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Behaviour of the Angoumois grain moth (*Sitotroga cerealella* Oliv.) in different grain substrates and assessment of losses

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Abstract

The Angoumois grain moth, *Sitotroga cerealella*, is a primary stored grain pest, which development occurs within a single grain. The respond of the pest to various offered grain substrates was studied in no choice laboratory experiment (temperature 27±1ºC; relative humidity 60-80%), by rearing moth populations on entire grains (corn, wheat, barley, sorghum, millet, tall fescue and Kentucky bluegrass) and mechanically damaged grains (corn in fractions with/without embryo, polished rice). The pest behaviour was determined by observation of the entrance and exit hole position on different grains. The food consumption was estimated after adult emergence, by measuring of mass losses of infested grains. Mass losses were correlated with quantitative and qualitative grain parameters. The development was successfully accomplished in all grain substrates, except in Kentucky bluegrass. Strategies of larval penetration and exit hole position depended on morphological properties of grains. As a rule, the development of an individual was completed in a single grain, but in polished rice the transfer from one to another grain was observed. The highest loss of infested grain was recorded in corn grains (55.48 mg), the lowest in tall fescue grains (2.40 mg). Positive correlations were detected between the mass losses and protein, lipid and sugar content, negative in relation to cellulose and ash content.

Keywords: grain, Poaceae, *Sitotroga cerealella*, behaviour, losses.

Introduction

The Angoumois grain moth (AGM), *Sitotroga cerealella* (Olivier), is worldwide distributed primary stored grain pest. In tropical, subtropical and temperate regions with warmer climate it can also affect cereals in the field (VUKASOVIĆ, 1940). The complete development of an individual generally occurs inside the kernel and therefore the survival directly depends on quantitative and qualitative properties of the available food resources, provided by a single infested kernel. The females of *S. cerealella* lay the eggs by attaching them to the grain surface, being stimulated by mechanical contact of the abdomen with the tight interspaces between the kernels (thigmotactism). After hatching, the larva penetrates the kernel and once inside it continues to feed and develop. Before the pupation, the mature larva extends its feeding chamber to the outside of the kernel, leaving intact only the epidermis of the bran, a symptom of infestation that is visible from outside as a circular transparent "window". After emerging, the adult pushes the "window" and leaves a small characteristic round exit hole. The development and survival of an individual is strongly directly depending on the available food resources, which are determined and limited (quantitatively and qualitatively) by a single inhabited kernel, by itself.

The objective of the present study was to evaluate the convenience of different cereal species/types of grains (whole grains or mechanically modified grain kernels) as feed for the AGM by comparing penetration modes of neonate larvae in different offered grain types, positions of adult exit holes, as well as by evaluation of the final results of infestation: number of emerged adults, mass losses of overall infested grain substrates and single infested kernels, as well as to determine on which extent the survival of AGM and consecutive losses depend on quantitative and qualitatative properties of grains.

Materials and Methods

The respond of the pest to various offered grain substrates was studied in no choice laboratory experiment in controlled conditions of temperature (27±1ºC), relative humidity (60-80%) and photoperiod (16h/8h light/dark), by rearing AGM populations on grains of different Poaceae plant species, including whole and mechanically damaged kernels of the following plant species:

- Corn, *Zea mays* L. (NS SC 444), whole grains
- Corn, *Zea mays* L. (NS SC 444), fraction with embryo
- Corn, *Zea mays* L. (NS SC 444), fraction without embryo
- Wheat, *Triticum vulgare* Host (Balkan), whole grains
- Barley, *Hordeum sativum* J. (NS 27), whole grains
- Rice, *Oryza sativa* L. (population), polished (white) grains

- Sorghum, *Sorghum vulgare* Pers. (NS-šećerac), whole grains
- Millet, *Panicum mileaceum* L. (population), whole grains
- Tall fescue, *Festuca arundinacea* Schreb. (NS- visoki vijuk), whole grains
- Kentucky bluegrass, *Poa pratensis* L. (population), whole grains.

The two fractions of corn grains were obtained by cutting each grain transversally at the line approximately corresponding to the middle grain length.

