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Low temperature tolerance of *Plodia interpunctella, Sitophilus oryzae and Sitophilus zeamais* - the prevalent pests of stored maize in Serbia

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Abstract: Insect's bionomics and development are highly dependent on the environmental temperature. For centuries, this fact has been used for the control of storage pests. However, the temperature threshold depends on the species, life stage, acclimation and exposure period. This work assessed the effects of low temperatures (4, -4, -10, -15 and -18°C) and exposure period (10, 30, 60, 120 and 180 min) on the survival and development of *Plodia interpunctella* larvae, and adults of *Sitophilus oryzae* and *S. zeamais*, the prevalent maize pests in Serbia. Data were analysed using one-way and two-way ANOVA. Additionally, Probyt analysis was performed to determine the LT_{50} and LT_{99}. The first significant effects were recorded at -4°C for *S. oryzae* when the mortality was 41% after 120 and 52% after 180 min of exposure. At -10°C, the mortality of *S. zeamais* was significant after 180 min (52.5%) and increased with the exposure period. The significant mortality of *P. interpunctella* larvae was at -15°C after 10 min (55.5%). At -18°C, *P. interpunctella* larvae were the most susceptible and 98% of mortality was recorded after 10 min, while 77.5% of *S. oryzae* and 68% of *S. zeamais* was recorded after 10 min. Two-way ANOVA showed that both factors (temperature and exposure) significantly affected the mortality of tested species, but the first factor was the most influential. These results indicate that temperature and exposure period should be adjusted to specific pest, while in combined infestations the temperature should be adjusted to the most tolerant one.

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Introduction

Insect pests are a major factor of post-harvest losses of stored commodities over the world. The estimates on the post-harvest losses caused by insects are variable. According to FAO (2010), losses that result from poor post-harvest management, including insect infestations, were estimated at 20–30% and represent an important constraint to improving food security. APHLIS (2011) and Cao *et al*. (2002) reported the potential losses of 20-80% within a few months after harvest unless insects are controlled. Many authors emphasise that insects are prevalent pests of stored maize in Africa and Asia and can cause losses of up to 30% after six months of storage (Affognon *et al*., 2015; Boxall, 2002). In Serbia, maize is an important staple crop, cultivated on around 901.700-1.060.000 ha (Statistical Office of the Republic of Serbia, 2019), with stable annual production. However, in storages, maize is threatened by several pests which cause significant losses, both qualitative and quantitative. Major pests of stored maize in Serbia are Indian meal moth (*Plodia interpunctella* L.), rice and maize weevil (*Sitophilus oryzae* (L.) and *Sitophilus zeamais* (Motschulsky). *P. interpunctella* is a secondary pest that causes direct damages by feeding on germinal part of kernels (Vukajlović *et al*., 2017; Gvozdenac *et al*., 2018). Also, it causes the reduction of grain quality due to the presence of exuviae, feces, webbing, cadavers etc. The second two species are classified as the most important primary pests of stored maize, whose adults damage the whole grain. Their larvae inhabit and feed inside the kernels (Rees, 2004; Beckett *et al*., 2007), causing reduction of grain quantity, quality and production of a considerable amount of dust and frass. The most significant damages and losses occur in storages of high category of seeds, when both primary and secondary pests negatively affect germination, resulting in total loss of seeds value.

The control of storage pests is inevitable measure that ensures rentability and profitability of maize production. In storages, insects are mainly controlled by chemically synthetisized fumigants (phostoxine), seed treatments with insecticides (a.i. pyrimiphos methyl, deltamethrin, tefluthrin etc.), as well as by spray applications of different insecticidal products in empty storages and storages with packed goods. Due to the rising concern over the use of conventional pesticides in terms of health and environmental hazards, insect resistance and potentially restricted use of these compounds in the near future (WMO, 1994), there is a growing interest in alternative means of controlling storage pests and disinfesting food in storages (Fields, 1992). The most applied non-chemical techniques are aeration, use of diatomaceous earth or ash as seed and surface treatments, modified atmospheres and storing in insecticide-treated seed bags (De Groote *et al*., 2013; Tefera *et al*., 2011). Additionally, one of the most promising bio-rational pest management methods is the application of high and low temperatures. According to Stjeskal *et al*. (2019), temperature is one of the key

environmental factors affecting the physiological, life history, behavioral and population processes of arthropods. The use of extreme, particularly low temperatures, has been extensively used to control stored-product insects due to several advantages, like no residues, efficacy against insecticide resistant strains and low risks for operators (Fields, 2001). However, there are some restraints that limit widespread adoption of this method and large-scale applications. For instance, the treatment is dependent on cold ambient air or refrigeration equipment and the duration of treatment is longer that with high temperatures, including high energy costs. The success of cold treatment depends on a number of factors such as species, developmental stage of target insect, the level of cold acclimation, temperature attained, exposure and relative humidity. Subramanyam *et al*. (2011) emphasised that time of exposure to high temperatures is an important factor that has to ensure the penetration of heat in cracks in floor or wall spaces and into any processing equipment in order to kill insects that may be hiding. This principle can also be applied in low temperature treatments.

