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Effects of 50°C temperature on *Sitophilus* granarius (L.), *Sitophilus oryzae* (L.) and *Sitophilus zeamais* (Motsch.)

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SUMMARY

Adults of laboratory populations of granary weevil (S. granarius), rice weevil (S. oryzae) and maize weevil (S. zeamais) were exposed at the temperature of 50°C to determine lethal time (parameters LT_{20r} , LT_{50} and LT_{99}) and progeny production/reduction in F_1 generation at mentioned temperature. For each exposure and each species 25 adults aged 2-5 weeks, in four replicates, were used. In the first treatment, the insects were exposed for 6, 10, 14, 16, 18, 20, 22, 26 and 30 min in plastic dishes (V=200 cm³) with 1.8 \pm 0.2 g of untreated coarse wheat. In the second treatment, in dishes with 100 g of untreated wheat grain the adults were exposed for 90, 100, 110, 120, 130, 140, 150, 165 and 180 min. After the exposure, the adults were placed in 100 g of untreated wheat grain, in four replicates, at 25 ± 1 °C and 60 ± 5 % r.h., for recovery. After one, two and seven days of recovery, the weevils were separated by sifting of wheat, and the mortality was determined, and after total period of eight weeks from the exposure of parents, the effect on progeny in F_1 generation was determined. Lethal time for weevils was determined by probit analysis, and progeny production/reduction by analysis of variance. After weevils exposure in coarse wheat and after one, two and seven days of recovery, S. oryzae adults were the most susceptible (LT₅₀ 12.48-13.68 min), and the most tolerant were adults of S. granarius (LT₅₀ 17.79-20.89 min). After insects exposure in wheat grain, the most susceptible were S. granarius and S. oryzae (LT₅₀ 107.11-120.73 min), and the most tolerant S. zeamais (LT₅₀ 139.90-155.35 min). After exposure of parents of all three weevil species, in coarse wheat, progeny reduction at 100% level is after 22 min. However, after exposure of parents of all three weevil species in wheat grain, progeny reduction at 100% level in S. granarius is after 130 min, in S. oryzae after 150 min, and at 99.7% level in S. zeamais after 180 min. The investigations indicated that short-term exposure of weevils from Sitophilus genus at the temperature of 50°C adversely affects their survival and progeny production, as well as that there is a potential for its successful use as a physical measure in control of storage pest insects.

Keywords: S. granarius; S. oryzae; S. zeamais; 50°C temperature; Effects

INTRODUCTION

Weevils, Sitophilus granarius (L.), Sitophilus oryzae (L.) and Sitophilus zeamais (Motsch.), are classified in the most important primary pests of stored wheat, whose adults damage grains, and larvae inhabit and feed inside the grain (Rees, 2004; Beckett et al., 2007).

As chemical measures for pest insect control in storages, fumigants and contact (residual) insecticides are stil being used (Subramanyam and Hagstrum, 1996; Kljajić, 2008) as an essential part of integrated pest management. Among fumigants, methyl-bromide (in use until 2015) and phosphin are used, and among contact insecticides organophosphorus compounds (dichlorvos, malathion, chlorpyrifos-methyl and pirimifos-methyl) and pyrethroids (deltamethrin, bioresmetrin and cyfluthrin) are used. However, the problem of residues of contact insecticides, which remain in wheat after application, and altered susceptibility i.e. resistance in mentioned species, are the main limiting factors for their future wide use. Resistance to lindan, malathion, dichlorvos, fenitrothion, chlorpyrifos-methyl, pirimifos-methyl, deltamethrin and cypermethrin was found in S. granarius, in S. oryzae to na lindan, malathion, DDT, fenitrothion, pirimifos-methyl and deltamethrin, and in S. zeamais to lindan, malathion, DDT, fenitrothion, pirimifos-methyl, permethrin, deltamethrin and cypermethrin (Busvine, 1980; Subramanyam and Hagstrum, 1996; Kljajić and Perić, 2005).

