Life history of *Plodia interpunctella* Hübner on sunflower seeds: Effects of seed qualitative traits and the initial seed damage

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ABSTRACT

Sunflower seeds are regularly infested by *Plodia interpunctella* during storage. Although this pest prefers damaged seeds, in practice it can infest undamaged seeds as well. This research assessed the influence of the sunflower seed type (oil, protein for human consumption and bird-feed) and the initial seed damage during post-harvest processing (dehulled kernels, 10, 20, 30% of damaged seeds and undamaged seeds) on development of *P. interpunctella* (larval mortality, larval development, mean developmental duration, adult emergence and fecundity). Biochemical analysis of seeds, kernels and hulls detected the highest content of phenols in the seed and hull and tocopherols in the kernel of the oil type hybrid. The anti-oxidative activity was the highest in the seed, kernel and hull of the protein type for bird feed. The shortest development (39.5 days) and the highest fecundity (91.3) were on the oil type seeds, while the longest development (42.1 days) and the lowest fecundity (68.1) were on the seeds of the protein type for bird feed. The highest mortality of larvae was on the undamaged seeds of the protein type for bird feed and human consumption (21.3% and 14.0%, respectively). The type of sunflower and the level of initial damage affected larval mortality, developmental duration and fecundity. The mean developmental duration and the number of emerged adults were dependent only on the initial seed damage. Principal component analysis detected strong positive correlation between mortality and development with the tocopherol content on the undamaged seeds while fecundity was associated with the state of kernel and the amount of tannins, proteins and oil content in the seed. The undamaged seeds of the protein type for the bird feed were the least suitable for the development of this pest, while the oil type kernels were the most suitable.

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1. Introduction

The sunflower (*Helianthus annuus* L.) is the most important oil crop in the Republic of Serbia and one of the four major oil crops in the world (Anandhan et al., 2010; Balalić et al., 2012). According to FAO estimates, it is cultivated on over 26.2 million hectares in more than 70 countries (FAOSTAT, 2016). The EU sunflower seed production in 2016 was 34.0 million tonnes, a decrease of −14.8% compared to 2014, followed by an increase of 10.7% between 2015 and 2016 (FAOSTAT, 2016). Two basic types of sunflower seeds are cultivated: the oil type for the production of vegetable oils, and the non-oil type (protein, confectionery or nibbling), intended for the human consumption and bird-feed (Jocić et al., 2015).

The quality of sunflower seeds is influenced by the genetic traits inherited from their parent lines, germination and vigour, but also by the post-harvest processing and storage conditions (Zambolim, 2005; Prole et al., 2010). One of the main concerns about the long-term seed storage is the physical damage of the seeds, which

is not always visible. The sunflower seeds have an easily cracking shell, which makes them very susceptible for the development of pests in storages (Beratlief and Iliescu, 1997). Seed damage could occur in production, harvesting, drying or storing process, but could be also caused or enhanced by storage pests, and usually results in the reduction of the seed germination (Stejskal et al., 2014; dos Santos et al., 2016). Stored product pests can sense certain volatiles which are released by the damaged seeds (Trematerra et al., 2000, 2007, 2013, 2015, 2016; Athanassiou et al., 2006). Under these conditions, sunflower grains are regularly infested, mainly by secondary pests such as *Plodia interpunctella* (Hübner, 1813), *Oryzaephilus surinamensis* (L., 1758) *Cryptolestes ferrugienus* (Steph, 1893), *Tribolium castaneum* (Herbst, 1797) and *T. confusum* (Du Val, 1863), which prefer the damaged seeds, because they feed primarily on the germ or seed/grain dust (Storey, 1987; Golob et al., 2002; Silhacek and Murphy, 2005). Out of all mentioned species, *P. interpunctella* is one of the most important pests frequently found in sunflower storages, which often causes deterioration of the seeds (Atanasov, 1974; Beratlief and Iliescu, 1990; Gvozdenac et al., 2018).

The development of *P. interpunctella* is highly dependent on the nutritive quality of the available food (Locatelli and Limonta, 1998; Vukajlović and Pešić, 2012; Predojević et al., 2017; Vukajlović et al., 2017). Proteins, polyunsaturated fatty acids, vitamins and steroids in a diet are very important for the fast and successful development of this moth (Vukajlović and Pešić, 2012; Vukajlović et al., 2017; Predojević et al., 2017). Oilseeds, like the sunflower are rich in proteins and fats (Beratlief and Iliescu, 1997; Sarwar et al., 2013), which is why they are very suitable for a strong colonization of *P. interpunctella*. A protein-rich diet, such as a germ in seeds or an oilseed itself is very suitable for *P. interpunctella*, as it leads to short developmental duration and high fecundity (Almaši, 1984; Silhacek and Murphy, 2005, 2006; Onaolapo et al., 2017).

Most studies on the development of the *P. interpunctella* have been carried out on stored cereals or cereal products. However, the information on the development of this species on the sunflower is scarce. Several facts inspired this research. Namely, there is a constant increase in the sunflower production (from 167 000 ha to 231 000 ha) and the seed export (from 15 671 t to 136 580) in Serbia, in the last five to seven years, 2011—2018 (Annonimus, 1, 2017). At the same time, there is a registered increase of *P. interpunctella* population in Serbian storages which indicates the growing importance of this moth as a pest of sunflower seeds. The last published research on *P. interpunctella* as a pest of sunflower in Serbia date back from 1980s (Vukasović et al., 1966; Stojanović and Kosovac, 1974; Almaši, 1984). Therefore, the increase of economic importance and damages caused by this pest imposed a need for a more detailed study of *P. interpunctella* on sunflower. Therefore, the aim of this work was to assess the influence of different types of the sunflower seeds (oily and protein) and the level of the initial seed damage that occurs during the post-harvest processing, on *P. interpunctella* life history parameters.

