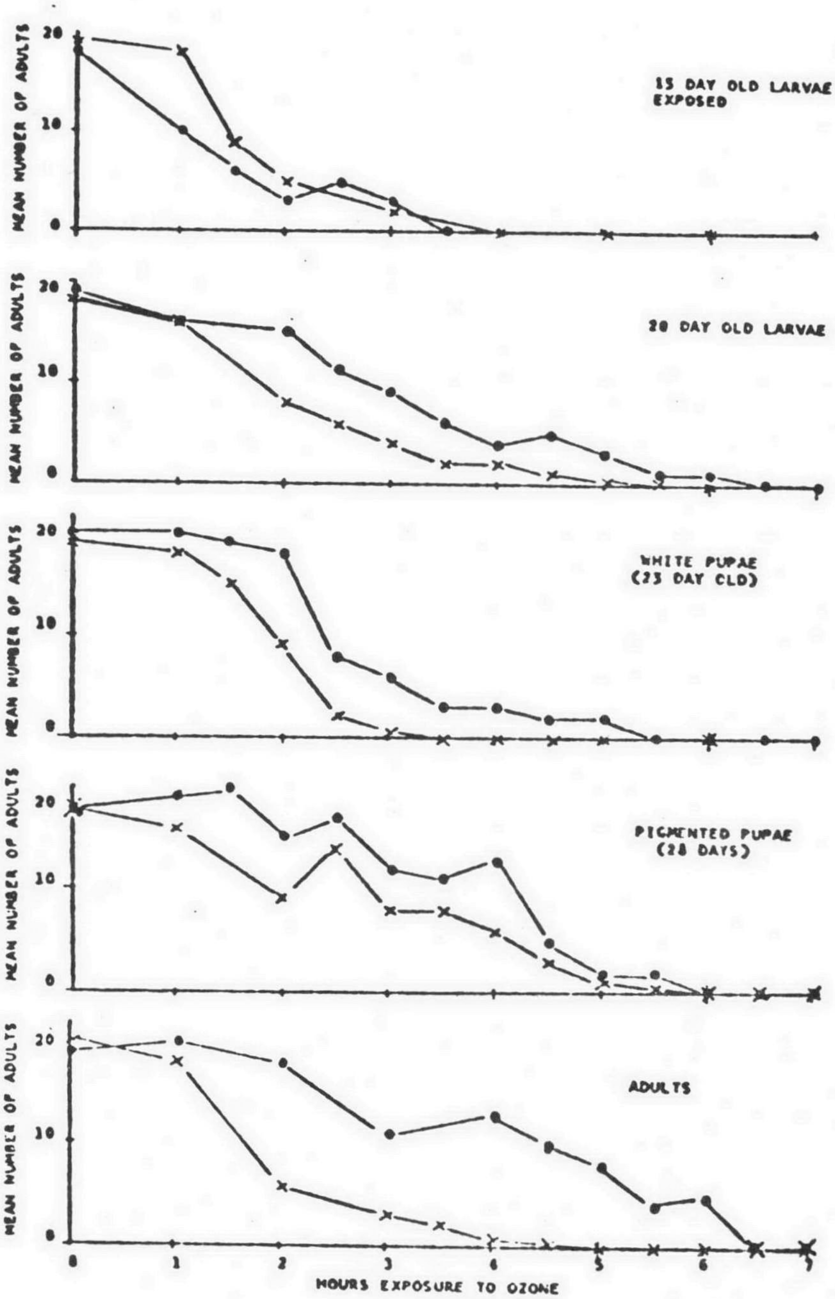

FIGURE 1. MEAN NUMBERS OF ADULTS OF THE FLOUR BEETLES, TRIBOLIUM CONFUSUM DU VAL (●) AND T. CASTANEUM HERBST (X) EXPOSED TO 450 PPM OZONE FOR VARIOUS TIME PERIODS AT SPECIFIC LIFE CYCLE STAGES.

species the youngest life cycle stage exposed (15 day old larvae) was most sensitive; the white pupal stage was the next most sensitive. These highly metabolically active stages in which immature tissues are changing into mature adult tissues reflect the greater sensitivity to ozone.

The percent mortality at the life cycle stages for specific durations of ozonation (Figure 2) showed that *T. confusum* could exploit an ozone-containing environment more successfully than *T. castaneum* could. All life cycle stages of *T. castaneum* exposed 2 hours or longer of ozone were comparatively sensitive.

The mean numbers of *T. confusum* adults obtained after ozonation of a life cycle stage (Table II) showed that sensitivity

Table II. Mean numbers of adults ± standard errors for *Tribolium confusum* duVal exposed to 450 ppm ozone for various periods of time at different life cycle stages

LIFE CYCLE STAGE IN DAYS

HRS OF O_3	LARVAE 15	LARVAE 20	PUPAE 23	PUPAE 28	STOCK ADULTS
0	18 ± 1	19 ± 1	20 ± .5	18 ± 1	19 ± 1
1	10 ± 3	16 ± 0	20 ± .5	19 ± 1	20 ± 1
2	3 ± 1	15 ± 1	18 ± .6	15 ± 2	18 ± 1
3	3 ± 0	9 ± 1	6 ± 2	12 ± 3	11 ± 1
3 1/2	0	6 ± 2	3 ± 1	11 ± 1	8 ± 2
4		4 ± 2	3 ± 1	13 ± 2	13 ± 1
4 1/2		5 ± 1	2 ± 1	5 ± 2	10 ± 1
5		3 ± 3	2 ± 1	2 ± 1	8 ± 2
5 1/2		1 ± .4	0	2 ± 1	4 ± .5
6		1 ± .4		0	5 ± 2
6 1/2		0			0

to ozone decreased with ontogeny from the larval through adult stages. The 20 day old larvae were unexplainably resistant to lethal effects from ozone. In general, this ozone response followed that of ionizing radiation induced lethality (21), namely, sensitivity decreased with increasing age.

