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THE USE OF LOW TEMPERATURES AND ICE-NUCLEATING BACTERIA AGAINST STORED-PRODUCT INSECT PESTS

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SUMMARY

Cold temperatures have been used for many years to control populations of stored-product insects. The aim of aeration was primarily to cool down the grain to prevent its deterioration by reducing the number of insects without eliminating the whole population.

The present study was undertaken to determine the survival at low temperature of non-cold-acclimated and cold-acclimated insects, and to evaluate the potential of using the ice-nucleating-active bacteria *Pseudomonas syringae* to increase the susceptibility of stored-product insects to cold treatments. We have choose to work with the granary weevil *Sitophilus granarius* (L.) and the saw-toothed grain beetle *Oryzaephilus surinamensis* (L.). They are both the most frequent stored-grain pests in Belgium.

Considering both species, *S. granarius* adults were more cold-hardy than *O. surinamensis*, but *O. surinamensis* adults compensated their cold-sensibility by a great ability to acclimate. *S. granarius* is able to survive the winter in Belgium because of its cold-hardiness, and *O. surinamensis* too because of its ability to acclimate to low temperatures. Application of 100 or 1000 ppm of *P. syringae* in wheat increased the mortality of the two species tested at -10°C (24-h exposure). In the saw-toothed grain beetle, all adults died after treatment with 100 ppm, whereas nearly all granary weevils died to 1000 ppm.

Different ways to increase the ice-nucleating activity of *P. syringae* are considered.

INTRODUCTION

Low temperatures have been used for many years to control populations of stored-product insects. The aim of aeration was primarily to cool down the grain and then to prevent its deterioration by reducing the number of insects but not to eliminate the whole population. In Belgium, the mild winters enable insects to survive to the next season. It is for this reason that the present study of the possibility of killing insects by aeration has been undertaken.

Physical methods of control such as aeration are indispensable to reduce the resistance to insecticides wich increases the cost of chemical control and as well as pesticides quantity in the environment. To evaluate the potential of using low temperatures as physical method of control, we have choose to work with the granary weevil *Sitophilus granarius* (L.) (Coleoptera: Curculionidae) and the saw-toothed grain beetle *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae). They are both the most frequent pests of stored-grains in Belgium. Letellier et *al.* (1994) mentioned that these two species make up more than 70% of insects infesting bins and elevators in Belgian farms.

The present study was undertaken, firstly to determine the survival of adult *Sitophilus granarius* (L.) and *Oryzaephilus surinamensis* (L.) at low temperatures, secondly, to assess the influence of thermal acclimation on their survival and, thirdly, to consider the potential of using the ice-nucleating-active bacteria *Pseudomonas syringae* as a means of increasing the susceptibility to cold treatments of stored-product insect pests.

MATERIALS AND METHODS

Insect cultures

The same strain of *S. granarius* (Av2:Anvers-Belgium) was used in the three experiments. Because of changes in the availability of laboratory stocks, two strains of *O. surinamensis* were used in the three groups of experiments: respectively OOt (Oteppe-Belgium) for the first experiment and OPe (Perwez-Belgium) for both other experiments.

Cultures of *S. granarius* and *O. surinamensis* were established with initials densities of about 250 adults, respectively per 500 g of wheat grains and 65 g of rolled oats. Both species were reared at 27±1°C at 70±5% RH.

Ice-Nucleating-Active Bacteria

The source of ice nucleators is a commercial product currently used to produce artificial snow. It was provided by Snomax Technologies Division of Genecor International (Rochester, N.Y., U.S.A.). To obtain this potent source of ice nucleators, *Pseudomonas syringae* (strain 31a), a common foliar bacterium isolated from corn leaves, is grown under conditions that maximize its ice-nucleating activity. Bacteria are then concentrated, freeze-dried, killed with electron beam irradiation and milled to a fine powder (Fields, 1993). The live end product has been shown to be non-toxic (acute oral LD50 for rats greater than 5 g/kg) and non-pathogenic to mammals or plants (Goodnow and *al.*, 1990a,b).

Low temperature survival

Schedules of survival were established for different temperatures for *S. granarius* (0-5-10-15-20°C) and *O. surinamensis* (0-5-10°C). 50 non-cold-acclimated insects were placed in ventilated 300 ml jars containing 250 g of wheat for both species. The number of surviving insects under each temperature regime was determined at various times. For each observation there were four replicates. The sexes of the beetles were not determined.

Laboratory cold acclimation

To compare the cold-hardiness of different cold-acclimated insects, *S. granarius* and *O. surinamensis* were placed at nine different cold acclimation temperature regimes (Table 1) before being tested for cold-hardiness by placing insects at 5°C for 2, 4 and 6 weeks, warming them up (30°C for 24 hours) and assessing their mortality.

