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Low-temperature as an alternative to fumigation to disinfest stored tobacco of the cigarette beetle, *Lasioderma serricorne* (F.) (Coleoptera: Anobiidae)

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Abstract

Time-temperature-mortality relationships for egg, larval, pupal, and adult stages of the cigarette beetle, *Lasioderma serricorne* were investigated to determine disinfesting conditions for stored tobacco. Susceptibility to low temperatures changed according to the developmental stage. Eggs were most susceptible to low temperatures that were higher than −5°C. Larvae acquired cold tolerance during exposure to 15°C for 1 mo. These acclimated larvae were the most tolerant of all. LT_{99} values of acclimated larvae were calculated as 7.2 h at −15°C, 23.7 h at −10°C, 376 h at −5°C, 1,140 h at 0°C, and 1,880 h at 5°C. As the most susceptible stage, egg susceptibility to moderately low temperatures (16–20°C) was specifically examined. At 20°C, most eggs (>80%) normally hatched within 4 wk, but all eggs died within 6 wk at temperatures less than 18°C. This fact indicates that the reproductive cycle can be blocked at temperatures less than 18°C, and that tobacco stored in such conditions will never become infested, even if eggs are deposited by invading adults. Consequently, if the tobacco temperature is reduced to 5°C for 3 mo in winter and is subsequently maintained below 18°C throughout the rest of the year, tobacco can become and remain pest-free without any chemical control.

Key words: Low temperature; tobacco; warehouse; cigarette beetle; *Lasioderma serricorne*

INTRODUCTION

The cigarette beetle, *Lasioderma serricorne* (Fabricius) (Coleoptera: Anobiidae), is the most important stored-tobacco pest in Japan. It is distributed in tropical to temperate areas worldwide. Cured tobacco leaves are generally stored for years before manufacturing cigarettes. The long storage period offers this pest an opportunity for infestation and reproduction. Pesticide application to stored tobacco and tobacco products is restricted. Phosphine fumigation is the only applicable method for disinfestation in warehouses. Nevertheless, resistant beetles have continued to spread, and present fumigation techniques are becoming less efficient (Rajendran and Narasimhan, 1994; Zettler and Keever, 1994). In addition to the emerging difficulty of resistance, public concern has been growing regarding the potential health and environmental hazards of pesticides. Consequently, non-chemical control methods have become increasingly sought as future pest management strategies.

The prevalence of this pest in Japan is conspicuous in southwestern areas, which are warmer, but prevalence is scarce in cooler northern areas. This fact suggests that the cigarette beetle is not fully adapted to temperate climates and that minor manipulation of storage temperature, i.e. low-temperature storage, should mitigate or prevent damage in temperate regions. Numerous studies have examined the lethal effects of low temperatures on this pest (Swingle, 1938; Howe, 1957; Childs et al., 1968, 1970; Mullen and Arbogast, 1979), but only fragmentary information is available. Storage facilities that cool the air to 4°C have been designed to annihilate this pest in the winter (Beard et al., 1983, 1986; Childs et al., 1983), but annual temperature programs to reduce its population growth or to eliminate infestation have not been established. To propose an annual cooling program for tobacco warehouses that prevents infestation without chemical control, we confirmed time-tempera-

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ture-mortality relationships for all developmental stages of the cigarette beetle. We determined the lowest temperature that allows the beetle to reproduce.

MATERIALS AND METHODS

Insects. The cigarette beetles used in this study were of our laboratory culture maintained on 10% yeast-added corn flour under 27°C and 60% R.H. For acclimation, breeding jars containing early fourth (final)-instar larvae (24–27 d after oviposition) were transferred to a 15°C chamber and kept for 1–3 mo. Adults (<1 d after emergence) were collected from the tops of the jars. Cocoa powder for oviposition was applied to the adults; and then they were kept at 27°C for 1 d (Test 1), or 16, 18, or 20°C for 3 d (Test 2) to prepare eggs. The deposited eggs were sieved with a 150-μm mesh screen. The late fourth-instar larvae (>0.5 mm head-capsule width) were sieved from the jars with a 1-mm mesh screen and selected using a microscope. Pupae in their cells were sieved with a 1-mm mesh screen and stripped off with a gentle stream of water.