Lethal effects of ozone on *T. castaneum* (Table III) occurred at 2 hours exposure and thereafter. The older larvae and pupae were more ozone resistant than the younger forms. Older larvae and pupae were more ozone tolerant than the adult stage. Pigmented pupae were the most resistant of all life cycle stages. The lethal trend for *T. castaneum* exposed to ozone did not follow that for ionizing radiation (21, 22).

One might postulate similar oxidative mechanisms for ozone and ionizing radiation induced lethalities for *T. confusum*. Radiomimetic effects of ozone were documented for Chinese hamster cells (23, 24), *E. coli* (11), *Vicia faba* root tip meristems (16)

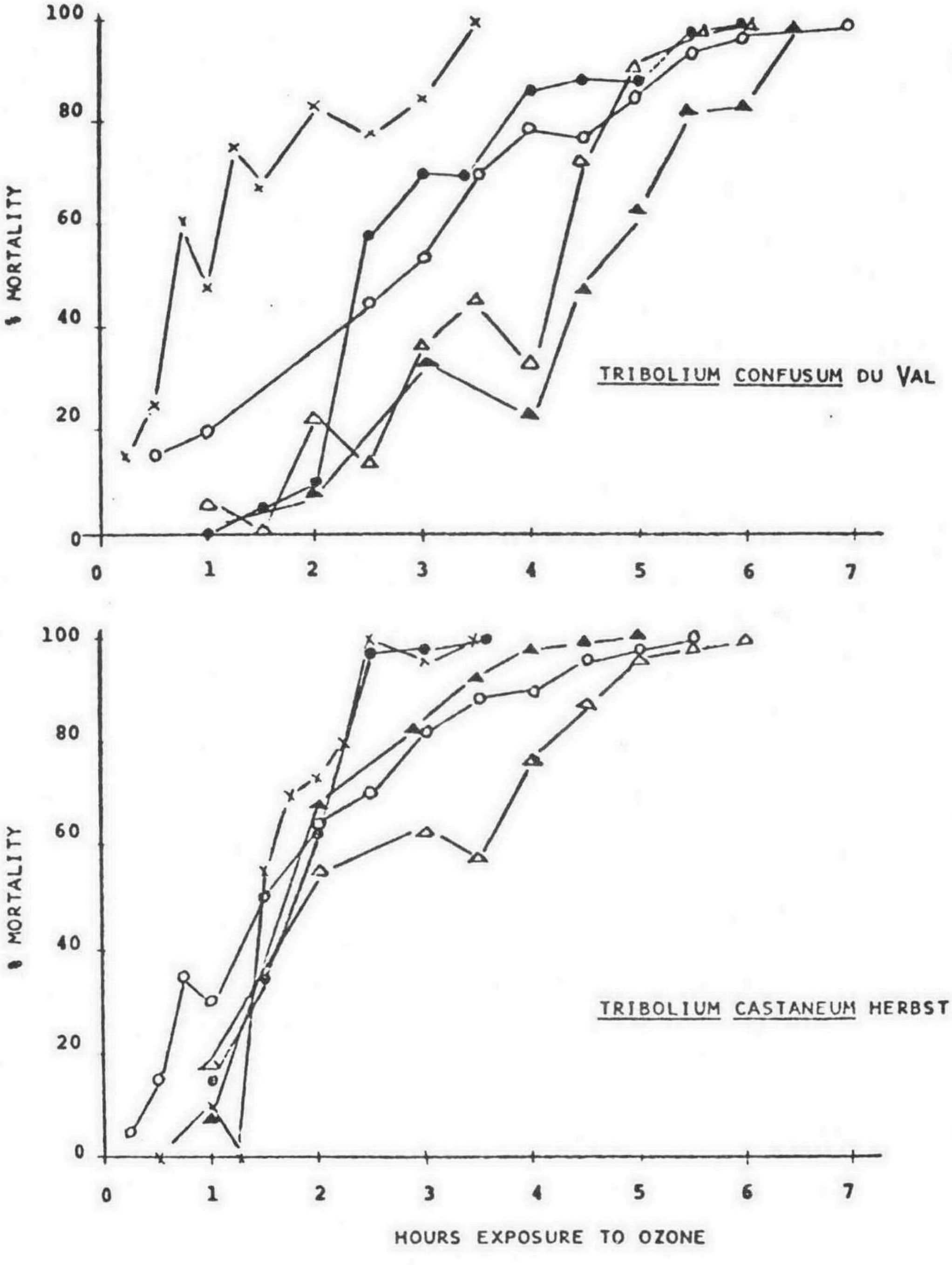

FIGURE 2. PERCENT MORTALITY OF FLOUR BEETLES EXPOSED TO 450 ppm OZONE FOR VARIOUS TIME PERIODS AT SPECIFIC LIFE CYCLE STAGES:

x 15-DAY OLD LARVAE
● 23-DAY OLD WHITE PUPAE
0 20-DAY OLD LARVAE
△ 28-DAY OLD PIGMENTED PUPAE
▲ ADULTS

rather than on older ones. This response is also a typical radiation response in studies featuring ontogeny (22).

