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SUPPRESSION OF INDIGO BUSH WITH POD PESTS

RENATA GAGIĆ-SERDAR[1*], Z. PODUŠKA[1], I. ĐORĐEVIĆ[1], G. ČEŠLJAR, SVETLANA BILIBAJKIĆ[1], LJ. RAKONJAC[1] and R. NEVENIĆ[1]

[1] *Institute of Forestry, University of Belgrade,* 11030, Belgrade, Serbia

Abstract - The recorded seed predators of *Amorpha fruticosa* L., indigo bush weevils and pteromalid wasps, were the subject of laboratory and field research studies in the period from 2006 to 2011. Sample analyses were carried out on more than 30 localities in Serbia with the aim of measuring the summarized pre-dispersal and post dispersal predation preferences. The percentages of the total pre-dispersal (max≈33%) and post-dispersal re-infested material (over 95%), make these insects serious candidates for host-plant suppression. Their bionomics were monitored through continuous collection, dessection and observation of infested seeds, in correlation with environmental parameters, especially water-level fluctuations in endangered forests.

Key words: Pods, *Amorpha fruticosa*, weevil, parasitic wasp, endophagous herbivores, Vojvodina (Serbia), Balkan Peninsula

INTRODUCTION

False indigo or indigo bush *Amorpha fruticosa* L. (Fabaceae: Papilionaceae: Astragalae) is a woody shrub that was introduced into Europe in 1724. It was brought to the Balkan Peninsula at the very beginning of the twentieth century, in 1900 (Petračić, 1938). This invasive species colonized native alluvial forests, ecosystems and habitats in large lowland river valleys (the rivers Sava, Danube, Drava and Tisa), posing a serious threat to the ecological balance (Krpan and Benko, 2009). Due to fundamental changes that occurred in the rare oases of relatively undisturbed wetlands and their direct influence on the continual shrinkage and disappearance of open water surfaces (water tables), there is a growing concern and need for international scientific cooperation in order to deal with this widespread aggressive species (Šefrová and Laštůvka, 2005; Hulme et al. 2008). Potential candidates for biological weed control were examined with the aim of protecting indigenous floristic diversity and providing indirect protection of the faunistic diversity of unprotected nature sites, as well as to prevent drainage and eutrophication caused by weed growth, i.e. increasing the abundance of indigo bush populations. Indigo bush reproduces generatively, with pods dispersed by water, and vegetatively with a strong sprouting power (Tucović and Isajev, 2000). Proof that its pod yields have natural chemical repellent and toxic influence on some insects (Tuda et al., 2001a), and demands for a combination of control measures requiring the systematic use of both chemical and mechanical practical measures (Tomović et al., 2008) make this woody plant challenging as a forest weed.

Research studies conducted in reestablished lowland forest ecosystems in Vojvodina confirmed that *A. fruticosa*, with its tendency to regenerate naturally, presents an extremely harmful biotic agent in plant

communities where autochthonous common oak and ash are dominant trees (Bobinac, 1999a). Lack of sunlight, space and soil depletion (Bobinac, 1999b) due to introduced plants, give the dominant native shrub and tree specimens only minimal chances to produce any offspring, since they cannot compete with indigo bush offspring. Native populations (in different habitats in North America, from the southern border of California to the Texas plains) are within the range of normal abundance. In recent decades, our region has experienced extreme water fluctuations and frequent droughts in the growing season. Climate change, in terms of increasing average air temperature and other adverse anthropogenic impacts, the most significant of which is the reduction of forest cover, contributes to the conditions favourable for the propagation and intense or even aggressive expansion of false indigo. Apart from forests, this woody weed spreads and grows as a component of different types of greenery. It is common in protective stands, line plantings (alleys) near roads and other corridor surfaces, and uncultivated agricultural land. It is also an edificatory species of ruderal communities.

MATERIALS AND METHODS

Emergence holes were identified on the pods collected in a number of localities in Serbia and a suitable methodology for field and laboratory research work was developed. The hypothesis of interspecies relations was formulated and the status of the parasitoids determined. The biology of the described species and genus was described, as well as the fact that parasitic wasps follow the occurrence of related endophagous herbivore seed weevils, which leads to the possibility of hyperparasitism (Mihajlović, 2008b). The collected hymenopterous specimens need to be determined with zoogeographical indicators.

The established host plant-seed predator linkage was studied with reference to different environmental parameters. Extremely flooded areas are characterized by water-level fluctuations in bank embankment stands and riparian cultures (Table 1). In the first pilot research season, racemes with mature pods were collected during the winter of 2006/07. Pods (100 pieces per locality) were dissected and the emerging adults were monitored for samples in eclector traps that clarify the bionomy of the species. Test tubes with 100 pods per locality provided data on each specimen's origin and development by examining the developmental stages after bruchid or wasp eggs had been laid (one pod - one glass tube). This method ensured measuring the duration of particular seed predator developmental stages for overwintered generations.

