COLD-HARDINESS OF EGGS OF MOTHS **EPHESTIA CAUTELLA** (WLK.), **E. KUEHNIELLA** (ZELL.), **PLODIA INTERPUNCTELLA** (HBN.) AND **CORCYRA CEPHALONICA** (STAINTON).

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ABSTRACT

The cold-hardiness of eggs of four stored-products moths exposed to low temperatures (next to and below 0°C) was investigated. Laboratory tests were carried out on eggs of different ages (ranging from 6 to 20 h and from 30 to 48 h) kept at +2°C, -2°C, -5°C, -10°C (±0.5°C) and 70 ±10% RH, for 3, 9, 18, 24 and 48 h. Five repetitions and five controls (200 eggs) of each group were tested at each cold exposure at different exposure period.

At +2°C, no significant reduction in the hatching of eggs of E. kuehniella was observed after a 48 h exposure, whereas a 80% mortality of eggs of P. interpunctella and more than 95% mortality for the other two species was obtained. Eggs hatching was lower than 5% for all the species except for E. kuehniella (85% hatching) after an 18 h exposure at -2°C.

At -5°C, a 3 h exposure is enough for a total inhibition of the hatching of P. interpunctella, whereas a 9 h exposure produced a mortality higher than 90% in E. cautella and C. cephalonica. At -10°C, no hatching was observed in E. cautella and C. cephalonica after a 9 h exposure, whereas a 24 and 48 h exposure produced a mortality respectively of 60% and higher than 95% of E. kuehniella eggs.

On the whole significant differences in mortality between mature or immature eggs were not reported. These results show that P. interpunctella is more susceptible to temperatures lower than 0°C, while E. kuehniella is much more tolerant to the cold.

INTRODUCTION

The control of stored-product pests by means of temperatures lower than the relative developmental thresholds requires extremely long periods of contact (Mathlein, 1961; Reichmuth, 1979; Stratil and Reichmuth, 1981a, b), but even with temperatures of around 0°C, exposure times of more than 15 days are nearly always

necessary (Burges, 1956; Cline, 1970; Torc'h, 1977; Stratil and Reichmuth, 1981a, 1981b, 1983, 1984). Shorter times are sufficient only if the insects are exposed to temperatures below 0°C (Adler, 1960; Tsvetkov, 1965; Süss and Moroni, 1982).

With regard to the cold-hardiness of some species of moths that frequently infest stored products, differences in ability to survive have been observed not only from one species to another, but also between the different stages of development (Torc'h, 1977; Krnjaic and Ilic, 1982).

In the case of Plodia interpunctella (Hbn.) the larvae have been found to tolerate temperatures of between 1 and 14°C better than the eggs (Stratil and Reichmuth, 1981a). Similar results have been observed at 2°C (Torc'h, 1977), while at -2°C the pupae of Ephestia cautella (Wlk.) proved to be more resistant than the other developmental stages (Burges, 1956).

In the case of eggs of Plodia interpunctella, Ephestia cautella, and E. elutella Hbn., kept at a variety of temperatures below 15°C, a progressive reduction in hatching was recorded in function of the age of the egg. Freshly-laid eggs were the most susceptible (Stratil and Reichmuth, 1983, 1984). Two hours at -10°C are enough to kill the eggs of Corcyra cephalonica (Stainton) when the eggs are only one day old, but three hours of exposure are necessary if the eggs are two days old (Torre Callejas and Diaz Azpiazu, 1980). Etman (1990), also working with a temperature of -10°C, observes total mortality of 24 and 48 hour-old eggs following 8 hours of exposure to this degree of cold, while 10 hours of exposure were needed to kill 72 and 96 hour-old eggs.

Eggs of Ephestia kuehniella (Zell.), when subjected to a temperature of -1°C, showed a susceptibility that varied according to the stage of embryonic development reached. Specifically, the most vulnerable were found to be those laid between 0-12, 15-17, and more than 96 hours previously. These intervals correspond respectively to the phases of division of the nucleus, to the invagination of the embryonic band, and to the complete formation of the embryo after blastokinesis. This final phase has proved to be the one most susceptible to adverse environmental conditions (Daumal et al., 1974).

