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STUDY OF *Rhinoncus pericarpius* (Linneus, 1758) (*Coleoptera: Curculionidae*) BIOLOGY, AN IMPORTANT PEST OF HERB PATIENCE AND RHUBARB IN ROMANIA

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Abstract

The new agricultural reform policy, which involves an upward trend in the organic farming system of medicinal and aromatic plants, but also plant protection program, raises more and more complicated problems. The protection of organically grown medicinal plants varies according to whether or not the interests of farmers are compatible with the need for introducing these plants grown in the production system. For farmers who cultivate large-grain cereal plants, spontaneous species of the genus Rumex are considered weeds and control measures are taken against them. For those who cultivate Rumex species for food or medical purposes, the protection of these crops raises a number of issues. The common denominator of this controversy is, for some, and for others, the possibility of biological control using biological agents represented by insect species of the Curculionidae family. The flora of Romania includes 25 species and 12 subspecies of the genus Rumex; among them only two species, Rumex patientia L. and R. rugosus Campd, are cultivated in small individual farms. Species of the genus Rumex are widely distributed on Romanian territory, although each species has specific life requirements. Corresponding to the species of the genus Rumex and Rheum are insect species that feed on different plant organs and cause varying degrees of attack. Many of these species are part of two genera of the Curculionidae family: Apion and Rhinoncus. Five species belonging to the Apion genus have been reported in Romania's fauna: Apion frumentarium Payk, A. miniatum Germ., A. cruentatum Steph., A. sanguineum DeGeer and A. rubens Steph. Only one, A. miniatum, which is spread throughout the country, could be used in the biological control of spontaneous Rumex species, or may be considered an important pests for cultivated species. Another important pests belong to the Rhinoncus genus, are three of the eight species occurring on Rumex acetosa L., namely Rhinoncus pericarpius L., Rh. castor F. and Rh. bosnicus Schultze, the last one very rare, was not found in the southern investigation areas. To assess the importance of Rh. pericarpius species both as a harmful species and as a biological control agent, the results obtained in the present paper, refer to the growth of the species under laboratory conditions on natural food. The work brings new data on the feeding trophic range, attack model, incubation duration, fecundity of the female and fertility of the eggs, the active duration of the female and the duration of the growth of the larvae, pupae and diapauses, as well as the spread on the Romanian territory.

Key words: *Rhinoncus pericarpius L., medicinal and aromatic plants, life cycle, laboratory mass rearing.*

INTRODUCTION

The family of *Curculionidae* (*Coleoptera*) with the 51,000 species and 4,600 genres represent, after Staphylinidae, one of the largest families of beetles from the world. Recently, some of taxonomists had included also the family *Scolytidae* like a subfamily *Scolytinae* into the superfamily *Curculionoidea*. Since the DNA barcoding method revealed that genetically bark beetles are very different from ‘’true weevils’’ the species of this group must be considered like a separated family, morphologically and genetically. From over the 13,000 species mentioned in the Palearctic area, in Romania only around 800 species are mentioned (Petri, 1912). After the Petri study few taxonomists they leaned forward to investigate and to classify into an exhaustive catalogue the fauna of such important species of coleopterans. Except the divers aspects regarding some pest species on the main crops from Romanian agriculture few papers are dealing with taxonomy of *Curculionidae* species (Teodor, 1993; Teodor, Dănilă, 1995; Teodor, Manole, 1996; Manole, Iamandei, 2002). More then 242 species are related to be important in the structure of coleopteran fauna from main agricultural crops (maize, wheat, sunflower, sugarbeet, fodder crops, fruit trees, grasslands and vegetables) (Manole, Iamandei, 2002). One of the remarkable aspect of the species of weevils behavior is the fact that the majority if not all are phytophagous feeding, and that is the explanation, like in the case of majority of *Chrysomelidae* species for which the main important pests are belong to the *Curculionidae* family. Regarding to the host plant of mostly species of weevils the aspects and position of experts are controversial. In the last century a widespread concern of industry from many countries are applied to recovery of a alimentary supplements from wild flora (i.e. medicinal and aromatic plants especially). In the usually community of weeds from main crops some of those plant species are considered weeds and would be target for weeds control methods (herbicides control). In some countries many of those species are cultivated on relatively large surfaces (example for genus Rumex which in China many hybrids of this plant are obtained and cultivated) (Li et al., 2001, 2002, 2003). Another relevant case is considering the invasive plant species. For instance, in Europe and also in Romania the species from genus *Centaurea* (*Asteraceae*) are cultivated in some little farms for many uses in pharmaceutical industry (Alexan et al., 1988; Păun, 1995; Bojor, 2003). In North America those species are invasive and USDA and other American Environmental Services established in the last years some programs for control using insect species like biological control agents (Wilson et al., 2005; Winston et al., 2005, 2010). As I said before in many of my author's scientific works, the problem of weeds control must be mainly well-balanced and environmentally addressed. For those species included in the wild associations of crops weeds, the herbicide control approach may be undesirable. Biological control in agricultural IPM systems became a subject of considerable current interest because of a perceived urgency to develop and adopt safe and efficient methods for managing agricultural pests. Problems associated with pest suppression (including environmental pollution, deleterious effects of pesticide on non-target organisms, pesticide resistance, resurgence of target pests, secondary pest outbreaks, and escalating costs of developing, producing and applying pesticides) all affect the vitality and profitability of agriculture and the well-being of our society. Some few studies carried out in Romania relate to this subject the possibility to use the insects like biological agents for weeds control (Perju, 1982; Perju et al., 1993; Perju et al., 1994; Perju et al., 1995). *Rh. pericarpius* and some species of genus *Rumex*, spontaneous or cultivated are both very important element of wild life in our country. It is in the same time the main pest of herb patience and rhubarb cultures, plants used both in food and in the pharmaceutical industry but in some others cases could be a very efficient agent of biological control of *Rumex* species. Nevertheless, the biology of the species is little known. Our field observations and techniques for mass rearing in laboratory under controlled conditions on natural food permits to obtain a series of new data on species biology, data complemented by observations in the field of species spreading in Romania, occurrence in the field and way of attack. To assess the importance of *Rh. pericarpius* species both as a harmful species and as a biological control agent, the results obtained in the present paper, refer to the growth of the species under laboratory conditions on natural food. The work brings new data on the feeding trophic range, attack model, incubation duration, fecundity of the female and fertility of the eggs, the active duration of the female and the duration of the growth of the larvae, pupae and diapauses, as well as the spread on the Romanian territory. *Rh. pericarpius* is a taxon native on palearctic biogeographically region and belongs to *Curculionidae*, *Ceutorhynchinae*, *Phytobiini*. Genus *Rhinoncus* was established by Schönherr, 1825 after the misidentification by Paykull 1792 and Stephens, 1831 (Huang, Collonelli, 2014). The genus *Rhinoncus* (Schönherr, 1825) is present with 8 species in Europe and 7 in North America from which 3 are invasive (Arnett et al., 2002).

