Effect of Low Oxygen Atmospheres on Mortality of Red and Confused Flour Beetles[1,2]

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ABSTRACT

Exposure to a generated low O_2 atmosphere required to kill 50 and 95% of developing confused flour beetle, *Tribolium confusum* Jacquelin DuVal, and red flour beetle, *T. castaneum* (Herbst), indicated that young larvae and adult stages were the most susceptible; pupae and eggs midway through development were the most tolerant. At 27°C, times of exposure for 95% mortality of the most tolerant ages of pupae were ca. 53 h for 3-day-old pupae of *T. confusum* and 46 h for 2-day-old pupae of *T. castaneum*. At 18°C, significantly longer exposures were required to kill developing stages, particularly 3 day old eggs of *T. confusum* (LT_{95} 132 h) and 2 day old pupae of *T. castaneum* (LT_{95} 113 h).

Studies have shown that low oxygen atmospheres produced by an exothermic inert atmosphere generator are effective against several species of stored product insects (Storey 1975 a, b, c). This generated atmosphere, a potential residue-free alternative to conventional chemical fumigation, would be useful particularly in the disinfestation of milled products. Since flour beetles are among the most abundant and injurious insect pests of flour and other grain products, a study was made of the effect of the oxygen deficient atmosphere on all stages of the confused flour beetle, *Tribolium confusum* Jacquelin duVal, and the red flour beetle, *T. castaneum* (Herbst).

MATERIALS AND METHODS.—The low oxygen atmosphere (oxygen <1%, CO_2 9–9.5%, and the balance principally N_2) was produced in a pilot inert atmosphere treatment system. The insects were exposed to a flow rate of 25 cc/min in test cages placed in 1-pt jars as described by Storey (1975a). Oxygen levels in the generated atmosphere were measured daily with a paramagnetic oxygen analyzer. The concentration of O_2 was generally between 0.1 and 0.25% and rarely exceeded 0.5%.

Adult and immature stages of the confused and red flour beetles were obtained by placing adult insects from laboratory cultures on white wheat flour for 24 h at 27°±1°C and 60% RH and then sifting eggs from the flour with a 60-mesh screen. Some eggs were placed in glass vial test cages (1.2×1.3 mm deep) fitted with a porous cellulose plug; others were placed on fresh flour and held for development to the larval, pupal, and adult stages. Eggs that were 1, 2, 3 or 4 days old, larvae that were 7, 14, 21, and 28 days posthatch, pupae that were 1, 2, 3, 4, and 5 days after pupation, and adults that were 14 days old were treated. Twenty-five insects of each stage-age combination were exposed to the inert atmosphere at 50% RH at 18° or 27°±1°C for periods ranging from 4–168 h. After exposure, they were transferred to small plastic boxes containing suitable food medium and stored at 27°±1°C and 60% RH until posttreatment mortality observations were completed. Mortality counts for each exposed stage began 42 days from the day that stage was collected as eggs and were continued over a 3-week period. All treatments (species, ages, and exposure times) were replicated 3 times. Parallel control insects were held similarly but in atmospheric air.

The time required to kill 50 and 95% of each age group was estimated by transforming mortality data to probits and calculating the regression of probits on time. Lethal times (LT) were then estimated by using the linear calibration technique (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION.—Table 1 reports the LT_{50} and 95 for each stage-age at 18° and at 27°C for the confused flour beetle, and Table 2 reports them for the red flour beetle.

Eggs.—LT_{95} values ranged from 132 h for 3-day-old confused flour beetle eggs at 18°C to only 25 h for 1-day old red flour beetle eggs at 27°C. No eggs survived 48 h of exposure at 27°C. Tolerance of eggs to the generated atmosphere increased after the 1st day of development and then either remained nearly the same or decreased slightly just before hatching the 4th day. Confused flour beetle eggs were significantly more tolerant than red flour beetle eggs at 18°C. Larvae from eggs surviving exposure appeared to develop normally, but the development time for the egg stage was prolonged.

