Article

Seed Pests of Woody Legume Seed Beetles (Bruhinae: Chrysomelidae, Coleoptera) and the Potential of Measures of Host Plant Biocontrol in the Republic of Serbia

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Abstract: In order to reduce negative effects of application of chemical agents, biological control is becoming increasingly important. This paper therefore focused on research of effects of certain types of seed beetles on control of invasive plant species in Serbia. Numerous sources suggest that for legumes and seed beetles Bruhinae: Chrysomelidae the rule of coexistence in pairs applies in all ecozones. The species rely on one another, primarily in terms of geographical origin or association, thus one plant species is frequently attacked by only one type of seed beetle. Being confirmed to be expressly monophagous, bruchins have proven to be excellent potential agents of control of invasive and harmful host plants (to date, false indigo-bush in Serbia), as is the case of the seed beetle of Persian silk tree, which has been registered as monophagous in Serbia for the first time, making it the number one potential agent in control of the host plant – Persian silk tree in Greece. Standard entomological methods were used, from sample collections, to experiments in photo-eclectors, during dissection counting and placing the seed for development inside by the method 1 flacon – 1 seed, to maintain precise records of the origin of the emerged insects and their numbers, within standard laboratory conditions. The conditions favoring the expansion of the plant have already developed due to climatic circumstances (changes in terms of heat extremes – global warming and precipitation), and it is now gradually becoming invasive. Parasitoid accumulation of roughly one-third (30%) of the total number of false indigo-bush pods makes the false indigo-tree seed beetle a viable candidate for the status of a bioagent. The species has the extreme potential for the application of biological measures which is now quickly gaining importance, all for the purposes of developing the integral control of the plants. Species *Acanthoscelides pallidipennis* and *Bruchidius terrenus* have been recorded in this research for the first time in Serbia, while calendars of development have been made for the researched bruchin species, which represents a significant scientific contribution.

Keywords: Bruhinae; seed beetles; biocontrol; legumes; parasitoids; Serbia

1. Introduction

In order to be defined as sustainable, management of forest resources must be primarily ecologically oriented, meaning that its negative effects on the environment must be properly assessed and reduced to a minimum (Sekulic, 2006). There are several programs for certification of forest resource management, which can in essence be classified under the program that satisfies pre-defined standards and the program that evaluates the quality of the management from the viewpoint of ecological policy.

In an effort to reduce the negative impact of application of chemical agents, biological control is becoming increasingly important. It is well-known that biological suppression is not a novel idea but dates back to the 19th century. For many years now, manufacturers have been conducting research, development and production of biological agents based on bacteria, viruses, fungi and insects (Rajkovic et al., 2010). Some of these useful organisms that proved to be more effective have recently been additionally developed and used as an alternative to the traditional, chemical agents (Marković et al., 2012). Mechanical

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suppression measures are also being avoided more and more frequently due to not only high labor costs, but also to strong sprouting capacity of certain plants – weeds.

A certain number of naturalized species that are extremely invasive, such as Black locust *Robinia pseudoacacia* L. 1753., have become economically significant species that are being utilized in forestry (Sitzia et al. 2016).Some leguminous plant species, which were introduced to the Balkans for their aesthetical value and which are commonly grown in parks and maintained sown areas, have become extremely aggressive and invasive species, the typical example being the introduction of Persian silk tree (ILDIS, 2002).

Without an adequate population growth control mechanism, invasive plant species in nature spread their areal very quickly, extending over the ecological niches of other species (Johnson 1981; 1987; 1989; 1990; Story et al., 2010, Tuda 2001). For the majority of invasive leguminous plant, spermatophagous from the subfamily Bruchinae were found, in which polyphagia is rare; bruchins are most often monophagous (Johnson, 1987, 1989, 1990; Jermy & Szentesi, 2003), however some of them may also be olygophagous Bhowmik, 1997).

In the temperate and continental climate zone, weevils inside a pod hibernate in one of the larva stadiums, feed on seeds (Tuda et al., 2005), while the pod provides double protection [15, 16, 17] (Hulme,1994; Howe & Oliver, 1987; Gressel, 2002) – pods are hidden but they also offer micro-climatic safety (seed membrane and pod skin) (Jordan et al., 2003; Praseeda et al. 2010). In species that are characteristic for southern climatic regions (*Albizia jullibrissin* Durazz.), the imago hibernates inside cracks of the bark or in hidden places. As for the form of leguminous plants, among herbaceous plants (legumes that shed the seed before winter), bruchids also hibernate inside the seed pod as eggs, whereas in woody legumes it is mostly one of the larva stadiums in seed pods (Harrison et al., 2003). False indigo-bush weevil was introduced to Serbia with the host plant *Amorpha fruticosa* L.(Fabaceae).