2. Materials and methods

2.1. Insect culture

The *P. interpunctella* culture used in this research originates from the population reared for ~50 generations in transparent plastic containers for mass rearing (5 L), in a thermostat chamber, at 28 ± 1 °C, r.h. 60 ± 10% and photoperiod 14:10 (L:D), on standard a laboratory diet (Silhacek and Miller, 1972), consisted of ground dog meal (10%), rolled oats (4%), white cornmeal (26%), whole wheat flour (23%), wheat germ (2%), brewers' yeast (5%), glycerol (16%), and honey (14%). 100 pairs of one-day-old adult males and females *in copuli* were isolated with an entomological aspirator from the

containers for mass rearing and placed into 1 L glass jars where the females laid eggs. The one-day-old eggs used in the experiment were carefully removed from the ovipositional jars with a brush made from fine hairs. Before the experiment, eggs were observed under a binocular microscope to eliminate those with obvious deformities. Only undamaged, whole one-day-old eggs were used in the experiment.

2.2. Sunflower seeds as a feeding nutrient

The seeds of three sunflower types, namely hybrids, were used in this research. Hybrid Leone is the oily type (OT) with a high oil (46—48%) and low protein content in seeds (20—22%). NS Colonel is a protein type hybrid intended for human consumption (PT) with the lower oil content (35%) and higher protein content (26%). Lactal is a protein hybrid intended for bird-feed (PBF) with a low content of oils (34—38%) in kernels and white hulls. The seeds were obtained from 2016 vegetation season, from The Institute of Field and Vegetable Crops, Novi Sad, Republic of Serbia.

2.3. Qualitative biochemical analysis of sunflower seeds

The biochemical analysis encompassed the determination of the content of total phenols, total tannins, flavonoids, phenolic compounds and tocopherol. Whole seeds (achenia), hulls and kernels (1 g) were grounded to a fine powder in a mill and extracted after 24 h with 50 mL of 70% methanol. The extracts for the spectrophotometric analysis (*Thermo Scientific* Evolution *220 spectrophotometer, Madison WI, USA)* were vacuum-filtered through the sintered glass funnel and kept refrigerated until the analysis. All extractions were performed in triplicates (Bereksi et al., 2018).

The total phenolic content (TP) was determined using a Folin-Ciocalteu colorimetric method (Nagavani and Raghava Rao, 2010) and the results were expressed in milligrams of quercetin equivalents per 1 g of a plant material (mg QE/g). Extract (20 µL) was mixed with 3.4 mL of deionized water, 0.4 mL of 20% sodium carbonate and 0.2 mL of Folin-Ciocalteu reagent, which was previously diluted (1:2) with distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 720 nm using an UV/VIS spectrophotometer. The data are reported as means for three replications. The total tannins (TT) content was determined by the Folin-Ciocalteu procedure, after the removal of tannins by their adsorption on an insoluble matrix PVPP (polyvinylpolypyrrolidone). Briefly, 1 mL of extract, in which the total phenolics were determined, was mixed with 100 mg PVPP, vortexed, kept for 15 min at 4 °C and then centrifuged for 10 min at 3000 rpm. In the clear supernatant the non-tannin phenolics were determined the same way as the TP. The calculated values were subtracted from the TP content, and the TT content was expressed in milligrams of quercetin equivalents (QE) per 1 g of the plant material (Nagavani and Raghava Rao, 2010).

DPPH (1,1-Dyphenyl-2-picrylhydrazyl) radical scavenging activity. Scavenging of free radicals was tested in a DPPH (2,2-diphenyl-1-picrylhydrazyl) acetone solution (Lai and Lim, 2011). DPPH is a stable free radical and accepts an electron or hydrogen to become a stable diamagnetic molecule. Plant extracts (200 µL) were added to 2.0 mL of 50 µM acetone DPPH solution. The mixture was left in the dark for 30 min before reading the absorbance at 517 nm with 70% methanol as blank. Radical scavenging activity was expressed as mg trolox equivalents (TE) per gram of plant material (mg TE/g). The ferric-reducing antioxidant power (FRAP) assay was carried out according to the procedure described in the literature (Valentão et al., 2002).

FRAP assay is based on the ability of antioxidants to reduce Fe $3 +$ into Fe $2 +$ in the presence of 2,4,6-tri (2-pyridyl)-s-triazine

(TPTZ), forming a blue Fe 2+ -TPTZ complex. This reaction is pH-dependent, with an optimum at pH = 3.6. The absorbance increase is proportional to the antioxidant content in samples. The working FRAP reagent was prepared daily by mixing 10 vol of 300 mM acetate buffer, pH = 3.6, with 1 volume of 10 mM TPTZ in 40 mM hydrochloric acid and with 1 volume of 20 mM ferric chloride. The standard curve was constructed using different concentrations of trolox, and the results were expressed as mg trolox equivalents per gram of the plant material (mg TE/g).

