Low temperature to control *Plodia interpunctella* and *Stegobium paniceum*

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Abstract

Plodia interpunctella (Indianmeal moth) and *Stegobium paniceum* (drugstore beetle) are two of the most common insects in dried fruits, nuts, grain products, herb teas or spices. All the stages of both these insects were held at -10, -14 and -18°C for 5 to 480 min and the survival noted. The following times and temperatures were required to control all stages: *P. interpunctella*; 480 min at -10°C (1% survival of eggs), 240 min at -14°C, 60 min at -18°C; *S. paniceum*; over 480 min at -10°C, over 240 min at -14°C, 60 min at -18°C. For *P. interpunctella,* eggs were the most cold hardy stage. For *S. paniceum*, adults were the most cold hardy stage with the exception of –14°C, where about 10% of the eggs but no adult beetles survived 240 min of exposure.

Keywords: Cold disinfestation, Freezing, Control, *Plodia interpunctella, Stegobium paniceum.*

1. Introduction

The Indianmeal moth *Plodia interpunctella* (Huebner) and the drugstore beetle *Stegobium paniceum* (L.) are among the most common stored product pests, and they often occur in grain and grain products, nuts, dried fruit, fruit, herb teas and spices. If the products are dried in the open, it is difficult to prevent infestation. To control infestation in storage and processing, fumigation has been a common control option for decades. After the phase-out of methyl bromide (CH_3Br), world-wide dependency on phosphine (PH_3) increased dramatically. At the same time, reports on the development of resistance to phosphine in various stored product insects (Chaudry, 1999; Collins, 2006) have lead to concerns and increased the need to search for alternatives to fumigants.

Looking at non-chemical techniques, the utilization of extreme temperatures may also lead to complete control of pests, and it does not pose a risk to workers or involve the application of potentially hazardous agents (Fields, 1992; Burks et al., 2000).

Artificial cooling or freezing has been described as a means of pest control by various authors (David et al., 1977; Evans, 1987; Hagstrum and Flinn, 1994; Lasseran and Fleurat-Lessard, 1990; Dohino et al., 1999). Freezing dry stored products between - 10°C and - 20°C is an option for rapid disinfestation of high-value goods. While this process is fast, leaves no residues, has little or no negative effect on product quality and is comparatively safe for workers, the costs for the construction of a cooling chamber and fairly high energy costs are drawbacks that limit widespread adoption of this method. On a commercial scale, an organic dried fruit processing company with locations in Turkey, the USA, and Germany, places all dried fruits in a cooling chamber at -20°C for 24 h, prior to processing. Furthermore, a herb tea and spice producer in Germany has utilized a cooling chamber for more than 15 years to freeze all products upon reception, until a core temperature of -18°C is reached. Cooling is achieved by adding liquid nitrogen and chamber temperatures are allowed to drop as low as -90°C. Product is held for 12 to 36 h depending on the heat conductivity, the bale size and the volume of the treated product. Depending on product and treatment time, 3000 – 5000 L of liquid nitrogen are needed for a treatment, and costs are roughly estimated to be 1000 Euro/t (Tallafus, personal communication).

The aim of this study was to test which exposure times are required at -10, -14 and -18°C to control all the developmental stages and adults of *P. interpunctella* and *S. paniceum*.

2. Materials and methods

2.1. Insects

Insects came from cultures kept for many years at the Institute for Ecological Chemistry, Plant Analysis and Stored Product Protection at 25 ± 1°C and 65 ± 5 % r.h. *Plodia interpunctella* was cultivated weekly by placing approximately 500 eggs into a mixture of 500 mL wheat bran and approximately 15 g of broken almonds. *S. paniceum* was cultivated by placing 200 adult beetles for two wk onto 150 mL wheat bran. After removing the beetles a further 200 mL of wheat bran were added together with a dried white bread roll. The insects used were not pre-adapted to cold prior to treatment.

