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Alfalfa winter cutting: Effectiveness against the alfalfa weevil, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae) and effect on its rate of parasitism due to *Bathyplectes* spp. (Hymenoptera: Ichneumonidae)

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ABSTRACT

The alfalfa weevil *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae) is a major pest of alfalfa worldwide. In Spain, insecticide sprayings are mainly used for control with low efficacy. As a potential more sustainable control method, we determined the effectiveness of an alfalfa winter cutting for reducing pest populations in the spring and its interaction with parasitism rate due to *Bathyplectes* spp. (Hymenoptera: Ichneumonidae). Forty-two commercial fields were split in two parts and one was mowed during winter. Larval abundance in each part of the field was sampled by sweep netting in 2019 and 2020 before the first alfalfa spring cutting, when damage is caused. The rates of parasitism due to *Bathyplectes anura* (Thomson) and *Bathyplectes curculionis* (Thomson) were estimated by rearing larvae in the laboratory. Winter cutting significantly reduced the spring larval weevil populations and favored the rate of larval parasitism. Our results suggest that winter cutting can be a useful cultural method for alfalfa weevil control that has potential to be a component of an integrated pest management program.

1. Introduction

Alfalfa, *Medicago sativa* L., is the world's most valuable cultivated forage crop (Orloff, 1997). In Spain, alfalfa is a traditional component of crop rotations. Plant stands remain in the field from 3 to 6 years. Alfalfa covers more than 250000 ha, accounting for approximately 20% of the alfalfa cultivation area in Europe (Delgado and Lloveras, 2020). Spain is the main European country exporting alfalfa (dehydrated or pellets), particularly to the Middle East and China (Capistrós, 2020). Alfalfa management in Spain consists of periodic cutting during the growing season (usually five cuttings from the end of April to the end of September in 30–40 days intervals).

The alfalfa weevil, *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae), is a highly destructive pest in most world regions where alfalfa is cultivated (Goosey, 2012; Saeidi and Moharramipour, 2017), including Spain (Pons and Núñez, 2020). After a summer aestivation, females lay eggs in clusters inside alfalfa stems (Hoffmann, 1963). Studies in Spain have shown that the main egg laying period occurs during the autumn and winter (Pons and Núñez, 2020; Levi-Mourao et al., 2021). The resulting larvae, hatched from the end of winter to the beginning of spring, feed on leaves and new plant buds, thus reducing forage yield and the quality of the first alfalfa intercut, and causing economic losses (Pons et al., 2011; Pons and Núñez, 2020). Damages caused by the larvae can account for 25–40% of the yield (Alfaro, 2005). At the end of the fourth instar larval development, the insects pupate between leaflets in white cocoons. Emerging adults only cause negligible damage. An additional incomplete generation can sometimes occur (Pons and Núñez, 2020).

Alfalfa is a reservoir of natural enemies that contribute to minimizing primary and secondary pest outbreaks not only in alfalfa but also in surrounding crops (Summers, 1998; Madeira et al., 2019). These natural enemies can play an important role in reducing populations of the alfalfa weevil (Summers, 1998; Soroka et al., 2020). *Hypera postica* larvae can be parasitized by *Bathyplectes anura* (Thomson) and *Bathyplectes curculionis* (Thomson) (Hymenoptera, Ichneumonidae). These species are native to Europe and other regions of the Old World (Kingsley et al., 1993; Kuhar et al., 1999; Radcliffe and Flanders, 1998). They were successfully introduced in the USA to control alfalfa weevil (Radcliffe and Flanders, 1998) but have been more effective in eastern than in western USA (Rand, 2013). In Spain, the incidence of these parasitoids

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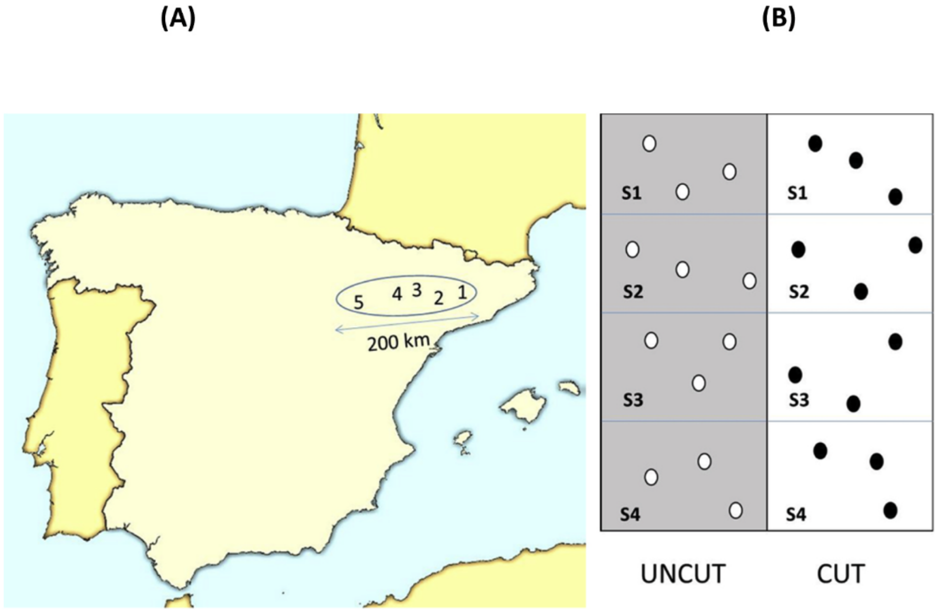

Fig. 1. (A) Map of areas where sampled fields were located in 2019 and 2020. Counties within the Ebro Valley: 1. Urgell; 2. Segrià; 3; Baja Cinca; 4. Monegros; 5. Zaragoza Central. (B) Schematic of one sampled field, each divided into two sections (UNCUT and CUT) of similar area, with each divided into four sectors (S1, S2, S3 and S4). In each sector, three points consisting of five net sweeps were sampled (total sampling points per section = 12).

has not been studied in depth and the few data available show highly variable parasitism rates (Pons and Núñez, 2020).

Chemical control against the alfalfa weevil should be avoided to preserve the ecological services of predators and parasitoids. However, farmers in Spain use one to three applications of pyrethroids (the only authorized class of insecticide) against the alfalfa weevil during the period when most damage occurs (Pons and Levi-Mourao, 2020; Pons and Núñez, 2020). Beyond their detrimental effects on natural enemies, the efficacy of these treatments is questionable and may lead to the development of resistance (Rethwisch et al., 2019). Therefore, alternative control methods should be used to implement integrated pest management (IPM), which has been mandatory in Spain since 2014.