2. Material and Methods

The biology of these seed pests has been studied in Serbia in detail on over 50 sites in the period 2006-2022, when results on the presence of infested host plant seeds in the total seed of host plant pods were obtained. The research also focused on other representatives of another four species of this sub-family (Bruchinae), on which there are few references in this country and very little data. Specifically, the finding of false indigo-bush weevil was followed by confirmation of the existence and results of studying the biology of other discovered seed beetles that hibernate in mature pods. Standard entomological methods were used, photo-eclectors, dissection and placing the seed for development inside by the method 1 flacon – 1 seed, to maintain precise records of the origin of the emerged insects and their numbers. Host plant samples were transported to the laboratory in portable refrigerators. The dissected seeds were inspected with stereo magnifier (Leica Wild M3Z). Breeding of the insects, establishment of the dimensions, and monitoring of the duration of certain development stadiums were done in glass flacons or in Petri dishes. Eggs, larvae, pupae and imagoes of insects were collected and kept for the purposes of experiments, preservation, and photography. To determine the dimensions, 300 imago specimens were measured with the appropriate scale ruler on the ocular of the stereo magnifier that was also used in monitoring the embryonic and larval development of many samples (groups) of false indigo-bush and other species weevils under the laboratory conditions. Beetles were reared on their natural host seeds in 1 L glass containers kept in climate chambers set to reproduce their natural climate and light conditions, at 29◦ C and 50% (±10%) relative humidity (RH) undera12h:12h light : dark cycle. All beetles were fed with 20% saccharine solution, and water.

3. Results and Discussion

The false indigo-bush is spreading over the areas in which lowland forests regenerate naturally, negatively impacting the offspring of the dominant tree species. The biggest threat, however, is to natural regeneration of lowland, alluvial flooded forests of common oak and narrow-leaved ash, where it forms the understory (Bobinac, 1999).In practice, false indigo-bush is systematically eradicated through a combination of chemical treatments and intensive mechanical measures (Tomovic et al., 2008). This invasive species, characterized as an aggressive weed of lowland forests, is dominant in ruderal communities and a factor of serious hazard to the already undermined biodiversity in various sites. The false indigo-bush spreads over a wide biotope range, from valuable industrial common oak and ash forests in early stages of natural restoration, through coastal and flooded alluvial autochthonous plant communities, to damp sites, or forests ruled by strict conservation regimens (Vukicevic, 1996).

Acanthoscelides pallidipennis: false indigo-bush seed beetle. Its occurrence was noted for the first time in Hungary in late 1980s, while the first results of a comprehensive research on the species were published in 1990s (Szentesi, 1999). Like most other researched bruchin species, the false indigo-bush seed beetle completely destroys the inside of the pod through feeding. The maximum recorded percentage of seed infestation in Hungary is 61%, while in the USA (California, Nevada and New Mexico), where both the host plant and the seed beetle are autochthonous, the infestation rises to 87% (Szentesi, 1999) which makes this seed beetle a very attractive bio-agent in control of an invasive weed species – false indigo-bush. In 2001, records were made of the false indigo-bush introduction to Japan, along with the first finding of its parasitoid from the genus *Eupelmus* (Tuda et al., 2001).

In Serbia, multiple parasitoid species of the seed beetle are recorded, mostly from Chalcidoideae superfamily. It was established that the populations of false indigo-bush weevil in Japan were introduced from China and Korea (Tuda et al., 2001). In Japan the generation is double, the first one occurring in mid-July and the second one in mid-September (Tuda et al. 2001); in Serbia, the insect species was also found to be bivoltine (Gagic et al., 2008), with the first generation occurring as early as the end of March, and the second one in June (Gagic & Mihajlovic, 2009, Gagic-Serdar et al., 2013). The pod infestation according to season and bionomy is presented in Graph 1 and on Figure 1. Certain types of bruchins are economically important storage pests, while only several species are utilized in biological control programs for curbing the invasive plant species (Syrettet et al.,1999; Redmonet et al.,2000; Radford et al.,2001; Kingsolver, 2004).

