The Feasibility of Using Modified Atmospheres to Control Insect Pests in Museums

Michael K. Rust
Janice M. Kennedy

GCI SCIENTIFIC PROGRAM REPORT
MARCH 1993

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TABLE OF CONTENTS

Introduction 1

Test system 4

Insects 5

1. The Webbing Clothes Moth 9
   Results 12
   Conclusions 13

2. The Furniture Carpet Beetle 19
   Results 22
   Conclusions 22

3. The Firebrat 28
   Results 31
   Conclusions 32

4. The Cabinet Beetle 38
   Results 41
   Conclusions 42

5. The Larder Beetle 46
   Results 50
   Conclusions 50

6. The Cigarette Beetle 55
   Results 59
   Conclusions 59

7. The Confused Flour Beetle 65
   Results 68
   Conclusions 68

8. The American Cockroach, Brownbanded Cockroach and German Cockroach 74
   Results 79
   Conclusions 80

9. The Powderpost Beetle 104
   Results 108
   Conclusions 108

10. The Western Drywood Termite 111
    Results 114
    Conclusions 115

Summary 122

Acknowledgments 123

References Cited 124

INTRODUCTION

Surveys of natural history and art museums indicate that beetles belonging to the families Anobiidae and Dermestidae and moths belonging to the family Tineidae are major pests (Tables 1 and 2). Schrock (1988) has provided a list of commonly damaged materials in museums and the associated pests. Other groups such as termites and silverfish may also be extremely important, especially in southeast Asia (Quek et al. 1990). Current control recommendations include the use of space fumigation or surface residual spraying. In addition to the need to train museum personnel to use these insecticides, there is always the potential for damage to rare antiquities and artifacts (Florian 1988, Dawson 1988). Preslock (1988) has provided an annotated bibliography of literature pertaining to pest control in museums.

The primary objectives of our study were to determine: (1) if controlled or modified atmospheres were lethal and (2) to establish the minimum time required to provide 100% kill of all developmental stages of insects likely to infest materials, objects, and artifacts in museums.

Table 1. Survey of natural history museums of North America[a]

	% of total respondents	
Pests	Pests encountered	Greatest threat
Dermestid beetles	70	49
Silverfish	30	2
Cockroaches	26	3
Anobiid beetles	13	6
Tenebrionid beetles	12	2
Mites	4	
Moths	0	11
Unspecified	30	

[a]Compiled from Bell and Stanley (1981).

Considerable research has been conducted with the use of modified atmospheres (MA) or controlled atmospheres (CA) to control insect pests of stored grains and food (reviews by Bailey and Banks 1980, Fleurat-Lessard 1990). Modified atmospheres include all cases in which atmospheric gases composition has been altered, and controlled atmospheres are modified atmospheres that are usually produced artificially and maintained by additionally generating the desired gases (CO_2 and N_2) or by purging with these gases from pressurized cylinders (Calderon 1990). The three methods of producing MA are: the constitution of low O_2 atmospheres with nitrogen purge, establishment of toxic CO_2 atmospheres, and the generation of low O_2 atmospheres with 9-15% CO_2 by oxygen conversion in a gas burner (review Fleurat-Lessard 1990). In most studies, the lowest range of O_2 concentrations tested were 0.6-0.9%.

Marzke et al. (1970) found that as O_2 concentrations decreased from 21.0% to 0.6%, the mortality of adults and larvae of Trogoderma glabrum (Herbst) increased. Mortality increased with increasing temperature. Three-day exposures at 0.5% O_2 at 26.7°C provided 100% kill of adults and larvae. Adults were more susceptible than larvae. Navarro (1978) found that exposure time was the critical factor for certain species (such as the rice weevil, Sitophilus oryzae L.), being almost independent of O_2 concentrations below 3%. However, as O_2 concentrations decreased, the time required to kill Tribolium castaneum (Herbst) also decreased. In low oxygen atmospheres generated by an exothermic inert atmospheric generator (1% O_2, 9-9.5% CO_2, balance N_2), 120-hour exposures were required to kill late-instar larvae and pupae of the Angoumois grain moth, Sitotroga cerealella (Olivier). When CO_2 was added to atmospheres containing 2-5% O_2, there was a significant increase in mortality of red flour beetle adults, T. castaneum. Mortality also increased when the temperature was increased from 26° to 30°C.

Table 2. List of common insect pests found in museums in North America.[a/]

Order	Family	Species	Common name
Coleoptera	Anobiidae	Lasioderma serricorne	cigarette beetle
		Stegobium paniceum	drugstore beetle
		Anobium punctatum	furniture beetle
		Xestobium spp.	deathwatch beetle
	Bostrichidae	Dinoderus minutus	bamboo powderpost beetle
	Cleridae	Necrobia rufipes	redlegged ham beetle
	Dermestidae	Anthrenus flavipes	furniture carpet beetle
		Anthrenus verbasci	varied carpet beetle
		Attagenus megatoma	black carpet beetle
		Dermestes lardarius	larder beetle
		Thylodrias contractus	odd beetle
		Trogoderma inclusum	cabinet beetle
	Lyctidae	Lyctus spp.	powderpost beetle
	Tenebrionidae	Tribolium confusum	confused flour beetle
Lepidoptera	Tineidae	Tinea pellionella	casemaking clothes moth
		Tineola bisselliella	webbing clothes moth
Dictyoptera	Blattellidae	Blattella germanica	German cockroach
		Supella longipalpa	brownbanded cockroach
Thysanura	Lepismatidae	Lepisma saccharina	silverfish
		Thermobia domestica	firebrat

[a/] Compiled from Kingsolver (1981) and Beauchamp et al. (1981).

Jay et al. (1971) found that as the relative humidity decreased, the mortality of three stored-product insect species exposed to low O_2 atmospheres (0.8-0.97%) increased. Jay and Cuff (1981) found that mortality and water loss with T. castaneum was low at 97% N_2 and 3% O_2, but high at 99% N_2 and 1% O_2, suggesting that water loss is the major cause of death at high N_2 atmospheres. Similarly, they found that the effects of weight loss and mortality vary among life stages within a given species. Navarro (1978) found that as the O_2 concentration decreased there was an increased daily percent weight loss for flour beetle adults. In simulated combustion atmospheres, faster kill was observed at lower RH (Soderstrom et al. 1990).

In studies with insect pests frequently encountered in museums, Gilberg (1989) found that 7-day exposures at 30°C and 65-70% RH killed webbing clothes moths, cigarette beetles, drugstore beetles, carpet beetles, and powderpost beetles. The same insects exposed for 3 weeks in plastic bags were also killed (Gilberg 1990). Preliminary studies by Valentin (1990) showed that exposures to 1.0% O_2 atmospheres for 20 days killed deathwatch and powderpost beetles. Valentin and Preusser (1990) found that 30-hour exposures to 0.5% O_2 and 99.5% N_2 atmospheres killed 100% of fruit flies. Exposure time decreased as the temperature at which the exposures were conducted increased. Exposures of 15 days provided complete kill of West Indian drywood termites, Cryptotermes brevis (Walker), inside pieces of infested wood.

Test System

In the current study, controlled atmospheres (CA) were achieved by purging the chambers with pre-purified nitrogen. The test system consisted of 12 acrylic chambers (42,475 cm^3, 1.5 ft^3) that could be independently flushed with nitrogen and sealed to provide stable low-oxygen atmospheres. A controllable humidifying apparatus for adjusting relative humidity (RH) and nitrogen flow was inserted between the prepurified nitrogen cylinder (99.999% N_2, < 20 ppm O_2) and the input manifold to the chambers. To allow analytical sampling of the atmosphere in any given chamber without opening it, a gas chromatography septum was installed in each chamber (Fig. 1).

Each chamber, constructed of 0.63-cm thick methacrylate (35.6 by 45.7 by 26.7 cm), had a 14.6-cm diameter opening that could be quickly closed and sealed. An O-ring-fitted door was placed over the opening and tightened to the chamber with six capscrews. Although each chamber was connected to the common nitrogen supply line with 0.63 cm copper tubing and an exhaust line, each of the inlet and outlet ports for the chambers was controlled by Whitey valves (Swagelok), making each chamber an independent experimental unit.

To humidify the nitrogen before flushing the chambers, the gas was passed through a solution of magnesium nitrate (Fig. 1). After passing through the initial on/off valve, the nitrogen flow was split into two streams; one (controlled by the "wet" metering valve) bubbled through the first bottle (A), and the other diverted to a T-tube where it joined the "wet" nitrogen stream exiting the water bottle. The ratio of wet to dry nitrogen was varied with the two metering valves. The combined flow of nitrogen entered a mixing bottle (B), fitted with a septum through which air samples were obtained for analysis. From the mixing bottle, the nitrogen passed to a third bottle (C) equipped with a RH sensor. The nitrogen then passed to the chambers (Fig. 2).

To analyze the amount of oxygen in the mixing bottle or the chambers, a Teledyne oxygen analyzer was used. The Teledyne analyzer was standardized by calibrating it with air and zeroing it with samples from the nitrogen mixing bottle. The needle was then inserted through the septum into the chambers and an oxygen reading determined. If the reading exceeded 0.1% above the nitrogen zero, the chamber was reflushed with nitrogen.

To determine the RH of the mixed nitrogen flow, the RH sensor was plugged into a temperature and humidity transmitter (General Eastern Type 850-232) and LCD Digital Multimeter (Micronata). The accuracy was about ± 1%.

Insects

The various insects and developmental life stages tested are listed in Table 3. The cockroaches, firebrats, and termites have incomplete metamorphosis and lack a pupal stage.

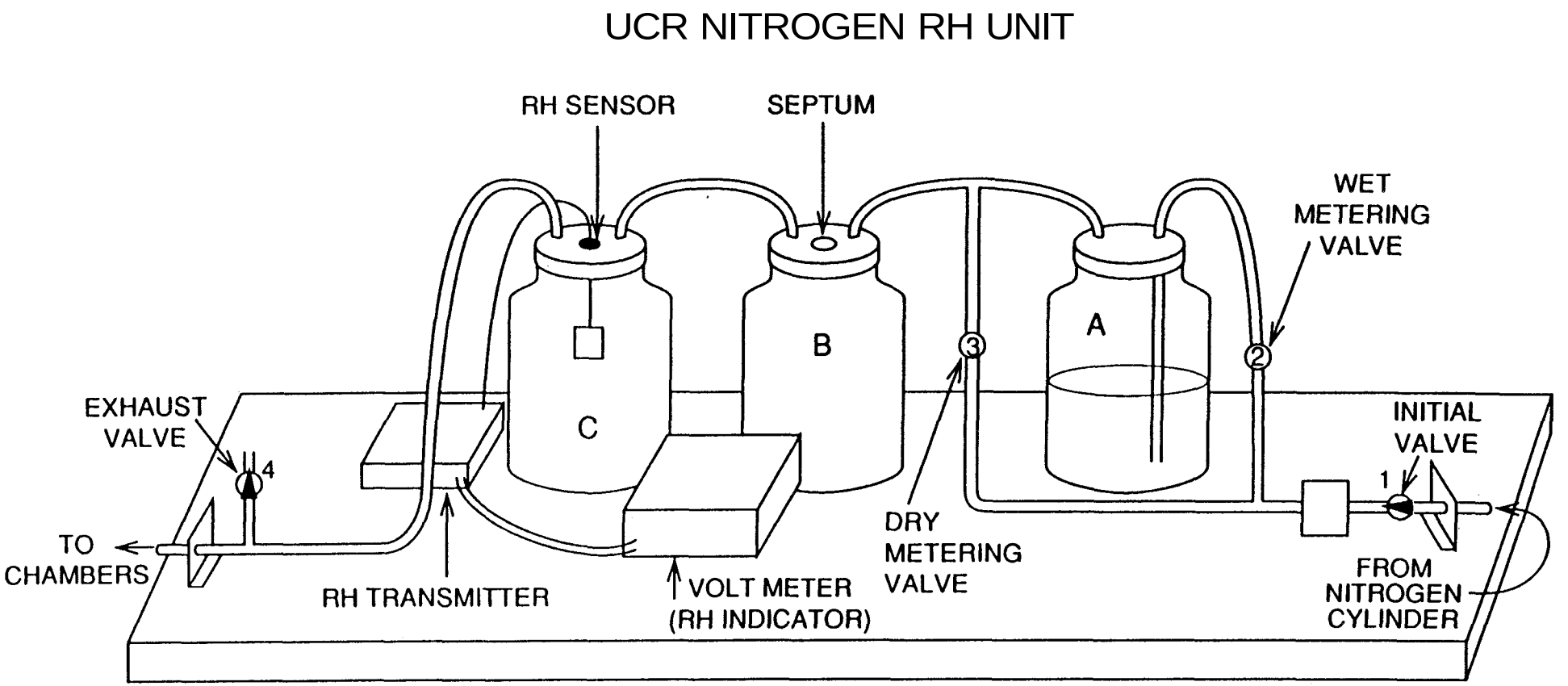

Fig. 1. Apparatus for humidifying the nitrogen flow and monitoring O_2 concentrations.

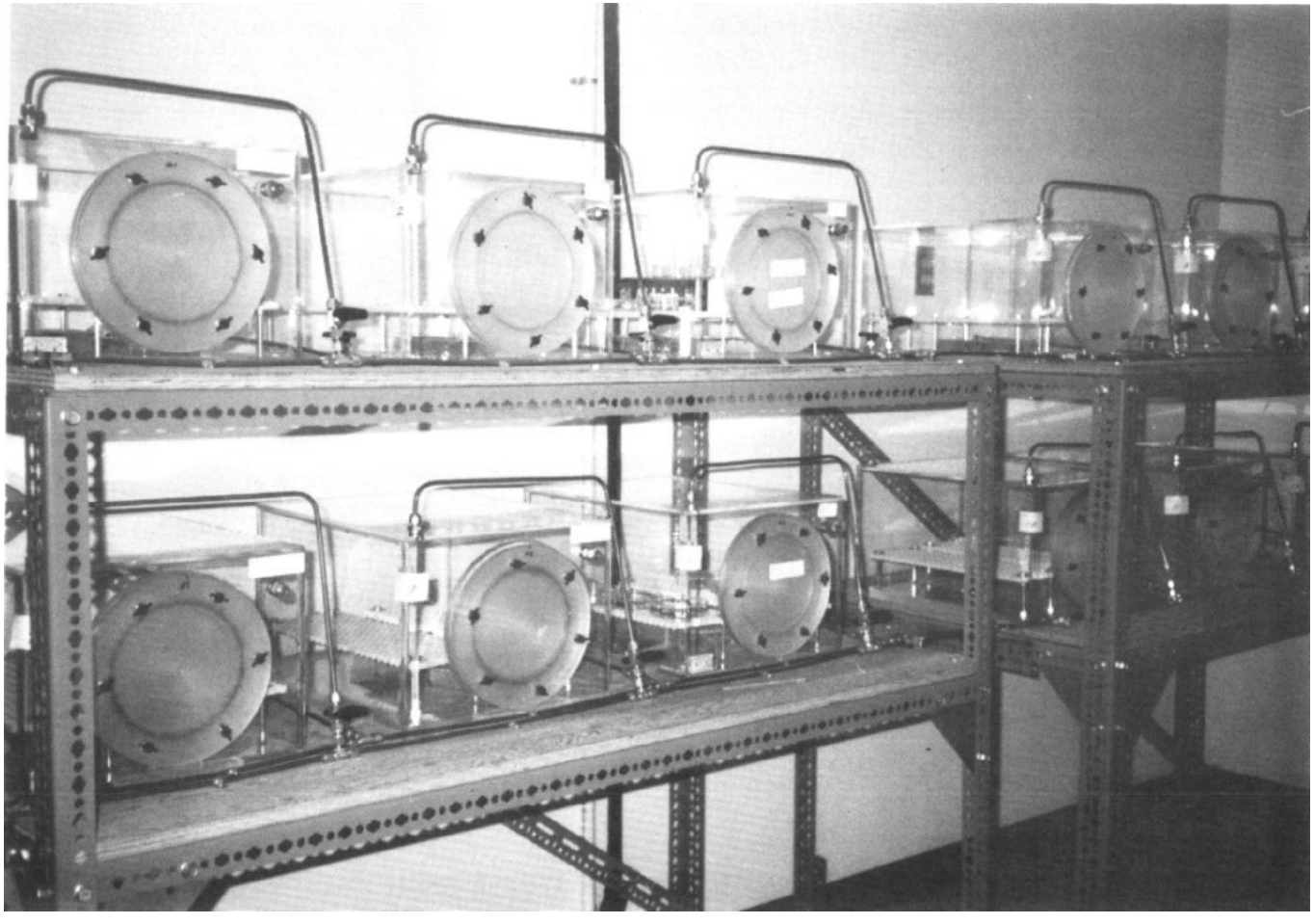

Fig. 2. The chambers used to expose insects with the container of saturated magnesium nitrate and Ageless oxygen scavenger.

Table 3. List of insects and life stages tested.

Species	Common name	Life stage tested			
		egg	larva/ nymph	pupa	adult
Lasioderma serricorne	cigarette beetle	X	X	X	X
Anthrenus flavipes	furniture carpet beetle	X	X	X	X
Dermestes lardarius	larder beetle	X	X	X	X
Trogoderma inclusum	cabinet beetle	X	X	X	X
Lyctus spp., Trogoxylon	powderpost beetle	X	X	X	X
Tribolium confusum	confused flour beetle	X	X	X	X
Tineola bisselliella	webbing clothes moth	X	X	X	X
Thermobia domestica	firebrat	X	X		X
Blattella germanica	German cockroach	X	X		X
Periplaneta americana	American cockroach	X	X		X
Supella longipalp	brownbanded cockroach	X	X		X
Incisitermes minor	western dry wood termite	X	X		X

1. THE WEBBING CLOTHES MOTH

The webbing clothes moth, Tineola bisselliella (Hummel), is the most common clothes moth found in the United States. Unlike most moths, adult clothes moths are not attracted to lights and, consequently, the adults are not usually seen. Most clothes moths that fly are males, which are capable of flying fairly long distances. Females fly only after laying their eggs and die shortly thereafter. Males often live several weeks longer. The larvae are the destructive stage, feeding on hair, feathers, fur, wool, upholstered furniture, piano felt, natural bristles, and lint. In museum storage, it is not uncommon to find active infestations inside acid-free boxes, hollinger boxes, textile storage boxes, and even cabinetry (Ebeling 1975, Parker 1990).

The average number of eggs laid by a female is between 40 and 50. Most of the eggs are deposited the first few days after emergence in loosely-woven materials such as yarns, carpets, rugs, woolens, cotton, and silk. The ovoid eggs are ivory white and about 1 mm long. Environmental conditions affect egg hatch; eggs hatch in 4 to 10 days in warm weather, but may not hatch for 30 days at cooler temperatures. Newly emerged larvae can enter fabrics or materials through openings greater than 0.01 mm. The larvae, without apparent cause, may enter a resting stage or cocoon. These dormant periods may extend from 8 to 24 months. The life cycle can vary from 55 days to 4 years depending on environmental conditions. Higher humidities favor rapid development (Ebeling 1975, Parker 1990).

Insects

The insects were reared at 26.6°C (80°F) and 55% RH, on a diet consisting of wheast (whole wheat fortified with brewer's yeast) and ground dog food (1:1). About 3 cm of the diet was placed in the bottom of a 0.9-1 glass jar. Twenty to 30 adults were added to each jar, along with a small piece of folded paper towel. A disk of filter paper was placed on the top of each jar, secured with a screened lid. A new rearing jar was set up every other week.

Cocoons and larvae were selected one day prior to testing. The collection was usually made from jars dated 6 to 8 weeks earlier. Gaseous CO_2 was used to anesthetize the adults. After

removing the adults, the remaining contents were emptied on a large 12-mesh screen to remove all small larvae, diet, and debris. The remaining larvae and cocoons were then transferred to a large petri dish.

All selections were made with soft forceps and the aid of a microscope. Only large, active larvae were selected. Cocoons containing dormant larvae and pupae were selected for testing when available. Twenty insects were placed in plastic vials (7 cm tall by 3 cm diameter) filled to the midpoint with sifted diet (20 mesh). The vials were covered with 50-mesh screened lids. Adults were scarce and were not selected for treatment, with the exception of those treated in the rearing jars. Most adults were saved for egg laying.

To collect eggs, approximately 100 adults were placed in a 0.45-liter jar covered with a 20-mesh screened lid. The jar was inverted over a petri dish, which was lightly sprinkled with presifted diet (60 mesh). The eggs were laid through the screen and collected in the petri dish below.

One day prior to testing, 1- to 3-day-old eggs were collected with the aid of a microscope. The eggs were individually transferred with a fine camel hair brush to a piece of double-stick transparent tape attached to a piece of black construction paper. The black background provided contrast so that eggs could easily be inspected. The number of eggs was totalled.

All the vials and pieces of tape were transferred to a large, covered plastic box. The relative humidity inside the box was maintained at 55% with a small container of a saturated solution of magnesium nitrate. The holding box and insects were placed in an environmental chamber maintained at 25.5°C (78°F) and 55% RH for one day prior to testing.

Phase II Testing

The vials containing insects and eggs to be tested were transferred to the chambers. The relative humidity inside the chamber was maintained at 55% with 25 ml of a saturated solution of magnesium nitrate in a small plastic box. Excess magnesium nitrate (25 g) was added to the saturated solution so that any excess moisture in the chamber was absorbed. Two packets (7 g) of Z-1000 AGELESS were placed in each chamber. AGELESS is a chemical oxygen scavenger

prepared from powdered iron oxide (Mitsubishi Gas Chemical Co., Inc.). After transferring all insects to the test chamber, the chamber door was loosely sealed. The chamber was flooded for 30 minutes with 99.999% pure nitrogen preconditioned to 55% RH. The chamber was tightly sealed and the flow of nitrogen discontinued.

If the oxygen content inside the chamber was greater than 0.1%, the chamber was flushed again for an additional 5 to 10 minutes. The oxygen content was again analyzed. Every morning the chambers were analyzed for oxygen content; whenever the level exceeded 0.1%, the chamber was flushed as described above.

The five exposure periods selected for testing were 3, 24, 48, 72, and 96 hours. The number of insects tested varied with each exposure because of the difficulty in obtaining certain stages. In the untreated control, all stages were held in the 55% RH holding box on top of the testing chamber during exposures. After the various exposures, the insects and eggs were transferred back to the holding boxes and placed in the environmental chamber.