MATERIALS AND METHODS

This study was carried out in the period 2000-2002 at the laboratory of entomology from RDIPP Bucharest and part of field observations were been made at RDIVF Vidra, Giurgiu district and biological material were collected at the country level in the period 1982-2018.

Experimental setup in laboratory

Insects. The insects individuals that formed the starting colony for laboratory gains (G_0) were collected from the field in two localities in the adult stage at the begining of spring when the plants had 2 or 3 leaves growth, in the period of normal appearance of the adults of *Rh. pericarpius* (between 09-15 May 2000). One lot was collected from rhubarb crops (*Rheum officinale* Baill.) from RDIVF Vidra and another lot from a little surface cultivated with *Rumex acetosella* L., at RDIPP Bucharest. The other field observations were been conducted apart of the main study of *Rh. pericarpius* growing to investigate the dispersal of the species population on the Romanian territory. The adults population was introduced in some special plastic recipients with cylindrical shapes and next dimensions: 25 cm Ø and 30 cm height. The bottom of the vessel was covered with a filter paper roll to prevent the moisture on the vessel walls. The adults were feed with leaves of herb patience fresh collected from the field and removed after 72 hours. The insects were daily observed and when the copulation begin the couples of male and female were separated in the other vessels (15 cm Ø and 15 cm height).

Adult growth. The adults were fed until they naturally died and the eggs were collected soon after the female deposit the bunch of eggs on the leaf surface or on the rods of the leaves.

Incubation. The eggs collected from one couple represent a new colony of the insect species and after collection were incubated in plastic Petri dishes with 9 cm diameter at ± 25°C. Inside the Petri dishes, the humidity required for the hatching process was ensured by two methods: a) Petri dishes with daily wetted filter paper; b) Petri dishes with cotton swabs soaked in water and moistened for 48 hours; after hatching the larvae were translated into special glass Petri dishes on the plant food.

Larvae growth. Growth and development of larvae was carried out in Petri dishes of 8 cm, 9 cm and 10 cm Ø. The larvae were fed with the rods of the herb plant (fragments harvested from ribs or young stalks) in which an incision was made for the penetration of neonate larvae. At intervals of 48-72 hours (as the case may be) the vegetal fragments were replaced by fresh ones by two methods: a) direct passage of the larvae one the new fresh stem with a fine paint-brush; b) free migration of larvae after the degradation of the vegetal material to the new fresh stem; In each Petri dish for larvae growth only 2 fragments of plant stem was introduced but the larvae were between 5 and 10 individuals.