Eggs of the same age, with ongoing embryogenesis confirmed by the detection of the formed larva under the transparent chorion, were transferred to plastic jars (500 ml), filled with a grain substrate (10 different substrates, 4 replications, 100 eggs per unit). The successful hatching was confirmed by observations of empty transparent chorions.

Each of the 40 experimental units contained 37.5 g of a grain substrate, equivalent to the average weight of 100 undamaged corn grains, which as being the largest grain species could hypothetically assure the development of at least 1 larva/grain.

Quantitative and qualitative parameters of each grain species/type regarded: the measurement of the mass of uninfested kernels (with precision of 0.01 g), determination of sugar content (mg/g of dry matter), protein, lipid, cellulose and ash content (% of dry matter) and energy value (J/g) of each grain substrate before exposition to AGM. Standard methodology was applied for determination of protein content (Kjeldhal method), lipid content (Soxlet method) and cellulose content (Sharner-Kurschuer method), while the ash content was determined by heating at 550°C. Sugar content was determined by the method of liquid chromatography (Van Riel and Olieman, 1989) and the energy value by the method of calorimetry described in Krajcovic and Regal (1976).

Emerged adults were extracted from the jars on daily basis and the total number of emerged adults was recorded in each experimental unit. The termination of the eclosion was assured by absence of newly hatched adults in the period of 14 days after the last recorded adult emergence.

The pest behaviour in relation to different offered grain species/type was determined by visual observation of the mode of penetration of neonate larva into akernel (i.e. position of entrance holes), as well as by the position of the exit holes of the emerged adults. Determination of infested kernels was conducted under magnification of binocular microscope Wild M400.

After the termination of adult eclosion, the measurements of the mass of infested substrates (expressed in grams) and single infested kernels (in milligrams; up to 50 kernels with a single exit hole/unit) were conducted in each experimental unit and compared with the related values obtained before the infestation in order to evaluate the mass losses. Average mass loss of an infested kernel (expressed in mg and %) represented directly the total feed consumption (i.e damage) of a single individual in larval stage that successfully developed and survived until adult emergence. Furthermore, the obtained data on mass losses of infested grain substrates and number of emerged adults served for calculation of average mass losses per survived individual (adult). Comparison of the results for each obtained quantitative paramether (number of adults, mass losses of grain substrate, mass losses of grain substrate/adult and mass losses of infested kernel) was conducted by Duncan Multiple Range Test for significance level of $p = 0.05$.

Finally, the impact of qualitative grain paramethers to the expression of the number of emerged AGM adults and to the mass loss paramethers (mass losses of grain substrate, substrate/adult and infested kernels) were identified by determination of correlation coefficients and the level of their significance. The statistical analyses were conducted in Statistica 13.2 software (Dell Inc. Dell Statistica (data analysis software system) version 13. 2016. (http://software.dell.com).

Results

The development of AGM was successfully accomplished in all tested grain substrates (with whole and mechanicaly damaged kernels), except in Kentucky bluegrass. Neonate larvae were not able to bore the entrance hole in Kentucky bluegrass, probably because of the structure of the husk. Indeed, during the carefully observation of the behaviour of neonates on Kentucky bluegrass grains under the microscope magnification, it was remarked that the trichomes on the husk represent the mechanical barreier limiting the larval movement and causing letal injuries to larval body. Thus, Kentucky bluegrass may not be considered by us as a host plant species.

Observations of exit holes on infested kernels of other grain species/types demonstrated that usually only one AGM individual can complete the development in a single kernel. An exception was recorded in the substrate with whole corn kernels, where appart the most frequent combination of one individual per single kernel, also two AGM individuals could inhabit the same kernel and successfuly accomplish the development.