Table 1: Cold acclimation temperature regimes tested on *Sitophilus granarius* and *Oryzaephilus surinamensis*.

Acclimation regimes	Temperature regimes
1.1	3 weeks at 25°C
1.2	3 weeks at 15°C
1.3	3 weeks at 10°C
1.4	1 week at 20°C, 1 week at 15°C and 1 week at 10°C
2.1	6 weeks at 25°C
2.2	6 weeks at 15°C
2.3	6 weeks at 10°C
2.4	3 weeks at 15°C et 3 weeks and 10°C
3.1	1 week at 20°C, 3 weeks at 15°C and 3 weeks at 10°C

For each cold-acclimation-temperature regime, 600 beetles of each species, in twelve groups of 50, were held in ventilated 300-ml jars containing wheat for *S. granarius* and rolled aots for *O. surinamensis*. After acclimation and transfer at 5°C, mortality after 2, 4 and 6 weeks was observed on 200 beetles.

Field cold acclimation

One bin containing 300 kg of wheat was infested in May 1994 by 4000 *S. granarius* and 4000 *O. surinamensis*. From October onwards, 200 *S. granarius* and 200 *O. surinamensis* were taken monthly and transferred at 5°C for 6 weeks. After this period, their mortality were observed.

Effect of *P. syringae* on low temperature survival

To evaluate the effect of ice-nucleating-active bacteria on the cold tolerance of insects, 50 beetles of the two species were added to 200 g of wheat inoculated respectively with 100 and 1000 ppm *P. syringae* and held at 30°C for 24 hours. The treatment doses are expressed as the ratio of the weight of dry powdered *P. syringae* to the weight of grain. There were 4 replicates of each treatment. The samples were transferred at -10°C for 24h and back again at 30°C for 24 h. The proportion of dead insects was then determined. The experiment was done three times for *S. granarius* and two times for *O. surinamensis*.

RESULTS

Low temperature survival

In grain cooled at 10°C, about 90% of the beetles of both species survived after 13 weeks (Fig. 1 and 2). More than 90% of *S. granarius* were still alive after 17 weeks. The mortality rates at this temperature were very low. They were higher at all other temperatures. After 7 weeks at 25°C, the mortality rate of *S. granarius* was about 65%. For both species, the mortality of adults at 5°C after 7 weeks was higher than 90%.

O. surinamensis adults were more sensitive to low temperature than *S. granarius*. At 0°C, no adults of *O. surinamensis* survived 2 weeks, and 6 weeks for *S. granarius*. A much higher percentage of adult granary weevils than adult saw-toothed grain beetles survived after 5 weeks at 5°C.