Improving and getting adults. After larval development, larvae which reached maturity (L_3) were collected and passed to the hump in two variants: a) Petri dishes on dry filter paper on both lids of the vessel; b) in black plastic plastic sapphires, on filter paper. All stages experimental bioassay for laboratory rearing were carried out in one single variant (2 adults (♀♂) on natural food, leaves and stems from *R.acetosella*) and 10 replications for each stage. The host plant preference test bioassay was performed with 5 variants and 14 replicates. The laboratory conditions were different and variable with the stage of the insect. In the adult case the temperature in the feeding rooms was of 20 ± 2^0C and RH of 75-80% at the daylight photoperiod of 16:8 light/dark. The eggs were incubated at 25 ± 2^0C in the special conditions of RH mentioned above. The pupal stage was maintained at the lower temperature compared with the soil temperature in the May-June period (i.e. $10\text{-}12^0$C) in special rooms in the dark conditions. The replicates designed for growth period bioassay were also performed with 10 variants including each one couple of adult insects (♀♂).

Experimental setup in the field. Plant host were collected and conserved in laboratory herbarium and for identification Illustrated flora of Romania (Ciocîrlan, 2000) was used. *Rh. pericarpius* feeding on the herb patience were observed in the field to evaluate leaf damage index. A four-degree injury scale was used where (Piesik, Lamparski, 2006):

0 - no injury;
1 - up to 10% injured leaf area;
2 - 11-20% injured leaf area;
3 - 21% injured leaf area;
4 - 31-50% injured leaf area.

Following a five-degree injury scale, leaf damage index was calculated, basing on Townsend and Heuberger equation:

$$IP\% = \frac{\sum_0^i (n*v)}{i\,x N} * 100, \text{ where:}$$

n = number of leaves in consecutive injury-degree;

v = injury degrees from 0 to 1 (the highest in scale);

N = number of examined leaves.

RESULTS AND DISCUSSIONS

The study represent an first attempt to enlarge the knowledge about the biology and behaviour of the species of weevil *Rh. pericarpius*, insect belonging to a group of species very quite widespread in Romania.

In Europe, to which is native, the species is enlarge distributed in all countries (Figure 1). The results obtained on the first step indicate that the colonies of species could be mass reared in controlled conditions to be used in many purposes i.e.like an efficient biological control agent in some cereal crops.

Figure 1. European distribution of species *Rhinoncus pericarpius* L.

Food regimen. *Rh. pericarpius* belong to the family of *Curculionidae* which include only phytophagous insects feeding on a large diversity of plants.

In our study, for the first time in Romania, the host plants (spontaneous or cultivated) of the species had been registered but the insect was, also present on other plant species without detection the sign of consumption or attack on plant organs (Table 1).

In our laboratory experiments bioassay test his feeding preferences were registered (Table 2). *Rh. pericarpius* feed only on a small number of species belonging to the *Polygonaceae* family from genus *Rumex* (*R. acetosella* L., *R. crispus* L., *R. acetosa* L., *R. obtusifolium* L., *R. hydrolapathum* Huds.), *Polygonum* (*P. persicaria* L., *P. lapathifolium* L.), and *Rheum* (*Rh. officinale* Baill., *Rh. rhaponticum* L.). From this point of view his feeding behavior can be framed in oligophagous category. The consumption of the plants organs are: leaves, stems, buds and rods in this order of preferences in the adult stage, only rods or stems fragments in case of larvae.Host plant preference was, after our investigations at the country level (Figure 2), herb patience, *R. acetosella* (with 100% frecquency of presence on this plant). *Rh. officinale* is another prefered host plant but in this case the damages are highest in the rhubarb crops then in herb patience cultures. *R. acetosella* appear to be more resistant to the insect attack. Consecutively, the level of the attack was highest (5.7) then in the case of preferenced host *R. acetosella* (4.6) (Table 2).

Table 1. Localities and plant species where *Rhinoncus pericarpius* L. was collected

LOCALITY	HOST SPECIES	OBSERVATIONS
Bucharest	*Rumex acetosella* L *Rumex acetosa* L. *Rumex crispus* L. *Rumex patientia* L. *Rumex acetosa* L. subsp.*hortensis* Dierb.*	Pairing, eggs deposit, larval hatching, damages of the attack
Vidra	*Rheum officinale* Baill.* *Rheum rhaponticum* L.* *Rheum palmatum* L.* *Rumex acetosa* L. subsp.*hortensis* Dierb.*	Pairing, eggs deposit, larval hatching, damages of the attack
Comana	*Rumex crispus* L. *Rumex hydrolapathum* Huds. *Polygonum persicaria* L. *Polygonum lapathifolium* L. *Rumex aquaticus* L. *Rumex palustris* Sm.	Pairing, eggs deposit, larval hatching, damages of the attack
Arad	*Rumex acetosa* L. subsp.*hortensis* Dierb.*	Pairing, eggs deposit, larval hatching, damages of the attack
Curtici	*Rumex acetosa* L. *Rumex acetosella* L	Pairing, eggs deposit, larval hatching, damages of the attack