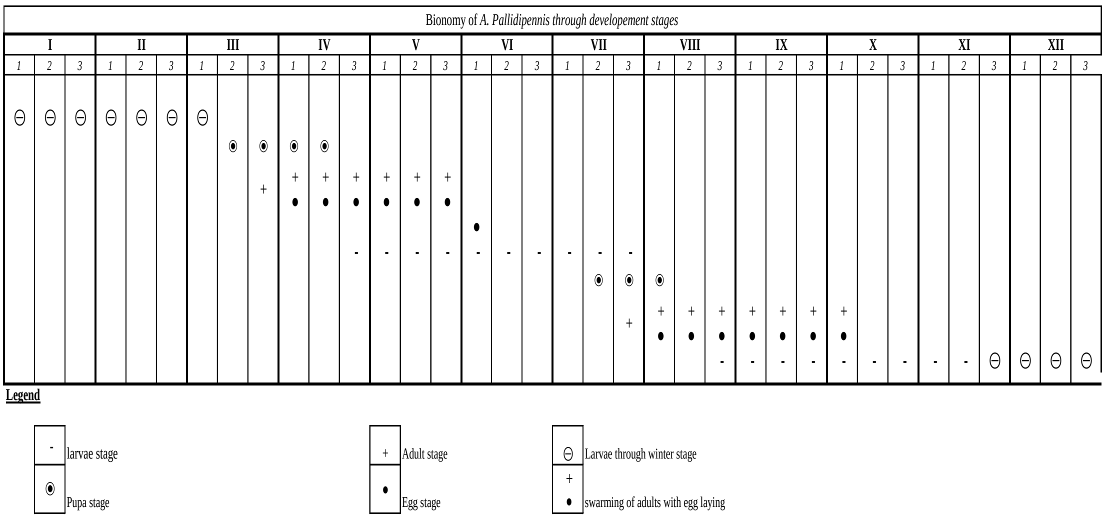
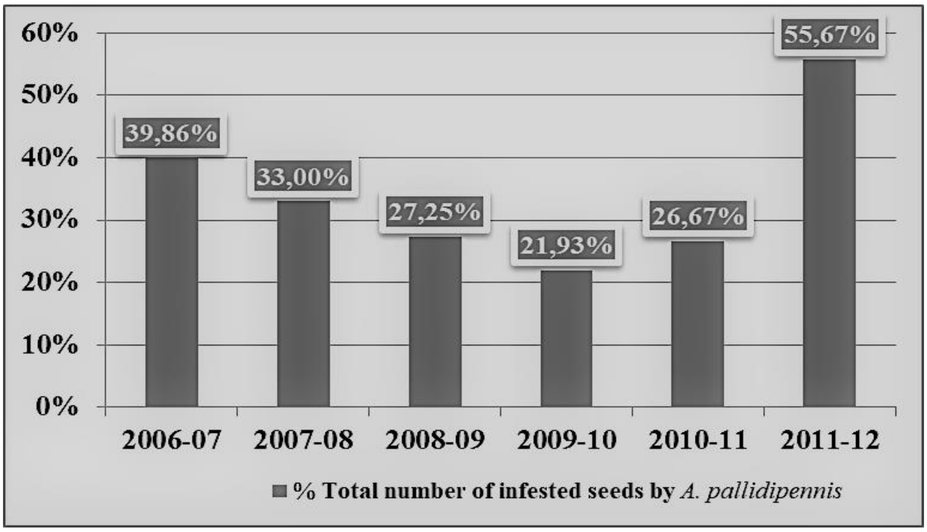


Figure 1. Bionomy of *Acanthoscelides pallidipennis* through development stages, one average year, tree decades per month.



Graph. 1 Infestation of seeds of *A. fruticosa* with *A. pallidipennis* (2006 – 2012).

The proof of that false indigo-bush weevil is monophagous was obtained by offering the population of 30 pairs the seeds of various legumes (*Phaseolus vulgaris* L., *Pisum sativum* L., *Glycine max* (L.) Merr., *Lens culinaris* Medikuss), but also the woody legume family (*Gleditcia triacanthos* L., *Wisteria sinensis* (Sims) DC. and *Robinia pseudoacacia* L.). There was no parasitoid accumulation and the insects died without feeding. According the most significant discovery during few past seasons is the confirmation that the one weevil we had investigated permanently (from *Gleditsia triacanthos* L. seeds), has a common bean seeds, *Phaseolus vulgaris*, as a host, which is completely new. Results are only estimated at 2% of total seed observed as infested, but confirmation is secure (Gagic-Serdar et al., paper in press).