Overall, ozone had a greater adverse effect on population performance of *T. castaneum* than that of *T. confusum* but significantly reduced population size for both species. The reproductive potential of *T. confusum* seemed more conservatively determined by the genotype thus maintaining a gene pool less easily changed by environmental conditions. Supportive evidence was found in these two examples: (1) When temperature (33) was an environmental factor *T. confusum* performed better in mixed-species culture than in single-species culture; (2) *T. confusum* also was more productive than *T. castaneum* at 32°C under chronic gamma radiation of 49 R/day and 170 R/day (34).

Unexpectedly, during the first 5-6 months more larvae were observed in the A-C-F and C-F-O populations of *T. confusum* than in the controls (Figure 4A) indicating a stimulation of

TABLE III. Mean number of adults ± standard errors for *Tribolium castaneum* Herbst. exposed to 450 ppm ozone for various periods of time at different life cycle stages

LIFE CYCLE STAGE IN DAYS

HRS OF O_3	LARVAE 15	LARVAE 20	PUPAE 23	PUPAE 28	ADULT
0	19 ± 1	18 ± 1	19 ± 1	18 ± 1	20 ± .4
1	18 ± 1	16 ± 1	18 ± 1	16 ± 2	18 ± 1
2	5 ± 1	8 ± 2	9 ± 2	9 ± 2	6 ± 2
3	2 ± 1	4 ± 1	1 ± .3	8 ± 2	3 ± .6
3 1/2	0	2 ± .5	0	8 ± 2	2 ± .8
4		2 ± 1		6 ± 2	1 ± 2
4 1/2		1 ± .7		3 ± .7	.2 ± .1
5		.4 ± 1		1 ± .7	0
5 1/2		0		.5 ± .5	
6				0	

and microsporocytes (25), chick fibroblasts (15) and nucleic acids (26, 27). Similar type chromosome aberrations were reported for barley (28) exposed to hydrogen peroxide 'precursors' which are active radicals (OH and HO_2) formed by high-energy radiations in water (29) or protoplasm and by the decomposition of ozone in solution (30).

The failure of *T. castaneum* to respond to ozone in a way similar to that of ionizing radiation like *T. confusum* did remains an enigma. One would expect that similarities of morphology and ecological requirements of these two species were endowed by similar metabolism and physiology which would respond to environmental stimuli in much the same way. Such was not the case at least for the postulated mechanism of reaction for ozone and ionizing radiations.

Observations indicated that any resulting adults were able to reproduce, but no quantitative data were recorded. Since surviving adults were fecund and fertile, a lack of gonad differential sensitivity during development to ozone is in opposition to the ionizing radiation response for these species (22). Ozone exposure for 8 hours (no concentration given) had slight effect on egg hatch and no effect on larvae or pupae of three dipteran species (31). In another series of experiment (32) the presumed ozone concentration reached 0.3 ppm or greater and after 22-26 days continuous exposure, no effects were noted for cockroaches, *Periplaneta americana (L.)*, *Nauphocta cinerea* (Oliver) and the red fire ant, *Solenopsis invicta* Buren.

Insects are the most ozone tolerant organisms of which I know; nevertheless, their control by ozonation seems promising. They are also among the most radiation resistant multicellular organisms, insofar as induced sterility and/or lethality is concerned.

Ozone effects on single species populations. Counts of the mean numbers of larvae in all *T. castaneum* populations indicated that all treatments had an effect. These data presented as percent of control (Figure 3A) showed: (1) all treatments reduced the numbers of larvae; (2) A-C-F populations had the larval segment reduced to approximately 60 percent; however, an increase was noted after the 12th month; (3) the C-F-O populations persisted after month 4 with about 35 percent of control values; (4) the larval component of the O-C-F groups was severely reduced to less than 10 percent of the control. After 4 or 6 months, the larval populations reached the carrying capacity which remained relatively constant for the specific environment until the experiment was terminated except in the A-C-F populations in which an increase occurred.

The adult data presented as percent of control (Figure 3B) for *T. castaneum* reflected the performance of the larvae for a given set of experimental conditions. Apparently the adverse effects of air and/or ozone were on a younger life cycle stage