Cultural control such as early harvesting of first crop, autumn cutting, intercropping or grazing have been proposed to combat the alfalfa weevil many years ago, particularly in North American (Pellissier et al., 2017). Onstad and Shoemaker (1984) claimed that a robust strategy is to always harvest early. Studies conducted in northern Catalonia in Spain concluded that this strategy is effective in reducing the impact of *H. postica* (Pons and Núñez, 2020). However, under the common production system in most of the Ebro Valley in Spain, this strategy is not easily applied, because most farmers/producers sell the alfalfa to dehydrating forage companies who decide and manage when to cut according to their needs. Dowdy et al. (1992) reported that late autumn cutting in the USA reduced the number of alfalfa weevil eggs by half. However, no data of the effectiveness of cutting during the overwintering period of *H. postica* in Spain or Europe exist, apart from those reported in Núñez et al. (2015). The Núñez et al. (2015) study was conducted in a single county in small fields (<2 ha) using a laser mower, which only rarely used today. Our study was conducted over a broader geographical range in larger fields using a disk mower in most of the fields. Intercropping is not used in commercial alfalfa production in Spain. Grazing was another effective practice reported in some countries as useful to reduce weevils in alfalfa (Gossey, 2012; Sanaei and Seiedy, 2016; Wynn-Williams et al., 1991). This practice is currently in disuse in Spain, mainly because of the decrease in sheep flocks and intestinal bloating risk by foraging (Delgado, 2020). Therefore, cutting the alfalfa during winter to eliminate overwintering population of the pest needs to be further investigated as a control method under Spanish crop conditions.

The aim of this work was to determine the efficacy of one winter alfalfa cutting as a cultural strategy to reduce the population of the alfalfa weevil under Spanish crop conditions and to evaluate the interaction of this management method with parasitism rates due to the parasitoid *Bathyplectes* spp. We expected that winter cutting would remove a substantial number of eggs and larvae of *H. postica*, thus resulting in significantly lower spring populations (Hypothesis 1). Because host density can have cascading effects on parasitoids, with positive (Eveleigh et al., 2007) and negative (Costamagna et al., 2004) effects reported, and because higher host densities may enhance the parasitism of *Bathyplectes* spp. (Rand, 2013), we predicted a higher level of parasitism in fields without winter cutting management (Hypothesis 2).

2. Materials and methods

2.1. Field site description

The study was performed in the Ebro Valley region, where 60% of Spanish alfalfa is cultivated (Delgado and Lloveras, 2020), mostly under irrigation. The Ebro Valley is a geographic region of Northeast of the Iberian Peninsula. Mean temperatures range from 1 °C in winter to 30 °C in summer. Annual rainfall is variable and ranges from 200 to 800 mm, and is mainly concentrated in spring and autumn. Mean altitude is 200 m (asl).

A total of 42 commercial fields in five counties in the region (Urgell, Segrià, Baja Cinca, Monegros and Zaragoza Central) were selected during 2019 and 2020 crop seasons (Fig. 1A). When possible, sampling was repeated in the same fields in the two study years. The fields in each county were separated by at least 2 km. Most of the selected fields were 2 or 3 years old, and were sown with the Aragon variety. This commercial variety, obtained from the ecotype Aragon, has been cultivated in the Ebro Valley for decades. In addition to tolerating temperatures down to −15 °C, it has a short dormancy period, fast development in spring and after cutting regrowth, and it may be cut 5–6 times under irrigation (Lloveras et al., 2020; Delgado 2020). No insect resistance traits are known for this variety. The field size ranged from 1 to 7 ha, and fields were sprinkler or blanket irrigated. During the study period no pesticides were used. Field characteristics are shown in Table S1.

2.2. Sampling plan

Each field was divided into two sections with approximately equivalent area, and each section was randomly assigned to one of the cutting management treatments (Fig. 1B). One section was not subject to any management practice during the winter (UNCUT, hereafter), whereas in the other section, alfalfa was cut in winter once, and the forage was removed after cutting (CUT hereafter). Alfalfa was cut as short as the mowing machinery allowed (always below 4 cm in height). In most fields in Zaragoza Central, alfalfa cutting was performed with a laser mower (a mower guided by a laser land level equipment), whereas fields in the other counties were cut with a disc mower (Table S1). Cutting dates depended on the weather conditions and farmer availability, and varied among fields from the beginning of January to the middle of February (Table S1). Each section of the field (UNCUT and CUT) was divided into four sectors of approximately equivalent size (Fig. 1B).

All fields were sampled before winter cutting management (to determine whether differences existed between the experimental sections) and during the first alfalfa intercut (period between the beginning of the vegetative growing season and the first spring alfalfa cutting; see Pons et al., 2011 for details), when *H. postica* larvae damage the crop. Sampling before winter cutting management was performed in 2019 (during January) with a sweep-net (procedure described below), but very few records of the occurrence of larvae and eggs were obtained (stem pieces occasionally collected with net sweeping were dissected for that purpose). Therefore, in 2020, the sampling method (from middle December to the second fortnight of January) consisted of collecting 25 stems in each of the four sectors (100 stems in each UNCUT and CUT management section) and gently excising them from the plant crown with scissors. Stems were brought to the laboratory of entomology of the University of Lleida, kept in a refrigerator at 5 °C and dissected during the next 2 weeks.

In the following spring, samplings were performed by conducting 180° sweeping with a 38 cm diameter net. In each UNCUT and CUT section, three samples, consisting of five sweeps for each of the four sectors, were collected. Therefore, for each field, 12 samples were obtained for each section (Fig. 1B). Because of the small size of the fields in Zaragoza Central County, only six samples per field section were collected. Spring sampling was performed twice in 2019, with the first at the beginning of alfalfa vegetative growing (mid-March) and the second when alfalfa was well developed (mid- or second fortnight of April). Because of the COVID-19 pandemic, only one sampling was performed in 2020. Collected field samples were transported to the laboratory and frozen at −20 °C until processing.

In addition to the sampling pattern described, on the same sampling date, we collected an additional sample consisting of 20 sweeps in each management section. Within the next 24 h, 25–150 L3 or L4 instar larvae, depending on the larval abundance of *H. postica* per sample, were selected (usually 100–150). Larvae were kept in 500 ml rearing polyethylene cages (maximum 50 larvae/cage), covered by a mesh to facilitate aeration. Fresh alfalfa was provided every 2 days. Larvae were maintained until pupation in a climatic chamber at 22 °C, with an 8:16 (light: dark) photoperiod and 50% relative humidity.

