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The effect of sub-zero temperature on the mortality of *Ephestia elutella* (Hübner)

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Abstract

The effects of sub-zero temperature (-10, -15 and –20 °C) on the most cold tolerant stages (eggs and diapausing larvae) of *Ephestia elutella* were assessed in the laboratory as alternatives to the use of chemical control methods for the disinfestation of stored tobacco. Test insects were exposed for five exposure periods at each test temperature following a gradual acclimation to simulate practical freezing of a tobacco bale. Mortality was evaluated after a gradual return to and incubation at 25 °C. Eggs were less cold tolerant than diapausing larvae with hours of exposure being required to produce complete mortality as opposed to days. Complete mortality of eggs was achieved after 1 and 7 hours at -15 °C and -10°C respectively, whereas complete mortality of diapausing larvae was achieved after 1, 3 and 22 days at -20 °C, -15 °C and -10 °C respectively.

Key words: Ephestia elutella; sub-zero temperatures; cold tolerance; tobacco storage.

Introduction

The warehouse moth, (also known as the tobacco or cacao moth) *Ephestia elutella* (Hübner), is a major pest of stored products, particularly in warehouses in temperate regions. The larvae feed on a wide range of commodities, including tobacco. As well as the physical damage caused by feeding, the larvae contaminate commodities with excreta and produce large amounts of silk webbing that bind food particles together and can clog processing machinery.

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 temperature, but is well adapted to cooler climates with a developmental range of 10-30 °C (Bell, 1975). It is further able to survive cold temperature by the ability of fully-grown larvae to enter diapause in response to short day lengths and low temperature (Bell, 1983). Diapause is a state of dormancy that allows insects to survive adverse conditions, such as extreme temperature, 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 temperature if the day length falls below 14 hours (Bell, 1976, 1991). The occurrence of diapause greatly prolongs the developmental period and can last up to nine months in northern Europe (Bell, 1983). Eggs are also tolerant of the cold with 1-2 day old eggs less tolerant than those aged 0-1 and 2-3 days old (Bell, 1975; Bovingdon, 1933).

Insect control in stored tobacco has relied on the use of fumigation and contact pesticides, however increasing concerns over the use of toxic

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compounds, linked to health and environmental fears, as well as the ineffectiveness of fumigations below 16 °C and the development of resistant populations, has fuelled the need to find alternative control methods. The use of sub-zero temperature to freeze the commodity is one option and has been used to disinfest tobacco (ICPT, 1995). Five days at -20 or -30 °C has been used to treat samples and manufactured tobacco resulting in 100% mortality of all stages of *E. elutella* and *Lasioderma serricorne* (ICPT, 1995). However, this prolonged exposure time may be an over-estimate of what is actually required to produce complete mortality. Unpublished information suggests the use of 48-hour exposures at –21 °C for the treatment of tobacco might suffice.

A literature review, commissioned by the CORESTA sub-group on pest and sanitation management in stored tobacco, investigated the use of extreme temperature to control *E. elutella*. Little data was available on the use of sub-zero temperatures, with no information at -20 °C, the temperature generally encountered during commercial freezing. The review also highlighted difficulties interpreting data from different insect strains and methodologies. Bell (1991) found that it took 28 days at -10 °C to completely kill diapausing larvae of a laboratory strain that had been acclimated on a weekly regime, whereas it took Bovingdon (1933) only 6 hours to kill all larvae, when the diapause status was not stated and there was no acclimation. Against eggs, Bovingdon (1933) found that none hatched after 18 and 12 hours exposure to -10 to -11 °C and -15 to -16 °C respectively, but again there was no acclimation.

It is known that carefully phased acclimation procedures can greatly increase the cold tolerance of insects (Fields, 1992; Bell, 1991; Burks et al., 2000). Cold acclimation has the potential to reduce the effectiveness of low temperature which can result in an up to ten-fold increase in survival time, depending on the rate of temperature fall (Fields, 1992). Insects developing in the centre of large bales of tobacco are likely to experience gradual changes in temperature during the cooling process, compared to those nearer the surface. Data supplied by B.A.T., South Africa has shown that it takes approximately 5 days for a case of lamina to reach –15 °C from +15 °C and another day to reach –20°C, with 6 days required to return to +15 °C.