The insects were examined after two months. The contents of each vial were dumped into a 20-mesh screen to separate the larvae, pupae, and adults from the diet. The insects were transferred to a petri dish to be counted with the aid of a microscope. Adults and larvae were individually probed with a pair of soft forceps; those that moved were scored as live. All larvae that pupated and adults that emerged from cocoons were scored as live. Resting larvae in cocoons that pupated or ultimately emerged into adults were scored as live. The remaining cocoons were opened. If they moved they were scored as live. An egg was scored as live if it had hatched and a larva could be found near the egg shell on the sticky tape. Unhatched eggs dehydrated. Approximately 650 larvae, 350 cocoons, 80 eggs, and 2 rearing jars were tested in Phase II, plus controls.

Phase III Testing

The three exposure times tested in Phase III were 48, 72, and 96 hours. When the 72-hour exposure produced less than 100% mortality, a 96-hour exposure was added. The procedure for sorting and preparing the webbing clothes moths was identical to those in Phase II except that

vials were placed in 0.9-liter glass jars and covered with 10 to 13 cm of flour to simulate conditions where the insects might be buried underneath articles or items. The paper containing the tape with eggs was rolled up and placed in a vial and covered with snap-on lid with a 50-mesh screen insert.

The various life stages of the webbing clothes moth are extremely susceptible to disturbances, resulting in increased mortality. To lessen the chance of disturbing the moths, the inside of the rearing jar was gently but firmly packed with flour. Rearing jars without flour were tested as well. Only three jars were placed in each chamber. A rearing jar packed with flour was held outside the chamber to serve as the control. After each exposure, the vials were removed from the jars, placed in large, covered plastic holding boxes, and held in an environmental chamber. Approximately 400 larvae, 1,270 eggs, 540 cocoons, and three rearing jars were tested in Phase III, plus controls.

RESULTS

Phase II

The larvae and cocoons (containing resting larvae and pupae) were readily killed when exposed to the low oxygen atmospheres for 24 hours (Figs. 3 and 4). Three-hour exposures killed 71-75% of the larvae and cocoons (Table 1.1). A 48-hour exposure provided 100% kill of the eggs.

Phase III

The 48-hour exposures provided 100% kill of all stages tested (Table 1.2). However, only 98.7% of the cocoons were killed with a 72-hour exposure. All stages were killed with a 96-hour exposure.

CONCLUSIONS

1. In Phase II, exposures of 24 hours killed 100% of the cocoons and larvae. A 48-hour exposure was required to produce 100% kill of eggs.
2. The 72-hour exposure in Phase III resulted in less than 100% mortality of the cocoons, indicating the possibility of a slight leak in the chamber even though there was a 100% mortality of larvae and eggs in the same chamber. The resting or quiescent larvae in cocoons may be more tolerant to low oxygen atmospheres than the pupae or larvae and this aspect should be explored further.
3. Ninety-six-hour exposure in Phase III produced 100% mortality with all life stages.

Table 1.1. Percent mortality of all life stages of the webbing clothes moth, Tineola bisselliella, 21 and 60 days after exposure to low oxygen atmospheres in Phase II.

Stage[a/]	Exposure Time (hrs)	No. Chambers Tested	$\bar{X}$ Percent Mortality ± SD[b/] 21 Days	60 Days
Cocoons	3	1	75.0 ± 0.0	88.8 ± 1.77
	24	2	100	100
	48	1	100	100
	72	1	100	100
	Control	1	NA	18.7 ± 6.29
Larvae	3	1	71.0 ± 7.98	90.0 ± 5.0
	24	3	100	100
	48	1	100	100
	72	1	100	100
	96	1	100	100
	Control	1	NA	6.2 ± 7.50
Eggs	48	1	NA	100
	Control	1	NA	0
Rearing Jars	96	1	100	100
	120	1	100	100

[a/] Rearing jars contained all life stages.

[b/] NA = not available.

Table 1.2. Percent mortality of all life stages of the webbing clothes moth, Tineola bisselliella, 60 days after exposure to low oxygen atmospheres in Phase III.

Stage[a/]	Exposure Time (hrs)	No. Chambers Tested	X Percent mortality ± SD
Cocoons	48	1	100
	72	1	98.7 ± 1.89
	96	2	100
	Control	1	0
	Control	1	21.2 ± 4.79
Larvae	48	1	100
	72	1	100
	96	2	100
	Control	1	5.0 ± 7.07
		1	10.0 ± 13.23
Eggs	48	1	100
	72	1	100
	96	5	100
	Control	1	1.2 ± 1.77
Rearing jars	96	3	100
	Control	1	Low[b/]

[a/] Rearing jars contained all life stages.

[b/] Low mortality, all life stages were observed alive.

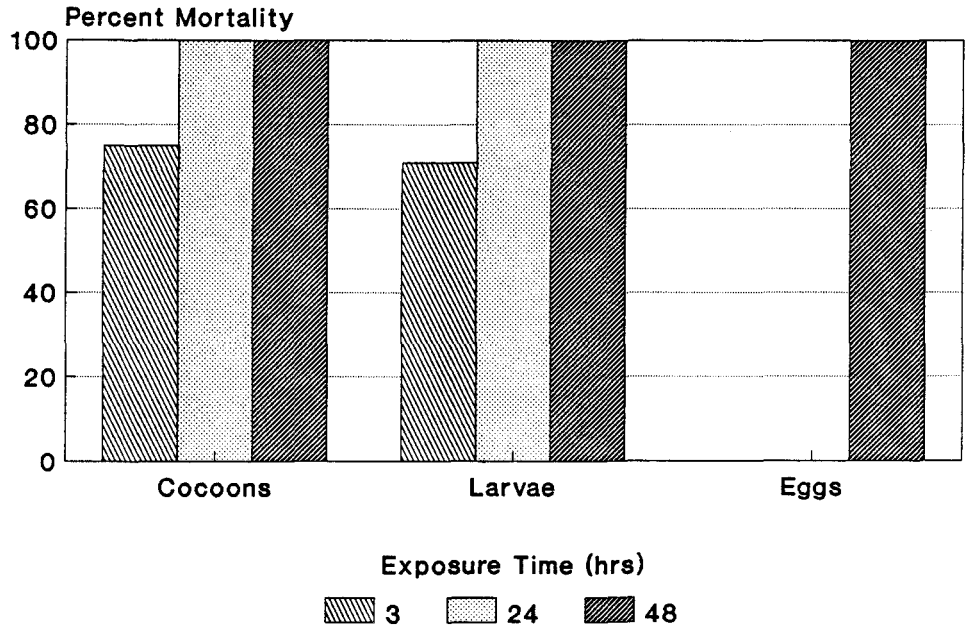

Fig. 3. Percent mortality of cocoons, larvae and eggs of the webbing clothes moth, Tineola bisselliella, 21 days after exposure to low oxygen atmospheres (< 0.1% O_2) for varying periods in Phase II.

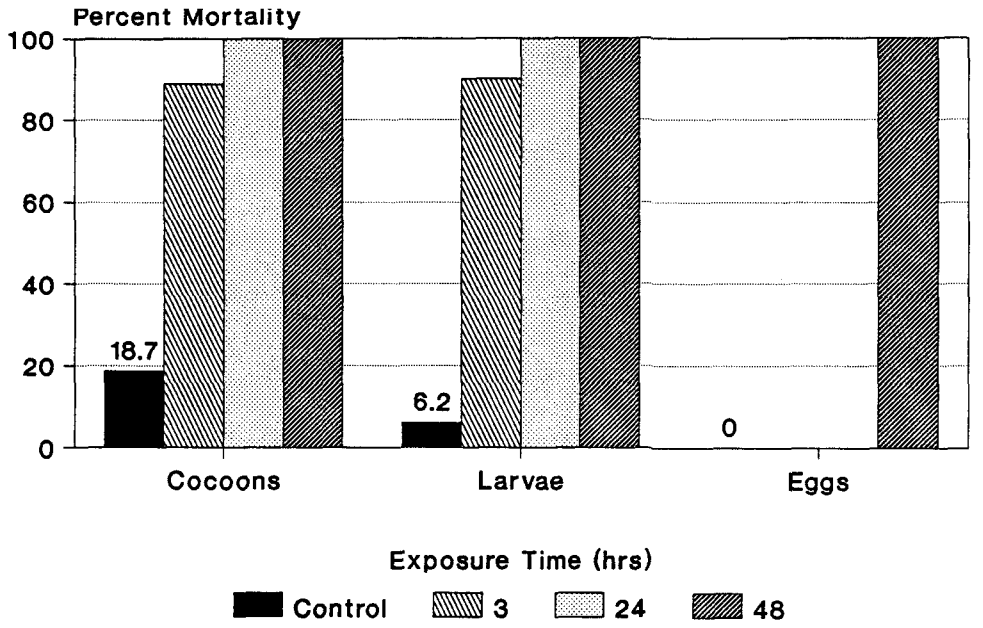

Fig. 4. Percent mortality of cocoons, larvae and eggs of the webbing clothes moth, Tineola bisselliella, 60 days after exposure to low oxygen atmospheres (< 0.1% O_2) for varying periods in Phase II.

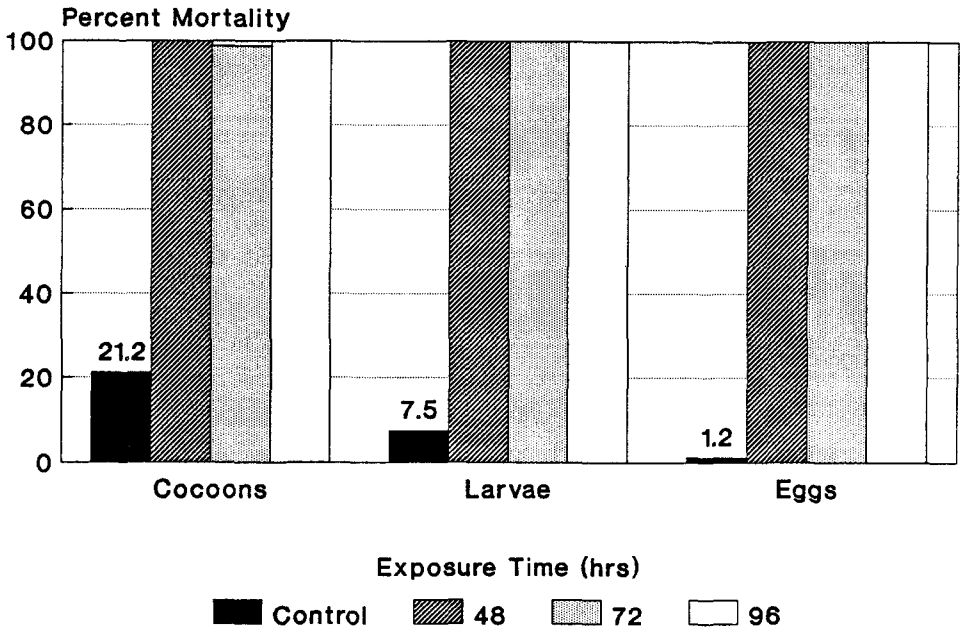

Fig. 5. Percent mortality of cocoons, larvae and eggs of the webbing clothes moth, Tineola bisselliella, 60 days after exposure to low oxygen atmospheres (<0.1% O_2) for varying periods in Phase III.

2. THE FURNITURE CARPET BEETLE

The furniture carpet beetle, Anthrenus flavipes LeConte, is destructive to a wide variety of household and museum articles made of animal products. In particular, they attack upholstered furniture, hair, feathers, natural brushes, and carpets. Other materials damaged include wool, fur, leather bindings of books, and glue of book bindings. Fabrics such as silk and cotton may be attacked if they are contaminated with perspiration, blood, or urine. Adult beetles are attracted to flowers and feed on pollen and nectar, whereas the larvae are responsible for all the damage to fabrics, materials and other items (Ebeling 1975).

The typical life cycle of the furniture carpet beetle at optimal rearing conditions is about 93 to 126 days from egg to adult. The female lays from 37 to 96 eggs over her lifetime. The eggs require about 1.5 to 3 weeks to hatch at optimal conditions. The larval stage is about 10 to 13 weeks. Adults may live for 30 to 60 days (Ebeling 1975, Hinton 1945).

Insects

The carpet beetles were reared in 3.8-liter glass jars, half filled with chicken feathers, at 26.7°C (80°F) and 55% RH. Under our rearing conditions, eggs hatched in about 3 weeks. The larval developmental period was about 11 to 12 weeks. About 15 ml (1 tablespoon) of Brewer's yeast was added to each jar to encourage adults to lay eggs. Feathers were added to each jar once every two weeks or whenever needed. A cloth treated with 18.5% dicofol held over the mouth of each jar by two rubber bands was used to cover the lid to prevent mite infestations.

To collect eggs, a separate rearing jar was set up every 3 days. A small amount of chicken feathers with Brewer's yeast was placed in a 0.9-liter jar. Young adults and pupae ready to emerge were added to the jar. Eggs were collected for testing by shaking the feathers from rearing jars over a petri dish every three days. The eggs were removed individually with a camel-hair brush and transferred to a piece of double-stick tape, one side of which was attached to a piece of black construction paper. The number of eggs on the tape was counted.

One day prior to testing, adult, pupae, and larvae were selected from rearing jars established 11 to 12 weeks earlier. The contents of the jar were dumped onto a 12-mesh screen, allowing all small larvae and fine debris to pass through. The remaining contents were then transferred to a 8-mesh screen and placed on a tray for 30 minutes in room light. Adults and larvae migrated through the screen, leaving the pupae on the screen. Only young pupae were selected. Aged pupae containing partly formed adults were added to the rearing jar for egg production. Only large, active larvae (3-4 mm long) and active adults were selected for testing. Twenty insects were placed in a 10-ml plastic vial provisioned with small pieces of feather. The vials were capped with screened lids. All vials were transferred to a large, covered plastic holding box. The RH inside the box was maintained at 55% with a small container with a saturated solution of magnesium nitrate. The large holding box and insects were placed in an environmental chamber maintained at 25.5°C (78°F) and 55% RH for one day prior to testing.

Phase II Testing

The vials containing insects to be tested were transferred to the test chambers. The relative humidity inside the chamber was maintained at 55% with 25 ml of a saturated solution of magnesium nitrate in a small plastic box. Excess magnesium nitrate (25 g) was added to the saturated solution so that all the moisture in the chamber was absorbed. Two packets (7 g) of Z-1000 AGELESS were placed in each chamber. AGELESS is a chemical oxygen scavenger prepared from powdered iron oxide (Mitsubishi Gas Chemical Co., Inc.). After transferring all insects to the test chamber, the chamber door was loosely sealed. The chamber was flooded for 30 minutes with 99.999% pure nitrogen conditioned to 55% RH. The chamber was tightly sealed and the flow of nitrogen discontinued.

If the oxygen content inside the chamber was greater than 0.1%, the chamber was flushed again for an additional 5 to 10 minutes. The oxygen content was again analyzed. Every morning the chambers were analyzed for oxygen content. Whenever the oxygen level exceeded 0.1%, the chamber was flushed as described above.

Five exposure periods were tested with all life stages: 6, 16, 24, 48 and 72 hours. Each test chamber contained four vials of adults, pupae, and larvae with 20 insects per vial, for a total of approximately 240 insects per test, plus the control insects. The number of eggs varied with each test. Each test was replicated three times, on three different dates. The control insects were held at 55% RH in a holding box, which was placed on top of the test chamber. After the exposure tests were completed, the test insects were transferred to the holding boxes and placed in an environmental chamber.

The pupae, larvae, and egg stages of carpet beetles were examined three weeks after being exposed to the low oxygen atmospheres. The number of larvae adhered to the tape was counted. The difference between the number of larvae and total number of eggs was considered to be the egg mortality. Live larvae and pupae were counted; the difference from the initial number was the mortality during the test. Similarly, the difference between the emerged adults and live pupae from the initial number was the pupal mortality. Adults were examined at 1, 2, and 7 days. The contents of each vial were dumped into a petri dish and examined with a microscope. The adults were very sensitive to light, and a bright light evoked more of a response than probing. If the adults moved, they were scored as live. When in doubt, the adult was dissected, as dead insects are dehydrated and brittle.

Phase III Testing

The only exposure time tested in Phase III was 72 hours, which was the minimum time required to produce 100% mortality in Phase II. The procedure for sorting and preparing the carpet beetles was the same as described in Phase II above except that the vials were placed in 0.9-liter glass jars and covered with 10 to 13 cm of flour. The construction paper containing the tape with the eggs was rolled up, placed in a vial (7 cm tall by 3.5 cm diameter), and covered with a screened lid before being transferred to the glass jars. The flour was firmly packed on top of the vials. Three jars packed with vials and flour were placed in each chamber. Approximately 630 adults, 190 pupae, 650 larvae, and 200 eggs were tested in Phase III, plus control insects. The unexposed insects were held in identical jars on top of the chambers during the tests.

After each exposure the vials were removed from the jars, placed in large, covered plastic holding boxes, and held in an environmental chamber. The insects were examined and mortality determined as described in Phase II.

RESULTS

Phase II

Exposures for 24 hours provided 100% kill of adult carpet beetles (Table 2.1). The adults are short-lived; 25% were dead at day 7 in the untreated controls (Table 2.2). The egg was the most tolerant stage, with only 50% killed with a 24-hour exposure (Table 2.3). Exposures for 48 hours were required to provide 100% kill of the eggs and pupae (Fig. 5). Larvae were the most susceptible immature stage, requiring only a 24-hour exposure to provide 100% kill.

Phase III

When all developmental stages were exposed for 72 hours, there was complete kill (Table 2.4).

CONCLUSIONS

1. In Phase II, exposures of 24 hours killed 100% of the adults and larvae. The pupae and eggs were the most resistant stages, requiring exposures of 48 hours to provide 100% kill.
2. In Phase III, exposures of 72 hours provided 100% mortality. The use of 72-hour exposures should provide sufficient margin of error to insure complete kill of all life stages.

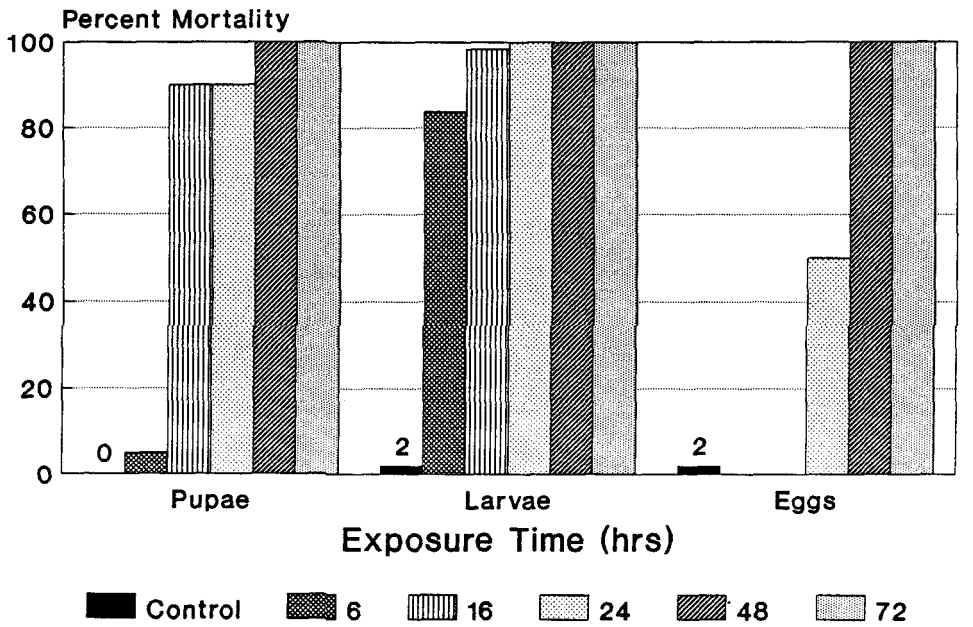

Fig. 6. The mortality of three life stages of the furniture carpet beetle, Anthrenus flavipes, 3 weeks after exposure to low oxygen atmospheres in Phase II.

Table 2.1. The percent mortality of the adult furniture carpet beetle, Anthrenus flavipes, three weeks after exposure to low oxygen atmospheres in Phase II.

Exposure Time (hrs)	No. Chambers Tested	$\bar{X}$ Percent mortality ± SD[a/]
6	1	85.0 ± 12.91
24	1	100
48	3	100
72	3	100
untreated	7	75.0 ± 33.29

[a/] 2-4 replicates tested per treatment.

Table 2.2. Percent mortality of the adult furniture carpet beetle, Anthrenus flavipes, to 24-hour exposures to low oxygen atmospheres in Phase II.

Days after exposure	$\bar{X}$ Percent mortality ± SD	
	Exposed	Controls
1	100	27.5 ± 3.53
2	100	22.5 ± 3.53
6	100	25.0 ± 7.07
7	100	25.0 ± 7.07

[a/] 2-10 replicates tested per period after exposure.

Table 2.3. The percent mortality of the immature stages of the furniture carpet beetle, Anthrenus flavipes, 3 weeks after exposure to low oxygen atmospheres in Phase II.

Stage	Exposure Time (hrs)	No. Chambers Tested	$\bar{X}$ Percent Mortality ± SD[a/] Control	Exposed
Pupae	6	1	0.0	5.0 ± 7.07
	16	1	0.0	90.0 ± 14.14
	24	3	0.0	90.0 ± 14.14
	48	4	0.0	100
	72	3	0.0	100
Larval	6	1	10.0	83.8 ± 6.29
	16	2	0.0	98.4 ± 2.33
	24	1	0.0	100
	48	2	1.0 ± 1.41	100
	72	1	0.0	100
Eggs	24	1	2.9	50.0
	48	1	0.0	100
	72	2	1.6 ± 2.33	100

[a/] 2-10 replicates tested per exposure period.

Table 2.4. The percent mortality of all life stages of the furniture carpet beetle, Anthrenus flavipes, three weeks after exposure to low oxygen atmospheres in Phase III for 72 hours.

	$\bar{X}$ Percent Mortality ± SD	
Stage	Control	Exposed[a/]
Adults	83.0 ± 11.31	100
Puape	2.5	100
Larvae	0	100
Eggs	0	100

[a/] 2-4 replicates tested per life stage.

3. THE FIREBRAT

The firebrat, Thermobia domestica (Packard), is a primitive, wingless insect belonging to the order Thysanura. Firebrats feed on numerous items found in museums and libraries, especially those made with starch, paste, glue (as in bookbindings), and starched cotton, linen, rayon, or lisle. They are pests of paper, especially those with a glaze or sizing consisting of starch, dextrin, casein, gum, or glue. Occasionally synthetic items will be attacked if they are coated with sizings or paste (Carr 1990).

The typical life cycle of firebrats at optimal rearing conditions (37-39°C) is about 1.5 to 3.5 months. Females lay about 50 eggs in a lifetime. The eggs require about 14-18 days to hatch at optimal conditions (37°C, 76-85% RH). The adults may live for as long as two years (Ebeling 1975).

