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Parasitism of the Alfalfa Weevil *Hypera postica* (Gyllenhal) by *Bathyplectes* sp. in Spain †

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Abstract: *Hypera postica* is currently the main pest of alfalfa over the world. In Spain, damages are produced during alfalfa first cutting, its control is not resolved and little is known about biological control. Parasitoids of the genus *Bathyplectes* sp. occurs along the weevil larval development. The rates of parasitism by *Bathyplectes anura* and *Bathyplectes curculionis* were recorded during eleven years. They varied between fields and years but *B. anura* was the prevalent species. Yearly total parasitism rates ranged from 1% to 31%, averaging from 13.8% to 6.2% at the beginning and the end of April, respectively.

Keywords: *Bathyplectes anura*; *Bathyplectes curculionis*; biological control; integrated pest management

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1. Introduction

The alfalfa weevil, *Hypera postica* Gyllenhal (Col., Curculionidae) is currently the main pest of alfalfa over the world. In Spain, the weevil produces damages during the first alfalfa cutting and its control is not resolved. Only pyrethroids are allowed to be sprayed and they show generally low efficacy. Beside this, knowledge on the efficacy of alternative methods is scarce. Among alternative methods, the efficacy of early or winter cutting and the role of natural enemies are being studied in our research group. Both methods can play an important role in the control management [1,2].

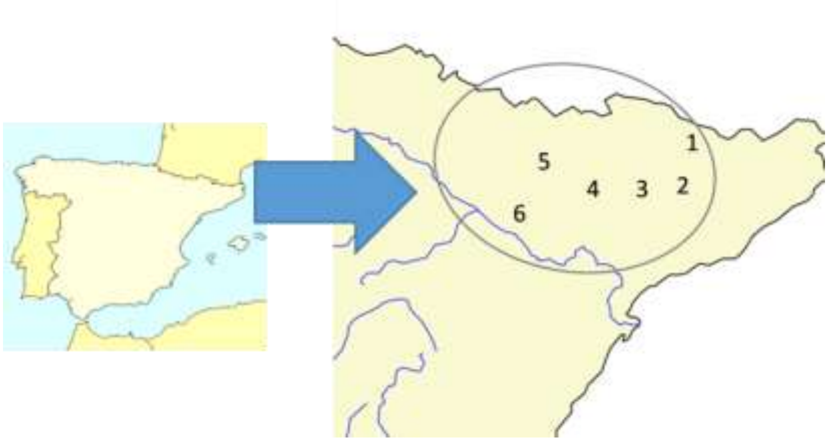
Larvae of the alfalfa weevil are parasitized by wasps of the genus *Bathyplectes* sp. (Hymenoptera, Ichneumonidae). Although eight species of *Bathyplectes* sp. have been recorded in Spain [3], only two have been reported from alfalfa, *Bathyplectes anura* (Thomson) and *Bathyplectes curculionis* (Thomson) [4]. These two species were successfully introduced in North America for controlling the alfalfa weevil [5]. However, in spite of the Eurasian origin of the genus [5–8], very few studies dealing with the biology and the control capacity of these parasitoids have been developed in Europe. Some data coming from isolated studies in Northeaster Spain (Catalonia and Aragon) revealed that the rates of parasitism are variable along the years [4].

According to several North-American studies [9], *B. anura* and *B.curculionis* are solitary endoparasitoids of the alfalfa weevil larvae. Puparia allows a rapid and secure way to distinguish both species since, in the case of *B.anura*, larvae create a hard dark-brown puparium with a narrow, raised white horizontal band and have to capability of flipping. In the case of *B.curculionis*, the puparium is also brown but with a white diffuse flat horizontal band.

The objective of the present study was to determine the parasitism rate of *B. anura* and *B. curculionis* and to establish these parasitoids phenology during larvae weevil development period, under Spanish alfalfa crop conditions. To know the capacity of both parasitoid species for controlling the alfalfa weevil, will allow design strategies for conserving these natural enemies and achieve a more sustainable control of this pest.

2. Material and Methods

The study was conducted in the Ebro Valley region (North East of Spain), where 60% of Spanish alfalfa is cultivated [10]. Untreated commercial fields, distributed in 6 zones of the region (Figure 1) were sampled eleven years, within the period of 2007 to 2020, at the beginning and at the end of April (April 1 and April 2, respectively), the period of major occurrence of *H.postica*. Available number of fields were sampled each year and the total number within this period was 129.