Having in mind that insects are poikilotherm organisms, and that their development and survival greatly depend on environmental temperature, high temperatures (as a physical measure) alone, or in combination with some other control measures against storage pests are being used in the last few years (Mahroof et al., 2003a, 2003b; Roesli et al., 2003; Kljajić and Andrić, 2010). Temperature of 50°C is considered to be the most effective (Wright et al., 2002; Mahroof et al., 2003a, 2003b; Roesli et al., 2003), because relatively fast, without use of chemicals and risk of resistance development a total disinfestation is obtained (Beckett et al., 2007). There is a lot of literature data on the effects of high temperatures: on survival of storage insects (Kirkpatrick and Tilton, 1972; Fields, 1992; Kljajić et al., 1996; Beckett et al., 1998), on development of insects (Fields, 1992; Beckett et al., 1998; Wright et al., 2002; Mahroof et al., 2003b; Loganathan et al., 2011), on toxicity of contact insecticides in combined application (Kljajić et al., 2009), as well as on physiological, biochemical, and other consequences that occur when storage insects are exposed to high temperatures (Fields, 1992; Neven, 2000).

The objective of this paper was to investigate and compare the effects of 50°C temperature on S. granarius, S. oryzae and S. zeamais under laboratory conditions, in coarse wheat (1.8 g) and wheat grain (100 g), by determination of lethal parameters (LT $_{20}$, LT $_{50}$ and LT $_{99}$), as well as effects on progeny production/reduction in F_1 generation.

MATERIAL AND METHODS

Test insects

Adults of laboratory populations of *S. granarius*, *S. oryzae* and *S. zeamais* reared in insectarium, according to methods described by Harein and Soderstrom (1966) and Davis and Bry (1985), were used in the study. Weevils, *S. granarius* and *S. oryzae* were reared on soft whole grain wheat, and *S. zeamais* on the whole grain maize, moisture of 12%, in glass jars (volume 2.5 dm³), at $25 \pm 1^{\circ}$ C and $60 \pm 5\%$ r.h.

Adult insects aged two to five weeks in the unknown sex ratio, were used in the experiment.

Bioassay

Effects of 50°C temperature were investigated in two treatments according to adjusted method used by Mahroof et al. (2003b).

First treatment – in plastic dishes ($V=200~cm^3$) 1.8 \pm 0.2 g of untreated coarse wheat grains was added, to avoid direct contact of insects with the dish and too rapid reaction of insects to the heat, i.e. conditions of emty storages were imitated. Exposure intervals of adult test insects were 6, 10, 14, 16, 18, 20, 22, 26 and 30 min.

Second treatment – in plastic dishes (V=200 cm³) 100 g of untreated wheat grain was added with an average temperature of 25 \pm 1°C and moisture content of 11.5 \pm 0.5%. Exposure intervals of adult test insects were 90, 100, 110, 120, 130, 140, 150, 165 and 180 min.

Both investigated treatments were set up in four replicates with 25 insects, for each exposure interval, after which they were put in the incubator (MRC, Israel) at $50 \pm 1^{\circ}$ C and $11 \pm 1\%$ r.h.

After each exposure, the weevils were put in dishes with 100 g of untreated wheat grains, under conditions of 25 \pm 1°C and 60 \pm 5% r.h. for recovery. Weevil mortality was assessed after one, two and seven days of recovery. On the seventh day, all the weevils (alive and dead) were removed by sifting, to determine the effects on progeny production/reduction in F_1 generation after eight weeks of exposure of parents. The same procedure was applied to control weevils except that they were not exposed at 50°C temperature.

Data analysis

The mortality data were corrected for those in controls using the Abbott (1925) formula and were processed by probit analysis according to method described by Finney (1971) using computer software developed by Raymond (1985). Before analysis progeny number in F_1 generation was transformed using log (x + 1) and then were statistically compared using one-way ANOVA and the significance of mean differences between treatments and control was determined by Fisher's LSD test at P < 0.05 (Sokal and Rohlf, 1995). However, untransformed

means and standard errors are shown in the tables. The reduction in progeny size against the control is shown as a percentation (PR %) according to formula used in similar studies by Tapondjou et al. (2002).

RESULTS

The results obtained for lethality (LT₂₀, LT₅₀ and LT₉₉) to weevils after different recovery periods from their exposure at 50° C in coarse wheat are shown in Table 1, and in wheat grain in Table 2.