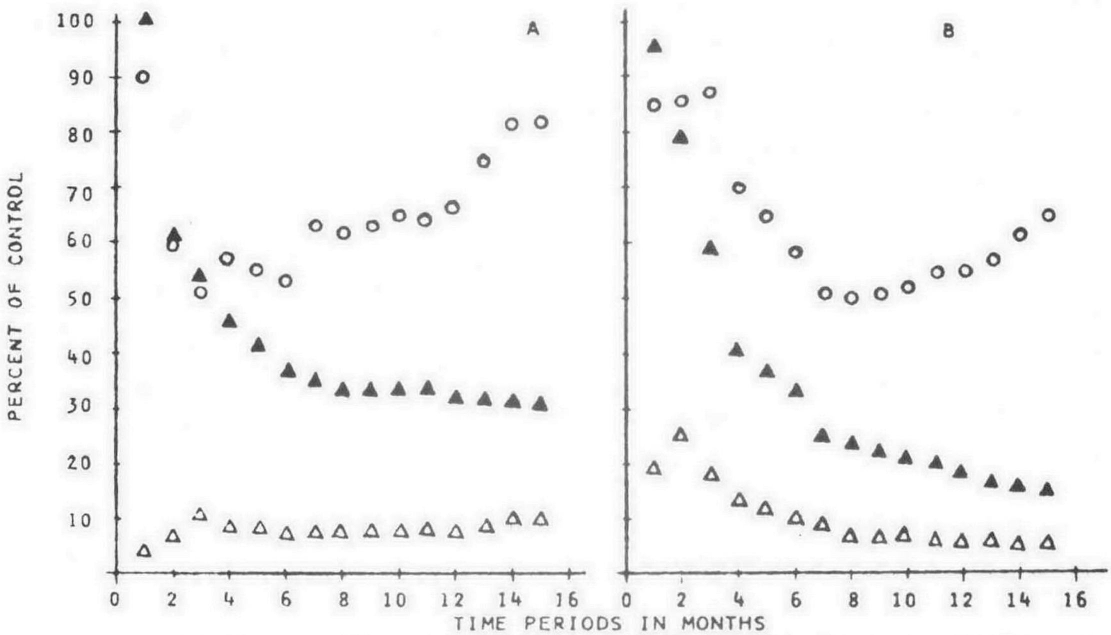

FIGURE 3. LARVAE (A) AND ADULTS (B) AS PER CENT OF CONTROL FOR THE FLOUR BEETLE, FOR TRIBOLIUM CASTANEUM, HERBST IN SINGLE-SPECIES POPULATIONS CENSUSED MONTHLY AND SUBJECTED TO THESE CONDITIONS:

○ AIR - CENSUS - NEW FOOD (A-C-F)

△ OZONE - CENSUS - NEW FOOD (O-C-F)

▲ CENSUS - NEW FOOD - OZONE (C-F-O)

AIR OR 450 PPM OZONE WERE GIVEN FOR 5 HOURS EACH MONTH AT 0.3 ℓ/MIN.

oviposition. This response was also reported for house flies (31, 35). During this time if competition had been lessened by providing more food, perhaps more larvae would have developed into adults. Such a stimulated reproductive response would be economically attractive to insect culture facilities.

By month 9, C-F-O populations again showed an increase in numbers of larvae over control values. The larvae in the O-C-F populations were consistently approximately 35-40 percent of control values.

Numbers of *T. confusum* adults in A-C-F populations were comparable to those of controls (Figure 4B). Numbers of adults in

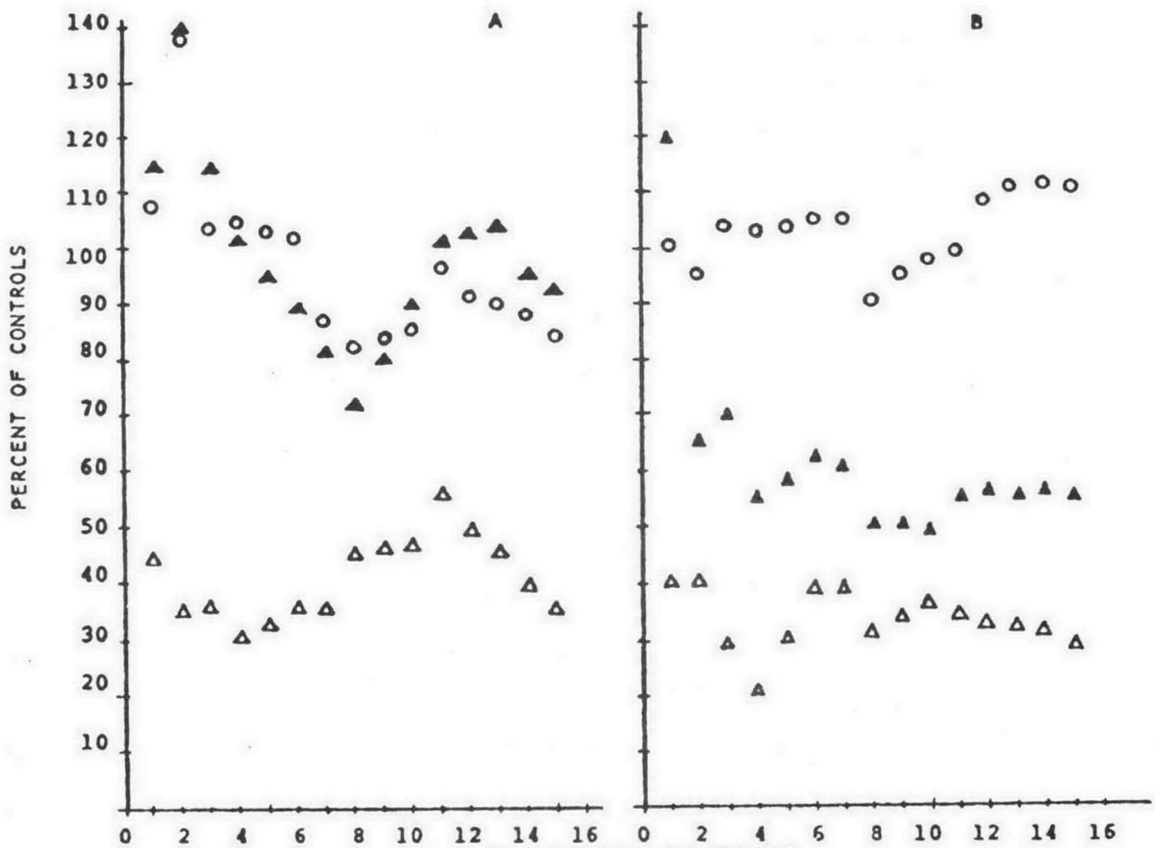

FIGURE 4. LARVAE (A) AND ADULTS (B) AS PER CENT OF CONTROL FOR THE FLOUR BEETLE, FOR TRIBOLIUM CONFUSUM DU VAL, IN SINGLE-SPECIES POPULATIONS CENSUSED MONTHLY AND SUBJECTED TO THESE CONDITIONS:

○ AIR - CENSUS - NEW FOOD (A-C-F)

△ OZONE - CENSUS - NEW FOOD (O-C-F)

▲ CENSUS - NEW FOOD - OZONE (C-F-O)

AIR OR 450 PPM OZONE WERE GIVEN FOR 5 HOURS EACH MONTH AT 0.3 ℓ/MIN.