It has also been observed that, with adverse thermic conditions, greater or lesser cold-hardiness depends directly upon the quantity of lipids present in the organism. Hence the crystallization point of the tissues is a function of the ratio existing between free fluid and fluid bound to lipidic colloids (Mullen and Arbogast, 1984; Baust and Rojas, 1985; Bale, 1989).

Unfortunately, the various authors of much of the research carried out up to now on the cold-hardiness of moth eggs have been taking into consideration parameters so different that they cannot easily be correlated. We therefore felt that it would be useful to evaluate the resistance to temperatures of around 0°C, or even lower, of the eggs of four species that commonly infest foodstuffs: specifically, Ephestia cautella, Ephestia kuehniella, Plodia interpunctella and Corcyra cephalonica.

MATERIALS AND METHODS

The eggs used in the study were collected from Petri dishes

placed under special plexiglass egg-laying cylinders, 15 cm in diameter and 40 cm high, the base fitted with an 18-mesh metal net through which the eggs can fall.

Each cylinder, containing 50 pregnant females, was kept at 26°C (±1) in a thermostatically regulated cell, at 60% ±5 relative humidity (RH), and with 12 hours of light alternating with 12 hours of darkness. Tests were carried out on eggs laid 6-20 and 30-48 hours previously, preserved in environmental conditions identical to those provided for the laying.

After collection and a stereoscopic examination, the eggs were placed in special plastic containers (Ø 11 mm x h. 7 mm), which were then glued to a tray. The trays, in their turn, were inserted into a polystyrene foam container (30 x 30 cm x h. 12 cm) fitted with a temperature and a humidity gauge. The humidity gauge was connected to a display screen mounted outside the air-conditioned cell in which the tests were performed.

Each test involved 5 repetitions of 20 eggs each, and an equal number of control batches.

Tests were performed at +2, -2, -5, -10°C (±0.5) for a variety of contact times, respectively 3, 9, 18, 24 and 48 hours.

In order to reduce to a minimum the time taken to reach the desired temperature (from a minimum of 20 min. at +2°C to a maximum of 40 min. at -10°C) we calibrated the thermostat of the cell to initial values of between -15°C and -20°C. Exposure times were measured from the instant at which the desired temperature was reached. Inside the polystyrene container the temperature remained nearly constant, with oscillations of only ±0.5°C. The humidity values recorded corresponded to 70% ±10 RH.

On completion of exposure, the egg containers were fixed to the centre of plastic Petri dishes (Ø 5,5 cm x h. 1 cm), and 5 ml of paraffin were added to prevent any possible migration of newly hatched larvae beyond the edge of the dish.

The treated eggs and their relative control batch were then placed in a thermostatically controlled cell at a temperature of 26 ±1°C and 60% ±5 RH.

For a period of 14 days the eggs were examined every second day under a stereoscope to see if any larvae had hatched.

The results were elaborated statistically by variance analysis, and subsequently by application of the Tukey test.

RESULTS

The hatching percentage in control egg-batches not exposed to low temperatures varies; for Ephestia kuehniella it ranges between 81 and 95%; for E. cautella between 80 and 91%; for Plodia interpunctella between 83 and 97 % and finally, for Corcyra cephalonica, between 79 and 91%.

On the whole no particular variations in mortality-rate were observed between mature or immature eggs.

Variance analysis demonstrated that the interaction between lenght of exposure to cold and the actual temperature was of significance for both groups of eggs, which were characterized by differences in embryonic evolution.

The results are illustrated: in table I for all the species, in figure 1 for Ephestia kuehniella and E. cautella, in figure 2 for Plodia interpunctella and Corcyra cephalonica.