Bruchidius terrenus Sharp,1886.; Distribution of monophagous species *A. pallidipennis* and *B. terrenus* follows the distribution of host plants (due to climatic factors it is spreading northbound, thus threatening to be invasive), which was confirmed here for these two species recorded for the first time in Serbia by this research. The species is monophagous both in Serbia and worldwide [33, 34, 35, 36,] (Meng, 1992; Kergoat et al.,2007a; Hoebeke et al., 2009; Moutet et al., 2016; Yus-Ramos et al., 2019) and accumulates parasitoids in a significant percentage (almost 1/4 of the total seed in pods of *A. julibrisinn*) [37] (Gagic-Serdar, 2020), becoming attacked by parasitoids from the Braconidae family [37] (Gagic-Serdar, 2020). Table 1 showed total number of infested seeds and percentage of infested seeds, for all species (besides indigo-bush weevil detailed) with legend used during application of research methodology.

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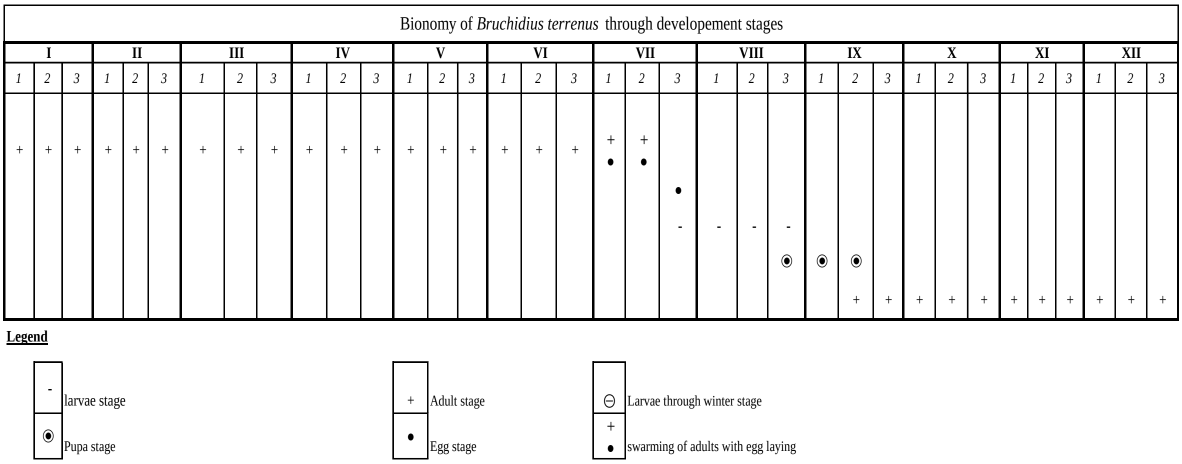

Figure 2. Bionomy of *Bruchidius terrenus* through development stages, one average year, tree decades per month.

Table 1. Total number of infested seeds and percentage of infested seeds, for all species with legend used during application of research methodology; samples with 300 seeds (dissection and one per flacon); Experimenting period 2012-2022.

Seed beetle on host plant		TSE	TIS	EH	A	PA	PP
		(1)	(2)=(2)/(1)	(3)=(3)/(1)	(4)=(4)/(1)	(5)=(5)/(1)	(6)=(5)/(2)
Bruchidius terrenus Albizia julibrissin	Sum	300	265	246	207	39	-
	%	-	88%	82%	69%	13%	15%
Bruchidius villosus Laburnum anagyroides	Sum	300	69	55	43	12	-
	%	-	23%	18%	14%	4%	17%
Bruchidius siliquastri Cercis siliquastrum	Sum	300	202	146	135	11	-
	%	-	67%	49%	45%	4%	5%
Megabruchidius tonkineus Gleditchia triacanthos	Sum	300	140	135	25	0	-
	%	-	47%	45%	8%	0%	0%
Megabruchidius tonkineus Gimnocladus dioicus	Sum	300	48	33	21	0	-
	%	-	16%	11%	7%	0%	0%

Seed beetle on host plant

Legend:

1)TSE: Total number of seeds inspected;
(2) TIS: Total number of infested seeds and percentage of infested seeds;
(3) EH: Emerge holes - number on a certain number of seeds;
4) A:Number of imagoes that flew out;
(5) PA:Number of parasitoids - imago of parasitoids (Pteromalidae, Eupelmidae, Eulophidae; Chalcidoidea) Braconidae wasps on *B.villosus* and *B.terrenus*
(6) PP: (5) PA divided by (2) TIS (in %)