Table 1. Lethal time (LT) for *S. granarius, S. oryzae* and *S. zeamais* adults after exposure at 50°C in coarse wheat

Species	After recovery (days)	LT ₂₀ (min) FL (0.05)	LT ₅₀ (min) FL (0.05)	LT ₉₉ (min) FL (0.05)	Slope <i>lt-p</i> line (±SE)
S. granarius	1	17.18 (15.74-18.74)	19.67 (18.37-21.04)	28.55 (23.95-34.14)	14.37±2.40
8	2	17.40 (16.17-18.65)	20.89 (19.76-22.07)	34.58 (29.68-40.68)	10.62± 1.15
	7	15.27 (14.07-16.51)	17.79 (16.83-18.78)	27.12 (23.62-31.37)	12.70±1.62
S. oryzae	1	11.46 (9.99-13.06)	13.68 (12.50-14.93)	22.31 (18.93-26.50)	10.96±1.50
	2	10.28 (9.63-10.83)	12.48 (11.97-12.93)	21.29 (20.06-22.98)	10.88 ± 1.28
	7	11.00 (9.72-12.36)	13.15 (12.12-14.23)	21.52 (18.72-25.02)	10.03 ± 0.73
S. zeamais	1	15.38 (13.63-17.32)	17.83 (16.42-19.36)	26.87 (21.63-33.52)	13.07±2.44
	2	13.62 (11.68-15.83)	17.00 (15.38-18.79)	31.42 (25.19-39.44)	8.73±1.30
	7	13.19 (11.41-15.18)	15.56 (14.11-17.14)	24.57 (19.56-31.07)	11.73 ± 2.14

Table 2. Lethal time (LT) for S. granarius, S. oryzae and S. zeamais adults after exposure at 50°C in wheat grain

Cmanian	After recovery	LT_{20} (min)	LT ₅₀ (min)	LT ₉₉ (min)	Slope lt-p
Species	(days)	FL (0.05)	FL (0.05)	FL (0.05)	line (±SE)
S. granarius	1	103.51 (98.65-108.35)	117.39 (113.29-121.61)	166.25 (153.63-180.91)	15.40±1.38
	2	105.62 (99.94-111.46)	119.87 (115.28-124.61)	170.09 (155.46-186.67)	15.31±1.65
	7	101.50 (98.94-103.73)	114.32 (112.38-116.21)	158.87 (153.56-165.62)	16.28±0.93
S. oryzae	1	101.40 (94.50-108.59)	120.73 (115.20-126.49)	195.63 (174.14-220.63)	11.10±1.18
	2	99.87 (91.92-108.31)	117.14 (110.58-124.05)	182.11 (159.86-208.19)	12.14±1.47
	7	89.99 (82.11-98.25)	107.11 (101.00-113.44)	173.34 (152.94-197.68)	11.13±1.30
S. zeamais	1	125.71 (113.75-138.73)	152.98 (141.63-165.26)	263.27 (211.61-328.99)	9.87±1.47
	2	122.85 (112.08-134.33)	155.35 (144.62-166.99)	297.33 (237.80-375.22)	8.25 ± 1.04
	7	106.37 (93.57-120.57)	139.90 (128.31-152.55)	298.43 (225.99-397.39)	7.07±1.00

According to nominal values of lethal parameters at levels LT_{20} , LT_{50} and LT_{99} , realized after exposure in dishes with coarse wheat, regardless recovery period, it can be concluded that *S. oryzae* adults are the most susceptible (LT_{50} 12.48-13.68 min), and the most tolerant are adults of *S. granarius* (LT_{50} 17.79-20.89 min). However, given the confidence intervals for obtained LT values, there are no statistically significant differences among adults of all three weevil species at 50°C. Also, after insects exposure to wheat grain, according to nominal values at LT_{20} ,

 LT_{50} and LT_{99} levels, it can be concluded that *S. granarius* (LT_{50} 114.32-119.87 min) and *S. oryzae* (LT_{50} 107.11-120.73 min) are the most susceptible, and the most tolerant (LT_{50} 139.90-155.35 min) is *S. zeamais*, but the differences, regardless recovery period, are not statistically significant.

After different exposures of parent weevils at 50°C, significant progeny reduction in both tested treatments was recorded, and the results obtained i.e. average number of offsprings and calculated values of progeny reduction (PR %) are shown in Tables 3 and 4.