Exposure to low temperatures. We put 50 eggs (2–4 d after oviposition), 30 larvae, 50 pupae (unsexed), and 50 adults (unsexed), respectively, into polystyrene vials (2.5 cm diameter×5.0 cm high) with ca. 1 g of corn flour. The insects in vials were exposed to −20, −15, −10, −5, 0, or 5°C (Test 1), or 16, 18, or 20°C (Test 2) in a climatic test chamber (CRH-210; Tabai Espec Corp.). For Test 2, the eggs were exposed to the same temperatures as those deposited. Temperatures were monitored with a thermistor, recorded with a thermo recorder (RT-11; Tabai Espec Corp.) and controlled at the set points ±0.2°C. For exposure to temperatures higher than 0°C, the vials were held in closed containers over a saturated NaCl solution to maintain ca. 75% R.H. Two replications were conducted for each stage.

Assessment of mortality. After exposure, the insects in vials were held under the rearing conditions until their viability was checked. The respective viabilities of eggs, larvae, pupae, and adults were determined 14, 28–42, 14, and 2 d after treatments. For eggs, larvae, and pupae, the criteria for viability assessment were transitions to their respective subsequent stages of development; for adults it was normal movement (paralyzed adults were considered as dead). The 50% and 99% lethal times (LT_{50} and LT_{99}) were calculated with the PriProbit (ver. 1.63) computer program developed by Sakuma (1998), which was downloaded from URL: http://bru.gmprc.ksu.edu/sci/throne/.

RESULTS AND DISCUSSION

Lethal effects of 'extremely low' temperatures on eggs, larvae, pupae and adults (Test 1)

Table 1 shows the lethal times of low temperatures (−20–5°C) on the insects at each developmental stage. Results obtained in this study are consistent with those of most preceding papers, as summarized in Howe (1957). Susceptibility to low temperatures differed according to the developmental stage. Eggs were most susceptible to temperatures higher than −5°C. Eggs are usually reported as the most susceptible stage (Fields, 1992; Mason and Strait, 1998); Dohino et al. (1999) demonstrated that eggs were the most tolerant below freezing temperatures for five of six major stored-product pests. Eggs of this species also exhibited relatively higher tolerance to temperatures less than −10°C. This alteration of the susceptibility of eggs beyond freezing temperatures may be partly attributable to their higher supercooling capacity because of the eggs' smaller size (Denlinger and Lee, 1998).

Exposure to a sublethal low temperature (15°C) before exposure to lethal low temperatures (−20–5°C) increased the survival times of larvae. Enhanced cold tolerance through acclimation has been described for many other insect species including stored-product pests (Fields, 1992). The cold-tolerance of acclimated larvae increased 2–4-fold; the larvae were more tolerant than insects of any other stage. The acclimated larvae required >11 wk for eradication at 5°C, whereas other stages were eradicated within 5 wk. This result coincides well with field observations reporting that only larvae survive the winter in tobacco warehouses in temperate regions (Takaoka and Nakazawa, 1956; Fletcher et al., 1973).

Lethal effects of 'moderately low' temperatures on eggs (Test 2)

Table 2 shows egg susceptibility to 'moderately low' temperatures (16–20°C); the egg stage is the

Table 1. Lethal effects of 'extremely low' temperatures on *Lasioderma serricorne* eggs, fourth instar larvae, pupae, and adults

Stage	Temperature (°C)	LT_{50} (fiducial limit) (h)	LT_{99} (fiducial limit) (h)	100% mortality [a] (h)
Egg	−20	—	—	1
	−15	0.22 (0.00–0.50)	2.48 (1.72–11.13)	4
	−10	1.29 (0.92–1.62)	7.72 (5.54–13.65)	12
	−5	4.92 (2.93–6.51)	34.02 (25.43–58.65)	48
	0	39.07 (19.16–53.77)	259.0 (193.2–494.1)	240
	5	137.6 (121.5–151.5)	447.2 (380.4–565.6)	336
Larval	−20	—	—	1
	−15	0.98	2.77	3
	−10	5.20 (4.50–5.72)	11.31 (9.30–17.24)	12
	−5	35.29 (23.85–47.53)	134.7 (85.28–441.3)	96
	0	105.9 (96.16–115.4)	244.6 (216.7–286.2)	288
	5	112.9 (85.04–139.4)	409.1 (327.5–556.9)	336
Acclimated larval [b]	−20	—	—	1
	−15	2.54	7.17	6
	−10	10.90 (8.57–12.47)	23.71 (20.05–33.21)	24
	−5	155.7 (115.9–181.9)	375.8 (295.0–695.0)	504
	0	492.2 (448.5–532.7)	1,137 (1,020–1,309)	1,176
	5	517.8 (431.7–609.1)	1,877 (1,441–2,807)	1,680
Pupal	−20	—	—	1
	−15	1.16 (1.06–1.28)	1.44 (1.30–1.59)	2
	−10	2.44 (2.28–2.59)	4.05 (3.73–4.59)	4
	−5	3.00 (0.65–4.99)	30.86 (19.95–108.9)	48
	0	153.5 (127.4–171.2)	306.3 (258.7–442.2)	288
	5	365.1 (323.4–397.0)	759.2 (671.0–928.4)	840
Adult	−20	—	—	1
	−15	0.64 (0.55–0.75)	1.00 (0.87–1.15)	2
	−10	1.21 (1.14–1.29)	1.47 (1.38–1.57)	2
	−5	25.84 (22.92–28.36)	76.21 (63.87–99.76)	72
	0	126.8 (119.6–133.8)	283.9 (255.6–325.3)	288
	5	457.6 (437.5–479.5)	800.5 (738.2–893.2)	840