Insects

The firebrats were reared in specially modified Coleman ice chests. The bottom of the ice chest was lined with a layer of unrolled cotton. Four small water jars with cotton dental wick through the lids were placed inside the chest, one in each corner. A lamp with a 15 watt light bulb was placed inside an inverted clay flower pot to provide heat. A thermostat attached to the lamp maintained the temperature at 33 ± 1°C (92°F). Six or seven small pieces of corrugated cardboard (3 by 5 cm) stacked together were placed on the cotton to provide harborage. Three or four cardboard harborages were placed in each ice chest. The firebrats were fed dry dog chow.

Nymphs were selected one day prior to testing. Firebrats were dumped on a 20-mesh screen, and very small nymphs (< than 4 mm long) and debris collected underneath the screen were returned to the ice chests. The contents on the screen were transferred to a 12-mesh screen and the screen was placed on a tray for 30 minutes in room light. Firebrats capable of passing through the screen were retained and placed into containers. These were considered to be small nymphs, usually around 5 mm in length. The firebrats remaining on the screen were dumped on a 10-mesh screen and allowed to let stand over a metal tray for 30 minutes. The nymphs (6 to 8 mm

in length) that passed through the screen were collected and placed in containers. They were considered to be medium size. Those remaining on the screen (8 to 10 mm in length) were placed in another container; the majority of these firebrats were adults. All three sizes were carefully inspected and any odd-sized insects removed.

The firebrats were carefully handled (with soft forceps) only when necessary. Ten nymphs or adults were poured into plastic holding vials (7 cm tall by 3 cm diameter) provisioned with 3 g of ground dog food. Eggs were collected by peeling off a thin top layer of the exposed cotton from the ice chests. The cotton was teased apart to dislodge the eggs which were collected in a petri dish. The petri dish was placed over a piece of black paper and, with the aid of a microscope, the white- and pearl-colored eggs were discarded, as these were ready to hatch. The eggs were picked up individually with a fine camel-hair brush and transferred to a piece of transparent double-stick tape, one side of which was attached to a piece of black construction paper. The number of eggs on the tape was counted. Each exposure usually contained 4 vials of small and medium nymphs, adults, and tapes with eggs.

All the vials and pieces of tape were transferred to a large, covered plastic box. The relative humidity inside the box was maintained at 55% with a small container of a saturated solution of magnesium nitrate. The large holding box and insects were placed in an environmental chamber maintained at 25.5°C (78°F) and 55% RH for one day prior to testing.

Phase II Testing

The vials containing insects to be tested were transferred to the chambers. The relative humidity inside the chamber was maintained at 55% with 25 ml of a saturated solution of magnesium nitrate in a small plastic box. Excess magnesium nitrate (25 g) was added to the saturated solution so that all the moisture in the chamber was absorbed. Two packets (7 g) of Z-1000 AGELESS were placed in each chamber. AGELESS is a chemical oxygen scavenger prepared from powdered iron oxide (Mitsubishi Gas Chemical Co., Inc.). After transferring all insects to the test chamber, the chamber door loosely sealed. The chamber was flooded for 30

minutes with 99.999% pure nitrogen conditioned to 55% RH. The chamber was tightly sealed and the flow of nitrogen discontinued.

If the oxygen content inside the chamber was greater than 0.1%, the chamber was flushed again for an additional 5 to 10 minutes. The oxygen content was again analyzed. Every morning the chambers were analyzed for oxygen content. Whenever the oxygen level was greater than 0.1%, the chamber was flushed as described above.

Nymphs and adults were exposed for 0.5, 1, 2, 3, 16, and 24 hours. Eggs were exposed for 3, 16, and 24 hours. In the control, the nymphs, adults, and eggs were held in the holding box at 55% RH on top of the testing chamber during the exposures. After each exposure was completed, the insects and eggs were transferred back to the holding boxes, placed in the environmental chamber, and maintained at 55% RH.

To determine if the exposures were lethal, the contents of each vial were dumped into a petri dish. Nymphs and adults were examined 24 and 168 hours after exposure. Each nymph or adult was examined under a microscope and probed with a pair of soft forceps. If it moved, it was scored as live. An egg was considered alive if it had hatched and the emerged nymph was adhered to the tape next to the empty egg shell. If not, the egg was scored as dead. Eggs were inspected at 24, 48, and 168 hours after exposure. The eggs were held for an additional 9 weeks before a final count was made. Approximately 1,040 nymphs, 750 adults, and 1,500 eggs were tested in Phase II.

Phase III Testing

To determine if the addition of materials interfered with the exposure to low oxygen atmospheres, firebrats were buried in flour to simulate conditions in the field where they might be confined in garments or fabrics. The only exposure time tested in Phase III was 48 hours, which equalled the minimum time required to produce 100% mortality in Phase II, plus 24 hours. The procedure for sorting and preparing the firebrats was identical to those in Phase II except that the vials were placed in 0.9-liter glass jars and covered with 10 to 13 cm of flour. Each paper containing tape with eggs was rolled up, placed in a vial (3.5 cm diameter by 7 cm), and covered

with a screened lid before being transferred to the glass jars. The flour was firmly packed on top of the vials. Three jars packed with vials and flour were placed in each chamber for testing. Control insects were held in identical jars on top of the chambers during the tests. After each exposure, the vials were removed from the jars, placed in large covered plastic holding boxes and held in an environmental chamber. The tests were replicated three times. The insects were examined for mortality as described in Phase II. Approximately 150 nymphs, 250 adults, and 120 eggs were tested.

RESULTS

Phase II

Firebrat nymphs and adults were readily killed when exposed to the low oxygen atmospheres (Fig. 7). As short as a 3-hour exposure killed all the stages within 24 hours. Briefer exposures resulted in about 20-78% mortality. The average percent mortality of small nymphs exposed for 0.5 hours was 47.2% and 93.2%, after holding the nymphs for 24 and 168 hours, respectively. Mortality was consistent among chambers at 24 hours, whereas there were some differences at 168 hours (Table 3.1). There were no significant differences in mortality between medium nymphs and adults when exposed for 0.5 or 1 hour (Table 3.1). When the firebrats were examined 168 hours after exposure, the 0.5- and 1-hour exposures provided 80-90% kill of all three stages (Fig. 8).

In most chambers, 0.5- or 1-hour exposures provided a significant increase in mortality at 168 hours (Table 3.1), suggesting that the short exposures to the oxygen deficient atmospheres have a latent mortality effect. The cause of this mortality is unknown.

The mortality of firebrat eggs exposed for 24 hours was 97.1% (Table 3.2). As exposure time increased, there was a direct linear increase in mortality ($y = 2.6x + 12.6$, $t = 26.6$, $\mathbf{P} = 0.02$, SEM slope = 0.098; Fig. 9). Consequently, exposures of 29.8 hours should provide 100% kill of the egg stage.

Phase III

The 48-hour exposure produced 100% kill of all stages. The flour did not significantly increase the time required to kill all stages of the firebrat. Therefore, 48-hour exposures should provide complete kill.

CONCLUSIONS

1. In Phase II, exposures as short as 3 hours killed 100% of the nymphs and adults. Eggs were the most resistant life stage, requiring exposures of 29.8 hours to provide complete kill.
2. Brief exposures of 0.5 or 1 hour provided significant latent mortality when firebrats were examined after 168 hours. The mode of action is unknown.
3. In Phase III, 48-hour exposures provided 100% kill of all stages. Oxygen was quickly displaced from the flour and replaced with nitrogen in the chambers.
4. The use of 48-hour exposures should provide sufficient margin of error to insure complete kill of all stages.

Table 3.1. Percent mortality of firebrats, Thermobia domestica, exposed to low oxygen atmospheres in Phase II, at 24 and 168 hours after exposure.

Stage[b/]	Exposure Time (hrs)	Chamber (1-3)	$\bar{X}$ Percent Mortality[a/] 24 Hrs		168 Hrs	
M. Nymphs	0.5	1	26.4 ± 21.58	def	89.9 ± 7.52	ab
		2	4.4 ± 5.20	ef	97.5 ± 5.00	ab
		3	31.7 ± 3.96	cde	68.5 ± 10.78	cd
	Control	--	0.0	f	1.2 ± 1.44	e
	1.0	1	51.8 ± 14.54	bcd	68.0 ± 6.06	cd
		2	68.0 ± 4.88	b	100	a
		3	61.9 ± 28.6	bc	100	a
	Control	--	0.0	f	1.2 ± 1.44	e
	3.0	1	100	a	100	a
		2	100	a	100	a
		3	100	a	100	a
	Control	--	0.0	f	1.2 ± 1.44	e
Adults	0.5	1	13.9 ± 16.66	ef	80.1 ± 10.18	bcd
		2	10.8 ± 9.24	ef	89.2 ± 12.78	ab
		3	31.8 ± 12.84	cde	65.8 ± 13.70	d
	Control	--	0.0	f	1.2 ± 1.44	e
	1.0	1	70.7 ± 8.28	ab	83.6 ± 8.90	abc
		2	63.9 ± 13.98	b	97.2 ± 5.56	ab
		3	62.7 ± 24.20	bc	100	a
	Control	--	0.0	f	1.2 ± 1.44	e
	3.0	1	100	a	100	a
		2	100	a	100	a
		3	100	a	100	a
	Control	--	0.0	f	1.2 ± 1.44	e

[a/] Four lots of insects tested per chamber. Averages followed by the same letters in each column are not significantly different $P < 0.05$ (Tukey's HSD).

[b/] M. Nymph - medium sized.

Table 3.2. Percent mortality of firebrat, Thermobia domestica, eggs nine weeks after exposure to low oxygen atmospheres in Phase II.

Exposure time (hrs)	No. Chambers Tested	$\bar{X}$ Percent Mortality ± SEM[a/]
3	3	36.1 ± 4.33
16	3	89.0 ± 1.33
24	3	97.1 ± 0.52
Control	6	18.8 ± 2.81

[a/] A minimum of 3 replicates for each exposure and chamber.

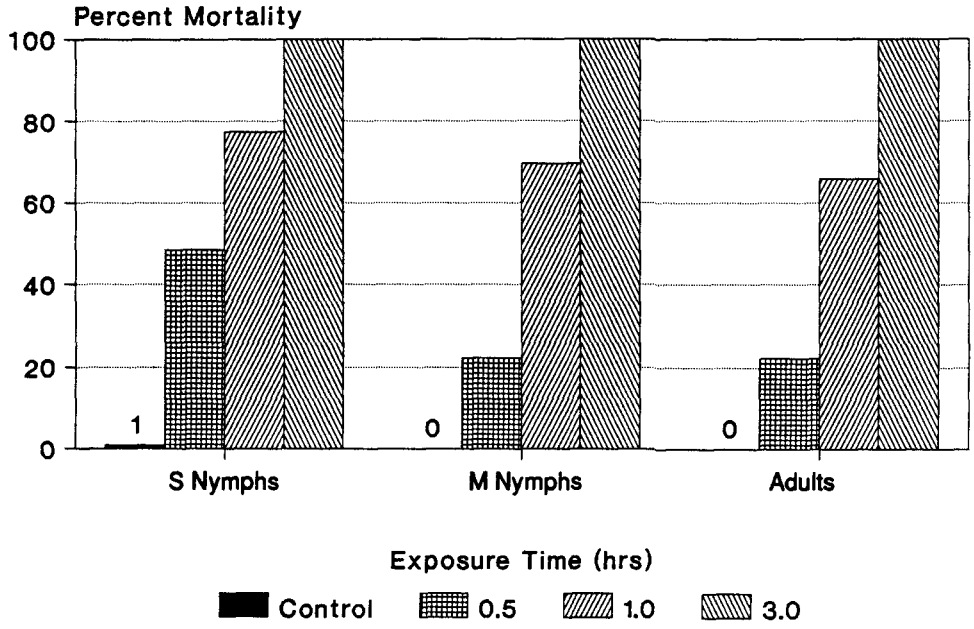

Fig. 7. The mortality of nymphal and adult firebrats, Thermobia domestica, 24 hours after exposure to low oxygen atmospheres (<0.1% O_2). S Nymph - small sized nymphs; M Nymph - medium sized nymphs.

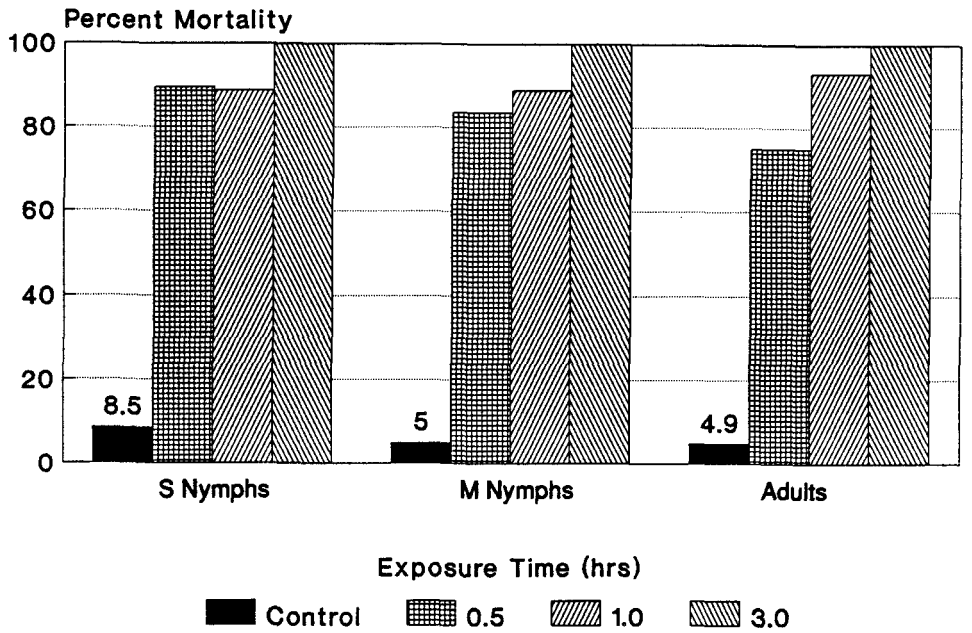

Fig. 8. The percent mortality of nymphal and adult firebrats, Thermobia domestica, 168 hours after exposure to low oxygen atmospheres (<0.1% O_2). S Nymph - small sized nymphs; M Nymph - medium sized nymphs.

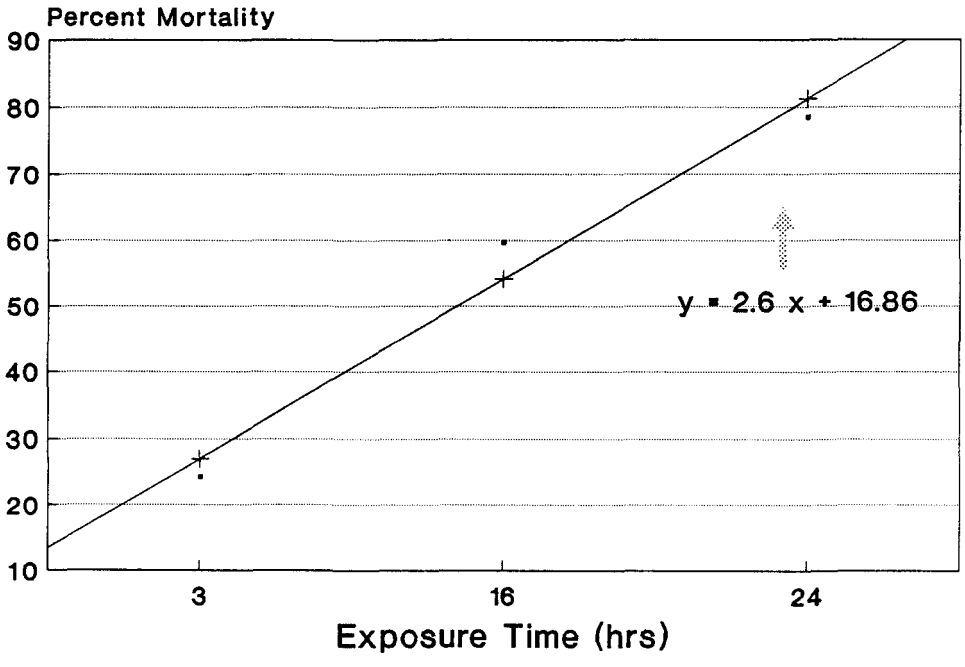

Fig. 9. The direct linear relationship of percent egg mortality (arcsin transformation) and exposure time to low oxygen atmospheres (<0.1% O_2).

4. THE CABINET BEETLE

The larvae of the cabinet beetle, Trogoderma inclusum LeConte, damages insect collections, hides, skins, wool, and feathers. The larvae are more likely to be a pest of processed dry foods, animal feeds, and storage facilities than a serious pest of stored grains. The distribution of T. inclusum is influenced by climate; they are encountered in areas with a relative humidity in excess of 35% (Strong 1975).

The typical life cycle of cabinet beetles under normal rearing conditions is about 5.5 to 7 months. Females lay up to 85 eggs in a lifetime. The eggs require about 8 to 12 days to hatch at room temperature. Under optimal conditions, the time from egg hatch to adult maturation is about 24-92 days. The larval period can extend from 5 months to 4.5 years, depending on food availability. Adults may live for up to 90 days (Strong 1975).

Insects

Cabinet beetles were reared at 25.6°C (85°F) and 30% RH on a diet consisting of whole wheat fortified with Brewer's yeast. Eggs hatched in 4 to 6 days. The time required for eggs to mature into adults under these conditions was about 13 to 15 weeks. The pupal stage lasted about 12 to 14 days. Six to 8 cm of the diet was placed on the bottom of a 3.9-liter glass jar and provisioned with young adults and pupae. A new jar was set up once every month. A cloth treated with 18.5% dicofol, held over the mouth of each jar by two rubber bands, was used to cover the lids to prevent mite infestations.

One day prior to testing, adults, pupae, and larvae were selected from rearing jars established 13 to 15 weeks earlier. The contents of the jar were sifted through a 12-mesh screen to remove all small larvae and diet. The remaining contents were dumped onto a tray, and all large debris were removed. Aged pupae become dark inside before emerging as adults; these were discarded. Light colored pupae were selected individually with the aid of a microscope and removed with soft forceps. Active adults and large larvae (3-4 mm long) were selected for testing. Twenty insects of each life stage were placed in plastic vials (7 cm tall x 3 cm diameter).

Approximately 3 g of sifted diet was added to each vial before covering with a snap-on lid with a screened insert.

A separate rearing jar was set up every 3 days for egg collections. About 20 ml of diet was sifted through a 60-mesh screen, and the finely ground diet was transferred to a 3.9-liter jar with young adults. Eggs were collected by passing the contents of the jar through a 20-mesh screen to remove the adults, and then through a 60-mesh screen to remove the eggs. The eggs were transferred individually with a camel-hair brush to a piece of double-stick tape, one side of which was adhered to a piece of black construction paper to make the eggs readily visible. The emerging larvae were stuck to the sticky surface of the tape. The number of eggs on the tape was counted. The tapes with the eggs were placed inside the plastic vials and sealed with a snap-on lid with a screen insert. To provide additional confirmation of the exposure time required for 100% mortality, large numbers of eggs were collected and placed in a small petri dish with sifted diet. The dishes and eggs were exposed along with the tape strips with eggs.

All the vials, pieces of tape with eggs, and petri dishes were transferred to a large, covered plastic box. The relative humidity inside the box was maintained at 55% with a small container of a saturated solution of magnesium nitrate. The large holding box and insects were placed in an environmental chamber maintained at 25.5°C (78°F) and 55% RH for one day prior to testing.

Phase II Testing

The vials containing insects to be tested were transferred to the test chambers. The relative humidity inside the chamber was maintained at 55% with 25 ml of a saturated solution of magnesium nitrate in a small plastic box. Excess magnesium nitrate (25 g) was added to the saturated solution so that all the moisture in the chamber was absorbed. Two packets (7 g) of Z-1000 AGELESS were placed in each chamber. AGELESS is a chemical oxygen scavenger prepared from powdered iron oxide (Mitsubishi Gas Chemical Co., Inc.). After transferring all insects to the test chamber, the chamber door was loosely sealed. The chamber was flooded for 30 minutes with 99.999% pure nitrogen conditioned to 55% RH. The chamber was tightly sealed and the flow of nitrogen discontinued.

If the oxygen content inside the chamber was greater than 0.1%, the chamber was flushed again for an additional 5 to 10 minutes. The oxygen content was again analyzed. Every morning the chambers were analyzed for oxygen content. Whenever the oxygen level was greater than 0.1%, the chamber was flushed as described above.

Five different exposure periods were selected for testing with the pupal, larval, and egg stages: 24, 48, 72, 96, and 120 hours. Adults were only exposed for 24 hours. The number of insects varied with each exposure, according to availability. In the untreated control, all stages were held at 55% RH in holding chambers during the exposures. After each exposure the adults, pupae, larvae, and eggs were transferred back to the holding boxes and held in the environmental chamber.

To determine the mortality for each stage, the contents of each vial were passed through a small 20-mesh screen to separate the insects from the diet. The pupae, larvae, and eggs were examined 3 weeks after the nitrogen exposures. The adults were examined at 24, 48, and 168 hours because of high control mortality. With the aid of a microscope, an individual adult was picked up with soft forceps and dropped; if there was no detectable movement, it was scored as dead. Light colored pupae were considered dead. Dark colored pupal cases were dissected and the pupa was probed with soft forceps. If there was no response, the pupa was scored as dead. Dead pupae were usually dehydrated. If adults emerged, the pupae were considered as live. Each larva was probed with soft forceps and if it moved, it was scored as live. If pupae were present, the larvae were also scored as live. Eggs were considered alive if they hatched and larvae could be found near the eggs adhered to the sticky tape. If not, the eggs were scored as dead. Approximately 290 adults, 670 pupae, 1,500 larvae, and 1,640 eggs were tested in Phase II, plus controls.

Phase III Testing

The two exposure times tested in Phase III were 96 hours and 120 hours (the minimum time required to produce 100% mortality determined in Phase II). The tests were replicated three times.

The procedure for preparing the various stages of cabinet beetles was identical to those in Phase II except that the vials were placed in 0.9-liter glass jars and covered with 10 to 13 cm of flour. The paper strips containing the eggs were rolled up, placed in vials (7 cm tall by 3.5 cm diameter), and covered with screened lids before being transferred to the glass jars. The flour was firmly packed on top of the vials. Three jars packed with vials and flour were placed in each chamber for testing. Unexposed control insects were held in identical jars on top of the chambers during the tests. After each exposure, the vials were removed from the jars, placed in large, covered plastic holding boxes, and held in an environmental chamber. The insects were examined for mortality as described in Phase II. Approximately 270 adults, 530 pupae, 1,150 larvae, and 740 eggs were tested in Phase III, plus controls.