The ABTS (2,2'anizonbis (3-ethylbenzothiazoline-6-sulfonic acid)-diammonium salt)) assay was based on a method developed by Miller et al. (1993). 10 mg ABTS radical cation (ABTS•+) was dissolved in 2.6 mL destilated water and reacted with 1.72 mgpotassium persulfate during 24 h. The ABTS solution was diluted with spectroscopic grade methanol to absorbances of 0.900 ± 0.05 at 734 nm. Different volumes of the plant sample were added to 2.5 mL ABTS solution and producing inhibition of the blank solvent between 20% and 80%. Absorbance values were measured after 2 h. The methanol solution of known trolox concentrations were used for the calibration and the results were expressed as mg trolox equivalents per g of the plant material (mg TE/g).A reducing power assay (total reduction capacity-TRC) was performed by the method of Saha et al. (2013). Plant extract solutions (200 μL) were mixed with 3 mL of potassium ferricyanide (1% w/v) in phosphate buffer (pH = 6.6) and incubated at 50 °C for 20 min. 1.5 mL of trichloroacetic acid (10% w/v) was added and centrifuged for 10 min 3 mL of supernatant was mixed with 500 μL of ferric chloride (0.1% w/v) and absorbance was measured at 700 nm. The standard curve for the total reducing capacity was plotted using the trolox solution (mg TE/g).

The total tocopherol (TTo) content was determined according to Emmerie and Engel (1938) in the conditions of inert atmosphere obtained using a nitrogen generator N2 MICRO (Claind, Italy). The content was determined spectrophotometrically on a Cary 300 UV−Vis (Agilent, USA) spectrophotometer. The total tocopherol content was calculated as follows:

$$\text{Tocopherol amount mg/kg} = (A_1 - A_0 \times V \times 10^4) / 39.2 \times v \times m$$

A_1 – sample absorbance
A_0 – absorbance of reference blank
V – volume of benzene solution of unsaponifiable matter (50 mL)
v – volume of solution for development of colour (4 mL)
m – sample weight for analysis (g)
39.2 is absorptivity coefficient for pure α-tocopherol

2.4. Experimental design

The experiment was set in a randomized $3 \times 5 \times 4$ block design. Three types of sunflower seeds were used (OT, PT and PBF) and five levels of initial seed damage: dehulled seeds - kernels; 10%, 20% and 30% of the mechanical seed damage and undamaged seeds (uncracked hull). Damaged seeds were obtained after the standard post-harvest processing. Different levels of damaged seeds (%) were created by making proper proportions, for example: 10 g of damaged + 90 g of undamaged seeds for the treatment "10% damaged seeds". Each seed type and damage level were repeated four times (four replicates), with a total of 60 assays. The seeds were not treated with insecticides after the harvest or before setting up the experiment.

Each assay contained 100 g of the specific seed type (moisture content 9%) and the damage level. The seeds were placed into 0.25 L glass jars with 50 one-day-old *P. interpunctella* eggs. The jars were sealed with a cotton swab and coated with a cotton cloth for proper aeration. The experiment was carried out in the same environmental conditions (28 ± 1 °C, r.h. 60 ± 10% and photoperiod 14:10 (L:D) as for the rearing of parental population.

2.5. Experimental procedure

Ten days after setting up the experiment, the content of jars was examined for hatched larvae. Each week, larval mortality was monitored, and the final mortality was recorded after 21 days. The observations, the counting of larvae and monitoring of life cycle parameters were carried out weekly, until the last larva pupated. The larval developmental duration (LDD) and mean developmental duration (MDD) was expressed as the average time in days, from the beginning of the experiment until all adults emerged. For the adult emergence, the jars were monitored every 12 h. Once the emergence began, the number of adults and their gender was recorded based on the genital structure. The freshly emerged males and females *in copuli* from the same assay were isolated in separate test tubes to obtain fecundity data (defined as a total number of eggs laid after the mating).

2.6. Statistical analysis

The data were statistically analysed using a software package IBM SPSS Statistics 21. Two-way ANOVA was used to analyse the influence of two factors: the type of sunflower seeds and the level of initial seed damage, as well as their interaction, on life history parameters and the development of *P. interpunctella*. Dunnett's multiple comparison test (probability 95%) was used to analyse the difference between these parameters in different treatments.

Additionally, Principle Component Analysis (PCA) was employed to estimate the influence of sunflower qualitative traits on life history parameters of *P. interpunctella*. This multivariate statistical method summarizes the variation within the sample from a large number of interrelated variables, into a set of values of linearly uncorrelated variables called the principal components (PCs). Only principal components with an eigenvalue greater than 1 were considered significant, and variables with a factor loading greater than ±0.6 were interpreted as meaningfully correlated with the PCs. A biplot of PCA was used to graphically display the position of the observations in space defined by biochemistry and qualitative traits of different sunflower seed types and *P. interpunctella* life history parameters.

3. Results

3.1. Qualitative biochemical analysis of sunflower seeds

The qualitative parameters of different types of sunflower seeds are presented in Table 1. In the whole seeds (achenia), the highest content of TT was detected in OT and PFB hybrids (Table 1). The highest antioxidative activity, based on DPPH, FRAP, ABTS and TRC were registered for PFB seeds, while the highest tocopherol content (210.9 mgkg^{-1}) was in the seeds of OT hybrid.

The hull of OT hybrid contained the highest levels of TP and had the highest DPPH value. The highest antioxidative activity (based on FRAP and ABTS) was registered in PFB hulls, as well as TRC. No tannins were detected in the hull of all three varieties.

