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ORIGINAL ARTICLE

Nutrient content restructuring and CT-measured density, volume attritions on damaged beans caused by *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae)

Sándor Keszthelyi[1]*, Helga Bosnyákné Egri[1], Dávid Horváth[2], Ádám Csóka[3], György Kovács[4], Tamás Donkó[5]

[1] Department of Plant Production and Plant Protection, Faculty of Agricultural and Environmental Sciences, Kaposvár University, H-7400 Kaposvár, Guba S. str. 40, Hungary

[2] Department of Entomology, Faculty of Horticulture Science, Szent István University, H-1118 Budapest, Villányi str. 29-43, Hungary

[3] Health Center, Kaposvár University, H-7400 Kaposvár, Guba S. str. 40, Hungary

[4] Analytical Minds Ltd., H-4032 Debrecen, Civis str. 8, Hungary

[5] Institute of Diagnostic Imaging and Radiation Oncology, Kaposvár University, H-7400 Kaposvár, Guba S. str. 40, Hungary

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*Corresponding address:
ostrinia@gmail.com

Abstract

Leguminous plant products have great nutritional and economic importance in the European Union, which is reflected by its protein policy. These harvested yields are risked by stored product pests, such as *Acanthoscelides obtectus* Say, which can cause up to 50–60% loss in stored bean items. The bean weevil causes both quantitative and qualitative damage to seeds. We aimed to map the qualitative damage of this devastating pest, which deteriorates the nutritional content of bean kernels. Furthermore, our purpose was to determine accurately the decrease in the volume and density alteration in beans caused by this important stored product pest using CT-assisted imaging analysis. Our results showed that the nutritional arrangement in damaged beans was caused by *A. obtectus*. The measured nutrient content increment in damaged samples can be explained by the presence of extraneous organic material which originates from perished specimens of the bruchin pest. This is a negative phenomenon in bean items used as forage, because of the loss of valuable proteins and rancidity in herbal oils. Weight loss triggered by developing larvae was 49.42% in examined bean items. The use of 3D technologies has greatly improved and facilitated the detailed investigation of injured seeds. The density (75,834 HU; 41.93%) and the volume (296.162 mm^3; 26.21%) values measured by CT of the examined samples were significantly decreased. The decreasing of tissue density in damaged beans can be accounted for by the consumption of starch present at a high ratio and that of the dense reserve components in the cotyledons.

Key words: *Acanthoscelides obtectus* Say, bean, nutrient attrition, CT-assisted analysis, pests, storage

Introduction

Insects are major post-harvest pests of crops both at the farmers' and consumers' levels especially in tropical circumstanses (Babarinde *et al.* 2008). Storage of legumes is an integral part of post-harvest procedures over the course of the "field to table" yield management. An estimated 5–15% total weight loss of all plant-derived produce occurs during post-harvest events (Harris and Lindblad 1978). Therefore, there is an increasing necessity to prevent foodstuff-depreciation from occurring particularly in the form of quality deterioration and weight loss during storage. According to Singh (2010), feeding and waste production by insects are some of the primary factors leading to quantitative and qualitative damage in stored grains. This is exacerbated by many types of storage-insects' becoming cosmopolitan, which has been gradually occurring since the commencement of harvesting and storing foodstuffs. This tendency is even more aggravated via world-wide transportation practices and distribution of agricultural produce (Kozár 1997).

The bean weevil, *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae) is an important pest causing substantial damage in terms of both quantitative and qualitative traits of stored grain stock (Baier and Webster 1992). The former is accounted for by grain weight loss due to insect feeding (Gołebiowska 1969), whereas the latter is explicable by product alterations such as the decrease in nutritional value, higher levels of rejections in the grain mass, and depravation of stocks' industrial characteristics.

Acanthoscelides obtectus is originally native to Central America. It was introduced into Europe at the end of the 19th century, from where it has subsequently spread around the globe. It has escalated in Europe, Asia, North and South America, Africa, Australia and elsewhere. Its global spreading is attributable to both global warming, which supports field overwintering, and changes in crop storage (Alvarez *et al.* 2005; Oliveira *et al.* 2013).