Entrance and exit holes

The position of the entrance hole of newly hatched larvae (neonate) on the kernel surface of the offered grain species/type indicated that larvae perform different strategies of penetration depending on grain the properties. In whole, undamaged grains (corn, barley, wheat, sorgum, millet, tall fescue), as a rule, the entrance holes were detected in the zone of the germ (embryo). In such cases, the exit holes were always located at the opposite end (latero-terminally), indicating that during the feeding a larva is following the direction of the longitudinal axis of the grain. Moreover, in some of the infested wheat kernels, the entrance hole was also detected on the dorsal side, in the zone of endosperm and in such cases the exit hole was located at the oposite, ventral side of the kernel. In millet grains, covered by tightly adhering husk (*palea* and *lemma*), which represents a hard, insuperable mechanical barrier, the precise location of the entrance hole coincided with the micropyle, as a naturaly present opening. In tall fescue, the larvae were able to bore the entrance hole through the husk covering at the ventral side of the kernel, whether in the zone of the germ (down below or through the *rachila*) or in the zone of endosperm, and the exit holes were detected on the terminal side of the kernel, exclusively. The hull present on barley kernels did not represent any kind of barrier for larval penetration in the germ zone.

The position of the entrance hole in the zone of the germ was also detected in mechanicaly alterated corn kernels containing the germ, and in this case the the exit hole was also located on the oposite side laterally, but never directly on in the transfersal cut.

In mechanicaly damaged corn kernels without the germ (fraction without embryo), the penetration of a larva occured in the zone of the transfersal cut, through the space left after the removal of the germ. The exit holes in this grain type were always located on the latero-terminal, intact part of the grain, as in the case of the entire corn grains.

Finally, polished white rice (free of husk and bran), the entrance hole of AGM was found on different parts of the kernel, with no particular rule, but in this substrate type the transfer of the larva from one to another kernel was remarked, suggesting that the food resource obtained by a single grain is not satisfying the food requirements of a larva to complete the development. After consuming the most of the single kernel, a larva was able to pass to the next one in order to continue the feeding. The transfer from one kernel to another occurred within the silky tunnel produced by the larva, which served as protective „bridge" between the kernels. In some cases few (up to 6) kernels were connected with silky threads and some of them were damaged only superficially. The pupation occured whether inside or outside the kernel in the silky cocoon.

Losses

The suitability of different offered grain species/types for AGM was estimated by the number of emerged adults, mass losses of the grain substrates, including the mass losses calculated per emerged AGM individual (Tab.1) and mass losses of infested kernels (Tab. 2).

As demonstrated in Tab. 1 highly significant differences ($p<0.01$) in mass losses of grain substrates, number of emerged adults and estimated consumed mass of substrate per emerged (i.e. survived) individual were recorded among different grain species/type.

Tab. 1 Mass losses of different grain substrates caused by *Sitotroga cerealella* infestation and estimation of the individual consumption based on the number on emerged adults

Grain substrate		Mass losses of grain substrate*			Number of emerged adults**		Consumed mass of substrate per individual	
		Mean (g)	Sd	Mean (%)	Mean	Sd	Mean (mg)	Sd
Corn		4.86 a	0.59	12.97 a	79.75 b	2.75	60.99 a	7.24
Corn fraction without embryo		4.16 b	0.09	11.09 b	68.75 c	6.75	60.82 a	4.80
Corn fraction with embryo		4.24 b	0.13	11.30 b	88.25 a	4.57	48.11 b	2.76
Barley		2.71 c	0.19	7.22 c	86.75 ab	9.78	31.33 c	1.49
Wheat		2.05 d	0.08	5.46 d	84.00 ab	5.72	24.42 d	1.10
Rice-polished		0.94 e	0.08	2.49 e	19,50 d	1.91	48.08 b	2.96
Sorghum		0.97 e	0.04	2.59 e	70.00 c	4.24	13.93 e	0.97
Millet		0.16 f	0.03	0.42 f	19.25 d	2.87	8.17 f	0.27
Tall fescue		0.09 f	0.01	0.25 g	21.00 d	2.45	4.41 f	0.27
Kentucky bluegrass		0.00 f	0.00	0.00 h	0.00 e	0.00	.	.
Analysis of variance	F	337.40		863.61	199.31		181.44	
	p	<0,01		<0,01	<0,01		<0,01	

*Each experimental unit initially contained 37.5g of grain substrate

**Each experimental unit was initially infested with 100 individuals in egg stage

Mean values labeled with the same letters are not significantly different according Duncan Multiple Range Test for significance level of p = 0.05. In the process of statistical analyses the % values were transformed in arcsin √%

