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OVERVIEW OF THE COMPETITION BETWEEN TWO SPECIES (Bruchinae; Chrysomelidae) – HONEY LOCUST SEED BEETLE (*Megabruchidius tonkineus* Pic) AND BEAN WEEVIL (*Acanthoscelides obtectus* Say) AS THE MOST IMPORTANT PESTS OF BEAN SEED STOCK

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ABSTRACT

A multidisciplinary approach considers integral control and favors biological control measures, whereby it is crucial to conduct a comprehensive examination of the existence of potential candidates to prevent the spread and destroy generative propagation of insects, in particular the invasive ones. Such an integral approach makes it possible to create conditions required for restoration and protection of stocks of seeds and forest cultures, as well as seed stocks for human consumption. This would ensure the future use of species whose seeds are kept for restoration of rare species, and most importantly, for keeping stocks with enormous economic and social importance. This paper provides an overview of occurrence and use of potentially harmful Bruchinae insect, honey locust seed beetle, the use of which in practice would be compliant with the principles of sustainable development, given the proven competition with bean weevil. The researched Bruchinae species *Megabruchidius tonkineus* (Pic, 1904) has recently been proven not to be monophagous in Serbia, and to attack and infest bean seeds in addition to Kentucky coffee tree seeds. During a three-year research (in the period 2019 – 2021), analyses were conducted on data from statistically important samples from 3 localities across the county (3 experimental host plants per locality). The second Bruchinae beetle is the best-known bean pest *Acanthoscelides obtectus* Say. The two noted seed predators were over this period subjected to various laboratory examinations in order to collect data on their general impact on one another. Such a combination or community may form the key for adoption of the host to the pests, modifying the plants themselves phenotypically, genetically and chemically, and in turn the probability of attack by a known seed predator.

KEYWORDS:
Seed Beetles, bean weevil, *Megabruchidius tonkineus*, competition

INTRODUCTION

Insects represent a limiting factor in cultivation of beans (*Phaseolus vulgaris* L.), and in most regions of production they are frequently the main cause of low productivity. During the vegetation, beans and other legumes are susceptible to attacks by different pests, among which the bean weevil is the most significant and the most dangerous one [1]. The majority of insects feeding on plants use a narrow range of plant species as hosts for nutrition and/or laying eggs, compared to the available number [2, 3]. A possible explanation for this specialized pattern of host plant selection is the presence of genetic compromise in the ability of larvae to efficiently use plants that are differently chemically enriched. This constraint could prevent expansion of the host range for insects through the pressure of competition, which as a process favors specialized behavior. This as a wide-spread rule could explain why specialization of the host is generally developed in insects [3, 4, 5].

Mechanisms of transition to a new host in phytophagous are insufficiently explained and understood. Among many proposed processes, sexual selection "via semiochemicals" has recently been proposed. We tested this hypothesis on *Acanthoscelides obtectus* Say, olygophagous bean seed beetle (*Phaseolus vulgaris* L.), which can be trophically endangered compared to the competition with honey locust seed beetle [6], [7]. After the larvae of *A. obtectus* in the first stage infest the seed (*neonates)*, their development is primarily directed through the chemical composition of the endosperm of bean cotyledons [8]. The hypothesis that this may be the difference in bionomics and physiological properties between the reared populations of *A. obtectus* and *M. tonkineus* (the main difference is in the degree of infestation as an indicator) is being continuously researched but the conclusions so far remain incomplete. Therefore, before potential release of any biocontrol agent, it would be crucial to get acquainted with its trophic potential and preferences, so that they would contribute to the objectives of preserving the sustainable development biodiversity. Research on insects carried out in the laboratory for over a decade represents a key contribution to findings on biological and ecological characteristics of *M. tonkineus* and the infestation of bean seed recorded here for the first time (under the conditions of the experiment).