The kernel of PFB hybrid contained the highest levels of TP, TT and also expressed the highest antioxidative activity (DPPH, ABTS and FRAP). TRC did not differ significantly between the kernels of different hybrids. The highest tocopherol content was in the kernels

Table 1
The biochemical analysis of the tested sunflower hybrids.

Seed part	Component	OT	PT	PFB	F value
Seed	TP (mg quercetine/g)	6.3 ± 0.13 a	5.5 ± 0.18 b	6.0 ± 0.16 ab	32.01**
	TT (mg quercetine/g)	0.9 ± 0.17 a	0.9 ± 0.11 a	1.0 ± 0.09 a	0.54NS
	DPPH (mg trolox/g)	19.9 ± 0.65 a	17.4 ± 0.39 b	19.5 ± 0.60 a	34.66**
	FRAP (mg trolox/g)	78.8 ± 0.37 b	76.5 ± 2.18 b	83.0 ± 3.55 a	7.07**
	TRC (mg trolox/g)	50.1 ± 0.33 a	47.4 ± 1.67 a	50.0 ± 1.07 a	6.42*
	ABTS (mg trolox/g)	24.2 ± 0.43 a	21.0 ± 0.21 b	22.2 ± 0.12 ab	119.98**
	TTo (mgkg^{-1})	128.5	162.4	210.9	/
Hull	TP (mg quercetine/g)	1.1 ± 0.04 a	0.9 ± 0.02 b	1.0 ± 0.12 b	12.27**
	TT (mg quercetine/g)	/	/	/	/
	DPPH (mg trolox/g)	2.3 ± 0.09 a	1.5 ± 0.05 c	1.7 ± 0.10 b	110.68**
	FRAP (mg trolox/g)	6.3 ± 0.73 b	4.5 ± 0.23 c	8.4 ± 0.22 a	99.95**
	TRC (mg trolox/g)	12.5 ± 0.65 b	13.9 ± 0.86 b	16.1 ± 0.78 a	12.08**
	ABTS (mg trolox/g)	4.8 ± 0.24 b	3.8 ± 0.16 c	5.1 ± 0.21 ab	40.08**
Kernel	TP (mg quercetine/g)	8.3 ± 0.19 b	7.5 ± 0.18 c	9.0 ± 0.21 a	71.23**
	TT (mg quercetine/g)	1.6 ± 0.18 b	0.9 ± 0.11 c	2.2 ± 0.15 a	95.11**
	DPPH (mg trolox/g)	32.9 ± 0.92 b	31.6 ± 0.87 b	36.7 ± 0.60 a	29.27**
	FRAP (mg trolox/g)	1117.3 ± 9.77 b	116.1 ± 6.04 b	131.5 ± 3.81 a	6.91**
	TRC (mg trolox/g)	52.4 ± 0.53 a	52.9 ± 0.63 a	52.9 ± 0.48 a	1.62NS
	ABTS (mg trolox/g)	43.4 ± 0.65 b	42.4 ± 1.61 b	45.6 ± 0.73 a	11.74**
	TTo (mgkg^{-1})	154.0	75.2	118.2	/

OT − oil type (Leone); PT − protein type for human consumption (NS Colonel); PFB − protein type for bird feed (Lactal).
TP − total phenols; TT - total tannins; TTo − total tocopherols; TRC − total reduction capacity.
Value ± SD; F value; Values with the same letter in the column are on the same level of significance for the confidence interval 95%; **- $P < 0.01$; *- $P < 0.05$; NS - $P > 0.05$.

of OT hybrid (154.0 mgkg^{-1}).

3.2. P. interpunctella larval mortality

In all treatments, all inserted eggs (50) hatched. The highest mortality of *P. interpunctella* larvae was recorded on PFB seeds (8.9%). It was significantly higher compared to the other two types, regardless on the levels of seed damage (F = 12.11*; 12.11*, $P < 0.05$, respectively). The results are presented in Table 2.

When observed in the context of the initial seed damage, the highest mortality on OT was in the treatments with the undamaged seeds and 10% of damage (6.7%, 10.6%, respectively). On PT and PFB, the highest mortality was also on the undamaged seeds (14.0% and 21.3%, respectively). The difference is statistically highly significant between larval mortalities (%) in all three types of the sunflower

seeds (F = 10.43**; 38.88**; 48.19**, $P < 0.01$, respectively).

3.3. Larval developmental duration (LDD)

The shortest larval development was on OT (32.4 days), followed by PT (32.5 days) while the longest development, regardless of the level of the initial damage, was on PBF (in average 35.9 days). However, the differences between the treatments are not statistically significant (F = 1.39ns, $P > 0.05$). The results are presented in Table 2.

When observing the LDD values, depending on the seed damage level, the slowest development, in all examined sunflower types, was on the undamaged seeds, followed by 10 and 20% of the initial damage of seeds. LDD ranged from 22.7 to 38.7 days on OT, 27.7 to 39.7 PT, and from 29.3 to 39.0 days in PBF, respectively. The

Table 2
P. interpunctella larval mortality and the larval developmental duration (LDD) on sunflower seeds.