RESULTS

Phase II

Exposures of 72 hours provided 100% kill of adult beetles. Adult mortality in the untreated control was 5% at day 7 and >95% by day 21. Strong (1975) reported that mated adult males and females survived 10-18 days at 22.7°C. The longevity of adults increased as the temperature decreased.

Figure 10 shows that the 24-hour exposure resulted in only 62% kill of pupae, 8% kill of larvae and 90% kill of eggs. A 48-hour exposure to the low oxygen atmosphere killed 100% of the pupae (Table 4.1). Exposures of 72 hours killed 92.7% of the larvae. An exposure of 72 hours was necessary to insure 100% kill of the eggs. The larvae were the most tolerant stage, requiring 120-hour exposure to kill 100%.

Phase III

The 96-hour exposures killed 100% of the adults and eggs, 98% of the pupae, and 93% of the larvae (Table 4.2). The 120-hour exposures resulted in complete kill of all stages. The

flour did not significantly increase the time required to kill the larvae and other stages of the cabinet beetle.

CONCLUSIONS

1. The cabinet beetle was the only species tested in which the larvae was the stage most tolerant to low oxygen atmospheres. Larvae required 120-hour exposures to produce 100% mortality, compared with 48 hours for pupae and 72 hours for eggs.
2. Adults were killed with a 72-hour exposure. Additional tests with adults exposed for 24 and 48 hours need to be conducted, and exposed adults should be examined every 48 hours for mortality.
3. With 24-hour exposures, 55% of the pupae and 50% of the eggs were killed. The 72-hour exposures killed 100% of both stages.
4. In Phase III, the 120-hour exposures provided 100% kill of all life stages. The flour did not increase the exposure time required to provide complete kill.

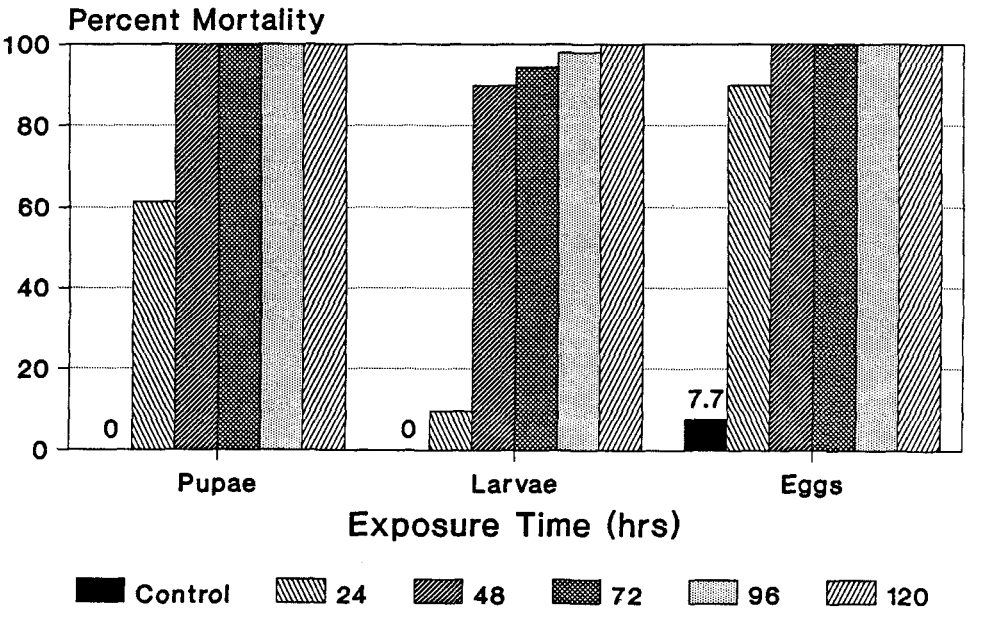

Fig. 10. The percent mortality of all tests with pupae, larvae, and eggs of the cabinet beetle, Trogoderma inclusum, 3 weeks after exposure to low oxygen atmospheres in Phase II.

Table 4.1. The percent mortality of the pupae, larvae, and eggs of the cabinet beetle, Trogoderma inclusum, exposed to low oxygen atmospheres in Phase II, three weeks after exposure.

Stage	Exposure Time (hrs)	No. Chambers Tested	$\bar{X}$ Percent Mortality + SEM[a/]
Pupae	24	2	64.2 ± 19.15
	48	1	100
	72	6	100
	96	3	100
	120	2	100
	--	4	0
Larvae	24	3	8.4 ± 2.98
	48	1	90.0
	72	5	92.7 ± 3.92
	96	3	98.6 ± 1.40
	120	2	100
	--	9	0
Eggs	24	2	64.2 ± 19.15
	48	1	100
	72	6	100
	96	3	100
	120	2	100
	--	6	7.7 ± 1.97

[a/] 2-4 replicates tested per chamber.

Table 4.2. The percent mortality of immature stages of the cabinet beetle, Trogoderma inclusum, exposed to low oxygen atmospheres in Phase III, at three weeks after exposure.

Stage	No. Chambers Tested	Exposure (hrs)	$\bar{X}$ Percent Mortality ± SD Exposed	Controls
Pupae	2	96	98.0 ± 4.0	0
	2	120	100	0
Larvae	2	93	93.1 ± 6.20	0
	2	120	100	0
Eggs	2	96	100	0
	2	120	100	0

[a/] 3-16 replicates were tested per chamber and exposure.

5. THE LARDER BEETLE

The larder beetle, Dermestes lardarius (L.), feeds on ham, bacon, dried beef or fish, cheese, hair, horn, feathers, fur, and carcasses. With the discontinuance of curing meats at home, the larder beetle has become less important in recent years. However, the presence of the larder beetle may indicate rodent or bird carcasses in buildings. In museums, it is frequently used to clean flesh from bones. The typical life cycle from egg to adult is 60 to 90 days. Eggs hatch in 2.5 to 12 days, usually 7 to 8 days. The larval period is from 15 to 80 days. The pupal period lasts from 8 to 15 days, depending on the temperature. Full-grown larvae will leave the food to search for a place to pupate. The larvae will tunnel into almost any compact material, such as wood, books, cork, and vegetable fiber, frequently damaging it. Adults may live up to 1.5 years. In our rearing conditions, eggs hatched within 10-12 days held at 25.5°C (78°F) 55% RH. The life cycle (egg to adult) was 28 to 35 days (Hinton 1945).

Insects

The larder beetles were reared in 3.8-liter glass jars provisioned with two separate diets: (A) 4 parts whole wheat flour and 1 part fish meal; (B) 0.8 liter of chicken laying mash mixed with 200 ml honey and 200 ml glycerin. One scoop of diet B was placed on the bottom of a jar and one-half scoop of diet A was added on top. A large disk of filter paper (12.5 cm diameter), covered on one side with aluminum foil the edges of which were folded over the filter paper, was placed in the jar with the foil side down. A short, wide-mouth bottle (100 ml) was filled with water. A piece of wet filter paper was placed over the mouth of the jar and it was covered with a piece of tight-fitting aluminum foil. Three pin holes were made through the foil and filter paper. The water bottle was inverted on the filter paper in the rearing jar, allowing moisture to slowly escape into the diet. Thirty to 40 adults were placed in the jar. A cloth treated with 18.5% dicofol was placed over the mouth of each jar and secured by two rubber bands to prevent mite infestations. The jar was placed in a rearing chamber set at 26.6°C (80°F), 55% RH.

Once every week a new jar was set up. The dead adults from the previous jar were removed and live ones added. The water bottle and filter paper were also removed and replaced with 120 ml of both diets. Moist Burger Bit dog food wrapped in foil was added to the diet for moisture, eliminating the need for a water bottle and decreasing the likelihood of mites and mold. Usually no additional maintenance was required.

A separate jar with adults was set up for egg collections every 3 days. About 60 ml of diet A, plus a pinch of Brewer's yeast, was sifted through a 60-mesh screen before transferring it to a 3.8-liter jar. A water bottle and disc of filter paper were prepared as previously described. Moist Burger Bits and about 30 ml of diet B were wrapped in foil before placing in the jar, to stimulate adults to lay eggs and to make it easier to sift the diet A to remove eggs.

To select 1- to 3-day-old eggs, one day prior to testing, the water jar and filter paper in the jar were removed. The adults were removed with a 12-mesh screen, leaving the finely screened diet containing the eggs. The diet was sifted with a 60-mesh screen, removing the rod-shaped eggs. Under a microscope the eggs were transferred individually with a camel-hair brush to a piece of double-stick tape, one side of which was attached to a piece of black construction paper. The number of eggs was counted. The piece of tape with the eggs was transferred to a petri dish containing a small amount of sifted diet.

Larder beetles were selected for tests 1 day prior to testing. Usually a rearing jar set up 5 weeks prior would contain the necessary young adults, pupae, and large larvae needed for testing. Collecting the beetles was time-consuming because the various life stages could not be sifted out of the crude diet as with most other species. The contents of the jar were dumped and spread out on a large tray. Adults and pupae were individually selected and removed with soft forceps. The diet and remaining immature stages were sifted with a 10-mesh screen. Pupae and large larvae (11-12 mm long) still remaining on the screen were removed with soft forceps. Each adult, pupa, and larva was placed in a small, individual glass container (2 cm tall by 2 cm diameter), as adults have a strong tendency to become cannibalistic. The small containers were made by cutting off the

bottom inch of 15-ml glass vials. Each vial was provisioned with about 0.5 g of diet (1A:1B), plus a piece of Burger Bit dog food.

The small vials were placed in petri dishes, which were then transferred to a large, covered plastic holding box. The relative humidity inside the box was maintained at 55% with a small container of a saturated solution of magnesium nitrate. The large holding box was placed in the environmental chamber set at 25.5°C (78°F), 55% RH and held for one day prior to testing.

Phase II Testing

The vials containing insects were transferred to the chamber. The relative humidity inside the chamber was maintained at 55% with 25 ml of a saturated solution of magnesium nitrate in a small plastic box. Excess magnesium nitrate (25 g) was added to the saturated solution so that all the moisture in the chamber was absorbed. Two packets (7 g) of Z-1000 AGELESS were placed in each chamber. AGELESS is a chemical oxygen scavenger prepared from powdered iron oxide (Mitsubishi Gas Chemical Co., Inc.). After transferring all insects to the test chamber, the chamber door was loosely sealed. The chamber was flooded for 30 minutes with 99.999% pure nitrogen conditioned to 55% RH. The chamber was tightly sealed and the flow of nitrogen discontinued.

If the oxygen content inside the chamber was greater than 0.1%, the chamber was flushed again for an additional 5 to 10 minutes. The oxygen content was again analyzed. Every morning the chambers were analyzed for oxygen content. Whenever the oxygen level was greater than 0.1%, the chamber was flushed as described above. Seven exposure periods were selected for testing: 6, 18, 21, 24, 48, 72, and 96 hours. The number of insects tested varied with each exposure according to availability. On the day of testing, control insects were held at 55% RH in a holding box placed on top of the test chamber. After exposures were made, the tested insects were transferred to the holding box with the controls. The holding box was then placed in the environmental chamber set at 25.6°C (78°F), 55% RH.

After holding all stages for three weeks, the insects were inspected for mortality. Tests varied with life stages. All observations were made with the aid of a microscope. Adults and

pupae were picked up with a pair of soft forceps and dropped. If they moved they were considered alive. Pupae that did not emerge as adults were usually darker and dehydrated. The difference in the number of dead or live emerged adults from the initial number of pupae tested was the pupal mortality. If larvae pupated, they were considered to have survived the exposure. Eggs were considered alive if the egg hatched and a larva could be found. Larva usually managed to crawl off the sticky tape to the diet in the petri dish. All Unhatched eggs were dark and dehydrated. Approximately 650 adults, 320 pupae, 650 larvae, and 750 eggs were tested in Phase II, plus controls.

Phase III Testing

The only exposure time tested in Phase III was 96 hours, which was twice the maximum time required to produce 100% mortality determined in Phase II.

The procedure for selecting the larder beetles was identical to selecting in Phase II except for the holding containers and the number of insects tested per container. The individual glass containers were replaced with vials with screened snap-on lids (12 dram). Each vial was half filled with diet mix (1A:1B). Ten insects of each life stage were placed in individual vials. A few pieces of moist Burger Bit dog food were added for a moisture and food source. Eggs were collected and placed in vials with sifted diet, as well as adhered to tape as described above. The vials containing the insects were placed in 0.9-liter glass jars and covered with 10 to 13 cm of packed flour. Three jars packed with vials and flour were placed in each chamber for testing. Control insects were held in identical jars on top of the chambers during the tests. After each exposure, the vials were removed from the jars, placed in large, covered plastic holding boxes, and held in an environmental chamber. The insects were examined for mortality as described in Phase II.

Approximately 100 adults, 200 pupae, 560 larvae, and 500 eggs were tested in Phase III, plus controls.

RESULTS

Phase II

The adults were the most susceptible life stage tested, with 16-hour exposures resulting in 100% kill (Table 5.1). The pupal stage was the most tolerant; 24-hour exposures resulted in 70.1% mortality, compared with 98.9% kill of larvae and complete kill of eggs. All stages were killed with 48- or 72-hour exposures (Fig. 11).

Phase III

The only exposure tested in Phase III in which all stages were buried in flour, was 96-hours. All life stages were killed (Table 5.2).

CONCLUSIONS

1. Adults were the most susceptible stage. Sixteen-hour exposures resulted in 100% kill.
2. In Phase II, 48-hour and 72-hour exposures produced 100% mortality with all life stages.
3. In Phase III, 96-hour exposures killed 100% of all stages buried in flour.
4. Some additional tests with 72-hour exposures should be conducted in Phase III. However, exposures of 72 to 96 hours should provide 100% kill of all stages.

Table 5.1. Percent mortality of larder beetles, Dermestes lardarius (L.), exposed to low oxygen atmospheres in Phase II, 3 weeks after exposure[a/].

Stage[a/]	Exposure Time (hrs)	No. Chambers Tested[b/]	$\bar{X}$ Percent Mortality ± (SEM)
Adults	6	1	74.1
	16	2	100
	18	4	100
	21	3	100
	24	5	100
	48	5	100
	72	4	100
	Controls	5	32.1 ± 8.47
Pupae	16	1	47.0
	21	1	50.0
	24	4	70.1 ± 14.23
	48	5	100
	72	4	100
	Controls	6	2.8 ± 2.78
Larvae	6	1	29.5
	16	1	64.7
	18	4	97.4 ± 2.57
	21	3	94.9 ± 2.61
	24	3	98.9 ± 1.73
	48	5	100

Table 5.1. (cont.)

Stage[a/]	Exposure Time (hrs)	No. Chambers Tested[b/]	X Percent Mortality ± (SEM)
Larvae	72	4	100
	Controls	5	0.5 ± 0.52
Eggs	24	2	90.9 ± 4.25
	48	1	100
	72	1	100
	Controls	7	4.0 ± 2.07

[a/] Insects were held in individual containers to prevent cannibalism.

[b/] No. of individual containers per chamber for each stage varied from 8-165 depending on availability.

Table 5.2. Percent mortality of larder beetles, Dermestes lardarius (L.), exposed to low oxygen atmospheres in Phase III, 3 weeks after exposure[a/].

Stage	Exposure Time (hrs)	No. Chambers Tested[b/]	$\bar{X}$ Percent Mortality ± (SEM)
Adults	96	5	100
	Control	1	50
Pupae	96	6	100
	Control	2	0
Larvae	96	6	100
	Control	5	3.9 ± 3.00
Eggs	96	6	100
	Control	4	0

[a/] Insects held in individual containers per treatment to prevent cannibalism.

[b/] No. of individual containers per chamber for each stage varied from 8-165 depending on availability.

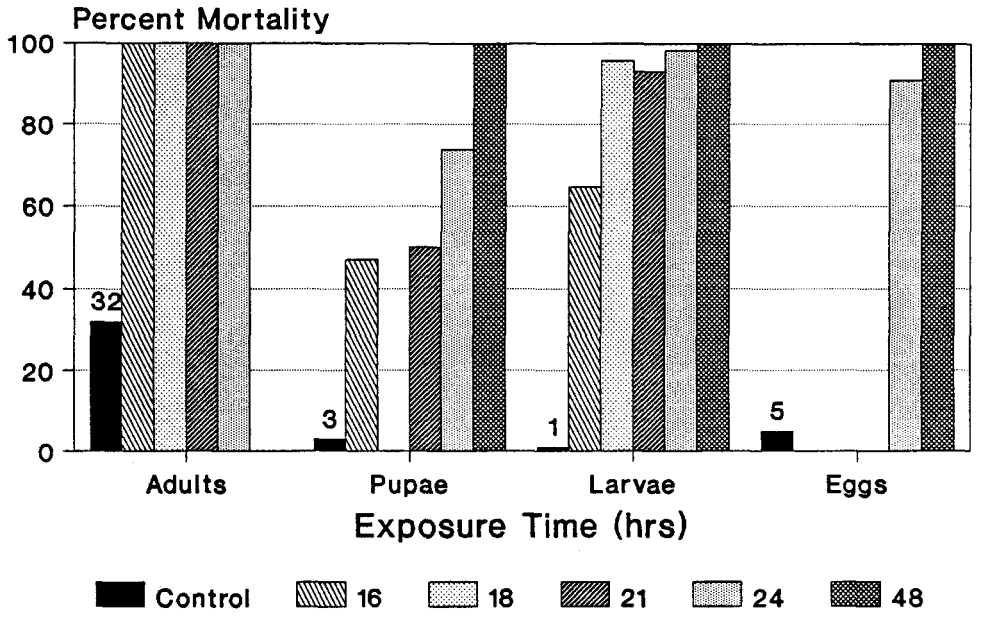

Figure 11. The percent mortality of all life stages of the larder beetle, Dermestes lardarius, 3 weeks after exposure to low oxygen atmospheres (<0.1% oxygen).

6. THE CIGARETTE BEETLE

The cigarette beetle, Lasioderma sericorne (F.), is the most destructive pest found in stored tobacco, but will attack a variety of stored products. Other items it feeds on include seeds, paper, spices, drugs, grain, cereal products, botanical specimens, insect specimens, silk, rodent bait, and dried plants (Ebeling 1975). The larvae feed on upholstered furniture, particularly stuffing. It is an important pest of books, damaging the binding and leaves (Walter 1990).

The life cycle (egg to adult) is 7 to 14 weeks. At 29°C (85°F), eggs hatch in 6 to 8 days, the larval period lasts 30 to 70 days, and the pupal period is 14 to 21 days. Typically the life cycle is 40 to 50 days long, about 3 to 6 generations per year. At 16°C (60°F) larvae become dormant and hibernate. Late instar larvae incorporate bits of rearing media into the cocoons, creating a crisp carton of diet on the surface of the media. The adult remains in a cocoon until sexually mature. Emerged adults live from 7 to 42 days. Females lay an average of 40 eggs in a lifetime (Ebeling 1975, Walter 1990).

Insects

The cigarette beetles were reared at 27.8 ± 0.5°C (82 ± 2°F), 70 ± 5% RH on a diet consisting of 3.9 liters ground Kebble 5 dog food and 15 ml (1 tablespoon) Brewer's yeast. Eggs hatched in 6 to 10 days and the life cycle for treatment was 28 to 42 days.

About 500 ml of diet were placed in a 3.9-liter glass jar, and each jar was provisioned with 100 adults. A new jar was prepared every two weeks. A cloth treated with 18.5% dicofol was used to cover the lid of the jar to prevent mite infestations.

A separate rearing jar for egg collections was prepared every three days. About 30 g of diet was sifted through a 60-mesh screen and placed in a 3.9-liter jar along with young adults. One day prior to testing, 1- to 3-day-old eggs were collected by dumping the contents of the jar into a 20-mesh screen to remove the adults. The media was then sifted through a 60-mesh screen to remove the eggs. Under a microscope the eggs were transferred individually with a camel-hair brush to a

piece of double-stick tape, one side of which was adhered to black construction paper. The eggs were then totalled.

Cocoons containing adults, pupae, and larvae were also tested. Mortality of adults and pupae outside cocoons was too high in the untreated controls. Adults and cocoons were collected from clusters of cocoons found on the top 1.5 cm of diet from a jar established 6 weeks prior to testing. Gaseous CO_2 was used to anesthetize the young adults. A chunk of the carton material containing the cocoons was broken and removed from the jar. Cocoons were dissected to determine if both larvae and pupae were present. The selected cocoons represented both life stages, with a small percent of adults also present. To keep the ratio even, larval cocoon collections were made from jars dated 2 weeks prior to the jars containing the adult and pupal cocoons. All cocoons were removed and weighed. Sixty grams of cocoons were placed in large (8.5 cm tall by 4 cm diameter) plastic vials with screened lids. Screened diet (20-mesh) was added to each vial. Young adults were generally unlikely to fly, and they were removed with soft forceps and placed in vials (n = 20 per vial). Plastic vials (7 cm tall by 3 cm diameter) were half filled with screened diet (20-mesh). Snap-on lids with 20-mesh screens covered each vial.

To collect pupae and larvae without cocoons, a jar was selected with clusters containing mostly larvae. The top 2.54 cm of diet containing the clusters was broken up, removed from the jar, and passed through a 10-mesh screen to remove all large pieces of food and a 20-mesh screen to remove the finely ground diet. The remaining contents in the sieve were allowed to stand for 30 minutes in room light. All active larvae migrated through the sieve screen, leaving the large (3 mm long), inactive larvae and pupae. The active larvae on the bottom were then collected and placed in a 28-mesh sieve. The pupae and larvae were removed with soft forceps and twenty of each were placed in vials to which screened diet was added.

All the individual vials and pieces of tape were transferred to a large, covered plastic box. The relative humidity inside the box was maintained at 55% with a small container of a saturated solution of magnesium nitrate. The large holding box and insects were placed in an environmental chamber maintained at 25.5°C (78°F) and 55% RH for one day prior to testing.

Phase II Testing

The vials containing insects to be tested were transferred to the chambers. The relative humidity inside the chamber was maintained at 55% with 25 ml of a saturated solution of magnesium nitrate in a small plastic box. Excess magnesium nitrate (25 g) was added to the saturated solution so that any excess moisture in the chamber was absorbed. Two packets (7 g) of Z-1000 AGELESS were placed in each chamber. AGELESS is a chemical oxygen scavenger prepared from powdered iron oxide (Mitsubishi Gas Chemical Co., Inc.). After transferring all insects to the test chamber, the chamber door was loosely sealed. The chamber was flooded for 30 minutes with 99.999% pure nitrogen conditioned to 55% RH. The chamber was tightly sealed and the flow of nitrogen discontinued.