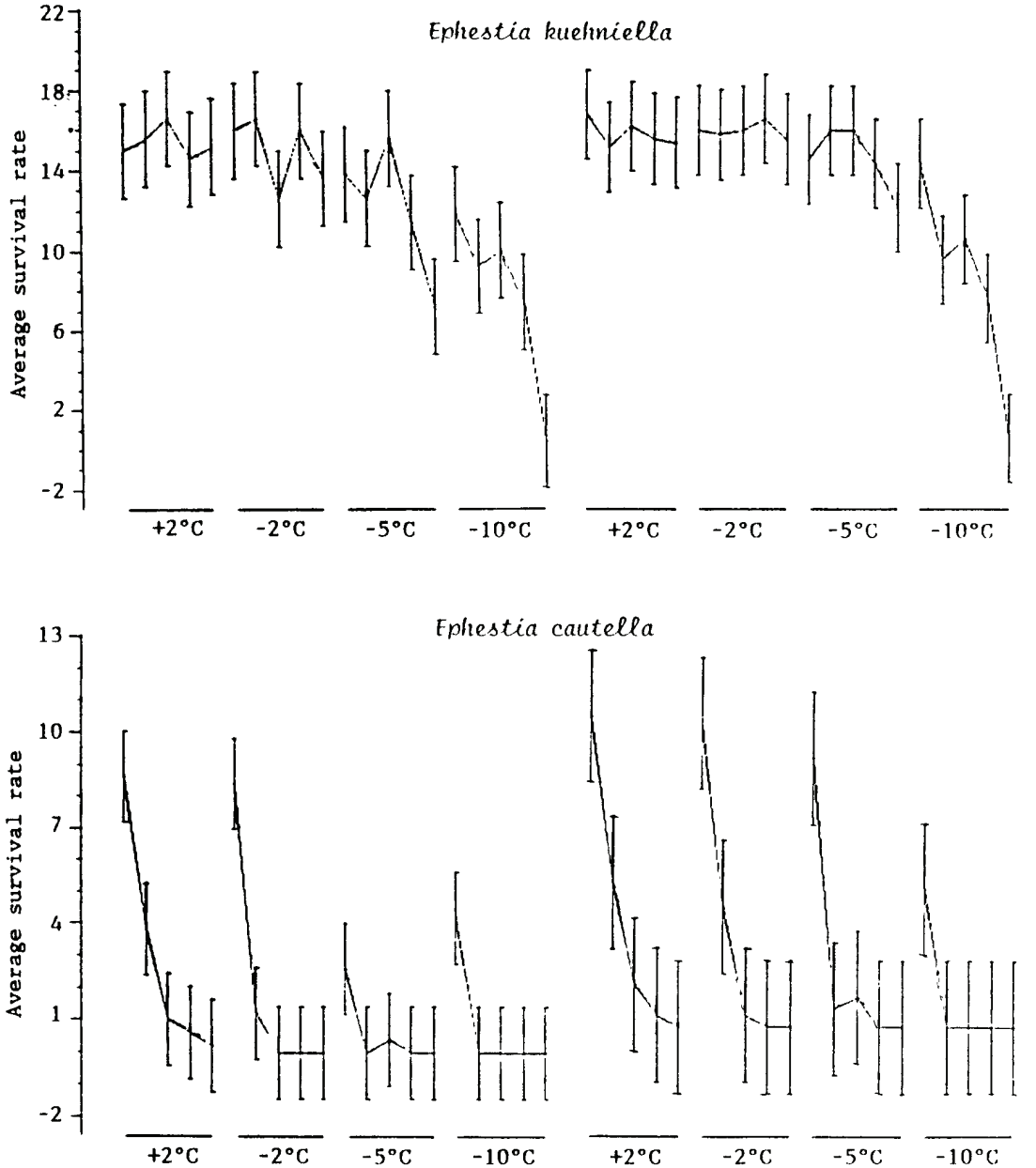

Fig. 1 - Average survival rate for the eggs of Ephestia kuehniella (Zell.) and Ephestia cautella (Wlk.) 6-20 hours old (l e f t) and 30-48 hours old (r i g h t), subjected to different temperatures and a variety of exposure times.
(Temperatures: +2, -2, -5, -10°C; Times: 3, 9, 18, 24, 48 hours)