The aim of these experiments was to determine the exposure periods required to kill two cold tolerant stages (eggs and diapausing larvae) of *E. elutella* at sub-zero temperature. Two relatively recently isolated strains of moths were compared, as it has been shown that different strains vary in their responses and laboratory strains can be less tolerant than field strains. 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). Two temperature levels were initially proposed, -20 °C and -15 °C, the first the temperature at which commercial freezers operate and the second to investigate if the total treatment time could be reduced by starting the exposure period before the tobacco reached -20 °C. However, an additional temperature of -10 °C was also assessed in response to initial findings. Most of the treatment time in freezing tobacco bales consists of the time taken for the tobacco to be cooled to the target temperature and then returned to ambient conditions. Therefore any reduction in the minima temperature would have a marked impact on total treatment time. The aim of this study was to make a more realistic efficacy assessment of the impact of the modified freezing treatments on insects, than is currently possible using existing data sources.

Materials and Methods

Test insects

Two strains of *E. elutella* (E4P and E50) were used, originating from warehouse infestations in mid-2000 and mid-2001 respectively. The strains

were reared in the laboratory on a diet of wheatfeed, yeast and glycerol (10:1:2) in conditions of 25 ± 2 °C and 60 ± 5 % rh with a 15 h light and 9 h dark light regime. For the egg tests, three batches of 10 tubes (75 x 25 mm, half-filled with laboratory diet) each containing 5 eggs (approximately 1-3 days old), were prepared for each strain and exposure period including controls. For the diapausing larvae tests, 2 batches of 15 tubes (75 x 25 mm, half-filled with laboratory diet) each containing 2 diapausing larvae (14 weeks after hatch) were prepared per strain and exposure period.

Experimental apparatus

In order to acclimate the insects gradually at a realistic rate, the eggs and larvae were exposed to the test temperatures in the glass tubes contained within boxes made from domestic insulating material (Thermawrap). The material was approximately 3 mm thick but equivalent to 65 mm of polystyrene. Two sets of boxes were constructed. In the first set the bottoms of the boxes measured approximately 32.5 x 15 x 9 cm and the tops measured approximately 33 x 15.5 x 9 cm. The tops were constructed so that they fitted closely over the bottoms. The second set of tops and bottoms were constructed to be slightly bigger than the previous set so that the first box fitted inside the second. This meant that four layers of insulating material at the sides and two layers at the top and bottom insulated the test insects. This allowed for a gradual acclimation to the target temperatures at a slower rate than would occur if the tubes were merely put into the test conditions unprotected.

Experimental set up

The diapausing larvae and eggs were assessed separately. The required numbers of tubes of each strain were placed into the experimental insulation boxes. Batches were separated from each other by a piece of insulation material. Six boxes were prepared for each experiment with one box for each of the 5 exposure periods, together with a control. An additional tube with food was placed into the centre of each exposure box and a thermocouple (Type-T with a beaded tip and PTFE insulation (-50 to +250 °C)) placed into the food. This was to monitor the temperature inside the boxes and to determine when the test temperature was reached and exposures began. A thermocouple was also attached to the outside of one of the boxes to record the interior temperature of the environmental cabinet and freezers. The thermocouples were connected to a chart recorder (MobileCorder Model MV230, Yokogawa Martron Ltd., Wooburn Green, U.K.) and the temperatures of the tubes were recorded continuously throughout the exposure period. The boxes containing the tubes were then sealed with insulation tape and were ready to commence the acclimation regime.