If the oxygen content inside the chamber was greater than 0.1%, the chamber was flushed again for an additional 5 to 10 minutes. The oxygen content was again analyzed. Every morning the chambers were analyzed for oxygen content. Whenever the oxygen level was greater than 0.1%, the chamber was flushed as described above.

In the untreated controls, adults, pupae, larvae, eggs, and cocoons were held at 55% RH in a plastic holding box on top of the testing chamber during the exposures. Five exposure periods were selected for testing with the adult, pupal, and larval life stages: 48, 72, 120, 144, and 168 hours. In addition, the egg stage was exposed for 192 hours. Cocoons were exposed for 168 hours only. The number of insects and eggs tested varied with each exposure according to availability. Most exposures were replicated 2-6 times, all on different dates. After various exposure tests were completed, the insects were transferred back to the holding boxes and placed in the environmental chamber.

To examine the insects for mortality, the contents of each vial were dumped in a 20-mesh screen to remove the insects from the diet. Adults, pupae, and larvae were examined under a microscope with a high intensity, narrow light beam. While under the light beam, those that did not respond were individually probed with soft forceps; if they moved, they were scored as live. Light usually evoked a response faster than probing. All pupae that emerged as adults were scored

as live. All larvae that pupated were scored as live. Each vial containing cocoons was dumped into a large petri dish and cocoons were carefully probed with soft forceps for any signs of movement. The number of larvae adhered to the tape was counted.

To verify the exposure period required to kill 100% of the eggs, 1-day-old eggs were collected, weighed (6 g), and placed in small petri dishes with sifted diet. Groups of eggs were also placed on tape as described above.

Adults, pupae, larvae, and cocoons were inspected for mortality 3 weeks after exposure to low oxygen atmospheres. Eggs were inspected at 5 weeks. Approximately 920 adults, 1,050 pupae, 1,590 larvae, 3,000 eggs, and 60 grams of cocoons were tested.

Phase III Testing

The only exposure time tested in Phase III was 192 hours, which equalled the minimum time required to produce 100% mortality in Phase II, plus 24 hours. The tests were replicated twice on different dates.

The procedure for sorting and preparing the cigarette beetles was identical to those in Phase II except that the vials were placed in 0.9-liter glass jars covered with 10 to 13 cm of flour. The paper strips containing the eggs were rolled up, placed in plastic vials (>3.5 cm diameter), and covered with a screened lid before being transferred to the glass jars. Flour was firmly packed on top of the vials. Three jars packed with vials and flour were placed in each chamber for testing. Control insects were held in identical jars on top of the chambers during tests. After each exposure the vials were removed from the jars, transferred to the large, covered plastic holding boxes, and held in an environmental chamber. The insects were examined for mortality as described in Phase II. Approximately 190 adults, 140 pupae, 125 larvae, 392 eggs, and 143 cocoons were tested in Phase III, plus controls.

RESULTS

Phase II

Adult carpet beetles were killed with a 120-hour exposure to low oxygen atmospheres (Fig. 12). Mortality in the untreated control was extremely high at 3 weeks (>97%). Exposures of 144 hours were required to kill 100% of the pupae; mortality in the untreated control was >60% at week 3. Larvae required exposures of 144 hours to kill 100% (Table 6.1). The egg stage was the most tolerant, requiring 192 hours to provide complete mortality (Table 6.2). When cocoons containing adults, pupae, and late-instar larvae were tested, 168-hour exposures provided complete kill (Table 6.3).

Phase III

Exposures of 192 hours provided 100% kill of all stages of cigarette beetle (Table 6.4).

CONCLUSIONS

1. All of the adult and pupal cigarette beetles were killed with 144-hour exposures in Phase II. Control mortality was extremely high at 3 weeks. Some additional exposure tests should be conducted and mortality counts made every few days after exposure.
2. The egg is the most tolerant stage tested, requiring 192 hours to provide 100% kill.
3. All stages of the cigarette beetle were killed with 192-hour exposures in Phase III.

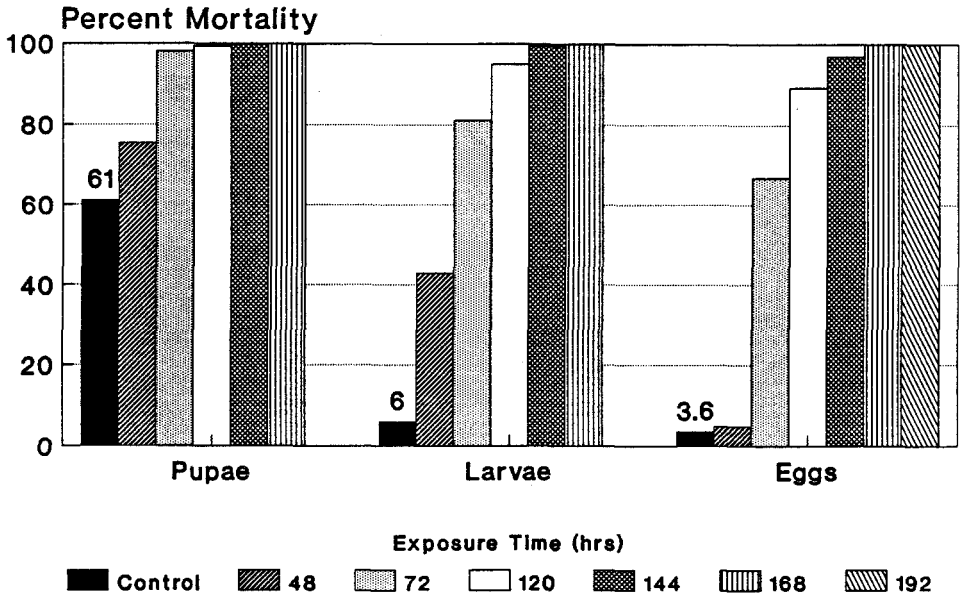

Figure 12. The percent mortality of immature stages of the cigarette beetle, Lasioderma serricorne, 3 weeks after exposure to low oxygen atmospheres (<0.1% O_2) (5 weeks with eggs).

Table 6.1. Percent mortality of adult cigarette beetles, Lasioderma sericorne, exposed to low oxygen atmospheres in Phase II at 3 weeks after exposure.

Exposure Time (hrs)	No. Chamber Tested[a/]	$\bar{X}$ Percent Mortality ± (SEM)
48	5	43.5 ± 2.89
	Control	13.0 ± 2.60
72	1	86.2
	Control	0
120	3	93.6 ± 3.03
144	6	100
	Control	13.7 ± 1.25
168[b/]	1	100

[a/] 3-10 replicates were tested per chamber.

[b/] 6 g of cocoons were exposed.

Table 6.2. Percent mortality of the egg stage of the cigarette beetle, Lasioderma serricorne, exposed to low oxygen atmospheres in Phase II 5 weeks after exposure.

Exposure Time (hrs)	No. Chambers Tested[a/]	$\bar{X}$ Percent Mortality ± (SEM)
48	2	3.7 ± 3.70
	Control	1.3 ± 0.84
72	3	46.6 ± 23.98
	Control	4.6 ± 3.35
120	2	93.6 ± 3.65
144	6	97.7 ± 2.45
	Control	3.5 ± 0.50
192	1	100
	Control	2.4 ± 2.4

[a/] 1-6 replicates tested per chamber.

Table 6.3. Percent mortality of the cocoon stage of the cigarette beetle, Lasioderma sericorne, exposed to low oxygen atmospheres in Phase II 3 weeks after exposures[a/].

Exposure Time (hrs)	No. Chamber Tested	Percent Mortality
168	1	100
	Control	0

[a/] Cocoons containing adults, pupae, and larvae. 60 g of cocoons were exposed.

Table 6.4. Percent mortality of all life stages of the cigarette beetle, Lasioderma sericorne, exposed to low oxygen atmosphere (<0.1% O_2) for 192 hours in Phase III at 3 weeks after exposure[a/].

Stage	No. Chambers Tested[b/]	X Percent Mortality ± (SEM)
Adult	2	100
Pupae	2	100
Larvae	2	100
	Control	0
Egg	2	100
	Control	2.2 ± 1.41
Cocoons[c/]	3	100
	Control	0

[a/] Eggs examined 5 weeks after exposure.

[b/] 1-3 replicates tested per chamber.

[c/] Cocoons contained adults, pupae, and larvae. 120-180 g of cocoons tested.

7. THE CONFUSED FLOUR BEETLE

The confused flour beetle, Tribolium confusum Jacquelin du Val, is considered to be one of the most important pests of stored food. Confused flour beetles feed on broken grains, beans, nuts, spices, drugs, and herbarium and museum specimens. They are unable to feed on unbroken grams, but readily attack processed foods.

The typical life cycle of T. confusum in heated warehouses or structures is about 3 months, with 4 to 5 generations of beetles yearly. Eggs hatch in 5 to 12 days, depending on the temperature. The larvae may go through 5 to 12 instars before pupating, with the majority having 7. This period may be as short as 21 days or as long as 5 months if the larvae overwinter. The pupal period is approximately 8 days. Adults are extremely long-lived and may survive for more than 2 years. A female deposits 2-3 eggs each day, totalling some 400 to 500 in her lifetime. Generally, 80-95% of the eggs hatch (Khalifa and Badaway 1955).

Donahaye (1990) has shown that the red flour beetle, Tribolium castaneum (Herbst), developed resistance to hypoxia when selected for 40 generations in 99.5% N_2 and 0.5% O_2 atmospheres. The relative humidity was held at 95% to reduce the effects of desiccation.

Insects

The confused flour beetles were reared at 26.7°C (80°F) and 55% RH on a diet consisting of whole wheat flour, corn meal, wheat germ, nonfat dry milk, and wheat. The time required for eggs to develop into adults was about 5 weeks. About 400 ml of the diet were placed in the bottom of a 3.9-liter jar and infested with 100-200 adult beetles. To provide sufficient numbers of all life stages, a new rearing jar was set up every three days. The adults were removed from the media with a 20-mesh screen and the remaining media was transferred to a new rearing jar.

All life stages were collected one day prior to testing. The 3- to 6-day-old adults were selected from the earliest dated jar filled with adults; usually the jar dated just before this one contained only a few emerged adults (0-3 days old). The youngest pupae and active, late instar larvae were selected in the same manner. The adults, pupae, and larvae were collected by sifting

the media and insects through a 14-mesh screen. With a pair of soft forceps, 20 adults and 20 pupae were placed in small separate glass dishes, usually with three replicates per test. Because of high tolerance found in some fully developed larvae of various other species, only active, last instar larvae were selected for testing. One to 2 grams of sifted diet were added to each dish. All filled dishes were then transferred to a large, covered plastic holding box. The relative humidity inside the box was maintained at 55% with a small container of a saturated solution of magnesium nitrate. The large holding box was then placed in an environmental chamber set at 25.6°C (78°F) and held for one day.

Egg Collections

A separate rearing jar was set up for egg collections every three days. About 30 ml (2 tablespoons) of the above diet plus 0.25 ml of Brewer's yeast (1/2 teaspoon) was sifted through a 60-mesh screen before transferring it to a clean 3.9-liter glass jar. Adults were added to the jar. Eggs were collected for testing by first sifting the contents of the jar through a 20-mesh screen to remove the adults and then through a 60-mesh screen to remove the eggs. The eggs were transferred individually with a camel-hair brush to a piece of double-stick transparent tape, one side of which was attached to a piece of black construction paper. The eggs were counted and the tape with the eggs was placed in a holding box with the adults, pupae, and larvae.

Phase II Testing

After the chamber was flooded and sealed, the oxygen content inside the chamber was analyzed. If the oxygen content was found to be greater than 0.1%, the chamber was flushed again for an additional 5 to 10 minutes. The oxygen content was again analyzed. Every morning the flooded chambers were analyzed for oxygen. Whenever the oxygen level was exceed 0.1%, the chamber was flushed as described above.

Six exposure periods were selected for testing with all life stages: 6, 18, 21, 24, 48 and 72 hours. Three to four open dishes (20 insects per dish) of each life stage were placed in the test chambers. The relative humidity inside the chamber was maintained at 55% with 25 ml of a

saturated solution of magnesium nitrate in a small plastic box. Excess magnesium nitrate (25 g) was added to the saturated solution so that any excess moisture in the chamber was absorbed. Two Z-1000 AGELESS packets (7 g) were placed in each chamber. AGELESS is a chemical oxygen scavenger prepared from powdered iron oxide (Mitsubishi Gas Chemical Co. Inc.). After transferring all insects to the chamber, the chamber door was loosely sealed. The chamber was flooded for 30 minutes with 99.999% pure nitrogen preconditioned to 55% RH.

Insects were examined for mortality at 24 hours and again after 3 weeks. At 24 hours, mortality was determined by picking up individual adults, pupae, or larvae with a pair of soft forceps while under a microscope; if the insects moved they were scored as alive. Eggs were not examined at 24 hours. After three weeks the procedure for determining mortality varied with each life stage. Adults were examined as described for 24 hours. The pupae were scored as live if they emerged into adults. Those not developing into adults were assumed to be dead. The larvae that successfully pupated were scored as live. If newly emerged larvae were stuck on the double-stick tape, eggs were scored as live. Unhatched and desiccated eggs were scored as dead. Dead pupae and larvae were usually dehydrated and discolored.

Phase III Testing

The only exposure time tested in Phase III was 72 hours. This was the minimum exposure time required to produce 100% mortality of all stages tested in Phase II.

The collection of insects and testing procedures in Phase III were identical to those in Phase II except that vials were placed in 3.9-liter jars and covered with 10 to 13 cm of packed flour before being placed in the chambers. Three jars were placed in each chamber. Control insects were kept in jars identical to the tested insects, held on top of the flooded chambers during the testing period. Each exposure period was replicated three times. After each exposure, the insect vials were removed and the insects transferred to the holding boxes. Each stage was examined at 24 hours (except eggs) and 3 weeks, as described above.

RESULTS

Phase II

There was a significant increase in latent mortality between day 7 and 21 for pupae and larvae exposed for 21 hours (Table 7.1). Consequently, in all subsequent tests the insects were examined 3 weeks after exposure to determine the mortality.

The adults and larvae were completely killed with 48-hour exposures (Table 7.2), whereas pupae and eggs survived. All developmental stages of the confused flour beetle were killed with 72-hour exposures to low oxygen atmospheres (Fig. 13).

Phase III

All stages of the confused flour beetle were killed with 96-hour exposures to low oxygen atmospheres (Table 7.3).

CONCLUSIONS

1. The reason for the significant increase in mortality of larvae and pupae between 7 and 21 days after exposure is unknown. All developmental stages were killed with a 72-hour exposure.
2. In Phase III, all stages were killed with 96-hour exposures to low oxygen atmospheres.
3. It is extremely unlikely that T. confusum will rapidly develop resistance to low O_2 treatments. Donahaye (1990) exposed red flour beetles for 40 generations at 95% RH to reduce the effects of desiccation. It is unlikely that this would ever be achieved under practical conditions.

Table 7.1. Percent mortality of adults, pupae, and larvae of the confused flour beetle, T. confusum 1, 7, and 21 days after 8- and 21-hour exposures to low oxygen atmospheres[a/].

Stage	Exposure (hrs)	Avg. Percent Mortality ± SEM at Day 1	7	21
Adults	8	15.4 ± 1.67	20.4 ± 1.67	21.2 ± 2.89
	21	91.1 ± 3.39	99.4 ± 0.56	99.4 ± 0.56
	Control	0.6 ± 0.60	1.2 ± 0.0	1.9 ± 0.65
Pupae	8	1.2 ± 0.72	5.8 ± 0.83	9.5 ± 1.67
	21	0.6 ± 0.57	18.3 ± 6.67	31.4 ± 7.10
	Control	0.6 ± 0.60	3.8 ± 1.20	5.7 ± 3.05
Larvae	8	0	0	2.9 ± 0.83
	21	74.4 ± 5.65	81.1 ± 2.41	86.7 ± 0.95
	Control	3.1 ± 3.12	3.1 ± 3.12	3.1 ± 3.12

[a/] Twenty insects were tested in each of 3 replicates.

Table 7.2. Average percent mortality of all life stages of the confused flour beetle, Tribolium confusum, in Phase II three weeks after exposure to low oxygen atmospheres.

Stage	Exposure (hrs)	No. Chambers[a/] Tested	Avg. Percent Mortality ± SEM
Adults	6	2	0
	18	3	71.2 ± 17.06
	21	3	85.5 ± 14.46
	24	3	99.6 ± 0.42
	48	3	100
	72	3	100
	Control	13	0.2 ± 0.19
Pupae	6	2	0
	18	3	15.8 ± 0.83
	21	3	38.1 ± 7.68
	24	3	32.4 ± 19.42
	48	3	73.1 ± 6.75
	72	3	100
	Control	13	1.9 ± 1.24
Larvae	6	2	4.9 ± 4.90
	18	3	67.5 ± 16.31
	21	3	97.1 ± 2.99
	24	3	87.1 ± 7.68
	48	3	100
	72	3	100
	Control	13	4.1 ± 2.69

Table 7.2. (cont.)

Stage	Exposure (hrs)	No. Chambers[a/] Tested	Avg. Percent Mortality ± SEM
Eggs	6	1	15.0
	18	2	24.8 ± 0.16
	21	3	13.1 ± 9.06
	24	3	44.2 ± 6.51
	48	1	37.5
	72	3	100
	Control	7	9.7 ± 1.94

[a/] Number of chambers tested. Each chamber with 3 to 4 replicates of 20 insects per stage.

Table 7.3. Average percent mortality of all stages of the confused flour beetle, Tribolium confusum, in Stage III three weeks after exposure to low oxygen atmosphere for 4 days.

Stage	No. Chambers[a/] Tested	No. tested	Exposure	Exposure Avg. Percent Mortality ± SEM
Adults	3	870	yes	100
	4	550	no	0
Pupae	5	1,280	yes	100
	5	670	no	0
Larvae	4	860	yes	100
	4	510	no	0
Eggs	3	390	yes	100
	2	114	no	0

[a/] Number of chambers tested with 3-5 replicates per chamber for each life stage.

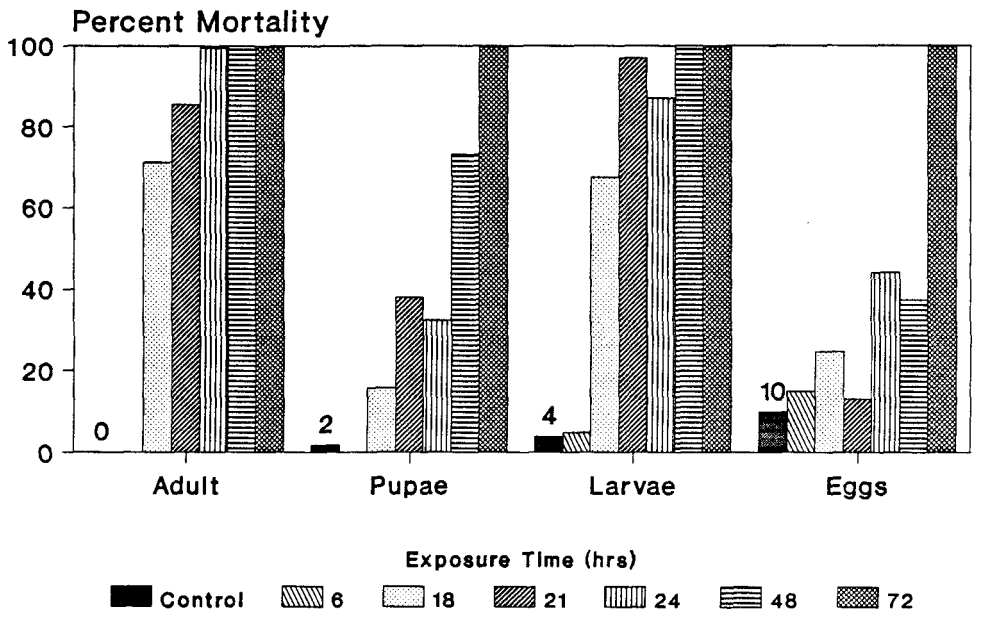

Fig. 13. Average percent mortality of all life stages of the confused flour beetle, Tribolium confusum, three weeks after various exposures to low oxygen atmospheres.

8. THE AMERICAN COCKROACH, BROWNBANDED COCKROACH, AND GERMAN COCKROACH

The American cockroach, Periplaneta americana (L.), is the largest of the common structure-infesting cockroaches; it is 38 mm (1 1/2 inches) long, with fully developed reddish-brown wings. The American cockroach is found most commonly in restaurants, grocery stores, bakeries, and wherever food is prepared and stored. They are also attracted to fermenting liquid. They are occasionally found in museums in North America and commonly found in museums in Southeast Asia (Quek et al. 1990). In museums they feed on starchy materials, sugary or fermented foods, leather, and parchment. In addition to sighting live insects, they can be detected by feeding damage, excrement, and egg cases.

The brownbanded cockroach, Supella longipalpa (F.), is a cosmopolitan cockroach pest, occurring both outdoors and indoors. In Egypt, this species is often referred to as the furniture cockroach. The brownbanded cockroach is tan to brown with light yellow crossbands on the dorsal side, especially prominent in nymphs. The adult is small (up to 12.5 mm long), active and flies readily when disturbed. It prefers warm temperatures and is found in areas of the structure where temperatures exceed 36.5°C (80°F) for most of the year. It prefers high locations such as shelves, behind picture moldings, etc. The damaging stages are the nymphs and adults. In museums they feed on starchy materials, sugary or fermented foods, leather, and parchment.

The egg capsule is yellowish- or reddish-brown in color and is 5 mm (3/16 inch) in length. The female carries the oothecae (egg capsule) for 24 to 36 hours before attaching it to some object, usually near the ceiling where temperatures are higher. The average number of young to emerge from a capsule was 13.2 at room temperature. The complete life cycle takes an average of 161 days.

The German cockroach, Blattella germanica (L.), is a cosmopolitan species occurring primarily indoors in areas where food is prepared or served. Adult German cockroaches are about 16 mm (5/8 inch) in length, brown in color, with two dark longitudinal streaks on the pronotum.

breed throughout the year indoors, especially in a humid environment averaging approximately 21°C (70°F).

Female German cockroaches carry their oothecae until the eggs are ready to hatch. Often, the capsule becomes as large as the female's abdomen. The number of eggs in a capsule varies from 30 to 40. The eggs may hatch while still held in the ovipositor of the female, or the capsule may be dropped several hours, or even a day, before hatching. Capsules removed from the female do not hatch unless removal is made only a day or two before hatching. An individual female may produce an average of 4 to 5 capsules in her lifetime; some females live more than 200 days. The life cycle (egg to adult) varies from 55 to 68 days.

