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Low Temperature as an Alternative to Fumigation for Disinfesting Stored Products

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Abstract: To develop a quick and safety alternative quarantine treatment for postal item or hand baggage to methyl bromide fumigation, the mortality effects of low temperature exposure to −18℃ were investigated on all developmental stages of six stored-product insects, Granary weevil, *Sitophilus granarius* (LINNE), Rhodesian bean seed beetle, *Callosobruchus rhodesianus* (PIC), Mexican bean seed beetle, *Zabrotes subfasciatus* (BOHEMAN), khapra beetle, *Trogoderma granarium* EVERTS, Indian meal moth, *Plodia interpunctella* (HUBNER) and Mediterranean flour moth, *Ephestia kuehniella* ZELLER. The most cold tolerant stage was egg stage in all insects tested except *T. granarium*. The estimated time for each species to produce 99% kill of the egg stage were 143.8, 124.1, 151.7, 71.5 and 290.7 min for *S. granarius*, *C. rhodesianus*, *Z. subfasciatus*, *P. interpunctella* and *E. kuehniella*, respectively. In *T. granarium*, the cold treatment at −18℃ did not provide rapid kill, especially the larval stage. Thus, the low temperature around at −18℃ was considered as an available quarantine treatment for stored products imported by postal matter.
Key words: Insecta, low temperature, stored product, quarantine treatment

Introduction

Imported agricultural commodities infested with stored-product insects are mostly subjected to methyl bromide fumigation under the plant quarantine law in Japan even they are postal matters or hand baggages. Few owners go through the receipt procedures and the others resign since it usually takes about 3 - 7days to post or return the fumigated products to their owners.

Since methyl bromide was regarded as an ozone depleting substance in the 4th Meeting of Montreal Protcol in November 1992, it is urgently required to develop the alternatives to methyl bromide for disinfestation of quarantine pests (CHAMP, 1998). Japan should make an effort to reduce the quantity of methyl bromide for plant quarantine treatment.

Numerous studies have been made on the influence of low temperature on stored-product insects and found that sub-freezing temperatures provided rapid kill (ADLER, 1960; SWAIN, 1975; JOHNSON and WOFFORD, 1991; FIELDS, 1992; DONAHAYE, *et al.*, 1995). FIELDS (1992) reported that supercooling point was useful because it caused rapid death on insects in a matter of minutes.

Therefore, in the present study, the efficacy of cold temperature at −18℃ was investigated on six important stored-product insects, Granary weevil, *Sitophilus granarius* (LINNE), khapra beetle, *Trogoderma granarium* EVERTS, Indian meal moth,

Plodia interpunctella (HUBNER) and Mediterranean flour moth, *Ephestia kuehniella* ZELLER, which were regarded as most cold-tolerant species (FIELDS, 1992) and Rhodesian bean seed beetle, *Callosobruchus rhodesianus* (PIC) and Mexican bean seed beetle, *Zabrotes subfasciatus* (BOHEMAN), which susceptibilities were not reported, in order to develop an alternative quarantine treatment with swift and safety for postal item or hand baggage to methyl bromide fumigation.

Materials and Methods

Test insects

Sitophilus granarius

Eggs, larvae, pupae and adults of *S. granarius* were obtained from a laboratory culture maintained on wheat and yeast under the rearing conditions, 25℃, 70% r.h. and a photoperiod of 16:8h (L:D). Culture obtained from Australia in 1993 (Import permit No.5Y2258) was used.

Callosobruchus rhodesianus and *Zabrotes subfasciatus*

All stages of both species were obtained from laboratory cultures maintained on small red bean under the rearing conditions as described above. Cultures were obtained from South Africa in 1978 (Import permit No.52Y599) and the Argentine Republic in 1976 (Import permit No.51Y1897) for *C. rhodesianus* and *Z. subfasciatus*, respectively.

Trogoderma granarium

This species was reared on wheat-flour under the rearing conditions as described above. Culture was obtained from the Sudan in 1976 (Import permit No.51Y1897).

Plodia interpunctella and *Ephestia kuehniella*

These species were reared on a medium of wheatbran, yeast and glycerine under the rearing conditions as described above.