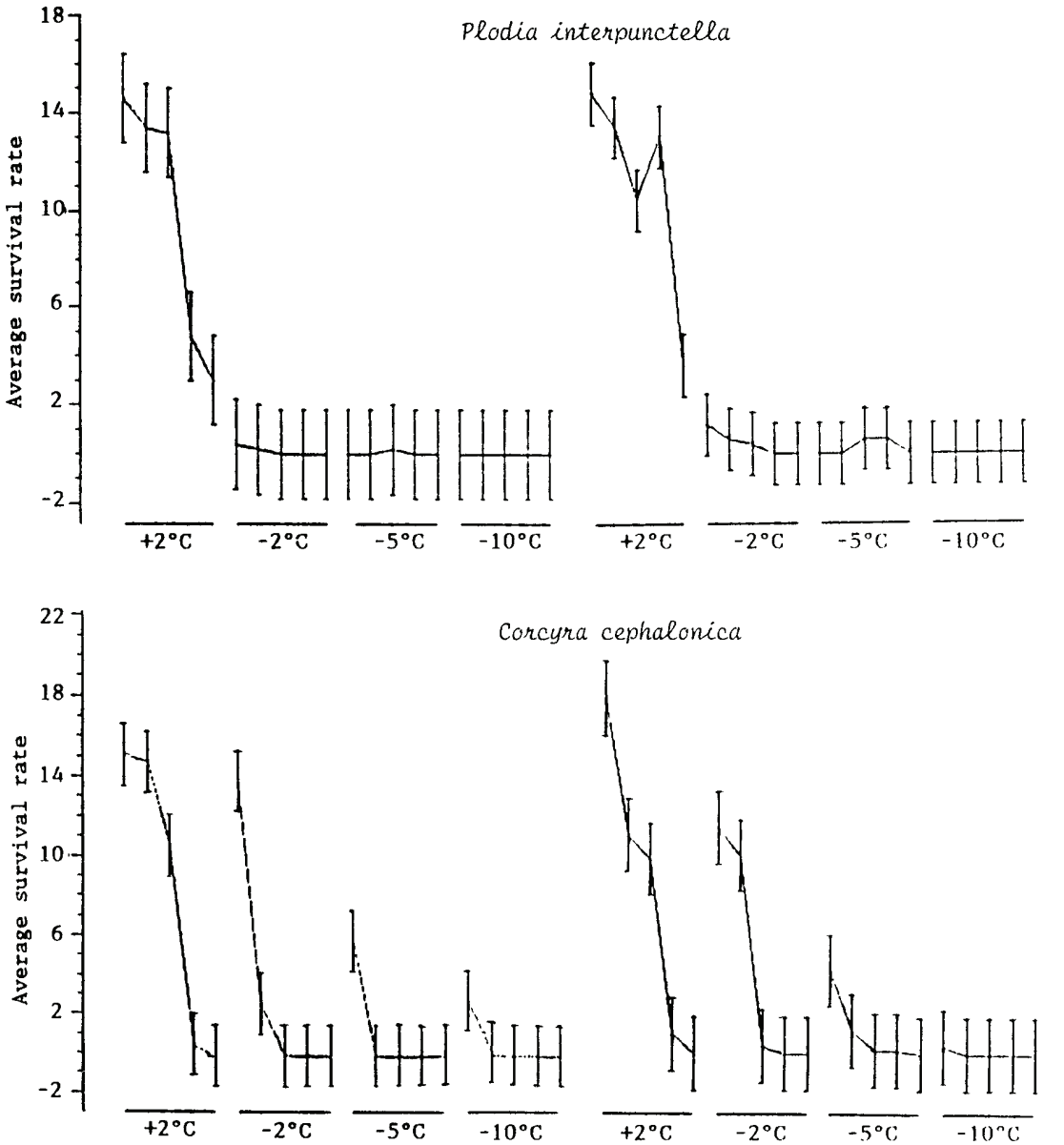

Fig. 2 - Average survival rate for the eggs of Plodia interpunctella (Hbn.) and Corcyra cephalonica (Stainton) 6-20 hours old (l e f t) and 30-48 hours old (r i g h t), subjected to different temperatures and a variety of exposure times.
(Temperatures: +2, -2, -5, -10°C; Times: 3, 9, 18, 24, 48 hours)

Table I - Mortality at different temperatures and times, found for the eggs of Ephestia kuehniella (Zell.), Ephestia cautella (Wlk.), Plodia interpunctella (Hbn.) and Corcyra cephalonica (Stainton) from 6 to 20 and from 30 to 48 hours after laying.