Artificial cooling or freezing has been described as a mean of pest control by various authors decades ago (David *et al*., 1977; Evans, 1987; Hagstrum and Flinn, 1994; Dohino *et al*., 1999). Freezing dry stored products between - 10°C and - 20°C is an option for rapid disinfestation of high-value goods. For example, experiences from Germany show that, herb tea and spices producers have been successfully using 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 can drop as low as -90°C. In Japan, Nakakita and Ikenaga (1997) reported that the commercial use of low temperature storage of rice has enabled the reduction of conventional fumigants since 1991. Nowadays, the method of grain preservation by cooling has been used worldwide, primarily in developed countries while in underdeveloped countries its use is not so spread, due to expensive power source and construction of storage facilities. However, there is increasing use of cooling treatment of dry grains by applying stationery or mobile devices of «Granifrigor» type in Serbia and neighbouring countries, as Salha *et al*. (2010) reported for Croatia.

According to Stjeskal *et al*. (2019), low temperatures affect both the individual and population development and all physiological and behavioral activities. It is well known that with decreasing/increasing temperature, insects' development gradually slows down, and at a certain point stops completely. Although the influence of temperatures on insect bionomics, relationship between the developmental rate and temperature as well as the minimal temperature requirements have been well elaborated by several authors (Honek and Kocourek, 1990; Kiritani, 1997; Jarosik *et al*., 2011) and generally it has validity for all arthropods, there are significant differences in the absolute values of specific minimum and maximum thresholds. The knowledge about pests thermal requirements, especially threshold temperatures at which development and other activities of harmful species cease (development, flying, walking, reproduction, respiration etc.), is of crucial importance for the protection of stored products and for the food industry in general (Stjeskal *et al*., 2019). In practice, the knowledge on the

specific thresholds must be implemented in order to achieve efficient cooling treatments and successfully disinsect commodities.

As stated by Stjeskal *et al*. (2019), the research on the effects of temperature on insects has been documented since the 18th century, providing ample scientific results that give substantial insights into the physiology, ecology distribution of certain pests as well as a background for many practical applications, such as pest monitoring and control. However, this author has identified the problem of the high variability of already existing data for identical species (referring to *P. interpunctella* in particular) which introduces uncertainty. This fact indicates the necessity of conducting studies to complete the missing data and to validate already existing but highly variable data under various conditions.

This work aimed to contribute to the above-mentioned goal, by assess the tolerance, namely the survival thresholds, of three predominant pests of stored maize (*P. interpunctella*, *Sitohpilus oryzae* and *S. zeamais*), to low temperatures depending on the exposure period.

Material and methods

Insect cultures

The most prevalent pests of maize were used in this study: Indian meal moth (*P. interpunctella* L.), rice weevil (*Sitohpilus oryzae* L.) and maize weevil (*S. zeamais* Motschulsky). Specimens of all three species originate from laboratory populations reared in the Laboratory for phytopathology at Sunflower department, Institute of Field and Vegetable Crops, Novi Sad, Serbia.

P. interpunctella laboratory population was reared for 10 generations on a Standard laboratory diet (SLD) for this pest (Silhachek and Miller, 1972) in an environmental growth chamber at 27±2°C, 65% relative humidity (RH), and 14:10 h L:D photoperiod, in Laboratory for phytopathology, at Institute of Field and Vegetable Crops, Novi Sad, Serbia. *S. oryzae* parenteral population was maintained in glass jars (2.5 L) on wheat kernels (variety Simonida) for one year, while *S. zeamais* was reared on dent type maize kernels (NS 640). Kernels were not treated with insecticides.

This work tested the susceptibility and tolerance levels of larval stage (mature larvae L_4, L_5) of *P. interpunctella* and Sitophilus weevils to different low temperatures, at different exposure periods. Mature instars were used because they were hypothetically less vulnerable to extreme environmental conditions (Johnson *et al*., 2003).