Table 3. Average number of offsprings (No. \pm SE) and progeny reduction (PR, %) <i>S. granarius</i> , <i>S. oryzae</i> and <i>S. zeamais</i> in F_1
generation after exposure of parents in coarse wheat at 50°C

Exposure	S. granarius		S. oryzae		S. zeamais	
interval (min)	No.±SE	PR (%)	No.±SE	PR (%)	No.±SE	PR (%)
Control	282.3±39.9 a*	_	238.0±6.4 a	-	225.3±41.8 a	-
6	187.0±22.5 a	33.7	233.5±32.3 a	1.9	237.8±37.1 a	-5.5
10	130.0±16.8 a	53.9	131.5±31.4 a	44.7	156.5±23.1 a	30.5
14	89.3±10.0 ab	68.4	43.0±19.1 b	81.9	108.3±43.2 a	51.9
16	48.5±26.7 bc	82.8	9.3±2.8 b	96.1	80.3±27.0 a	64.4
18	22.0±15.2 cd	92.2	1.8±1.8 c	99.3	9.0±5.7 b	96.0
20	3.5±3.5 de	98.8	1.8±1.8 c	99.3	2.5±1.5 bc	98.9
22	0.0±0.0 e	100	$0.0\pm0.0 \; c$	100	$0.0\pm0.0 \; c$	100
26	0.0±0.0 e	100	$0.0\pm0.0 \; c$	100	$0.0\pm0.0 \; c$	100
30	0.0±0.0 e	100	$0.0\pm0.0 \; c$	100	$0.0\pm0.0 \; c$	100

^{*} For each species, means within columns followed by the same letter are not significantly different (p<0.05)

Table 4. Average number of offsprings (No. \pm SE) and progeny reduction (PR, %) *S. granarius*, *S. oryzae* and *S. zeamais* in F_1 generation after exposure of parents in wheat grain at 50°C

Exposure	S. granarius		S. oryzae		S. zeamais	
interval (min)	No.±SE	PR (%)	No.±SE	PR (%)	No.±SE	PR (%)
Control	282.3±39.9 a*	_	238.0±6.4 a	_	225.3±41.8 a	-
90	90.5±19.1 b	67.9	133.5±19.3 ab	43.9	159.3±19.1 ab	29.3
100	52.0±12.5 c	81.6	65.5±5.7 bc	72.5	84.3±18.5 bcd	62.6
110	42.3±11.3 c	85.0	38.0±13.9 c	84.0	123.5±11.9 abc	45.2
120	22.8±6.3 d	91.9	3.8±2.6 d	98.4	15.5±4.6 fg	93.1
130	0.0±0.0 e	100	0.5±0.5 de	99.8	71.0±23.7 cd	68.5
140	0.0±0.0 e	100	2.0±1.7 de	99.2	50.0±19.7 de	77.8
150	0.0±0.0 e	100	0.0±0.0 e	100	12.5±3.9 g	94.5
165	0.0±0.0 e	100	0.0±0.0 e	100	24.5±3.5 ef	89.1
180	0.0±0.0 e	100	0.0±0.0 e	100	$0.8\pm0.8h$	99.7

^{*} For each species, means within columns followed by the same letter are not significantly different (p<0.05)

According to the results shown in Table 3, after 10 min exposure nominally the lowest number of offsprings in all three species of weevil parents in coarse wheat was in *S. granarius* and *S. oryzae* (130.0; 131.5; PR=53.9 and 44.7%), and the highest in *S. zeamais* (156.5; PR=30.5%), but the differences between them were not significant. After exposure of 20 min, progeny reduction was 98.8-99.3% in all three weevil species, and after 22 min of exposure 100%. After 10 min exposure of *S. granarius* parents in coarse wheat at 50°C, the average number of offsprings (130.0) was 2.2-fold lower compared to average number of offsprings in control (282.3), and after 20 min even 80.6-fold lower. In *S. oryzae* after 10 min of parents exposure the average number of offsprings was 1.8-fold lower compared to

control (238.0), and after 20 min even 132.2-fold lower. In *S. zeamais* after 10 min exposure the average number of offsprings was 1.4-fold lower compared to control (225.3), and after 20 min 90-fold lower.