(a) (b)

Figure 1. Field sampled zone location. (a) Iberian Peninsula; (b) sampling areas in the NE of Spain.

Each field was divided in four sectors and three samples per sector were collected following the central part of one of the main diagonals and at least 25 m apart [11]. Samples were taken with a 38 cm diameter sweep net sweeping the net from side to side five times in a 180° arc. Collected field samples were transported to the laboratory and kept in the refrigerator at 6°C until processing. Within the next 24 hours, 25 to 150 third and mainly fourth instar larvae, depending on the larval abundance of *H. postica*, were selected (usually 100–150). Larvae were kept in rearing polyethylene cages of 500 ml capacity (maximum 50 larvae/cage), covered by a mesh to facilitate aeration. Fresh clean alfalfa was provided every two days. Larvae were maintained in a climatic chamber at 22 °C, 8:16 (light: dark) photoperiod and 60 % relative humidity until pupation. Then, the number of pupae of *H. postica* and puparia of *B. anura* and *B. curculionis*, as well as the number of dead larvae were recorded daily and withdrawn from the cage. Both species of *Bathyplectes* sp. were distinguished by the characteristics of the puparium, described before. The experiment finished when all initial larvae pupate to *H. postica* or to *Bathyplectes* sp. The rates of parasitism for each parasitoid species were calculated as: [(Number of puparia of each *Bathyplectes* species / number of *H. postica* larvae reared)] * 100

The last four years of the study (2017–2020), after selecting larvae for estimating the rate of parasitism, the samples were frozen at −20 °C and the number of adults of *Bathyplectes* sp. present in each sample recorded. Species were not identified because slight morphological differences make their distinguishing very difficult. Puparia of *B. anura* and *B. curculionis* in the remaining sample were also recorded and identified.

3. Results

3.1. Bathyplectes sp. parasitism Rate

Total parasitism rates were variable among years and ranged from less than 3 % to more than 30 %, averaging from 13.8 % to 6.2 % at the beginning (April 1) and the end of April (April 2), respectively. The 80 % of the puparia recorded belonged to *B. anura*, resulting in this species to be the prevalent over *B.curculionis* (Table 1). The rate of parasitism by *B. anura* during the whole studied period was higher in the beginning than in the end of April, averaging 13 % and 4 %, respectively, but some years and in some zones the rate of parasitism arose more than 20 % (Table 1). Conversely, the rates of parasitism due to *B.curculionis* were slightly lower at the beginning than at the end of April, averaging 1 % and 2.3 %, respectively (table 1). Only in one zone of one year the rate was above 10 %.

Table 1. Yearly average rate of parasitism (%) by *B. anura* (Ba), *B. curculionis* (Bc) and total (TOTAL) in larval laboratory rearing, at the beginning (April 1) and the end (April 2) of April, in the different zones of the study region. Dashes indicate the absence of data for the sampling date due to low field weevil larvae abundance or high laboratory mortality by unknown factors.

YEAR	ZONE	Field number	April 1			Field number	April 2		
			Ba	Bc	TOTAL		Ba	Bc	TOTAL
2007	1	8	1.03	3.3	4.33	8	0.39	5.11	5.5
2008	1	8	2.44	3.84	6.28	8	1.58	3.58	5.16
2009	1	4	6.86	0.88	7.74	8	5.85	2.55	8.4
2010	1	3	3.32	0.68	4	5	4.79	0.67	5.46
2014	2	5	20	0	20	5	6.5	0	6.5
2015	2	5	13	0	13	5	4	0	4
2016	2	12	30.8	0.91	31.71	0	-	-	-
2017	3	4	28.42	0.75	29.17	0	-	-	-
2017	3	16	15.54	1.87	17.41	0	-	-	-
2018	3	12	23.75	0	23.75	0	-	-	-
2018	3	6	8.02	0.72	8.74	0	-	-	-
2019	4	4	20.52	0	20.52	0	-	-	-
2019	5	16	14.52	0.11	14.63	2	13.5	0	13.5
2019	6	4	14.97	1.08	16.05	4	3.67	12.25	15.92
2020	3	8	3.36	0.81	4.17	2	0	0	0
2020	4	0	-	-	-	6	2.5	0.83	3.33
2020	5	8	8.46	1.2	9.66	8	0	0	0
2020	6	6	2.29	0.51	2.8	6	-	-	-
TOTAL 11 YEARS		**129**	**12.78**	**0.98**	**13.76**	**67**	**3.89**	**2.27**	**6.16**