Experimental conditions and protocol

Test was carried out in different laboratory freezers and refrigerators, providing desirable temperatures. In order to minimize the loss of cold air when opening the door, the freezing chamber had been sealed with a plastic sheet leaving enough space to fit in the tray with test samples and data loggers for temperature recording.

Insects were subjected to following temperatures: 4°C; -4°C; -10°C; -15°C and -18°C and exposure periods: 10, 30, 60, 120 and 180 min. Insects were placed in Petri

dishes (50 specimens per dish) and exposed to each temperature, without seeds. After the given exposure period, the samples were removed from the freezer or refrigerator and transferred to a temperature-controlled chamber at 25°C and 65±5% RH to be checked for survival two days after the exposure. Each temperature–time combination was replicated four times, while the control Petri dishes that were kept at room temperature (25°C and 65±5% RH.), were used to determine the natural mortality of insects.

The tolerance to low temperatures was assessed based on the mortality of test insects. The data of mortality were presented as percentage, corrected for natural mortality in untreated controls according to Abbott (1925).

Statistical analyses

Results on the mortality, after Abbott's correction, were processed with one-way ANOVA, using Bonferroni test, to test the differences between the mortality among treatments. Additionally, two-way ANOVA was used to determine the influence of two factors on the mortality of different factors (temperature, exposure) and their interaction (temperature*exposure) on mortality of *P. interpunctella* larvae, *S. oryzae* and *S. zeamais* weevils.

The exposure–mortality data of each species were subjected to Probyt analysis for estimating the time required to kill 95% and 99% (LT_{50} and LT_{99}) of the exposed insects at corresponding temperature.

Statistical analysis was performed in SPSS19, software (IBM SPSS, 2017).

Results

The mortality (%) of *P. interpunctella* larvae, *S. oryzae* and *S. zeamais* weevils after the exposure to low temperatures are presented in Table 1. The first effects of low temperatures were visible at -4°C, when specimens of all three species were immobile after 180 min of exposure. However, the mortality at this temperature was recorded only for *S. oryzae* after 60 min of exposure (23.5%) and next two exposure periods, 120 and 180 minutes (41.0, and 51.0%, respectively). At -10°C, the highest mortality of *P. interpunctella* larvae was at the longest exposure, 180 min (36.5%). Sitophilus weevils were less tolerant to temperature of -10°C, so the 30 min exposure caused 18.0 and 19.0% mortality (*S. oryzae* and *S. zeamais*, respectively). At longer exposure periods, it ranged from 22.5 – 67.0% for *S. oryzae*, and 19.0 – 55.2% for *S. zeamais*. The differences between mortality among different test insects, at specific exposure periods (10, 30, 60, 120 and 180 min) is highly significant (F=661.32**, 448.7**, 1408.7**, 398.7**, $p<0.01$, respectively).

The highest mortality after all exposure periods was recorded for *S. oryzae*. Temperature of -15°C caused high mortality of all tested species. For *P. interpunctella* larvae it ranged from 55.5% at 10 min exposure to 100% after 180 minutes. *S. oryzae* mortality was 78.0% at lowest exposure (10 min) up to 100% after 80 min. *S. zeamais* mortality ranged from 68.0% to the total (100%) after 180 min. Significant difference in

mortality between species at -15°C was after 10, 30 and 60 min of exposure (F=138.6*, 154.8*, 19.2*, p<0.05, respectively).

Table 1. Mortality of *P. interpunctella* larvae, *S. oryzae* and *S. zeamais* weevils at different low temperatures depending on the exposure period