Nădlac	*Rumex acetosa* L.	Pairing, eggs deposit, larval hatching, damages of the attack
Timişoara	*Rumex acetosa* L.	Pairing, eggs deposit, larval hatching, damages of the attack
Lugoj	*Rumex obtusifolius* L. *Rheum rhabarbarum* L.*	Pairing, eggs deposit, larval hatching, damages of the attack
Deva	*Rumex obtusifolius* L. *Rumex thyrsiflorum* Fingerh.	Only presence on the plant
Oradea	*Polygonum aviculare* L.	Only presence on the plant
Zalău	*Polygonum persicaria* L.	Only presence on the plant
Blaj	*Rumex obtusifolius* L.	Only presence on the plant
Turda	*Rumex obtusifolius* L.	Only presence on the plant
Braşov	*Rumex alpestris Jacq.* *Rumex alpinus* L. *Rumex confertus* Willd.	Only presence on the plant
Codlea	*Rumex confertus* Willd.	Only presence on the plant
Sibiu	*Rumex acetosa* L. *Rumex acetosella* L	Pairing, eggs deposit, larval hatching, damages of the attack
Tîrgu Mureş	*Rumex confertus* Willd.	Only presence on the plant
Darabani	*Polygonum lapathifolium* L.	Only presence on the plant
Botoşani	*Rumex acetosa* L. *Rumex acetosella* L	Pairing, eggs deposit, larval hatching, damages of the attack
Iaşi	*Rumex obtusifolius* L. *Rumex acetosa* L. *Rumex acetosella* L	Only presence on the plant
Huşi	*Rumex acetosa* L. *Rumex acetosella* L	Only presence on the plant
Roman	*Rumex obtusifolius* L.	Only presence on the plant
Piatra Neamţ	*Euphorbia spp.*	Only presence on the plant
Bicaz	*Rumex palustris* Sm.	Only presence on the plant
Focşani	*Rumex acetosa* L. *Rumex acetosella* L	Only presence on the plant
Măcin	*Rumex obtusifolius* L.	Only presence on the plant
Tulcea	*Rumex tuberosus* L. *Rumex aquaticus* L. *Rumex dentatus* L.subsp. *halácsyi* Rech.** *Rumex maritimus* L.	Only presence on the plant
Sulina	*Rumex tuberosus* L. *Rumex aquaticus* L. *Rumex palustris* Sm. *Rumex maritimus* L.	Only presence on the plant
Medgidia	*Rumex maritimus* L.	Only presence on the plant
Slobozia	*Saponaria officinalis* L. *Brassica oleracea* L.	Only presence on the plant
Ţăndărei	*Rumex acetosa* L. *Daucus carota* L.	Only presence on the plant
Jilava	*Rumex acetosa* L. *Solanum lycopersicum* L.	Only presence on the plant
Giurgiu	*Rumex palustris* Sm. *Saponaria officinalis* L. *Allium cepa* L.	Only presence on the plant
Şimnicul de jos	*Rumex tuberosus* L.	Pairing, small attack
Drobeta Turnu-Severin	*Rumex longifolius* D.C. *Saponaria officinalis* L.	Only presence on the plant
Herculane	*Rumex hydrolapathum* Huds. *Rumex palustris* Sm. *Polygonum lapathifolium* L. *Polygonum persicaria* L.	Pairing, eggs deposit, larval hatching, damages of the attack

*cultivated
**very rare

Regarding to the *Rh. pericarpius* plant hosts there is few contributions in world entomological literature. This wasn't the main purpose of the study but some aspects need to be clarified. For instance, in Romania our study brings for the first time almost exhaustive data about but we can find some most cited host species in other papers. In the world literature Reitter, 1916 cited for host plants only genus *Polygonum* and *Rumex*. Hoffmann, 1954 cited the species *Rh. pericarpius* on *R. obtusifolius* and *R. acetosa* in all France territory. Morris, 1967 had signaled the species *Rh. pericarpius* like common in Ireland on *Rumex* genus species: *Rumex acetosa, Rumex obtusifolius, Rumex crispus, Rumex acetosella, Rumex conglomeratus* Murr. In U.K., Read, 2002 mentioned the weevil species on genus *Rheum*, which are non-native in England. Walsh and Dibb, 1954, noted that only *P. amphibium* was host species for *Rh. pericarpius* and finnally Scherf, 1964, find *Rh. pericarpius* only on *R. hydrolapathum*. In district Nova Scotia (Canada) Majka et al., 2007, signaled the presence of *Rh. pericarpius* like a non-native faunistic element feeding on *R. crispus*, *R. maritimus* and *R. acetosa*. Piesik, 2004, mentioned in his study about using insects like biological control agents host plant for *Rh. pericarpius* the weed *R. confertus* which seems to be a big problem in Poland in pastures as his high amounts of oxalic acid. When consumed in large quantities the lethal poisoning of animals can occur. In China, where the herb patience is cultivated on a large surfaces used in consumption or like medicinal plant, Li et al., 2001, 2002 and 2003, had mentioned the species *Rh. pericarpius* on forage *Rumex* hybrid K-1 in Xinjiang region. Another controversial problem was shows by the studies carried out in Japan by Katsumata et al., 1930, and Harada, 1930, which mentioned the species *Rh. pericarpius* like on the most injurious pest on hemp. No mention in the world literature appear about some species of *Phytobiini* (and we can even say from all *Curculionidae* family) on cultivated or spontaneous hemp plants. In Romania the hemp crop was cultivated (a single species, *Cannabis sativa* L.), with two subspecies *Cannabis sativa* L. subsp. *sativa* Fr., and *Cannabis sativa* L. subsp. *spontanea* Serebr. on a large area in Banat and Transylvania region and even in some localities from southern of country but now the plant were not cultivated yet on the large surface.

