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The effect of high temperature on the mortality of *Lasioderma sericorne* (F.)

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Abstract

The effects of high temperature (45 and 50 °C) on the mortality of heat tolerant stages (eggs and cocoons) of *Lasioderma sericorne* (F.) were assessed in the laboratory as alternatives to the use of chemical control methods for the disinfestation of machinery in tobacco manufacturing premises. Insects were rapidly heated to each test temperature, exposed for seven exposure periods then rapidly cooled to 30 °C, to allow calculation of the lethal times (LT) for 50 % (LT_{50}) and 99 % (LT_{99}) mortality. At 45 °C in tubes, LT_{50} and LT_{99} values were 14.3 and 52 h and 13.4 and 50.3 h for the eggs and cocoons respectively, with no survivors after 54 h exposure. In jars at 45 °C, the LT_{50} and LT_{99} values for cocoons were 17.5 h and 67.5 h respectively with survivors at 48 h. At 50 °C in plastic dishes, LT_{50} and LT_{99} values were 3.4 and 10 hours and 1.4 and 6.6 hours for eggs and cocoons respectively, with no survival of cocoons after 12 h exposure. In tubes at 50 °C, the LT_{50} was 3.4 h and LT_{90} 8.6 h for eggs. The recommended treatment times for practical treatments are 72 h at 45 °C and 18 h at 50 °C.

Key words: *Lasioderma sericorne*; high temperatures; heat tolerance; tobacco storage.

Introduction

The use of high temperature for the control of storage pests is well documented and has been used to protect tobacco (Burks et al., 2000; ICPT, 1995). At extremely high temperature stored-product insects are killed. More moderate high temperature is less lethal, but can still prevent population increase and thus protect commodities.

Generally temperature from 38 °C upwards can kill the insect as long as the exposure period is sufficient (Howe, 1957). Literature suggests that *L. sericorne* is one of the most heat tolerant storage insects (Fields, 1992). This may be due in part to its developmental range. The upper temperature for development is 37 °C (Lefkovitch, 1967) and even a period of a week at 40 °C has been survived, as long as there was a return to normal developmental temperature, and this ability increased with larval age (Howe, 1957). Published literature indicates that 4th instar larvae and pupae of *L. sericorne* will be the most tolerant stages and that this tolerance is probably due to their pupal cells (Runner, 1919) as this adds a barrier between the pupae and the adverse environment which would delay heat gain and water loss. However, eggs have also been reported to be the most tolerant (Powell, 1931), which was supported by Adler (2002b) in experiments at 50 °C. However, this trend was reversed at 45 °C (Adler, 2002a). There have been other published works on heat with tobacco summarised in Howe (1957) with minimal insulation from tobacco with temperature ranging from 60-83 °C and exposure times giving 100 % mortality varying from 5 to 45 minutes. However,

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the times did not decrease with increasing temperature as would be expected. Further work has looked at infested tobacco bales and boxes with a range of temperature from 44 to 60°C and exposure times of 600 to 30 minutes required for 100% mortality (Howe, 1957; ICPT, 1995).

The aim of this study was to find the exposure times required to achieve complete mortality of the juvenile stages of *L. serricorne* at moderate temperature of 45 and 50 °C. This information will be used to assist with the heat treatment of tobacco processing machinery, which cannot withstand high temperature. This machinery requires disinfestation, as there is a build up in tobacco deposits within the machinery that would provide potential areas of harbourage for *L. serricorne*. For such large-scale operations it is recommended that a temperature of 50 °C should be maintained for 24 to 36 hours for effective control (Cabrera, 2005). To guarantee disinfestation, the time required to heat up to the target temperature must be added to the time necessary to kill the insects. The temperature must be carefully monitored so that the centre reaches the required temperature for required duration.

Materials and methods

Test Insect

A strain (Origin NSW, Australia) of *L. serricorne* with a high fecundity was used so that there would be no problems collecting the required numbers of eggs. The strain was kept on ground tobacco throughout the experimental period and was maintained in a controlled environment room at 30 ± 2 °C and 60 ± 5 % r.h. Eggs aged 0-2 and 2-4 days, IV instar larvae and cocoons were used in the tests.

Preparation of developmental stages

The eggs were placed separately in batches of 50 or 60 and the IV instar larvae and cocoons in batches of 30, in glass tubes (25 mm diameter x 75 mm high) on a layer of 0.5 g of sieved tobacco. A further layer of 0.5 g sieved tobacco was placed on top of them. Each tube was covered in nylon mesh held in place with a perforated plastic stopper. In a further experiment at 45 °C, the cocoons were brushed gently in batches of 50 or 60, into glass jars (60 mm diameter x 65 mm high) that contained 10 g of ground tobacco. Each jar was covered in nylon mesh that was held in place with a metal top with its middle removed.