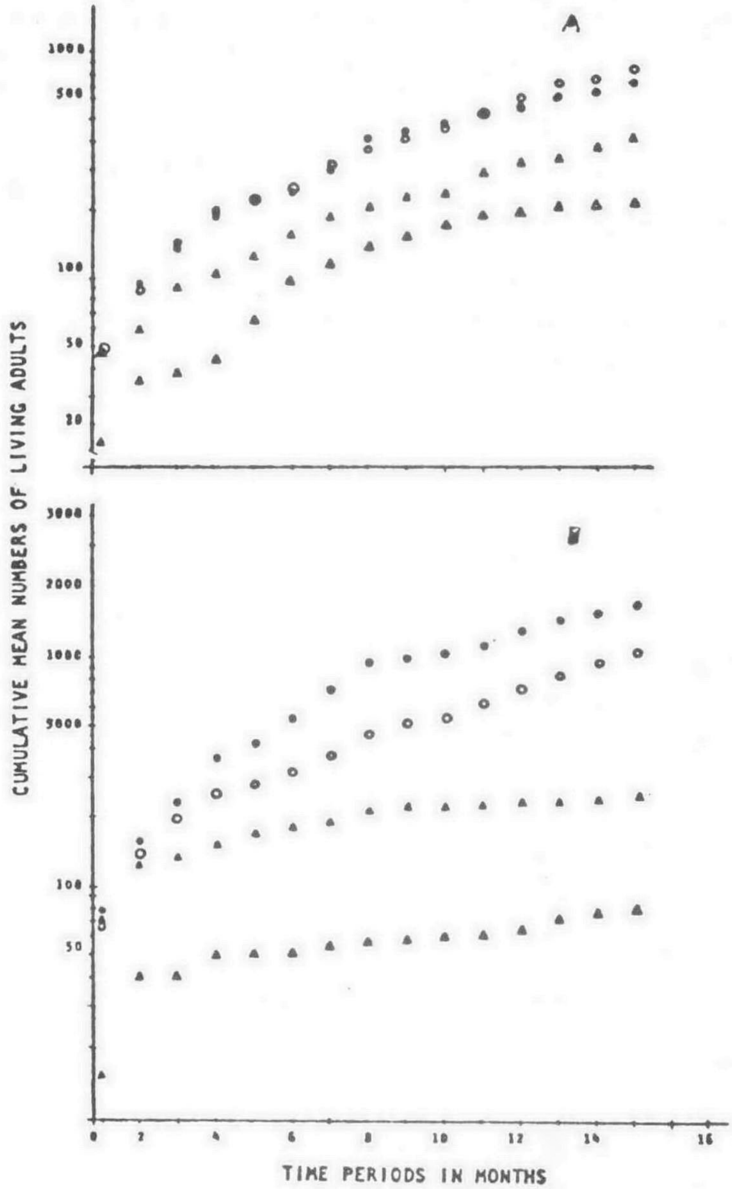

FIGURE 5. CUMULATIVE MEAN NUMBERS OF LIVING ADULTS IN SINGLE-SPECIES POPULATION OF FLOUR BEETLES, TRIBOLIUM CONFUSUM DU VAL (A) AND T. CASTANEUM HERBST (B). AIR OR 450 PPM OZONE WERE GIVEN FOR 5 HOURS MONTHLY AT WHICH TIME CENSUSES WERE TAKEN. EXPERIMENTAL CONDITIONS WERE:

- ● NO EXPOSURE - CENSUS - NEW FOOD (CONTROL)
- ○ AIR - CENSUS - NEW FOOD (A-C-F)
- △ OZONE - CENSUS - NEW FOOD (O-C-F)
- ▲ CENSUS - NEW FOOD - OZONE (C-F-O)

the O-C-F and C-F-O populations were significantly below control levels and maintained their numbers at 30 and 55 percent of controls, respectively, during the 15 months.

Based on progressively higher values for cumulative numbers of live adults within a species and between different environmental conditions, the populations could be ranked for *T. confusum* Figure 5A): O-C-F<C-F-O<A-C-F = control; for *T. castaneum* (Figure 5B) O-C-F<C-F-O<A-C-F<control. These data showed that 5 hours' exposure to 450 ppm O_3 reduced numbers of adult flour beetles in populations of either species; and, ozonated populations of *T. confusum* continued to increase in numbers of adults during the experimental 15 months; whereas, those of *T. castaneum* persisted at relative constant levels: 50 to 70 for O-C-F and 150 to 200 for C-F-O.

The total numbers of living forms for the populations of *T. confusum* (Figure 6A) and *T. castaneum* (Figure 6B) presented on a cumulative basis showed several trends which are important in an insect control technique program. Under these experimental conditions ozone is not effective as a control agent; although it reduced numbers in the population, it did not eliminate the populations or reduce their numbers to economically insignificant values. The ozone stimulation of productivity indicated by the *T. confusum* larvae (Figure 4A) was not reflected in the total numbers of living forms. Perhaps ozone had a residual toxic effect which occurred before the pupal stage.