Acanthoscelides obtectus has five to six generations per year. Both larvae and beetle can overwinter, usually only in storehouses (Alvarez *et al.* 2005; Wightman and Southgate 1982). The insects migrate to fields at 20°C. Initially, they feed on vetch, pea, lupin, alfalfa, and other leguminous cultures; they then move on to haricot bean, bean, soybean, lentils (Baier and Webster 1992; Tucić *et al.* 1997). Larvae penetrate grains, eating their content completely. Several larvae (even up to 50) can develop in one grain. The larvae usually consume the entire pod content, decreasing the yield by 50–60% (Padin *et al.* 2002). Partially damaged grains lose their germinating power and taste quality (Tucić *et al.* 1997; Odagiu and Porca 2003; Guzzo *et. al.* 2015).

The use of novel technologies, especially computer-based three-dimensional reconstructions and CT have greatly improved and facilitated the detailed investigation of injured grain, other plant part assays and insect anatomy (Ribi *et al.* 2008). Furthermore, the burrowing behaviour of insects has already been studied using CT technology (Harrison *et al.* 1993).

The aim of this study was to understand the effect of *A. obtectus* qualitative damage, which disrupts kernel development in beans and what it entails concerning the composition of bean forage, particularly its protein, starch, fat and ash content. Furthermore, our purpose was to determine accurately the decrease in volume and density alterations in beans caused by this important stored product pest using computer-based three-dimensional reconstruction technology.

Materials and Methods

Sample collecting and nutrient content analysis

Damaged and intact bean samples were collected from the Iregszemcse storehouse of the Forage Crop Research Institute of the Kaposvár University (the GPS coordinates are: N 46°41'18.79", E 18°10'50.76"), in winter 2016. Before collecting the samples, there was no insecticide treatment on the territory. The damage was easily discernible due to perforated bean coats caused by the pest. The intact and damaged samples were taken to the laboratory, where, following cleaning and sieving, they underwent Weende analysis.

The determination of raw protein, raw fat, ash, and raw fibre content was carried out in the Physiological and Biochemical Laboratory of the Agricultural and Environmental Sciences Faculty of the Kaposvár University (Kaposvár, Somogy County, Hungary). The raw protein content, the fatty acid composition (Hungarian Standard 1979), the crude fibre content (EC 2009) and the raw ash content (Hungarian Standard 1992) of the samples were examined on damaged and intact samples in four replicates (Hungarian Standard 2009).

The emergence holes were counted on randomly-selected 30 bean seeds, from which the average larva number ($\bar{x} \pm SE$), and consumed vegetal weight were calculated. This calculation was based on Jermy's (1952) study, according to which imagos of this pest leave the bean usually through a separate hole, and one larva consumes exactly 4.78-times the weight of the freshly hatched imago (5.3–5.7 mg) during their postembryonic development.

Computed tomography assisted imaging analysis

The CT measurements were performed covering the whole been seeds using a Siemens Somatom Definition Flash CT scanner (Siemens Ltd., Erlangen, Germany) at the Institute of Diagnostic Imaging and Radiation Oncology of the Kaposvár University (Hungary). The 54 healthy and 54 destroyed, randomly selected seeds were positioned in a 3 × 3 × 6 matrix in a polystyrene rack on the examination table (Fig. 1). The following scanning parameters were used for data collection purposes: 100 kV, 300 mAs and spiral data collection with pitch 0.6. Axial scans were reconstructed every 0.1 mm with 0.6 mm slice thickness and 55 mm field of view using medium soft convolution kernel: B30s and abdomen window. The images were archived in DICOM (Digital Imaging and Communications in Medicine) format and each