Preparation of stages for exposure to treatment

S. granarius

Egg stage (0-4 days after oviposition), 3 larval stages (early-, mid- and late-aged; 8-12 days, 16-21 days and 24-28 days after oviposition), pupal stage (30-34 days after oviposition) and adult stage were exposed to treatment.

About 1,500 adults were allowed to oviposit on the wheat (70 g) in a plastic cage (90×180×45mm) for 4 days. A mixtured medium of 15 g wheat infested with eggs and 35 g uninfested wheat was placed into a plastic Petri dish (15 mm in height, 90 mm in diameter) and then either treated immediately for egg stage, or held under the rearing conditions described above until treatment for larval and pupal stages.

For adult stage, 100 individuals with uninfested wheat (50 g) in a Petri dish were exposed to treatment.

C. rhodesianus and *Z. subfasciatus*

Egg stage (0-4 days after oviposition in *C. rhodesianus* and 0-6 days after oviposition in *Z. subfasciatus*), 3 larval stages (early-, mid- and late-aged: 6-10 days, 12-16 days and 19-23 days after oviposition in *C. rhodesianus*; 6-10 days, 11-15 days and 17-21 days after oviposition in *Z. subfasciatus*), pupal stage (27-31 days after oviposition in *C. rhodesianus* and 22-26 days after oviposition in *Z. subfasciatus*) and adult stage were exposed to treatment.

About 400 adults were allowed to oviposit on the beans (70 g) in a plastic cage for 4-6 days. A determined quantity of beans (7 g beans infested with eggs and 48 g uninfested beans) was placed into a Petri dish and then either treated immediately for egg stage, or held under the rearing conditions until treatment for larval and pupal stages.

For adult stage, 100 individuals with uninfested beans (55 g) in a Petri dish were treated.

T. granarium

Two egg stages (young and mature egg; 0-7 days and 8-14 days after oviposition), 3 larval stages (early-, mid- and late-aged; 21-28 days, 35-42 days and 49-56 days after oviposition), pupal stage and adult stage were exposed to treatment.

About 500 adults were allowed to oviposit on the wheat-flour (70 g) in a plastic cage for 7 days. A determined quantity of medium (14 g wheat-flour infested with eggs and 6 g uninfested wheat-flour) was placed into a Petri dish and then either treated immediately for egg stage, or held under the rearing conditions until treatment for early-aged of larval stage.

For the other stages, 100 individuals with uninfested medium (20 g) in a Petri dish were treated.

Plodia interpunctella and *Ephestia kuehniella*

Egg stage (0-3 days after oviposition), 2 larval stages (early- and late-aged: 11-13 days and 18-20 days after oviposition in *P. interpunctella*; 12-14 days and 23-25 days after oviposition in *E. kuehniella*), pupal stage and adult stage were exposed to treatment.

About 150 moths were placed inside oviposition jars (280 mm in height, 130 mm in diameter) with mesh (49 squares per cm^2) and allowed to oviposit for 3 days. Eggs laid within the jar fell through the mesh into a Petri dish. In a Petri dish, 150 eggs were placed with wheatbran (20 g) and then either treated immediately for egg stages, or held under the rearing conditions until treatment for early-aged of larval stage.

For late-aged of larval stage and pupal stage, 100 individuals with uninfested medium (20 g) in a Petri dish were treated.

Twenty adults in a polyethylene cup (40 mm in height, 100 mm in diameter) were treated.

Low temperature treatment

Experiments were conducted with cold chamber (Sanyo MDF-U441) set at −18℃ and the exposure time was 30, 60, 90, 120, 150, 180 and 210 minutes depending on the test insects. Starting time of the treatment means that the Petri dish or polyethylene cup with insects were placed inside the cold chamber. Air temperature inside the chamber and the temperature of infested stored product in the Petri dish or cup were measured with calibrated thermistor probes. Temperature recordings were taken every minute during the treatment with a data stocker (Shinyei TRH-DM3).

Assessment and statistical analysis

Following treatment, egg, larval and pupal stages in the Petri dishes were held under the rearing conditions until assessment. Storage period was determined from the preliminary developmental examination. The number of newly emerged adult was recorded since the criterion for survival was transition to adult stage irrespective of stage (from egg to pupa) and species. For adult stage, assessment was carried out within 1 day after treatment.