Treatments

Preliminary tests were done to assess the effect of the presence of tobacco in the treatments with regard to the time taken to achieve the target temperature. The aim was to achieve rapid heating to the required temperature and rapid cooling after treatment to 30 °C, the lower end of the optimum temperature range for development. The tests demonstrated that the quantity of tobacco was an important factor in determining the rate at which a sample reaches the target temperature. Tobacco was present to provide some heat buffering for the insect, which would be provided by tobacco residues in the tobacco processing machinery. A variety of test containers were evaluated to provide a range of information. Plastic Petri dishes were found to have the best heat transfer, glass tubes were more practical experimentally and glass jars allowed for a larger tobacco mass to be assessed as may occur in different types of machinery.

Tests at 50 °C

The contents of each glass tube were transferred to a plastic Petri dish (50 mm diameter x 10 mm high with lid covered in nylon mesh (130 mm apertures)). Each dish had a thermocouple (Type-T with a beaded tip and PTFE insulation (-50 to +250 °C)) whose end was glued to the middle of floor of each Petri dish. There were three dishes for each treatment time and three controls. A shelf midway up the oven (420 mm from the floor) was used and a

position at the mid-left of the shelf gave the shortest time to the target temperature for the sample.

All three replicates were exposed at the same time. The temperature of the oven was ascertained prior to the input of the samples by a further Type-T thermocouple, which was attached to the oven shelf adjacent to where the dishes were placed. The temperature of the dishes and the oven were recorded continuously on a chart recorder (MobileCorder Model MV230, Yokogawa Martron Ltd., Wooburn Green, U.K.). The contents of each dish were returned into the tubes with the addition of nylon mesh tops for incubation at 30 ± 2 °C and 60 ± 5 % r.h. after heating. An additional 5 g of sieved tobacco was added to ensure sufficient food was present to allow the completion of development to adult and the tubes monitored for the emergence of adults.

The heat assessments were completed initially in a 225 l fan-assisted oven (Model IPR225.XX1.5, Sanyo Gallenkamp plc, Loughborough, Leics, UK). There were seven different exposure times (8.00, 5.40, 4.00, 2.50, 2.00, 1.25 and 1.00 h) with three replicates for each exposure. Three control replicates were also used and kept at the same conditions as the experimental tubes before treatment (25 ± 2 °C and 60 ± 5 % % r.h.) during heat exposure and then at 30 ± 2 °C and 60 ± 5 % r.h. after testing with the treated tubes.

Additional tests were required as there had been survival at 8 h in the first test and therefore it was necessary to extend the exposure time to 12 h to ensure 100 % mortality. A further set of tests were therefore carried out at this temperature using a 294 l versatile environmental cabinet (MLR-350H, Sanyo Gallenkamp plc, Loughborough, Leics, UK). There were seven different exposure times (12.00, 8.30, 6.00, 4.15, 3.00, 2.00 and 1.30 h) with four replicates for each exposure time and four controls. The tests in these chambers used glass tubes (25 mm diameter x 75 mm high) filled with 1 g of ground tobacco.

Tests at 45 °C

Three versatile environmental cabinets were used for these treatments to limit the number of times their doors had to be opened and thus better maintaining temperature. The additional units required extra chart recorders for temperature monitoring (Hybrid Recorder Model HR2300, Yokogawa Martron Ltd., Wooburn Green, U.K.). An initial experiment with beetle cocoons in glass jars (60 mm diameter x 65 mm high) was done at 45 °C using seven different exposure times (40, 28, 20, 14, 10, 7 and 5 h) with four replicates for each exposure time. As there was survival at 40 h, a further test was done with seven different exposure times (54, 38, 28, 20, 15, 10 and 7 h) with four replicates for each exposure time. These were done in glass tubes with 1 g of tobacco set up in the same way as for the experiments at 50 °C.

Data analysis for heat

A combined mean heating time to temperature, mean temperature during treatment and subsequent mean cooling time, was produced. The mortality data was plotted against each time interval using probit analysis software (Version 7a, Central Science Laboratory, York UK). This produced a straight-line relationship between time and mortality, where the former was converted to Log10 and the latter to probits. This allowed for the prediction of the exposure time required to achieve a certain percentage of mortality and calculation of the lethal time (LT) required to produce 50 % (LT_{50}) and 99 % (LT_{99}) mortality of each stage.

Results and discussion

Tests at 50 °C

From the initial results (Table 1) it appears that the eggs are the most tolerant stage with higher LT_{99} values. However there was emergence from cocoons and 2-4 day old eggs after 8 h, the highest exposure period.