To provide bio-test samples, eggs, larvae, pupae and adults were counted into separate cages. Fifty moth eggs (0-2 d old) were counted into small glass tubes (diameter: 13 mm, length: 13 mm) with a nylon mesh (0.3 mm width) at one end and closed with a rubber cork at the other end. This small glass tube was then fitted into a round cage made of wire mesh gauze (diameter: 14 mm, length: 50 mm) filled with 5 mL of wheat bran in a way that the nylon gauze touched the wheat bran. This was done to keep eggs separate from substrate for the evaluation of hatch while providing them with insulation comparable to the other stages. Fifty larvae of *P. interpunctella* were counted into 5 mL of wheat bran and filled directly into the wire mesh cages. The larvae were third instar, approximately 4 wk after placing eggs onto fresh substrate. Fifty pupae were taken from cultures 6 wk after placing eggs onto fresh substrate, and they were placed with substrate into cages just like the larvae. Young adult moths were counted in batches of 50 into a wide glass tube (diameter: 40 mm, length: 105 mm) without substrate and closed on both ends with cotton cloth fixed onto the tube with a rubber band (Fig. 1).

Figure 1 Glass tubes for exposure of adult moths after treatment with -14°C.

In the case of *S. paniceum*, 50 eggs (0-1 d old) were counted into a glass tube (diameter:13 mm, length:17 mm) in which one end was sealed with a nylon mesh (0.3 mm width), covered from inside with a 2 mm thick layer of black felt, from the other end with a rubber cork. As in the case of moth eggs, the glass tube was inserted into a wire mesh cage with substrate before treatment (Fig. 2). Larval and adult drugstore beetles were counted into batches of 50 individuals that were filled together with 5 mL of wheat bran into a wire mesh cage that was then closed with a rubber cork. In preliminary tests, *S. paniceum* pupae had shown high mortality in untreated controls when removed from the webbings for counting. This is why in just one replicate 80 eggs, 0-1d old, were placed into the wire mesh cages with 5 mL of substrate and the cages were kept for 34 d at 25°C and 65% r.h. until the majority of control samples showed development into pupal stages. After treatment, hatching of adult beetles was counted as survival.

Figure 2 Small glass tubes for exposure of drugstore beetle eggs, and egg cages enclosed into wire mesh cages for testing.

2.2. Testing and evaluation

Test were carried out in a laboratory freezer (Rumed 3501, Rubarth Apparate GmbH, Laatzen, Germany) equipped with a PT 100 thermo couple and an accuracy of ±0.5°C. In order to minimize the loss of cold air when opening the door, the freezing chamber had been sealed with a plastic sheet leaving just sufficient space to fit in the tray with test samples and data loggers for temperature recording.

Temperatures and exposure times tested were:

- -10°C for exposure times of 30, 60, 120, 240 and 480 min,
- -14°C for exposure times of 10, 30, 60, 120 and 240 min, and
- -18°C for exposure times of 5, 10, 30, 60, and 120 min.

After the given exposure times, the samples were removed from the freezer. The minute cages with *P. interpunctella* eggs were placed into small glasses with 2 mL of wheat bran and covered with an insect-proof plastic cap allowing gas exchange. Hatch of larvae was counted as survival while darker colouration and shrinking of eggs due to water loss were signs of mortality after 7 d at 25°C and 65±5 % r.h. Moth larvae and wheat bran were transferred into Petri dishes, pupae and their substrate into 200 mL glass jars. Both were checked for hatch of adult moths. *P. interpunctella* adults retrieved from the freezer were kept in the glass vials. They like all other test insects were transferred to a temperature-controlled chamber with 25°C and 65±5 % r.h. to be checked for survival 2 d after exposure (Fig 1).

Stegobium paniceum eggs were transferred into a small plastic Petri dish (diameter 35 mm) without substrate and checked for larval hatch after 7 and 14 d at 25°C and 65±5 % r.h. For untreated controls, batches of 50 eggs were directly counted into Petri dishes. Larvae and their substrate were transferred into small Petri dishes and checked for survival after 2 and 7 d. Pupal stages were transferred from cages into a 200 mL glass jar with wheat bran and checked after 7 and 14 d for hatch of adults. Adult beetles and their substrate were placed into small Petri dishes and checked for survivors after 2 and 7 d. All data were collected and calculated into % mortality correcting for natural mortality in untreated controls according to Abbott (1925). Mean values were calculated and plotted against exposure time.