The first research task was to determine the percentage of pods or seeds infested by the detected legume seed predators. Accessible study areas were selected according to several key criteria. The localities were singled out based on the forests' practice to choose areas in which the host plant populations are developed to the extent that they start to cause problems. Populations of indigo bush formed a healthy habitus with notably thick canopies, which made *A. fruticosa* capable of adapting to intensive technological management treatments (combination of herbicides and mechanical measures of suppression) in alluvial forests in general. The material was collected from the plants that are obviously in competitive relations with the native forest trees in the early phases of natural regeneration. In order to make the studies comparable, the seed material was collected in river-flooded areas and in forelands, both from branches and from the ground during late spring and early summer (because there was a possibility that seed predators had drowned in the floods). Pods were collected and host plants monitored during the summer months in order to establish the precise number of insect bionomic generations. Pods were collected from ruderal communities, suburban areas near roads, from extremely dry places and from lakeshores. They were kept outside, in paper and fabric bags, and glass dishes covered with cloth. Pteromalid wasps rarely occurred on the host plant pods in laboratory conditions.

RESULTS

A. fruticosa was recognized as a host to the seed predator, *Acanthoscelides pallidipennis* Motschul-

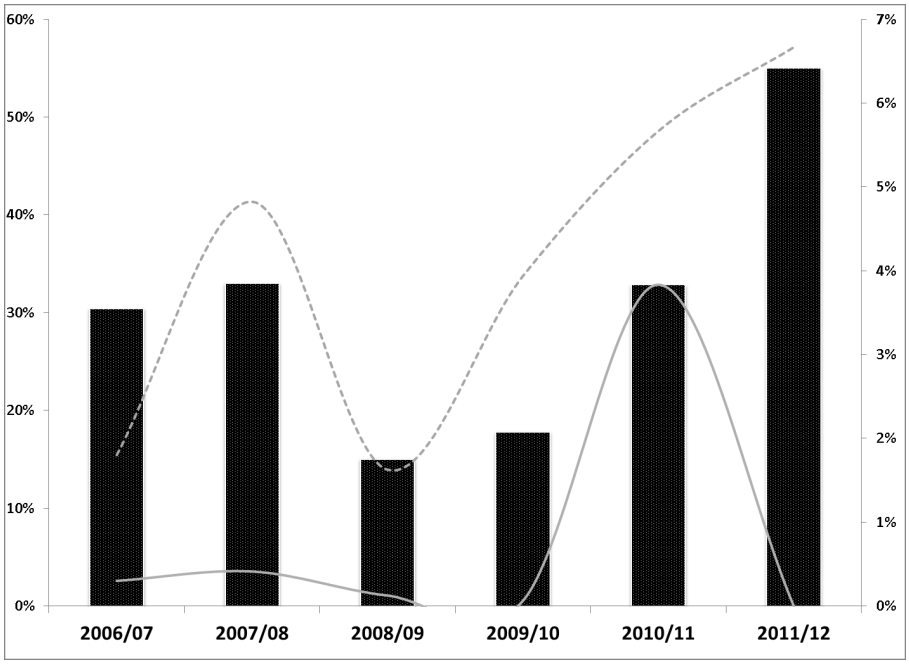

Fig. 1. Pre-dispersal seed predation on *Amorpha fruticosa* L. per seasons (total number of inspected seeds).

Legend key Figure 1

Mean value of infested pods percentage
Mean value of *Oedaule* sp. percentage
Mean value of weevil parasitoids percentage

Data table for Figure 1.

Season	Mean value % of infested pods	Mean value % of weevil parasitoids	Mean value % of *Oedaule* sp.
2006/07	30.4%	0.3%	1.8%
2007/08	33.0%	0.4%	4.8%
2008/09	15.0%	0.1%	1.6%
2009/10	17.8%	0.1%	3.9%
2010/11	32.8%	3.8%	5.7%
2011/12	55.0%	0.0%	6.7%

sky. Coleoptera: Bruchidae: Bruchinae (Tuda et al., 2001a) were recorded in Serbia in 2006 and are commonly referred to as indigo bush weevil (Mihajlović, 2008a). Investigations of seed predation on *A. fruticosa* in the Republic of Serbia have been performed intensively since 2006. At first, they were focused on recording certain bruchid beetles found feeding in the pods of this plant. They were assessed as a highly aggressive species in Serbia and several countries in Central and southeastern Europe. A massive eclosion of *A. pallidipennis* adults started in the third decade of April 2007. The data on mean infestation

Table 1. Average percentages of infested seeds (with causes of infestation identified by dissection in March, during six years of research) for three main different types of sampling areas, categorized by water fluctuation.

Infested seed \ water regime	**Flooded** ***(%)***	**Periodically flooded** ***(%)***	**Dry** ***(%)***
# bruchid larvae	1.83	13.14	21.12
# bruchid pupa	2.56	2.62	2.62
# bruchid adult	3.11	5.05	5.31
# parasitoid adult	1.17	0.05	0.08
# parasitoid larvae	0.11	0.14	0.04
# emergence holes	1.89	5.38	1.58
# parasitoid pupa	0.11	0.10	0.08
Oedaule sp. all dev. stages	0.61	0.62	0.42

Table 2. Parasitoid and predator complex of indigo bush weevil (or of Pteromalid endophagous wasp) as potential biological threatening agents for this introduced legume seed pest.