MATERIALS AND METHODS

In order to facilitate collection of insect stocks of *M. tonkineus* adults, 50-100 pods of *Gleditsia triacanthos* L. with infested seeds from three localities were transported in transparent plastic MARKIZET bags so as to gather sufficient insect stocks to be used in the experiments. The selection focused on vital specimens in 30 pairs, primarily those with clear phenotypic characteristics. During the winter seasons of 2019/20, 2020/21 and 2021/22 it was attempted to obtain a laboratory confirmation of the important fact that from now on the olyphagous *M. tonkineus* can also be a predator on seeds of another plant host – bean, which is widespread in Serbia (*Phaseolus vulgaris* L.). Adult *Megabruchidius tonkineus* (groups of males and females) collected immediately after the eclosion were released into paper bags with 100 bean seeds each (about 35 bags annually). Laboratory conditions in the improved version were slightly modified after the first attempt almost a decade ago (different pods or legume seeds in plastic bags were offered to groups of seed beetles, which were first confirmed as monophagous). Three more types of seed beetles, which are all but one invasive for Serbia, were in the same way offered seeds of several species of Fabaceae family. Insects were reared on the host seeds in one-litter glass receptacles inside climatic chambers. The conditions were set to reproduce the following climatic conditions: they were kept at 30°C and 50% (±10%) relative humidity (RH) and a 12h:12h light : dark cycle. All insects were fed with 1/3 solution of saccharine and water. Pods of host plants were collected during late fall and winter in order to collect insect stocks for the experiments. Pods of *Gleditsia triacanthos* L. were collected from tree lines, rural communities, suburban areas in the vicinity of roads, at the external temperature. In the very first season, in March and April 2019, 30 pairs of adult specimens of *Acanthoscelides obtectus* Say were selected and placed in previously laid bags containing *M. tonkineus.* Insect stocks were collected from the same number of selected plants (3). All seeds were dissected in the spring, seeds were extracted, and emergence holes on the pods were counted. Damage on seeds of *Phaseolus vulgaris* L. caused by *M. tonkineus* was finally registered during the spring. The seed was joined together and deliberately exposed to these two species for the purposes of comparison and confirmation of competition or competitive conditions. The research and collection of material in the 2018/19 season were aimed towards adjustments and improvements of the methodology, primarily to avoid damage to cultures caused by laboratory predator insects – dust mites, which have so far represented the primary cause of damage to cultures. The results on all observed damages to seeds were statistically processed in 2019, 2020 and 2021 in order to determine the number of generations and to make final conclusions. T and Z tests were used for comparison of the arithmetic mean of two types of specimens (bean seeds infested by *A. obtectus* and *M. Tonkineus).* The null hypothesis was formulated (the possibility that bean infestation with *A. obtectus* would be greater without *M. tonkineus*, and that one population influences the other). In addition, parasitoids were isolated by rearing the seeds with confirmed infestation in flacons, but they were also caught – collected during the final examination of specimens.

Host Plants (*Phaseolus Vulgaris* L. And *Gleditsia Triacanthos* L.). Although the efforts of bean breeders to create genotypes with higher yields and improved nutritive qualities have been fairly successful, in practice the yields often fail to reach the genetic potential [9]. Low productivity is attributed to various factors, including harmful insects. The most significant is the seed pest – bean weevil (*Acanthoscelides obtectus* Say, Coleoptera: Chrysomelidae; Bruchinae), in terms of the losses it causes [10]. Literature sources provide ample information on bean weevil populations that had different levels of specialization on the conditionally natural host – beans, with the focus on behaviour of a new bean seed predator, *Megabruchidius tonkineus,* and on testing the patterns of reproductive behavior in their mutual competition.

Gleditsia triacanthos L. also belongs to the family Fabaceae, and it is starting to be treated as invasive in Serbia although it has been naturalized. It originates from America, and has long been economically used in Serbia's forestry.

Seed Beetles. *Megabruchidius tonkineus* (Pic, 1904) and *Acanthoscelides obtectus* Say. The emphasis in the description of species is placed on the similarity of both species primarily in bionomia, which indicates a greater possibility for competition and rivalry (Table 1). The species *Megabruchidius tonkineus* (Pic, 1904) has one generation annually in Serbia and the world [11]. During the research in

Serbia, the species *Megabruchidius tonkineus* L., noted as a predator of honey locust seeds, was in the nature found to feed on seeds of Kentucky coffee tree *Gimnocladus dioicus* (L.) K. Koch. seeds as well. We can therefore determine that in our conditions honey locust seed beetle is an olyphagous species. It was individually noted here in the seeds of Kentucky coffee tree, whose seed coat is extremely hard.