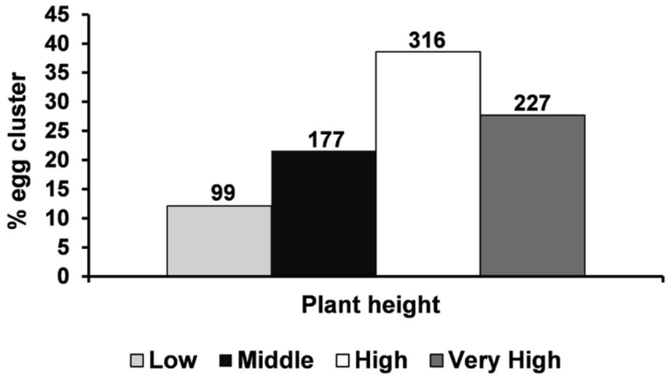

Fig. 2. Percentage of *H. postica* egg clusters found at different height intervals in alfalfa stems: low (below ¼ stem height), middle (between ¼ and ½ stem height), high (between ½ and ¾ stem height) and very high (above ¾ stem height). Data from all fields were combined. Total numbers of clusters are shown above the bars.

Table 1
ANOVA statistics for the fixed effects in the mixed model for the abundance of *H. postica* larvae.

Source	df	Approximate df denominator	F	*p*
County	4	26	4.07	0.0107
Year	1	23	6.66	0.0167
County*Year	4	22	2.90	0.0501
Sampling date [Year]	1	15	17.87	0.0007
Sampling date * County [Year]	4	15	6.53	0.0032
Winter Cutting	1	23	34.80	<0.0001
County * Winter Cutting	4	23	2.79	0.0502
Year * Winter Cutting	1	20	2.42	0.1349
County * Year * Cutting	4	19	3.53	0.0254
Sampling date * Winter Cutting [Year]	1	13	0.01	0.9327
Sampling date * County * Winter Cutting [Year]	4	13	4.19	0.0209

2.3. Data collection

Before winter cutting, the numbers of egg clusters and larvae per sampling point (in 2019) and per stem (in 2020) in each field were recorded. We also recorded the height of the stem and the height from the stem base at which the egg clusters were found. Based on the relative height location of the egg cluster on the stem height, we classified the location of the egg cluster as low (below ¼ stem height), middle (between ¼ and ½ stem height), high (between ½ and ¾ stem height) and very high (above ¾ stem height). These samplings were made between middle December until middle January.

From the sweep net samples taken after winter cutting we recorded 1) the number of *H. postica* larvae; 2) the number of larvae that were dead or with disease symptoms; 3) the number of adults of the parasitoid *Bathyplectes* spp. (species were not identified, because slight morphological differences make identification difficult).

From the rearing cages, we recorded the number of *H. postica* pupae and those of *B. anura* and *B. curculionis* puparia; dead larvae were removed from the cage, and their number was recorded daily. Both species of *Bathyplectes* spp. can be easily distinguished on the basis of the characteristics of the puparium. This additional sampling could not be performed in the Zaragoza Central fields.

2.4. Data analysis

For ANOVA analysis data from the samples taken in each section of the field (UNCUT and CUT) were averaged and the field was considered as a replication. Box-Cox's lambda was used to verify normality and homoscedasticity of variance and data of abundances. Data of abundance were square root transformed before analysis. Percentage data were transformed to arcsine $(\times /100)^{1/2}$. Comparisons within statistically significant factors ($p < 0.05$) were performed by Tukey HSD test. All analyses were performed with JMP PRO 15 software (JMP,).

1) Hypera postica
   1.1) Before winter cutting management, abundance of *H. postica* eggs and larvae was analyzed by a multifactorial mixed model ANOVA where county and winter cutting were considered fixed factors. Fields were nested to counties and all field interactions

Table 2

H. postica larval abundance (mean ± se) after winter cutting in UNCUT and CUT sections of the 2019 and 2020 fields (n = 12). Efficacy of the cutting management was calculated as ((1-C/NC)*100), where C and NC are the abundance of larvae in CUT and UNCUT sections.

Year													
2019								2020					
County	Field	Sampling 1 (March)			Sampling 2 (April)			County	Field	Sampling 1 (March/April)			
		UNCUT	CUT	Efficacy	UNCUT	CUT	Efficacy			UNCUT	CUT	Efficacy	
Urgell	1	60.9 ± 3.8	45.6 ± 4.5	25	654.6 ± 50.3	337.6 ± 26.7	48	**Urgell**	1	777.8 ± 49.1	220.3 ± 11.9	72	
	2	13.5 ± 2.9	8.9 ± 1.4	34	246.1 ± 18.9	143.4 ± 11.1	42		2	296.3 ± 32.8	148.6 ± 8.1	50	
	3	307.2 ± 43.1	106.7 ± 9.6	65	372.9 ± 31.2	149.9 ± 12.3	60		3	391.9 ± 32.8	210.5 ± 17.7	46	
	4	90.5 ± 11.5	29.5 ± 6.4	67	523.0 ± 34.6	118.2 ± 8.9	77		4	121.0 ± 19.1	159.9 ± 35.1	−32	
	5	9.8 ± 1.2	4.9 ± 1.2	50	196.2 ± 17.8	83.3 ± 15.8	58		5	187.5 ± 44.8	178.4 ± 21.6	5	
	6	80.2 ± 5.6	45.2 ± 3.3	44	263.7 ± 15.2	162.7 ± 6.1	38		6	176.2 ± 24.0	181.7 ± 19.3	−3	
	7	4.1 ± 1.0	2.4 ± 0.8	41	149.9 ± 13.3	84.28 ± 6.7	44						
	8	33.4 ± 3.5	24.3 ± 3.9	27	79.7 ± 7.9	48.9 ± 6.1	39	**Segrià**	7	819.4 ± 56.1	462.9 ± 65.2	44	
	9	71.4 ± 8.1	23.9 ± 3.5	67	222.8 ± 19.4	152.7 ± 11.9	31		8	133.2 ± 20.8	238.7 ± 33.8	−79	
Segrià	10	12.3 ± 3.3	12.2 ± 3.9	1	322.2 ± 29.5	202.7 ± 22.7	37		9	152.7 ± 34.1	142.7 ± 23.4	7	
	11	11.7 ± 1.7	6.6 ± 1.2	44	102.5 ± 11.3	55.8 ± 6.9	46	**Baja Cinca**	10	348.7 ± 37.1	222.3 ± 24.9	36	
	12	23.9 ± 3.3	15.5 ± 2.4	35	259.9 ± 26.4	176.2 ± 13.7	32		11	175.6 ± 7.8	261.2 ± 15.3	−48	
	13	5.3 ± 1.3	1.7 ± 0.5	67	93.9 ± 10.6	48.4 ± 6.5	48		12	318.1 ± 43.6	135.3 ± 8.3	57	
	14	20.0 ± 4.6	7.2 ± 1.6	64	229.7 ± 17.9	100.7 ± 8.4	56	**Monegros**	13	71.8 ± 7.4	43.5 ± 5.0	39	
	15	66.8 ± 3.3	49.5 ± 5.2	26	221.0 ± 20.0	184.8 ± 17.3	16		14	78.0 ± 8.2	7.2 ± 1.3	91	
Baja Cinca	16	151.1 ± 16.6	132.6 ± 11.9	12	318.9 ± 24.3	289.0 ± 32.2	9		15	110.8 ± 12.3	39.0 ± 3.7	65	
	17	557.0 ± 33.2	173.5 ± 14.6	69					16	136.7 ± 21.2	71.8 ± 9.9	47	
Monegros	18	109.8 ± 7.4	53.1 ± 5.2	52	291.6 ± 14.5	202.8 ± 16.8	30		17	124.8 ± 16.1	56.3 ± 7.4	55	
	19	67.8 ± 8.6	31.2 ± 3.2	54	91.9 ± 9.3	82.9 ± 7.0	10						
Zaragoza Central (n = 6)	20	25.0 ± 3.5	4.2 ± 0.9	83	2.5 ± 0.4	1.2 ± 0.3	52	**Zaragoza Central** (n = 6)	18	31.3 ± 4.1	9.7 ± 2.4	69	
	21	98.8 ± 9.8	20.3 ± 4.2	79	1.5 ± 0.6	0.2 ± 0.1	87		19	342.7 ± 19.7	4.33 ± 0.4	99	
	22	90.0 ± 15.2	29.2 ± 10.7	68	57.8 ± 12.6	25.8 ± 4.1	55		20	321.3 ± 23.1	21.8 ± 5.5	93	