Data for pupae were not given because in all groups the numbers of pupae ranged between 2 and 10. This was expected because the pupal stage is very brief compared to larval and adult stages.

One might anticipate that ozone would react with and cause the flour to have an adverse effect on those who ingested it; however, for both species the opposite response was observed (Figure 6), that is, C-F-O populations performed better than O-C-F populations. Perhaps the increased activity (metabolism) of census taking immediately after ozone exposure in the O-C-F populations accentuated the toxic effects of ozone.

Each ecological condition resulted in a different population maintenance pattern for the flour beetles (Figure 6). The separation of population census curves was more pronounced for *T. castaneum* (Figure 6B) than for *T. confusum* (Figure 6A). Control populations of *T. castaneum* contained more adults than those of *T. confusum* under these experimental conditions; however, ozone exposure more adversely affected populations of *T. castaneum* than those of *T. confusum*.

The population reduction by air exposure was unexpected. One might postulate a physical effect due to particles of flour and/or yeast interferring with beetle respiration because the air or ozone was given such that a constant flow at 0.3 liter per minute went through the flour containing the flour beetles. Such

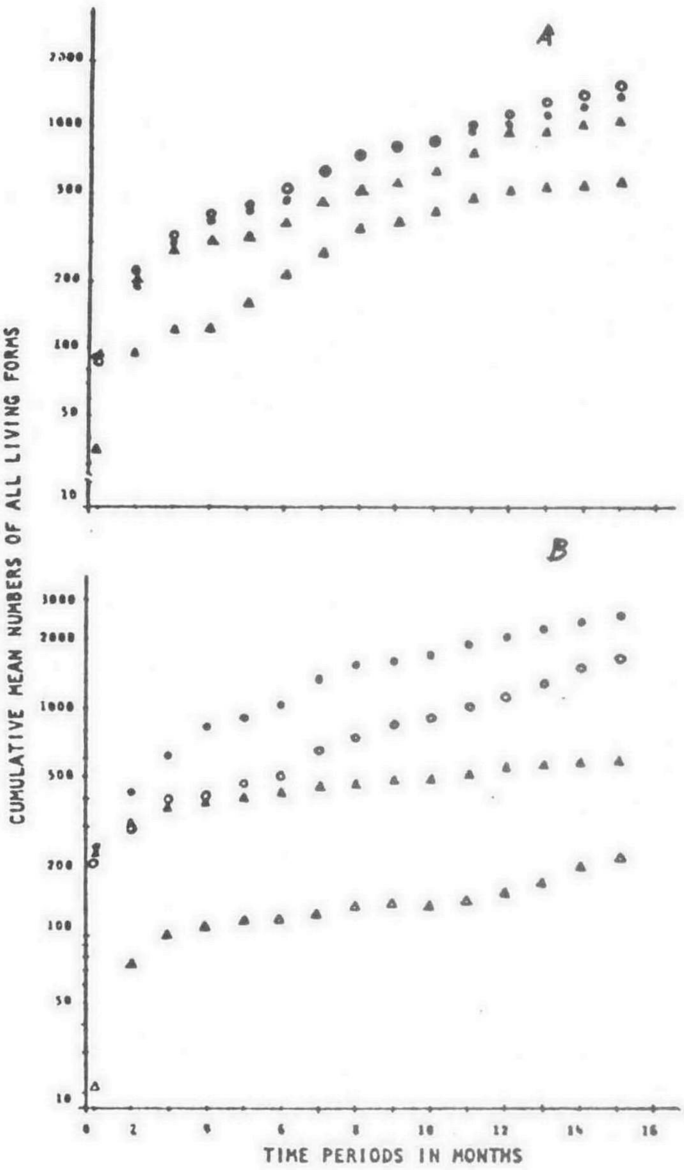

FIGURE 6. CUMULATIVE MEAN NUMBERS OF ALL LIVING FORMS (LARVAE, PUPAE, ADULTS) IN SINGLE-SPECIES POPULATION OF FLOUR BEETLES, *TRIBOLIUM CONFUSUM* DU VAL (A) AND *T. CASTANEUM* HERBST (B). AIR OR 450 PPM OZONE WERE GIVEN FROM 5 HOURS MONTHLY AT WHICH TIME CENSUSES WERE TAKEN. EXPERIMENTAL CONDITIONS WERE:

- ● NO EXPOSURE - CENSUS - NEW FOOD (CONTROL)
- ○ AIR - CENSUS - NEW FOOD (A-C-F)
- △ OZONE - CENSUS - NEW FOOD (O-C-F)
- ▲ CENSUS - NEW FOOD - OZONE (C-F-O)

a hypothesis was not supported by the number of dead individuals. The numbers of dead *Tribolium* seemed to cluster in two groups for *T. confusum* (Figure 7A), namely, the control and A-C-F populations