The calculation of mass losses of the whole substrates took in account the losses caused by entire population of hatched larvae which consumed the feed, including also those that eventually died during the development. The highest mass loss was recorded in substrate with entire corn kernels (4.86 g; 12,97%), followed by corn in fractions without and with embryo, barley, wheat, sorghum and polished rice, millet and finally tall fescue with the lowest registered mass loss (0.09 g; 0.25%). In the substrate with Kentucky bluegrass, neither emerged adults nor mass losses were recorded. The highest average number of emerged adults was recorded in substrates with corn fraction with embryo, barley and wheat (88.25, 86.75 and 84.00, respectively), followed by the substrate with entire corn grains (79.75), which was not significantly different from the number of adults recorded in barley and wheat. Significantly lower number of adults were recorded in sorghum and corn fraction without embryo (70 and 68.75), the lowest in tall fescue and millet (21 and 19.25, respectively).

Another general picture of the infestation consequences in each substrate is demonstrated by estimation of the consumed mass of the substrate per emerged AGM individual (Tab.1). Here, the ordination of the substrates following the decreasing values is similar as in the case of comparison of mass losses of the substrate, with the highest consumption/AGM individual recorded in the case of corn entire grain and fraction without embryo (60.99 mg and 60.82 mg, respectively), the lowest in the cases of millet and tall fescue (8.17 mg and 4.41 mg, respectively). Surprisingly high consumption of grain supstrate per AGM individual was recorded in polished rice (48.08 mg) where low number of adults emerged. The estimated individual consumption in rice was not significantly different from the value recorded in the population reared in corn fraction with embryo (48.11 mg), where high number of adults emerged. In order to survive larvae of AGM reared in polished rice, had to consume as high quantity of feed as in corn fraction with embryo and therefore the consumption of more rice grains was required and obtained by the transfer from one rice kernel to another.

The most precise quantification of the larval damage was provided by calculation of the mass losses of single infested kernels (Tab.2), that were determined before the measurement by the presence of both entrance and exit holes. The average mass of a kernel before and after the infestation, as well as the resulting average mass loss of single infested kernel were significantly different depending on the grain species/type. All offered grain species/types had significantly different mass before infestation, with the highest value recorded in corn with entire kernel (375.03 mg), the lowest in Kentucky bluegrass (0.40 mg). As previosly mentioned, in Kentucky bluegrass the infestation of the kernel was not observed and no losses in substrate mass were recorded. Therefore, within the serial of tested species/types recognized to host AGM, the lowest kernel mass that provides sufficient food resources for the successful development of AGM was recorded in tall fescue (2.80mg).

Tab. 2 Mass losses of single infested kernels of different grain species/type caused by *Sitotroga cerealella*

Grain species/type		Mass of a single kernel				Loss of a single kernel mass		
		Before infestation		After adult emergence				
		Mean (mg)	Sd	Mean (mg)	Sd	Mean (mg)	Sd	Mean (%)
Corn		375.03 a	0,29	319.55 a	7,67	55.48 a	7.61	14.75 a
Corn fraction without embryo		227.30 b	2,12	175.00 b	1,40	52.30 a	1.67	23.01 b
Corn fraction with embryo		143.28 c	0,79	95.60 c	2,51	47.68 b	2.67	33.26 c
Barley		51.43 d	0,17	21.90 e	0,68	29.53 c	0.66	57.41 f
Wheat		50.10 e	0,08	26.60 d	0.33	23.50 d	0.37	46.90 d
Rice-polished		19.63 g	0,30	5.73 g	0.17	13.90 e	0.20	70.83 g
Sorghum		20.75 f	0,06	9.75 f	0,10	11.00 e	0.14	53.01 e
Millet		7.43 h	0,05	1.95 gh	0.06	5.48 f	0.10	73.74 h
Tall fescue		2.80 i	0,00	0.40 h	0.00	2.40 f	0.00	85.71 i
Kentucky bluegrass		0.40 j	0.00	.	.	.	.	.
Analysis of variance	F	114831.83		6328.5		223.15		1472.8
	p	<0,01		<0,01		<0,01		<0,01

