ratar. povrt.

original scientific paper
Ratar. Povrt. 2021, 58(1): 14-22
doi: 10.5937/ratpov58-31204
Received: 8 March 2021
Accepted: 17 April 2021

# Archaeoentomological assessment of weevil (Coleoptera, Bruchidae) infestation level of pea (*Pisum sativum*) at the Late Bronze Age settlement Hissar

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**Summary:** A find of 2572 charred seeds of pea (*Pisum sativum* L.) was detected at the Late Bronze Age tell settlement Hissar near Leskovac, in Serbia, belonging to the Brnjica cultural group, 14–10 cent. BC. Two types of pea seeds were observed: apparently healthy seeds and seeds damaged by the activity of a weevil (Coleoptera, Bruchidae). At least two-fifths of all finds have apparently been infested most probably by pea weevil (*Bruchus pisorum* L.), one of the most important pea pests worldwide, especially in medium-moist and dry climates, such as Southern Europe and Australia. A large amount of infested pea seeds indicates a developed pea production on small plots, strongly indicating that cultivating this ancient pulse crop must have been well-rooted in field conditions. Previous DNA analyses of charred pea placed the ancient Hissar pea at an intermediate position between extantly cultivated pea (*P. sativum* L. subsp. *sativum* var. *sativum*) and a wild, winter hardy, 'tall' pea (*P. sativum* subsp. *elatius* (Steven ex M. Bieb.) Asch. et Graebn.). Based on an assumption of its late harvest time and combined with pea weevil life cycle stage in charred seeds, it was possible to estimate the season during which the seeds were carbonized, namely, the second half of July or the first days of August at the latest. Older, final weevil instars were predominant before seed carbonization. The pea infestation rate at Hissar is one of the highest noted among pulses in the Old World and the highest among peas, so far.
**Key words:** *Bruchus pisorum* L., Hissar, infestation, Late Bronze Age, mass find, pea, Pea weevil

## Introduction

Legumes, along with cereals, have always been a very important component of the human diet (Kislev, 1991). They belong to primary crops. It has been debated that people started cultivating legumes, e.g. lentil (*Lens* spp.), even before wheat (*Triticum* spp.) and other cereals (Weiss et al., 2006). The farming of legumes was practiced in the southern Levant as early as 10.240–10.200 (1σ) ago (Caracuta et al., 2017). The legumes identified at the Early Pre-Pottery Neolithic B site in Israel include lentil, inconspicuous vetchling (*Lathyrus inconspicuus* L.), Jerusalem vetchling (*Lathyrus hierosolymitanus* Boiss.), pea, bitter vetch (*Vicia ervilia* (L.) Willd.), faba bean (*Vicia faba* L.) and Narbonne vetch (*Vicia narbonensis* L.). Pea has been one of the most significant crops in Serbia and the Balkans since 6th millennium BC (Medović & Mikić, 2015). There is strong evidence of deliberate collection of 'tall' pea (*Pisum sativum* L. subsp. *elatius* (M. Bieb.) Asch. & Graebn. var. *elatius* (M. Bieb.) Alef.) as food at pre-agricultural and early agricultural sites across southwest Asia (Wallace et al., 2019). Tall pea is regarded as a direct progenitor of a cultivated pea (Zaytseva et al., 2017) and has been reassessed in the flora of Serbia, especially in the upper flow of the river Pčinja (Mikić et al., 2014).

Several thousand years later, at the multi-layered archaeological tell settlement Hissar near Leskovac, which includes layers that belong to the transitional period between the Late Bronze Age and the Early Iron Age (Brnjica cultural group, 14–10 cent. BC) a mass find of 2572 charred peas was discovered (Medović, 2012). A

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Acknowledgements:
We thank Dr. Gérard Duc from INRA Dijon, France, for inspiring this work and Dr. Dalibor Živanov from the Institute of Field and Vegetable Crops in Novi Sad for his support. In memory of Predrag Medović (1930–2021).