Probit analysis of time-mortality relationships was done using the program of POLO-PC (LeOra Software, 1987).

Results and Discussion

The susceptibilities of six stored-product insects to −18℃ with corrected mortalities are shown in Table 1-6. Estimated exposure time to produce 50% and 99% kill (LT_{50} and LT_{99} level) for egg stage of each test insect are shown in Table 7 although chi-square values showed a poor fit of data by probit analysis model.

In *S. granarius*, the egg was the most cold tolerant stage since newly emerged adults were obtained from eggs treated for 90 min. However, the other stages were completely killed by 90 min after placement inside cold chamber set at −18℃ (Table 1). The LT_{99} of egg stage was 143.8 min (Table 7).

In *C. rhodesianus* and *Z. subfasciatus*, the egg was the most tolerant stage and there was no significant difference in the sensitivity to −18℃. The LT_{99} of *C. rhodesianus* was 124.1 min and that of *Z. subfasciatus* was 151.7 min (Table 2, 3 and 7). Both species were killed within 150 min.

T. granarium was the most tolerant species among 6 species tested. The egg and larval stages, especially larval stages, were hard to kill for short time exposure (Table 4 and 7). Additionally, when late-aged of larval stage was treated for further exposure, treatment for 360 min provided 72.4% mortality and even for 1,440 min (24h) provided 82.3% mortality (data are not shown in Table 4).

P. interpunctella egg was more susceptible than *E. kuehniella* egg, in which the LT_{99}, for the former was 71.5 min and for the latter was 290.7 min (Table 5, 6 and 7). Similar response to −18℃ was observed in the other stages between these two species.

Cooling rate of cold treatment affects the mortality of stored-product insects (BAUST and ROJAS, 1985). In our study, the temperature of infested medium in the

Table 1. Corrected mortality of *S. granarius* to −18℃

Stage	No. of insects[1]	Exposure time (min)			
		30	60	90	120
Egg[2]	405	48.2	70.1	97.5	100
Larva(early-aged)[2]	389	75.1	100	100	100
Larva(mid-aged)[2]	401	78.6	100	100	100
Larva(late-aged)[2]	406	65.0	97.5	100	100
Pupa[2]	395	44.8	78.7	100	100
Adult[3]	300	8.7	100	100	100

1) Total number of insects in 3 replicates.
2) Corrected mortality(%)=
100−(No. of adults from treatment / No. of adults from control×100).
3) Corrected mortality(%)=
100−(Survival ratio in treatment / Survival ratio in control×100).

Table 2. Corrected mortality of *C. rhodesianus* to −18℃

Stage	No. of insects[1]	Exposure time (min)					
		30	60	90	120	150	180
Egg[2]	473	14.8	53.5	98.3	99.8	100	100
Larva(early-aged)[2]	522	11.3	93.5	99.6	100	100	100
Larva(mid-aged)[2]	383	0	78.9	100	100	100	100
Larva(late-aged)[2]	483	0	49.5	100	100	100	100
Pupa[2]	519	0	57.4	100	100	100	100
Adult[3]	300	23.0	68.1	100	100	100	100

1) Total number of insects in 3 replicates.
2) Corrected mortality(%)=
100−(No. of adults from treatment / No. of adults from control×100).
3) Corrected mortality(%)=
100−(Survival ratio in treatment / Survival ratio in control×100).

Table 3. Corrected mortality of *Z. subfasciatus* to −18℃

Stage	No. of insects[1]	Exposure time (min)					
		30	60	90	120	150	180
Egg[2]	396	26.0	57.3	99.8	99.8	100	100
Larva(early-aged)[2]	323	19.5	72.5	100	100	100	100
Larva(mid-aged)[2]	323	27.6	83.3	100	100	100	100
Larva(late-aged)[2]	323	18.9	88.5	100	100	100	100
Pupa[2]	316	19.3	55.4	100	100	100	100
Adult[3]	300	23.0	96.6	99.0	100	100	100

1) Total number of insects in 3 replicates.
2) Corrected mortality(%)=
100−(No. of adults from treatment / No. of adults from control×100).
3) Corrected mortality(%)=
100−(Survival ratio in treatment / Survival ratio in control×100).