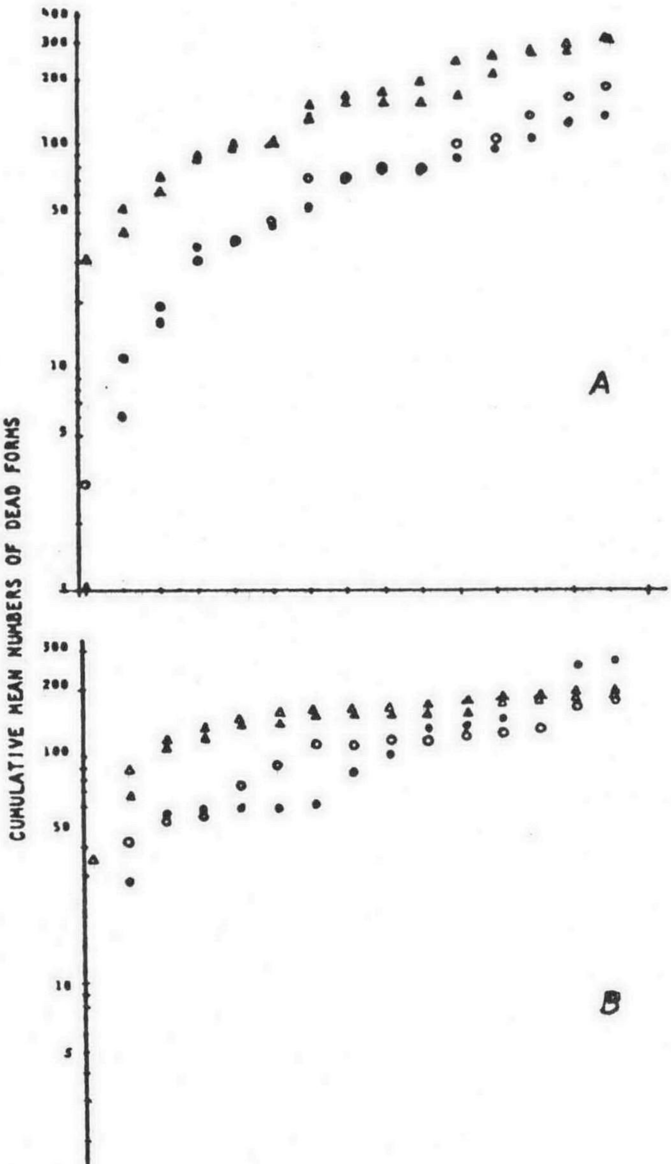

FIGURE 7. CUMULATIVE MEAN NUMBERS OF DEAD INDIVIDUALS IN SINGLE-SPECIES POPULATION OF FLOUR BEETLES, TRIBOLIUM CONFUSUM DU VAL (A) AND T. CASTANEUM HERBST (B). AIR OR 450 PPM OZONE WERE GIVEN FOR 5 HOURS MONTHLY AT WHICH TIME CENSUSES WERE TAKEN. EXPERIMENTAL CONDITIONS WERE:

- ● NO EXPOSURE - CENSUS - NEW FOOD (CONTROL)
- ○ AIR - CENSUS - NEW FOOD (A-C-F)
- △ OZONE - CENSUS - NEW FOOD (O-C-F)
- ▲ CENSUS - NEW FOOD- OZONE (C-F-O)

and the O-C-F and C-F-O populations with the latter group containing more dead individuals than the former. One would conclude that ozone was more toxic than air or no treatment. These two groups were not as clearcut for *T. castaneum* (Figure 7B) as they were for *T. confusum.*

These experiments showed that flour beetles, an economically important pest in stored food products, were resistant to high concentrations (450 ppm) of ozone given for long periods of time (5 hours). Perhaps ozone would predispose flour beetles to radiations for an effective control program; their action could be synergistic as reported in mouse studies concerned with C 3 H-tumors (36) and mortality (18) and in *E. coli* repair of DNA (11).

Another aspect of ozone in the control of harmful insect species in stored-products concerns the effects of ozone on the stored-product per se. Perpaps a highly oxidizing agent like ozone would change the stored-product making it objectionable to taste, in appearance and for marketability.

CONCLUSIONS: 1. Although 5 hour monthly exposures to 450 ppm O_3 at 0.31/min. reduced the numbers of individuals in populations of *T. confusum* and *T. castaneum,* they did not result in elimination or a significant decrease in numbers which would be useful for a control program of these flour beetle species.

2. *T. castaneum* was more ozone sensitive than *T. confusum* at the life cycle stages investigated.

3. Induced lethality during ontogeny of *T. confusum* exposed to ozone followed that induced by ionizing radiations; consequently a similar oxidative effect mechanism is hypothesized. No comparable trends were noted for *T. castaneum,* except that the oldest life cycle stage (adult) was more ozone resistant than the youngest stage (15 day old larvae).

4. During the first five months of culture, ozone had a stimulating effect on productivity of *T. confusum;* a similar effect was not noted for *T. castaneum.*

5. One hour exposure to 450 ppm ozone at a flow rate of 0.3 l/min had no effect on either species as 15 day old larvae or older stages.

6. Within seven hours exposure to this ozone, lethality to all living forms including adults of both species was observed.

7. Control of flour beetles by ozone in stored products seems unlikely; however, ozone in an integrated control program might prove beneficial.

REFERENCES:

(1) Burrell, N. J., Grain cooling studies-II: Effect of aeration on infested grain bulks. J. Stored Prod. Res. 3 (1967) 145.

(2) Sutherland, J. W., Control of insects in a wheat store with an experimental aeration system. J. Agric. Eng. Res. 13(3) (1968) 210.

(3) Navarro, S., Aeration of grain as a non-chemical method for the control of insects in the grain bulk, Proc. First Internat. Work. Conf. on Stored-Product Entomol., Savannah, Georgia (1974) 341.