NOTES: Mean values labeled with the same letters are not significantly different according Duncan Multiple Range Test for significance level of p = 0.05. In the process of statistical analyses the % values were transformed in arcsin √%

In accordance with the availability of the mass of food resources determined by a single kernel (Tab.2), the highest average mass loss of an infested kernel (i.e. larval consumption) was recorded in grain species/types having the highest kernel mass (whole corn grain and in corn-endosperm fraction, 55.48 mg and 52.30 mg, respectively), the lowest in millet and tall fescue (5.48 mg and 2.40 mg, respectively). Despite the low number of emerged adults in kernels of low mass (e.g. millet and tall fescue), it was demonstrated that AGM is able to survive with remarkably limited amount of feed, consuming about 10-23 times lower mass of kernel than in optimal conditions provided by wheat, barley or corn. The lowest average mass loss of an infested kernel expressed in percentages was recorded in whole corn kernels (14,75%), the highest in grains of tall fescue where 85.71% of grain mass was consumed. Obviously, the utilization of food resources in grains of low kernel mass is significantly higher.

Statistically highly significant correlations were detected between the paramethers of chemical composition of the grain substrates and number of emerged adults (Tab.3). Sugar, protein and lipid content had positive influence to the development of AGM, while cellulose and ash content had negative influence to the number of emerged adults. Highly significant positive correlation was also detected between the energy value of a substrate and number of emerged adults. Similarly, higly significant influences were also recorded when chemical composition parameters, as well as the energy value of grain substrates were correlated with mass losses of grain substrate, and with mass losses of single infested kernels. The impact of each of the tested chemical composition parameter to the mass loss of the supstrate per emerged adult (i.e. consumption of a survived individual), was also higly significant: positive when it regarded the influence of sugar, protein and lipid content and negative regarding the influence of cellullose and ash content. The only not significant correlation coefficient was established between the energy value of the substrate and mass losses of the grain substrate per emerged adult.

Tab. 3 Qualitative parameters of different grain species/types and correlations with the number of emerged adults of *Sitotroga cerealella* and mass losses parameters

Grain species/type	Chemical content					Energy value
	Sugar (mg/g)	Protein (% d.m.)	Lipid (%d.m.)	Cellulose (% d.m.)	Ash (% d.m.)	(kJ/g)
Corn	15.45	10.10	3.88	2.02	2.54	17.437
Corn - fraction without embryo	16.60	14.01	2.56	1.75	3.36	18.566

Corn - fraction with embryo		16.03	16.52	3.22	1.50	3.08	18.911
Barley		15.00	11.66	2.39	3.52	4.09	17.334
Wheat		14.56	12.53	2.10	2.33	2.81	16.196
Rice - polished		11.53	9.11	1.95	2.36	3.36	14.866
Sorghum		13.56	10.51	2.11	2.21	2.16	17.258
Millet		10.02	11.49	2.76	3.28	5.21	16.487
Tall fescue		9.23	5.06	1.47	3.71	6.83	16.911
Kentucky bluegrass		9.85	4.21	1.64	3.55	5.91	16.722
Correlation coefficients	Number of emerged adults	0.91**	0.76**	0.59**	-0.60**	-0.73**	0.56**
	Mass losses of grain substrate (g)	0.91**	0.68**	0.79**	-0.73**	-0.61**	0.65**
	Mass losses of substrate/adult (mg)	0.72**	0.47**	0.60**	-0.71**	-0.53**	0.32
	Mass losses of infested kernel (mg)	0.88**	0.61**	0.75**	-0.70**	-0.51**	0.66**

d.m.- dry matter ** highly significant (p<0.01)

Discussion

AGM is worldwide distributed oligophagous pest species that usually attack cereal grains in extensive storage conditions. In temperate regions, as demonstrated by TREMATERRA (2015) in Southern Italy, the infestations with AGM occurre both during preharvest plantation and postharvest storage, and therefore the author highlighted that warehouses, field-plots and wild hosts distributed on the teritory can each serve as sources of both reproduction and aggregation, depending on the time of the year. Stored grains of plant species that are frequently reported as hosts of AGM are corn, wheat, barley, sorghum, rice, ray, oatand millet, but apart these most usually cultivated cereals it can also develop in grains of some spontaneous Poaceae species of few genera, such as *Lolium* L., *Eleusine* Gaertn., *Phalaris* L. and *Echinochloa* Palisot de Beauvois (BALACHOWSKY, 1966; DAKSHINAMURTHY AND REGUPATHY, 1988). So far, the only available report on successful development of AGM in grains of tall fescue (*Festuca arundinacea*) is given by IGNJATOVIĆ ĆUPINA (2001).