Table 4. Corrected mortality of *T. granarium* to −18℃

Stage	No. of insects[1]	Exposure time (min)						
		30	60	90	120	150	180	210
Egg(young)[2]	128	0	27.3	60.9	83.6	69.2	85.4	89.2
Egg(mature)[2]	117	10.3	19.7	59.8	96.6	96.4	100	100
Larva(early-aged)[2]	100	3.0	0	12.0	90.0	74.0	100	100
Larva(mid-aged)[2]	300	0	16.0	42.0	81.7	30.7	41.0	53.0
Larva(late-aged)[2]	300	2.4	27.9	53.7	72.4	27.2	37.1	51.4
Pupa[2]	400	0	0	14.6	50.3	94.7	98.1	98.1
Adult[3]	300	5.4	14.9	62.2	71.8	100	100	–

1) Total number of insects in 3 or 4 replicates.
2) Corrected mortality(%)=
100−(No. of adults from treatment / No. of adults from control × 100).
3) Corrected mortality(%)=
100−(Survival ratio in treatment / Survival ratio in control × 100).

Table 5. Corrected mortality of *P. interpunctella* to −18℃

Stage	No. of insects[1]	Exposure time (min)			
		30	60	90	120
Egg[2]	360	21.1	95.3	100	100
Larva(early-aged)[2]	346	76.9	100	100	100
Larva(late-aged)[2]	300	84.5	100	100	100
Pupa[2]	300	97.3	100	100	100
Adult[3]	300	100	100	100	100

1) Total number of insects in 3 replicates.
2) Corrected mortality(%)=
100−(No. of adults from treatment / No. of adults from control × 100).
3) Corrected mortality(%)=
100−(Survival ratio in treatment / Survival ratio in control × 100).

Table 6. Corrected mortality of *E. kuehniella* to −18℃

Stage	No. of insects[1]	Exposure time (min)						
		30	60	90	120	150	180	210
Egg[2]	346	18.6	49.5	61.7	70.0	97.1	99.2	99.5
Larva(early-aged)[2]	420	74.5	100	100	100	100	100	100
Larva(late-aged)[2]	300	89.3	99.7	100	100	100	–	–
Pupa[2]	300	37.3	46.8	91.0	100	100	–	–
Adult[3]	300	99.7	100	–	–	–	–	–

1) Total number of insects in 3 replicates.
2) Corrected mortality(%)=
100−(No. of adults from treatment / No. of adults from control × 100).
3) Corrected mortality(%)=
100−(Survival ratio in treatment / Survival ratio in control × 100).

Table 7. Estimated LT_{50}, LT_{99} and 95% C.L. for egg stage of six stored-product insects

Species	LT_{50} (min)	LT_{50} conf.limits	LT_{99} (min)	LT_{99} conf.limits
S. granarius	33.8	–	143.8	–
C. rhodeshianus	49.4	38.3 – 61.1	124.1	92.5 – 243.6
Z. subfasciatus	46.4	34.8 – 56.7	151.7	110.9 – 287.9
T. granarium (young)	87.7	65.2 – 108.2	376.9	250.3 – 960.2
T. granarium (mature)	71.9	51.4 – 95.1	215.3	140.8 – 843.6
P. interpunctella	37.5	36.3 – 38.8	71.5	66.8 – 77.6
E. kuehniella	62.2	41.1 – 80.7	290.7	187.3 – 838.6

Petri dish declined from initial temperature (25℃) to target temperature (−18℃) with the cooling rate 1.23, 1.03, 1.26 and 1.28℃/min for wheat, small red bean, wheat-flour and wheatbran, respectively. Comparison of the response of each species was allowed with LT_{99} in spite of the different cooling rate due to the difference of medium except between *C. rhodesianus* and *Z. subfasciatus*, and between *P. interpunctella* and *E. kuehniella*. The most tolerant stage and species to −18℃ were *T. granarium* larva, *T. granarium* egg (LT_{99} was 376.9 min), *E. kuehniella* egg (290.7 min), *Z. subfasciatus* egg (151.7 min), *C. rhodesianus* egg (124.1 min), *S. granarius* egg (143.8 min) (Table 7). This result fully recomfirmed the difficulty to kill or control *T. granarium* in short exposure by low temperature as MATHLEIN (1961) reported that a certain percentage of the larval stage were able to survive exposure at −19℃ for 10 days. The other swift treatment should be used to this species when it is intercepted by plant quarantine. While, the most susceptible stage and species was *P. interpunctella* adult.