In the follow-up experiment the exposure periods were increased to 12 h (Table 2). There was no statistical analysis for cocoon data as there were too few to allow more than three different exposure times, however there was survival at 8.5 h, but not at 12 h. Eggs aged 2-4 days had a slightly longer LT_{99} than in the initial experiments but the difference was not significant and there was survival at the highest exposure time of 12 h (Table 2). This indicates that eggs are the most tolerant stage and that it would advisable to use an exposure time of 18 h at 50 °C to ensure complete kill of all stages in a practical treatment.

The finding that the egg is the most tolerant stage is supported by Adler (2002b), however that work is unable to support other findings of this study as the highest time used was only 4.6 h with a mortality of ~50%. The only other study using a comparable temperature (49 °C) was by Kirkpatrick and Tilton (1972) but only adults were tested, with 65 % survival after 20 s exposure.

Tests at 45 °C

The initial test used 10 g of ground tobacco as a contrast to the initial tests at 50 °C to show the importance of the quantity of tobacco present. There was survival at the highest exposure time of 48 h (Table 3). In the next test, the original treatment vessels and 1 g of ground tobacco were used while the exposure time was increased to 54 h. There was no survival of either stage at the highest exposure time and there was little difference in tolerance between 0-2 day eggs and cocoons, with LT_{99}s of 52 and 50.3 h respectively (Table 4). This meant that there was no stage with overall tolerance and that it would be advisable to use an exposure time for practical treatments from the initial test to give a safety margin. Therefore an exposure time of 72 h should be used at 45 °C to ensure complete kill of all stages in a practical treatment. This is supported by data from a practical treatment of a tobacco factory containing bales treated using with ThermoNox heaters. Temperatures between 50-60 °C were achieved and held from 24-50 h, but there was still some survival (Cate et al., 2003).

Adler (2002a) achieved complete control of all stages within 40 h at 45 °C. Pupae and late larval stages within the cocoon were more heat tolerant than other stages. There were differences between the present study and Adler's (2002a) as the latter used a water bath with pre-heated exposure tubes. This would have ensured a more constant temperature and improved heat transfer. The choice of oven and heated cabinets for the present study ensured a more representative result for use in a practical treatment.

Table 1. The Lethal Times (Hours) after treatment of various stages in plastic dishes at 50 °C in an oven.

	Lethal Time				
Stage	LT_{50}	s.e.	LT_{99}		s.e
Egg 0-2 days	3.35	1.02	10.06	1.07	Survival at 5.40 h
Egg 2-4 days	3.12	1.03	7.99	1.08	Survival at 8.00 h
IV instar Larva	0.56	1.09	4.26	1.09	No survival at 5.40 h
Cocoon	1.40	1.03	6.56	1.07	Survival at 8.00 h

Table 2. The Lethal Times (Hours) after treatment as eggs (2-4 days) at 50 °C in glass tubes in a versatile environmental chamber.

	Lethal Time				
Stage	LT_{50}	s.e.	LT_{99}		s.e
Eggs 2-4 days	3.35	1.04	8.59	1.11	Survival at 12 h

Table 3. The Lethal Times (Hours) after treatment as cocoons at 45°C in glass jars in a versatile environmental chamber.

	Lethal Time				
Stage	LT_{50}	s.e.	LT_{99}	s.e	
Cocoon	17.53	1.03	67.46	1.07	Survival at 48 h

Table 4. The Lethal Times (Hours) for after treatment of various stages at 45°C in glass tubes in a versatile environmental chamber.

	Lethal Time				
Stage	LT_{50}	s.e.	LT_{99}	s.e	
Egg 0-2 days	14.34	1.07	52.00	1.18	No survival at 54 h
Egg 2-4 days	15.04	1.05	34.22	1.07	No survival at 40 h
Cocoon	13.44	1.02	50.30	1.06	No survival at 54 h

Conclusions

The results of these experiments have shown that the recommended exposure times required to achieve complete mortality of the eggs and cocoons of *L. serricorne* are 72h at 45 °C and 18h at 50 °C. Previous recommendations suggest that a temperature of 50 °C should be maintained for 24 to 36 hours for effective control (Cabrera, 2005). The current results, however, suggest that this may be an over-estimate and that the exposure period could be reduced, thereby saving on treatment time and costs.

These results form an initial laboratory assessment of the impact of this heat treatment and field studies may have to undertaken to assess how, for example, the heat capacity of the machinery for treatment and the residual tobacco will buffer the treatment's impact on *L. serricorne*. The volume of machinery will differ and therefore the heating time to reach the target temperature will vary. It will therefore be very important to monitor the temperature of all areas within the treatment to ensure that the exposure time is associated with the area with the slowest heating. It is also important to note that any strains encountered in the field may be more or less heat tolerant than that assessed in these experiments and may therefore require longer or shorter exposure periods. The effect of heat treatments on a strain that is exposed to naturally high temperatures should be assessed in the future, to determine any heat tolerance effects.

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