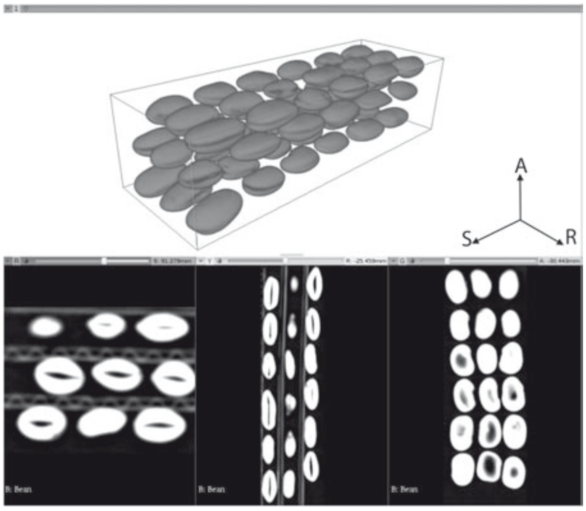

Fig. 1. 3D reconstruction and three plane cross section images of beans based on CT examination using Slicer 3D program. A – anterior, S – superior, C – right lateral

of the series was subsequently converted to MINC metafiles (Medical Imaging NetCDF). The resolution of the meta-images was nearly isotropic: 0.107 × 0.107 × 0.1 mm^3.

The images obtained were evaluated by means of OpenIP software package (Kovács *et al.* 2010). The first step was the separation and identification of the individuals and the allocation of them into files. The seeds were segmented by threshold method using the –300 HU (Hounsfield Unit) lower limit. The average radiodensities and volumes of the seeds were calculated using the voxels belonging to the seeds. Next, the coordinates of those seed voxels which were adjacent to other grains were extracted and considered to be the coordinates of the surface points of the seeds. Based on these 3D point clouds the surfaces of individual seeds were reconstructed through triangularization. The 3D models of seeds were made by Slicer 3D program (Fedorov *et al.* 2012) for measuring the surface size and visualisation of the holes and the channels caused by *A. obtectus*.

Statistical analysis

In order to test the nutrient content ($n \leq 50$) and the density and cavity values ($n > 50$), the Shapiro-Wilk and the Kolmogorov-Smirnov tests were used, respectively. For the survey of the normal distribution of data ($p < 0.05$), the method of Ghasemi and Zahediasl (2012) was employed. Nutrient content, cavity volume and density values of different samples were statistically analysed by one-way ANOVA by using the SPSS for Windows 11.5. software package. Mean values were separated by using the Tukey (HSD) test, at $p \leq 0.05$.

Results

The nutritional change caused by *A. obtectus* has been unequivocally confirmed by the results of Weende analysis of this study (Table 1). Interestingly, each examined nutrient content of damaged items was higher than the same parameters of intact samples. Especially, the alteration of raw protein percentage triggered by the arthropod pest was remarkable.

Shapiro-Wilk normality test indicated that the nutrient content data obtained were of normal distribution, $p > 0.05$. In contrast, different statistical relations could be evinced by variance-analysis. The raw protein value ($p = 0.042$), raw fat ($p = 0.038$) and raw fibre ($p = 0.002$) deviated significantly from the healthy items. The formation of raw ash showed no statistically significant deviation ($p = 0.519$), i.e. the damaged items did not change compared to the intact ones.

The calculated average larva number based on emergence holes per seed was 8.93 ± 1.83. The estimated weight of organic matter destroyed by the developing larvae was 234.76 ± 8.54 mg, which is 49.42% weight loss in the case of the examined leguminous items.

The kernel tissue densities expressed by HU of intact and damaged beans are shown in Table 2. The damaged samples have much lower radio-density values,

Table 1. Statistical data of Weende-analysis [%] of intact and damaged bean samples by *Acanthoscelides obtectus* (n = 4)

Bean samples		Mean [x̄]	Standard deviation	± SE	95% CI for mean	
					lower bound	upper bound
Raw protein	intact	27.033	0.152	0.088	26.654	27.413
	damaged	31.133	0.057	0.033	30.990	31.277
Raw fat	intact	1.045	0.041	0.024	0.952	1.138
	damaged	1.233	0.046	0.033	1.090	1.377
Raw fibre	intact	4.633	0.208	0.120	4.116	5.150
	damaged	5.233	0.251	0.145	4.608	5.858
Raw ash	intact	4.312	0.113	0.062	4.254	4.370
	damaged	4.433	0.033	0.052	4.290	4.577

Table 2. Statistical data of CT-assisted volume and density values of intact and damaged bean samples by *Acanthoscelides obtectus* (n = 54)