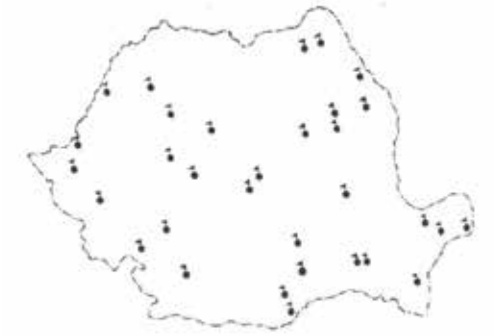

Figure 2. *Rhinoncus pericarpius* L., spread in Romania

Mode of the attack. The attack shape of the adult stage consist in holes punching on the leaves on the entire surface of the leaf limb but concentrated mainly on the centre and on the margins of the leaves. Adults are feeding sometimes (especially on the driest period of weather) with the rods or young stalks causing small cavities on their surface, the way of some pathogens entrance. The larvae feeding behaviour, soon after his emergency was related with the organ were the eggs are deposit. They crunch the surface of the stalks or stems to enter inside of the parenchyma and they dig mining the stem to the roots and to the soil for pupation. The larval gallery could be the entrance way for some plant pathogens and other saprophytic organisms.

Eggs and incubation. After pairing and copulation, which could be after a period of feeding or in some cases during the feeding, the female laid daily the eggs, in small bunches, usually on the young stalks, on the rods or on the leaf petiole (Figure 3).

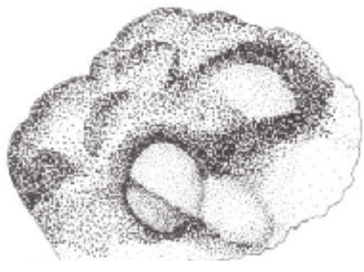

Figure 3. Eggs batch covered with specific glandular agglutinat deposit on *R. acetosella* stem

The eggs were regularly deposit on the leaves petiole at the stem insertion or relatively frequent on the young stalks. In very rare situation (one single case in laboratory) the eggs were laid on the lower face of the leaf. In the field, in conditions of southern part of Romania the female laid the eggs between earlier first week of May and the last eggs were laid on the end of May in very rare cases in the beginning of June. The egg batch had a dark brown colour and count between 5 and 30 eggs agglutinated in the secretion of the female glands situated on the copulatory bursa on the last terga of the abdomen. Eggs have a discoid, flattened shape with length of 1.04±0.39 and width of 0.54±0.1 (Table 5).

The incubation period in the laboratory conditions was registered between 8 and 16 days after deposit on the plant organs. The results are presented in the Table 3.

Table 2. Testing the host preferences of *Rhinoncus pericarpius* L. on spontaneous weeds and culture plants

VARIANT	TESTED SPECIES	FOOD CONSUMPTION		ATTACK DEGREE	OSERVATIONS
		Adults	Larvae		
1 adult ♀	*Rumex crispus* L.	+	++	0.5	Pairing, eggs deposit, larval hatching
1 adult ♂	*Rumex acetosella* L	+++	+++	4.6	Pairing, eggs deposit, larval hatching
2 adults (♀+♂)	*Rumex acetosa* L.	++	++	2.8	Pairing, eggs deposit, larval hatching
10 adults (5♀ + 5♂)	*Rumex hydrolapathum* Huds.	+	-	1.8	Pairing
	Rumex obtusifolium L.	+	-	0.2	Pairing
	Polygonum aviculare L.	+	-	-	-
	Polygonum persicaria L.	+	-	0.6	-
	Polygonum lapathifolium L.	+	-	0.8	-
	Rheum officinale Baill.	++	++	5.7	Pairing, eggs deposit, larval hatching
	Rheum rhaponticum L.	++	++	2.3	Pairing, eggs deposit, larval hatching
	Brassica oleracea L.	-	-	-	-
	Daucus carota L.	-	-	-	-
	Solanum lycopersicum L.	-	-	-	-
	Allium cepa L.	-	-	-	-

Table 3. Eggs incubation duration of species *Rhinoncus pericarpius* L. in laboratory conditions on *Rumex acetosella* L.