Megabruchidius tonkineus was first described by Tonkin [12] as *Laria tonkinea*. Four years later [13], *Megabruchidius tonkineus* was classified into this genus. Presence of *Megabruchidius tonkineus* in Europe was first recorded by Wendt [14] in Germany. Earlier studies had shown two species of bruchid beetles developing in the seed of woody plants in Hungary: *Bruchidius villosus* (Fabricius, 1792) on *Laburnum anagyroides* [15, 16], while specimens of larger bruchid beetles were identified as *Megabruchidius tonkineus* [17, 18, 19].

Bean weevil, *Acanthoscelides obtectus* Say is the most significant pest attacking bean seeds. It was brought into Serbia after the First World War and spread extremely quickly through all bean cultivation areas. The greatest damage occurs in the warehouse, where it can devastate up to 50% of seeds [20]. Besides beans, it feeds and develops on seeds of other legumes (peas, soy, field peas, etc.). Damaged seeds lose the market value and cannot be used for human consumption [21]. Until recently it was believed that bean weevil is a warehouse pest, however it has now been established that the attack in fact starts in the field. With adjustments of temperature and air humidity there may be several generations per year, while in the field, where there are constant oscillations in temperature and humidity, only one generation develops annually. According to Lopez-Moreno et al. [22], there may be up to 20 larvae in a single bean. Characteristic holes or openings occur on the bean, while during a stronger attack the entire seed may be pierced so that it remains hollow. The imago is 3.5 – 5 mm long, grayish-brown in color, with the head bent downwards. It lays 50-80 eggs on the average, but this number may reach as many as 150, depending on the warehouse conditions [20]. Optimal development is at temperatures of around 27°C. Larvae emerge from the eggs and pierce holes in the seed through the seed coat. The development lasts between 30 and 80 days, depending on the temperature [21].

RESULTS AND DISCUSSION

Given that both *M. tonkineus* and its host *Gleditcia triachanhos* L. are characterized as invasive, the starting assumption was that examination of trophic preferences towards related types of legumes would be required for each insect species considered as a potential biological agent [23]. Damaged seeds of the host plant **(***Phaseolus vulgaris* L.) were processed from spring of the first season (2019), from summer to winter, in order to determine the insect bionomic and the exact number of generations. Pods of *Gleditcia triachanhos* L. were collected to rear adult specimens to the commencement of insect stocks. The most significant result is the importance and relevance of trophic preference of the species *Megabruchidius tonkineus* (Pic, 1904) towards beans. Here there are two competing species of Bruchinae beetle, which is quite interesting as domestic beans are not the first choice of the honey locust seed beetle. The research results obtained in the future will include assessment of risk of uncontrolled spread of invasive plants, in particular their seed pests. A significant contribution would be preservation of diversity of the domestic natural ecosystems, protected from aggressive expansion of invasive species through control in the seed dispersal phase. During three seasons (2018/19, 2019/20 and 2020/21), examination of trophic preferences for species *A. obtectus* and *M. tonkineus* was performed under laboratory conditions, on the same materials and side by side. Pairs of the species *Acanthoscelides obtectus Say,* obtained from the bean seeds immediately upon eclosion, were additionally fed ripe seeds of *Phaseolus vulgaris* L., and the same was also done with *Megabruchidius tonkineus* pairs obtained from pods of Kentucky coffee tree and honey locust. The total lifecycle of the specimens lasted for approximately 90 days, the same as for the bean weevil (Table 1).

The majority of the emerging first stage mobile larvae (*neonatus)* of honey locust did not transition to the next stage and died. However, a small number survived, attained the infestation, and imagoes emerged. The first larval stage (*neonatus* or L1) dies unless they find a suitable base for the development of the vulnerable and much less mobile remaining specimens [23]. Although they can maintain the energy for a long time after emergence, the majority of *M. tonkinues* larvae wanderers eventually died. The maximum number of emerging larvae occurred in the second season, when the number of emerging holes for *A. obtectus* was about 50%. Only a few larvae wanderers of honey locust seed beetle managed to find an alternative source of nutrition, roughly 3% over three seasons, which is an extremely small number. This result suggests that bean is not a suitable host for them, although infestation was attained. This was also reflected in the condition, and the imagoes used for supplemental nutrition mostly died within the period of 3-7 days.