Apart the cereal species commonly known as hosts of AGM (corn, wheat, barley, sorghum), this study confirmed the adaptability of the pest to survive in small grain species, such as millet and tall fescue, as well as in mechanically damaged kernels of common host species (corn grain fractions with and without embryo, polished rice), but not in Kentucky bluegrass. Despite the less favorable quantitative and qualitative conditions in host species of small grains, the survival of AGM was still evident at different extent, reflected by lower number of emerged adults and lower mass losses of the infested substrate.

Usually a single grain kernel is infested by only one, single AGM larva. The best conditions for the development of AGM are provided by corn grains, which offer enough food resources for the development of even more than one AGM individuals (up to 3) per single kernel, and such behavior was rarely recorded also in wheat grains (GRANDI, 1951; BALACHOWSKY, 1966; VUKASOVIĆ *et al*., 1972; MANOJLOVIĆ, 1987). According PRAKASH *et al*. (1982), the grain resistence to pest infestation depend on physical and biochemical grain properties, as well as on the pest feeding and/or oviposition preference.

Different modes of penetration of AGM neonate larvae into the kernel are described depending on the plant host species. In corn grains the penetration takes palce in the germ zone (VUKASOVIĆ *et al*., 1972), where the bran is thinnest and additionally such strategy provide the most nutritious matters contained in the germ during the initial feeding of the young larva. The same strategy of penetration in the germ zone in sorghum kernels was reported by WONGO (1990) and WONGO AND PEDERSEN (1990).

In the present research the boring of larvae in the germ zone was observed in grains free of hull (corn, corn-fraction with embryo, sorghum, wheat), but also in husked grains (barley, millet and tall fescue). According to VUKASOVIĆ *et al*. (1972) penetration of AGM larvae in the germ zone of wheat kernels seems quite unusual. Penetration in the zone of endosperm in wheat kernels was detected in the present study, but not exclusively (some larvae preferred the germ zone for penetration).

Few authors observed that the hull of rice kernels represents an important protective structure that prevent the penetration of pest insects into the kernel and hard, thick and intact hull represents a resistance factor that affect the penetration of AGM larve (Russell and Cogburn, 1977; Cogburn and Bollich, 1986; Ragumoorthy and Gunathilagaraj, 1988; Sauphanor, 1988; Cogburn *et al*., 1989; Takeshita and Imura, 1990).

In grains completely covered by intact hull, which reperesents a mechanical barrier, the penetration may occure through the abscission scar of the central vascular bundle, as described in rough rice kernels by Cogburn *et al*., (1983). In this research, such beheviour was observed in tall fescue kernels. Similar strategy of penetration was observed in millet kernels thightly enclosed by *palea* and *lemma*, where the larva also chose the natural opening (micropyle) to bore into the kernel. However, the hairy hull structure of Kentucky bluegrass represented the insuperable mechanical barrier for larval penetration into the kernel. The larval mortality of 100% in this grain substrate was obviously attributed to the husk structure, not to the grain size (i.e. food resources).

The imperfect hulls of barley kernels did not represent a barrier for penetration of larvae in the germ zone. Similar observation was reported by Cogburn *et al*. (1983) who stated that rice varieties with imperfect hull favored the infestation by AGM.