Bean samples		Mean [x̄]	Standard deviation	± SE	95% CI for mean	
					lower bound	upper bound
Density [HU]	intact	180.630	28.048	3.817	172.974	188.285
	damaged	104.796	65.885	8.966	86.813	122.779
Volume [mm³]	intact	1129.857	243.859	33.185	1063.296	1196.418
	damaged	833.695	253.573	34.507	764.483	902.907

HU – Hounsfield unit

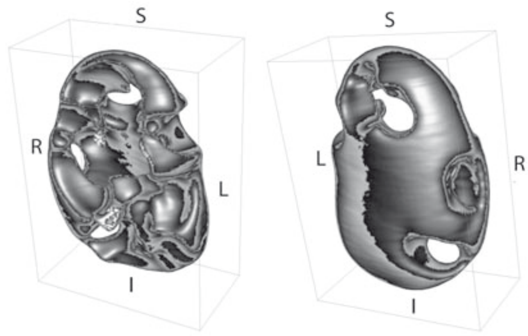

Fig. 2. Computer-based three-dimensional reconstructions of damaged bean seed by *Acanthoscelides obtectus*. L – left, R – right, I – interior, S – superior

than intact samples. The mean changing density of the damaged bean was 75,834 HU and 41.93%. Eventually, the dense tissue formation disappeared from the bean kernel, the cause of which may be due to mastication by *A. obtectus*.

The volumes of the examined bean samples parallel with the density results were measured by CT. The volume of the examined intact samples was 1129.857 ± 33.7185 mm^3. The change in the volume of the damaged samples was 296.162 mm^3 on average, (26.21% change).

The Kolmogorov-Smirnov normality test indicated that our CT-measured data are of a normal distribution, $p > 0.05$. Uniform significant differences ($p = 0.000$) both in the density and volume data were proven by statistical analysis of intact and damaged samples.

Computer-based three-dimensional (3D) reconstructions of bean kernels damaged by *A. obtectus* can be seen in Figure 2. The pericarp always remained intact, only emergence holes of adults are conspicuous. The destruction of the cotyledon, which is located in the central part of the seed, is discernible based on the location of apparent cavities revealed by CT. The reserve nutrient content necessary for embryo and juvenile plant development was entirely destroyed by the larvae.

Discussion

The total weight loss of stored leguminous seed can reach up to 40% (Midega *et al.* 2016). According to Adams (1976), there are two types of stored plant product weight losses; an apparent loss in which only the change in the weight of a sample is considered and a real loss which is the apparent loss plus the weight of fragments remaining in the kernel. According to our findings, the damage caused by *A. obtectus* altered the raw protein, raw fat, raw fibre, raw ash and moisture content of bean in a verifiable manner. The increased nutrient values of damaged samples can be explained by perished specimens of the bruchin pest (even 29 larvae/kernel, as in our case), which was located in the kernel's cavity. Unfortunately, this change does not cause an unequivocally positive consequence in bean items used as forage, because of the decrease or loss of valuable proteins and the rancidity of herbal oils as well as complication of assembly of forage additives (Nielsen 1988; Nowaczyk *et al.* 2008). So, using beans damaged by insect pest as protein components for forage items is highly questionable and raise concerns (Kucska *et al.* 2016).

Volume and density deficiencies of damaged bean samples were unequivocally proven by computed tomography examination. The decrease of tissue density in damaged beans can be expounded by the consumption of high starch content and dense reserve components in the cotyledon (Vose *et al.* 1976; Bonants and Witt 2017). Naturally, stored product-insects may damage the seed embryos, causing a decrease in germination (Baier and Webster 1992).

The results provided by CT can be used for both entomology and plant physiology as well. This method provides additional data for biological and ecological information studying the hidden lifestyles of covertly developing insects. Therefore, it can greatly contribute to the realisation of IPM criteria in stored product protection as well.