After 90 min exposure of all three species of weevil parents in wheat grain (Table 4), nominally the lowest number of offsprings was recorded in *S. granarius* (90.5; PR=67.9%), about 48% more than in *S. oryzae* (133.5; PR=43.9%), and the highest (80% more) in *S. zeamais* (159.3; PR=29.3%), yet with no significant differences. After 180 min exposure, progeny reduction was high in all three weevil species, 99.7% in *S. zeamais*, and 100% in *S. granarius* and *S. oryzae*. In *S. zeamais* a certain variability in progeny production can be observed, which is not in direct connection with exposure intervals of weevil parents.

DISCUSSION

In general, the results obtained in this experiment are in agreement with results of recent studies on the efficacy of high temperatures in storage insects control, as well as with the claim that temperature of 50°C is minimum effective temperature for direct use in protection of stored products, particularly in empty storage facilities. Although it has to be pointed out that this is a very costly measure with a high risk of damaging equipment and sensitive materials at longer heating intervals (Fields and White, 2002; Beckett and Morton, 2003; Mahroof et al., 2005; Arthur, 2006; Tilley et al., 2007b).

In previous studies (Kirkpatrick and Tilton, 1972) it was found that the most tolerant to high temperatures is S. zeamais, less S. oryzae, and the least S. granarius, which is in agreement with the results obtained in our experiment. Kljajić et al. (1996) found that after 24 h recovery from exposure of *S. granarius* adults, in empty glass dishes covered with Petri dishes, at high temperatures (45, 50, 55 and 60°C), LT₅₀ is 31 min at 50°C, and LT_{95} 46 min. This is about 1.6-fold slower action than in our experiment where S. granarius was exposed in coarse wheat, and about 3.7-fold faster than when granary weevils were exposed in 100 g of wheat grain. Besides use of plastic dishes without cover in this investigation, the reason for expressed differences could be a higher relative air humidity (20 \pm 2%) in the incubator. Beckett et al. (1998) by exposing S. oryzae adults at 48°C in wheat grain found that for 99% mortality it takes 38 min, which is about 5.1-fold faster than in our investigation where the weevils were exposed in 100 g of wheat grain. The same authors found that the second larval stage of rice weevil is the most tolerant to high temperatures (LT₅₀ = 144 min).

Findings on effects of high temperatures on other species of storage insects are different. For example, Fields (1992) and Fields and Muir (1996) found that for survival of 2% of *Tribolium castaneum* (Herbst) adults and 3% of *Rhyzopertha dominica* (F.) adults at temperature of 57°C it takes 32 seconds. Mahroof et al. (2003b) investigated effect of high temperatures on different development stages of *T. castaneum* and found that young larvae were the most tolerant to temperatures above 50°C and that for 99% mortality it took even 7.2 h. The same authors recorded a negative impact of 50°C temperature on progeny production of *T. castaneum*. From the studies conducted by Arthur (2006) and Tilley et al. (2007a) it can be concluded that exposures shorter than 2 h at 51°C and 50°C cause

a 100% mortality of all development stages of *T. castaneum* and *Tribolium confusum* (DuVal) and adults of *T. castaneum*, *S. oryzae* and *R. dominica*.

In broader context of consideration of advantages and disadvantages of 50°C temperature use, the optimization of costs and risks of equipment and sensitive materials damaging is also very important, and therefore we point to some research results on direct effects of short-term exposure of storage insects to high temperatures, where populations of different densities and with altered susceptibility to insecticides were also included. From the literature data summarized by Fields (1992) it can be seen that 30% of S. granarius adults recover after exposure of 20 s at 49°C, and 10% after 5 s exposure at 52°C, while 62% of S. oryzae specimens recover after 108 seconds exposure at 50°C, and 33% after 30 s exposure at 52°C. Kljajić et al. (1996) after the exposure of laboratory population of S. granarius, from population densities of 500, 1000 and 2000 adults/1500 g grains, found that LT₅₀ is 19-26 min at 50°C after recovery of 24 h, and LT₉₅ 22-37 min, which is in agreement with our experimental results if confidence intervals are cosidered (Table 1). Kljajić et al. (2009) investigated the effect of 50°C temperature on insecticide toxicity to S. granarius adults (one laboratory and two field populations resistant to deltamethrin and pirimifos-methyl) and found that because of altered susceptibility to pirimifos-methyl for total paralysis of the field weevils it took 22% more time than for laboratory population. This reaction was explained by slower physiological-biochemical processes in speciments with altered susceptibility to insecticides, and that the dynamics is not significantly affected by prior direct short-term exposure at 50°C.