Furthermore, the hardness and thickness of the bran also contribute the resistance to penetration of insects into the kernel, as demonstrated in sorghum kernels where the lowest infestation occurred in varieties with the hardest bran layer (Shazali, 1985). In the present research, the penetration hole in corn fraction without embryo was always detected on the side of the transfersal cut (free of the bran), where the larvae penetrated through the crevice left after removal of the germ. The possibility of larvae to penetrate mechanically damaged corn kernels suggests that such substrates are equally susceptible to infestation by AGM as whole grains. Allotey and Moloko (2015) recorded higher emergance rate of AGM reared in substrates with whole grains of maize varieties than in mechanically altered grains (cut and ground grains). However, in the present study where the fractions with and without embryo were separately considered, the obtained results demonstrated the highest adult emergence rate in corn fraction with embryo, followed by whole corn grains and fraction without embryo. In contrast, in sorghum and millet varieties Allotey and Moloko (2015) recorded the highest number of adults in substrates with broken grains and such results were explained by the earlier exposure of endosperm for larval feeding and easier exit path between the broken grains. In the present study, small grain species, were offered to AGM only as whole grains. Nevertheless, the emergence rate of 70% of AGM reared in sorghum whole grains was similar to the values recorded by Allotey and Moloko (2015) in both whole or broken sorghum grains (66,3% and 68,8%, respectively), depending on the tested variety.

The most interesting behavior of AGM was observed in polished rice, where no particular rule of larval penetration was observed. Lacking the external mechanical barrier in this type of mechanically processed substrate the larvae were able to pass from one kernel to another by producing the silky tunel between the kernels, and additionally during the transfer some of the surrounding kernels were damaged superficially. Superficial damages on adjacent kernels were reported in rare cases when the food resource provided by a single kernel is scarce (Balachowsky, 1966). Lacking the epidermis layer in polished rice kernels, the exit holes of AGM aduls did not have a typical "window" appearance. Whether the transfer to another kernel was conducted with ultimate pupation inside the kernel, or the pupation occurred outside in the interspace between the kernels in the cocoon, the silky threads connecting the kernels and frass were evident. At a first appearance, such infestation symptom might be incorrectly linked to some other stored product pests that are feeding externaly, such as *Nemapogon granella* (Linnaeus) or *Plodia interpunctella* (Hübner).

Appart the positive association of the integrity of the rice husk with the resistence to pest infestation, COGBURN AND BOLLICH (1986) emphasized that hardness and texture of the kernel surface are of a crucial importance for oviposition and further development, and suggested that nutritive compounds also play a role in grain resistance. However, several aspects are involved in grain resistence to infestation with store products pests, such as the absence of preferences (for oviposition and/or feeding), physical and chemical grain properties and changes during the grain processing (PRAKASH *et al*., 1982). Furthermore, the grain resistence to infestation depends on the grain size, as observed among different cultivars of pearl millet (SEIFELNASR AND MILLS, 1985). Numerous studies were conducted in order to estimate the survival rate and mortality of AGM in grains of different plant species, as well as in different varieties of the same plant species. According MANOJLOVIĆ (1987), the mortality rate during the post-embryonic developlent of AGM reared under the same conditions in corn hybrids of larger grain size ranged between 36.2% and 40.05%, while in smaller size hybrid it was 41.46%. In the same study, the mortality rate in wheat variety of smaller grain size was also higher (46.02%) than in the varieties of larger kernels (36.2-40.05%). The research conducted by COGBURN (1989) demonstrated that the survival rate of AGM reared in different species of rice with deliberately broken hulls ranged between 0.0% and 40.2% and significantly depended on the size and mass of the kernel. The resistence of different stored rice varieties, expressed through the number of emerged AGM adults was positively and highly significantly correlated with the weight loss (RIZWANA *et al*., 2011). In different tested varieties of sorghum, SRIVASTAVA (1996) determined significantly different mortality rates during the postembryonic development ranging between 7.47% and 41.64%.