3. Results

At -10°C, all stages of *P. interpunctella* except eggs could be controlled in 480 min. In *S. paniceum* none of the tested stages was controlled completely at this temperature and exposure time. Results of the treatment of *P. interpunctella* or *S. paniceum*, respectively, are given in Figures 3 and 4. Pupal stages of *S. paniceum* at -14°C were controlled within 120 min and at -18°C within 60 min. As recorded by data loggers, 10-15 min were required to reach target temperatures after the insect samples had been placed into the freezer. In untreated pupae of *S. paniceum*, an average of 58 ± 7 adult beetles emerged from 80 eggs in 11 replicates that had been placed with 5 mL of substrate into wire mesh cages.

4. Discussion

At -10°C, *P. interpunctella* was controlled at the longest exposure time of 8 h. In one of three replicates few eggs survived the treatment. Therefore, possibly 10-12 h could be a safe treatment time. Stage and cold acclimation can greatly increase the cold tolerance. Under similar conditions, Fields and Timlick (2010) found that fifth instar *P. interpunctella* had 98% mortality after 48 h at -10°C. For *P. interpunctella* that were cold acclimated and in diapause, after 14 d there was 88% mortality. In comparison, *S. paniceum* was not controlled at -10°C, and the adult beetles seem to be least affected by cold. In this stage there is no obvious dependency between mortality and exposure time with zero mortality in two of three replicates after 480 min exposure, while in egg and larval stages mortality increased with exposure time (Fig. 3). It is thus obvious that much longer times are required to achieve control at -10°C. Ryan (1995) reports on practical treatments of tobacco infested with the closely related tobacco beetle *Lasioderma serricorne* (F.) that 100% mortality was achieved at -10°C within 28 d. From literature data available, Reichmuth and co-workers (2007) calculated for this species a lethal time of approximately 18-20 d at -10°C.

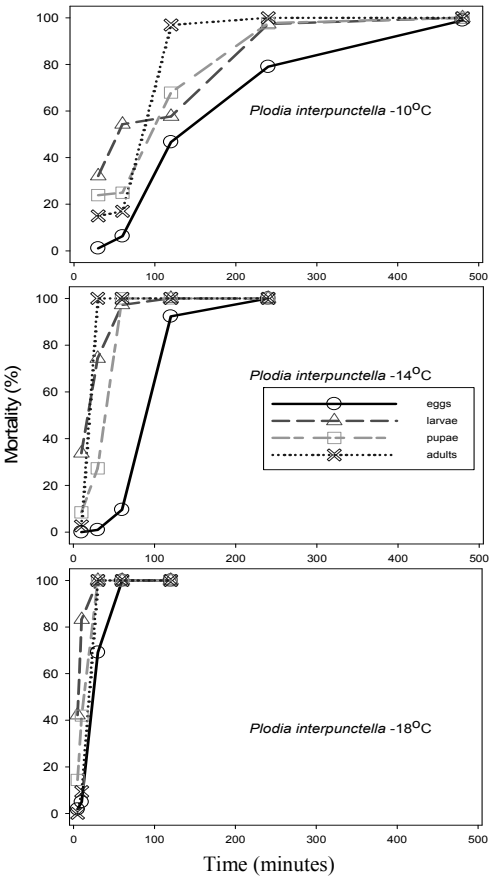

Figure 3 Mortality of eggs, larvae, pupae and adults of *Plodia interpunctella* after exposure to –10, -14, and –18°C.