Emergence holes of *Acanthoscelides obtectus* and *Megabruchidius tonkineus* on *Phaseolus vulgaris* were processed as the number of emerged imagoes and presented by mean value and standard deviation.

Host bean / female Year Spring (μ;σ) Summer(μ;σ)

TABLE 1
Duration of individual life stages of *Megabruchidius tonkineus* in laboratory conditions (In days)

Year of research	Egg stage	Larva stages	Pupa stage	Imago stage	Total duration of lifecycle
2019	15 – 20	35 – 39	18– 20	15– 18	83 – 96
2020	19 – 21	37 – 41	17 – 21	14 – 18	87 – 95
2021	19 – 22	38 – 40	18 –22	13 – 15	90 – 99

P. vulgaris/ A. obtectus 2019 29.9; 4.5 2.5;0.5

P. vulgaris/ A. obtectus 2021 39.9; 5.5 2.6;1.2

P. vulgaris/ A. obtectus /bean 2022 24.5; 2.5 5.5; 1.4

Mean number of emergence holes and standard deviation

Host bean / female Year Spring (μ;σ) Summer (μ;σ)

P. vulgaris/ M. tonkineus (1st gen.) 2019 0.4;0.2 1.4;0.4

P. vulgaris/ M. tonkineus /bean (2nd gen.) 2020 0.5;0.5 1.0;0.5

P. vulgaris/ M. tonkineus (3rd gen.) 2022 2.5;0.5 1.5;0.5

Total infested seeds (TIS)

The maximum number of emergence occurs in the second season, when the number of emergence holes for *A. obtectus* is around 51% (Figure 1).

In order to determine the impact of two seed beetle populations, as well as potential competition or any type of mutual relation, T and Z tests were performed.

T test was calculated on the reliability level α=0.05 and absolute value of t-statistics was calculated at │-7,992 │, higher than the critical value of t-statistics of 4.0067709. It was therefore concluded that the null hypothesis should be rejected with the risk of 5% (Table 2). The alternative H_1 hypothesis is accepted, that there is a significant statistical difference between mean values on two pests to infestation of seeds (there is a possibility that infestation of beans with *A. obtectus*, without *M. tonkinues*, would be greater).

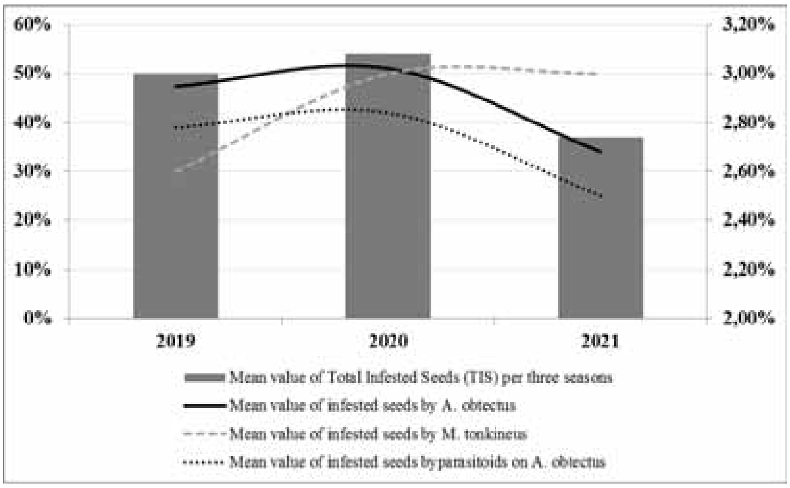

FIGURE 1
Percentage of infested seeds of *A. obtectus,* together with parasitoids in infested seed *M. tonkineus*

TABLE 2
Testing of mean values of *P. vulgaris* seed infestation with *M. tonkineus* (T test)

T test	*M. tonkineus*	*A. obtectus*
Mean value	1.430606061	40.72121212
Variance	9.055871212	246.0473485
Observations (samples pcs.)	33	33
Pearson correlation	0.000957941	
Hypothetical difference between means	0	
df (Degrees of freedom)	32	
T statistics	-7.992427869	
P(T<=t) /One tailed test - distribution	2.0033909	
T Critical value for one tailed test	1.693888748	
P(T<=t) two tailed	4.0067709	
T Critical value for two tailed distribution	2.036933343	