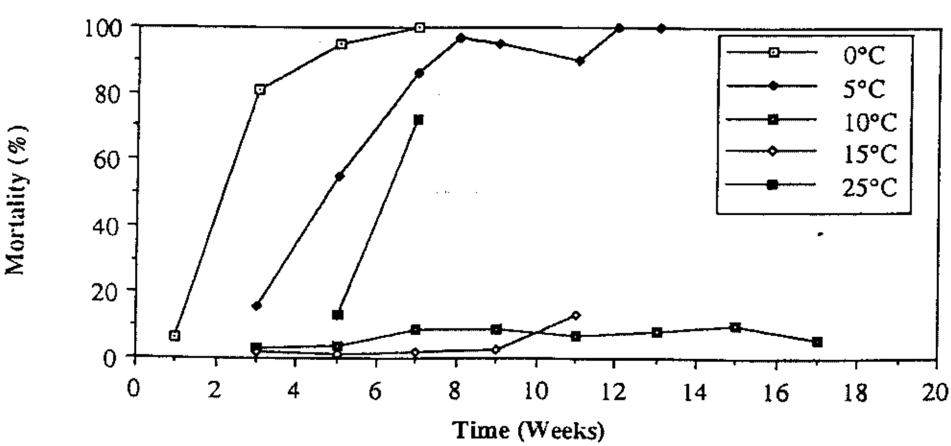

Figure 1: The mortality of *Sitophilus granarius* at five different temperatures

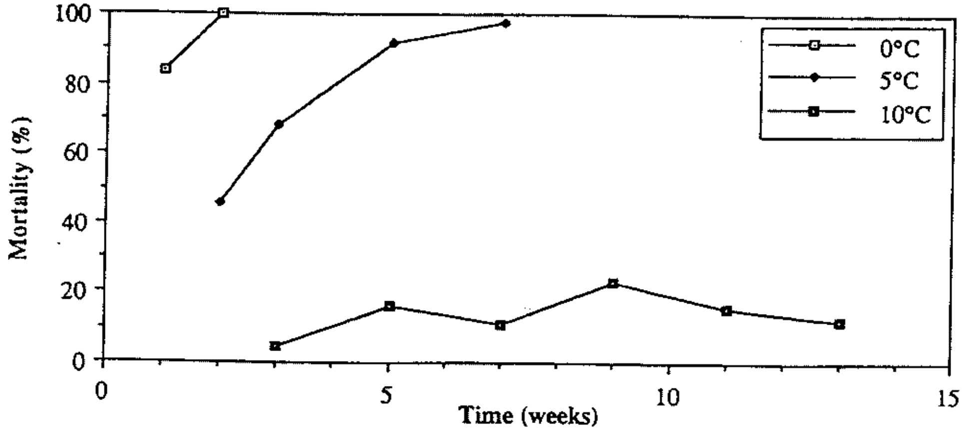

Figure 2: The mortality of *Oryzaephilus surinamensis* at three different temperatures

Laboratory cold acclimation

Figure 3 indicates a significant reduction in mortality of *S. granarius* after the 3.1-cold-acclimation-temperature-regime. After one week at 20°C, three weeks at 15°C and three weeks at 10°C (3.1-regime), the ability of adults to survive exposure at 5°C was increased (about 30% mortality after 6 weeks). For 1.1 and 2.1-regimes, no adults survived an exposure of 6 weeks at 5°C. Those regimes were considered as non-acclimated samples (control).

Figure 3 (below) shows that various acclimation regimes (1.2-1.3-2.2-2.4-regimes) allowed for acclimating of *O. surinamensis* (about 30% mortality after 6 weeks'exposure at 5°C). Therefore *O. surinamensis* adults are more able and more easy to acclimate than *S. granarius* adults.

Field cold acclimation

The percentages of individuals of each species dying at 5°C after 6 weeks are shown in Figure 4. There was no difference between the mortality of both species during the October-December period. After this period, the mortality rates of *O. surinamensis* adults progressively decreased. However, there was no variation of the mortality rates of *S. granarius*.

These observations result from acclimation occured in *O. surinamensis*.

Effect of *P. syringae* on low temperature survival

Of the untreated insects of the control, only *S. granarius* exhibited low mortality rates (Table 2). *O. surinamensis* had a high level mortality (99,4%) for one of the replicates. 100 or 1000 ppm of *P. syringae* in weath increased the mortality of the two species tested at -10°C. In the saw-toothed grain beetle, all adults died in the treatment with 100 ppm, whereas hardly any granary weevils survived to 1000 ppm.