Variant / Replicate	1 wetted filter paper	2 cotton swabs soaked
	Eggs incubation duration (days)	Eggs incubation duration (days)
1	10	8
2	13	8
3	10	8
4	16	8
5	10	8
6	11	8
7	11	8
8	14	9
9	13	9
10	13	9
Mean	**12.1**	**8.3**

In the first variant of incubation bioassay, on the wetted filter paper the incubation duration was longer then on the cotton swabs soaked, respectively. In the first variant the incubation period was between 10 and 16 days with the average of 12.1 days. In the second variant the same incubation process were count an average of 8.3 days, between 8 and 9 days maximum. The incubation were influenced by temperature conditions and also by the moisture and relative humidity that could be the explanation of the short period of the incubation process in the second variant.

Female fecundity. On the laboratory conditions the female of *Rh. pericarpius* were laid the number of eggs counting between of 75 and 830 eggs on the entire of the active period (Table 4). The daily average of the eggs deposit in those 10 replicates used in the experiment was between 3.38±1.0 and 18.04±2.1 (Table 4).

Eggs fertility. In laboratory conditions the eggs fertility was relatively high compared with the values obtained in the field. For instance in our study the fertility was between 86.19% and 100.00% compared with the 10 replicates from the field where the fertility reach the values of 45.55 and 70.24% of the hatching larvae. It seems to be some conditions of natural infection from the soil vehiculated by the ants (like gregarine, and some other parasitic organisms) but the small values of the larvae hatching in the field could be explained also by the eggs consumption by some predatory species of ants (*Lasius fuliginosus* Latr., *L. flavus* F. and so on) (Table 4).

Table 4. Prolificity of female and eggs fecundity to species *Rhinoncus pericarpius* L. in laboratory conditions on *Rumex acetosella* L.

Replicate	Prolificity		Fecundity	
	Eggs number	Daily mean	Larvae number	% of hatching
1	105	3.38	105	100.00
2	384	9.60	331	86.19
3	75	3.75	75	100.00
4	98	6.53	92	93.87
5	319	9.66	316	99.05
6	830	18.04	810	97.59
7	188	6.26	188	100.00
8	80	5.33	80	100.00
9	161	5.36	160	99.37
10	132	4.40	131	99.24

Growth development in laboratory conditions.

Larval stage. The larva is grub with length dimensions of 1.8±0.4 in media, apodous, eucephalous type, white in colour, with well-developed head capsule with functional mandibles with act transversely, maxillae stemmata and antennae. Through measure the wide of head capsule of larvae, the larval development covers 3 instars (Table 5). In literature there are few studies regarding the morphology of adult and larvae of the species *Rh. pericarpius*, the only one paper which refer to the larval development were written by Li et al., 2001. The values obtained in this study for head capsule of larvae measurement (L_1 = 0.305±0.0105 mm; L_2 = 0.516±0.0105 mm; L_3 = 0.796±0.0083 mm) was similar with those obtained in the present study. Larval stage duration in laboratory conditions on natural food was covered a period of 6 to 8 days with the average of 6.9 days (Table 7). No other mention in the world literature can be found related to the larval growth and development.

Table 5. Dimensions of cephalic capsule, larvae and egss of *Rhinoncus pericarpius* L., reared in laboratory conditions on natural food

Larvae length (mm)	Head capsule of 3 instars (mm)	Egg (mm)	
		Length	Width
1.8±0.4	0.310±0.01 L_1	0.60±0.3	0.55±0.12
1.9±0.3	0.518±0.01 L_2	0.55±0.2	0.50±0.1
2.0±0.6	0.851±0.08 L_3	0.81±0.3	0.48±0.2
2.1±0.8		0.98±0.2	0.58±0.3
2.3±0.9		1.5±0.8	0.58±0.1
1.6±0.1		1.2±0.5	0.53±0.2
1.8±0.2		1.0±0.2	0.47±0.1
1.6±0.4		1.4±0.4	0.59±0.2
2.0±0.5		1.3±0.5	0.58±0.1
1.5±0.1		1.1±0.5	0.58±0.2
1.8±0.4		**1.04±0.39**	**0.54±0.1**

Adult stage. The adult body of species *R. pericarpius* count after our study measurements between 2 - 3.5 mm with small variations of length between 0.5 - 0.1 mm (no visual sexual dimorphism but generally the female had the body bigger then male) (Figure 4). In the classical studies of taxonomy of *Curculionidae* the species are mentioned by Reitter, 1916, and Hoffmann, 1954, with the body length of 2 - 3.5 mm and Freude et al., 1981, with body length of 2.5 - 3.4 mm. In laboratory conditions the feeding and matting behaviour was studied and confirmed by the field observations.