were considered as random factors. Analysis was performed for 2019 and 2020 separately, since sampling methods were different between both years.

1.2) After winter cutting management, *H. postica* abundance in spring was analyzed by a multifactorial mixed model ANOVA where county, year, and winter cutting were considered as fixed factors. As samplings were performed at different times in 2019 and 2020, the sampling date was nested to year and considered as a fixed factor. Fields were nested to counties and all interactions involving the random term field [counties] were also considered random.

1.3) Winter cutting efficacy, for each field, was calculated according to Abbot (1925) as [(1-C/UC)*100], where C and UC were the abundance of alfalfa weevil larvae in the CUT and UNCUT sectors, respectively. The efficacy was analyzed through a multifactorial mixed model ANOVA where county and year were considered as fixed factors. Sampling date was nested to year and field was nested to county. All field interactions were considered as random factors. The 4 fields where the efficacy was negative were not included in the analysis. The relationship between efficacy and area of a field was analyzed by Pearson correlation. The relationship between efficacy and mowing method and irrigation type, since they were binary variables, were analyzed by a Point-biserial correlation (see Table S1).

2) *Bathyplectes* spp.

The abundance of collected adults in field sampling and the rate of parasitism were analyzed with the same ANOVA model described in 1.2.

3) Fungal disease

Because of an unexpected epizootic of *Zoophthora phytonomi* (Arthur) (Zygomycetes: Entomophthorales) in 2020, the influence of this fungal disease on the efficacy of the cutting management and on the rate of parasitism of *Bathyplectes* spp. was evaluated for this year.

3.1) The proportion of larvae showing symptoms of infection by *Z. phytonomi* in the UNCUT and CUT sections of each field sampled in 2020 was calculated as (Li/Lt*100), where Li is the number of larvae with symptoms of infection, and Lt is the total number of larvae in the sample. This rate was analyzed by

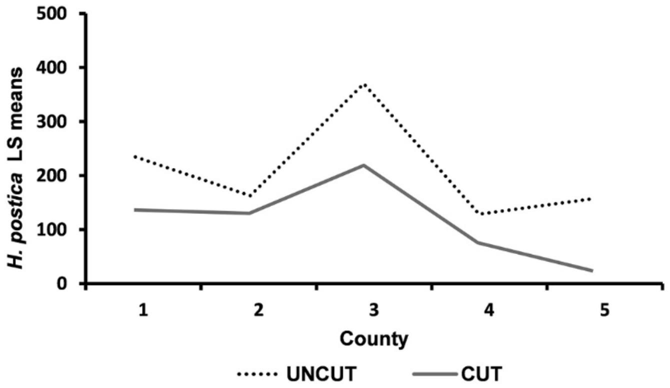

Fig. 3. Abundance of *H. postica* larvae expressed as LS means in UNCUT and CUT sections in each of the five sampled counties: 1. Urgell; 2. Segrià; 3; Baja Cinca; 4. Monegros; 5. Zaragoza Central.

ANOVA with county and cutting as fixed factors. Field factor was nested to county and considered as random.

3.2) The relationship between the rate of parasitism by *Bathyplectes* spp. and the proportion of larvae showing symptoms of infection by *Z. phytonomi* was determined by Spearman correlation.

3. Results

3.1. Effect of winter cutting management on H. postica

H. postica data before winter cutting are presented on Table S2. In both the 2019 (sweep net) and the 2020 (stem) samplings, there were no significant differences between CUT and UNCUT sections in the abundance of *H. postica* eggs (2019: $F_{1,\ 15} = 0.032$, $p = 0.86$; 2020: $F_{1,\ 13} = 0.39$, $p = 0.54$) and larvae (2019: $F_{1,\ 17} = 0.0035$, $p = 0.95$; 2020: $F_{1,\ 15} = 0.033$, $p = 0.63$). Most of the egg clusters (around 65%) were found on the upper part of the stem, mainly between the middle and the three quarter parts of their height (Fig. 2). Only around 10% of egg clusters were located in the first quarter of the plant.

After winter cutting, the most significant factor determining abundance across years, county and field was the winter cutting management ($p < 0.0001$, Table 1). The abundance of *H. postica* larvae in UNCUT sections was very high in April 2019 and 2020 (Table 2), exceeding the economic threshold of 20 larvae/sweep (Martin et al., 2020). In CUT sections the abundance was lower and in some cases below the economic threshold (Table 2). Although the effects of sampling date, year and county were also significant, the cutting management factor interacted with these other factors in a quantitative manner, as can be seen for the interaction County * Cutting (Fig. 3).

