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The effect of sub-zero temperatures on different lifestages of *Lasioderma serricorne* (F.) and *Ephestia elutella* (Hübner)

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ABSTRACT

The effect of sub-zero temperatures on different lifestages of *Lasioderma serricorne* and *Ephestia elutella* was investigated as a means of disinfesting stored tobacco. Eggs, unacclimated cocoons and acclimated cocoons of *L. serricorne* were exposed to −10 °C, −15 °C and −20 °C in insulated boxes. There was no adult emergence from eggs or unacclimated cocoons following exposure to the respective temperatures for 4 h, 2 h and 1 h. With acclimated cocoons there was no adult emergence after 2 h at −15 °C and 1 h at −20 °C, but at −10 °C, there was adult emergence after 8, 12 and 24 h exposures.

In field-scale experiments, cold acclimated fourth-instar larvae of *L. serricorne* were inserted into cases of leaf tobacco and boxes of finished product, put into commercial freezers and exposed to minimum temperatures of −10 °C, −18 °C or −25 °C. Critical temperatures were measured at the core of the commodity. No adults emerged from the commodity when exposed to at least −18 °C for periods ranging between 3.75 h and 39.25 h or when exposed to at least −25 °C for between 2.4 h and 3.7 h. At a minimum of −10 °C, 3 live adults emerged after 24 h exposure.

With *E. elutella*, diapausing larvae were inserted into small scale tobacco bales and exposed to −10 °C, −13 °C, −15 °C or −20 °C. No emergence of adults and no larval survival was achieved after 21 d, 3 d and 2 h exposure at −10 °C, −15 °C and −20 °C respectively. At −13 °C, there was no adult emergence after 2 and 5 d exposure, but live larvae remained after 24 weeks incubation at 25 °C.

Minimum conditions of −18 °C for 24 h and −25 °C for 4 h are recommended for the control of *L. serricorne* and −20 °C for 24 h for the control of *E. elutella* in stored tobacco (to fit with operational logistics).

1. Introduction

Lasioderma serricorne (F.) (the cigarette beetle) and *Ephestia elutella* (Hübner) (the warehouse, tobacco or cacao moth) are the most destructive pests of stored tobacco, infesting the commodity during storage, manufacture and at retail outlets. Damage is primarily caused by larval feeding, with moth larvae being heavier feeders than beetle larvae (Anonymous, 1995). Product contamination with excreta, frass and webbing also occurs. It is conservatively estimated that the tobacco industry loses approximately US $300 million worth of tobacco lamina annually due to infestations caused by both pests (Blanc et al., 2002).

Fumigation (for treating tobacco) and contact pesticides (for treating structural surfaces) have been the main chemical control tools used for insect control in stored tobacco. Increasing concerns over the use of toxic compounds, linked to health and environmental fears, as well as the ineffectiveness of fumigations below 16 °C and the development of resistant insect populations (Zettler and Keever, 1994; Savvidou et al., 2003), have fuelled the need to find alternative control methods.

The use of low temperatures for the control of storage pests is well documented and has been used to disinfest bulk tobacco and finished products (Runner, 1919; Swingle, 1938; Reed and Vinzant, 1942; Tenhet et al., 1957; Childs et al., 1968; Fletcher et al., 1973; Anonymous, 1995; Imai and Tsuchiya, 2007). However, it is often difficult to make comparisons between studies because of differences in acclimation, temperatures and exposure duration (Fields, 1992).

Five days at below −20 °C has previously been used to treat samples and manufactured tobacco resulting in 100% mortality of all stages of *L. serricorne* and *E. elutella* (Anonymous, 1995). Twenty-four hours at −20 °C was found to be sufficient, but the additional 4 days was suggested to err on the side of caution and ensure kill of any diapausing (overwintering) stages (Anonymous, 1995). This

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prolonged exposure time may be an over-estimate of what is actually required. Unpublished information suggests the use of 48-h exposures at −21 °C (once core temperature had reached −21 °C) for the treatment of tobacco. Any treatment time that is over and beyond what is actually required could have serious economic and financial impacts due to the treatment costs.

It is known that insects acclimated to low temperatures are more cold tolerant, which can result in an increase in survival time (Bell, 1991; Fields, 1992; Burks et al., 2000). Cold acclimation has the potential to increase survival time by up to ten-fold at low temperatures, depending on the rate of temperature fall (Fields, 1992; Imai and Harada, 2006). Insects developing in the centre of packaged commodities are likely to experience gradual changes in temperature during the cooling process, compared to those nearer the surface. Larvae of *L. serricorne* can penetrate deeply into loosely packaged commodities, but remain in the peripheral 50 mm in tightly packed tobacco strips, whereas *E. elutella* can burrow into tobacco, begin feeding and remain unseen for 2–3 months (Anonymous, 1995).

Different strains are also known to vary in their responses and strains reared in the laboratory can be less tolerant to extreme temperatures than field strains (Fields, 1992). The geographical origin of strains, together with the number of generations reared in the laboratory before testing can affect cold tolerance, and studies on laboratory strains often underestimate cold tolerance (Fields and Muir, 1995).

