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Efficacy of heat against the mediterranean flour moth *Ephestia kuehniella* and methods to test the efficacy of a treatment in a flour mill

***C.S. Adler*[1]**

Abstract

The Mediterranean flour moth *Ephestia kuehniella* is a typical pest in German flour mills and was tested in laboratory experiments at 45 °C, 50 °C and 55 °C for the efficacy of heat. Eggs, pupal and young larval stages were found most tolerant surviving up to 60 min at 45 °C, up to 7 min at 50 °C and up to 5 min at 55 °C, respectively. Fitting a log trend through the data gave lethal exposure times of 660 min at 45 °C, 27 min at 50 °C and 7.2 min at 55 °C. Differences between the most tolerant and most sensitive developmental stages became smaller with increasing temperature. Results are compared with those of stored product beetles, such as the red flour beetle *Tribolium castaneum*, the granary weevil *Sitophilus granarius* and the lesser grain borer *Rhizopertha dominica.* Among the grain pests this latter species was by far the most heat tolerant. All developmental stages and adults of *R. dominica* were used as bio-indicator in a practical heat treatment in a flour mill. Insect survival was detected in most samples where temperature did not exceed 50 °C as recorded by data loggers. Infrared-thermography was found a helpful tool to detect areas of heat loss from outside and cold bridges that might allow the survival of insects from inside. These are the areas that should be heated first in order to drive out insects towards zones where they are more easily controlled later on. Insulating materials such as large amounts of grain, straw and husks, flour dust or packaging materials should be removed from buildings prior to treatment.

Key words: heat disinfestation, stored product insects, control, *Ephestia kuehniella*, *Rhyzopertha dominica*.

Introduction

In December 2004, after the phase-out of methyl bromide, sulphuryl fluoride (SO_2F_2) was registered in Germany for the disinfestation of empty structures such as flour mills. Being used to the high levels of control and low costs of methyl bromide, some millers complain about insufficient efficacy of this new fumigant and the costs of a treatment. Due to its molecular structure, SO_2F_2 easily penetrates materials such as wood (Bell, 2004). This advantage in the control of structural pests may turn into a disadvantage in food-producing plants like flour mills when product such as grain, millet, bran or flour is stored in a treated plant because residues of the fumigant, mainly fluoride, may build up in the product. Moreover, no warning smell protects man in case of leaks and no filter is available for gas masks that could retain SO_2F_2, which forces fumigatiors to use compressed air

[1] Federal Biological Research Centre for Agriculture and Forestry (BBA), Institute for Stored Product Protection, Koenigin-Luise-Str. 19, D-14195 Berlin, Fax +49-30-83042503, Email: c.adler@bba.de

to enter a treated building.

An alternative to fumigation is the application of high temperatures. However, there are concerns about the efficacy of a heat treatment against various pests and about the difficulties to secure a successful and complete disinfestation. The present paper describes laboratory experiments to determine the efficacy of 45, 50 and 55 °C against all developmental stages of the Mediterranean flour moth *Ephestia kuehniella* Zeller and a practical heat treatment carried out in a German flour mill in 2005. During this practical treatment, all stages of the Lesser grain borer *Rhizopertha dominica* F. were used as bio-essay, temperature and relative humidity were recorded.

Material and methods

a) Laboratory experiment on control of *E. kuehniella* in substrate

E. kuehniella from a laboratory culture were cultivated on wheat bran. Each week 2 mL of moth eggs (approx. 500) were placed onto 250 mL of fresh uninfested wheat bran made from wheat with a moisture content of 14 ± 1 % in a glass vial with a volume of 2 L. In order to have sufficient numbers of individuals available, at least 2 glasses were prepared simultaneously each week. Cultures were stored at 25 ± 1 °C and 65 ± 5 % r.h. After 5 weeks of cultivation, this gave the following stages for testing: stage 1 (eggs), stage 2 (young larvae), stage 3 (medium size larvae), stage 4 (fully grown larvae), stage 5 (pupae).Two mL of wheat bran together with either 50 eggs, 50 neonate larvae, 30 medium sized larvae, 30 grown larvae or 30 pupal stages, respectively, were given through a funnel into a glass tube (diam. 1.8 cm), two thirds of which were submerged in a water bath at controlled temperature of 45 °, 50 °, and 55 ± 0.1 °C. After filling in the test insects the glass tubes were closed with a stop cock to avoid cooling by convection (for further details see Adler, 2002). The substrate was simulate product residues in a mill with cracks and crevices broom cleaned prior to a heat treatment. Potential mistakes in temperature control (± 0.1 °C), thermometers (± 0.1 °C), and reading (± 0.1 °C), are estimated to a max. of ± 0.3 °C. In preliminary experiments exposure times had been varied until survival and complete control was found. Each exposure time was tested in at least three replicates. In all experiments, untreated controls were prepared for each exposure time tested. Results from hatching were transferred into per cent mortality, with mean numbers of hatched individuals in controls (all exposure times of a given experiment) taken for 100 %. The data were subsequently depicted in an MS Excel graph. For preliminary evaluation a log trend curve was fitted into the data to calculate lethal exposure times.