In the present study a series of different grain species/types were tested to AGM infestation. Appart the external physical characteristics of the kernel that influenced the larval penetration, quantitative and qualitative grain properties had significant impact to the survival of AGM (i.e. number of emerged adults) and consecutive mass losses, as expected. Substrates with higher mass of kernels favored the development of AGM resulting in higher number of adults, higher mass losses of the infested kernels and higher mass losses of grain substrates as a whole. In kernels of lower mass, the number of emerged adults was significantly lower, but in such kernels the available food resources were efficiently utilized by the survived individuals (e.g. consumed kernel mass of 73.74% and 85.71% in mellet and tall fescue, respectively). The mass of consumed feed by an individual in such small kernels (5.48 mg and 2.40 mg in mellet and tall fescue, respectively) was significantly lower than in optimal conditions, such as provided by whole corn kernels where the consumption was 55.48 mg. Despite the low mass of consumed feed in sorghum kernels (11 mg/larva; 53,01% of kernel mass), the survival rate (i.e. number of emerged adults) in this small grain substrate was surprisingly high (70%), not significantly different from the survival rate recorded in corn fraction without embryo, but still significantly lower than in corn fraction with embryo, whole corn grains, barley and wheat. Similarly to our results, BORZOUI *et. al* (2017) also recorded significantly lower food consumption per larval individual in sorghum grains (about 27 mg), than in wheat and maize grains (about 52 mg and 65 mg, respectively), as well as significantly lower survival rate of immature stages reared in sorghum (about 46%) than in maize (65%), barley (68%) and wheat (90%). Our results demonstrated the high adaptability of AGM populations to survive in limited conditions of available feed, determined by the mass of kernels. Nevertheless, the food resource available in polished rice kernel was not sufficient to an individual, and therefore, appart the consumation of 13.90 mg of internaly infested kernel (70.83 % of the kernel mass), the larva was able to move to the next kernel and continue the feeding (supeficially or internaly). Therefore, the mass losses of polished rice substrate calculated per number of emerged individual was as high as recorded in corn fraction without embryo. However, the ability of AGM to transfer and infest more polished rice kernels did not result in high number of survived individuals.

Obvuiously, the chemical composition and the energy value of the feed were also significantly involved in the survival of AGM and consequently had the impact on mass losses parameters (mass losses of whole grain substrate, mass losses of grain substrate per adult and mass losses of infested kernels). Reserches conducted by some authors on different varieties of the same plant species were not able to identify significant correlations between the chemical composition parameters (sugar, lipid, protein, starch, ash content) on one side and mass losses or pest survival rate (or index of population growth) on the other side. Such results were obtained in the studies conducted by PANDEY AND PANDEY (1983) in varieties of corn, as well as in the research of RIZWANA *et al.* (2011) in rice varieaties. However, DEMISSIE *et. al* (2015) also tested different varieties of corn for susceptibility to AGM and found significant positive correlation between the ash content and the number of emerged adults, while the impact of the moisture and phenolic content to the number of total progeny emerged was significantly negative. In addition, the same authors also have not found statistically significant correlations between other studied parameters of biochemical composition of the feed (i.e. content of crude oil, crude carbohydrate, crude proteins, crude fibre, amylose and amylase) and the total emerged progeny, as well as no significant correlations between biochemical composition paramethers and the percentage of weight loss, with the exception of crude proteins and amylose content that had significantly positive impact to the grain weight loss. Recent research of SAFIAN MURAD AND BATOOL (2017) demonstrated that varieties of wheat with higher protein and carbohydrate content, higher grain weight and lower grain hardness were more susceptible to AGM, since in such varieties significantly higher number of AGM adults emerged, and higher values of percent damage and percent weight loss were recorded. In our present research, where different plant species/types of grains were compared in relation to AGM development and consecutive losses, the impact of biochemical composition of the feed became clearly evident. Sugar, protein and lipid content were positively and highly significantly correlated with the number of emerged adults and mass losses parameters, while negative, also highly significant correlation was detected with the cellulose and ash content. The energy value of the feed had also statisticaly significant positive impact to the number of emerged adults, to the mass losses of the whole substrate and mass losses of infested kernels.

Based on the presented results, it can be concluded that kernels of small grain species with intact hulls, higher cellulose and ash content, lower sugar, lipid and protein content (e.g. millet and tall fescue), as well as mechanically/morphologically dammaged kernels (e.g. polished rice) had negative impact to the development of AGM populations, which resulted in lower number of survived individuals and lower mass losses of grain substrates. However, in such substrates AGM individuals could survive by consuming surprisingly low mass, but high percentage of available feed limited by a single kernel, as demonstrated in millet and tall fescue, or by successful transfer and infestation of more than one kernel in order to compensate the insufficient food resources, as observed in the substrate with polished rice.

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Progeny production by *Stegobium paniceum* in different spices

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