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References

Adams J.M. 1976. Weight loss caused by development of *Sitophilus zeamais* Motsch. in maize. Journal of Stored Products Research 12: 269–272. DOI: https://doi.org/10.1016/0022-474X(76)90043-6

Alvarez N., McKey D., Hossaert-McKey M., Born C., Mercier L., Benrey B. 2005. Ancient and recent evolutionary history of the bruchid beetle, *Acanthoscelides obtectus* Say, a cosmopolitan pest of beans. Molecular Ecology 14 (4): 1015–1024. DOI: https://doi.org/10.1111/j.1365-294x.2005.02470.x

Babarinde S., Sosina A., Oyeyiola E. 2008. Susceptibility of the selected crops in storage to *Sitophilus zeamais* Motschulsky in Southwestern Nigeria. Journal of Plant Protection Research 48 (4): 541–550. DOI: https://doi.org/10.2478/v10045-009-0003-z

Baier A.H., Webster B.D. 1992. Control of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) in *Phaseolus vulgaris* L. seed stored on small farms. II. Germination and cooking time. Journal of Stored Products Research 28: 295–299. DOI: https://doi.org/10.1016/0022-474X(92)90012-F

Bonants P., Witt R.T. 2017. Molecular Diagnostics in Plant Health. p. 175–202. In: "Molecular Diagnostics" (E. van Pelt-Verkuil, W. van Leeuwen, R.T. Witt, eds.). Springer, Singapore, 237 pp. DOI: https://doi.org/10.1007/978-981-10-4511-0_9

EC. 2009. Commission Regulation (EC) No 152/2009 of 27 January 2009 laying down the methods of sampling and analysis for the official control of feed (Text with EEA relevance) Annex III/I., Determination of crude fibre, OJ EU, L 54/40–42

Fedorov A., Beichel R., Kalpathy-Cramer J., Finet J., Fillion-Robin J.C., Pujol S., Bauer C., Jennings D., Fennessy F.M., Sonka M., Buatti J., Aylward S.R., Miller J.V., Pieper S., Kikinis R. 2012. 3D Slicer as an image computing platform for the quantitative imaging network. Magnetic Resonance Imaging 30 (9): 1323–1341. DOI: https://doi.org/10.1016/j.mri.2012.05.001

Ghasemi A., Zahediasl S. 2012. Normality tests for statistical analysis: A guide for non-statisticians. International Journal of Endocrinology and Metabolism 10 (2): 486–489. DOI: https://doi.org/10.5812/ijem.3505

Gołebiowska Z. 1969. The feeding and fecundity of *Sitophilus granarius* (L.), *Sitophilus oryzae* (L.) and *Rhyzopertha dominica* (F.) in wheat grain. Journal of Stored Products Research 5: 143–155. DOI: https://doi.org/10.1016/0022-474X(69)90056-3

Guzzo E.C., Vendramim J.D., Chiorato A.F., Lourenção A.L., Carbonell S.A.M., Corrêa O.M.B. 2015. No correlation of morpho-agronomic traits of *Phaseolus vulgaris* (Fabaceae) genotypes and resistance to *Acanthoscelides obtectus* (Say) and *Zabrotes subfasciatus* (Boheman) (Coleoptera: Chrysomelidae). Neotropical Entomology 44: 619–625. DOI: https://doi.org/10.1007/s13744-015-0315-4

Harris K.L, Lindblad C.J. 1978. Post-harvest grain loss assessment methods. A manual of methods for the evaluation of post-harvest losses. American Association of Cereal Chemists. Office of Nutrition, U.S. Agency for International Development. Available on: http://pdf.usaid.gov/pdf_docs/PNAAG842.pdf

Harrison R.D., Gardner W.A., Tollner W.E., Kinard D.J. 1993. X-ray computed tomography studies of the burrowing behaviour of fourth-instar pecan weevil (Coleoptera: Curculionidae). Journal of Economical Entomology 86: 1714–1719. DOI: https://doi.org/10.1093/jee/86.6.1714

Hungarian Standard. 1979. Animal feeding stuffs. Determination of nutritive value. Determination of crude fat content. Hexane extract method. MSZ 6830-19:1979

Hungarian Standard. 1992. Animal feeding stuffs. Determination of crude ash. MSZ ISO 5984:1992

Hungarian Standard. 2009. Animal feeding stuffs. Determination of nitrogen content and calculation of crude protein content. Part 2: Block digestion and steam distillation method (ISO 5983-2:2009)). MSZ EN ISO 5983-2:2009

Jermy T. 1952. A babzsizsik (*Acanthoscelides obtectus* Say): táplálékfogyasztása. [The food consumption of the bean weevil, *Acanthoscelides obtectus* Say.] Annales Instituti Protectionis Plantarum Hungarici, Budapest 5: 305 (in Hungarian)

Kovács Gy., Ivan J.I., Panyik A., Fazekas A. 2010. The open IP open source image processing library, pp. 1489–1492. Proceedings of the 18th ACM Multimedia International Conference, 25–29. October 2010. Firenze, Italy. DOI: https://doi.org/10.1145/1873951.1874255

Kozár F. 1997. Insects in a changing world. Acta Phytopathologica et Entomologica Hungarica 32: 129–139.