In our result except *T. granarium*, the most tolerant stage was mostly egg, however, FIELDS (1992) reported that eggs were usually the most cold-susceptible stage. The probability indicates that cold tolerant stage depends on the target temperature. *Tribolium castaneum* adult was more tolerant than egg at −6℃, however, the egg was more tolerant than adult at −12℃ (NAGEL and SHEPARD, 1934). Similar phenomenon that the tolerant stage varied by temperature was reported in *Ephestia cautella*, in which the tolerant stage was shifted around at −10℃ (DONAHAYE, *et al.*, 1995)

Making direct comparisons of cold tolerance of stored-product insects on the published studies, in which the target temperature was close to −18℃, are difficult since the experimental methology consisted of many factors such as initial temperature, acclimation and target temperature etc. were not uniformity (BACK and COTTON, 1924; ADLER, 1960; MATHLEIN, 1961; SWAIN, 1975; MULLEN and ARBOGAST, 1979; JOHNSON and WOFFORD, 1991; DONAHAYE, *et al.*, 1995). A variety of factors such as temperature duration of exposure, species, stage of development,

froze (supercooling point) of *P. interpunctella* larva and *S. granarius* adult. The supercooling point of *P. interpunctella* was −10.3℃ and that of *S. granarius* was −15.7 ℃ in their experiments of which initial temperature were 23℃ closed to our initial temperature (25℃). Their results seem to be in support of our data that *S. granarius* was more cold tolerant than *P. interpunctella*. The coincidence of response to cold between LEE *et al.*(1992) and ours may stimulate measuring the cold tolerance of stored-product insect with supercooling point as an indicator of the cold-hardiness. Actually, in early studies of insect cold hardiness, it was often assumed that the supercooling point was equivalent to a measure of the lethal temperature (LEE, 1991). However, supercooling point was affected by acclimation, developmental stage, age within a single developmental stage, food and feeding/starvation (SMITH, 1970; LEE, *et al.*, 1992; FIELDS, 1992; CHAUVIN and VANNIER, 1997). Nevertheless measuring the supercooling point might be useful for comparing the cold tolerance of stored-product insects and disinfesting stored products by low temperature in quarantine treatment as well as CHAUVIN and VANNIER (1997) measured the supercooling point at all stages of the webbing clothes moth, *Tineola bisselliella* with the aim of finding parameters for destroying the insect by freezing infested material.

In our study, the response of 6 insects to −18℃ from an initial temperature at 25 ℃ was shown. Further mortality data with variable initial temperature i.e. 15, 20 and 30℃, needs to be investigated since the temperatures of stored products imported by postal matter or hand baggage depend on the season. Accumulating the mortality data at low temperatures will contribute to clarify the range of target temperature and stored-product insects (species, stage and diapausing stage) in order to develop low temperature treatment as a quick and safety quarantine treatment.

Literature Cited

ADLER, V. E. (1960) Effects of low temperatures on the eggs of the Angoumois grain moth, the Indian-meal moth, and the confused flour beetle. *J. Econ. Entomol.* **53**: 973-974.

BACK, E. A. and R. T. COTTON (1924) Relative resistance of the rice weevil *Sitophilus oryzae* L., and the Granary weevil, *S. granarius* L., to high and low temperatures. *J. Agric. Res.* **28**: 1043-1044.

BAUST, J. G. and R. R. ROJAS (1985) Review − Insect cold hardiness: facts and fancy. *J. Insect Physiol.* **31**: 755-759.

CHAMP, B. R. (1998) Alternative chemicals for methyl bromide. Report of the 5th JIRCAS international symposium on postharvest technology in Asia. 9-10 Sep. 1998, Tsukuba, Japan.

