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Biology and natural enemies of spotted ash looper, *Abraxas pantaria* (Lepidoptera, Geometridae) in Krka National Park

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List of nonstandard abbreviations:
CFRI – Croatian Forest Research Institute
NPK – Krka National Park
SAL – Spotted Ash Looper

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Abstract

Background and Purpose: *Spotted ash looper (*Abraxas pantaria*) is a forest present in Krka National Park, Croatia with occasional mass occurrence. The caterpillars of this pest have completely defoliated leaves of Narrow-leafed ash (*Fraxinus angustifolia*) in the upper flow of river Krka in the period from 2008–2010. We have researched the biology of spotted ash looper which is first comprehensive study of this pest in Croatia.*

Materials and Methods: *The research of spotted ash looper included field trials through all the years and laboratory experiments. Caterpillars were reared and daily weighted in laboratory. Fresh and dry Narrow-leafed ash leaves have been weighted and factor of dry matter in leaves defined. For each individual sex, moment of onset of particular larval instar, prepupa, pupa and butterfly was noted in order to identify the duration of each stage and possible differences. Duration of larval stages and consummation of food were analysed separately by gender. A total of 200 pupae of spotted ash looper were transferred to the laboratory for the identification of natural enemies. Each pupa was placed in a separate glass tube and incubated in laboratory.*

Results and Conclusions: *Our results show biology of this pest in Croatia. Caterpillars feed on Narrow-leafed ash and have not been found on Manna ash (*Fraxinus ornus*). They have moulted only 3 times and pass through 4 larval instars in laboratory conditions. Average caterpillars of 4^{th} larval instar gained their maximum growth on the 4^{th} day from the beginning of that stage and maximum weight on the 8^{th} day, three days before entering the prepupal stage. The difference between males and females starts to develop during the 4^{th} larval instar when females consume significantly more food, gain significantly more weight and form significantly heavier pupae. The average 4^{th} larval instar consumes between 0.3329 and 0.3673 g of fresh leaf. Research shows that number of pupae diapauses during the winter, while some of them have no diapause. This indicates that the pest has at least two generations a year, which was also shown by observations in the field. Pathogen fungus* Beauveria bassiana*, parasitoids* Pales pavida, Cratichneumon cf. fabricator *F. and unidentified species from the genus* Coelichneumon *were natural enemies found in pupae.* Beauveria bassiana *as a pupal pathogen plays an important role as the most important natural enemy and the existence of its alternative host, ash weevil (*Stereonychus fraxini*) which occupies the same overwintering niche is also important.*

INTRODUCTION

Spotted Ash Looper (SAL), *Abraxas pantaria* L. (Lepidoptera: Geometridae) is a well-known pest of ash (*Fraxinus* spp) in the Mediterranean area. It was described as a pest of Narrow-leafed ash (*Fraxinus angustifolia* Vahl.) in Croatia *(1)*, common ash (*Fraxinus excelsior* L.) in Portugal and Spain *(2)* and Turkey *(3)*. It is listed as a species in fauna of northern Caucasus *(4)*, Armenia and Georgia *(5)* and the north-eastern part of Russia *(6)*. Narrow-leafed ash is an important forest species in Croatia *(7)* and it can be also often found as a horticultural species in parks and avenues.

In the Mediterranean region, in river valleys, where there is plenty of soil moisture and air humidity, forest vegetation that resembles the continental flood exposed vegetation is growing. It differs only in addition of some Mediterranean and sub-Mediterranean elements. Such forests are found along the river Krka. The tree species represented in these forests are: Narrow-leafed ash, willow (*Salix* spp), poplar (*Populus* spp), and black alder (*Alnus glutinosa* L.) *(8)*. Within the Krka National Park (NPK) grow forests that belong to the order *Alnetalia glutinosae* TX. 1937 and *Alnion glutinosae* Maculit 1929, and in NPK they are present in the narrow but uninterrupted belt of Narrow-leafed ash along the river Krka. These forests are characterized by long-term stagnation of flood water. Narrow-leafed ash is growing in the area next to the stream of Roški waterfall to the monastery of Archangel. On the plateau Skradinski buk individual Narrow-leafed ash trees are growing. Manna ash (*Fraxinus ornus* L.) is abundant species in the NPK.