Soon after his appearance the female, start feeding on the *R. acetosella* leaves and they then was matting. Many observations look like they first start matting and after they begin to feed after or in the same time with copulation.

Figure 4. *Rhinoncus pericarpius* L., adult stage

I think that it could be possible to feed on another plant and to fly after on the new host plant and start matting. In laboratory conditions the active period from female life cycle were rigorous registered. After the matting process which count no more then 2 hours or almost 1 day began the preovipositary period which mean between 2 and 15 days (mean of 6.4 days). After the preovipositary period the female stop feeding and begin to lay eggs on the plant organs closed to the stem or at the insertion of the shells on the stem. The period calls ovipositary period and in conditions of our study this count 15-46 days (with the mean of 29 days) (Table 6).

Table 6. Active period of the female to species *Rhinoncus pericarpius* L. in laboratory conditions on *Rumex acetosella* L.

Replicate	Preovipositary period	Ovipositary period
1	2	31
2	5	40
3	10	20
4	2	15
5	2	33
6	8	46
7	9	30
8	15	15
9	5	30
10	6	30
Mean	**6.4**	**29.00**

Pupal stage. After browsing last instar (L_3) the larvae build a puparium from vegetable tissue debris agglutinated with some special secretions from the last abdominal segment and sterilized in the same time with this secretion paste and inside this pupal lodge the pupal stage was spend. The duration of pupal stage in conditions of our experiment was between 6 and 8 days (mean 6.9 days) (Table 7).

Life cycle duration. In laboratory conditions on natural food the adult life duration was registered contained between 32 and 45 days (mean of 34.1 days). After the pupal stage the adult's emergency and the number of adults obtained from all ten replicates means in percent about 75.26 (similar with the percent observed in field) and 100.00% but the mean value was 92.45% (Table 7). The whole period of life cycle of *Rh. pericarpius* in laboratory conditions beginning to egg stage and ending with the adult dead was contained between 63 and 96 days (mean of 73.6 days). In the field conditions the observations in the field cages were difficult to make because of the insect small size and they had usually the habit to enter in the soil or to hide on the vegetation's from the soil. Only after some estimation could be reasonable to suppose that this duration reach maximum of 75-80 days.

Table 7. Growth duration of the species *Rhinoncus pericarpius* L., in laboratory conditions on natural food

Replicate	Growth duration	Larval stage duration	Pupae stage duration	Adults %
1	43	27	7	93.33
2	45	27	8	75.26
3	33	17	6	93.33
4	32	15	7	81.63
5	40	23	7	94.04
6	34	19	8	96.50
7	36	18	7	95.74
8	34	19	6	100.00
9	44	21	7	96.27
10	34	19	6	98.48
Mean	**34.1**	**20.5**	**6.9**	**92.45**

Using species *Rh. pericarpius* like biological control agent. Beginning with this study the efficacy of insect species in biological control of the host plants from Polygonaceae was revealed. The attack of adults cause significant damages on leaves when the numerical density was more then 5-10 individuals/plant. The most important attack of the adult (especially of the female) is manifest in the flowering and in the period of seeds formation when the adults completely destroy the fructifications. In our previous paper (Manole, Iamandei, 2002) we establish for the first time the important role which he can play in weeds biological control (especially on *R. obtusifolius*). Other than our study form 2002 a much documented review of this subject was published by Herrick and Kok, 2010. The only limit of this study is related with the weeds definition which, in conception of the authors for the study was "an alien plant species that is non-native to the ecosystem under consideration and whose introduction causes or is likely to cause economic and/or environmental harm, and/or harm to human health". And in this study it is revealed that the insects from family *Curculionidae* (and *Chrysomelidae*) include the most potential biological control agents against weed plants (Table 8). Several species of beetles in the family *Curculionidae* have played a vital role in the suppression of invasive weeds throughout the world (Julien et al., 1984; Julien, Griffiths, 1998; Coulson et al., 2000). Our study established that over 242 species could be used in Romania like biological control agents but Herrick and Kok, 2010, successfully used as biological control agents in classical biological weed control. In the actual ecological context biological control programs using insects became very desirable. First problem which rises to the scientists all over the world is the problem of alien invasive species, plants including. In many countries that kind of biological control program became very attractive correlated with the ecological gains. Some technical problems need to be resolved in the future according with the costs reduction. First step in this direction consist in development of the systematic studies about some candidates of biological control agents. Present study brings new knowledge's about the biology and ecology of *Rh. pericarpius* which could be mass reared in controlled conditions and released in control of some weeds in horticultural and agricultural crops. In Europe and also in North America, Canada the species *Rh. pericarpius* were used in some programs against the weed plants from *Polygonaceae* family. Piesik (2004) conducted one field research in vicinity of Bydgoszcz and Toruń in period 1997-2001, using some insect's species among those *Rh. pericarpius* against one important weed, *Rumex confertus* Willd. (mossy sorrel). The same mention about this host plant for *Rh. pericarpius* appear in the book written by McPortland et al., 2000. In Asia, Russian Far East, China, Korea and Japan *Rh. pericarpius* is considered invasive species but another weevil belong of Subfamily *Ceuthorhynchinae*, native in the area could be used like biological control agent against some weeds from *Polyganaceae* family. Herrick and Kok, 2010, mentioned that species *Rhinoncomimus latipes* Korot., could be used for biological control of mile-a-minute weed, (*Polygonum persicaria* L., syn. *Persicaria perfoliata* (L.) H. Gross) in Africa. The species had however used already in biological control program in Asia by augmentation practices and in North America by mass rearing and released in the field (Hough-Goldstein et al., 2009; Lake et al., 2011; Paynter et al., 2015). A very intense concern regarding the biological control programs using insects against weeds were to evaluate the effectiveness of the method and implicit the costs of programs. After of that evaluation Herrick and Kok, 2010, mentioned that although the initial investment in a classical biological control programme is expensive, the monetary gain after programme implementation is greater. Classical biological control programme with *Curculionidae* is a viable option for weed control because of its sustainability. Morin et al., 2009, had also made a very documented economic analysis of effectiveness and costs-benefits balance and in conclusion stated that the problem to highlighted the stakeholders is most important to negociate the long-term benefits. The ussual trend is to evaluate the agent effectiveness soon after their release and establishment in the field but practitioners must understand to undertake long-term post-release evaluation. Paynter et al., 2015, concluded in his study in the same manner that is a necessity to make a cost-benefit analysis before implementing the program but the duration of host-range testing agents released is the long-term element for economical analysis.