Insects

All cockroaches were obtained from laboratory cultures, reared on dry dog chow with a water source, maintained at 25.5 ± 2°C, 50 ± 10% RH, exposure to 12-hour light:dark photoperiod. Several thousand American cockroaches were reared in 120-liter rubbish bins. The brownbanded and German cockroaches were reared in 3.9-liter jars.

Cockroaches were selected from lab colonies one day prior to testing. All cockroaches were lightly anesthetized with CO_2 during selection. The oothecae were collected and inspected with the aid of a microscope; only uniform, undamaged oothecae were selected. The following life stages for each species were tested:

a. American cockroach

Each exposure test consisted of 10-20 glass vials of adult males, adult females without oothecae, large nymphs approximately 20 mm long, and small nymphs approximately 6 mm long. One cockroach was placed in each vial, with the exception of small nymphs, which were placed 5 per vial. The number of oothecae tested varied, with two capsules per vial. Fifty to 100 cockroaches of each life stage were tested in one day, totalling approximately 250 to 500 cockroaches, including controls.

b. Brownbanded cockroach

Adult males, adult females, large nymphs approximately 8 mm in length, small nymphs approximately 3 mm in length, and egg capsules were selected for testing. Five cockroaches or two oothecae were placed in individual plastic vials. Fifteen to 20 cockroaches of each life stage were selected for each low oxygen exposure, totaling 75 to 100 cockroaches of each life stage tested each day. Four hundred to 500 cockroaches, including controls, were tested.

c. German cockroach

The selection procedure was identical to that described for brownbanded cockroaches, with the exception of one additional adult stage. Since the German female carries the egg capsule until one to two hours before it is ready to hatch, females with egg capsules were also selected in addition to isolated egg capsules.

All specimens were placed in plastic vials (7 cm tall by 3 cm diameter). Each vial was covered with a plastic snap-on lid with a 7 mm hole in the center. A piece of dental cotton wick approximately 3.5 cm long was inserted in the hole and moistened to serve as a water source. The egg capsules were placed in vials and covered with a snap-on lid with a fine screen insert.

Phase II Testing

The plastic snap-on lids with the water wicks were removed and replaced with screened lids on the day of testing. The vials containing insects to be tested were transferred to the test chambers. The relative humidity inside the chamber was maintained at 55% with 25 ml of a saturated solution of magnesium nitrate in a small plastic box. Excess magnesium nitrate (25 g) was added to the saturated solution so that all the moisture in the chamber was absorbed. Two packets (7 g) of Z-1000 AGELESS were placed in each chamber. AGELESS is a chemical oxygen scavenger prepared from powdered iron oxide (Mitsubishi Gas Chemical Co., Inc.). After transferring all insects to the test chamber, the chamber door was loosely sealed. The chamber was

flooded for 30 minutes with 99.999% pure nitrogen conditioned to 55% RH. The chamber was tightly sealed and the flow of nitrogen discontinued.

If the oxygen content inside the chamber was greater than 0.1%, the chamber was flushed again for an additional 5 to 10 minutes. The oxygen content was again analyzed. Every morning the chambers were analyzed for oxygen content. Whenever the oxygen level exceeded 0.1%, the chamber was flushed as described above. The control roaches were held in the 55% RH holding box, which was placed on top of the nitrogen testing chamber.

Exposure Periods

Five exposure periods were selected for testing with adults and nymphs of P. americana: 1, 4, 6, 8, and 24 hours. Egg capsules of American cockroaches were also exposed for 92 and 120 hours. For brownbanded cockroaches, five exposure periods were selected for testing with all nymphs and adults: 1, 2, 3, 6 and 24 hours. Egg capsules were subjected to additional exposure periods of 96 and 120 hours. Exposure periods of 1, 3, 6, and 24 hours were selected for testing with all life stages of B. germanica.

After each exposure, a few grams of ground dog food were added to each vial before transferring them to the holding boxes which were placed in an environmental chamber set at 25.5°C (78°F), 55% RH. The number of live and dead cockroaches was counted after holding the exposed cockroaches for 1 and 2 days. The oothecae were examined 1, 2, and 3 months after exposure. Each vial containing exposed insects was gently tapped and rotated. If the cockroaches moved, they were scored as live. All life stages of the brownbanded and German cockroaches, and the small nymphs of the American cockroach, were inspected for mortality with the aid of a microscope.

To determine if there was any recovery and to confirm 100% mortality after the 2-day mortality counts, the cockroaches were transferred to a 0.9-liter jar, covered with a screened lid, and provisioned with dry chow and a water source. The upper inside 6 cm of the jar was coated with a thin layer of petroleum jelly to prevent cockroaches from escaping. The jars with unexposed and exposed cockroaches were held in the environmental chamber for one month, after

which the jars were checked for survivors. The contents of the jars were dumped onto a tray to check for survivors. The oothecae were held in the testing vials (2 egg capsules per vial) without food or water. Treatments were considered to be lethal if egg capsules failed to hatch.

Phase III Testing

The procedure for sorting and preparing the cockroaches was identical to that described in Phase II except that vials were placed in 0.9-liter glass jars and covered with 10 to 13 cm of packed flour before being placed in the chambers. Three filled jars were placed in each chamber for testing. Control cockroaches were kept in jars identical to those being exposed to the low O_2 atmosphere, held on top of the chambers during the testing period.

Only the oothecae of the American cockroach were tested in Phase III, because it was the most tolerant stage. Exposure periods of 72, 96, and 120 hours were selected for testing. Four replicates were tested at the 120-hour exposure.

Only the egg capsules of the brownbanded cockroach were tested in Phase III, with exposure periods of 96 and 120 hours. The oothecae were the most tolerant of stage of brownbanded cockroach examined in Phase II. Exposures were replicated twice.

In Phase III, 70 female and male adult German cockroaches were exposed for 48 hours. Females with oothecae and large and small nymphs were tested with 10 to 20 insects per life stage per chamber. Tests were replicated 5 times.

After each exposure, the vials were removed from the jars, placed in large, covered plastic holding boxes, and held in an environmental chamber. The cockroaches were examined for mortality as described in Phase II.

RESULTS

Phase II

Figures 14 and 15 show that an 8-hour exposure provide 100% kill of all nymphal and adult stages of P. americana. Exposures of 4 and 6 hours killed 52.6 and 94.3% of adult females (Table 8.1) and 56.3 and 93.3% of adult males 1 day after exposure (Table 8.2). Large nymphs were somewhat more tolerant, only 6.7 and 68.9% being killed with 4- and 6-hour exposures (Table 8.3). The 4- and 6-hour exposures killed 20.0 and 85.6% of the small nymphs within 24 hours after the exposure (Table 8.4). There was no substantial increase in mortality 2 days after the exposure (Fig. 15).

The developing P. americana nymphs in the oothecae were killed with a 5-day exposure to low oxygen atmospheres (Table 8.5). Shorter exposures provided 25.4 to 32.4% hatch within 3 months after exposure. About 40% of the egg capsules failed to hatch in the control. Many of these probably contained non-fertile eggs that desiccated before the nymphs could hatch.

Six-hour exposures to low oxygen atmospheres killed 100% of the adult male and female brownbanded cockroaches (Figs. 16 and 17). There was no noticeable increase in mortality after 24 hours (Tables 8.6 and 8.7). The nymphs were not as susceptible as the adults, with 6-hour exposures killing 93.3 and 97.8% of the large and small nymphs, respectively (Table 8.8 and 8.9).

Oothecae exposed for at least 72 hours failed to develop and there was complete inhibition of emergence (Table 8.10). About 60% of the control oothecae selected hatched within 3 months.

Figures 18 and 19 show that 6-hour exposures to low oxygen atmospheres were lethal to nymphal and adult B. germanica. All stages had about equal susceptibility to low oxygen atmospheres (Tables 8.11 to 8.15). Exposures of 24 hours were required to kill all the nymphs developing inside the oothecae (Table 8.16).

Phase III

Exposures of 120 hours were necessary to kill 100% of the P. americana oothecae (Table 8.17). With 72- and 96-hour exposures there was some survival and subsequent hatch. There was no apparent effect of burying the capsules in the flour (Tables 8.5 and 8.17).

CONCLUSIONS

1. Adult cockroaches are the most susceptible stage to low oxygen atmospheres, requiring 6-hour exposures to provide 100% kill of all 3 species tested.
2. The nymphal cockroaches of all 3 species are somewhat more tolerant of low oxygen atmospheres, requiring 8-24 hours to kill 100%.
3. The oothecae are the most tolerant life stage. Exposures of 120, 72, and 24 hours were required to kill 100% of the developing nymphs of P. americana, S. longipalpa, and B. germanica, respectively.

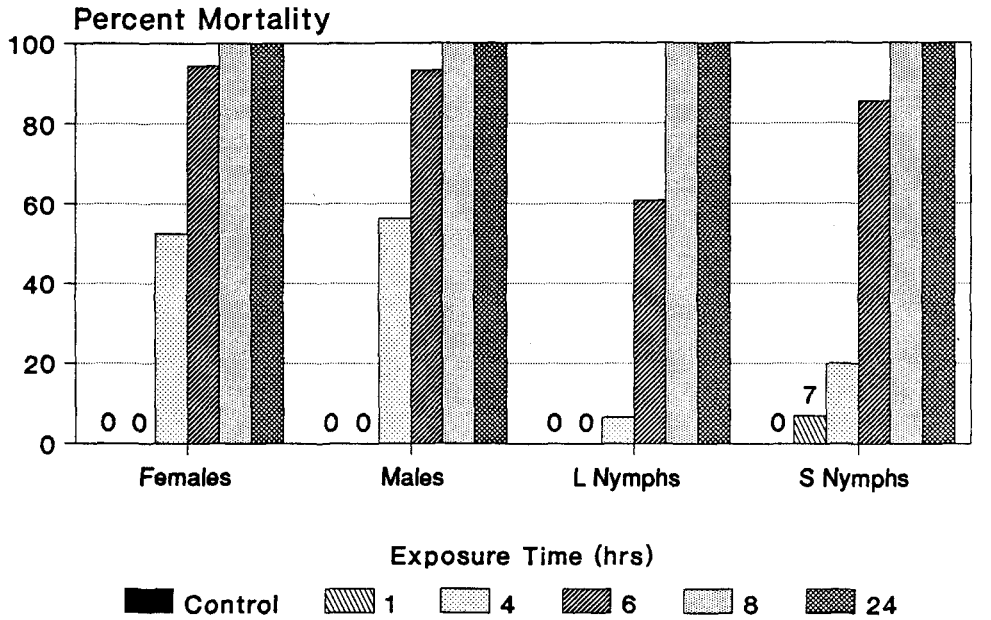

Fig. 14. The percent mortality of female and male adults and large (L) and small (S) nymphs of the American cockroach, Periplaneta americana, 1 day after exposure to low oxygen atmospheres (<0.1% O_2).

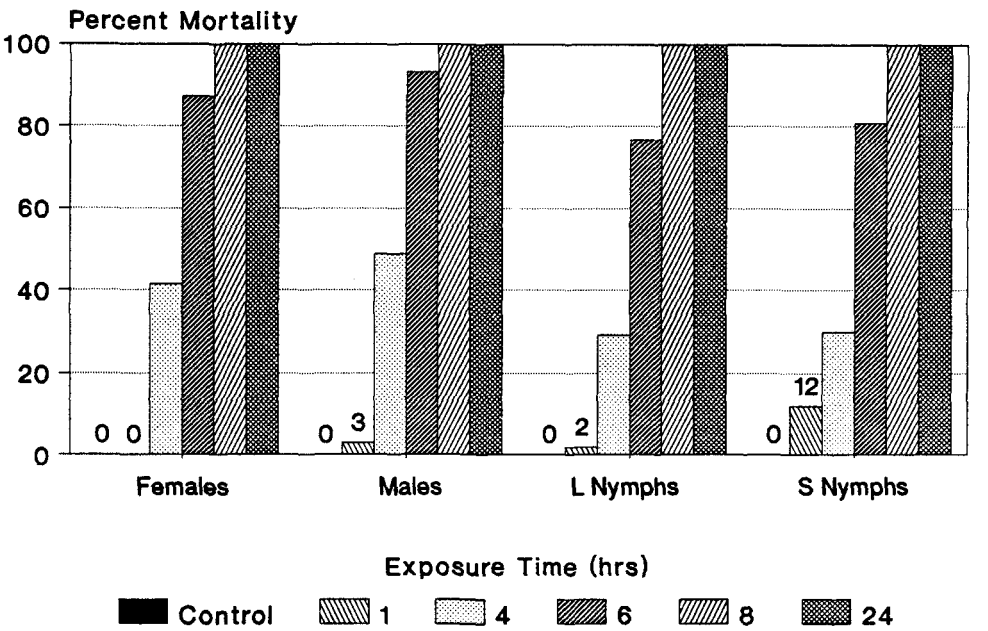

Fig. 15. The percent mortality of of female and male adults and large (L) and small (S) nymphs of the American cockroach, Periplaneta americana, 2 days after exposure to low oxygen atmospheres (<0.1% O_2).

Table 8.1. Percent mortality of female adults of the American cockroach, Periplaneta americana, exposed to low oxygen atmospheres in Phase II tests at 1 and 2 days after exposure.

Exposure Time (hrs)	No. Chambers Tested[a/]	$\bar{X}$ Percent Mortality ± SEM	
		1 day	2 days
1	3	0	0
4	5	52.5 ± 20.56	41.5 ± 13.07
6	4	94.3 ± 2.98	87.3 ± 5.75
8	3	100	100
24	1	100	100
Controls	4	0	0

[a/] Number of chambers tested. Number of insects tested varied per treatment, with 3 to 4 replicates each.

Table 8.2. Percent mortality of male adults of the American cockroach, Periplaneta americana, exposed to low oxygen atmospheres in Phase II tests at 1 and 2 days after exposure.

Exposure Time (hrs)	No. Chambers Tested[a/]	$\bar{X}$ Percent Mortality ± SEM	
		1 day	2 days
1	3	0	3.3 ± 3.33
4	5	56.3 ± 16.85	48.8 ± 8.75
6	4	93.3 ± 6.68	93.3 ± 6.68
8	3	100	100
24	1	100	100
Controls	4	0	0

[a/] Number of chambers tested. Number of insects tested varied per treatment, with 3 to 4 replicates each.

Table 8.3. Percent mortality of large nymphs of the American cockroach, Periplaneta americana, exposed to low oxygen atmospheres in Phase II tests at 1 and 2 days after exposure.

Exposure Time (hrs)	No. Chambers Tested[a/]	$\bar{X}$ Percent Mortality ± SEM	
		1 day	2 days
1	3	0	1.9 ± 1.85
4	4	6.7 ± 6.67	29.2 ± 10.30
6	4	68.9 ± 15.57	76.7 ± 11.71
8	3	100	100
24	1	100	100
Controls	4	0	0

[a/] Number of chambers tested. Number of insects tested varied per treatment, with 3 to 4 replicates each.

Table 8.4. Percent mortality of small nymphs of the American cockroach, Periplaneta americana, exposed to low oxygen atmospheres in Phase II tests at 1 and 2 days after exposure.

Exposure Time (hrs)	No. Chambers Tested[a/]	$\bar{X}$ Percent Mortality ± SEM	
		1 day	2 days
1	3	6.7 ± 6.67	11.7 ± 6.01
4	5	20.0 ± 10.95	30.0 ± 17.32
6	4	85.6 ± 8.54	80.8 ± 3.44
8	3	100	100
24	1	100	100
Controls	4	0	0

[a/] Number of chambers tested. Number of insects tested varied per treatment, with 3 to 4 replicates each.

Table 8.5. Percent egg hatch of American cockroach oothecae exposed to low oxygen atmospheres in Phase II tests for 24, 96 and 120 hours. Oothecae examined 1, 2 and 3 months after exposure.

Exposure Time (hrs)	No. Chambers Tested[a/]	$\bar{X}$ Percent Hatched ± SEM at Months		
		1	2	3
24	2	18.3 ± 78.3	32.4 ± 10.95	32.4 ± 10.95
96	3	19.9 ± 12.48	25.4 ± 8.73	25.4 ± 8.73
120	2	0	0	0
Controls	3	35.5 ± 20.21	59.4 ± 5.80	59.1 ± 5.80

[a/] Number of chambers tested. Two oothecae per vial tested; the number of vials tested per treatment varied.

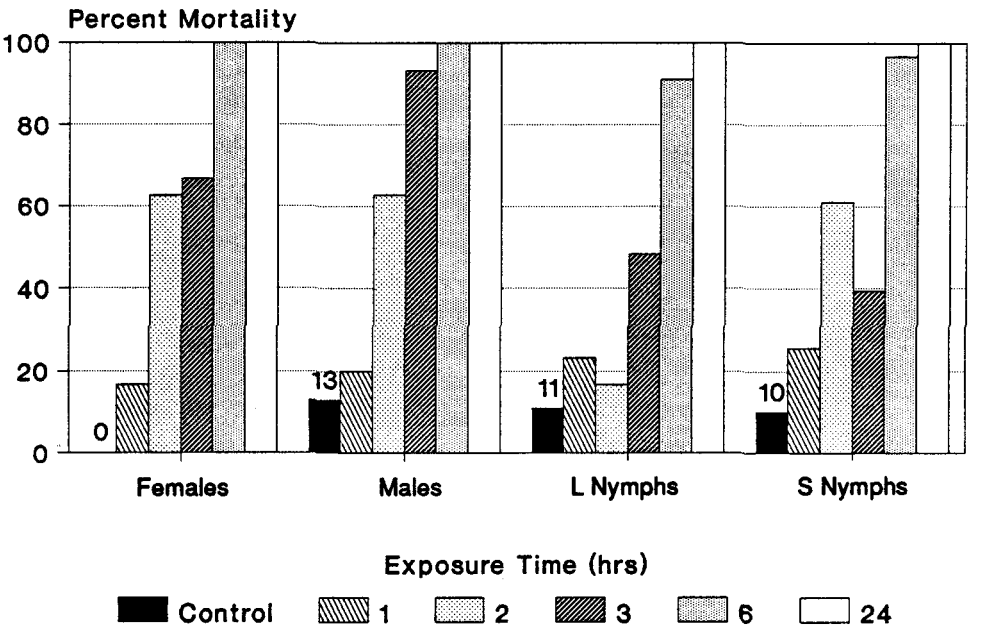

Fig. 16. The percent mortality of female and male adults and large (L) and small (S) nymphs of the brownbanded cockroach, Supella longipalpa, 1 day after exposure to low oxygen atmospheres (<0.1% O_2) for varying periods.

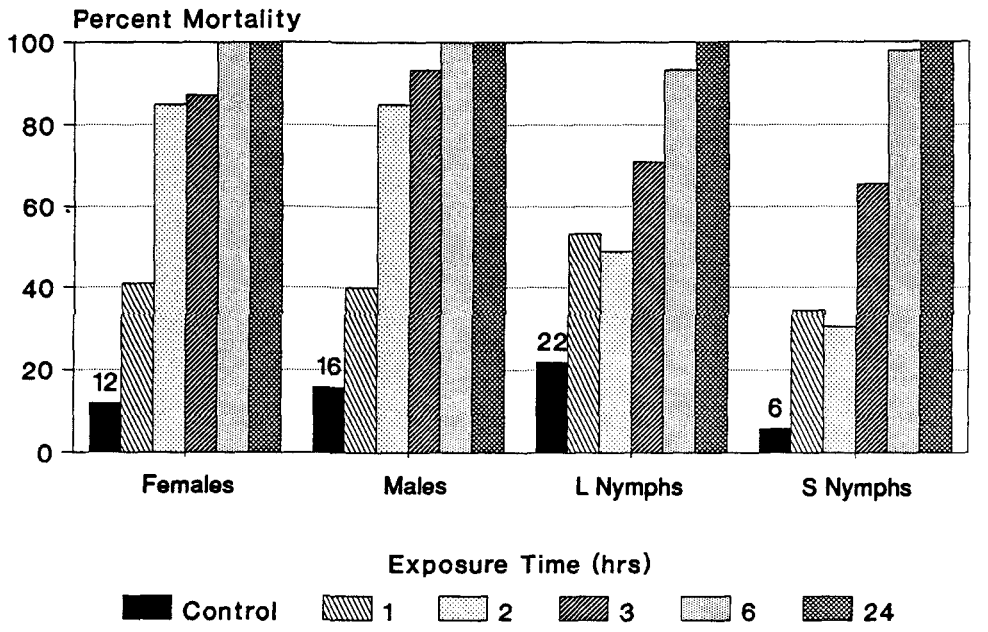

Fig. 17. The percent mortality of female and male adults and large (L) and small (S) nymphs of the brownbanded cockroach, Supella longipalpa, 2 days after exposure to low oxygen atmospheres (<0.1% O_2) for varying periods.

Table 8.6. Mortality of female adults of the brownbanded cockroach, Supella longipalpa, exposed to low oxygen atmospheres in Phase II tests at 1 and 2 days after exposure.

Exposure Time (hrs)	No. Chambers Tested[a/]	$\bar{X}$ Percent Mortality ± SEM	
		1 day	2 days
1	3	16.7 ± 16.67	41.0 ± 26.29
2	3	62.7 ± 19.72	85.0 ± 12.58
3	3	66.7 ± 24.04	87.2 ± 10.37
6	3	100	100
24	2	100	100
Controls	3	0	12.5 ± 4.33

[a/] Number of chambers tested. Number of insects tested varied per treatment, with 3 to 4 replicates each.

Table 8.7. Mortality of male adults of the brownbanded cockroach, Supella longipalpa, exposed to low oxygen atmospheres in Phase II tests at 1 and 2 days after exposure.

Exposure Time (hrs)	No. Chambers Tested[a/]	$\bar{X}$ Percent Mortality ± SEM	
		1 day	2 days
1	3	20.0 ± 11.55	40.0 ± 23.09
2	3	62.8 ± 19.64	85.0 ± 12.58
3	3	93.3 ± 6.67	93.3 ± 6.67
6	3	100	100
24	2	100	100
Controls	3	13.3 ± 13.33	16.1 ± 12.19

[a/] Number of chambers tested. Number of insects tested varied per treatment, with 3 to 4 replicates each.

Table 8.8. Mortality of large nymphs of the brownbanded cockroach, Supella longipalpa, exposed to low oxygen atmospheres in Phase II tests at 1 and 2 days after exposure.