SAL can cause defoliation resulting in loss of vitality and aesthetic value of attacked ash trees. In areas with high frequency of visitors, such as national park, social nuisance is an important negative effect, disrupting the movement of visitors due to the large number of caterpillars hanging on silky threads and falling excrements. Outbreaks of this pest in NPK are temporal; the last was recorded in the period 2008–2010, in the area of Roški waterfall up to the monastery of St. Archangel *(9)*. In the same period, in the most visited area of NPK Skradinski buk, no caterpillars have been found *(9)*. Last gradation was recorded in this area in the late nineties of the 20th century *(1)*.

Biological pest control in protected areas is very valuable, and the first step in the study of its application is a research of hosts and their natural enemies *(10, 11, 12)*. Natural enemies of insects can, in general, be classified into several groups: insect predators, parasitoids, nematodes and pathogens (viruses, bacteria, protozoa, fungi) *(e.g. 13, 14)*. *Beauveria bassiana* (Balsano) Vuillemin is mentioned as an antagonist of many insect species, which application as a biological agent has been researched on some species from the family Curculionidae *(10, 15)*.

Pupation of SAL which takes place in the soil when the soil temperature and moisture are optimal for the development of pathogenic fungi such as *B. bassiana*, has opened up the question of how much is this pathogen involved in the reduction of population through infecting pupae. The presence of ash weevil (*Stereonychus fraxini* De Geer) in NPK *(9)* as an alternative host of *B. bassiana* underpins this question.

Furthermore, many studies of natural enemies of forest pests emphasize parasitoids as significant factor in population decrease *(16)*. Parasitoids of SAL are mentioned by Bathon and Tirry 2005 *(17)* and Ozbek and Calmasur 2010 *(3)*. Parasitoids of ash weevil, which could play a role as alternative host for parasitoids of SAL, have been mentioned by several authors *(18, 19, 20, 21, 22, 23)*.

As SAL is relatively unknown ash pest in Croatia, the aim of this research was to study its morphological characteristics and biology, as well as its natural enemies. Pupal parasitoids could be a significant population reduction factor and this data together with the knowledge of biology could be used in prognosis and biological control of SAL. Special importance is given to *B. bassiana* as pupal pathogen. The possibility of use of parasitoids in SAL control has not yet been studied, and the first step in this direction is the identification of the parasitoid species found.

MATERIALS AND METHODS

Biology and natural enemies of SAL in the NPK were studied from 2009 to 2012 at several locations along the river Krka from Roški slap (waterfall) (43° 54' 32" S, 15° 58' 45" S) to the Monastery of St. Archangel (43° 57' 47" S, 15° 59' 31" I) and the lake Visovac (43° 51' 37" S, 15° 58' 04"). This area was chosen because total defoliation of ash was observed in the area from 2008 to 2010.

The research of SAL biology included field observations once a month in the period July–October during the years of research as well as comparison with data from the references. The intensity of defoliation and life stage of the insect were noted in the field. During the pupal stage the area under the damaged trees was searched and the upper soil layer on the plots 20x20 cm dug out and searched for pupae. In total 10 plots were dug out from which the pupae were collected which were used for rearing and identification of parasitoids. The depth of the soil where the pupae were found and collected was measured.

For additional laboratory studies caterpillars were collected along Lake Visovac on 18/07/2012 and brought to the entomological laboratory of Croatian Forest Research Institute (CFRI) for pupation. Pupae were placed in a glass tube and covered with cellulose gauze. The experiment was carried out in climate chamber in controlled conditions of temperature (22 °C) with a relative humidity of 60% and the ratio of light: dark (L: D) 18:6.

After the emergence of butterflies, ash twig with leaves has been added to the tube. Over the next few days the butterflies have mated and females have laid egg masses on ash leaflets as well as on the cylinder sides and cellulose cover.

For the palatability test 30 caterpillars were collected, they were individually placed in glass Petri dishes to which food was regularly added. The caterpillars were weighted and fresh food was added daily. Every day a sample of five ash leaflets was weighted in fresh conditions, then dried for 24 hours on 70 °C and then weighted again for defining the dry matter coefficient in leaves. The coefficient was calculated according to the formula:

$$\text{Dry weight coefficient} = \frac{\text{fresh weight of leaves}}{\text{dry weight of leaves}}$$

The remains of leaves and excrement were taken out of Petri dishes in separate containers after pupation and dried for 24 hours at 70 °C and then weighted. Pupae were weighted three days after moulting. All weighting was done on analytical balance Acculab ATL-224-I to four decimal places of grams.

For each caterpillar sex and the moment of onset of individual stage was recorded: prepupa, pupa and butterfly, in order to define the duration of each stage and eventual difference.