Table 8. Target plant species worldwide, grouped by habitat, using *Curculionidae* as biological control agents (after Herrick, Kok, 2010, modified)

Habitat type	Plant species targeted worldwide	% of species	Number of insects weevils used
Terrestrial-herbaceous	44	65.7	47
Terrestrial-arborescent	17	25.3	18
Aquatic or semi-aquatic	6	9.0	10
Total	**67**	**100.00**	**75**

CONCLUSIONS

In Romania *Rh. pericarpius* is a well-distributed species associated with some host plants from genus *Rumex* and *Polygonum* which could be used in biological control programme together with other species frequently present in this associations like *Gastroidea polygoni* L., *G. viridula* Deg., *Hypera rumicis* L., *Apion miniatum* Germ., *A. frumentarium* Payk., and *Pegomya nigritarsis*

Zett. In conditions of Romania in the field the life cycle had a single generation/year, diapausing in soil in the adult stage and pairing and oviposing beginning with the first week of May. For the first time new data about ecology and biology of this species were registered. The distribution at the country level and the worldwide status of species was established. The food regimen is phytophagous related with plant associations of *Rumex*, *Polygonum* and cultivated *Rheum* genres in Romania 9 host plant were recorded (*R. acetosella* L., *R. crispus* L., *R. acetosa* L., *R. obtusifolium* L., *R. hydrolapathum* Huds., *P. persicaria* L., *P. lapathifolium* L., *Rh. officinale* Baill., *Rh. rhaponticum* L.). The attack shape of the adult consist in holes punching on the leaves and the larvae were minning the stems. In laboratory conditions the species was reared on natural food on leaves and stems of *Rumex acetosella*. The female had two active period of life cycle: pre-ovipositary which was in our experiment between 2 and 15 days (mean of 6.4 days) and ovipositary period which count 15-46 days (mean of 29 days). Female deposit after copulation daily between 5 and 30 the eggs usually on young stalks or on the leaf petiole. Eggs have a discoid, flattened shape with length of 1.04±0.39 and width of 0.54±0.1. The incubation period in the laboratory conditions was registered between 8 and 16 days after deposit on the plant organs. The larva is grub with length dimensions of 1.8±0.4 in media, apodous, eucephalous type, white in colour, with well-developed head capsule with functional mandibles with act transversely, maxillae stemmata and antennae. Through measure the wide of head capsule of larvae, the larval development covers 3 instars. After browsing last instar (L_3) the larvae build a puparium from vegetable tissue debris agglutinated with some special secretions from the last abdominal segment and sterilized in the same time with this secretion paste and inside this pupal lodge the pupal stage was spend. The duration of pupal stage in conditions of our experiment was between 6 and 8 days (mean 6.9 days). The adult body of species *R. pericarpius* count after our study measurements between 2 - 3.5 mm with small variations of length between 0.5 - 0.1 mm (no visual sexual dimorphism but generally the female had the body bigger then male). In laboratory conditions on natural food the adult life duration was registered contained between 32 and 45 days (mean of 34.1 days). The study establish for the first time the important role which he can play in weeds biological control (especially on *R. obtusifolius*).

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