Exposure Time (hrs)	No. Chambers Tested[a/]	$\bar{X}$ Percent Mortality ± SEM	
		1 day	2 days
1	3	23.3 ± 8.82	53.3 ± 24.03
2	3	16.7 ± 8.82	48.9 ± 8.90
3	3	48.3 ± 8.3	71.1 ± 14.58
6	3	91.1 ± 8.9	93.3 ± 6.67
24	2	100	100
Controls	3	10.7 ± 6.19	21.7 ± 11.67

[a/] Number of chambers tested. Number of insects tested varied per treatment, with 3 to 4 replicates each.

Table 8.9. Mortality of small nymphs of the brownbanded cockroach, Supella longipalpa, exposed to low oxygen atmospheres in Phase II tests at 1 and 2 days after exposure.

Exposure Time (hrs)	No. Chambers Tested[a/]	$\bar{X}$ Percent Mortality ± SEM	
		1 day	2 days
1	3	25.6 ± 14.44	34.4 ± 8.68
2	3	61.1 ± 20.03	30.5 ± 17.64
3	3	39.4 ± 24.73	65.6 ± 19.67
6	3	96.7 ± 3.33	97.8 ± 2.23
24	2	100	100
Controls	2	10.0 ± 10.00	5.8 ± 0.85

[a/] Number of chambers tested. Number of insects tested varied per treatment.

Table 8.10. Percent of egg hatch of the brownbanded cockroach, Supella longipalpa, exposed to low oxygen atmospheres in Phase II at 2 and 3 months after exposure.

Exposure Time (hrs)	No. Chambers Tested[a/]	$\bar{X}$ Percent Hatched	
		2 months	3 months
2	1	56.3 ± 6.25	56.3 ± 6.25
3	1	100	100
6	3	49.2 ± 14.46	67.5 ± 7.50
24	2	22.5 ± 2.50	36.2 ± 13.75
72	1	0	0
96	2	0	0
120	2	0	0
Controls	3	56.7 ± 23.33	63.3 ± 15.75

[a/] Number of chambers tested. Number of oothecae tested varied per treatment because of availability.

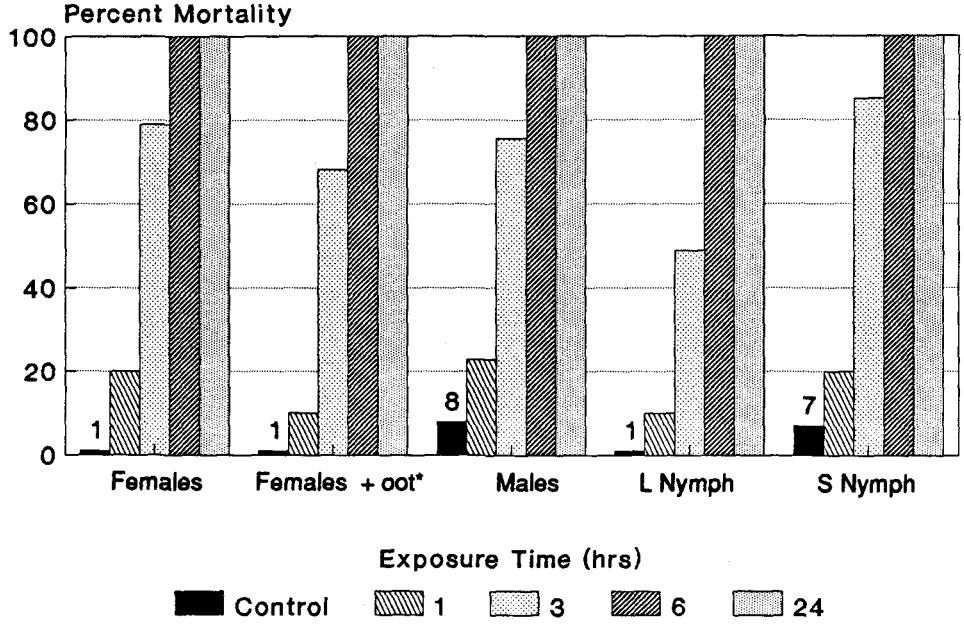

Fig. 18. The percent mortality of females, *females with oothecae, males, and large (L) and small (S) nymphs of the German cockroach, Blattella germanica, exposed to low oxygen atmospheres in Phase II at 24 hours after exposures.

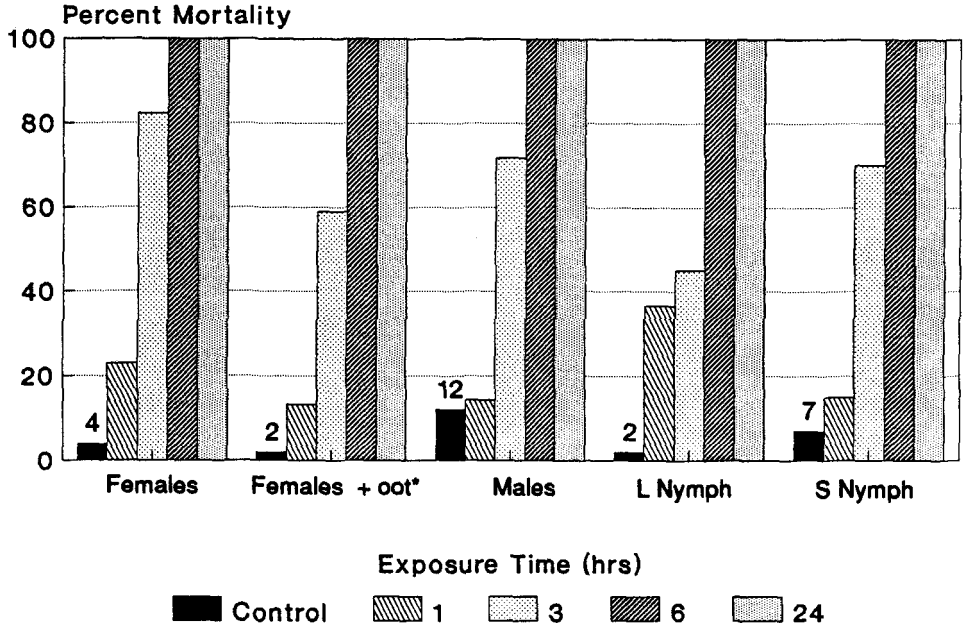

Fig. 19. The percent mortality of females, *females with oothecae, males, and large (L) and small (S) nymphs of the German cockroach, Blattella germanica, exposed to low oxygen atmospheres in Phase II at 48 hours after exposures.

Table 8.11. Mortality of female adults without oothecae of the German cockroach, Blattella germanica, exposed to low oxygen atmospheres in Phase II tests at 1 and 2 days after exposure.

Exposure Time (hrs)	No. Chambers Tested[a/]	$\bar{X}$ Percent Mortality ± SEM	
		1 day	2 days
1	1	20.0	22.9
3	3	78.9± 9.50	82.2 ± 9.68
6	3	100	100
24	4	100	100
Controls	4	1.3 ± 1.32	3.5 ± 3.50

[a/] Number of chambers tested. Number of insects tested varies per treatment.

Table 8.12. Mortality of female adults with oothecae of the German cockroach, Blattella germanica, exposed to low oxygen atmospheres in Phase II tests at 1 and 2 days after exposure.

Exposure Time (hrs)	No. Chambers Tested[a/]	$\bar{X}$ Percent Mortality ± SEM	
		1 day	2 days
1	1	10.0	13.3
3	3	68.3 ± 24.55	58.9 ± 27.50
6	3	100	100
24	4	100	100
Controls	4	1.2 ± 1.25	1.8 ± 1.77

[a/] Number of chambers tested. Number of insects tested varied per treatment.

Table 8.13. Mortality of male adults of the German cockroach, Blattella germanica, exposed to low oxygen atmospheres in Phase II tests at 1 and 2 days after exposure.

Exposure Time (hrs)	No. Chambers Tested[a/]	$\bar{X}$ Percent Mortality ± SEM	
		1 day	2 days
1	1	22.9	14.3
3	3	75.6 ± 24.43	71.7 ± 22.49
6	3	100	100
24	4	100	100
Controls	4	7.9 ± 4.27	11.7 ± 4.41

[a/] Number of chambers tested. Number of insects tested varied per treatment.

Table 8.14. Mortality of large nymphs of the German cockroach, Blattella germanica, exposed to low oxygen atmospheres in Phase II tests at 1 and 2 days after exposure.

Exposure Time (hrs)	No. Chambers Tested[a/]	$\bar{X}$ Percent Mortality ± SEM	
		1 day	2 days
1	1	10.0	36.7
3	3	48.9 ± 25.64	45.0 ± 25.98
6	3	100	100
24	4	100	100
Controls	4	1.2 ± 1.25	2.2 ± 2.23

[a/] Number of chambers tested. Number of insects tested varied per treatment.

Table 8.15. Mortality of small nymphs of the German cockroach, Blattella germanica, exposed to low oxygen atmospheres in Phase II tests at 1 and 2 days after exposure.

Exposure Time (hrs)	No. Chambers Tested[a/]	$\bar{X}$ Percent Mortality ± SEM	
		1 day	2 days
1	1	20.0	15.0
3	3	85.0 ± 15.00	70.0 ± 10.00
6	3	100	100
24	4	100	100
Controls	4	7.2 ± 4.74	6.7 ± 6.67

[a/] Number of chambers tested. Number of insects tested varied per treatment.

Table 8.16. Percent of egg hatch of the German cockroach, Blattella germanica, exposed to low oxygen atmospheres in Phase II tests at 30 days after exposure.

Exposure Time (hrs)	No. Chambers Tested[a/]	$\bar{X}$ Percent Mortality ± SEM
1	1	survivors
3	2	survivors
6	2	survivors
24	2	0
48	1	0
72	1	0
96	1	0
Controls	3	$\simeq$0

Table 8.17. Percent egg hatch of American cockroach oothecae, Periplaneta americana, exposed to low oxygen atmospheres in Phase III at 1, 2 and 3 months after exposure.

Exposure Time (hrs)	No. of Chambers[a/]	$\bar{X}$ Percent Hatched ± SEM		
		1	2	3
72	1	2.5	2.5	10.0
96	2	0.8 ± 0.83	0.17 ± 1.67	1.7 ± 1.67
120	4	0	0	0
Controls	3	15.3 ± 11.37	72.1 ± 27.92	75.8 ± 24.17

[a/] Number of chambers tested. Two oothecae per vial tested; the number of vials tested per treatment varied.

9. THE POWDERPOST BEETLE

Beetles belonging to the family Lyctidae are collectively referred to as true powderpost beetles. The larvae of powderpost beetles feed primarily on hardwoods such as ash, oak, hickory, mahogany, walnut, wild cherry, locust, poplar, sycamore, orange, eucalyptus, and other open-grained woods. Articles made of bamboo are also frequently infested. Infestations are commonly found in hardwood floors, furniture, antiques, tool handles, gunstocks, picture frames, packing crates, and ornamental pieces such as grape canes and figurines (Ebeling 1975).

Powderpost beetles can be recognized by small round emergence holes (1-2 mm diameter) in wood and by the presence of fine dust (trass) produced by larvae. Powderpost beetle frass is extremely fine, similar in consistency to facial talc, and does not feel gritty or coarse like that of many other wood-destroying beetles. The greatest feeding activity occurs in wood with 10-20% wood moisture content. Relative humidities of 40-80% favor development (Gay 1953). Lyctid larvae utilize the starch, sugar, and proteins in wood; they cannot digest the cellulose in cell walls.

Most species lay about 50 eggs, which are deposited in the open vessels of wood. However, Lyctus brunneus (Stephen) may lay up to 221 eggs (Gay 1953). Covering all exposed surfaces with paint, varnish, or wax will prevent adult beetles from laying eggs. The eggs hatch in 8 to 12 days. The larvae develop from 2 to 9 months, depending on the temperature as well as the moisture and starch content of the wood. The pupal period ranges from 12 days to 3 weeks. Adult Lyctids are nocturnal, living about 3 to 6 weeks. Adults readily fly and are attracted to light (Parkin 1934, Christian 1940, 1941). The entire life cycle of the powderpost beetle ranges from 3 months to a year or more. Under natural conditions, adult emergence usually occurs from June to August in northern temperate climates. Environmental conditions and resource quality affect the longevity of development.

Three species used in the tests were Lyctus brunneus (Stephens), Lyctus linearis (Goeze), and Trogoxylon prostomoides (Gorham). Lyctus brunneus and L. linearis are cosmopolitan species, found in a wide variety of hardwoods. T. prostomoides has been found infesting toys, furniture, herbwood, and bamboo.

Insects

The powderpost beetles were reared on a hard baked diet consisting of 255.6 g long-fibered cellulose, 51.6 grams yeast extract (Wheast), 204 grams wheat flour, and 600 ml water. The dry ingredients were mixed with water to form a dough, which was pressed into a 5-cm-thick sheet in a greased baking pan. The dough was baked at 60°C for 48 hours until hardened. The sheet was broken into small pieces about 3 cm square, and 0.9-liter jars were half filled with media (Ito and Hirose 1980). Two 12.5-cm filter papers were placed around the inside of each jar. The adults were added to the jar and a cloth treated with 18.5% dicofol was affixed to the lid by two rubber bands, to prevent mite infestations. The jars were held at 25.5°C (78°F), 30-45% RH. The colonies were small and it was difficult to increase the numbers of adults obtained. The life cycle for L. brunneus and L. linearis was about 3 months; it was 3 to 4 months for T. prostomoides.

Once every two weeks the adults were collected from the rearing jars. Only adults were available for testing with Lyctus planicollis LeConte and Trogoxylon prostomoides. The adult, larval, and pupal stages were available with L. brunneus and L. linearis. To obtain adults less than 1 week old, adults were removed from jars one week prior to testing.

The adult, pupal and larval collections were made one day prior to testing Lyctus spp. The contents of each jar were dumped into a white metal pan. When pieces of diet were trapped on the sides of white metal pan, all life stages of L. brunneus and L. linearis were dislodged from the diet, making it possible to collect them. The collection was sifted through a 20-mesh screen to remove all fine debris. Only uniformed-sized adults, pupae, and larvae were selected for testing. It was not possible to collect larvae and pupae of T. prostomoides. Twenty insects of each stage were placed in individual glass vials (7 cm tall by 3 cm diameter) with diet and were covered with screened lids. The number of insects used in the tests varied according to availability.

The selected insects were transferred to a large, covered plastic holding box. The relative humidity inside the box was maintained at 55% with a small container of a saturated solution of magnesium nitrate. The holding box was then placed in an environmental chamber set at 25.5°C (78°F) and held for one day prior to testing.

Phase II Testing

The vials containing insects to be tested were transferred to the chambers. The relative humidity inside the chamber was maintained at 55% with 25 ml of a saturated solution of magnesium nitrate in a small plastic box. Excess magnesium nitrate (25 g) was added to the saturated solution so that any excess moisture in the chamber was absorbed. Two packets (7 g) of Z-1000 AGELESS were placed in each chamber. AGELESS is a chemical oxygen scavenger prepared from powdered iron oxide (Mitsubishi Gas Chemical Co., Inc.). After transferring all insects to the test chamber, the chamber door was loosely sealed. The chamber was flooded for 30 minutes with 99.999% pure nitrogen conditioned to 55% RH. The chamber was tightly sealed and the flow of nitrogen discontinued.

If the oxygen content inside the chamber was greater than 0.1%, the chamber was flushed again for an additional 5 to 10 minutes. The oxygen content was again analyzed. Every morning the chambers were analyzed for oxygen content; whenever the level exceeded 0.1%, the chamber was flushed as described above.

Three exposure periods were tested with the adult T. prostomoides: 48, 96, and 120 hours. The adult L. planicollis were exposed for 24, 120, and 144 hours. Control insects were held in the 55% RH holding box, which was placed on top of the testing chamber. After the exposure tests were competed, the insects were transferred back to the holding boxes and placed in the environmental chamber.

To examine the insects for mortality, the contents of each vial were dumped into a petri dish. The insects were removed from the diet. All observations were made with the aid of a microscope at 24 and 48 hours after each exposure. Each adult, pupa, and larva was probed with a pair of soft forceps. If they moved, they were scored as live. All larvae that pupated and pupae that emerged as adult were scored as live. After examining for mortality after 48 hours, the contents of each vial were transferred to a 0.9-liter jar half filled with diet to encourage development if at all possible. Controls were transferred to identical jars. Additional observations were made at 1, 2, 3, and 4 months for survivors.

Approximately 50 adult T. prostomoides were tested in Phase II. About 50 adults, 20 larvae, and 20 pupae of the Lyctus spp. were tested.

Phase III Testing

A simple wooden holding block was designed to confine the beetles deep inside the blocks during exposure. Pieces of wood (8 by 8 by 14 cm) were cut from a post of Douglas fir. Each block was cut in half and the center was hollowed to hold a 10 ml glass vial, leaving 1.27 to 2.54 cm of solid wood surrounding each insect vial. Two holes were drilled through the two blocks so that bolts could pass through both blocks (Fig. 20). A sheet of parafilm was placed on either block; pressing the two halves firmly together sealed the blocks like a sandwich. The blocks were held tightly together with 2 1/2-inch bolts and wing nuts. Six blocks were placed in each chamber for each test.

The procedure for sorting and preparing the powderpost beetles was identical to those in Phase II, except that beetles were placed in 10 ml vials with 20-mesh screen bottoms and lids. Small pieces of diet were added to each vial and ten adults were placed in each one. The two exposure times tested in Phase III were 120 and 144 hours. Only 10 adult T. prostomoides were tested in Phase III, plus controls. Ten each of adults, pupae, and larvae of the Lyctus spp. were tested in Phase III, plus controls.

RESULTS

Table 9.1 shows that in Phase II tests a 48-hour exposure produced 97% kill of adult beetles. A 96-hour or longer exposures produced 100% mortality. The only exposure period tested in Phase III was 144 hours, which provided 100% kill.

Table 9.2 shows a 90% kill of the Lyctus larvae within 24 hours in Phase II. All other life stages were completely killed. Two exposures were tested in Phase III, 120 and 144 hours, all resulting in 100% kill.

CONCLUSIONS

1. The colonies were too small to allow testing of sufficient replicates. Although very encouraging, results can only be considered preliminary.
2. At least a 96-hour exposure in Phase II with Trogoxylon prostomoides was necessary to provide 100% kill.
3. In Phase II, a 120-hour exposure produced only a 90% kill of the Lyctus larvae, indicating a need for a longer exposure period to achieve 100% mortality.

Table 9.1. Percent mortality of adults of the powderpost beetle, Trogoxylon prostomoides.

Exposure	No. Chambers	X Percent Mortality (weeks)		
Time (hrs)	Tested	0.1	3	12
Phase II				
48	1		97	
96	1	100	100	
120	1	100	100	100
144	1	100	100	100
Controls		0	100	
Phase III				
144	1	100	100	100

Table 9.2. Percent mortality of adults, pupae, and larvae of Lyctus spp.

Exposure Time (hrs)	No. Chambers Tested	Life[a/] Stage	$\bar{X}$ Percent Mortality (weeks) 0.1	4	12
Phase II					
24	1	A			100
	2	A			100
	3	A			100
120	1	P	100	100	100
		L	90	100	100
144	1	A	100	100	
5 Day Controls	---	A	100	100	100
	---	P	0	0	
	---	L	50	100	
Phase III					
120	1	P	100	100	100
		L	100	100	100
144	1	A	100	100	100

[a/] A, adults; L, larvae; P, pupae.

10. THE WESTERN DRYWOOD TERMITE

The western drywood termite, Incisitermes minor (Hagen), is the most destructive drywood termite in the United States. Drywood termites are aptly named, because they establish themselves in wood that is not decayed or in contact with ground moisture. They frequently attack perfectly dry softwoods used in framing structures, but they occasionally infest hardwoods used in furniture construction. Western drywood termites range from Northern California to central Mexico and from the Pacific Coast inland to New Mexico (Ebeling 1975).

Drywood termite colonies develop very slowly. Like all truly social insects, termite workers care for the young, there is a division of labor (caste system), and queens live for many generations. The newly established colony consists of a king, queen, and a small number of immature insects, usually less than 12 for the first year. The young colony will consume only 0.5 to 2.5 cc of wood within the first 15 months. During the second year the young colony increases to a maximum of about 12 to 15 individuals. Generally, more than a year is required for a nymph to develop from egg to alate (winged reproductive) or soldier, depending on environmental conditions. Newly emerged immature insects cannot survive without workers, because they lack the hindgut protozoa necessary to digest wood. Consequently, control strategies such as fumigation that kill only workers and reproductives will eliminate colonies, even though the eggs survive (Ebeling 1975).

Insects

Heavily infested wood was collected from various areas in Riverside County and stored outdoors next to the laboratory. Two to fourteen days prior to testing, termites were collected by splitting infested logs and gently extracting the termites. The termites were placed in airtight plastic tubs provisioned with thin wafers of Douglas fir. The termites were divided into two groups: small nymphs (up to 3.5 mm long), and reproductives, soldiers and the large nymphs (> 3.5 mm long). The large nymphs were selected for testing. Termites are exceedingly sensitive to the slightest difference in the relative humidity and will very quickly move to areas of greater moisture

content: consequently, the large nymphs were held in individual airtight, plastic containers. A sheet of paper towel covered the bottom of each container, and a few layers of the thin wafers of Douglas fir wood were placed on the paper. A 10 ml vial filled with water and cotton wick was added for moisture. The airtight plastic boxes were stored in the dark in an environmental chamber set at 25.5°C (78°F).

One day prior to testing, individual nymphs were transferred with soft forceps to small disposable petri dishes (10 mm tall by 35 mm diameter) lined with a disk of paper towel. A small piece of wood was added to each dish before covering the termites with a second disk of paper. One drop of water was applied to the paper before replacing the lid.

The termites were held in the airtight plastic holding box. The relative humidity inside the box was maintained at 55% by a small container of a saturated solution of magnesium nitrate. The holding box was placed in the environmental chamber set at 25.5°C (78°F) and held for one day.

Phase II Testing

On the day of testing, the petri dish lids were removed and the petri dishes with termites were transferred to the test chambers. The relative humidity inside the chamber was maintained at 55% with 25 ml of a saturated solution of magnesium nitrate in a small plastic box. Excess magnesium nitrate (25 g) was added to the saturated solution so that all excess moisture in the chamber was absorbed. Two packets (7 g) of Z-1000 AGELESS were placed in each chamber. AGELESS is a chemical oxygen scavenger prepared from powdered iron oxide (Mitsubishi Gass Chemical Co., Inc.). After transferring all insects to the test chamber, the chamber door was loosely sealed. The chamber was flooded for 30 minutes with 99.999% pure nitrogen conditioned to 55% RH. The chamber was tightly sealed and the flow of nitrogen discontinued.