Temp (°C)	Exposure	*P. interpunctella*	*S. oryzae*	*S. zeamais*	F value
4	10 min	0 ±0.00 a	0 ±0.00 a	0 ±0.00 a	0.00ns
	30 min	0 ±0.00 a	0 ±0.00 a	0 ±0.00 a	0.00ns
	60 min	0 ±0.00 b	2.5 ±0.50 a	0 ±0.00 b	12.00*
	120 min	2.5 ±0.50 b	7.0 ±1.00 a	1.5 ±0.50 b	22.45*
	180 min	7.1 ±1.90 a	6.5 ±1.50 a	0 ±0.00 b	64.50*
-4	10 min	0 ±0.00 a	0 ±0.00 a	0 ±0.00 a	0.00ns
	30 min	0 ±0.00 b	6.5 ±1.5 a	0 ±0.00 b	108.0**
	60 min	0 ±0.00 b	23.5 ±4.5 a	0 ±0.00 b	765.6**
	120 min	1.0 ±0.00 c	41.0 ±3.00 a	25.5 ±4.50 b	2127.9**
	180 min	8.50 ±1.5 c	51.0 ±5.00 a	32.5 ±2.50 b	2765.0**
-10	10 min	0 ±0.00 a	6.0 ±0.00 a	0 ±0.00 a	7.70*
	30 min	3.0 ±0.00 b	18.0 ±3.00 a	19.0 ±1.00 a	661.32**
	60 min	3.0 ±0.00 b	22.5 ±5.50ba	19.0 ±2.00 a	448.7**
	120 min	12.5 ±3.5 c	47.5 ±0.50 a	31.5 ±1.50 b	1408.7**
	180 min	36.5 ±4.50 c	67.0 ±3.00 a	52.5 ±3.50 b	398.7**
-15	10 min	55.5 ±2.50 c	78.0 ±4.00 a	60.0 ±2.00 b	138.6*
	30 min	77.5 ±3.50 b	82.0 ±4.00 a	76.5 ±2.50 b	154.8*
	60 min	90.0 ±7.00 a	92.0 ±0.00 a	84.0 ±7.00 b	19.2*
	120 min	96.5 ±7.50 a	94.5 ±6.50 a	93.0 ±2.00 a	2.22ns
	180 min	100 ±0.00 a	100 ±0.00 a	100 ±0.00 a	0.00ns
-18	10 min	98.0 ±0.00 a	77.5 ±8.50 b	68.0 ±3.00 b	351.9**
	30 min	100 ±0.00 a	88.5 ±5.50 b	81.0 ±5.00 b	659.1**
	60 min	100 ±0.00 a	100 ±0.00 a	100 ±0.00 a	0.00ns
	120 min	100 ±0.00 a	100 ±0.00 a	100 ±0.00 a	0.00ns
	180 min	100 ±0.00 a	100 ±0.00 a	100 ±0.00 a	0.00ns

At -18°C, larvae of *P. interpunctella* were the least tolerant, hence the mortality was 98.0% after the shortest exposure (10 min) and 100% after 30 min and longer exposure. Mortality of *S. oryzae* was 77.5% after 10 min and 88.5% after 30 min. *S. zeamais* was the most tolerant to low temperatures. At -18°C, after 10 min the mortality was 60.0%, and 81.0% after 30 min. The differences between the mortality of three species was highly significant only after the first two exposure periods, 10 and 30 min (F=351.9**, 659.1**, $p<0.05$, respectively).

The Probyt analysis (Table 2) determined the lethal period needed to achieve 50 and 99% of mortality (LT_{50} and LT_{99}) of *P. interpunctella* larvae, *S. oryzae* and *S. zeamais* weevils at each applied temperature. As presented, the shortest period at 4ºC since the mortality was too low, the Probyt calculation was not relevant, therefore was not put in the Table. In other temperature treatments, it took the shortest time, at each temperature to kill 50% of *S. oryzae* population compared to other two species, while at -18ºC, the time need to achieve the mortality of 50% of *P. interpunctella* was 1.9 min and for *S. zeamais* (2.2 min) did not differ significantly. Lethal time for 99% mortality depended on the temperature (Table 2).

Table 2. LT_{50} and LT_{99} at different temperatures for *P. interpunctella* larvae, *S. oryzae* and *S. zeamais* weevils

Insect species		**Temperature**				
		4 °C*	**-4 °C**	**-10 °C**	**-15 °C**	**-18 °C**
P. interpunctella	**LT50**	x	542.1	296.3	9.2	**1.9**
	LT99	x	978.6	306.0	190.50	12.80
S. oryzae	**LT50**	x	**159.6**	**122.4**	**4.3**	**2.1**
	LT99	x	394.1	309.1	23.5	15.6
S. zeamais	**LT50**	x	216.7	190.7	7.4	6.8
	LT99	x	655.8	356.4	16.3	13.2

*Due to very low mortalities (0-7.1%) it was impossible to calculate the relevant LT values, therefore these values were omitted from the table

Two-way ANOVA determined the influence of two factors (temperature and exposure) and their interaction (temperature*exposure) on the mortality of *P. interpunctella* larvae, *S. oryzae* and *S. zeamais* weevils. The results presented in Table 3 indicate that both factors and their interaction significantly affected mortality of all three species. However, based on the values of the Sum of Squares, the most influential factor in all cases was the temperature, followed by exposure period and interaction.