Acclimation

The insects were acclimated and exposed to the test temperatures by using a combination of CE rooms, environmental cabinets (MLR-350H, Sanyo Gallenkamp plc, Loughborough, Leics, UK) and freezers. The acclimation regime was selected so as to mimic as closely as possible the cooling and subsequent return to ambient conditions of tobacco in a practical situation and was as follows +20 °C (24 hours) → +15 °C (24 hours) → +10 °C (24 hours) → +5 °C (24 hours) → 0 °C (12 hours) → -5 °C (12 hours) → -10 °C (exposures started when –10 °C was reached or insects were acclimated for a further 24 hours) → -15 °C (exposures started when - 15 °C was reached or insects were acclimated for a further 24 hours) → -20 °C (exposures started when –20 °C was reached) → -15 °C (24 hours) → -10 °C (12 hours) → -5 °C (12 hours) → 0 °C (12 hours) → +5 °C (24 hours) → +10 °C (24 hours) → +15 °C (24 hours) → +20 °C (24 hours). Following acclimation, exposures and return to 20 °C, the boxes were opened, tubes removed and placed into a CE room at 25 ± 2 °C and 60 ± 5 % rh with a 15 h : 9 h light regime in order to break diapause or induce egg hatch.

The boxes containing insects which acted as controls, were not acclimated, as the decline in temperature may have affected mortality, but were put immediately into the CE room at 25 ± 2 °C and 60 ± 5 % rh with a 15 h : 9 h light regime. These boxes were opened and the tubes were removed when the first cold exposed insects were put into the room.

Exposure periods

There were 5 exposure periods at each test temperature. Exposures commenced once the target temperature had been reached (± 0.5 °C).

For the egg experiments at -10 °C, exposure periods of 2, 4, 6, 8 and 24 hours were assessed with 1, 3, 5, 9 and 24 hours assessed at -15 °C. In the first test at -10 °C, the insulated boxes took varying amounts of times to reach -10 °C, depending on their shelf position in the environmental cabinet and it was therefore difficult to assess when the exposures started. As a result, the experiments at 2, 4 and 6 hours were repeated. However, due to insufficient numbers of eggs, only 2 batches of 10 tubes were prepared per strain per exposure period. There were no experiments at -20 °C because the acclimation regime down to -20 °C included a 24-hour period at -15 °C, which our results indicated would have killed the eggs before reaching the target temperature.

For the diapausing larvae experiments, exposure periods of 7, 10, 14, 22 and 28 days; 2, 3, 6, 10 and 14 days and 1, 2, 3, 4 and 7 days were assessed at -10 °C, -15 °C and -20 °C respectively.

Assessments

The eggs and diapausing larvae were assessed on a weekly basis after at least 7 weeks and 4 weeks incubation at 25 °C respectively. Assessments continued well beyond the time when the control insects had emerged as adults, in case the cold had affected development time. The tubes were inspected visually and physically and the numbers of larvae, pupae and adults in each tube were counted and mortality calculated.

Results and discussion

The intended and actual temperatures achieved for each experiment are shown in Table 1, together with the exposure periods. In all the experiments the required temperatures were achieved with means ranging to within ±1.5 °C of the target temperature (Table 1). Unavoidable larger temperature fluctuations did occur during the longer exposure periods, which were due to variations within the cabinets and freezers e.g. due to the defrosting procedure. However the defrosting procedure only disrupted the temperatures for about 3 hours a day. In general the intended exposure periods were achieved, but in some instances slightly shorter or longer exposures were assessed due to difficulties in determining precisely when the target temperature had been reached (Table 1). However, these variations did not have any significant impact on the study's results or its interpretation and did not affect the aim which was to determine the minimal exposure periods required to provide complete mortality of each stage.

Egg experiments

In experiments at -10 °C, there was 73 and 61 % adult emergence of strains E50 and E4P respectively in the controls (Table 2). Complete mortality of each strain was achieved after 2, 7, 8 and 27 hours exposure, however 1 live pupa of strain E50 was observed following 4 hours exposure at -10 °C and 6 weeks incubation at 25 °C (Table 2). The reason why there should be survival at 4 hours and not at 2 hours is unknown, but may be due to age, as it is known that 0-1 and 2-3 days old eggs are more cold tolerant than those aged 1-2 days old (Bell, 1975). Bovingdon (1933) found the last hatch of 12-hour old eggs occurred after 17.9 hours exposure at -10 to –11 °C, whereas the last hatch of 36-hour old eggs occurred after 5.4 hours; no eggs of any age hatched after 18 hours exposure. Therefore it may be that the survivor in these experiments originated from an egg that was slightly older or younger than the majority of those tested.