3.2. Bathyplectes sp. Field Abundance

Adults of *Bathyplectes* sp. occur along the major period of weevil larval development. Wasps were found since the last week of January until the first week of July. The highest capture number was recorded during the last week of April (Figure 2), some days before the first alfalfa commercial cutting. After this, the number of *Bathyplectes* sp. decreased considerably. Puparia from both species were mainly found in March and April (Figure 2), but *B.anura* was the first being recorded during early spring and this represented the 70 % of the collected puparia.

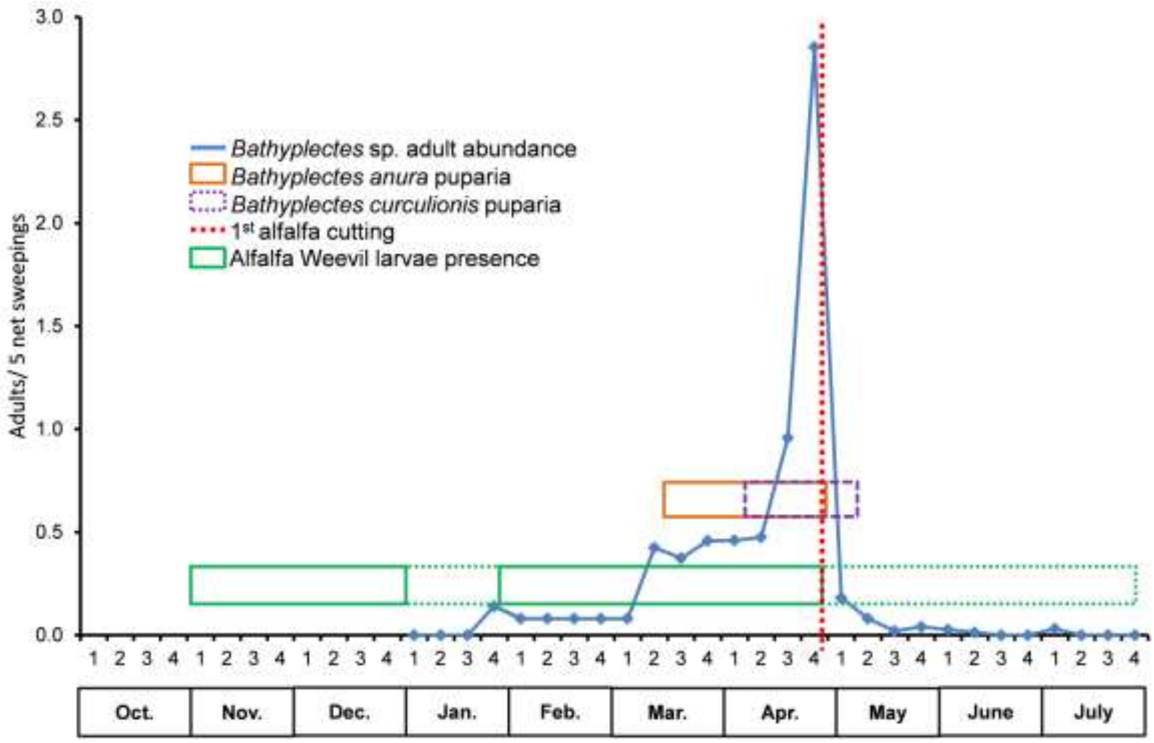


Figure 2. *Bathyplectes* sp. phenology in NE Spain. The blue line shows the field abundance of *Bathyplectes* sp. adults. The orange bar shows the occurrence *B. anura* puparia and the blue-dashed bar the occurrence of *B. curculionis* puparia. Solid green bar indicates the period of the weevil larval development and the dashed green bar indicates the presence of *H. postica* larvae but few or even no development, due to abiotic (January) or cultural factors (May – July). Red pointed line indicated the usual date of the first alfalfa cutting in the region. Numbers above months indicates the four weeks of each month.