Table 3. The influence of different factors (temperature, exposure) and their interaction (temperature*exposure) on the mortality of *P. interpunctella* larvae, *S. oryzae* and *S. zeamais* weevils

Insect species	Source	SS	df	F
P. interpunctella	Corrected Model	144947.580[a]	24	35.75**
	temperature	134531.613	4	**199.089****
	exposure	4151.813	4	**6.14****
	temperature * exposure	6264.153	16	**2.31***
S. oryzae	Corrected Model	113750.880[a]	24	8463.61**
	temperature	98419.680	4	**43937.35****
	exposure	10680.780	4	**4768.20****
	temperature * exposure	4650.420	16	**519.02****
S. zeamais	Corrected Model	117391.380[a]	24	3650.23**
	temperature	104947.980	4	**19579.85****
	exposure	8668.380	4	**1617.23****
	temperature * exposure	3775.020	16	**176.07****

Df – degrees of freedom; SS – Type III Sum of Squares

Discussion

Back in 1934, Nagel emphasized that in order to obtain the most economical combination of the temperature and exposure for storage pest control, it is necessary to obtain actual data on the time required to kill a certain insect species at a certain low temperature. Driven by this thought, this work assessed the low temperature tolerance of three most important pests of stored maize, *P. interpunctella* larvae, *S. oryzae* and *S. zeamais* weevils, at different exposure period. The idea derives from the fact that in practise, the joint infestations are a common scenario in maize storages in Serbia, and it is difficult to choose the adequate temperature for cold treatment that could effectively reduce the population of all three species.

Typically, stored product insects are more tolerant to temperature treatments than other insects because their habitats and are less prone to sudden temperature change (Mullen and Arbogast, 1979). According to Lewthwaite *et al*. (1997), the amount, method and rate of temperature, exposure, acclimation and relative humidity can influence insect response application (Fields, 1992; Brokerhof and Banks, 1993). Additionally, other factors such as diet, population density, developmental stage and gender may affect temperature tolerance of individual insects (Cline, 1970; Fields, 1992). This work proved that weevils of *S. zeamais*, as thermophilic species, were the most susceptible to low temperatures, followed by *P. interpunctella* larvae. Additionally, according to Fields (1992) and Stjeskal *et al*. (2019), the survival of extremely low temperatures by stored product beetle pests is associated with their cold

acclimation, the relative humidity of the air and the commodity moisture content. Extremely low temperatures (i.e., frost) are known to kill sensitive and non-acclimatized pests in hours or days. Extreme temperatures are rapidly effective but can be economically and technologically demanding. However, it was recognized that prolonged exposure to suboptimal temperatures may provide substantial levels of control within weeks or months (Beckett, 2011).

According to Fields (2001), larvae of Lepidoptera species (moths) that infest stored products can only penetrate a few centimetres into the grain, therefore are exposed to some of the most extreme temperatures in the grain bulk. Nevertheless, Lepidoptera are some of the most cold-hardy insects among storage pests. Adler (2010) reported that at -10°C, *P. interpunctella* was controlled at the longest exposure time of 8 h. Stage and cold acclimation can greatly increase the cold tolerance. Under similar conditions, Fields and Timlick (2010) found that fifth instars of *P. interpunctella* had 98% mortality after 48 h at -10°C. For cold acclimated diapausing larvae of *P. interpunctella*, after 14 days, the mortality was 88%. In this work, we did not use diapausing or acclimated larvae, which made them more susceptible to low temperatures. However, the longest exposure to this temperature lasted only 180 min and caused mortality of 36.5%, but according to Probyt analysis, it would take 306.0 min (5.1 hours) to reach 100% mortality of this pest. At -18°C, larvae of *P. interpunctella* were controlled after an exposure time of 30 min in study performed by Adler (2010), while in our study, after 10 min of exposure, it was 98.0%, and after 30 min, total mortality was achieved. Johnson *et al*. (1997) report that postharvest storing of dried fruits and nuts at low temperatures, is one of the alternatives to methyl bromide fumigation for controlling *P. interpunctella*. Long-term exposure to 10°C lengthened the life of adults; 50% mortality was reached after 49 days of exposure, adult mortality reached 90% after 70 days of exposure. Exposure to 10°C for greater than 25 days reduced egg production by more than half and reduced the number of viable eggs by nearly 90%. Eggs were most susceptible to 10°C; the exposure time estimated to obtain 95% egg mortality was 11.6 days. Based on these data it was estimated that clean product that has been under storage at 10°C and undisturbed for at least 4 weeks should be relatively free of this pest (Johnson *et al*., 1997). In our work, we assessed only the effect of much lower temperatures but on larvae of *P. interpunctella*. According to Probyt analysis it would take 1.58 days (2278.1 min) at 4°C to reduce the population for 50 %, while to achieve 99% of mortality it would take 3.93 days (5670.0 min).