(4) Broadwater, W. T., et al., Sensitivity of three selected bacterial species to ozone. Appl. Microbiol. 26(3) (1973) 391.

(5) Burleson, G. R., et al., Inactivation of viruses and bacteria by ozone, with and without sonication, Appl. Microbiol. 29(3) (1975) 340.

(6) Berg, G., Removal of viruses from sewage, effluents and waters. Bull. Wld. Hlth. Org. 49 (1973) 451.

(7) Carazzone, M. M., Vanini, G. C., Richerche Sperimentali circa L'Azione dell'ozono sui virus, G. Batt. Virol. 62 (1969) 828.

(8) Majumdar, S. B., et al., Communication: Inactivation of polio virus in water by ozonation. J. Water Pollution Control Fed. 46 (8) (1974) 2048.

(9) Ewell, A. W., Ozone and its application in food preservation, Refrig. Eng. (1950).

(10) Hibben, C. R., Stotzky, G., Effects of ozone on the germination of fungus spores. Can. J. Microbiol. 15 (1969) 1187.

(11) Hamelin, C., Chung, Y. S., The effect of low concentrations of ozone on *Escherichia coli* chromosome, Mutat. Res. 28(1) (1975) 131.

(12) Duggar, B. M., et al., Physiological and biochemical effects of atmospheric oxidants on plants. J. Air Pollut. Cont. Assn. 16 (1966) 467.

(13) Brinkman, R., et al., Radiomimetic toxicity of ozonized air. Lancet 7325 (1964) 133.

(14) Dungworth, D. L., et al., Effect of ambient levels of ozone on monkeys, Federation Proceedings 34 (1975) 1670.

(15) Sachsenmaier, W., et al., Wirkung von Ozon auf Mauseascitestumorzellen und auf Hühnerfibroblasten in der Gewebekultur, Zeitschrift für Krebsforschung 67 (1965) 113.

(16) Fetner, R. H., Ozone-induced chromosome breakage in human cell cultures, Nature 194 (1962) 793

(17) Maclean, S., et al., Effects of ozone-treated seawater on the spawned, fertilized, meiotic and cleaving eggs of the commercial American oyster, Mutation Res. 21 (1973) 283.

(18) Veninga, T. S., Toxicity of ozone in comparison with ionizing radiation, Strahlentherapie 134 (3) (1967) 469.

(19) Menzel, D., Toxicity of ozone, oxygen, and radiation, Ann. Rev. Pharmacol. 10 (1970) 379.

(20) Flamm, D. L., Analysis of ozone at low concentrations with boric acid buffered KI, Environ. Sci. and Tech. 11 (10) (1977) 978.

(21) Erdman, H. E., Comparative x-ray sensitivity of *Tribolium confusum* and *Tribolium castaneum* (Coleoptera: Tenebrionidae) at different development stages during their life cycle, Nature 195 (1962) 1218.

(22) Erdman, H. E., Ontogeny and comparative radiation sensitivity in the confused flour beetle and strains of the red flour beetle, J. Econ. Entomol. 61 (1) (1968) 123.

(23) Zelac, R. E., et al., Inhaled ozone as a mutagen, I. Chromosome aberrations induced in Chinese hamster lymphocytes, Environ. Res. 4 (1971) 262.

(24) Zelac, R. E., et al., Inhaled ozone as a mutagen, II. Effect on the frequency of chromosome aberrations observed in irradiated Chinese hamsters, Environ. Res. 4 (1971) 325.

(25) Janakiraman, R., Harney, P. M., Effects of ozone on meiotic chromosomes of *Vicia faba*, Can. J. Genet. Cytol. 18 (1976) 727.

(26) Christensen, E., Giese, A. C., Changes in absorption spectra of nucleic acids and their derivatives following exposure to ozone and ultraviolet radiation, Arch. Biochem. Biophysica 51 (1954) 208.

(27) Prat, R., Effets de l'hypochlorite de sodium, de l'ozone et des radiations ionisantes sur les constituants pyrimidiques de *Escherichia coli*, Ann. Inst. Pasteur 114 (1968) 595.

(28) Phillips, L. L., Effects of free radicals on chromosomes of barley, Science 124 (1956) 889.

(29) Allen, A. O., The radiation Chemistry of Water and Aqueous Solutions, D. Van Nostrand Co., Inc., Princeton, N.J. (1961)

(30) Weiss, J., Investigations on the radical HO_2 in solution, Trans. Farad. Soc. 31 (1935) 668.

(31) Levy, R., et al., Effects of ozone on three species of Diptera, Environ. Entomol. 1 (5) (1972) 608.

(32) Levy, R., et al., Tolerance of three species of insects to prolonged exposures to ozone, Environ. Entomol. 3 (1) (1974) 184.

(33) Erdman, H. E., The influence of temperature on the performance of single and mixed species populations of flour beetles, Res. Pop. Ecol. 12 (1) (1970) 61.

(34) Erdman, H. E., Effects of chronic Co-60 (Gamma) radiation, dose rate, temperature and cohabitation on population performance of flour beetles, *Tribolium confusum* Duval and *T. castaneum* Herbst (Coleoptera: Tenebrionidae), Env. Entomol. 2 (1973) 41.

(35) Beard, R. L., Observation on house flies on high-ozone environments, Ann. Entomol. Soc. Am. 58 (1965) 404.

(36) Teske, H. J., et al., Tierexperimentelle Untersuchungen uber die Ozonewirkung auf Tumorwachstum und Bestrahlungseffekt, Strahlentherapie 145 (2) (1973) 155.