TABLE 3
Testing of mean values of parasites in seeds *P. vulgaris* by *M. tonkineus* (Z test)

Z- test	*M. tonkineus*	*A. obtectus*
Mean value	1.430606061	40.72121212
Known variance	1.32233	15.6859
Observations (samples pcs.)	33	33
Hypothetical difference between means	0	
z/з statistics	-27.2607051	
P(Z<=z) One-tailed (one-tailed assumed distribution)	0	
Z critical value for one tailed test -one tailed distribution	1.644853627	
P(Z<=z) two-tailed	0	
Z critical value for two tailed distribution (test with two tailed distribution)	1.959963985	

Since the number of specimens exceeded 30, the standardized Z-test was also performed. The results of verification of the null hypothesis tested with the Z-test are presented in Table 3.

Z-test confirms the alternative hypothesis that there is a significant statistical difference between the two pests to seed infestation. It would be unnecessary to seek further statistical proof of the set hypothesis through coefficient of determination and correlation coefficient (the possibility that bean infestation with *A. obtectus* without *M. tonkineus* would be greater, does exist).

T and Z tests were used for comparison of the arithmetical means of two types of specimens (bean seeds infested with *A. obtectus* and with *M. tonkineus)*. The null hypothesis was formulated (the possibility that bean infestation with *A. obtectus* without *M. tonkineus* would be greater) and proven as accurate; therefore, one population definitely has the influence on the other.

H_0: $\underline{x}_1 = \underline{x}_2$ is that the means of the two sets do not differ with statistical significance compared to the alternative H_1: $\underline{x}_1 \neq \underline{x}_2$ and that the difference is present. Since the arithmetic mean of the set is the mean value of 1.75% of seeds infested with *M. tonkineus,* accepting H_0 would suggest that the set plays no role in the degree of infestation of bean pods with *A. obtectus.* Competition was proven to be significant. Results of verification of the null hypothesis tested with T-test are presented in Table 2. Since the number of specimens exceeded 30, the standardized Z-test was also performed. The results of verification of the null hypothesis tested with the Z-test are presented in Table 3. Infestation of seeds of *P. vulgaris* compared to the infestation by *M. tonkineus* is given in % and presented through basic statistical values (Table 4).

In examining the consequences of rearing *A. obtectus* and *M. tonkineus* side by side during 3 consecutive generations, there is a strong preference in the attack of bean weevil on the host offered (51% attained infestation in total, Figure 1). By comparison, our research suggests that in case of its closest phylogenetic relative – *A. pallidipennis,* which is a decidedly monophagous species [24], this does not apply to specimens of either *A. obtectus* or *M. Tonkineus.*

This was proven again in this research for *Megabruchidius tonkineus*, the species which was here very similar to A. *obtectus,* phenotypically rather than phylogenetically [25]. Most species of taxonomically close Bruchin genus (*Bruchidius* and *Sulchobruchus*) prefer one host plant from the family Fabaceae, but show preference for other species of the same plant family under altered conditions. Over three seasons and under laboratory conditions, ecloded specimens of *Megabruchidius tonkineus* were given, in addition to honey locust seeds, the option to feed on seeds and pods of Kentucky coffee tree *Gimnocladus dioicus.* The first stage larvae, which hatched out of the successfully laid eggs, pierced their way into Kentucky coffee tree pods or seeds (in a small number of cases). The percentage of ecloded imagoes of the generation that developed in the Kentucky coffee tree seeds was up to 5% [26].

TABLE 4
Infestation of seed *P. vulgaris* compared to infestation by *M. tonkineus* (%)

Seed infestation	Infestation by *M. tonkineus* per 100 bean seeds (%)
Minimum (min)	1
Maximum (max)	2.5
Mean value ($\underline{x}$)	1.75
Standard deviation (σ)	1. 32233
Number of samples	33 Σ (per 3 years)