Larvae.—LT_{95} values ranged from 70 h for 3-week-old red flour beetle larvae at 18°C to less than 7 h for 1-week-old confused flour beetle larvae at 27°C, the 1-week-old larvae being the most susceptible of each species. Tolerance to the inert atmosphere increased with each successive week through the 1st 3 weeks of development, but the largest difference was between 14-, and 21-day-old larvae. Younger larvae, killed by the inert atmosphere, died during the exposure and were collapsed and shriveled when they were removed from the treatment. Some mature larvae revived within 24 h after removal from the atmosphere, but they appeared partially paralyzed and incapable of full locomotion. Larvae, so stricken,

[1] Coleoptera: Tenebrionidae.
[2] Received for publication Oct. 29, 1976.
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Table 1.—Mortality of developing confused flour beetles exposed at 2 temperatures to an atmosphere[a] produced by an exothermic inert atmosphere generator.

Stage/days from oviposition pupation	LT_{50} (h) – (95% CI)		LT_{95} (h) – (95% CI)	
	18°±1°C	27°±1°C	18°±1°C	27°±1°C
Eggs (1)	53.9 (30.0–77.8)	9.8 (6.8–12.8)	95.2 (71.4–119.7)	30.2 (27.2–33.2)
Eggs (2)	93.1 (75.5–110.8)	24.1 (21.0–26.4)	128.3 (110.6–146.4)	38.7 (36.4–41.0)
Eggs (3)	100.5 (69.9–131.5)	30.3 (29.7–31.0)	131.7 (101.1–163.6)	39.6 (38.9–40.3)
Eggs (4)	101.7 (97.7–195.6)	29.9 (29.3–30.6)	124.4 (120.4–128.5)	39.3 (38.7–40.0)
Larvae (7)	16.9 (6.1–27.7)	3.0 (0–6.6)	24.3 (13.8–35.6)	6.6 (2.6–10.3)
Larvae (14)	25.4 (14.2–36.5)	4.4 (0–7.9)	37.8 (26.7–49.1)	7.6 (3.9–11.2)
Larvae (21)	37.5 (34.4–40.6)	8.2 (0–17.3)	53.0 (49.9–56.2)	20.0 (10.9–29.4)
Larvae (28)	39.4 (37.7–41.2)	6.5 (0–15.4)	54.9 (53.1–56.7)	18.2 (9.1–27.4)
Pupae (1)	62.1 (48.9–75.3)	28.7 (19.1–38.5)	73.6 (60.5–87.2)	37.1 (27.5–47.3)
Pupae (2)	86.1 (67.8–104.9)	37.0 (26.6–47.7)	99.0 (80.7–118.5)	48.3 (37.8–59.3)
Pupae (3)	87.3 (75.8–98.9)	41.0 (28.3–53.8)	99.3 (87.8–111.2)	53.3 (40.7–66.4)
Pupae (4)	69.3 (56.2–92.5)	35.5 (23.7–47.5)	89.7 (76.6–103.3)	48.1 (36.3–60.6)
Pupae (5)	59.0 (41.9–76.6)	13.0 (11.7–14.3)	77.0 (59.8–95.2)	24.0 (22.7–26.3)
Adults (14 days after emergence)	28.6 (19.4–38.0)	10.8 (9.9–11.6)	36.7 (27.5–46.4)	17.0 (16.2–17.8)

[a] Composition of the inert atmosphere > 1% O_2 and 9–9.5% CO_2, the balance principally N_2.

died without developing pupal characteristics. All larvae of both speices were killed by a 48-h exposure at 27°C.

Pupae.—LT_{95} values ranged from 113 h for 2-day-old red flour beetle pupae at 18°C to 17 h for 5-day-old red flour beetle pupae at 27°C. Pupae 2–4 days old were the most tolerant stage–age of both species except for confused flour beetle eggs at 18°C. Pupae were more susceptible at the beginning and near the end of the pupal period than during the middle period. Many adults emerging from exposed pupae were less than normal size and had rudimentary wings; others never developed adult characteristics in the posterior segments. Similar morphological abnormalities were reported by Ali Niazee (1971) when flour beetle pupae were exposed to 100% CO_2. In exposures at 27°C that killed ca. 50% of 1-, to 3-day-old red flour beetle pupae, some died while still in the pupal stage. When 4-, and 5-day-old pupae were exposed for the interval that produced 50% mortality, death generally occurred after the pupae had emerged as fully formed adults. Longer exposures (72 h) killed all pupae (no further development) regardless of age at time of exposure to the inert atmosphere. In both species, susceptibility of 5-day-old pupae was significantly increased over that of 4-day-old pupae. A similar increase in susceptibility of older red flour beetle pupae fumigated with methyl bromide was reported by Bennett (1969). Lindgren (1935) found the rate of respiration of developing confused flour beetle pupae to be high at first, lower for several days, and then rising again near the end of the pupal period. The response of both flour beetles to the low O_2 atmosphere during the pupal period therefore appears to follow this cyclic rate of respiration.