Cite this article:
Medović A. & Mikić A. (2021). Archaeoentomological assessment of weevil (Coleoptera, Bruchidae) infestation level of pea (*Pisum sativum*) at the Late Bronze Age settlement Hissar. *Ratar. Povrt.,* 58 (1), 14-22.

recent pan-European study on broomcorn millet (*Panicum miliaceum* L.) provided the possibility of indirect radiocarbon dating of the rich pea collection by the AMS C14 technique (Filipović et al., 2020). In the same deposit, more than 300 charred grains of broomcorn millet were identified (Medović et al., 2011). They represent unintended impurities of stored pea seeds. Broomcorn seeds derive from the same period as those of pea. Two dates were obtained: 2965 ± 35 and 2920 ± 35 years BP, i.e. 1280–1053 and 1218–1011 cal BC. The results coincided with the approximate date of the Brnjica cultural group (Stojić et al., 2007). Significant discoveries have been made by analyzing ancient DNA extracted from charred peas (Smýkal et al., 2014). It was assumed that the pea that grew on the plots of the ancient farmers at Hissar had colored flowers and the pigmented seed coat, similarly to today's field pea (*Pisum sativum* subsp. *sativum* var. *arvense* (L.) Poir.).

This paper was aimed at a combined archaeoentomological and palaeoagronomical assessment of the infestation of pea seeds from the Late Bronze Age settlement Hissar by Bruchidae weevils.

## Material and Methods

The fortified hill fort settlement Hissar in Leskovac was sampled by the archaeologists for the plant remains since 1999 (Medović, 2012). In the campaign of the year 2005, a rich pulse-crop sample was gathered from the deposits of the Brnjica cultural group. The sample size was 7 liters of soil substrate (Medović et al., 2011). It was subjectively taken from an archeological feature belonging to Phase IIa. The flotation took place in 2006 in the Museum of Vojvodina in Novi Sad. The sieve with a mash size of 0.25 mm was used for the hand flotation. Heavy pulse seeds don't float easily. Great care is needed to separate moist, at this stage the most fragile, charred pulse seeds from the substrate. The plant material had been drying slowly for several days in a dry, dark place to be analyzed later using low power (7×–45×) microscopes. Sieving is performed for the easier identification work in three fractions: 3 mm, 1.5 mm, and 0.25 mm. Through sieving plant material was submitted to mechanical force. It turned out that every reexamination of the same plant material was combined with mechanical damage on seeds affected by the activity of a phytophagous insect pest. The Harmfulness coefficient (q, %) was calculated by the formula used by Nikolova (2016a): $q = (a - b)/a \times 100$ (a—weight of 1000 apparently healthy seeds; b—the weight of 1000 damaged seeds).

## Results

During the archaeobotanical analysis, two types of pea seeds were observed: apparently healthy seeds and seeds damaged by the activity of a phytophagous insect pest (Fig. 1). Additionally, a charred fragment of a holometabolic insect larva was found. What also remained unclear was the assessment of the infestation level. After a re-examination of the charred plant material, it was calculated that the percentage of visibly damaged seeds was 38.31%, while seeds that did not show visible damages accounted for 61.69% of the total amount of charred peas. It can be assumed that at least two-fifths of all pea finds were infested. Additionally, by splitting the cotyledons, at least seven more charred larvae were discovered inside the seeds. One larva was 1.3 mm long and 0.8 mm wide and the other 1.4 mm long and 0.9 mm wide (Fig. 2). A larva from a multiple-infested seed was 1 mm long and 0.4 mm wide. The other larvae were deformed to such an extent that they became almost unrecognizable during charring. Damaged seeds indicated typical activity of the pea weevil larvae activity.

Fig. 1. At least two-fifths of all charred peas from the rich Late Bronze Age Hissar pea deposit were infested by bruchids

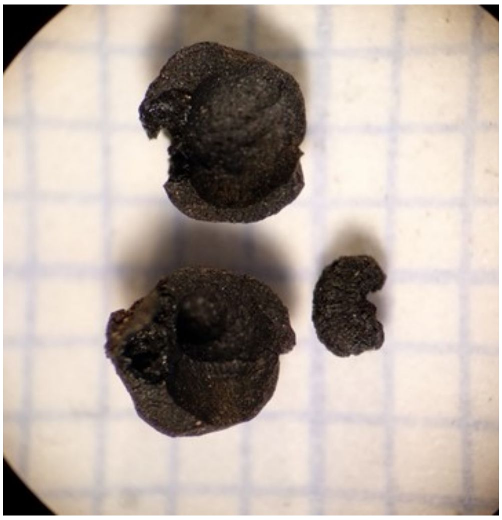

Fig. 2. Pea seed from the Late Bronze Age Hisar damaged by weevil larva

According to the visual observations of the external signs of infestation, internal damages on pea seed (Fig. 1 –5) and detected larvae inside some of the infested pea seeds, the incriminated causing agent of pea seed damages corresponded to a weevil species from the Bruchidae family, most probably the pea weevil *Bruchus pisorum* L. (Coleoptera, Bruchidae).