b) Heat treatment in a flour mill

R. dominica was cultivated on whole wheat grains at 25 ± 1 °C and 65 ± 5 % r.h. 150 adults where placed onto 500 mL of wheat with a water content of approx. 14 % to oviposit for one week. This was done in weekly intervals. A mix of all stages was provided by adding 50 young adults and 6 mL (approx. 2 g) substrate of each stage into a photo film tube with a fine wire mesh welded into the bottom and the lid of each tube. The film tube was then given into a linen bag of 20 X 30 cm. A total of 52 insect samples were prepared. 50 bags were numbered and 2 bags were used as untreated control of which one (U1) was taken along to the treatment, the other (U2) remained under laboratory conditions at 25±1°C and 65 ± 5 % r.h. At the end of the treatment, all samples were collected, taken back to the laboratory and checked weekly for beetle mortality and adults hatching from grain with immature stages for the following 12 weeks at 25 °C and 65 % r.h.

To determine the changes of temperature over time, 25 data loggers were added to each odd number of the 50 insect samples. These data loggers had been calibrated and programmed to

record ambient temperature and moisture contents. Subsequently all bags were closed with a Velcro fastener along the seam of each bag, the seam was patent-folded and fixed with a metal wire. The 50 insect samples and one untreated control sample were placed into a thermo-insulated bag and taken to the flour mill where the samples were distributed within the building. In addition to the data loggers used, an electric digital thermometer (GTH 1200) was utilised during inspection walks in the building while the heat treatment was carried out in order to determine temperature in the air, on surfaces, in cracks or crevices and in substrates.

Infra red (IR) thermographs were taken from the outside of the building to determine areas of heat loss in the outside walls of the building. Another set of thermographic pictures was recorded from inside in the seventh flour of both mills in order to visualise cooler zones that may allow insect survival.

Results

a) Laboratory experiment on control of *E. kuehniella* in substrate

The levels of mortality achieved by 45, 50 and 55 °C against the developmental stages of *E. kuehniella* are depicted in Figures 1-3. For reasons of legibility, the log trend curves are only given for the most and least heat tolerant stages. According to this method, the tested strain could be controlled in 5 mL of substrate at 45 °C within 660 min at 50 °C within 27 min and at 55 °C within 7.2 min.

Temperatures recorded in various bags with insect samples are given in Figure 4. Surviving *R. dominica* were found in 19 out of the 50 treated insect samples, and the majority of these had been deposited between produce and packaging materials in the packaging hall or certain parts of the mill building. All test insects deposited in machinery, on floors, or in the open were killed.

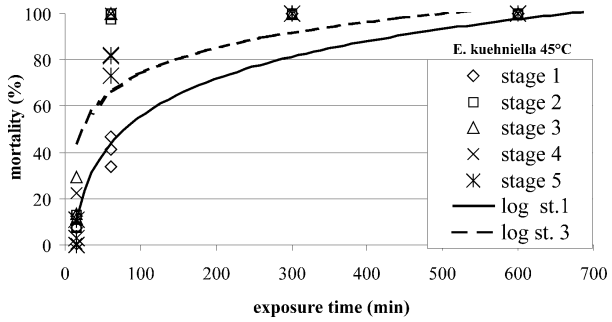

Figure 1. Mortality of various developmental stges of *Ephestia kuehniella* after exposure to 45 ºC in laboratory experiments (stage 1: eggs, stage 2-4: lavae, stage 5: pupae)

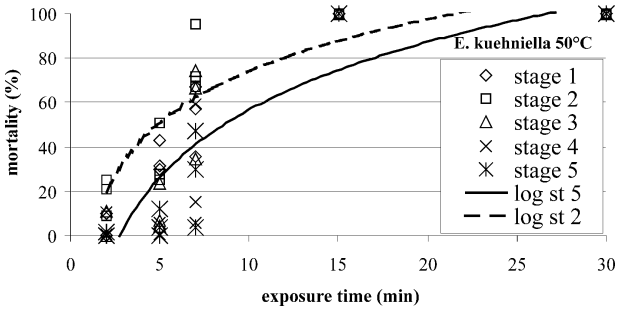

Figure 2. Mortality of various developmental stges of *Ephestia kuehniella* after exposure to 50 ºC in laboratory experiments (stage 1: eggs, stage 2-4: lavae, stage 5: pupae)