4. Discussion

According several worldwide literature, the alfalfa weevil is a very destructive pest that cause severe losses to alfalfa [12–15]. In Spain, is considered nowadays, the most important pest of this crop [4] and its control has not been solved. Despite insecticide sprayings, this pest continues causing damages every year. Furthermore, only pyrethroids are allowed to be applied with important risks of resistance development [16,17].

Biological control against the alfalfa weevil with *Bathyplectes* sp. has been reported in several places of the New World [18–21]. However, no data from European literature are available. Our results are the first taken in a regularly way and during a long period of time, allowing to make an approach of the phenology and the potential role of *Bathyplectes* sp. as a control agent in Spanish and European alfalfa crop conditions.

Even though yearly parasitism from *Bathyplectes* sp. varied, these larval parasitoids were present during the complete alfalfa weevil larval development period. The result of our study show that *B. anura* and *B. curculionis* are able of coexisting in the Spanish alfalfa crop conditions. However, there was a clear predominance of *B. anura* on *B. curculionis*, with the former representing more than 80 % of the total parasitoid individuals obtained from the laboratory experiment and a 70 % of the fields collected puparia. Our results confirm what was already suggested and initially demonstrated in some fields of the region [4].

It has been reported that in many regions of North America, where the two species live together, *B. anura* is able to be the predominant or even to displace *B. curculionis* because the former has a greater reproductive capacity, a more rapid search and handling and a more aggressive behaviour [20]. These studies report that after completing the puparium, *B.anura* larvae enter in summer diapause. In autumn, diapause is broken and the insects pupate and become adults, but remain in their puparia to pass the coldest months of winter [5]. However, *B. curculionis* has been reported as the main and the most effective parasitoid of the *H. postica* in some regions of USA and Canada [5,15,22]. Therefore, environmental or agronomic conditions could affect the dominance of one of the both species when both coexist in the same region.

Our results suggest that *B. anura* is somehow more efficient than *B. curculionis* in parasitizing *H. postica* along our studied region. The reasons for that may be: 1) *B.anura* tends to fly earlier than do *B. curculionis*; the emergence of adults of *B. anura* may happen in early winter (first catches of *Bathyplectes* sp. adults were recorded already by the end of January and increased very much at the beginning of March, when alfalfa weevil starts its larval development and there is enough host availability). 2) that females of *B. anura* oviposit mainly to 2^{nd} and 3^{rd} *H. postica* instars [23,24] which suggests that larval parasitoid development time is shorter than if parasitization occurs in weevil younger larval instars, giving capability of most of the population of *B. anura* to complete its larval development and forms the puparia before alfalfa harvesting, usually by the end of April in the study region [10].

Conversely to that of *B. anura*, parasitism rates also showed that *B.curculionis* occurs in slightly higher numbers at the end of April. This may suggest that a certain potential succession of both species may occur. However, crop conditions can seriously affect *B. curculionis* and its effectiveness be seriously compromised by the time when the first alfalfa cutting is done. Females of *B. curculionis* oviposit preferentially firsts instar larvae of *H. postica* and this is strictly related to a highest parasitoid larval survival [25,26]. Our results showed that puparia of *B. curculionis* are mainly found in late April, which coincides with the maximum occurrence of adults. The species composition of this peak is unknown but can be suggested that a greater proportion belongs to *B. curculionis*, which probably fly's later, accordingly to [20]. Taking into account that the availability of first instar weevil larvae is low at the end of April (when most of the *H. postica* larvae are near to complete their development), this can represent a negative dependence for *B. curculionis*. Additionally, the cutting of the alfalfa performed at the end of April, eliminates nearly all the population of *H. postica*. If this cutting is advanced in order to reduce yield losses, *B. curculionis* can be more seriously affected by the reduction of the host availability.

Our results show the potential of *Bathyplectes* sp., especially *B. anura*, as a control agent for *H. postica* in Spanish and European conditions. However, the cutting management of the alfalfa can be detrimental for *B. curculionis*. These results combined with cultural methods, such as winter cutting management [1] are promising tools for alfalfa weevil control than can be included in IPM strategies in Spain and potentially in other European regions.

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