Lasioderma serricorne is a pest of economic importance infesting many materials in most areas with tropical and subtropical climates, but its survival in cold climates is dependent upon entry into warm buildings. Larval activity ceases when the temperature falls below 15 °C and large larvae can remain dormant for many months and may overwinter in this stage in cool climates (Runner, 1919; Howe, 1957). Laboratory studies have shown that immature stages of *L. serricorne* are more tolerant of the cold than adults (Swingle, 1938; Mullen and Arbogast, 1979; Rassmann, 1980). Insects that annually face low winter temperatures tend to stop growing at a moderately low temperature when they reach the stage most resistant to cold (Howe and Hole, 1968), with development resuming as the temperature increases in the spring (Runner, 1919; Howe, 1957). Childs et al. (1968) reported that live larvae found infesting hogsheads of tobacco stored at a low temperature over an extended period, were usually in the fourth instar.

Ephestia elutella has a higher level of cold tolerance than *L. serricorne*. The species originated in temperate regions in the northern hemisphere, but has now spread through trade to temperate zones in the southern hemisphere (Cox and Bell, 1991). It is rarely found in the tropics because it cannot tolerate long exposures to high temperatures, but is well adapted to cooler climates with a developmental range of 10–30 °C (Bell, 1975). It is further able to survive cold temperatures by the ability of fully-grown larvae to enter diapause in response to short day lengths and low temperatures (Bell, 1983). Diapause is a state of dormancy that allows insects to survive adverse conditions, such as extreme temperatures, with development resuming when conditions improve. At 20 °C and below there is a strong tendency to enter diapause, but this also occurs at higher temperatures if the day length falls below 14 h (Bell, 1976, 1991). The occurrence of diapause greatly prolongs the developmental period, which can last up to 9 months in northern Europe (Bell, 1983).

Previous preliminary experiments (Collins et al., 2006) investigated the effects of sub-zero temperatures (−10 °C, −15 °C and −20 °C) on the eggs and diapausing larvae of *E. elutella* when exposed to test temperatures in insulated boxes. The diapausing larvae were more cold tolerant than the eggs, with days, as opposed to hours, of exposure required to produce complete mortality. Complete mortality of eggs was achieved after 1 and 7 h at −15 °C and −10 °C respectively, whereas complete mortality of diapausing larvae was achieved after 1, 3 and 22 d at −20 °C, −15 °C and −10 °C, respectively.

The aim of these experiments was to provide a more realistic practical scenario. For *E. elutella*, tests in small bales of tobacco were based on the results from the previous laboratory experiments (Collins et al., 2006), while for *L. serricorne*, field-scale tests in leaf tobacco cases and finished product followed exploratory laboratory tests at −10, −15 and −20 °C in insulated boxes. The most cold-tolerant stages (diapausing larvae of *E. elutella* and eggs and cocoons of *L. serricorne*, the latter containing late fourth-instar larvae and pupae) were tested at various temperatures below −10 °C in order to provide practical recommendations for the disinfestation of stored tobacco by freezing.

2. Methods

2.1. Laboratory experiments on L. serricorne

Two strains of *L. serricorne* were assessed. Strain Cor10 is a phosphine-susceptible strain which was collected in Australia in 1995, and strain SAIII is a phosphine-resistant strain collected in South Africa in 2006. Since arrival at the laboratory, both strains have been reared on a diet of wheatfeed and yeast in conditions of 25 ± 2 °C and 60 ± 5% relative humidity (r.h.), with a 15:9 h (L:D) lighting regime. For the purposes of these experiments, Cor10 was kept on ground tobacco throughout the experimental period (to provide a more realistic situation), whereas SAIII was maintained on the laboratory diet (due to poor development on tobacco).

Fifty 0–3 d old eggs were placed in glass jars (60 × 65 mm) containing 5 g of ground tobacco or laboratory diet. A further 5 g of the corresponding food was then added to the jars. Nine jars were prepared for each test temperature with three replicates for each exposure period and an additional five replicates for the controls. Each jar was covered with nylon mesh held in place with a screw top lid with its middle removed.

The unacclimated cocoons (containing late fourth-instar larvae and pupae) were prepared as above but were incubated in conditions of 25 ± 2 °C and 60 ± 5% r.h. until the correct age was reached. To obtain the acclimated cocoons (containing late fourth-instar larvae and pupae), once the correct age had been reached, the jars were transferred to conditions of 15 ± 2 °C and 80 ± 5% r.h. for at least one month before treatment.

In order to slow the rate of cooling to simulate a practical situation, the treatment jars were placed in boxes (220 × 100 × 100 mm) made from domestic insulating material (Thermawrap™, 4 mm thick and equivalent to 65 mm of polystyrene). A thermocouple (Type-T with a beaded tip and PTFE insulation (−50 to +250 °C)) was inserted into the middle jar, which was connected to a temperature recorder (MobileCorder Model MV230, Yokogawa Martron Ltd., Wooburn Green, U.K.). Another layer of insulating material was then placed around the initial layer.

The insects inside the boxes were cooled and exposed to the test temperatures using a combination of controlled environment (CE) rooms, environmental cabinets (MLR-350H, Sanyo Gallenkamp plc, Loughborough, Leics, UK) and freezers. There was a gradual decrease in temperature in 5 °C steps at 12 h intervals, until the test temperature was reached. Following the required exposure period, the boxes were returned to 25 °C in 5 °C steps at 12 h intervals.

There were three exposure periods at each test temperature. At −10 °C: 4, 8 and 12 h for eggs and unacclimated cocoons and 8, 12 and 24 h for acclimated cocoons; at −15 °C: 2, 4 and 6 h for all