Table 2.—Mortality of developing red flour beetles exposed at 2 temperatures to an atmosphere[a] produced by an exothermic inert atmosphere generator.

Stage/days from oviposition-pupation	LT_{50} (h) – (95% CI)		LT_{95} (h) – (95% CI)	
	18°±1°C	27°±1°C	18°±1°C	27°±1°C
Eggs (1)	42.3 (10.7–73.3)	7.9 (0–19.3)	75.9 (44.8–108.3)	25.1 (13.5–36.7)
Eggs (2)	70.5 (53.0–88.0)	30.3 (29.2–31.4)	95.2 (77.8–112.9)	39.5 (38.4–40.6)
Eggs (3)	70.8 (52.7–88.9)	32.4 (31.9–32.8)	96.1 (78.1–114.5)	39.6 (39.1–40.1)
Eggs (4)	42.8 (30.8–54.7)	19.8 (17.4–22.1)	63.6 (51.7–75.8)	33.4 (31.1–35.8)
Larvae (7)	21.2 (14.9–27.5)	5.7 (4.5–6.8)	30.7 (24.4–37.2)	8.4 (7.4–9.5)
Larvae (14)	37.2 (35.8–38.6)	6.7 (6.4–7.0)	50.0 (48.6–51.4)	9.1 (8.8–9.4)
Larvae (21)	50.6 (47.3–53.9)	11.0 (3.6–18.2)	69.9 (66.6–73.2)	16.3 (9.2–24.1)
Larvae (28)	44.9 (40.1–49.8)	13.2 (11.1–15.3)	65.8 (60.9–70.8)	23.4 (21.3–25.5)
Pupae (1)	76.1 (59.0–93.4)	21.7 (20.8–22.7)	95.0 (77.9–112.9)	28.0 (27.1–28.8)
Pupae (2)	93.6 (77.5–109.7)	34.7 (33.1–36.3)	113.1 (97.1–129.7)	46.5 (44.8–48.1)
Pupae (3)	95.2 (92.7–97.8)	30.8 (17.8–44.0)	107.9 (105.2–110.5)	42.3 (29.4–55.8)
Pupae (4)	76.2 (73.1–79.3)	28.7 (20.0–37.5)	98.9 (95.8–102.1)	42.6 (34.0–51.6)
Pupae (5)	42.5 (39.0–46.0)	10.2 (9.3–11.1)	67.5 (63.9–71.0)	16.6 (15.7–17.5)
Adults (14 days after emergence)	32.1 (25.2–38.9)	13.8 (13.7–14.0)	43.0 (36.1–50.0)	17.9 (17.7–18.0)

[a] Composition of the inert atmosphere > 1% O_2 and 9–9.5% CO_2, the balance principally N_2.

Exposure periods had to be significantly longer at 18° than at 27°C to obtain 95% mortality, but the differential varied for each stage. For example, the LT_{95} for confused flour beetle eggs exposed to 18°C averaged 83 h longer than the LT_{95} for eggs exposed to 27°C but the difference was only 20 h for adults, 29 h for larvae, and 46 h for pupae. With the red flour beetle, the differential was greatest (61 h) for pupae. Except for the egg stage, red flour beetles were consistently more tolerant of the inert atmosphere at 18°C than confused flour beetles but there was little difference between the 2 species at 27°C. At 18°C, the general order of tolerance for red flour beetles was pupae > eggs > larvae > adults; that for confused flour beetles was eggs > pupae > larvae >adults. At 27°C, the order of tolerance was the same for each species: pupae > eggs > adults > larvae. These data emphasize the critical relationships between temperature, time, and life stage that must be considered when extablishing treatment criteria for the use of generated low O_2 atmospheres in control programs.

ACKNOWLEDGMENT.—I am indebted to W. E. Blodgett of the Grain Marketing Research Center for assistance in conducting the tests and to G. D. Booth, ARS Statistical Laboratory, Ames, Iowa, for advice and for computer analysis of the data.

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