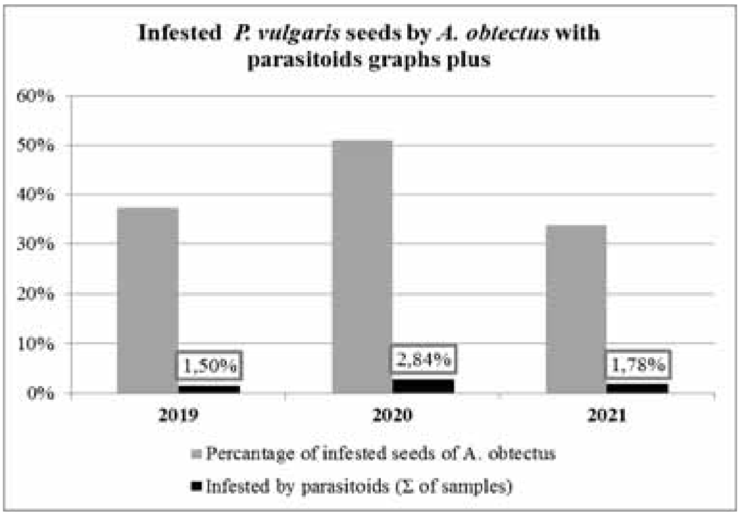

FIGURE 2
Percentage of infested seeds of *A. obtectus* plus Graphs of sum. of parasitoids in Σ samples

Based on the data obtained, it was established that the species *Megabruchidius tonkineus* can also develop on an alternative host, precisely *Phaseolus vulgaris* L., with the success rate of up to 3%, including ecloded imagoes (Figure 1). The results on dissected seeds are similar. Besides the above proof of preference for beans of the Bruchin species, the objective of this experiment was to examine patterns of mutually competing reproductive behavior of bean weevil (*Acanthoscelides obtectus* Say; Coleoptera: Bruchinae) populations that had different levels of specialization for their natural host – beans (*Phaseolus vulgaris* L.) and the new seed predator *Megabruchidius tonkineus.* The obtained asymmetrical pattern in reproductive behavior of different populations of the two species is frequently considered to be the initial phase in the process of development of new trophic relations in invasive species [27]. Our results can therefore be significant as a starting point for further, comprehensive investigations into the role of level of specialization to a host plant, as well as in the process of evolution of pre-reproductive mechanisms of reproductive isolation in insects. The same applies to the new seed predator *Megabruchidius tonkineus*.

As there were no parasitoids detected for honey locust beetle [28], their numbers are presented for *A. obtectus* (Figure 1). In the year 2019/20, the most abundant species in the parasitoid complex of black locust beetle were *Dinarmus acutus* (Thomson, 1878), *Eupelmus urosonus* Dalman 1820 and *Eupelmus vesicularis* (Retzius, 1783).

During the months of April and May of 2019 and 2020 seasons, 53 Hymenoptera imagoes were collected from the annual samples (39 in April and 14 in May), specifically specimens of species from the superfamily Chalcidoidea.

Percentage-wise, they make up a maximum of 2.84% of the infested seeds and 2.04% of the total examined seeds, and have various statuses – from parasitoids of different attacked development stages of Bruchinae, to hyperparasitoids (Figure 2).

Moreover, given that these results, as well as the results of previous studies conducted on *A. obtectus,* indicate the insect's great evolutionary potential for invasion and quick specialization to new host plants [29], they may serve as valid information in development of long-term strategies within the integral pest control programs, but primarily in protection of beans, since they can be stored in warehouses in large quantities and as such have exceptional economic and social importance for any country.

Plants that are sensitive and susceptible to one species, in the presence of another, would reduce the intensity of attack by another insect, or this would reflect on both [29]. Bean is among the most valuable and preserved stored plants, and this highlights the importance of knowing its current and potential pests in order to achieve successful conservation in the future, as the emerging topic of food sources is yet to be considered on a larger scale. The discussion included plants that certainly have a great impact (through the intensity of infestation) on the attributes of the above two species of seed beetles and their interaction. This reflects on the consequences for the condition and health of the seed material, as the dominant agents in the selection and transfer of attributes of plant resistance [30]. The basic concept, that seed predators have the ability to modify and cause adaptation of plants and selection of their attributes, will provide the approach to identification of those who initiate the reaction of selection in complex interactions and competition among multiple pests in natural and agricultural systems [31].