Damaged pea seeds at Hissar had visible cavities inside of cotyledons caused by the feeding activity of larvae. There were three types of holes seen from the outside: 'small', 'medium-sized' and 'large'. A small hole is made by the neonate larva while entering the seed. Normally, the entry hole is surrounded by a still intact seed coat patch (Fig. 3). It is head-closed during vegetation. In many cases, the entry hole is placed close to the top of the hemispherical dome of a cotyledon. A medium-sized irregular semicircular form, reminding one of a broken window, is produced by a larva in advanced development and would serve as the adult exit whole. It is placed normally not far away from the entry hole of the same cotyledon. It was also observed that the entry holes were placed closer to the base of the cotyledon while the medium-sized holes were spotted on the opposite side of the same cotyledon. Only a few large holes, typical exit holes of emerged adult weevils with a neat circular shape, could be observed (Fig. 4). There were also seeds with clear signs of multiple infestations (Fig. 5).

Thousand-grain-weight (TGW) of charred pea seeds damaged by bruchids at Hissar was 15.62 g, while the TGW of apparently healthy seeds was 25.5 g. Damaged grains were lighter by 38.75%. This still corresponds with the fact that as much as 30% of individual seed weight is lost from larval feeding (Hardie and Micic, 2020). The losses in seed weight vary depending on the cultivars from 14.6 to 35.0% because of harmful effects to pea weevil (Nikolova, 2016a). The loss in the weight of the cultivars with a higher TGW has lower rates of damage and lower values of a harmfulness coefficient (Nikolova, 2016a). The small-seeded cultivar had the smallest seed weight and the highest harmfulness coefficients.

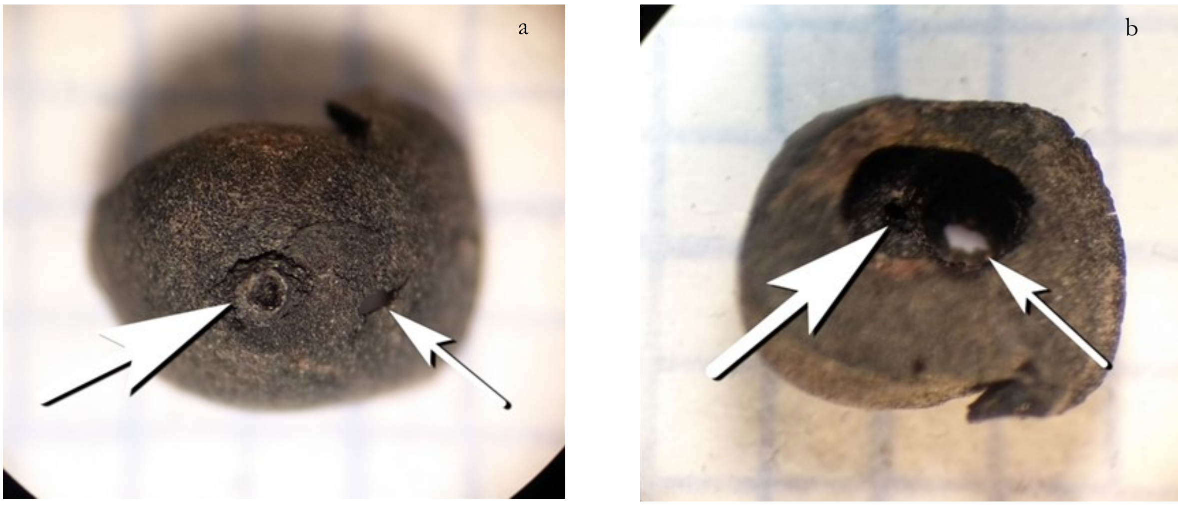


Fig. 3. Infested pea seed from the Late Bronze Age Hissar: a) Entering hole surrounded by a seed coat patch (thicker arrow) and *operculum* (thinner arrow) from outside; b) The inside of the cotyledon shows a cavity made by larva before carbonization with cavity made by entering larva (thicker arrow) and 'broken' *operculum* (thinner arrow).

Fig. 4. One of the few charred pea seeds with exit hole of an adult pea weevil.