At -14°C, eggs proved to be the most cold-hardy stage in both tested species. Eggs of *P. interpunctella* have the lowest supercooling points (-24°C) of all stages (Carillo and Cannon, 2005). At shorter exposure times of 60 and 120 min, adult *S. paniceum* were at least as or even more tolerant than eggs, but

no survivors were found at the longest exposure time (Fig. 4). This may hint to several and different factors being responsible for chill injury and mortality in eggs or beetles, respectively.

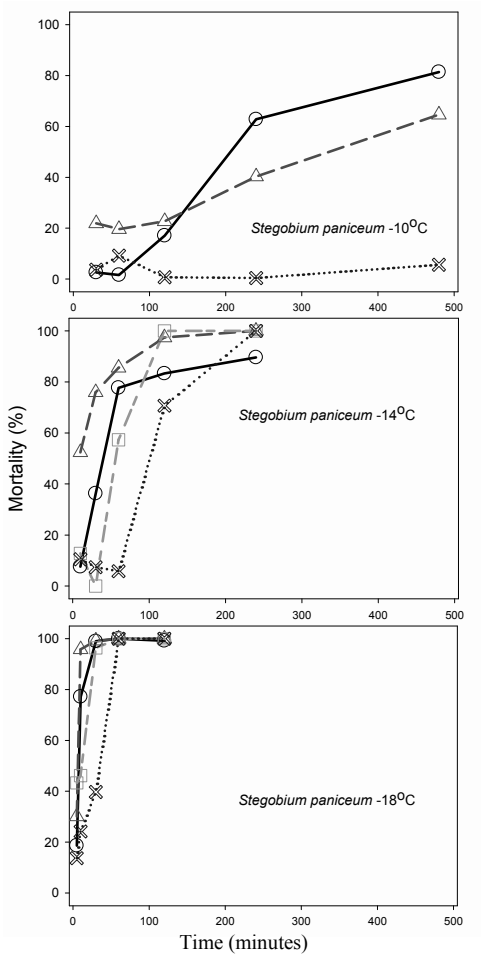

Figure 4 Mortality of eggs, larvae, pupae and adults of *Stegobium paniceum* after exposure to –10, -14, and –18°C.

At -18°C, eggs of *P. interpunctella* were controlled after an exposure time of 60 min the other tested stages could be controlled in half this time. Eggs of *S. paniceum* did not survive 60 min of exposure, but two survivors were found after 120 min exposure in one of five replicates. This effect may have been caused by a variation in genetic predisposition to cold hardiness. But also an artefact could be possible and further experiments are needed to add more precision to these data. More than 50% of the beetles survived up to 30 min exposure at -18°C. This is much more than in any other stage, while no survivors were found at longer exposure times. Dohino et al (1999) compared the efficacy of freezing at -18°C between various stored product beetles and moths including *P. interpunctella* and stated that the egg stage was most tolerant. Imai and Harada (2006) tested freezing treatments at various temperatures against *L. serricorne* and found that at -15°C eggs of unacclimated individuals were most tolerant and 100% mortality was reached after 5 h. On the contrary, Abdelghany et al. (2010) found that adults not eggs to be most cold tolerant stage. Variations in the cold hardiness of eggs may depend on age. The eggs used in this study were 0-24 h old and thus comparatively young.

For pupal stages of *S. paniceum*, just one replicate could be completed at this time, but together with other preliminary results, these data indicate that pupae are not the most tolerant stage. From the presented data one may conclude that *S. paniceum* is more tolerant to low temperatures than *P. interpunctella*. Compared to other developmental stages, young moth eggs are fairly tolerant to low temperatures at -10, -14, and -18°C. The same seems to be true for *S. paniceum* eggs that appeared less uniform in their tolerance to cold than adult beetles. At -10°C adult beetles are more cold-hardy. At this temperature, exposure times much longer than 8 h will be needed to control beetles.

Freezing with liquid nitrogen adds safety because most of a product is cooled to even lower temperatures until the core reaches -18°C. Subsequently, the bales are stored untouched. This prolongs treatment time in the core of a product and leaves little chance for insect survival. Temperature profile recordings and bio-assays could help to prove the reliability of this method.

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