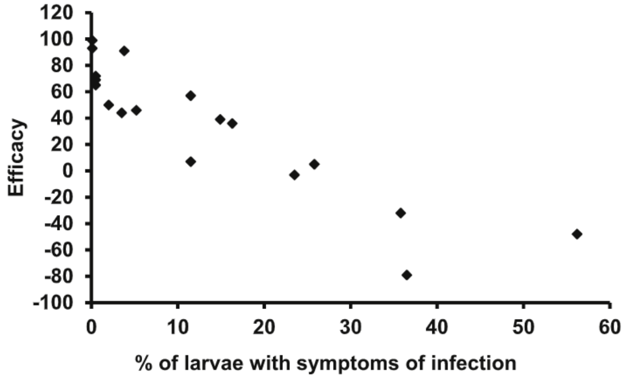

Fig. 4. Relationship between the percentage of *H. postica* larvae with symptoms of infection by *Z. phytonomi* in the UNCUT sections and the efficacy of the winter cutting management, calculated as [1-(C/UC)]*100, where C and UC are the abundance in CUT and UNCUT field sections (rho = −0.93).

Table 3
ANOVA statistics for the fixed effects in the mixed model for *Bathyplectes* spp. adult abundance.

Source	Df	Approximate Df denominator	F	*p*
County	4	10	62.02	<0.0001
Year	1	30	0.006	0.9375
County*Year	4	30	7.71	0.0004
Sampling date [Year]	1	27	22.02	<0.0001
Sampling date * County [Year]	4	26	12.02	<0.0001
Winter Cutting	1	31	12.44	0.0013
County * Winter Cutting	4	31	3.00	0.0333
Year * Winter Cutting	1	21	0.76	0.3919
County * Year * Cutting	4	20	2.63	0.0649
Sampling date * Winter Cutting [Year]	1	24	3.91	0.0598
Sampling date * County * Winter Cutting [Year]	4	22	0.81	0.5290

When the efficacy of the method was analyzed, the only significant factor was county ($F_{4,\ 21} = 5.67$, $p = 0.0029$; ANOVA table not shown) with higher efficacy in Zaragoza Central than in the other counties. The efficacy was associated with mowing method ($r = 0.54$; $p = 0.0004$) and to the field surface ($r = -0.32$; $p = 0.0351$) but not to the irrigation system ($r = -0.16$; $p = 0.2956$).

In some fields, the proportion of larvae with symptoms of *Z. phytonomi* infection exceeded 35% in 2020. The efficacy of the winter cutting was negatively correlated with the proportion of larvae showing symptoms of fungal infection (rho = −0.93; t = −10.09; $p < 0.0001$) (Fig. 4).

3.2. Effect of winter cutting management on Bathyplectes spp.

The total number of *Bathyplectes* spp. adults collected in the sweep net sampling was low but varied between the cutting management (Table 3). Although there was an interaction between county and cutting, this was once again quantitative and the abundance of *Bathyplectes* spp. adults was significantly higher in CUT than in UNCUT sections. No other interactions with cutting occurred.

H. postica collected in the field and reared in the laboratory resulted in variable rates of parasitism, ranging from 5 to more than 50%, and the prevalent parasitoid species was *B. anura*, with more than 90% of the puparia belonging to this species (Table 4). ANOVA results showed a significant effect of winter cutting, with higher rates of parasitism in CUT than in UNCUT sections (Table 5).

The rate of *H. postica* larvae with symptoms of fungal infection was evaluated in 2020, when the epizootic occurred. ANOVA results showed a significant effect of the winter cutting ($p < 0.0001$). Higher rates of infection were found in UNCUT than in CUT sections, resulting in a significant negative correlation (rho = −0.82; t = −8.14; $p < 0.0001$) between the rate of infection by *Z. phytonomi* and the rate of parasitism by *Bathyplectes* spp. (Fig. 5).

4. Discussion

In the study area, female alfalfa weevils start egg laying in the second fortnight of October. Consequently, some larvae are already present at the end of November and eggs can easily be found during January and February when our fields were sampled (Levi-Mourao et al., 2021). Although the natural abundance of *H. postica* markedly varied across fields and counties, the results of the comparison of UNCUT and CUT sections at the field and regional levels revealed the value of winter cutting in significantly reducing the *H. postica* larval population in the spring. The efficacy of the winter cutting was previously reported for some areas of the region (Núñez et al., 2015; Pons and Núñez, 2020), but the results presented in this study include a greater number of fields, located along the main Spanish alfalfa growing area. Therefore, we

Table 4

Rates of parasitism (%) by *B. anura* (Ba), *B. curculionis* (Bc) and total (Ba + Bc) in larval laboratory rearing in the UNCUT and CUT sections of the study fields in samplings of A) 2019 (March and April) and B) 2020. The rate of larval killing by *Z. phytonomi* is also shown (Zooph). Ni = initial number of larvae.