## Discussion

*Do the pea-finds from the territory of nowadays Serbia indicate traditional areas of legume cultivation?*

Pulses, in contrast to cereals, did not increase seed size for a long time compared to the earliest period of their cultivation. The increase in seed size in legumes has been delayed for 2000-4000 years. The use of plows pulled by animals provides selective pressure to increase seeds in legumes (Fuller, 2007). The gradual increase of legume seeds becomes noticeable in the Late Bronze Age, Iron Age, and Roman period. At the archeological site Feudvar in northern Serbia, the thousand-grain weight of pea in one sample from the Early Bronze Age is surprisingly low (7.27 g) (Kroll & Reed, 2016). TGW of pea more than doubled itself in the Middle Bronze Age (19.25 g). At Feudvar peas are mainly grown in the Early Bronze and Middle Bronze Ages, while its finds are becoming rarer towards younger periods. For comparison, a total of 3688 charred grains was found at Feudvar. The massive pea find from Hissar makes about 2/3 of the total peas recovered from Feudvar.

*Losses caused by pea weevil*

The main vector of pea weevil invasion is the unintended introduction during the transportation of pea seeds (Kaplin, 2020). It is considered that even today, an infestation is an inevitable part of food storage and handling. Pea weevil is one of the main pests of peas in the field and it is responsible for seed weight loss between 25 and 43% (Smith, 1990). Additionally, high percentages of seed infestation have been reported in Australia 10.6–71.5%, Spain 12.2–25.7%, and up to 64% in the USA (Clement et al., 2002). Weight loss is directly related to the development of the pea weevil. In the early larval stages, the extent of damage is limited. The greatest damage is caused by the fourth larval instar. In addition to yielding losses, pea weevil infestation results in a significant reduction in the quality of the harvested grain, which is unsuitable for human consumption, but also due to reduced germination, for subsequent sowing. Toxic cantharidin alkaloid accumulates in the pea grain damaged by the larvae, which leads to poisoning of domestic animals and humans when the damaged grain is eaten (Kaplin, 2020). Moreover, pea weevil can host parasitic itch mite (*Sarcoptes scabiei* L.) which can cause allergic skin reactions in humans (Armstrong & Matthews, 2005).

Damaged seeds with parasitoid emergence holes, together with healthy seeds, provide a very good opportunity for growth and development while plants from damaged seeds with bruchid emergence holes had poor germination and vigor and low productivity (Nikolova, 2016b). It was concluded that these seeds cannot provide the creation of well-garnished seeding and stable crop yields.

*Insect biology*

Seed beetles (Coleoptera: Bruchidae: Bruchinae) exhibit a strong relationship with their larval host plants (Delobel and Delobel, 2006). The genus *Bruchus* is almost exclusively associated with the tribe Vicieae Rchb. of the Fabaceae Lindl. (Kergoat et al., 2007). The degree of host specificity ranges from monophagy (at least ecological monophagy) to oligophagy (Jermy and Szentesi, 2003).

Pea weevil (*B. pisorum*) is a narrow oligophagous seed beetle, that specifically feeds on the *Pisum* genus (Kaplin et al. 2019).

Adult pea weevil is chunky, 5 mm long, brownish beetle flecked with black, grey, and white patches (Armstrong and Matthews, 2005). In entomological terms, it is not a true weevil (Coleoptera, Curculionidae), but its seed-feeding behavior is suitable to be called by its common name of pea weevil. It develops one generation annually, with adults, less frequently pupae or larvae overwintering inside pea seeds. Adults emerge from hibernation at 18°C and colonize pea crops during the budding and flowering phases. They can fly up to 5 km attracted by the scent of pea flowers, however, most pea weevils come from closer infestations from previous seasons (Armstrong and Matthews, 2005). Females are the first to appear in pea fields. Irrespective of how far the beetles fly to reach the crop, their movement within a flowering crop is generally restricted to the crop's edge. Pea weevil numbers will be highest around the edges (10 to 15 m) of the plot and around trees, and negligible in the center (Börner, 1997; Armstrong and Matthews, 2005). This pest prefers smaller plots under peas near settlements, but peas can be attacked near larger warehouses, near last year's crop heavily infested with weevils, as well as near forests and orchards (Kereši et al., 2019)

Approximately 2 weeks after arrival in the pea crop the females lay eggs on the developing pods. Eggs hatch in about 2–4 weeks and neonate larvae bore the seed directly from the egg, through the pod wall, and into the developing seed creating a small, dark entry hole, about 0.2 mm in diameter. There are four larval instars, with second through fourth instars consuming a large part of the seed (Smith, 1990). The larvae initially feed close to the surface of the seed and then move to the center to feed more extensively. After about 40 days of feeding inside the pea seeds, the larvae prepare a 2–5 mm circular exit hole, i.e. a 'window', by chewing partly through the seed coat. The larvae then pupate and after about 2 weeks develop into an adult beetle. By this time the seed has generally been harvested and some beetles will emerge from the neat circular holes to find suitable hibernation sites. The remaining beetles will stay within the seed until next spring or until they are disturbed by seed movement or vibration.