Time of exposure (h)	Eggs 6-20 h				Eggs 30-48 h			
	Temperature (°C)							
	+2	-2	-5	-10	+2	-2	-5	-10
	Ephestia kuehniella mortality (%)							
3	25a	20a	33ab	41a	16a	20a	27a	28a
9	22a	17a	37b	54a	24a	21a	20a	52b
18	17a	37a	22a	50a	19a	20a	20a	47b
24	27a	20a	43b	63a	22a	17a	28a	62b
48	24a	32a	64c	98b	23a	22a	39a	97c
	Ephestia cautella mortality (%)							
3	57a	58a	87a	79a	48a	49a	55a	77a
9	81b	95b	100b	100b	76b	80b	97b	100b
18	95b	100c	98b	100b	93bc	98bc	95b	100b
24	98c	100c	100b	100b	98c	100c	100b	100b
48	100c	100c	100b	100b	99b	100c	100b	100b
	Plodia interpunctella mortality (%)							
3	33a	98a	100a	100a	26a	94a	100a	100a
9	33a	99a	100a	100a	33ab	97ab	100a	100a
18	34a	100a	99a	100a	48b	98ab	97a	100a
24	76b	100a	100a	100a	34ab	100b	97a	100a
48	85b	100a	100a	100a	82c	100b	100a	100a
	Corcyra cephalonica mortality (%)							
3	24a	31a	71a	86a	11a	43a	79a	98a
9	26a	87b	100b	99b	45b	50a	94b	100a
18	47b	100c	100b	100b	41b	98b	99b	100a
24	97c	100c	100b	100b	95c	100b	99b	100a
48	100c	100c	100b	100b	100c	100b	100b	100a

$P < 0.05$

DISCUSSION AND CONCLUSIONS

The results of such research prove that at all temperatures the eggs of E. kuehniella show a greater cold-hardiness than the eggs of other species tested. In fact to obtain a mortality higher than 95% a 48 hours treatment is needed at -10°C, while at -5°C with the same period of exposure to cold 64% of mortality is obtained for eggs 6-20 hours old and 39% for the ones 30-48 hours old. Mathlein (1961) had already demonstrated with similar experiments that 7 days of treatment at -9,5°C were necessary to prevent hatching. The larvae and the pupae show an equally low susceptibility to cold, since after 12 days of exposure to temperatures varying between -4 and -10°C they are still able to complete their postembryonic development.

The exposure-times needed to prevent the hatching of the eggs of this species become longer if the treatment is carried out when the eggs are between 92 and 96 hours old. This period, correspondent to blastokinesis, is a particularly resistant phase (Daumal et al., 1974).

E. cautella is the most susceptible species, even at temperatures above 0°C. At the temperature of 2°C, after 18 hours, mortality is 95% (eggs 6-20 hours old) and 93% respectively (eggs 30-48 hours old); at lower temperatures a mortality higher than 95% is obtained particularly with 18 hours at -2°C, 9 hours at -5°C. Total mortality of the eggs of both groups is observed at -10°C after 9 hours. Our own egg mortality values agree with the results of similar experiments, in which hatching was evaluated at temperatures lower than those taken into consideration here, giving a mortality rate of 95% after 5 hours exposure at -15°C or after less than 2 hours exposure at -20°C (Mullen and Arbogast, 1979).

With regard to the larval stage, approximately 24 hours at -1°C are needed to prevent the complete development of mature larvae, which have been found to be more resistant than immature ones.

P. interpunctella, on the other hand, is the most vulnerable species at temperatures of below 0°C. In fact at -2°C a time of exposure of 3 hours is enough to cause a mortality equal at least to 94% in both groups of eggs, while total mortality is reached with 3 hours of cold at -5 and -10°C. Krnjaic and Ilic (1982), whose test results also agree with our own, gave an exposure of 5 hours at -4°C as being sufficient for the total prevention of larvae-hatching, whereas only one hour at this temperature does not prevent either the hatching of eggs or the subsequent development of larvae, even though the mortality rate is high.