Duration of individual stages was calculated on the base of the date of moulting, whereby for the younger larval stages only the minimum and the maximum duration was recorded because the development of individual caterpillars was monitored from the 4th larval instar (Table 1). Results of the larval instar duration and leaf consumption of individually monitored caterpillars were analyzed separately by the gender.

In September 2010 a total of 200 pupae of SAL were collected from the soil in NPK and they were transported to the entomological laboratory of CFRI for analyses of parasitism and infection rates by pathogens. One pupa was placed in one glass tube. The pupae were incubated in controlled conditions of temperature (22 °C) with a relative humidity of 60% and the ratio of light: dark (L: D) 18:6.

As this pest is unknown in Croatian literature we therefore present the basic morphological characteristics. With help of available references, our own observations and laboratory experiments we reconstruct the development cycle of SAL in NPK on smaller and for Croatia on a bigger scale.

RESULTS

SAL has been present with higher or lower intensity in NPK for several years (*1*, *9*), feeding on Narrow-leafed ash while it did not feed on Manna ash.

Morphological features and biology of the SAL

SAL butterfly has a wingspan of 40–44 mm for females and 38–44 mm for males. Head, thorax and abdomen are covered with yellowish hairs and scales, and on the abdomen dark brown spots are found (Fig 1a). It gives the impression of white, silky colour, eyes are black. Butterflies are starting to emerge in late June and early July. The largest number can be expected in the second half of July, their numbers declining in early August. Average life span is 10–15 days *(2, 3)*. Adults can be found on top of ash trees or on tree trunk. Butterflies are good fliers. Males emerge before females. Females lay eggs shortly after copulation on the underside of ash leaves from the main leaf vein to the edge. A female can lay up to 500 eggs, about 300 on average in several egg masses *(2, 3)*.

Egg size is 0.8x0.6x0.5 mm and laterally flattened. Freshly laid egg is clear-green, and before the end of embryonic development the colour changes to brown-gray with brown spots. Females lay varying number of eggs and stick them to the underside of ash leaves (Figure 1b). The eggs can be found from mid-July to mid-August. The egg stage lasts for about 10 days and changes colour during embryonic development *(2, 3)*.

The results of this experiment show that the egg stage lasts 4–6 days, the caterpillar eclosion ends after 3 days (18 to 20/08/2012.). Caterpillars do not eat the egg shell. First moulting (from 1st to 2nd larval instar) followed after 2 days, and 1st larval instar lasted on average 6–9 days (Table 1). The 2nd instar lasted 7–12 days (Table 1). Larvae of the first two instars eat lower epidermis and parenchyma tissue making furrows and small holes. They do

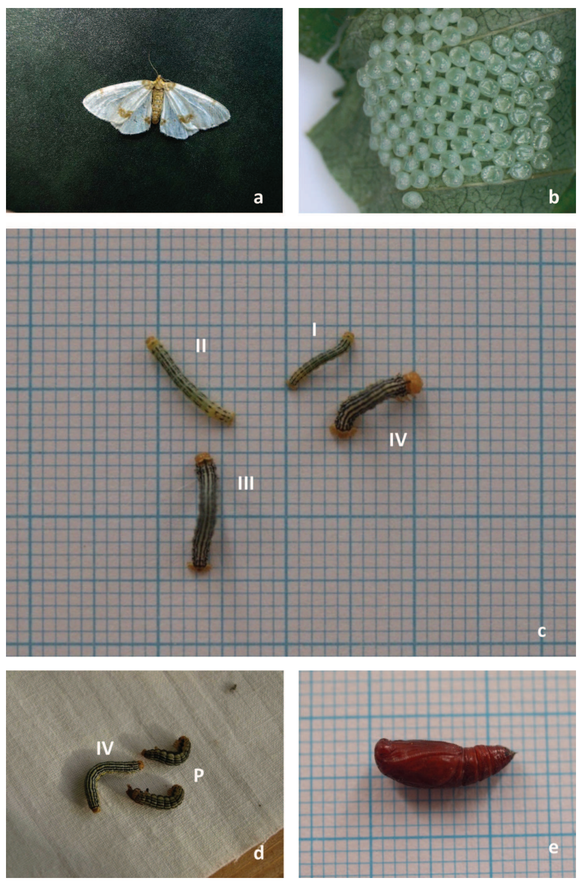

Figure 1. *Different development stages of spotted ash looper (*Abraxas pantaria*) a) buttefly b) egg mass c) 1st–4th larval stage d) 4th larval stage and prepupa (P) e) pupa.*

not consume leaf veins thus forming lacy appearance of foliage. After moulting to older instars they begin to consume large quantities of food and damage to the leaves becomes visible. The 3rd larval instar lasted 4–11 days and the 4th instar about 9 days (Table 1). These instars can completely defoliate ash trees.