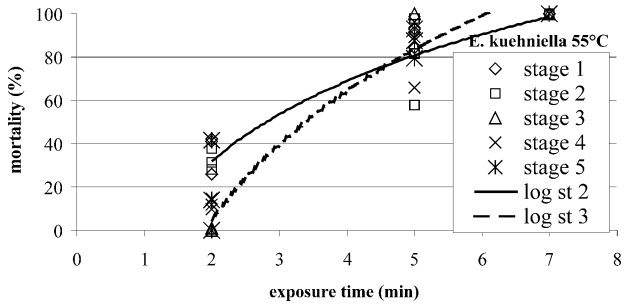

Figure 3. Mortality of various developmental stges of *Ephestia kuehniella* after exposure to 55 ºC in laboratory experiments (stage 1: eggs, stage 2-4: lavae, stage 5: pupae)

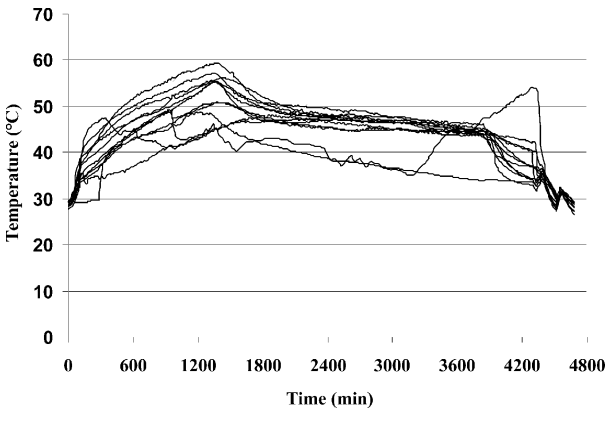

Figure 4. Temperature determined during a practical heat treatment at various points in the mill.

Discussion

a) Laboratory experiment on control of *E. kuehniella* in substrate

The lethal exposure times achieved with developmental stages of *E. kuehniella* in laboratory experiments show that complete control is possible in reasonably short times. In comparison with *Sitophilus granarius* (L), *S. zeamais* Motschulsky, *Cryptolestes pusillus* (Schoenherr), *Tribolium castaneum* (Herbst), *Lasioderma serricorne* (F.) and *R. dominica* tested under similar conditions (Table 1) (Adler 2003, 2004, Adler and Große 2004), *E. kuehniella* appears quite sensitive against heat at 50 °C and above. First results of similar experiments with *Plodia interpunctella* (Hübner) seem to indicate that pyralid moths in general may be rather sensitive. The differences between the least and the most heat tolerant developmental stages if expressed as percentage values of the most tolerant stage at each temperature, appear small and become smaller with increasing temperature (24.2 %, 18.5 % or 15.2 %, respectively). At 55 °C, one single value with low mortality of stage 2 leads to a different slope of the log trend compared to the most sensitive stage 3 and results in stage 2 being most tolerant. Further experiments will be needed to prove if this finding is significant or not.

That *R. dominica* is among the most heat tolerant species is supported by other literature data (Kirkpatrick and Tilton, 1972; Becket et al., 1998).

b) Heat treatment in a flour mill with *R. dominica* bioassay

Complete mortality of *R. dominica* was found in almost all insect samples exceeding temperature of 50 °C. This together with the laboratory findings (Table 1) somewhat contradicts the results of Mahroof et al. (2003) that a minimum of 7.2 h were needed to kill larvae of the rather heat sensitive *T. castaneum* at 50 °C. Differences may be due to experimental design, relative humidity, variations in the tolerance of insect strains and calculation models applied. Bartlett et al. (2005) mention that in treatments of mills, 2 or 0.5 h were needed to control the red and confused flour beetle. *T. castaneum* and *T. confusum*, as well as the Turkish flat grain beetle *Cryptolestes turcicus* at 49 or 51 ° C, respectively.