At the time of harvest (13% seed moisture content), the pea weevil larvae are generally still immature and have only completed 20 to 30% of their feeding. This means 70 to 80% of the seed damage has yet to be done (Armstrong and Matthews, 2005). At the earliest possible harvest date, 3rd and 4th instar larvae were predominant (Smith, 1990). In the central forest-steppe zone of Ukraine, the weevil was found during harvesting in pea seeds at the stage of pupa (57.9%) and larvae of the fourth instar (40.4%) and third instar (1.7%) (Kaplin, 2020).

A single larva develops in one seed. However, cases of multiple infestations are known (Smith, 1990). The percentage of multiple infestations increases with increasing total seed infestation. However, only one larva survives while the others are trained to die in the third larval stage. In the charred material from Hissar, we found several seeds with two larvae inside (Fig. 5).

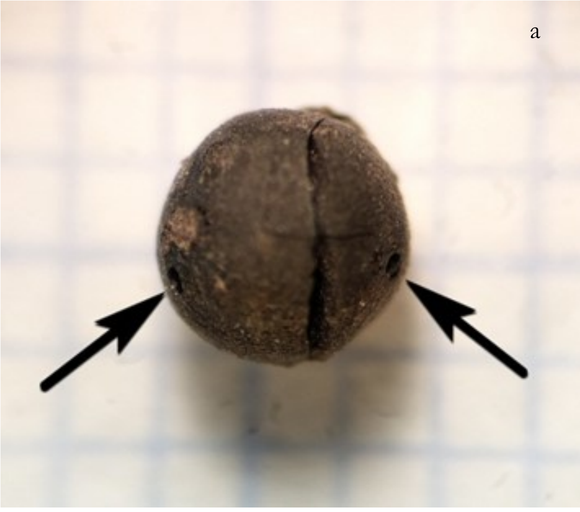



Fig. 5. Charred pea seed with two „broken“ *operculum* (a) and two larvae inside the seed (b) are clear evidence of multiple infestations with pea weevil at Hissar.

It must be also stressed, that field pea seed damaged by pea weevil can be confused with the damage caused by *Heliothis* spp. or *Helicoverpa* spp. (Armstrong and Matthews, 2005). *Helicoverpa* persists throughout the year only in the southernmost parts of Europe where winters are not too cold (EFSA 2014).

*Insect—Host-plant relationship*

A certain level of host conservatism prevails among West European Bruchinae. Like most of the other phytophagous insects, related species usually feed on related plants. (Delobel and Delobel. 2006). Other pests can attack pea seeds, such as *B. brachialis* Fahraeus although it is primarily a pest in bitter vetch (Kislev 1991). Also, in the Mediterranean area and Near East *B. tristiculus* Fahraeus, which primarily attacks species of the genus *Lathyrus*, can also attack pea seeds (Kislev, 1991; Delobel and Delobel, 2006).

If no adult insects are found in the archaeobotanical sample, it is still possible to find out the cause of the insect seed damage. The species of the host plant, as well as the region in which the find was made, can help determine the pest. The charred plant material generally lacks seed coat (testa), and many cotyledons are easily separated or are already separated. This reveals the drilling activities inside the seed and eases the pest identification of infested seeds.

*Experimental archaeobotany and the carbonization process*

Unlike charred cereals, charred legumes are less often found in archaeological strata. The reason should be sought in the different chemical compositions and higher temperature required for their carbonization. It has been experimentally determined that the remains charred at temperatures higher than 310°C have a better chance of being found in the archaeological collection. For charred pea residues to survive the natural decomposition processes, peas should be charred, at least, at 310°C (Braadbaart, 2004). At lower temperatures, e.g. 270°C, at which cereals grains carbonize, the carbonized remnant of peas is still dominated by polysaccharide and protein material. It was determined that a charred pea from an archaeological excavation was exposed to a temperature of not less than 440°C (Braadbaart, 2004). On the other hand, charring experiments suggest the preservation of DNA under conditions of low oxygen and low temperatures (below 200°C). Such temperatures exist in smoldering fires or below the surface, for example in storage pits (Schlumbaum et al. 2008). At temperatures above 250°C wheat seeds undergo a rapid decrease in weight with a total loss of detectable DNA after less than 2 h (Threadgold & Brown, 2003).