SAL caterpillar in the experiment had four larval instars. The 4th instar measured about 27–30 mm. It has distinct white, black and yellow longitudinal lines along the body, the head, chest and abdominal legs are distinctly yellow (Figure 1c).

When caterpillars complete their development they descend by silky threads to the soil. They search for a most suitable place for pupation, mainly near the trunk, underneath the leaf litter or under rocks. The soil depth where it pupates depends on the looseness of the substrate, and usually is about 4–5 cm deep. As soon as the caterpillar buries itself it enters into prepupal stage and then moults into pupae. In prepupal stage the caterpillars body shortens and becomes thicker (Figure 1d). The colour changes and becomes darker yellow. The pupa is first light brown and then becomes reddish brown as the cuticle hardens (Figure 1e) with a length of 1.3 to 1.5 cm.

It was observed in the field that the pupal stage lasts from September/October until June/July next year. This is the diapause stage. Our research has shown that pupal stage in summer lasts for about 16 days (Table 1), which shows that there was no diapause.

All caterpillars in the experiment have developed to the adult stage, the sex ratio was ♂ : ♀ = 1.5: 1. Analysis of duration of individual stages showed a statistically significant difference of the average duration of the 4th larval instar development between males and females (Table 2).

A great variability of analyzed traits in both males and females can be noticed and the results of F-tests show that groups compared at variance level do not differ. Furthermore, t-test shows that there is no statistically significant difference in the mean values of masses of initial 4th instar larvae between males and females, but shows significantly higher mean values of weight of the food consumed, gained weight of caterpillars and female pupae weight (Table 3). The mean gained mass of 4th instar female caterpillars is 0.0690±0.003 (n=12) with an average gain of 0.0061±0.0019 g / day, males 0.05745±0.002 (n=18) with an average gain of 0.0045±0.0020 g / day.

There is no difference between male and female larvae up to the beginning of 4th larval instar (Table 3). The difference starts to develop during the 4th larval instar when females consume significantly more food, gain significantly more weight and form significantly heavier pupae.

TABLE 1

Average duration of life stages of spotted ash looper (Abraxas pantaria) in laboratory conditions.

Life stage	Egg	1st instar	2nd instar	3rd instar	4th instar	Prepupa	Pupa	Adult
Duration [days]	4 – 6	6 – 9	7 – 12	4 – 11	8,9±0,2	1,9±0,1	16,8±0,1	n/a

TABLE 2

Analysis of individual stages by gender and results of statistical tests. For the F-tests the values of one-tailed statistical significance (p) are shown. An asterisk indicates a stage in which the t-test revealed a significant difference in mean values between the sexes.

Life stage	4th instar *		prepupa		pupa	
Sex	♂	♀	♂	♀	♂	♀
duration (days)	9,42±0,23	8,44±0,18	2±0,12	1,89±0,14	17±0,25	16,72±0,18
F-test	1,02; p = 0,470		1,87; p = 0,119		1,29; p = 0,308	
t-test	3,31; p = 0,001		0,60; p=0,288		0,92; p=0,177	

TABLE 3

Analysis of some developmental and nutritional parameters of males and females. An asterisk indicates a parameter which is significantly different in the mean value of compared groups (p<0,01).

Weight	Initial 4th instar caterpillar		Consumed food*		Gained weight*		Pupae*	
Sex	♂	♀	♂	♀	♂	♀	♂	♀
Weight	0,0282±0,001	0,0277±0,001	0,1582±0,007	0,1342±0,003	0,0690±0,003	0,05745±0,002	0,0811±0,003	0,0704±0,002
F-test	1,757; p=0,144		2,568; p=0,039		1,351; p=0,279		1,649; p=0,171	
t-test	0,303; p=0,383		3,279; p=0,002		3,294; p=0,002		2,996; p=0,004	