Insect	***Biology and host preference of A. fruticosa pod pests***
Eupelmus **and** ***Anastatus*** **(Hymenoptera: Chalcidoidea: Eupelmidae)**	ectoparasitoids of weevil larvae
Syntomaspis **sp. and** ***Torymus*** **sp. (Hymenoptera: Chalcidoidea: Torymidae)**	possibility of seed predation and hyperparasitism, both need to be proved
Tetrastichus **sp. (Hymenoptera: Chalcidoidea: Eulophidae)**	known to encompass parasitoids of the first and second order, so further research is needed in order to determine their status - hyperparasitism phenomena demands experimental "tricks"
(Hymenoptera: Proctotrupoidea: Scelionidae),	reared one specimen as fresh bruchid beetle egg parasite. Investigation needs to be continued with the aim of getting more specimens, data, status confirmation and species determination
(Hymenoptera: Proctotrupoidea: Diapriidae),	Hyperparasitoid of *Eupelmus* and *Torymus* genera; to date one specimen had been reared and prepared
Acari, Pyemotidae	Predators of weevil larvae and pupa
Pyemotes **spp. (=*Pediculoides*) verticosus, National Academy of Sciences 1978**	

for the season 2006/07 were obtained by dissecting the pods (30.4 %) before the observed adult insects were captured and returned to the same fabric bags. The pupal stage started in the first decade of June and ended in the first decade of September. At the end of the month, adult insects started to emerge in increasing numbers. Since the adults were not removed, re-infestation occurred in the bags. The pilot study, in which at least two generations overlapped, showed that the percentage of infested seed predation was higher than 95%. In October 2007, the percentage of seeds with exit holes was over 95% (F1+F2+F3…) in almost all samples. Damaged seeds included both pre-dispersal and post-dispersal seed predation.

A. pallidipennis infests mature pods of indigo bush. The degrees of seed infestation in the localities in Serbia were determined and are presented in Fig. 1.

The status of the genus *Oedaule* (Hymenoptera: Chalcidoidea: Pteromalidae) was established based on the collected and observed indigo bush seeds. The initially proposed hypothesis that these wasps were zoophagous-weevil parasitoids was rejected. The ensuing laboratory analyses proved that this species belonged to the group of endophagous herbivores, feeding on seedpods (like herbaceous insect). Factors that can have the greatest influence on the fluctuations of the indigo bush seed predation numbers are either abiotic (temperature, precipitation, presence of ground water and flooding) or biotic (inter-species relations between the host plant and other trees from the phytocoenological community or its parasitoids, hyperparasitoids and predators of seed pests). During seed dissection (2008), 3-4 larvae revealed the existence of mites *Pyemotes ventricosus* (Acari) at different stages of development. The parasitoid complex (parasitic wasps) and Acari predator of *A. pallidipennis* as reducing agents of this introduced legume seed pests are also presented in Table 2.

DISCUSSION

Scientifically based directives about the preservation of our autochthonous forests depend on an integral and easily applicable method, and on the results of our research of indigo bush entomofauna presented in this paper, which has identified potential candidates for aggressive plant biological control measures. The indigo bush weevil hibernates in the final larval or pupal stages in the pods that remain on branches. In a pod, the larva feeds on seed endosperm (Gagić and Mihajlović, 2009). The indigo bush weevil infests *A. fruticosa* pods after floral button formation. Autochthonous parasitoid entomofauna has successfully adapted to this chemical barrier. Egg laying coincides with the pod yield time and its final growth formation. The biology of the indigo bush weevil as a spermatophagous insect in Serbia is completely related to one host plant, and in infected pods, it passes through all larval stages and reaches the pupal stage. Larvae develop inside the pod with one seed, which prevents the plant from germinating, and after dissecting, the infested pods it was confirmed that the entire pod content was usually eaten. According to previous data, it does not have preferences for other related plant species, which was also confirmed by studies conducted in Hungary (Szentesi, 1999). During the winters of 2009/10 and 2010/11 we confirmed this in the laboratory by exposing pods and seeds of other host plants widely spread in Serbia: *Phaseolus* sp. *Gleditsia triacanthos* L., *Robinia pseudoacacia* L. and *Glycine max* (L.) Merr. to indigo bush weevil adults (groups of male and female individuals right after eclosion). The closely related Bruchinae subfamily members (*Megabruchidius*, *Bruchidius* and *Sulchobruchus*) are also related to one host plant from the Fabaceae family. The polyphagous performance of these insects is still under investigation, especially as they are potential biocontrol candidates (Tuda and Morimoto, 2004). The final conclusion is the identification of a host plant-seed predator system (*A. fruticosa -A. pallidipennis* and *Oedaule* sp.).

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