[a] Minimum exposure time observed to kill all of the tested insects.
[b] Larvae were exposed to 15°C for 1–3 mo before exposure to each low temperature.

most cold-susceptible stage in the life cycle. At 20°C, most eggs hatched normally within 4 wk, but all eggs died within 6 wk at temperatures less than 18°C. This result agrees well with those of Howe (1957), but not with those of Fletcher and Long (1976). The latter reported that ca. 1% at 15.6°C and ca. 60% at 18.3°C hatched, respectively, in 23–30 d and in 17–25 d. This discrepancy may result from those studies' different experimental procedures, the accuracy of temperature control and measurement, and the insect strains used. Disregarding the suggestion by Fields (1992) that test in-

Table 2. Mortality of eggs exposed to 'moderately low' temperature[a]

Temperature (°C)	Exposure time (wk)				
	1	2	3	4	6
16	10.2	19.3	28.4	87.5	100
18	0	11.4	26.1	90.9	100
20	3.4	4.5	6.7	7.9	—[b]

[a] Mortality (%) corrected according to the method of Abbott (1925).
[b] All viable eggs hatched normally within 4 wk.

sects should be from stocks cultured within 2 yr after field collection, this study used laboratory stocks that had been maintained for a decade. No information exists regarding variation in susceptibility to low temperatures between laboratory strains of the cigarette beetle, but it is necessary to apply field populations for validation before practical application.

Mortality in insects is dependent upon the final temperature and the cooling rate: a more gradual cooling rate may require a longer exposure time to achieve the same mortality because of elevated tolerance through acclimation (Mason and Strait, 1998). Larvae inhabiting the center of cased tobacco are likely to experience gradual changes of temperature during cooling procedures. Therefore, exposure to eliminate the acclimated larvae may be necessary to assure disinfestation of cased tobacco. Freezing temperatures of less than −10°C are required to disinfect tobacco as rapidly as phosphine fumigation. Our preliminary experiment indicated 86 h for the temperature at the center of cased fluecured tobacco (200 kg in a 73×110×72 cm cardboard case; 7°C initial temperature) to reach −10°C in a −25°C freezing container, whereas Rassman (1980) described 11 h to cool a 90×50×50 cm tobacco bale from 20°C to −12°C in a −20°C room.

Application of low suboptimal temperatures (13–20°C) can slow the rate of damage from mold and insects, thereby increasing the storage time of commodities even if infestation is not eliminated, possibly preventing further damage (Mason and Strait, 1998). Our results indicate that the reproductive cycle of the cigarette beetle can be blocked at 18°C. At that temperature, the stored tobacco will never be infested, even if eggs are deposited by invading adults. In general, cased tobacco undergoes winter conditions at least once in warehouses before being made into cigarettes, during which time insects are exposed to lethally low temperatures. That is, when tobacco is stored at 5°C for 3 mo in the winter and is then kept at <18°C throughout the rest of the year, such wintered tobacco would become and remain pest-free without any chemical control. This condition is achievable with cooling facilities that are commonly used for rice warehouses in Japan, in which temperatures are maintained at <15°C year-round (Nakakita and Ikenaga, 1997). This study gave no consideration to economic aspects, but it is inferred that low-temperature storage may present a feasible alternative to chemical fumigation of stored tobacco.

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