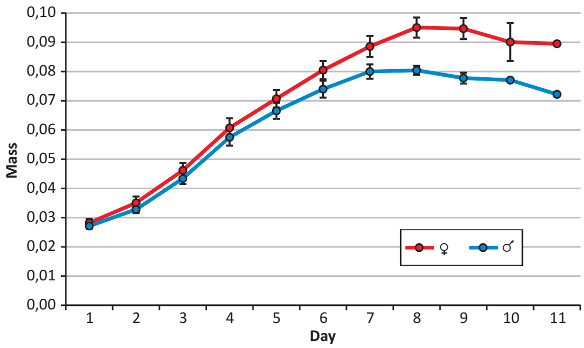

Figure 2. *Development rate of 4th instar caterpillars with ranges of standard error.*

Pearson correlation coefficient (r) shows that the hypothesis about correlation between initial mass of 4th instar larvae and weight of corresponding pupae cannot be rejected ($r = 0.617$, $p = 0.033$). The correlation is shown by linear regression in the graph (Figure 3) and

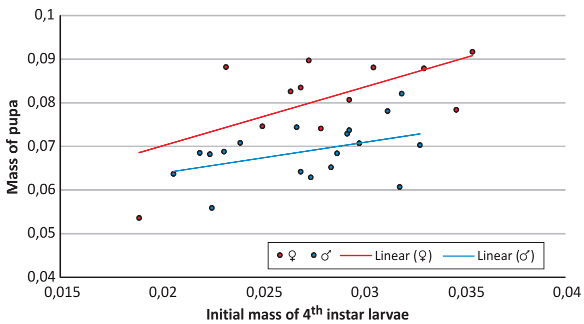

Figure 3. *Positive correlation of the weight of 4th larval instar and pupae shown with linear regression.*

the line slope of linear regression is steeper in females which is consistent with the hypotheses that the difference in weight is generated during the 4th larval instar.

Dry weight of leaves consumed during the 4th larval instar for the whole experiment has an average of 0.1438±0.004g, and the average coefficient of fresh leaf weight from dry matter is 2.445±0.052g which means that the average 4th instar caterpillar can consume between 0.3345 and 0.3691 g of fresh leaf.

Beauveria bassiana and pupal parasitoids of SAL

Very high mortality rate was found for the 200 pupae examined and only 2% of individuals completed their development (Table 5). Most of the pupae (70%) were sterile, i. e. development and metamorphosis stopped. A large number of pupae were infected (19%) with mycelia of fungus *B. bassiana*, parasitized were 9% (parasitoid wasps (5%) and parasitoid flies (4%)) (Table 5). Consequently, after gradation of SAL in 2009 and 2010 the population collapsed in 2011 and no defoliation was observed.

Pupal parasitoids were raised in the laboratory of CFRI from pupae collected in October 2010. One species of parasitic fly *Pales pavida* (Meigen) [Diptera: Tachinidae] (Figure 4) and parasitic wasps *Cratichneumon* cf. *fabricator* F. and *Coelichneumon* sp. (Hymenoptera, Ichneumonidae) were found.

DISCUSSION AND CONCLUSIONS

Our research has shown some new details of SAL biology in NPK on smaller and for Croatia on a bigger scale. SAL feeds on Narrow-leafed ash and it does not feed on Manna ash.

TABLE 4

Development of the Spotted ash looper *(Abraxas pantaria)*.

J	F	M	A	M	J	J	A	S	O	N	D
● ● ●	● ● ●	● ● ●	● ● ●	● ● ●	● ● ●	● ●					
					+	+ +	+				
						○ ○	○ ○				
						~ ~	~ ~	~ ~	~ ~		
							● ● ●	● ● ●	● ● ●	● ● ●	● ● ●

● pupa; + adult; ○ egg; ~ caterpillar

TABLE 5

Causes of pupal mortality of SAL in 2011.

	Pupae	Ecloded butterflies	Mortality	Sterile pupae	*Beauveria bassiana*	*Pales pavida*	*Coelichneumon* sp.	*Cratichneumon fabricator*
n	200	4	196	140	38	8	6	4
%	100	2	98	70	19	4	3	2

Figure 4. *Pupal parasitoid of spotted ash looper: Pales pavida*.

According to references caterpillars moult 4 times, have 5 larval instars and prepupal stage *(2, 3)*. In our research caterpillar moulted 3 times and had 4 larval stages after which a prepupal stage followed. The exact number of larval stages should be tested in field trials in natural conditions. The duration of entire larval stage of SAL is 40–45 days *(2, 3)*. In our research larval stage including the prepupa lasted 29–43 days.