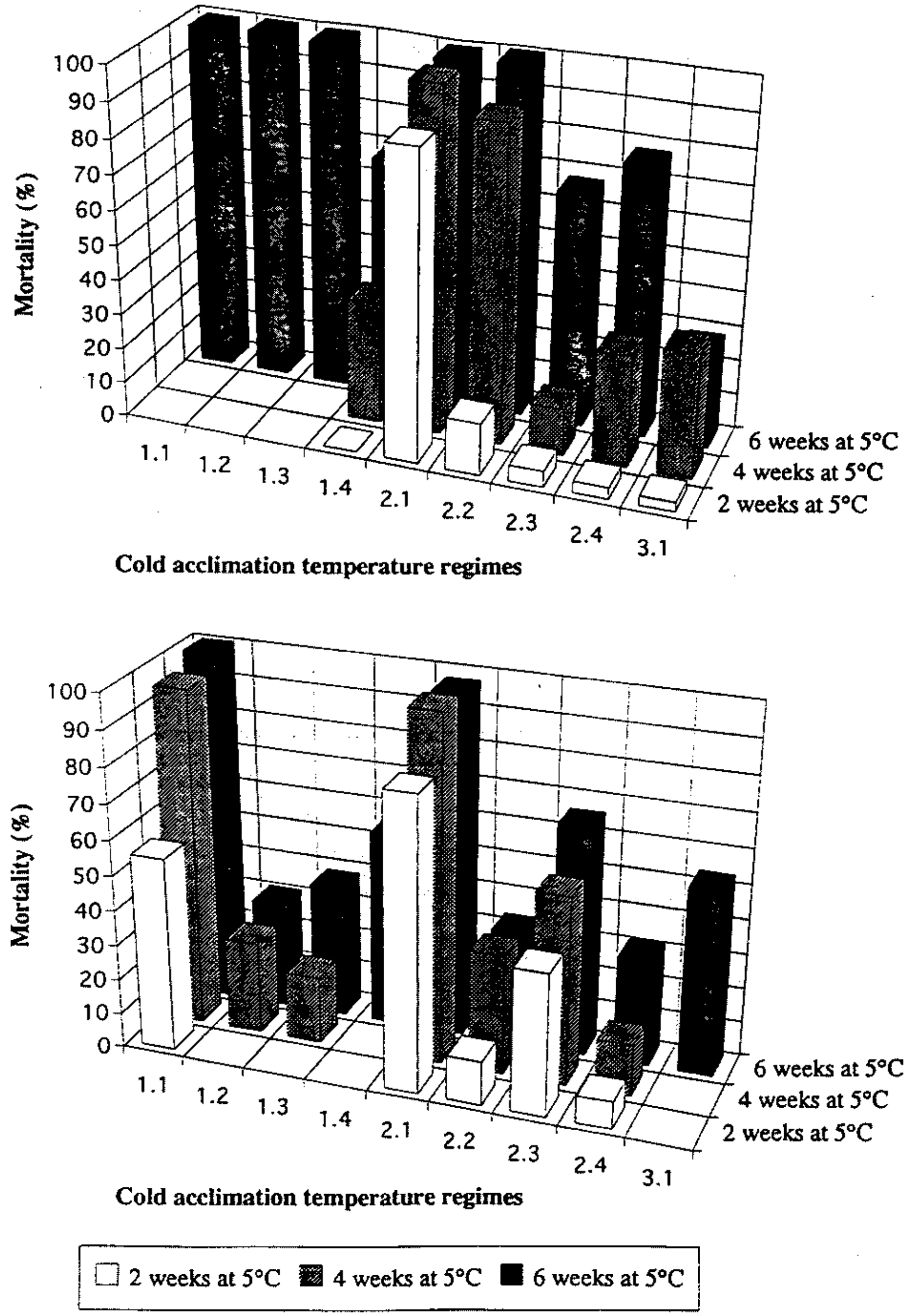

Figure 3: The mortality of laboratory-cold-acclimated *Sitophilus granarius* (above) and *Oryzaephilus surinamensis* (below) exposed at 5°C for 2, 4 and 6 weeks.

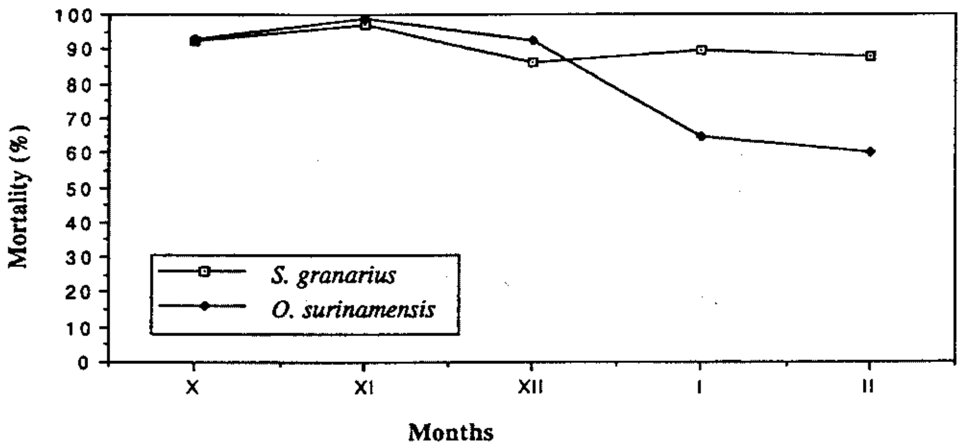

Figure 4: The mortality of *Sitophilus granarius* and *Oryzaephilus surinamensis* acclimated to cold in the field and transferred at 5°C for 6 weeks.

Table 2: The mortality of *Sitophilus granarius* and *Oryzaephilus surinamensis* exposed to wheat contaminated by various concentrations of dry powdered *Pseudomonas syringae* after an exposure of 24 h at -10°C.

	Sitophilus granarius			*Oryzaephilus surinamensis*		
Replicates	0 ppm	100 ppm	1000 ppm	0 ppm	100 ppm	1000 ppm
I	24,1	89,1	100	31,4	100	100
II	13,0	92,5	100	99,4	100	100
III	39,8	64,5	92,7	No Essays	No Essays	No Essays

DISCUSSION

The use of low temperatures to control insects in stored grain is not a new idea. Our strategy for control consists in using the ice-nucleating-active bacteria as biological insecticide. Ingestion or topical application of *P. syringae* cause a rapid and significant increase in the supercooling point of insects (Strong-Gunderson and *al.*, 1990 and 1992). In practice, these microorganisms would increase winter mortality by decreasing the effectiveness of natural mechanism of supercooling (Lee and *al.*, 1993).