Hybrid	Level of initial seed damage	Larval mortality	Average	Larval mortality (%)	Average	LDD (days)	Average
OT	Kernel	1.0 ± 0.01 c	2.5 b	4.9 ± 3.84 c	4.93 b	22.7 ± 1.52 d	32.4 a
	10%	3.3 ± 1.53 b		6.7 ± 3.06 a		36.0 ± 1.00 b	
	20%	1.7 ± 0.57 bc		3.3 ± 1.15 d		37.0 ± 1.00 ab	
	30%	1.0 ± 0.00 c		4.9 ± 3.84 c		27.7 ± 0.58 c	
	Undamaged	5.3 ± 0.58 a		10.6 ± 2.60 a		38.7 ± 0.56 a	
	F value	10.43**		10.43**		142.90**	
PT	Kernel	0.0 ± 0.00 c	3.2 b	0.0 ± 0.00 d	6.4 b	29.3 ± 1.53 c	32.5 a
	10%	3.3 ± 0.58 b		6.7 ± 1.15 b		37.7 ± 1.53 b	
	20%	4.3 ± 1.15 b		8.7 ± 2.31 b		29.0 ± 1.00 c	
	30%	1.3 ± 0.56 c		2.7 ± 1.15 c		27.7 ± 1.15 c	
	Undamaged	7.0 ± 1.00 a		14.0 ± 2.50 a		39.7 ± 1.52 a	
	F value	38.88**		36.83**		45.94**	
PFB	Kernel	1.00 ± 0.00 d	4.5 a	2.0 ± 7.08 d	8.9 a	31.0 ± 1.00 c	35.9 a
	10%	3.7 ± 0.56 bc		7.3 ± 1.15 bc		38.0 ± 1.00 b	
	20%	4.7 ± 0.57 b		9.3 ± 1.15 b		30.0 ± 1.00 c	
	30%	2.3 ± 0.56 cd		4.7 ± 1.15 cd		29.3 ± 1.53 c	
	Undamaged	10.7 ± 1.53 a		21.3 ± 2.00 a		39.0 ± 1.00 a	
	F value	48.19**	12.11*	48.19**	12.11*	162.45**	1.39NS

OT − oil type (hybrid Leone); PT − protein type for human consumption (hybrid NS Colonel); PFB − protein type for bird feed (hybrid Lactal).
LDD − larval developmental duration.
Value ± SD; F value; Values with the same letter in the column are on the same level of significance for the confidence interval 95%; **- $P < 0.01$; *- $P < 0.05$; NS - $P > 0.05$.

differences between the duration of the larval development among different levels of damaged seeds were highly significant, on all types of sunflower seeds (F = 142.9**, 45.94**, 162.45**, P < 0.01, respectively).

3.4. Mean developmental duration (MDD)

The shortest *P. interpunctella* development was on OT, 39.5 days and the longest on PFB, 41.7 days, although the differences (Table 3) were not statistically significant (F = 0.49ns, P > 0.05). When observed within groups (sunflower types) depending on the level of the initial seed damage, the shortest development was on kernels, and 30% of damaged seeds, for all three types of the sunflower seeds. The MDD on OT kernels was significantly the shortest (29.3 days) compared to MDD on PT and PFB (38.0 and 39.0 days, respectively). The differences between MDD on the seeds with different damage levels, differed significantly for all three types i.e. hybrids (F = 58.72**, 44.03**, 49.50**, P < 0.01, respectively).

3.5. The number of emerged adults

The number of emerged adults did not differ significantly regardless on the sunflower type or the initial seed damage (Table 3). It ranged from 45.5 to 47.7, in average (F = 47.4ns, 46.8ns, 45.5ns, P > 0.05, respectively).

3.6. Fecundity

The fecundity was the highest in all treatments with OT hybrid, ranging from 42.7 to 126.7 eggs per a female (Table 3). Within this group, the largest number of eggs was laid on the kernels and 30% of the damaged seeds and the difference within the same type is highly significant (F = 308.68**, P < 0.01). The average fecundity on PT was 79.2 eggs per a female, ranging from 42.7 eggs per a female on undamaged seeds up to 121.0 on kernels. Also, the difference between the fecundity among different damage levels is statistically highly significant (F = 509.07**, P < 0.01). The average number of laid eggs per a female on PFB was 68.1 and the highest fecundity was, as in previous cases, on kernels (118.0) and on 30% damaged seeds (84.7). The values varied significantly (F = 310.97**, P < 0.01). Also, the differences between the fecundity among different types

of the seeds are statistically significant (F = 1.82*, P > 0.01).

3.7. The influence of the sunflower type and the level of the initial damage on life parameters of P. interpunctella

The results of the Two-way ANOVA (Table 4) indicate that both, the type of sunflower and the level of damage, as well as their interaction, affected larval mortality (%), LDD and fecundity. However, MDD (egg to adult) and the number of emerged adults was dependent on the level of damage and interaction (type x damage). The most influential factor in all cases, based on the SS values, was the level of the initial seed damage.

3.8. Principal component analysis (PCA)

To identify the influence of the sunflower seed type and qualitative traits of seeds along with the seed state (kernels and undamaged seeds) on the development of *P. interpunctella*, PCA analysis was used. PCA extracted three principal components with eigenvalue greater than 1 (PC1: 6.12; PC2: 1.47; PC3: 1.05). The first two PCs were meaningfully correlated with variables and used in further analysis.

The first PC (principal component) explained the majority of variation (61.9%). It positively correlated MDD, LDD and mortality with the total tocopherol content. According to PCA biplot, PC1 indicates the influence of the seed state (kernel or undamaged seeds) on the larval development (Fig. 1). It implies that the higher level of larval mortality, LDD and MDD together with the higher level of total tocopherol were related with undamaged seeds of all three sunflower hybrids.