A) 2019											
March											
County	UNCUT						CUT				
	Field	Ni	Ba	Bc	Total	Zooph	Ni	Ba	Bc	Total	Zooph
Urgell	1	122	9	0	9	6.6	51	16	0	15.7	0
	2	100	13	2	15	0	100	19	0	19	0
	3	125	11	0	11.2	0	125	20	0	20	0
	4	142	11	2.1	12.7	0	42	33	17	50	0
	5	100	19	0	19	0	100	28	0	28	0
	6	39	44	0	43.6	0	54	19	0	18.5	0
	7	45	2.2	0	2.2	0	37	5.4	5.4	10.8	0
	8	84	4.8	0	4.8	0	53	5.7	3.8	9.5	0
	9	34	12	0	11.8	0	55	9.1	0	9.1	0
Segrià	10	50	6	2	8	0	38	18	0	18.4	0
	11	36	83	0	83.3	0	25	100	0	100	0
	12	25	60	0	60	0	25	60	15	75	0
	13	100	7	0	7	0	75	16	0	16	0
	14	100	3	0	3	0	100	9	0	9	0
	15	40	2.5	0	2.5	0	40	10	0	10	0
Baja Cinca	16	100	37	2	39	0	100	36	1	37	0
	17	150	19	0	18.7	0	150	22	0	22	0
Monegros	18	110	11	0	10.9	0	120	2.5	0.8	3.3	0
	19	115	12	0	12.2	0	105	43	0	42.9	0
April											
	UNCUT						**CUT**				
Urgell	1	150	1.3	0	1.3	0	150	11	0	10.7	0
	2	150	8.7	0	8.7	0	120	18	0	18.3	0
	3	150	4	0	4	0	100	23	0	23	0
	4	150	7.3	0	7.3	0	100	27	0	27	0
	5	150	21	0	21.3	0	109	30	0	30.3	0
	6	150	14	0	14	0	150	11	0	11.3	0
	7	100	17	0	17	0	40	18	0	17.5	0
	8	60	6.7	0	6.7	0	50	24	0	24	0
	9	110	2.7	0	2.7	0	100	12	0	12	0
Segrià	10	150	6.7	0	6.7	40	150	21	0	20.7	8.7
	11	100	24	0	24	0	75	36	0	36	0
	12	150	17	0	17.3	6.7	130	46	0	46.2	0
	13	100	23	0	23	0	75	33	0	33.3	0
	14	100	18	0	18	0	67	42	0	41.8	0
	15	100	5	0	5	64	100	13	0	13	0
Baja Cinca	16	140	23	0	22.9	0	140	38	0	37.9	0
	17	150	8.9	0	8.9	0	150	13	0	12.7	0
Monegros	18	60	8.3	13	21.7	0	150	2	19	20.7	0
	19	90	16	0	15.6	51	70	1.4	34	35.7	50
B) 2020											
	UNCUT						**CUT**				
County	**Field**	**Ni**	**Ba**	**Bc**	**Total**	**Zooph**	**Ni**	**Ba**	**Bc**	**Total**	**Zooph**
Urgell	1	150	8.7	2	10.7	43.3	150	17	1	18.7	18
	2	100	0	0	0	79	100	1	1	2	71.4
	3	25	4	0	4	75	28	7.1	0	7.1	57.4
	4	100	0	0	0	100	100	15	2	17	65
	5	100	0	0	0	100	60	1.7	0	1,7	80
	6	100	0	0	0	96	100	3	1	4,0	75
Segrià	7	150	13	3	16	58	150	17	3	20	22
	8	97	1	0	1	96.9	100	2	1	3	71
	9	100	0	0	0	100	100	11	2	13	75
Baja Cinca	10	26	3.9	0	3.9	96.1	58	1.7	2	3.4	81
	11	100	0	0	0	100	100	1	0	1	92
	12	150	4.7	1	6	84.7	40	2.5	0	2.5	97.5
Monegros	13	50	0	0	0	100	40	15	5	20	80
	14	25	0	0	0	100	25	0	0	0	83.3
	15	25	0	0	0	100	25	0	0	0	83.3
	16	115	7.8	0	7.8	14.8	95	7.4	2	9.5	0
	17	137	18	0	17.5	0	70	19	6	24.3	0

assume that the applicability of our results could be extended to other Spanish alfalfa crop conditions and potentially to other European regions.

The average efficacy of winter cutting in 2019 was approximately 50%, a similar value to the late autumn cutting efficacy reported by Dowdy et al. (1992) in USA. However, in our study, the efficacy varied across counties. The highest efficacies were recorded in Zaragoza Central, where a laser mower was used, which allows for cutting the alfalfa at the plant crown level and automatically collecting the cut plant material. In the fields where a disk mower was used, the plants were cut at 2–4 cm from the soil and left in the field for 1–2 days before collection. There was a significant correlation between efficacy and mowing

Table 5
ANOVA statistics for the fixed effects in the mixed model for the rate of parasitism by *Bathyplectes* spp.

Source	df	Approximate df denominator	F	*p*
County	4	14	0.58	0.6338
Year	1	54	15.60	0.0002
County*Year	4	52	0.3449	0.7930
Sampling date [Year]	1	22	0.01	0.9139
Sampling date * County [Year]	4	20	0.31	0.8181
Winter Cutting	1	12	20.74	0.0006
County * Winter Cutting	4	12	1.96	0.1747
Year * Winter Cutting	1	20	0.01	0.9196
County * Year * Cutting	4	20	0.27	0.8440
Sampling date * Winter Cutting [Year]	1	17	1.70	0.2091
Sampling date * County * Winter Cutting [Year]	4	16	0.57	0.6449

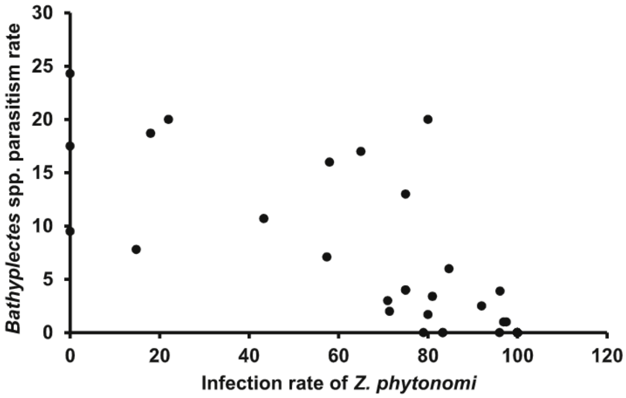

Fig. 5. Relationship between the parasitism rate of *Bathyplectes* spp. and the rate of larvae of *H. postica* showing symptoms of infection by *Z. phytonomi* (rho = −0.82).

method. Although both mowing methods cut stems less than 4 cm from the soil level and potentially remove most of the egg clusters (90% are located above the bottom quarter of the stem height; Fig. 2), laser mower cuts and collects nearly all stems, whereas the cutting efficiency of disc mower is lower. Furthermore, cut stems remain in the field for a longer time before collection with disc mower. Plant pieces remaining in the field or under the windrow may allow for the survival of eggs and small larvae (Summers, 1998; Blodgett et al., 2000; Blodgett and Lensen, 2004), even during the winter. Larvae already developed or emerging from the remaining eggs can infest new alfalfa stems. Furthermore, a significant correlation was found between the field size and the efficacy of the winter cutting, with lower efficacies in larger fields. In these larger fields, with their more variable topography and higher numbers of sprinkler supply pipes, whose bases hinder the cutting of alfalfa with the disc mower, the collection of alfalfa was less precise. In addition to local field management, the potential effect of nearby unmanaged alfalfa fields that can act as a source for adult alfalfa weevil immigration during the first crop intercut (Prokopy et al., 1967), should be further studied.

Epizootics of *Z. phytonomy* have been reported in Europe (Papierok et al., 1986), including in Spain (Pons and Núñez, 2020). The efficacy of the winter cutting method recorded in 2020 was lower than in 2019, and a negative relationship was observed between the efficacy of the method and the occurrence of *Z. phytonomy*. This fungus remains in the soil and infects larvae of *H. postica* when environmental humidity conditions are adequate (Radcliffe and Flanders, 1998). The infection is exacerbated in years with high rainfall during the winter and the beginning of spring, as occurred in 2020 (Table S3). These results indicate that in epizootic years, the effects of winter cutting management may be less evident. Additionally, the rate of infected larvae was higher in UNCUT than in CUT sections in several fields, suggesting that the epizootic was more severe where the density of alfalfa weevil larvae was higher (Los and Allen, 1983).