The percentage of weight loss during carbonization is 60% at a temperature of 310°C, while this percentage is already 75% at a temperature of 440°C. This means that the thousand-grain weight of damaged grains before carbonization was between 39.05 and 62.48 g and that the thousand-grain weight of undamaged seeds was between 63.75 and 102 g. This is, at best, half the weight of today's thousand-grain weights of a pea.

*Archaeobotanical and archaeoentomological finds of pea weevil*

Although insects damage crops by attacking different organs (root, stem, leaf, seed) at different stages of development (e.g. seedlings), only fruit borers or storage pests have been recorded in the archaeological literature. There is numerous archaeological evidence of infested major pulses of the Old World (Kislev, 1991; Kislev and Melamed, 2000; Panagiotakopulu, et al. 2013). The earliest report of damage to legumes (pea, faba bean) is almost 150 years old (Staub 1882). At the end of the last century, the authors dealing with pests, as a rule, botanists, were reserved when identifying insects: they were always reported as bruchid beetles (Bruchidae, sometimes the old family name Lariidae was used).

In theory, archaeobotanists should be able to document attacked seeds, but pulses are scanty in prehistoric archaeobotanical assemblages of many areas of Europe, and insect boreholes, when present, are not always reported (Antolín and Schäfer, 2020).

The primary distribution of Bruchinae must relate to coevolution with their host plants (Kergoat et al., 2007), although species may be further constrained by climatic conditions in their areas of origin (Tuda, 2011). On the other hand, it's been argued that the case for coevolution between the seed beetles and their hosts is weak. A comparison of the available taxonomic relationships (and presumed phylogenies) best fits a case of sequential evolution, with stronger phylogenetic conservatism in *Bruchus* species than in *Bruchidius* species (Jermy and Szentesi, 2003).

Both cultivated and wild peas can suffer from pea weevils. Two populations of tall pea were found in the Zagros Mountains in Iran (Kosterin et al., 2020). A quarter of all collected seeds (27.6% and 24.9 % respectively) were infested by the pea weevil. Evidence of *P. arvensis* infested with *Bruchus* sp. dates from as early as the seventh millennium BC at the pre-Pottery Neolithic site of Beidha in Jordan (Helbaek, 1970). The same author stated that many peas (such as *P. elatius*) were damaged by the larvae of genus *Bruchus* in the early Neolithic site at Haçilar in Turkey dated to 5400 BC. At the Early Bronze Age site on an island of western Anatolia, some cotyledons of a pea from a pithos dating to 2900–2700 BC have boreholes caused by pea weevils. It was a large stock of peas estimated at 95 million seeds (Dönmez, 2005). Also, from an Early Bronze Age in southeastern Turkey, there is a pea find with about 5962 seeds. About 200 seeds have larvae-formed holes (Oybak and Demirci, 1997). In the north of Hungary, in the Baradla cave, charred plant remains from the early Iron Age were found. Among a small number of charred pea seeds, several seeds had characteristic boreholes caused by *Bruchus* sp. (Staub, 1882; Kislev and Melamed, 2000). A large number of pea-damaged seeds dated to 3500 BC have been found in Ukraine (Kislev, 1991). Numerous larvae (23) of probably pea weevil were found in higher concentrations of peas dated to the Middle Bronze Age Troy in Asia Minor (Riehl, 1999). This indicates that pea cultivation must have been well-rooted in field production and underlines the great importance of peas in that period. Two fragments of pea beetle from the Early Bronze Age Troy could be also identified. It must be taken into account that the pea cultivation must have been well established already at that time. Intensification of pea production leads to a rapid increase in pea infestation (Riehl, 1999). The presence of pea weevil was documented in Zürich-Parkhaus Opéra, Switzerland, in a layer dated to ca. 3160 BC (Antolín and Schäfer, 2020). In total, 8 elytra of *B. pisorum* were identified.

*Indicator of pea weevil activity*

The fact that in charred plant material the seed-coat is generally missing and many cotyledons are easily separated, helps expose the infested seeds (Kislev 1991). The percentage of seed infestation is directly related to the size of the population of adult weevils in the pea crop in the spring. Pea cultivars with a lower protein and phosphorus content had a lower level of damage (Nikolova, 2016a). Therefore, it can be assumed that pea from Hissar had a higher protein and phosphorus content. A field trial regarding the resistance of 16 field pea genotypes to the occurrence of pea weevil was conducted in Croatia (Gantner et al., 2008). A high infestation can be attributed to the high number of natural pea weevil populations due to the long tradition of pea cultivation in the area and poor storage hygiene.