The higher female fecundity was associated within the kernel state only. According to the vector angles (a small angle indicates the variables are positively correlated, an angle of 90° indicates the variables are not correlated, and an angle close to 180° indicates the variables are negatively correlated), the fecundity was positively correlated with the amount of tannin, protein and oil content in the seed (Fig. 1).

The second PC accounted for 14.66% of variation and illustrated the dispersion of observations according to the number of emerged adults and the level of total phenols in the seeds (Fig. 1). These two variables were negatively correlated, which is shown by a vector

Table 3
The mean developmental duration (MDD), number of adults and female fecundity on sunflower seeds.

| Hybrid	Level of initial seed damage	MDD (days)	Average	Number of adults	Average	Female fecundity	Average
OT	Kernel	29.3 ± 1.90 d	39.5 a	49.0 ± 1.01 a	47.5 a	126.7 ± 2.01 a	91.3 a
	10%	42.0 ± 1.10 b		46.7 ± 1.00 ab		85.6 ± 1.52 d	
	20%	43.3 ± 0.56 c		48.3 ± 2.00 a		91.3 ± 2.08 c	
	30%	34.0 ± 1.15 d		49.0 ± 2.00 a		110.0 ± 3.60 b	
	Undamaged	45.7 ± 0.67 a		44.7 ± 0.58 b		42.7 ± 4.51e	
	F value	58.72**		11.45**		308.68**	
PT	Kernel	38.0 ± 0.56 cd	41.4 a	50.0 ± 2.08 a	46.8 a	121.0 ± 3.52 a	79.2 b
	10%	46.3 ± 1.14 b		46.7 ± 0.56 ab		43.7 ± 2.08 d	
	20%	38.0 ± 0.67 c		45.7 ± 2.08 b		90.0 ± 2.65 c	
	30%	36.9 ± 1.15 d		48.7 ± 0.58 ab		98.7 ± 2.08 b	
	Undamaged	48.0 ± 1.00 a		43.0 ± 1.53 c		42.7 ± 2.51 d	
	F value	44.03**		7.31**		509.07**	
PFB	Kernel	39.0 ± 0.67 c	42.1 a	49.0 ± 0.58 a	45.5 a	118.0 ± 5.56 a	68.1 c
	10%	47.0 ± 1.13 b		46.3 ± 0.58 b		36.3 ± 2.08 d	
	20%	38.3 ± 0.56 c		45.3 ± 0.58 c		67.0 ± 1.00 c	
	30%	37.0 ± 1.12 c		47.7 ± 1.53 b		84.7 ± 3.21 b	
	Undamaged	49.3 ± 1.15 a		39.3 ± 1.15 a		34.7 ± 3.51 d	
	F value	49.50**	0.49NS	28.93**	0.69NS	310.97**	1.82*

OT – oil type (hybrid Leone); PT – protein type for human consumption (hybrid NS Colonel); PFB – protein type for bird feed (hybrid Lactal).
MDD - Mean developmental duration.
Value ± SD; F value; Values with the same letter in the column are on the same level of significance for the confidence interval 95%; ** - P < 0.01; * - P < 0.05; NS - P > 0.05.

Table 4
The influence of the sunflower type and the level of the initial seed damage, and their interaction on *P. interpunctella* life history parameters.

Source	df	Larval mortality (%)		LDD (days)		MDD (days)		Adult number		Female fecundity	
		SS	F	SS	F	SS	F	SS	F	SS	F
Corr. model	14	1311.64[a]	28.49	1855.64[a]	96.20	669.24[a]	42.18	281.64[a]	11.32	45206.53[a]	340.29
Type	2	122.84	**18.68****	117.91	**42.79****	1.64	0.72NS	10.71	3.01NS	3644.40	**192.03****
Damage	4	1047.64	**79.63****	1417.64	**257.23****	625.91	**138.07****	153.42	**21.57****	38621.64	**1017.5****
Type* damage	8	141.16	**5.36****	320.09	**29.04****	41.69	**4.59****	117.51	**8.26****	2940.49	**38.74****