A clear predominance of *B. anura* over *B. curculionis* was observed in both study years; the former represented 90% of the total parasitoid individuals. This predominance has been observed previously in the study area (Pons and Núñez, 2020). In many regions where the two species live together, *B. anura* has been found to be predominant or even to displace *B. curculionis*, because the former has greater reproductive capacity, more rapid search and handling, and more aggressive behaviour (Harcourt, 1990).

Contrary to our hypothesis, winter cutting management did not negatively affect the rate of parasitism by *B. anura*. The rates of parasitism obtained were higher in the CUT than in UNCUT sections. These results further underline the value of winter cutting and suggest that this method may enhance conservation biological control of *B. anura*. Rand (2013) has reported that *B. curculionis* parasitizes a lower proportion of hosts at high alfalfa weevil densities, thus potentially explaining the results obtained with *B. anura* in our study. The mechanism through which high host density negatively affects *Bathyplectes* spp. is unknown but may occur through hindering the selection of hosts or increasing the host handling time. Specific studies should be performed to elucidate this relationship.

Parr et al. (1993) reported that *B. anura* and *Z. phytonomi* were able to coexist in the USA. Our results support these findings, because we have found both *H. postica* parasitoid species in our region for years. Although *Z. phytonomi* causes high mortality of *H. postica* larvae in wet seasons and is considered an important biological control agent (Harcourt and Guppy, 1991; Giles and Obrycki, 1997), our results show that fungal epizooties can negatively affect the role of *B. anura* by reducing the rate of parasitism. Because the disease also kills parasitized larvae of the alfalfa weevil (Giles et al., 1994; Kuhar et al., 1999), epizootics of *Z. phytonomy* disrupt the alfalfa weevil-parasitoid system.

5. Conclusion

The results show that winter cutting management can be a useful cultural tool that has potential as a component of an IPM program against the alfalfa weevil in Spain and potentially in other European regions. This cultural method not only reduces overwintering stages but also the larval density of this pest in the spring. Furthermore, the method increases the rate of parasitism of *Bathyplectes* spp., particularly *B. anura*, and can be considered a strategy for enhancing conservation biological control. However, despite this potential control capacity, the occurrence of *Z. phytonomy* epizootics, which strongly depends on weather conditions, may conceal the value of the winter cutting strategy, which should be applied in winter, before it is known whether a fungal epizootic will occur in the spring.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table S1
Sampled field coordinates and characteristics during a) 2019 and b) 2020.

a) 2019										
County	Field	Latitude	Longitude	Altitude (m)	Age (years)	Irrigation type	Type of mowing	Mowing height	Winter cut date	Surface (ha)
Urgell	1	41°41′22.36″N	1°3′58.83″E	302	2	Blanket	Disc	2–4 cm	February 05, 2019	3.35
	2	41°38′58.94″N	0°45′6.98″E	187	3	Blanket	Disc	≤2 cm	January 29, 2019	2.36
	3	41°39′9.61″N	0°44′15.41″E	183	3	Blanket	Disc	≤2 cm	January 29, 2019	4.87
	4	41°39′42.59″N	0°48′9.19″E	200	3	Blanket	Disc	≤2 cm	February 04, 2019	4.79
	5	41°40′59.75″N	0°49′44.68″E	205	3	Blanket	Disc	2–4 cm	February 07, 2019	2.94
	6	41°38′13.42″N	0°57′3.90″E	262	3	Blanket	Disc	2–4 cm	February 06, 2019	3.95
	7	41°42′20.05″N	0°58′50.76″E	256	3	Blanket	Disc	2–4 cm	February 05, 2019	6.34
	8	41°35′5.45″N	0°50′12.09″E	222	3	Blanket	Disc	2–4 cm	February 06, 2019	6.96
	9	41°39′16.48″N	0°55′15.53″E	246	2	Blanket	Disc	2–4 cm	February 06, 2019	4.12
Segrià	10	41°46′49.35″N	0°31′12.15″E	287	2	Sprinkler	Disc	2–4 cm	January 11, 2019	2.76
	11	41°43′42.73″N	0°20′54.59″E	187	2	Sprinkler	Disc	≤2 cm	January 04, 2019	2.58
	12	41°42′46.44″N	0°20′53.02″E	178	2	Sprinkler	Disc	≤2 cm	January 04, 2019	4.75
	13	41°47′30.82″N	0°16′52.37″E	259	3	Sprinkler	Disc	≤2 cm	January 09, 2019	1.24
	14	41°47′54.43″N	0°17′25.43″E	259	6	Sprinkler	Disc	≤2 cm	January 09, 2019	1.58
	15	41°44′9.59″N	0°47′17.84″E	216	3	Blanket	Disc	2–4 cm	February 06, 2019	2.47
Baja Cinca	16	41°57′17.82″N	0°3′14.55″E	315	2	Sprinkler	Disc	2–4 cm	February 09, 2019	4.60
	17	41°54′33.19″N	0°3′11.14″E	304	2	Sprinkler	Disc	2–4 cm	February 09, 2019	2.91
Monegros	18	41°28′12.75″N	0°4′27.75″W	315	2	Sprinkler	Disc	2–4 cm	February 15, 2019	4.7
	19	41°31′0.66″N	0°10′33.68″E	363	3	Sprinkler	Disc	2–4 cm	February 15, 2019	2.76
Zaragoza Central	20	41°33′30.90″N	0°41′32.29″W	179	3	Blanket	Laser	≤2 cm	February 06, 2019	0.9
	21	41°34′43.53″N	0°45′48.37″E	188	2	Blanket	Laser	≤2 cm	February 06, 2019	1.3
	22	41°42′21.63″N	0°51′11.01″E	217	2	Blanket	Disc	≤2 cm	February 08, 2019	1.52
b) 2020										
County	Field	Latitude	Longitude	Altitude (m)	Age (years)	Irrigation type	Type of mowing	Mowing height	Winter cut date	Surface (ha)
Urgell	1	41°39′9.61″N	0°44′15.41″E	183	4	Blanket	Disc	2–4 cm	February 14, 2020	4.87
	2	41°39′42.59″N	0°48′9.19″E	200	4	Blanket	Disc	2–4 cm	February 13, 2020	4.79
	3	41°40′59.75″N	0°49′44.68″E	205	4	Blanket	Disc	2–4 cm	February 13, 2020	2.94
	4	41°38′13.42″N	0°57′3.90″E	262	4	Blanket	Disc	2–4 cm	February 08, 2020	3.95
	5	41°35′5.45″N	0°50′12.09″E	222	4	Blanket	Disc	2–4 cm	February 11, 2019	6.96
	6	41°39′16.48″N	0°55′15.53″E	246	3	Blanket	Disc	2–4 cm	February 06, 2019	4.12
Segrià	7	41°46′49.35″N	0°31′12.15″E	287	3	Sprinkler	Disc	2–4 cm	January 17, 2020	2.76
	8	41°38′23.86″N	0°32′34.20″E	214	3	Sprinkler	Disc	2–4 cm	January 17, 2020	7.84
	9	41°47′30.82″N	0°16′52.37″E	259	4	Sprinkler	Disc	≤2 cm	February 11, 2020	1.24
Baja Cinca	10	41°57′17.82″N	0°3′14.55″E	315	3	Sprinkler	Disc	≤2 cm	February 27, 2020	4.60
	11	41°53′27.92″N	0°2′9.58″E	328	4	Sprinkler	Disc	≤2 cm	February 27, 2020	6.40
	12	41°54′33.19″N	0°3′11.14″E	304	3	Sprinkler	Disc	≤2 cm	February 27, 2020	2.91
Monegros	13	41°29′24.18″N	0°6′6.49″W	327	4	Sprinkler	Disc	2–4 cm		1.60