If the oxygen content inside the chamber was greater than 0.1%, the chamber was flushed again for an additional 5 to 10 minutes. The oxygen content was again analyzed. Every morning the chambers were analyzed for oxygen; whenever the oxygen level exceeded 0.1%, the chamber was flushed as described above. Five exposure periods were selected: 15, 24, 48, 72, and 96 hours, plus controls. Each test was replicated 3-5 times, on 3-5 different dates. The lids were

removed from each petri dish with untreated control insects and the petri dishes and termites held in an empty chamber without nitrogen.

After completion of the various exposures, the tested termites and controls were transferred back to the airtight holding boxes. Three drops of water were added to the paper in each dish before replacing lids.

The number of live insects was counted after holding for 1, 7, and 14 days. Under a microscope, each termite was probed with soft forceps; if it moved, it was scored as alive. Dead termites were removed. Three drops of water were added to each dish after it was inspected. Approximately 1,400 nymphs were tested in Phase II, plus controls.

Phase III Testing

A simple wooden block was designed to confine the termites deep inside the blocks during exposure. Pieces of wood (8 by 8 by 14 cm) were cut from a post of Douglas fir. Each block was cut in half, and the center was hollowed to hold a 10 ml glass vial, leaving 1.27 to 2.54 cm of solid wood surrounding each vial. Two holes were drilled through the two blocks so that bolts could pass through both blocks (Fig. 20). A sheet of parafilm was placed on either block; pressing the two halves together sealed the blocks like a sandwich. The blocks were held tightly together with 2 1/2-inch bolts and wing nuts. Six blocks were placed in each chamber for each test.

The procedure for selecting and preparing the nymphs was identical to those in Phase II, except that 20 nymphs were placed in a specially modified 10 ml glass vial. The bottom of each vial was cut off and replaced with a piece of 20-mesh screen. A hole was cut in each screw cap and covered with the screen mesh. A small piece of paper towel covered the inside of each vial. Small pieces of wood were added before adding the termites. Control nymphs were held in identical vials in wooden blocks and placed on top of the chambers during tests.

The exposure periods tested were 48, 72, and 96 hours. After each exposure, the insects were removed from the vials and placed in the small disposable petri dishes (10 by 35 mm diameter), 20 per dish, as in Phase II. The insects were examined for mortality as described in Phase II.

Each nitrogen exposure was replicated 4 times, on 4 different dates, with the exception of the 48-hour exposure. It was replicated twice, with only one vial per chamber. Approximately 1,650 nymphs were tested in phase III, plus controls.

RESULTS

Phase II

Table 10.1 shows that 96-hour exposures produce 100% kill of termites within 24 hours after exposure. Exposures for 72 hours provided 100% kill by day 14. Shorter exposures failed to provide 100% kill.

Mortality was consistent among chambers with the 15-hour exposures when compared at 1, 7, and 14 days. With 15-hour exposures, there was a 53% increase in mortality between day 1 and day 7, indicating a latent mortality effect. The increase in mortality was less than 8% between day 7 and day 14 (Fig. 21).

The 24-hour exposures provided somewhat more variable results. Exposures of 24 and 48 hours to low oxygen atmosphere also resulted in a higher percent increase in mortality in the first 7 days after exposure than between day 7 and day 14 (Fig. 21).

Phase III

Three exposures (48, 72, and 96 hours) were tested in Phase III. The 48-hour exposures provided 65% kill within 1 day following exposure and all termites were dead within 7 days (Table 10.2). When exposed for 72 hours, 85-97% of the termites were killed within 1 day after exposure (Fig. 22). Complete mortality was achieved within 14 days. The 96-hour exposure killed all the termites in 1 day after exposure.

Results were nearly identical with all three exposure tests in Phase II and III (Fig. 23). The wooden block did not significantly increase the exposure time required to produce 100% kill of drywood termites.

CONCLUSIONS

1. Exposures for 96 hours resulted in 100% kill of nymphs within 24 hours following exposure in Phase II. A 72-hour exposure killed 100% of the nymphs within 14 days after exposure.
2. When termites were exposed for 15, 24, or 48 hours, there was a significant increase in mortality between day 1 and day 7 after exposure. Clearly the exposure to low oxygen atmospheres produced some latent effects.
3. In Phase III, 48-, 72- and 96-hour exposures produced 100% within 14 days after exposure. The wooden block was not a barrier to the nitrogen.
4. In Phases II and III, the 96-hour exposure produced 100% kill within 24 hours after exposure.

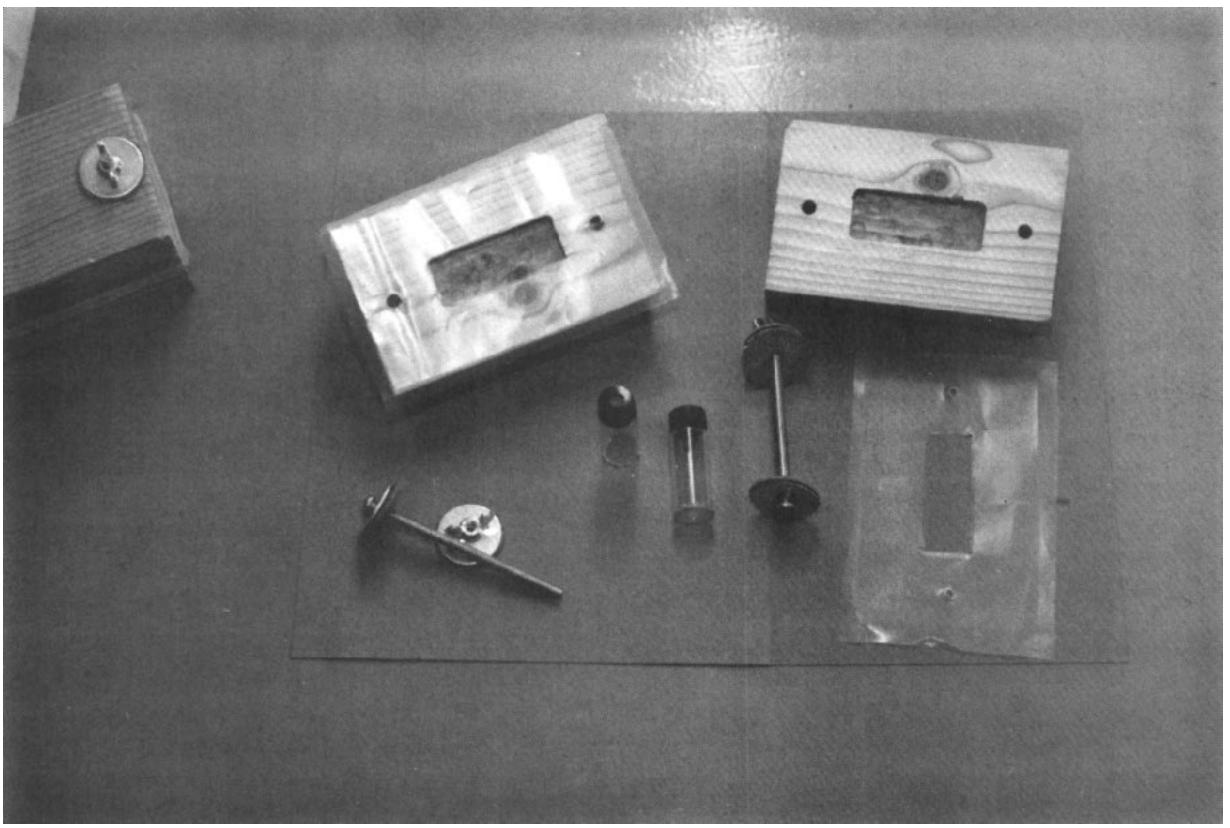

Fig. 20. The wood blocks used to confine drywood termites in Phase III.

Table 10.1. Percent mortality of nymphs of the western drywood termite, Incisitermes minor, in Phase II.

Exposure Time (hrs)	No. Chambers Tested	$\bar{X}$ percent mortality (+ SEM) at day[a/]		
		1	7	14
15	3	30.6 ± 4.83	65.3 ± 2.38	70.9 ± 1.82
24	3	48.2 ± 19.49	75.4 ± 15.71	95.0 ± 4.40
48	3	74.1 ± 0.85	96.7 ± 3.33	98.9 ± 1.10
72	3	94.0 ± 3.38	98.5 ± 0.96	
96	3	100	100	100
Controls		0.3 ± 0.27	2.2 ± 0.73	4.5 ± 0.79

[a/] 6 to 9 lots of insects tested per treatment.

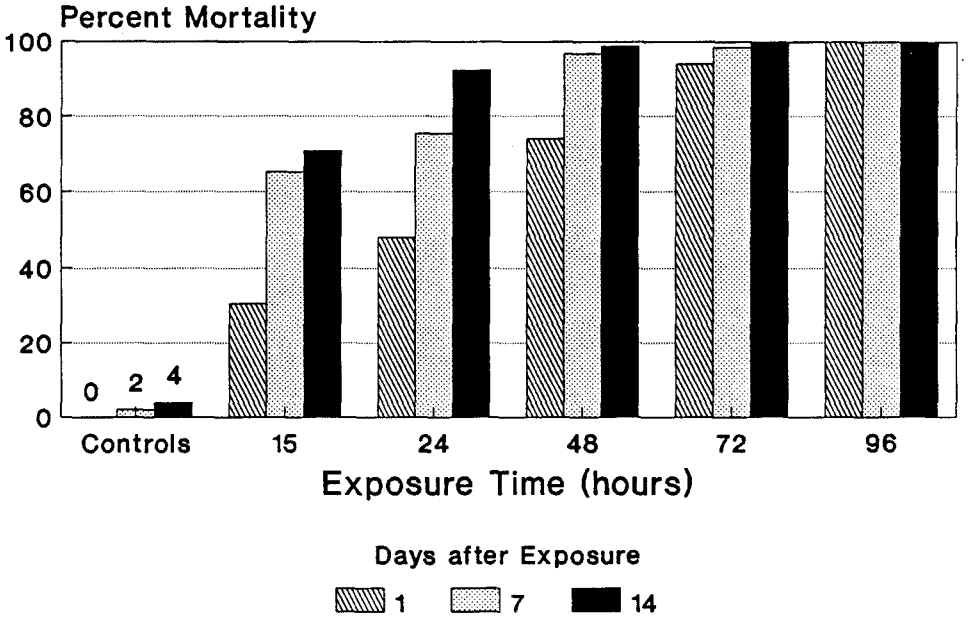

Fig. 21. The mortality of nymphs of the western drywood termite, Incisitermes minor, 1, 7, and 14 days after exposure to low oxygen atmospheres (<0.1% oxygen).

Table 10.2. Percent mortality of nymphs of the western drywood termite, Incisitermes minor, in Phase III.

Exposure Time (hrs)	No. Chambers Tested	$\bar{X}$ percent mortality (+ SEM) at day[a/] 1	7	14
48	2	65.0 ± 15.0	100	100
72	4	92.9 ± 2.47	99.6 ± 0.23	100
96	4	100	100	100
Controls	8	0	2.3 ± 1.04	3.2 ± 1.02

[a/] 5 to 6 lots of insects tested per treatment, except for one per treatment with the 48-hour exposure.

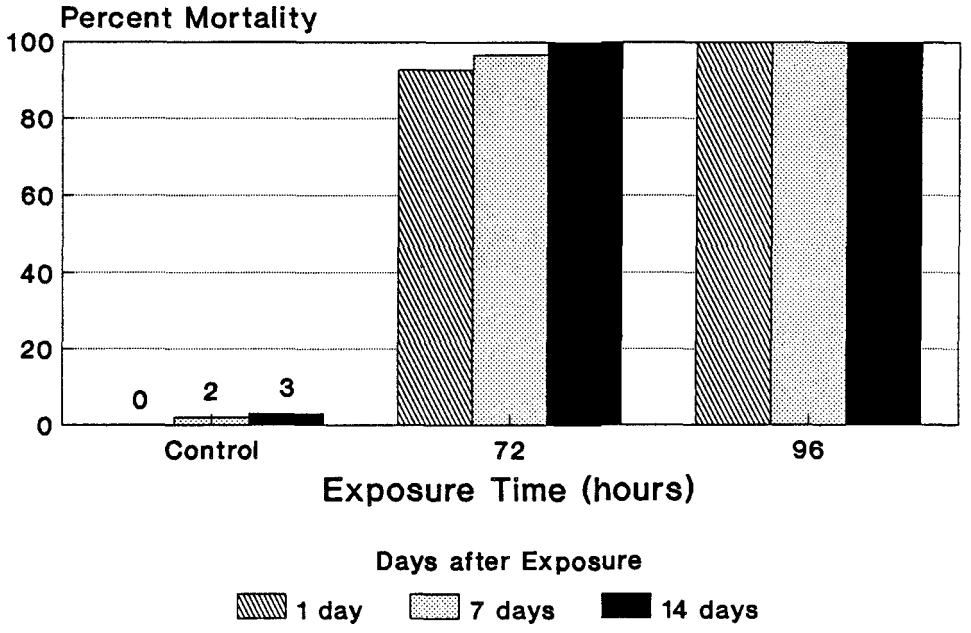

Fig. 22. The mortality of nymphs of the western drywood termite, Incisitermes minor, 1, 7, and 14 days after exposure to nitrogen atmospheres (<0.1% oxygen) in Phase III.

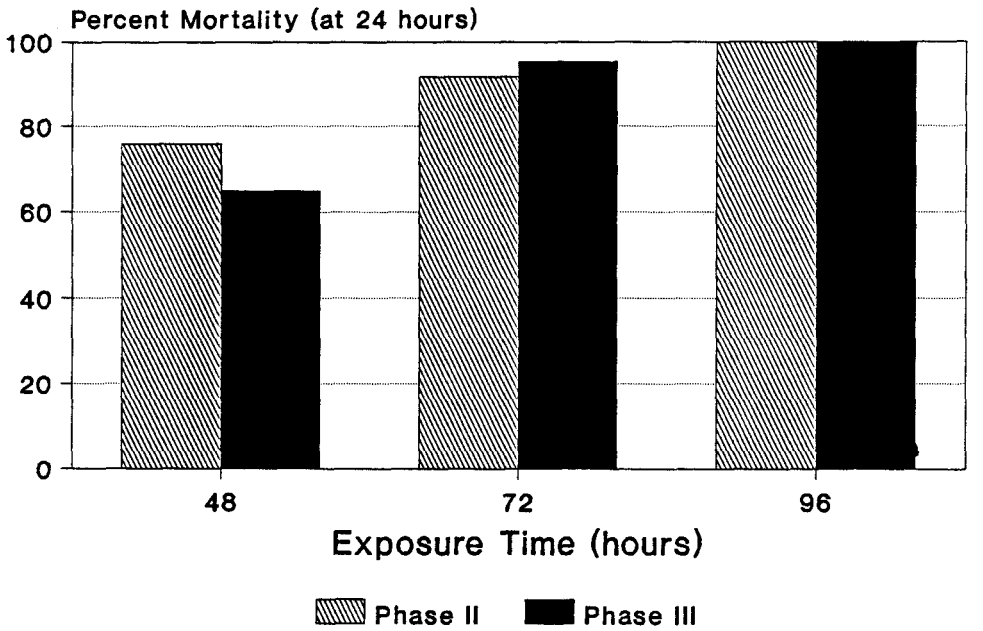

Fig. 23. Comparison of mortality of nymphs of the western drywood termite, Incisitermes minor, in Phase II and III, 24 hours after exposure to nitrogen atmospheres (<0.1% oxygen).

SUMMARY

The use of low oxygen atmospheres to control insect pests in museums looks extremely promising. It was possible to maintain low oxygen atmospheres (<0.1%) for at least 8-10 days with only an occasional flushing of pre-purified N_2 and several packets of AGELESS oxygen scavenger. The results showed that the time required to kill 100% of the insects varied among species and even among the developmental stages of a given species. For most insects tested, exposures of less than 72 hours were required to insure complete kill. Certain stages, such as eggs of cigarette beetles, may require up to 8-day exposures to insure complete kill. Preliminary tests indicated that the addition of CO_2 to the nitrogen slightly decreased the exposure time required to kill the insects. However, if increased temperatures or decreased relative humidities could be tolerated by the objects, they would probably have a much greater effect than using CO_2 and N_2 mixtures in reducing exposure times.

The time required to kill the most resistant stages of each insect tested did not significantly increase in Stage III testing. The oxygen was quickly displaced and removed by the AGELESS, killing the insects buried deep in the wood or flour. Consequently, it would probably not be necessary to specially prepare or stack items for treatment.

The future use of controlled atmospheres to control museum pests largely depends on the development of chambers or bags large enough to hold furniture, paintings and other objects. Special bags capable of enclosing larger items and still maintaining the low O_2 atmosphere are needed. If such bags and chambers could be developed, the use of low oxygen atmospheres will replace the current use of insecticide sprays and fumigants.

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REFERENCES CITED

Bailey, S. W. & H. J. Banks. 1980. A review of recent studies of the effects of controlled atmospheres on stored product pests, pp. 101-118. In J. Shejbal [ed.], Controlled atmosphere storage of grains. Elsevier Scientific Publishing Co., Amsterdam.

Beauchamp, R., J. Kingsolver, and T. Parker. 1981. Reference listing of museum pests, pp. C1-C5. In S. R. Edwards, B. M. Bell, and E. M. King [eds.], Pest control in museums: a status report (1980). Assoc. of Syst. Collections, Lawrence, Kansas.

Bell, B. M. & E. Stanley. 1981. Tabular results of the survey of museum pest control procedures, G1-G10. In S. R. Edwards, B. M. Bell, and E. M. King [eds.], Pest control in museums: a status report (1980). Assoc. of Syst. Collections, Lawrence, Kansas.

Calderon, M. 1990. Introduction, pp. 3-8. In M. Calderon and R. Barkai-Colan [eds.], Food preservation by modified atmospheres, CRC Press, Boca Raton, Florida.

Carr, R. C. 1990. Silverfish and booklice, pp. 87-99. In K. Storey [ed.], Handbook of Pest Control. Franzak & Foster Co., Cleveland, Ohio.

Dawson, J. 1988. The effects of insecticides on museum artifacts and materials, pp. 135-150. In L.A. Zycherman and J. R. Schrock [eds.], A guide to museum pest control, Assoc. of Syst. Collections. Washington, D.C.

Donahaye, E. 1990. Laboratory selections of resistance by the red flour beetle, Tribolium castaneum (Herbst), to an atmosphere of low oxygen concentration. Phytoparasitica 18: 189-202.

Ebeling, W. 1975. Urban entomology. Univ. California Div. Agr. Sci. Berkeley, California.

Fleurat-Lessard, F. 1990. Effect of modified atmospheres on insects and mites infesting stored products, pp. 21-38. In M. Calderon and R. Barkai-Colan [eds.], Food preservation by modified atmospheres, CRC Press, Boca Raton, Florida.

Florian, M.-L. E. 1988. Ethylene oxide fumigation: a literature review of the problems and interactions with materials and substances in artifacts, pp. 151-158. In L. A. Zycherman and J. R. Schrock [eds.], A guide to museum pest control, Assoc. of Syst. Collections. Washington, D.C.

Gilberg, M. 1989. Inert atmosphere fumigation of museum objects. Studies in Conservation 34: 80-84.

Gilberg, M. 1990. Inert atmosphere disinfestation using Ageless oxygen scavenger, pp. 812-816. In Ninth Tiennial Meeting of the Inner Council of Museum Committee for Conservation.

Hinton, H. E. 1945. A monograph of the beetles associated with stored products. British Museum (Nat. Hist.). Vol. I.

Jay, E. G., R. T. Arbogast, and G. G. Peraman, Jr. 1971. Relative humidity: its importance in the control of store-product insects with modified atmospheric gas concentrations. J. Stored Prod. Res. 6: 325-329.

Jay, E. G. & W. Cuff. 1981. Weight loss and mortality of three life stages of Tribolium castaneum (Herbst) when exposed to four modified atmospheres. J. Stored Prod. Res. 17: 117-124.

Khalifa, A. & A. Badaway. 1955. Biology and studies on Tribolium confusum Duv., Tribolium castaneum Hbst., and Latheticus oryzae Waterh. Bull. Soc. Entom. Egypte 39: 351-373.

Kingsolver, J. 1981. Illustrated guide to common insect pests in museums, B1-B20. In S. R. Edwards, B. M. Bell, and E. M. King [eds.], Pest control in museums: a status report (1980). Assoc. of Syst. Collections, Lawrence, Kansas.

Marzke, F. O., A. F. Press, Jr., and G. C. Pearman, Jr. 1970. Mortality of the rice weevil, the Indian-meal moth, and Trogoderma glabrum exposed to mixtures of atmospheric gases at various temperatures. J. Econ. Entomol. 63: 570-574.

Navarro, S. 1978. The effects of low oxygen tensions on three stored-product insect pests. Phytoparasitica 6: 51-58.

Parker, T. A. 1990. Clothes moths, pp. 347-375. In K. Storey [ed.], Handbook of Pest Control. Franzak & Foster Co., Cleveland, Ohio.

Preslock, K. 1988. Pest control in museums (1986): a partially annotated bibliography, pp. 177-198. In L. A. Zycherman and J. R. Schrock [eds.], A guide to museum pest control, Assoc. of Syst. Collections. Washington, D. C.

Quek, L. C., M. Razak, and M. W. Ballard. 1990. Pest control for temperate vs. tropical museums: North America vs. Southeast Asia, pp. 817-820. In Ninth Tiennial Meeting of the Inner Council of Museum Committee for Conservation.

Soderstrom, E. L., D. G. Brandl, and B. Mackey. 1990. Responses of codling moth (Lepidoptera: Tortricidae) life stages to high carbon dioxide or low oxygen atmospheres. J. Econ. Entomol. 83: 472-475.

Schrock, J. R. 1988. List of insect pests by material or apparent damage, pp. 113-120. In L. A. Zycherman and J. R. Schrock [eds.], A guide to museum pest control, Assoc. of Syst. Collections. Washington, D.C.

Strong, R. G. 1975. Comparative studies on the biologies of six species of Trogoderma: T. inclusum. Annals Ent. Soc. Am. 68: 91-104.

Valentin, N. 1990. Insect eradication in museums and archives by oxygen replacement, a pilot project, pp. 821-823. In Ninth Triennial Meeting International Council of Mus. Committee for Conservation.

Valentin, N. & F. Preusser. 1990. Insect control by inert gases in museums, archives and libraries. Restaurator 11: 22-33.

Walter, V. E. 1990. Stored product pests, pp. 501-580. In K. Storey [ed.], Handbook of Pest Control. Franzak & Foster Co., Cleveland, Ohio.

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