Corr. Model − corrected model; [a]R Squared = 0.930 (Adjusted R Squared = 0.897); NS - P > 0.05, *P < 0.05; **P < 0.01; SS-Type III sum of squares; df-degrees of freedom.
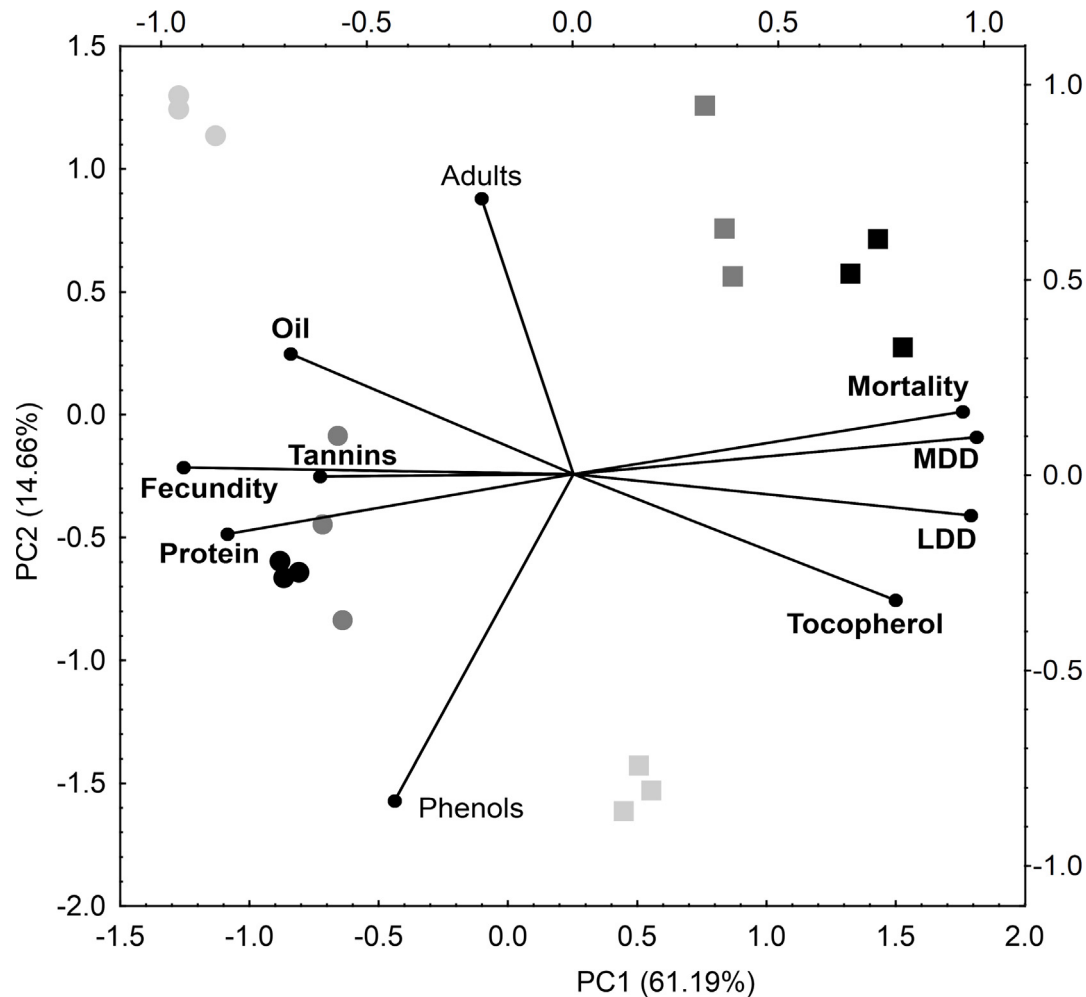
Fig. 1. PCA biplot of qualitative traits of different sunflower type seeds and *P. interpunctella* life history parameters. The scales of the upper x-axis and the right y-axis represent the correlation coefficient between the each variable and the first and second PCs, respectively. The scales of the lower x-axis and the left y-axis represent factor scores of each observation for the first and second PCs, respectively. A small vector angle indicates the variables are positively correlated. Angle of 90° indicates the variables are not correlated. Angle close to 180° indicates the variables are negatively correlated. ● - OT kernel; ■ - OT seed; ● - PT kernel; ■ - PT seed; ● - PBF kernel; ■ - PBF seed.

angle close to 180°. According to PC2, the highest number of emerged adults was registered in an OT kernel and within the seeds of two protein types (PT and PFB). Contrary to this, the observations with the highest level of total phenols (OT-seed, PT and PFB-kernel) were with the lower number of emerged adults.

The PC analysis showed that PBF and PT undamaged seeds were the least suitable for the larval development based on the highest larval mortality rate, longest LDD and MDD, and the lowest number of emerged adults (Fig. 1). This can be a result of higher levels of tocopherols in the entire seed and hull of PFB hybrid. The opposite results were obtained for an OT kernel, which was the most suitable for the larval development, as the values of mortality LDD and MDD were the lowest and contained the highest levels of tocopherols.

Also, the number of emerged adults was the highest in this treatment and the tocopherol and phenol content was the lowest. The same was registered for a PT seed and PFB seed that had the lowest number of emerged adults but the lowest phenol content.

The correlation analysis confirms the above presented results. Namely, LDD and MDD were in strong positive correlation with the tocopherol content (p = 0.845**; 0.694**, respectively). However, the strong negative correlation was between LDD and MDD with the female fecundity (p = −0.887**; −0.958**, respectively) and between the fecundity and tocopherol content (p = −0.623**). LDD and MDD were also negatively correlated with the oil content (p = −0.758**; −0.610**).

4. Discussion

Many studies have been carried out to determine the effect of different commodities on *P. interpunctella* life history parameters. However, data are lacking for some of the commodities that are commonly infested by *P. interpunctella*, such as bird seed, pet foods, spices, oil crops etc. According to Nansen and Phillips (2004), a promising approach of studying the feeding preferences and life history of *P. interpunctella* is to classify the food types into several broader categories, based on the different criteria, like the nutrient composition. In this work, the seeds were divided according to the oil and protein content.

The highest average mortality of *P. interpunctella* larvae was recorded on PFB seeds, regardless on the initial seed damage. According to the results of biochemical analysis, the undamaged seeds of PFB hybrid contained the highest levels of tocopherols while kernels contained the highest levels of and total phenols. Also, the highest values of DPPH, FRAP and ABTS (antioxidative activity indicators) were in the entire seeds, hulls and kernels of PFB hybrid. High values of the mentioned parameters indicate the presence of secondary metabolites, which can be responsible for the higher mortality of *P. interpunctella* larvae and can affect the development of insects (Arnason et al., 1992; Cox, 2004; Kubo, 2006; García-Lara and Bergvinson, 2014). The Two-way analysis showed that the larval mortality was influenced mainly by the level of the initial seed damage. This is in accordance with reports of LeCato (1976) and Predojević et al. (2017), who found that cracked and ground maize kernels were more favourable for the growth, development and reproduction of *P. interpunctella*. According to Kaliyan et al. (2005), a very low percentage of larvae survived on whole maize kernels (7—28%). This work, however, presents the first report of the influence of the sunflower seed damage on *P. interpunctella* larval mortality.