*Another weevil pest at Hissar*

In the mass find of bitter vetch at Hissar (Medović, 2012), at least five seeds have large exit holes made by the imago of the weevil at the place where the radicle was located. According to Kislev (Kislev, 1991), bitter vetch was possibly attacked by (*B. brachialis* Fahraeus) or (*B. signaticornis* Gyllenhal), or even (*B. ervi* Frölich). Besides, *B. rufipes* is the most common bruchid field pest in Continental Europe and the Mediterranean region (Panagiotakopulu, 2001) (Panagiotakopulu, 2001) which attacks all major pulses except chickpea (Kislev, 1991). This fits the description made by H. Kroll (1983) on the charred bitter vetches seeds found at the tell of Kastanas in northern Greece. Rich bitter vetch finds from Kastanas were defiled by seed beetles (Brachinae). The infestation was not considerable: 1–2%. An infestation of pulses by seed beetles was also recorded on several archaeological sites in Europe, Middle East, and Egypt (Kislev and Melamed, 2000; Panagiotakopulu, 2001). Both, bitter vetch and pea storages from Hissar, were infested by pests. The history of crops is closely related to the history of pests of stored products. With a fair record of pests on archaeological sites, one could be able to reconstruct original routes of their expansion through crop trade in the past. The earliest records of bruchids of bitter vetch in Israel and Greece are from the Bronze Age, e.g. from 12–11 cent. BC (Kislev and Melamed, 2000).

*Determining the season of the pea seeds carbonization*

Is it possible to determine the season or time of pea seeds carbonization at Hissar? The DNA analysis of charred peas at Hissar revealed that it was probably a winter-type pea (Smýkal et al., 2014). In the Archaeobotanical Garden of the Museum of Vojvodina in Novi Sad, Serbia tall pea from the Pčinja region is harvested since 2012. The harvest takes place, very late, at the beginning of July. During archaeobotanical analyses, it was observed that charred pea seeds were considerably damaged by the larval activity. There were many residues of larvae inside charred seeds. In the multiple infested seeds two larvae were found inside. This could be the indication of a 3rd larval instar? On the other hand, no pupae, or adult pee weevil could be identified in the seed collection. The vast majority of the infested seeds had two holes; one closed (entrance of larval hole was healed from outside), while the typical 'windows' (*operculum*) were small and not circularly rounded (Fig. 3). One could even suspect that these are the exit holes of the parasite from bruchid infected seeds (Ritcher, 1966; Annis & O'Keeffe, 1987). But, we have no proof for that. We assume that the fragile 'windows' were 'cracked opened' due to the carbonization process and also through the archaeobotanical treatment. After every archaeobotanical reexamination, the number of infested seeds had suspiciously increased. Inevitably, infested charred seeds were submitted to mechanical force solely by pouring them into a Petri dish. It seems that 'windows' were intact before carbonization. Due to damaged caused (almost 39% lighter seeds), it can be assumed that older, final weevil instars were predominant. Therefore, the carbonization must have occurred after harvest, shortly after the seeds were transported to the storage, in the second part of July, or at the latest in the first days of August.

Considering the high concentration and relative purity of pea seeds in this context it can be excluded that these seeds had been discarded and given to animals as fodder, as suggested in the case of fava bean with boreholes found in dung at Can Sadurní Cave, Spain (Antolín & Schäfer, 2020).

*Biological control and the use of natural substances as repellents*

In the recent research authors (Antolín & Schäfer, 2020) suggests that early farmers from Neolithic Europe were aware of the damages produced by pests. They even discussed the possible uses of dill and chickpea (besides for edible purposes) as pest repellents or trap crops. The use of natural substances as insecticides, which would have repelled pests during storage like at the Bronze Age settlement at Akrotiri (Panagiotakopulu et al., 1995; Panagiotakopulu et al., 2013), has not been noted among pea seeds at Hissar.

On the other hand, we noticed that typical 'windows' were suspiciously small and possibly „cracked opened" during the carbonization process and after/during archaeobotanical processing. Before pupation, the pea weevil larva excavates a circular hole in the seed through which the adult emerges. The exit hole remains covered by only the thin seed coat, which the adult weevil removes when ready to emerge. This exit hole for emergence can also use the adult of another insect, a natural enemy of the pea weevil, e.g. *Eupteromalus leguminis* Gahan. The evidence of exit of this parasitoid is a small hole in the seed coat approximately one-half the diameter of the pea weevil exit window. Survival of *E. leguminis* depends on the development of pea weevils to the fourth instar and, hence, damage to the seed. If the larval parasite kills its host before completion of the host's exit hole, the adult parasite is trapped within the seed (Annis & O'Keeffe, 1987).