Besides determined LT parameters, the results that show that 50°C temperature greatly affects weevils progeny production and that delayed effect on stored wheat can be obtained by its use are very important in this paper. Due to differences in the concept, objectives and methodologies used, it is difficult to adequately compare the results obtained in our experiment with the relevant data available in literature. However, generally it can be concluded that for all three species of Sitophilus genus the use of 50°C temperature is more effective when speciments are outside wheat grains, for example in an empty storage, than in substrate where the exposure of weevil parents has to be prolonged, in S. granarius for 110 min, in S. oryzae for 130 min and in S. zeamais for over 160 min, to achieve total progeny reduction.

With an aim to find better solutions in control of mentioned and other storage insects, in the future studies it is necessary to investigate the use of 50°C temperature in different substrate masses on several storage pests (primary and secondary) comparatively. To reduce the cost of 50°C temperature treatments and the risk of equipment and sensitive materials damaging, it is necessary to include use of temperature in combination with, for example insecticides of natural origin, especially with inert dusts as one more effective physical measure for pest insects management in stored products.

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Efekti temperature 50°C na Sitophilus granarius (L.), Sitophilus oryzae (L.) i Sitophilus zeamais (Motsch.)

REZIME

Da bi se utvrdilo letalno vreme (parametri LT₂₀, LT₅₀ i LT₉₉) na 50°C i produkcija/redukcija potomstva u F_1 generaciji, adulti laboratorijskih populacija žitnog (S. granarius), pirinčanog (S. oryzae) i kukuruznog žiška (S. zeamais) su izlagani pomenutoj temperaturi. Za svaku ekspoziciju i svaku vrstu je korišćeno po 25 adulta starosti 2-5 nedelja, u četiri ponavljanja. Insekti su u prvoj varijanti, u plastičnim posudama ($V=200 \text{ cm}^3$) sa 1,8 \pm 0,2 g krupno mlevene netretirane pšenice izlagani 6, 10, 14, 16, 18, 20, 22, 26 i 30 min. U drugoj varijanti, u posudama sa 100 g netretirane pšenice u zrnu adulti su izlagani 90, 100, 110, 120, 130, 140, 150, 165 i 180 min. Nakon navedenih ekspozicija adulti su stavljani na oporavak u 100g netretirane pšenice u zrnu, u četiri ponavljanja, na 25±1°C i 60±5% r.v.v. Posle jedan, dva i sedam dana oporavka, prosejavanjem pšenice su izdvojeni žižci i utvrđena je smrtnost da bi, posle ukupno osam nedelja od izlaganja roditelja bio utvrđivan uticaj na potomstvo u F_1 generaciji. Letalno vreme žižaka je određeno probit analizom, a produkcija/redukcija potomstva analizom varijanse. Izlaganjem žižaka krupno mlevenoj pšenici, a posle oporavka jedan, dva i sedam dana, najosetljiviji su adulti *S. oryzae* (LT₅₀ 12,48-13,68 min), a najtolerantniji adulti *S. granarius* (LT₅₀ 17,79-20,89 min). Nakon izlaganja insekata u pšenici u zrnu najosetljiviji su S. granarius i S. oryzae (LT₅₀ 107,11-120,73 min), a najtolerantniji S. zeamais (LT₅₀ 139,90-155,35 min). Posle izlaganja žižaka-roditelja sve tri vrste na lomljenoj pšenici, redukcija potomstva na nivou 100% je posle 22 min. Međutim, posle izlaganja žižaka-roditelja sve tri vrste žižaka na zrnu pšenice, redukcija potomstva na nivou 100% je kod *S. granarius* posle 130 min, kod S. oryzae posle 150 min, a 99,7% kod S. zeamais posle 180 min. Ovim ispitivanjima je utvrđeno da temperatura 50°C pri kraćoj ekspoziciji žižaka iz roda Sitophilus, nepovoljno deluje na njihovo preživljavanje i produkciju potomstva, kao i da postoji potencijal za uspešnu primenu iste, kao fizičke mere suzbijanja štetnih insekata u skladištima.

Ključne reči: S. granarius; S. oryzae; S. zeamais; temperatura 50°C; efekti