Bruchidius villosus, Fabricius, 1792 Golden chain seed beetle, utilized in integral control of the introduced and invasive weeds of genus *Laburnum*in North America, where it was purposely introduced from Europe [29] (Syrettet et al., 1999). The species was introduced for the same purpose to New Zealand, Australia and the Hawaiian Islands (Syrettet et al., 1999; Haines, 2004). The biological control of the generative potential of the host plant was not universally successful, as *B. villosus* modifies the trophic preferences towards non-target host plants, if given a choice and different food accessibility [38] (Haines, 2004). *B. villosus* has multiple genera within the legume family as secondary host plants (*Lupinus*, *Cytisus* etc.). A positive example of successful biological control is curbing the legume *Leucaena leucocephala* (Lam.) within a kindred species, i.e. seed beetle of *Acanthoscelides macrophthalmus* [39] (Schaeffer, 1907); [40] (Ramanand & Olckers, 2013). Adaptation and synchronization of insect species binomy was determined not only for *Acanthoscelides pallidipennis* [26] (Gagic et al., 2008) but also for the species *Bruchidius terrenus* in Serbia [41] (Gagić-Serdar et al. 2014) and for *Megabruchidius tonkineus,* as well as for *Bruchidius siliquastrii* (Figures 1 -5). Table 1 also present total number of infested seeds of this species and percentage of infested seeds with legend used during application of research methodology samples with 300 seeds (dissection and one per flacon).

Megabruchidius tonkineus (Pic 1904) (Coleoptera: Chrysomelidae; Bruchinae) –seed beetle of honey locust *Gleditsia triacanthos* L. and seed beetle of Kentucky coffee tree, *Gimnocladus dioicus* (L.) K. Koch. There are no recorded parasitoids of the species, either in this country or worldwide. *M. tonkineus* is a seed beetle infesting the seed of honey locust *Gleditsia triacanthos* L. (Fabaceae) where bruchinae is a predator of honey locust seed, significantly reducing its generative capability. Nevertheless, the host plant is already spreading uncontrollably in a wide biotope range, and although it has previously been cultivated in forestry and naturalized like Black locust, over the years it may become an introduced invasive element. *M. tonkineus* has so far been recorded in the available references only as a seed predator of the genus to which the honey locust belongs. Our research has for the first time established its new host plant – Kentucky coffee tree *Gimnocladus dioicus* (L.) K. Koch (Fabaceae), a woody species whose presence in Serbia is rare (mostly as decorative trees within the park greenery – e.g. in Novi Sad), while in Belgrade the material was collected from the Arboretum of the Faculty of Forestry (Gagic-Serdar, 2020). The established parasitoid accumulation of honey locust seed amounted to 47%, while the recorded infestation of Kentucky coffee tree seed was 5% (Table 1). Research of bruchins as legume seed predators demands additional observations due to their potential role in biological control of the introduced and invasive woody species, and especially due to the intriguing origin of host plants and seed predators (Table 2 and Figure 3).

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Table 2. Origin of seed beetles and host plants (bold letters are shown how host plant and seed beetle were matched in Serbia, no matter they originated from two sides of a globe).

Seed beetle	Zoogeographical zone of seed beetle origin	Host plant in Serbia	Host plant worldwide	Zoogeographical zone of host plant origin
Acanthoscelides pallidipennis	North America	*Amorpha fruticosa*	*Amorpha fruticosa*	North America
Megabruchidius tonkineus	**Indochina (Vietnam)**	*Gleditchia triacanthos*	*Gleditchia triacanthos*	Central and North America
		Gimnocladus dioicus	-	**North American Midwest**
Bruchidius siliquastri	Indochina	*Cercis siliquastrum*	-	Western part of the Asian continent and South of Europe
			Cercis chinensis	East Asia
			Cercis griffithii	Southern parts of Central Asia
			Cercis occidentalis	California
Bruchidius terrenus	East Asia	*Albizia julibrissin*	*Albizia julibrissin*	Southwest and East Asia
Bruchidius villosus	Europe, Eurasia	*Laburnum anagyroides*	*Sarothamnus, Cytisus, Spartius, Lupinus and Robinia*	Europe