Even though we don't have any proof of the existence of parasitoids of the pest, this topic should be opened for discussion. Parasitoids, especially those belonging to Hymenoptera, are important elements of the agroecosystems (Kavallieratos et al., 2019). Pea weevils are attacked by several species of natural enemies. Bruchid infestation levels can be also restricted by the braconid wasp *Triaspis thoracicus* Curtis, an endoparasitoid (Hymenoptera: Braconidae) that feeds on developing bruchid larvae and increase their mortality (Tsialtas et al., 2020). With few exceptions, *T. thoracicus* is a specialized parasitoid of the genus *Bruchus*, which includes various species of economic importance for stored legumes (Nikolova, 2016a; Reddy et al., 2018). *Triaspis thoracicus* attacks the early-stage larvae of the pest within the seed. It prefers pea varieties that are the most heavily infested by the bruchid (Khrolinskii & Malakhanov, 1979).

Another natural enemy of pea weevil is larval parasitoid *Dinarmus basalis* (Rond.) (Schmale et al., 2005; Nikolova, 2016a) and egg parasitoid *Uscana senex* Grese (Reddy et al., 2018).

## Conclusions

The pea species/variety found at Hissar was not resistant to a weevil attack, most probably to *Bruchus pisorum* L. It can be assumed that older, final weevil instars were predominant in the seeds before carbonization. The pea deposit was most probably carbonized in the second half of July or the first days of August at the latest. There is no evidence for using natural substances as repellents against pea weevil. It can be ruled out that these pea seeds had been discarded and given to animals as fodder.

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## Arheoentomološka procena nivoa zaraženosti žiškom (Coleoptera, Bruchidae) u zalihi graška na kasnobronzanodobnom, gradinskom naselju Hisar

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**Sažetak:** U jednoj zalihi ugljenisanog graška na lokalitetu Hisar kod Leskovca primećene su dve vrste semena: naizgled zdrava semena i semena oštećena ubušivanjem larvi, najverovatnije graškovog žiška. Najmanje dve petine svih nalaza su imale tipična oštećenja nastala ishranom larvi *Bruchus pisorum* L. Ovaj broj nije konačan jer se nakon svake naknadne analize broj formiranih „prozora“ na zaraženim semenima povećao i postao vidljiv. Visok procenat zaraženih semena graška na Hisaru ukazuje na razvijenu proizvodnju graška na malim parcelama. Na kraju bronzanog doba, uzgoj graška je sigurno bio dobro ukorenjen u ratarskoj proizvodnji stanovnika ovog naselja. Prethodne DNK analize postavile su ovaj drevni grašak u poziciju između gajenog (*Pisum sativum* L.) i divljeg, ozimog *P. sativum* subsp. *elatius* (Steven ex M. Bieb.) Asch. et Graebn. Na osnovu pretpostavke o kasnom vremenu žetve ozimog graška i utvrđene faze životnog ciklusa graškovog žiška, bilo je moguće proceniti deo sezone tokom kojeg je došlo do procesa ugljenisanja. Na osnovu skoro formiranih „prozora“, pretpostavljamo da su se larve sa Hisara nalazile u poodmakloj fazi razvoja, neposredno pred preobražaj u stadijum lutke. Svega nekoliko velikih izlaznih otvora odraslih žižaka je bilo uočeno. Proces ugljenisanja se stoga morao dogoditi u drugoj polovini jula, ili najkasnije tokom prvih dana avgusta. Ovaj rezultat predstavlja jedan od dosad najvećih nivoa infestacije mahunarki dokumentovanih u Starom svetu i najveći praistroijski nalaz infestiranosti graška. Nema dokaza o upotrebi prirodnih supstanci kao repelenata protiv graškovog žiška. Grašak uzgajan na Hisaru bio je sitnozrni. Na osnovu kombinacije morfoloških podataka i rezultata dobijenih eksperimentima, pretpostavljamo da je grašak imao veći sadržaj proteina i fosfora.

**Ključne reči**: arheoentomologija, *Bruchus pisorum* L., grašak, graškov žižak, Hisar, kasno bronzano doba, Leskovac