The magnitude of the increase in mortality is related to the concentration of bacteria and differs among insects(Lee and *al.*, 1992). Lethal temperature varies considerably and depends on the species, stage of development, acclimation and relative humidity (Evans, 1983; Fields, 1992). In the first experiment, survival at low temperatures differed between species, being shortest in *O. surinamensis* (100% mortality at 0°C after 2 weeks) and longest in *S. granarius* (100% mortality at 0°C after 7 weeks). This agrees with the observations of Evans (1983) but contradicts the results of Armitage and Llewellin (1987). This variation may be ascribed to the strain of insects. The proportion of insects dying at 10°C was very small (<20% after 3 months for both species).

Cold-hardiness in insects is affected by many factors. One of the most important is the degree of cold acclimation (Fields, 1990). Survival at low temperatures differed, sometimes considerably, between non-cold-acclimated insects and cold-acclimated insects. A temperature of 15°C was very important in acclimation process. This temperature was used successfully by different authors (Smith, 1990) (Evans, 1981) (Ohtsu and *al.*, 1993) (Fields, 1993).

The lowering of mortality rates reported earlier (in laboratory and field acclimations) indicated that exposure to gradually falling temperatures increased the cold tolerance of both species. The manifestation of this increased cold tolerance in terms of survival was studied by comparing the survival at 5°C of insects cooled gradually to that temperature with the survival of insects transferred from 25°C to 5°C. The increased cold tolerance was also studied by comparing the survival at 5°C between both insects cold-acclimated in field conditions. Only one cold-acclimation-temperature-regime (3.1) increased survival of *S. granarius*. On the other hand, 4 acclimation-regimes permitted a significant decrease in mortality of *O. surinamensis*. This species are more able to acclimate than *S. granarius*. This is confirm with the observations realised in a experimental bin. Mortality at 5°C decreased from around 90% to 60% for *O. surinamensis* and stayed permanent (around 90%) for *S. granarius*.

Considering both species (acclimated and non-acclimated), *O. surinamensis* adults compensated their cold-sensibility by a great ability to acclimate.

In Belgium, the mild winters enable both *S. granarius* and *O. surinamensis* to survive to the next season. The use of the ice-nucleating-active bacteria *Pseudomonas syringae* could increase their winter mortality. In the last experiment, survival at -10°C differed among species: *O. surinamensis* adults were more sensible to cold temperatures and to the bacteria. It was related to the concentration of the bacteria. *S. granarius* and *O. surinamensis* adults exposed to grain treated with the ice-nucleating bacteria suffered a higher mortality than the controls when exposed to -10°C.

Different ways to increase the ice-nucleating activity of the bacteria can be considered: the use of other strains, the purification of the ice-nucleating proteins linked to the bacterial membrane and the use of proteinase inhibitors to decrease the digestion of the ice-nucleating proteins into the gut of stored-product Coleoptera. Optimization of the culture medium is also essential for the production of a large biomass of high ice-nucleating-active bacteria (Blondeaux and Cochet, 1994a, b).

To summarize, this study shows that survival at low temperatures differed, sometimes considerably, between species, strains and between both cold-acclimated and non-cold-acclimated species. Future research should focus on determining the rôle of those factors on effectiveness of the ice-nucleating-active bacteria *Pseudomonas syringae*, and on how increase this effectiveness.

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RESUME

Lutte contre les insectes ravageurs des denrees entreposees a l'aide des basses temperatures et utilisation de bacteries glacogenes

Dans les denrées entreposées, le contrôle des ravageurs fait principalement appel aux pesticides. Leur utilisation répétée depuis plus de 30 ans a entraîné l'apparition d'individus résistants à tous les groupes d'insecticides utilisés. Les méthodes de lutte physique (principalement par le froid) sont à développer.

En Belgique, les deux ravageurs les plus fréquemment retrouvés dans les centres de stockage sont *Sitophilus granarius* et *Oryzaephilus surinamensis*. Une étude comparative de l'effet des basses températures sur ces deux espèces a permis de mettre en évidence l'importance des phénomènes d'acclimatation chez *Oryzaephilus surinamensis*.

Les conditions hivernales rencontrées dans notre pays semblent insuffisantes à elles seules pour éradiquer complètement les insectes présents dans les centres de stockage. L'utilisation d'une bactérie synergiste des basses températures (*Pseudomonas syringae*) permettrait de rendre efficaces les méthodes de lutte physique par le froid.