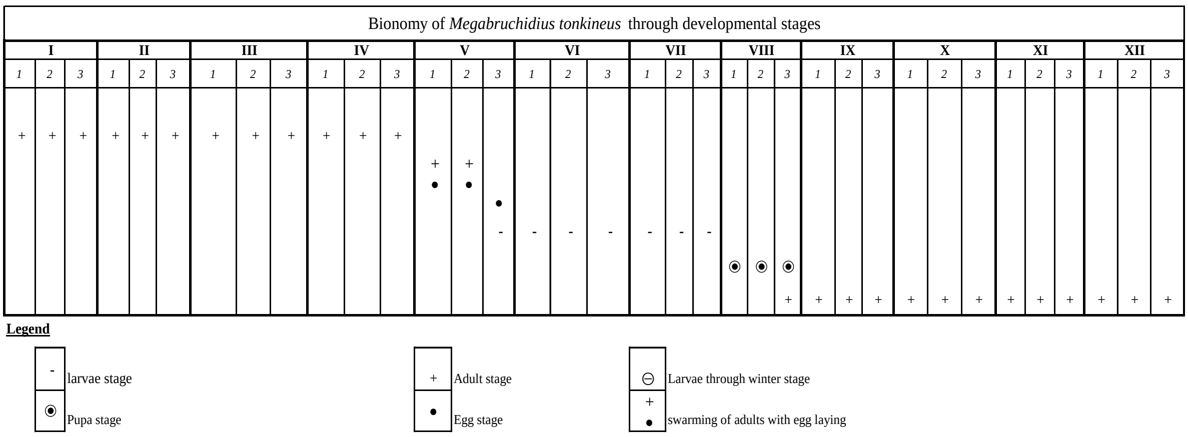

Figure 3. Bionomy of *Megabruchidius tonkineus* through development stages, one average year and sample, tree decades per month.

Bruchidius siliquastrii Delobel 2007 - seed beetle of Judas three, *Cercis siliquastrum* L. 1753 Species *B. siliquastrii* in Serbia is linked solely to *C. siliquastrum* and synchronized with its phenology (also proven for false indigo-bush weevil – seed of other leguminous plants was offered with no success or infestation, Figure 4). This type of bruchin has been internationally established to be oligophagous of the genus *Cercis*. Under conditions in this country, the species was found to have two generations annually and to hibernate in the larva stadium. At the end of the summer, as much as 80% of seeds (Table 1) in the pods are attacked by the larvae of its spermatophagous predators-parasitoids. The first generation imagoes start to swarm in the second decade of April and continue swarming until the first decade of May. Genus *Bruchidius* is represented in Serbia with three species:

Bruchidius siliquastri Delobel, 2007; *Bruchidius terrenus* Sharp,1886 and *Bruchidius villosus* Fabricius, 1792.