CHAUVIN, G. and G. VANNIER (1997) Supercooling capacity of *Tineola bisselliella* (Hummel) (Lepidoptera: Tineidae): its implication for disinfestation. *J. Stored Prod. Res.* **33**: 283-287.

DONAHAYE, E. J., S. NAVARRO and M. RINDNER (1995) Low temperature as an alternative to fumigation for disinfecting dried fruit from three insect species. *J. Stored Prod. Res.* **31**: 63-70.

EVANS, D. E. (1983) The influence of relative humidity and thermal acclimation on the survival of adult grain beetles in cooled grain. *J. Stored Prod. Res.* **19**: 173-180.

JOHNSON, J. A. and P. L. WOFFORD (1991) Effects of age on response of eggs of Indianmeal moth

and Navel Orangeworm (Lepidoptera: Pyralidae) to subfreezing temperatures. *J. Econ. Entomol.* **84**: 202-205.

FIELDS, P. G. (1992) The control of stored-product insects and mites with extreme temperatures. *J. Stored Prod. Res.* **28**: 89-118.

LEE, R. E. Jr. (1991) Principles of insect low temperature tolerance. In: LEE, R. E. Jr. and D. L. DENLINGER (eds), Insects at low temperature. Chapman and Hall, New York, pp17-46.

LEE, R. E. Jr., J. M. STRONG-GUNDERSON, M. R. LEE and E. C. DAVIDSON (1992) Ice-nucleating active bacteria decrease the cold-hardiness of stored grain insects. *J. Econ. Entomol.* **85**: 371-374.

LeOra Software (1987) POLO-PC.

MATHLEIN, R. (1961) Studies on some major storage pests in Sweden, with special reference to their cold resistance. Meddn. St. Vaxtsk Anst., Contribution **12:83**, 1-49.

MULLEN, M. A. and R. T. ARBOGAST (1979) Time-temperature-mortality relationships for various stored-product insect eggs and chilling times for selected commodities. *J. Econ. Entomol.* **72**: 476-478.

NAGEL, R. H. and H. H. SHEPARD (1934) The lethal effect of low temperatures on the various stages of the Confused flour beetle. *J. Agric. Res.* **48**: 1009-1016.

SMITH, L. B. (1970) Effects of cold-acclimation on supercooling and survival of the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Cucujidae) at sub-zero temperatures. *Can. J. Zool.* **48**: 853-858.

SWAIN, W. R. (1975) Cold tolerance in relation to starvation of adult *Rhyzopertha dominica* (Coleoptera: Bostrichidae). *Can. Ent.* **107**: 1057-1061.

和 文 摘 要

低温処理による貯穀害虫6種の殺虫試験

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郵便物及び携帯品で輸入される少量の穀類等に対する短時間消毒方法を確立する目的で，6種の貯穀害虫（グラナリアコクゾウムシ *Sitophilus granarius*，ローデシアマメゾウムシ *Callosobruchus rhodesianus*，ブラジルマメゾウムシ *Zabrotes subfasciatus*，ヒメアカカツオブシムシ *Trogoderma granarium*，ノシメマダラメイガ *Plodia interpunctella*，スジコナマダラメイガ *Ephestia kuehniella*）の卵，幼虫，蛹及び成虫を供試して，低温処理（−18℃）に対する感受性を調査した。

1) ヒメアカカツオブシムシについては，−18℃による低温短時間殺虫は困難であると考えられた。

2) ヒメアカカツオブシムシを除く5種では，全ての種において，卵が他のステージよりも低温耐性が高かった。

3) プロビット解析で卵のLT_{99}を推定した結果，スジコナマダラメイガでは290.7分間処理が必要であり，次いでブラジルマメゾウムシ（151.7分間），グラナリアコクゾウムシ（143.8分間），ローデシアマメゾウムシ（124.1分間），ノシメマダラメイガ（71.5分間）の順であった。

以上の結果から，郵便物検疫において，低温処理による消毒方法の導入は，消毒に要する日数の短縮及び害虫の分散防止の両面で，現状の消毒手続きを改善できると考えられた。