Use of bio control is often regarded as a progressive and ecologically acceptable way of pest control, given that it leaves no chemical residue that may have a harmful impact on people, other organisms, or the environment. When successful, bio control can provide permanent and widespread control with very favorable benefits – the benefit-cost ratio. Therefore, before releasing a bio control agent (or using another method), it is crucial to balance its potential so that it remains within the framework of preservation and sustainable development goals, while it is imperative that the damage-causing manner or potential be non-existent or limited [32]**.** It is important here to continue with the research of the host system (*Phaseolus vulgaris* L.) and of the two seed pests. There are numerous potential practical results that may be achieved further on, solely through the research of the limiting factor of the presence itself, i.e. by mixing two insect species on the same host plant (the first, widespread *A. obtectus,* and the second, new *M. tonkineus*). Both are considered to be pests, whereas one of them, although discovered relatively recently, is still in the invasive phase, and since it selects the invasive honey locust as its primary host, its classification as a pest is only provisional [32].

CONCLUSIONS

The important result in consideration of potential biocontrol through competence is that under laboratory conditions, besides the primary pests, bean seeds were infested with *M. tonkineus*. Unlike the bean weevil, it has no parasitism.

In examining the consequences of rearing *A. obtectus* and *M. tonkineus* side by side during 3 consecutive generations, there is a strong preference in the attack of bean weevil on the host offered (51% attained infestation by *A. obtectus* in total).

Based on the data obtained, it was established that the species *Megabruchidius tonkineus* can also develop on an alternative host, precisely *Phaseolus vulgaris* L., with the success rate of up to 3%, including ecloded imagoes. The species are very similar according to their bionomics; however bean weevil develops a higher number of generations, while honey locust seed beetle has maximum 3 generations under the laboratory conditions.

It is important to continue with the research of the host system (*Phaseolus vulgaris* L.) and of the two seed pests. There are numerous potential practical results that may be achieved further on, solely through the research of the limiting factor of the presence itself, i.e. by mixing two insect species on the same host plant (the first, widespread *A. obtectus,* and the second, new *M. tonkineus*).

T and Z tests were used for comparison of the arithmetic mean of two types of specimens (bean seeds infested by *A. obtectus* and *M. tonkineus).* The null hypothesis was formulated (the possibility that bean infestation with *A. obtectus* would be greater without *M. tonkineus)*, and proven to be true, hence the one population definitely influences the other.

As there were no parasitoids detected for honey locust beetles, their numbers are presented for *A. obtectus* (Figure 1). In the year 2019/20, the most abundant species in the parasitoid complex of the black locust beetle were *Dinarmus acutus* (Thomson, 1878), *Eupelmus urosonus* Dalman 1820, *Eupelmus vesicularis* (Retzius, 1783).

During the months of April and May of the seasons 2019 and 2020, 53 Hymenoptera imagoes were collected from the annual samples (39 in April and 14 in May), specifically specimens of species from the superfamily Chalcidoidea.

Percentage-wise, they make up a maximum of 2.84% of the infested seeds and 2.04% of the total examined seeds, and have various statuses – from parasitoids of different attacked development stages of Bruchinae, to hyperparasitoids.

Moreover, these studies have shown how plant attributes change and adapt to interactions with one insect species of seed beetles and how they can subsequently affect the interactions with other insects that also attack the same seed, which is a result of the association of the primary hosts in phylogeny – in this case, the legume meta family [29, 30]. Biological control is use of one species for control of another (weeds, insects and other arthropods). Here it has been demonstrated that in a potential attempt to battle, two species eventually interact, compete, share or otherwise impede the target pest species. Successful biocontrol programs significantly deplete the pest populations, and in some cases simply prevent the damage caused by other pests (i.e., by preventing them from feeding on valuable crops) without reducing the pest numbers, merely with the existence of one or more competing species [33].

This was proven by T and Z tests. Our results demonstrate a higher presence of the existing pest in the seed of the plant that had not been attacked by the other, in accordance with the previous findings on this system [34]. Plant seeds (beans in this case) have proven not to be susceptible in the same way to this dynamic but highly geno and phenotypically varied duo – community of two insect species which, as herbivores, mainly specialize for one host (monophagous or oligophagous). Such a combination or community may form the key for adoption of the host to the pests, modifying the plants themselves phenotypically, genetically and chemically, and in turn the probability of attack by a known seed predator.

The potential of bio agents is tested under strictly controlled laboratory conditions to avoid possible risk to the environment. This is particularly important when observing the introduced species – the goal is to evaluate the traits of a potential agent before it is released into nature [35].

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