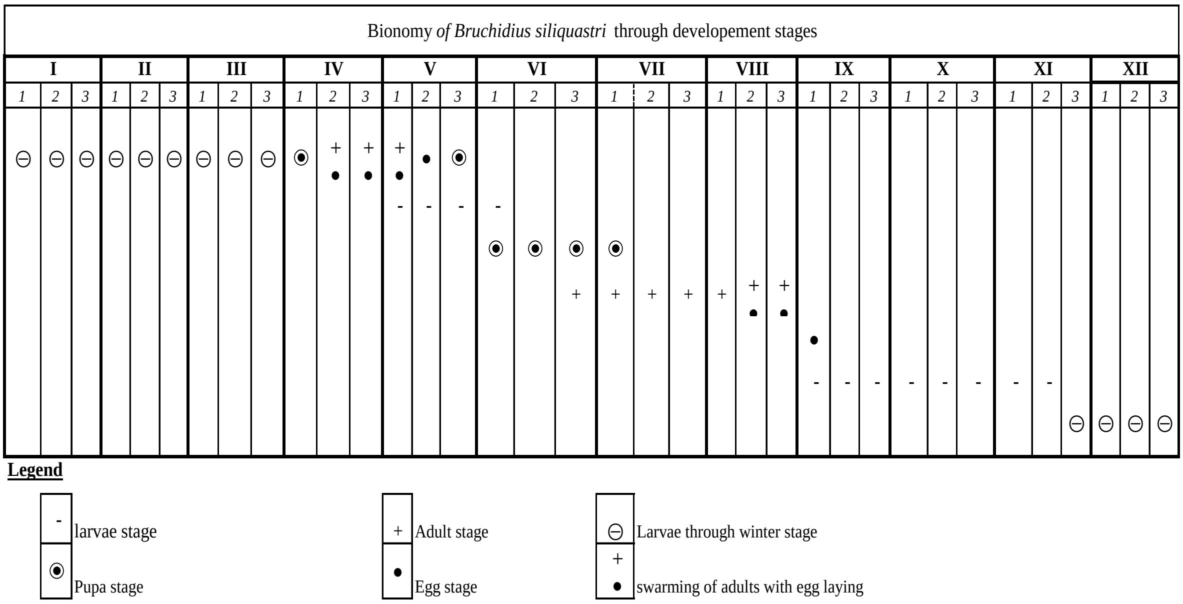

Figure 4. Bionomy of *Bruchidius siliquastrii* through development stages, one average year and sample, tree decades per month.

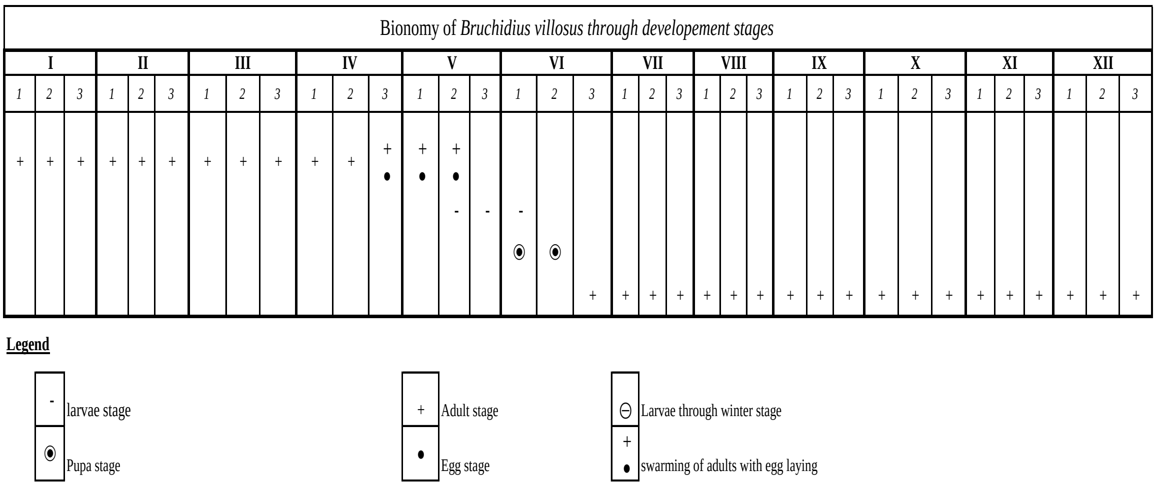

Figure 5. Bionomy of *Bruchidius villosus* through development stages, one average year and sample, tree decades per month.

In the following experiment and example, (results are for collected mass of 25 g of *Amorpha fruticosa* L., Fabaceae, pods are shown in Table 2), where seed beetles from the indigo bush sample (infested seed but threatened by parasitoids, presented in first part of connected tables) were counted, it can be seen that with all surviving seed beetles, the reduction capacity of indigo bush weevil would be the number at the end, and that was calculated based on the basic statistics and proportions. Status of the most common parasitoids genera from families Hymenoptera: Chalcidoidea; Pteromalidae and Eulophidae after intensive investigations has been trophicly identified, even there were determinated parasitoids of the second or third order - hyperparasitoids: Eulophid genera *Aprostocetus* and *Tetrastichus*. Here 443 would be the number of all opstructed usefull pests of seeds, by parasitoids from all levels (Figure 7), which leeds to at the end and 29,73% percentage of seed beetles capable for ruin the generative seed potential.

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Table 2. Reduction potentional of false indigo-bush seed beetle *Acanthoscelides pallidipennis*, sample statistics and basic statistics values.