In experiments at -15 ºC, there was 77 and 73 % adult emergence of E50 and E4P respectively in the controls and complete mortality of each strain was achieved at all exposure periods (Table 2). It was found that 7 hours at -10 ºC was sufficient to kill all eggs. As part of the acclimation regime at -15 ºC, the eggs were exposed to -10 ºC for 24 hours before going down to -15 ºC, so it is likely that the acclimation at -10 ºC produced 100% mortality before -15 ºC was reached. Bovingdon (1933) found that 5.2 % of young eggs hatched at between 1 and 4.9 hours exposure at -15 to -16 ºC, with no eggs hatching after 12 hours.

Diapausing larvae experiments

In experiments at -10 ºC, there was 89 and 98 % adult emergence of strains E50 and E4P respectively in the controls with the majority of emergence occurring within 7 weeks incubation at 25 ºC (Table 2). Complete mortality of E4P was achieved after 14 days exposure to -10 ºC with 22 days required for strain E50 (Table 2). The majority of survivors at the shorter exposure periods were still in the larval stage, even after 10 weeks incubation at 25 ºC. These larvae appeared to still be in diapause, moved very little and appeared not to feed even though conditions

Table 1. Intended and actual temperature and exposure periods achieved.

Target temperature (± 0.5ºC)	Test stage	Intended duration at target temperature	Actual duration at target temperature	Mean temperature temperature exposure at target temperature (ºC)	Temperature range during exposure during at target Temperature (ºC)
-10	Eggs	2 hours	2.2 hours	-10.1	-9.7 to -10.4
		4 hours	3.8 hours	-10.2	-9.5 to -10.9
		6 hours	6.8 hours	-10	-9.7 to -10.6
		8 hours	8.3 hours	-10.6	-9.7 to -11.1
		24 hours	27.5 hours	-9.9	-7.1 to -10.5
	Diapausing larvae	7 days	7 days	-10	-6.3 to -11.9
		10 days	9.6 days	-10	-6.8 to -12.5
		14 days	13.8 days	-10.1	-6.2 to -13.6
		22 days	21.7 days	-10.4	-5.1 to -14.3
		28 days	27.3 days	-10.2	-6 to -15.2
-15	Eggs	1 hour	1 hour	-15.2	-14.5 to -16
		3 hours	2.6 hours	-15.2	-14.6 to -15.5
		5 hours	3.9 hours	-14.9	-14.6 to -15.4
		9 hours	9.4 hours	-14.8	-14.5 to -15.2
		24 hours	24.1 hours	-16.5	-14.9 to -17.4
	Diapausing larvae	2 days	1.9 days	-15.9	-14.6 to -16.25
		3 days	2.9 days	-15.7	-12.2 to -16.8
		6 days	6.1 days	-15.9	-12.2 to -17.8
		10 days	6.8 days	-16.3	-14 to -17.7
		14 days	13.5 days	-15.4	-12 to -16.7
-20	Diapausing larvae	1 day	0.6 days	-19.5	-19.4 to -19.7
		2 days	1.6 days	-19	-18.3 to -19.9
		3 days	1.8 days	-20.5	-18.8 to -21.7
		4 days	4 days	-19.8	-18.4 to -21.7
		7 days	6.6 days	-20	-18.6 to -21.1

were suitable to break diapause (Bell, 1983). It is likely that these larvae would eventually die (see experiments at -15 °C) due to starvation.

Bovingdon (1933) found complete mortality of larvae after 5.8 hours at -10 to -11 °C, however, there was no acclimation and the diapause status was not mentioned. It is likely that Bovingdon tested larvae that were mostly too young to have entered diapause (Bell, 1991). In contrast, Bell (1991) found survival after one and two weeks at -10 °C with 4 weeks treatment being required to achieve 100 % mortality of diapausing larvae that had been acclimated on a weekly regime.