All parts of machinery were heated to levels providing complete control. However, some areas like the cellar or the room with the elevator heads in the 9th floor were not heated sufficiently. Other insects survived in a sample hidden in electric cables close to a small opening in the outside wall, as well as in insulating materials such as bags with flour, staples of corrugated cardboard for packaging, a dust bin filled with dust,

Table 1. Exposure times (min) needed for complete control of stored product insects with various temperatures in laboratory tests, and developmental stage found most tolerant to the respective temperature*

Insect species	45 °C		50 °C		55 °C	
Ephestia kuehniella	660 (11 h)	E	27	P	7.2	L
Sitophilus granarius	540 (9 h)	L	40	L	30	L
Sitophilus zeamais	660 (11 h)	L	45	A	30	A
Cryptolestes pusillus	1200 (20 h)	L	65	L	20	L
Tribolium castaneum	1800 (30 h)	L	35	L	20	L
Lasioderma serricorne	2400 (40 h)	L	370	E	45	E
Rhizopertha dominica	6000 (100 h)	L	370	L	45	L

E = egg stage, L = larval stage, A = adult stage.

*Tests in 10 ml of substrate in glass tubes heated in a water bath, 100% mortality determined graphically with a log trend curve.

straw and husks and below a bucket half filled with water in a corner close to the outside wall. This shows that the removal of such items is crucial for the success of a treatment and that a heat operator needs to communicate this to his client.

The relative humidity levels determined in the course of the heat treatment close to the test insect samples showed a dramatic drop in moisture, which of course is no surprise. However, in aereas where temperature did not reach 50 °C, prolonged periods of extremely low humidity may have been a cause for complete mortality as it was the case in a sample stored in the room above the elevator shaft that was indirectly heated but with a constant draft due to the opening for the elevator cables (< 30 % r.h. for more than 61 h). It could thus be that at lower temperature the dissication gains importance as a cause of mortality, while the destruction of proteins is the immediate cause of death at higher temperatures.

Surprisingly, one surviving *R. dominica* was found in the sample with the highest temperature and longest exposure time (Figure 4). This could either be an artefact occuring during the weekly evaluation of survival or a hint that at least the gene pool of *R. dominica* may still hold some surprises for heat treatments to come.

IR-thermography was useful to find spots of poor insulation in the outside walls of the buildings. Pictures taken from within the treated building showed cooler zones, which could be found mostly in the outside walls. Once identified, these areas could be heated first in future treatments, thus driving insects away from potential zones of survival.

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Reference

Adler, C., 2002. Efficacy of high temperatures to control *Lasioderma serricorne* and *Rhyzopertha dominica*. Proceedings of the meeting of the IOBC-WPRS study group ´Integrated Protection of Stored Products´, Lisbon, 3-5 September, 2001, IOBC-Bulletin 25, 187-191.

Adler, C., 2003. Efficacy of heat treatments against the tobacco beetle *Lasioderma serricorne* F. (Col., Anobiidae) and the lesser grain borer *Rhyzopertha dominica* F. (Col., Bostrychidae). Advances in Stored Product Protection, Proceedings of the 8th International Working Conference on Stored Product Protection, York 2002, CAB International Publishing: 617-621.

Adler, C., 2004. Control of *Cryptolestes pusillus* (Col., Cucujidae) and *Tribolium confusum* (Col., Tenebrionidae) at high temperatures. In: Integrated Protection in Stored Products. Proceedings of the meeting of the IOBC-WPRS working group ´Integrated Protection of Stored Products´, Kusadasi, 16-19 September 2003. IOBC Bulletin 27, 211-214.

Adler, C., Große, N., 2004. Wirkung hoher Temperaturen zwischen 45°C und 55 °C auf vorratsschädliche Insekten. In: 54. Deutsche Pflanzenschutztagung, Hamburg 20.-23. September 2004. Mitt. Biol. Bundesanst. Land-Forstw., Heft 396, 438-439.

Bartlett, D., Conyers, T., Bell, C.H., Watson, C., 2005. Further development of heat-based methods for desinfesting flour mills. Project Report Nº 378. HGCA, 62pp.

Beckett, S.J., Morton, R., Darby, J.A., 1998. The survival of *Rhizopertha dominica* (F.) (Col., Bostrychidae) and *Sitophilus oryzae* (L)(Col., Curculionidae) at moderate

temperatures. Journol of Stored Products Research 34, 363-376.

Bell, C.H., 2004. The use of sulphuryl fluoride in Europe for structure and commodity disinfestations. In: Batchelor, T. Alfarroba, F. (eds.). Proceedings of the 5th International Conference on Alternatives to Methyl Bromide, Lisbon, Portugal, 23-30 September 2004, 237-244.

Kirkpatrick, R.L., Tilton, E.W., 1972. Infrared radiation to control adult stored-product coleoptera. J. Ga. Entomol. Soc. 7, 73-75.

Mahroof, R., Subramanyam, Bh., Throne, J. E., Menon, A., 2003. Time-mortality relationships of *Tribolium castaneum* (Coleoptera: Tenebrionidae) life stages exposed to elevated temperatures. Journal of Economic Entomology 96, 1345-1351.