In percentage			
Statistics	**Healthy seed**	**Infested seed**	**Total**
Minimum (min)	36.00	1.00	100
Maksimum (max)	99.00	64.00	100
Mean(ȳ)	70.27	29.73	100
Standard deviation (σ)	17.51	17.51	0
Sample size (n)	5270	2230	7500
In Numbers of seed in sample			
Statistics	**Healthy seed**	**Infested seed**	**Total**
Sample size (n)	5270	2230	7500
Mean (ȳ)	70.27	29.73	

Season	2016-17	2017-18	2018-19	2019-20	2020-21	2021-22
Infested seed percentage	39.86%	33.00%	27.25%	21.93%	26.67%	55.67%
Percentage of parasitoids in infested seed	1.50%	1.78%	9.84%	10.60%	12.20%	13.47%

One sample reduction potencial

Total No. of seed beetles raered fron parasitoids **parasitoids**

2185 : 147 = X : 443

29.73%

Approx 6,584.73

Total number of all survived seed beetles from sample

29.73%

100%

6,584.73 29.73% X 100%

22,148.43

POTENTIAL OF 6,584.73 22,148.43 seeds

REDUCTION CAPACITY Seed beetles should destroy

Figure 6. Beetle *Bruchidius terrenus* after emerging, 2012, site Ruma (Original).

Figure 7. Parasitic, wasps fam. Eurytomidae (Hymenoptera: Chalcidoidea; Eurytomidae), by *A. julibrissin* weevil (Original).

4. Conclusions

Varied but relatively high percentages of parasitoid accumulation of seed in pods of other host plants by the above-listed weevils highlight the potential of species as biological control agents in curbing these legumes as introduced plants. Due to climatic changes, putting these species on the list of invasive species would represent an investment into the future. It has been established that the biology of the studied spermatophagous bruchinae species is linked exclusively to the host plant species and synchronized with its phenology. The flight dynamics of the black locust weevil was monitored in the outdoor conditions over a five-year research period. The unified overview of the dynamics of *A. pallidipennis* populations during the research period indicated the differences in the abundance of generations and length of their duration.

Development calendars were made for the researched bruchinae species, based on the established biology.

To determine the potential of black locust weevil as a bio-agent in suppression of false indigo-bush, the percentage of seed infestation was determined along with the percentage of germinability of the attacked seed. Dependence between the seed infestation and site category was also established. The highest percentages of pod infestation were noted in 2007 and amount to 39.86%, while in last stage the percentage was 55.67%, when there was no inundation at the beginning of the vegetation period. The false indigo bush weevil was found to show host plant specificity, mobility and adaptability to changing environmental conditions, all of which indicate its bio-potential for control of the host plant. In view of the established characteristics of the black locust weevil and the well-known features of the introduced and aggressive weed species such as the false indigo-bush, the assumption is that under the country's conditions the classical biological method would prove to be successful, which would entail protection against the introduced weed species by the introduced phytophagous insect species.

Due to the aforementioned features of the protected areas overrun by the false indigo-bush and their specificities, it is necessary to favor biological measures of control, in accordance with the sustainable development. The advantages of the biological over the chemical and mechanical control are financial affordability, ecological justifiability, and long-term effectiveness of this type of control.

In line with realistic possibilities (in cases of limited options of mechanical measures and shortage of labor), it is recommended to apply ecologically acceptable solutions in forest protection or to conduct combined application of pesticides and integral methods.

Status of genera from families Hymenoptera: Chalcidoidea; Pteromalidae and Eulophidae after intensive investigations has been trophicly identified. Those are *Dinarmus acutus* (Thomson, 1878), *Eupelmus urosonus* Dalman 1820, *Eupelmus vesicularis* (Retzius, 1783). Nevertheless, they were found by numerous species of Eulophidae (Hymenoptera: Chalcidoidea) as the base, as parasitoids of the second or third order-hyper-parasitoids: Eulophid genera *Aprostocetus* and *Tetrastichus*. Nothing less important for the abundance of germination of seeds is also mentioned parasitoids that have about 20-25 % of the total number of infested seeds (for *B. terrenus* and that is pretty high number). In general average procent of parasitoids in whole number of seed in experiments is closely to 3%, as mean for all species of weevils. Logically, for infested seeds it varying from 2 % to 15% in maximum cases. Information on the, biology, identity, phenology, distribution and impact of silk tree key parasitoid species in Serbia still need to be investigated in the future.

Within the framework of repressive measures, alternative methods of forest protection against harmful organisms are developed and introduced in order to formulate appropriate and acceptable protection measures, so as to resolve the problem of excluding the undesirable pesticides. It is therefore necessary to support scientific institutions in the research aimed at devising alternative methods that are less harmful to the environment and the biodiversity in forest ecosystems.

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Conflict of Interest: With results and conclusions presented in this manuscript, authors will not have any commercial gain or profits of any sort. Main objective of submission endeavors is possible opportunity of publishing final research findings of new established species during three years of integral pest management studies within the sub frames of ours expert scientific field directions, in yours honored journal.
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