Kucska B., Kabai P., Hajdú J., Várkonyi L., Varga D., Müllerné--Trenovszki M., Tatár S., Urbányi B., Zarski D., Müller T. 2016. *Ex situ* protection of the European mudminnow (*Umbra krameri* Walbaum, 1792): Spawning substrate preference for larvae rearing under controlled conditions. Archives of Biological Sciences 68 (1): 61–66. DOI: https://doi.org/10.2298/abs150428008k

Midega C.A.O., Murage A.W., Pittchar J.O., Khan Z.R. 2016. Managing storage pests of maize: Farmers' knowledge, perceptions and practices in western Kenya. Crop Protection 90: 142–149. DOI: https://doi.org/10.1016/j.cropro.2016.08.033

Nielsen S.S. 1988. Degradation of bean proteins by endogenous and exogenous proteases – a review. Cereal Chemistry 65 (5): 435–442.

Nowaczyk K., Obrępalska-Stęplowska A., Gawlak M., Olejarski P., Nawrot J. 2008. The RAPD analysis of genetic variability in the granary weevil (*Sitophilus granarius* L.) populations. Journal of Plant Protection Research 48 (4): 429–435. DOI: https://doi.org/10.2478/v10045-008-0052-3

Odagiu A., Porca M. 2003. The influence of the chemical composition of different origin beans (*Phaseolus vulgaris* L.) on tolerance to the bean weevil (*Acanthoscelides obtectus* Say) stroke. Journal of Central European Agriculture 4: 14–22.

Oliveira M.R.C., Corrêa A.S., De Souza G.A., Guedes R.N.C., De Oliveira L.O. 2013. Mesoamerican origin and pre- and post-Columbian expansions of the ranges of *Acanthoscelides obtectus* Say, a cosmopolitan insect pest of the common bean. Plos ONE 8: 1–12. DOI: https://doi.org/10.1371/journal.pone.0070039

Padin S., Dal Bello G., Fabrizio M. 2002. Grain loss caused by *Tribolium castaneum, Sitophilus oryzae* and *Acanthoscelides obtectus* in stored durum wheat and beans treated with *Beauveria bassiana.* Journal of Stored Products Research 38 (1): 69–74. DOI: https://doi.org/10.1016/S0022-474-X(00)00046-1

Ribi W., Senden T.J., Sakellariou A., Limaye A., Zhang S. 2008. Imaging honey bee brain anatomy with micro-X-ray-computed tomography. Journal of Neuroscience Methods 171 (1): 93–97. DOI: https://doi.org/10.1016/j.jneumeth.2008.02.010

Singh G. 2010. The Soybean. Botany, Production and Uses. CAB International, Oxfordshire, 512 pp.

Tucić N., Stojković O., Gliksman I., Milanović A., Šešlija D. 1997. Laboratory evolution of life-history traits in the bean weevil (*Acanthoscelides obtectus):* the effects of density-dependent and age-specific selection. Evolution 51 (6): 1896–1909. DOI: https://doi.org/10.1111/j.1558-5646.1997.tb05112.x.

Vose J.R., Basterrechea M.J., Gorin P.A.J., Finlayson A.J., Youngs C.G. 1976. Air classification of field peas and horsebean flours: chemical studies of starch and protein fractions. Cereal Chemistry 53 (6): 928–936.

Wightman J.A., Southgate B.J. 1982. Egg morphology, host, and probable regions of origin of the bruchids (*Coleoptera: Bruchidae*) that infest stored pulses – an identification aid. New Zealand Journal of Experimental Agriculture 10: 95–99. DOI: https://doi.org/10.1080/03015521.1982.10427850