As regards C. cephalonica it was observed for both groups of eggs that mortality is equal or higher than 94% at 2°C, after 24 hours of exposure, at -2°C after 18 hours, at -5°C after 9 hours; finally at -10°C a time of exposure of 9 hours for eggs 6-20 hours old and 3 hours for the ones 30-48 hours old are needed. Our evaluation of the ability of the eggs of C. cephalonica to resist low temperatures was investigated above all in function of the possibility of breeding Trichogrammatidae spp. in the eggs of this species. Singh (1969) reported that a temperature of -4°C maintained for a period of 16-76 hours would lead to total egg

mortality. Torre Callejas and Diaz Azpiazu (1980) report variations in hatching depending upon the age of the eggs. In particular, 11 days at +5°C or 2 hours at -10°C are sufficient to kill 24 hour-old eggs, whereas 15 days or 3 hours at the same temperatures are necessary to obtain the total mortality of eggs that are already 48 hours old.

Taken as a whole, the data obtained demonstrate that the eggs of these insects possess a marked resistance to low temperatures. In addition, it should be borne in mind that the values given here were obtained by exposing eggs to cold without the protection of the intervening layers of foodstuffs which would undoubtedly normally hamper diffusion of cold within the packaging. On this subject, Mullen and Arbogast (1979) showed, in tests carried out on limited quantities of foodstuffs (0,76 mc) of varying granulometries, that exposure for even 160 hours would be needed to reach an equilibrium between the external and internal temperatures of the mass. Since the time needed to achieve the mortality of the insect eggs must be added to this preparatory period, which will lengthen progressively for increased quantities of food, it is obvious that the application of cold will only be economically feasible when dealing with raw materials having a very high marketable value, such as cocoa, almonds, hazelnuts, or confectionary products ready for consumption.

Key words: Lepidoptera Phycitidae, eggs, cold-hardiness.

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EVALUATION DE LA RESISTANCE AUX BASSES TEMPERATURES DES OEUFS D'*EPHESTIA CAUTELLA* (WALK) *EPHESTIA KUEHNIELLA* ZELL., *PLODIA INTERPUNCTELLA* HB ET *CORCYRA CEPHALONICA* (STAINT.)

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RESUME

On a étudié la résistance au froid de quatre teignes de produits stockés exposés à des températures basses (aux environs ou au-dessus de 0° C). Des essais de laboratoire ont été menés sur des oeufs d'âge différents (allant de 6 à 20 heures et de 30 à 48 heures), conservés à +2° C, -2° C, -5° C, -10° C (± 0,5° C) et 70 ± 10 % HR, pour 3, 9, 18, 24 et 48 heures. L'expérience comporte cinq répétitions de cinq témoins (200 oeufs) de chaque groupe à chacun des niveaux de température et à différentes durées d'exposition.

A +2° C il n'y a pas eu de diminution prononcée du nombre d'éclosion des oeufs de *E. kuehniella* après 48 heures d'exposition, tandis qu'on obtenait une mortalité de 80 % des oeufs de *P. interpunctella* et plus de 95 % chez les deux autres espèces. L'éclosion était inférieure à 5 % chez toutes les espèces sauf pour *E. kuehniella* (85 % d'éclosion) après 18 heures d'exposition à -2° C. A -5° C, une exposition de 3 heures a été suffisante pour obtenir une inhibition totale de l'éclosion de *P. interpuntella* tandis qu'une exposition de 9 heures aboutissait à une mortalité de plus de 95 % chez *E. cautella* et *C. cephalonica*. A -10° C, aucune éclosion n'a été mentionnée chez *E. cautella* et *C. cephalonica* après 9 heures d'exposition, tandis qu'une exposition de 24 heures a provoqué une mortalité à peine supérieurec a 50% chez *E. kuehniella*. Il a fallu 48 heures pour inhiber complètement l'éclosion de ces espèces.

Les oeufs les plus âgés se sont montrés plus résistants, même si des différences sensibles entre les deux groupes n'ont pas toujours été observées. Ces résultats montrent que *P. interpunctella* est plus sensible aux températures inférieures à 0° C, tandis que *E. kuehniella* présente une plus grande résistance au froid.