In experiments at -15 °C, there was 90 and 94 % adult emergence of E50 and E4P respectively in the controls which occurred generally within 7 weeks incubation at 25 °C (Table 2). Complete mortality was achieved after 3 days exposure to -15 °C (Table 2). After 2 days exposure, the majority of larvae were dead after 4 weeks incubation at 25 °C. However, one larva of E50 and 3 larvae of E4P were still alive after 10 weeks, with one larva of each strain alive after 14 weeks incubation. These larvae appeared to still be in diapause, moved very little and appeared not to feed even though conditions were suitable to break diapause of larvae exposed to -10 °C (Bell, 1983). It may be that higher temperatures and longer day lengths are required to reactivate diapausing larvae held at temperatures lower than -10 °C, although there is no information available. The optimum low temperature for reactivation of diapausing larvae returned to 20 °C is 12.5 °C but for those returned to 25 °C and long days, it is 7.5 °C (Bell, 1983, 1989). The larvae did eventually die presumably due to internal factors such as weight loss. Bovingdon (1933) found complete mortality of larvae after 1 hour at -15 to -16 °C, however again there was no acclimation and the diapause status was uncertain.

In experiments at -20 °C, there was 95 and 97 % adult emergence of E50 and E4P respectively in the controls, with complete mortality at all exposure periods (Table 2).

Conclusions

These experiments have shown that the diapausing larvae are more cold tolerant than the eggs of *E. elutella*, with days of exposure

Table 2. Mean % mortality of each stage at each temperature and exposure period.

Target temperature (°C)	Test stage	Strain	Mean % control adult emergence	Mean % mortality at each exposure period				
-10	Eggs			2 hours	4 hours	7 hours	8 hours	28 hours
		E50	73	100	99	100	100	100
		E4P	61	100	100	100	100	100
				7 days	10 days	14 days	22 days	27 days
	Diapausing	E50	89	80.8	81.3	95.5	100	100
	larvae	E4P	98	84.2	95	100	100	100
-15	Eggs			1 hour	3 hours	4 hours	9 hours	24 hours
		E50	77	100	100	100	100	100
		E4P	73	100	100	100	100	100
				2 days	3 days	6 days	7 days	14 days
	Diapausing	E50	90	98.3	100	100	100	100
	larvae	E4P	94	98.4	100	100	100	100
-20	Diapausing			1 day	1.6 days	1.8 days	4 days	7 days
	larvae	E50	95	100	100	100	100	100
		E4P	97	100	100	100	100	100

required for complete mortality, compared to hours. Complete mortality of eggs was achieved after 1 and 7 hours at -15 °C and -10 °C respectively, whereas complete mortality of diapausing larvae was achieved after 1, 3 and 22 days at -20 °C, -15 °C and -10 °C respectively. In the field, tobacco samples have been treated for 5 days at -20 °C, however, the current experiments have shown that 24 hours at -20 °C or 3 days at -15 °C is sufficient. Any reduction in treatment temperatures and exposure periods will reduce the amount of time and costs required treat tobacco.

In these experiments the temperatures within the tubes were continuously monitored so that an accurate temperature profile could be determined. The use of such thorough recording is unlikely to have been used in previous work and fluctuations within experimental apparatus may not have been previously detected. In the field, the accuracy of the temperatures achieved will be dependant on the performance of the freezer. It is therefore vital that temperatures are recorded continuously and accurately so that it is known when and for how long the tobacco has been at the target temperature.

Although these experiments attempted to simulate the realistic conditions experienced during the freezing of a tobacco bale, the speed of cooling will be dependant on the actual size and volume of bale to be frozen. Larger bales may take longer to reach the target temperatures, which may affect the acclimation and survival of any moths contained within. It is also important to monitor the temperature at the centre of the bale as this will take the longest time to reach the desired temperature. The rate of cooling can be increased by the use of air circulation. Rassmann (1980) describes the cooling of tobacco bales by air circulation from 20 to -12 °C in 11 hours for the control of the cigarette beetle.

It is also important to consider that, although the strains used in these experiments were relatively recently collected from the field, they had been in laboratory culture for a few years. Any strains encountered in the field may be more or less cold tolerant than those assessed in these experiments and therefore may require longer or shorter exposure periods.

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