Analysis of duration of individual stages showed a statistically significant difference of the average duration of the 4th larval instar between males and females. Results also indicate that there is no difference in weight between male and female larvae up to early 4th instar, this difference develops during the 4th instar when females consume significantly more food, gain significantly more weight and form significantly heavier pupae. The 4th instar larvae consumed on average between 0.3329 and 0.3673 g of fresh leaf. The maximum growth of average caterpillar of the 4th instar of both sexes was gained on the 4th day after the beginning of that instar and maximum weight on the 8th day, 3 days before the onset of prepupal stage (Figure 2).

The results show that females have gained significantly more weight than males between 4th and 8th day. But large standard error of gained weight and absence of this data in later development period can be interpreted as result of great variability and relatively small sample. This error can be corrected by increasing the sample size in further research.

Adults of a new generation from the experiment were placed in a glass cylinder where females laid eggs after copulation. The fact that the caterpillars, which were brought from the field to the laboratory in mid-July, developed the cycle up to new egg masses, makes hypothesis that this insect can develop more than one generation per year possible. While most references cite one generation per year, on some localities in Spain two or three generations per year have been found *(2)*. However, new generation fully developed under laboratory conditions and thus a field monitoring has been carried out at the beginning of September. Heavy defoliation has been recorded on ash trees by Lake Visovac in NPK. Caterpillars have been found that were still feeding and descending to the soil for pupating. This observation leads to the hypothesis that there is more than one generation per year in NPK. The research has also shown that the pupal stage can last for 16 days without diapauses (Table 1) which additionally indicates the possibility of the existence of two generations per year.

After high mortality of pupae (98%) the prognosis for 2011 was favourable and no defoliation from SAL was recorded that year.

SAL pupae were killed mostly by mycosis caused by the fungus *B. bassiana*, which leads to the conclusion that the *B. bassiana* is the most important natural enemy, which leads to a rapid decline in gradation. It is known that *B. bassiana* can significantly reduce overwintering beetles of ash weevil *(24, 25)*, and its potential for use in biological control has been demonstrated in several cases *(10, 15, 26)*. The biggest problem with formulations based on *B. bassiana* is nonselectivity *(e.g. 15, 27, 28)*.

B. bassiana is the anamorph (asexually reproducing form) of *Cordyceps bassiana*, teleomorph (the sexually reproducing form) which has been described in 2001. This is a fungus that grows naturally in soils throughout the world and acts as a parasite on various arthropod species, especially in orders of Lepidoptera, Coleoptera, Hymenoptera, Homoptera, Hemiptera i Orthoptera *(29)*.

Positive results of laboratory and field treatment of insects from the family Curculionidae *(e.g. 30, 31)* are suggesting the need to study the possibilities of applying entomopathogenic fungi *B. bassiana* for control of SAL. *B. bassiana* as a pathogen of ash weevil, *(25)* which regularly feeds on ash in NPK has already been studied. That study shows that adults of this weevil, which overwinters in leaf litter, can be heavily infested by the pathogen. This makes ash weevil a potential alternative host for horizontal transmission of *B. bassiana* to SAL pupae. To prove this possibility an investigation of isolates of *B. bassiana* on each species is necessary.

Of the pupal parasitoids found in this research, *P. pavida* is important polyphagous parasitoid on many butterfly species *(32)*. It was recorded by Bathon and Tirry 2005 *(17)* in Spain and Ozbek and Calmasur 2010 *(3)* in Turkey. All 10 individuals of parasitic wasps were in the poor condition and it was difficult to identify them so *Coelichneumon* sp. was identified only to the genus level. Both species belong to polyphagous parasitoids, and have been described in Turkey *(3)* on SAL. Given the small proportion of parasitoids in relation to *B. bassiana* it can be concluded that they are less important in reducing populations of SAL at this gradation level. Although the overall pupal parasitism of 9% cannot be ignored their importance is probably higher in the latency and progradation.

This pest has not yet been recorded in other Croatian regions, so it can be concluded that it is adapted to the Mediterranean area and there is no threat for continental ash forests at present. Frequent climatic aberrations, drought and increased average yearly temperatures *(33)* could create favourable conditions for the movement of this pest towards north. Frequent ash defoliation by ash weevil in Croatia *(25)*, new pathogen *Chalara fraxinea* Kowalski *(34)* and the threat of quarantine pest *Agrilus planipennis* Fairmaire *(35, 36)* are threatening ash forests so the research on SAL and its natural enemies is important and really needed.

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