MDDs reported in this work are in accordance with several literature data. According to Rees (2004), lifecycle of *P. interpunctella* could be completed in 30 days under optimal conditions, or in 27—52 days depending on factors such as temperature, type of food, presence of oil, food odour etc., as reported by Onaolopo et al. (2017). The development duration depends on the quality and quantity of food (Almaši, 1984). Diet is the most important factor for determining the developmental period of the insects that feed on a wide range of food (Silhacek and Murphy, 2005) and as stated by Onaolopo et al. (2017), diets rich in fats (about 11.50%) support the growth of this insect. In this work, the level of the initial damage was the most influential factor for MDD, which was the longest on the undamaged seeds of PFB and PT hybrids. The results support Predojević et al. (2017), who showed that the mean development and can last from 34.0 to 47.0 days, but the longevity depends on the state of the maize kernel.

The pupation and adult emergence occurred sooner on OT and PT than on PFB. The biochemical composition of tested hybrids showed that OT seeds were rich in oils and the other two in proteins. Bouayad et al. (2008) reported that the commodity rich in proteins (wheat, barley and sorghum) can cause sooner pupation and adult emergence, compared with glucose rich commodity. The highest fecundity of *P. interpunctella* females was recorded on OT sunflower seeds. The most comprehensive study on the effect of nutrition on the fertility and a number of generations of *P. interpunctella* in Serbia was done by Almaši (1984). This author reported that among 43 tested food products, fecundity was the highest on the sunflower seeds. Nansen and Phillips (2003) reported that *P. interpunctella* has ovipositional preference towards the oil rich substrate. According to Vukajlović and Pešić (2012), protein and fat concentration in a larval diet is an important factor because it affects oogenesis. Also, it has been noted that

P. interpunctella larvae consume reproductive parts of seeds (germ), which is rich in proteins and fats (Alamši, 1984; Silhacek and Murphy, 2005). This assumption fully corresponds to the fact that adults do not feed at all, so larvae must collect (by eating) all necessary substances for the adult's living (Almaši and Srdić, 1988). The number of laid eggs in this research ranged from 64.2 to 91.3 on average, regardless of the initial seed damage but depending on the type of the sunflower. There are different data about the number of laid eggs, depending on the nutrient medium, starting from only 26 on wheat (Almaši, 1984), 54.9 on wheat flour (Deseÿ, 1976), 172.2 on split maize (Allotey and Goswami, 1990), 117—303 on garlic seeds (Peres-Mendosa and Aguilera-Peña, 2004) and 258—270 on walnuts, almonds, and wheat bran diet (Johnson et al., 1992), 288 on laboratory diet and up to 326 on dates (Boles and Marcke, 1966), while Johnson et al. (1997) reported the fecundity on wheat bran as 325—433 eggs per a female. According to Vukajlović and Pešić (2012), the highest fecundity was registered from females that fed as larvae on wheat germs (137.26), that were the richest in the protein content, compared to other components of a standard laboratory diet of rolled oats (94.82%) rich in fibres and whole wheat flour (rich in carbohydrates (66.04%)).

The results of this work indicate that the most influential factor for all the observed parameters of *P. interpunctella* life history and survival was the level of the initial seed damage. The main hypothesis of this work, that the hull condition could significantly influence the life history of *P. interpunctella*, was proven to be right. The presented results indicate the importance of a proper post-harvest management in the prevention of losses from this pest in storages. Shah (2013) reported that the mechanical damage during harvesting and threshing can result in bruised areas on grains, which may serve as centres for infections and a cause of deterioration. Results of many studies indicate that the mechanical state of a maize kernel has influenced some aspects of the development of this insect pest (Mbata, 1990; Kaliyan et al., 2005; Predojević et al., 2017), with broken kernels being more suitable for the development and survival of *P. interpunctella*, than the whole kernels. According to Kaliyan et al. (2005), cleaning the maize from broken kernels before storage is a very important measure that could reduce infestation caused by *P. interpunctella* to a tolerable level. Therefore, the precise separation of damaged seeds is of great importance. The presence of cracked seeds also positively favors the development of other major stored-product pests, i.e., *Trogoderma granarium* Everts, (Athanassiou et al., 2016; Athanassiou et al., 2017a), *Cryptolestes ferrugienus* (Steph, 1893), *Tribolium* spp. etc. Given that co-existence is a common phenomenon among stored-product insects (Athanassiou et al., 2014, 2017b; Kavallieratos et al., 2017) cracked grains could be the vehicle of a drastic increase in pests' population that can result in elevated infestation levels during storage.

Presumably, the pericarp may present a barrier to *P. interpunctella* larval attacks, which was also reported by Abdel-Rahman et al. (1968) and LeCato (1976) and Siwale et al. (2009) for insects in general. However, this presumption can also refer to sunflower seeds and indicate the importance of the hull preservation during the post-harvest processing. This is extremely important in the production of a high category seeds, particularly, to maintain the seed viability and germination.

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