In our work, *S. zeamais* was the most tolerant species to all applied temperatures and exposure periods. Salha *et al*. (2010) reported that by cooling stored maize grain at 6^{o}C showed different mortality of *S. zeamais* depending on exposure (days). Mean mortality value of 100% maize weevils was obtained after 43 days. According to results of mortalities from out study, the closest applied temperature was 4^{o}C. At this temperature, the LT values were not calculated because the mortalities were too low to obtain relevant results. However, the reduction of population at this temperature is very slow, as reported by Salha *et al*. (2010).

The situation in maize storages is usually complex and involves the presence of several species at the same time. Therefore, the application of physical control measures, especially low temperatures, must be carefully conceptualized, because each species has the temperature optimum conditioned by its origin (temperate regions, continental regions etc.), evolution and ecology. Hence, the lower developmental threshold and/or lethal temperatures for one species can be sub-lethal to other species, making it more acclimatized and tolerant. Also, it must be considered that some species, like *P. interpunctela* feeds outside the kernels, while weevil species develop inside, thus the kernel provides certain physical barrier. All the mentioned indicates that the temperatures and exposure period used for the purpose of controlling insects should be adjusted to the specific pest present in storage, and in combined infestations, the temperature should be adjusted to the most tolerant one.

Conclusion

This work assessed the effect of low temperatures and exposure period on the survival and development of three prevalent maize pests in Serbia. Tested species were differently susceptible to low temperatures. Depending on the exposure period, the susceptibility is as follows: *S. oryzae* > *P. interpunctella* > *S. zeamais*. This indicates that temperatures and exposure period should be adjusted to the specific pest, while in combined infestations the temperature should be adjusted to the most tolerant one.

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OSETLJIVOST NA NISKE TEMPERATURE *PLODIA INTERPUNCTELLA, SITOPHILUS ORYZAE I SITOPHILUS ZEAMAIS* – DOMINANTNIH ŠTETOČINA USKLADIŠTENOG KUKURUZA U SRBIJI

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Rezime

Razviće insekata je visoko uslovljeno temperaturom životne sredine. Vekovima je ova činjenica korišćena u kontroli brojnosti skladišnih štetočina. Međutim, temperaturni pragovi zavise od vrste, životnog stadijuma, aklimatizacije i trajanja ekspozicije. U ovom radu ispitivani su uticaji niskih temperatura (4, -4, -10, -15 i -18°C) i trajanja ekspozicionog perioda (10, 30, 60, 120 i 180 min) na nivo preživljavanja i razviće larvi *Plodia interpunctella* i imaga *Sitophilus oryzae* i *S. zeamais*, dominantnih štetočina uskladištenog kukuruza u Srbiji. Dobijeni podaci su analizirani korišćenjem one-way i two-way ANOVA. Dodatno, urađena je i Probyt analysis radi utvrđivanja LT_{50} i LT_{99}. Prvi značajni efekti niskih temperature registrovani su na -4°C za imaga *S. oryzae* sa registrovanim uginućem od 41% posle 120 i 52% posle 180 min ekspozicije. Na -10°C uginuće imaga *S. zeamais* bilo je značajno posle ekpozicije od 180 min (52,5%) i raslo je sa trajanjem ekspozicionog perioda. Značajna smrtnost larvi *P. interpunctella* registrovana je na -15°C posle 10 min (55,5%). Na -18°C larve *P. interpunctella* bile su osetljivije i 98% smrtnost registrovana je posle 10 min, a u istim uslovima 77,5% kod imaga *S. oryzae* i 68% kod *S. zeamais.* Two-way ANOVA ukazuje da oba faktora (temperatura i ekspozicija) značajno utiču na smrtnost testiranih štetočina, ali je temperatura uticajniji faktor. Dobijeni rezultati pokazuju da temperatura i ekspozicija predstavljaju manipulativne faktore koje treba prilagoditi vrsti, dok u kombinovanim infestacijama treba ih prilagoditi najtolerantnijoj štetočini.

Ključne reči: skladišne štetočine, bakrenasti plamenac brašna, pirinčani žižak, kukuruzni žižak, tretman niskim temperaturama, osetljivost.