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Table S1 (*continued*)

a) 2019										
County	Field	Latitude	Longitude	Altitude (m)	Age (years)	Irrigation type	Type of mowing	Mowing height	Winter cut date	Surface (ha)
									February 12, 2020	
	14	41°28′12.75″N	0°4′27.75″W	315	3	Sprinkler	Disc	2–4 cm	February 12, 2020	4.7
	15	41°29′2.29″N	0°5′46.44″E	282	3	Sprinkler	Disc	2–4 cm	February 12, 2020	5.0
	16	41°29′0.41″N	0°6′11.15″E	325	3	Sprinkler	Disc	2–4 cm	February 12, 2020	3.96
	17	41°32′25.74″N	0°9′42.93″E	372	4	Sprinkler	Disc	2–4 cm	February 12, 2020	2.07
Zaragoza Central	18	41°33′30.90″N	0°41′32.29″E	179	4	Blanket	Laser	≤2 cm	February 14, 2020	0.9
	19	41°34′43.53″N	0°45′48.37″E	188	3	Blanket	Laser	≤2 cm	February 14, 2020	1.3
	20	41°42′21.63″N	0°51′11.01″E	217	3	Blanket	Laser	≤2 cm	February 27, 2020	1.52

Table S2

Mean (±s.e.) abundance of larvae and eggs of *H. postica* in the UNCUT and CUT sectors of the alfalfa fields sampled before winter cutting management during 2019 and 2020. Samplings in 2019 were performed in five net-sweeps in 12 points (3 × 4), and alfalfa was collected and dissected to detect the occurrence of eggs. In 2020, 25 stems per sector of UNCUT and CUT sections (25 × 4 = 100 stems per section) were collected and dissected.

YEAR											
2019						2020					
County	Field	Larvae/5 net-sweeps		Eggs/5 net-sweeps		County	Field	Larvae/stem		Eggs/stem	
		UNCUT	**CUT**	**UNCUT**	**CUT**			**UNCUT**	**CUT**	**UNCUT**	**CUT**
Urgell	1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Urgell	1	0.00 ± 0.00	0.01 ± 0.01	5.34 ± 1.13	3.72 ± 1.45
	2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		2	0.00 ± 0.00	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00
	3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		3	0.03 ± 0.03	0.01 ± 0.01	0.39 ± 0.20	0.43 ± 0.19
	4	0.17 ± 0.17	0.17 ± 0.17	1.33 ± 0.90	0.00 ± 0.00		4	0.01 ± 0.01	0.02 ± 0.01	0.41 ± 0.20	0.50 ± 0.33
	5	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		5	0.05 ± 0.04	0.21 ± 0.16	2.52 ± 1.43	0.68 ± 0.31
	6	0.25 ± 0.18	0.33 ± 0.19	1.83 ± 1.33	4.00 ± 1.95		6	0.11 ± 0.08	0.02 ± 0.02	6.26 ± 0.75	7.69 ± 1.46
	7	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Segrià	7	0.52 ± 0.22	0.07 ± 0.06	1.83 ± 0.83	1.61 ± 0.78
	8	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		8	0.16 ± 0.10	0.02 ± 0.02	0.52 ± 0.30	2.09 ± 1.45
	9	0.01 ± 0.01	0.01 ± 0.01	0.00 ± 0.00	0.00 ± 0.00		9	0.19 ± 0.14	0.05 ± 0.03	0.37 ± 0.19	0.12 ± 0.12
Segrià	10	0.00 ± 0.00	0.00 ± 0.00	4.25 ± 3.66	0.92 ± 0.45	Baja Cinca	10	0.15 ± 0.08	0.06 ± 0.04	1.95 ± 1.04	2.03 ± 1.21
	11	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00						
	12	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		11	0.02 ± 0.02	0.06 ± 0.06	0.48 ± 0.16	0.38 ± 0.21
	13	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		12	0.12 ± 0.09	0.00 ± 0.00	1.68 ± 0.69	1.22 ± 0.77
	14	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Monegros	13	0.01 ± 0.01	0.00 ± 0.00	7.00 ± 1.20	5.85 ± 1.21
	15	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00		14	0.56 ± 0.28	0.11 ± 0.06	8.68 ± 0.83	8.65 ± 1.06
Baja Cinca	16	0.50 ± 0.19	0.58 ± 0.26	0.42 ± 0.42	2.33 ± 1.74		15	0.04 ± 0.01	0.01 ± 0.01	0.42 ± 0.24	0.18 ± 0.18
	17	0.75 ± 0.28	1.25 ± 0.39	1.50 ± 1.18	0.50 ± 0.50		16	2.50 ± 1.11	1.33 ± 0.80	4.17 ± 1.87	5.83 ± 1.90
Monegros	18	0.08 ± 0.08	0.00 ± 0.00	2.50 ± 1.50	2.25 ± 2.25		17	0.33 ± 0.20	0.00 ± 0.00	3.00 ± 2.3	1.00 ± 1.00
	19	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	Zaragoza Central (n = 6)	18	0.00 ± 0.00	0.00 ± 0.00		
Zaragoza Central (n = 6)	20	0.00 ± 0.00	0.00 ± 0.00				29	0.01 ± 0.01	0.00 ± 0.00		

(continued on next page)

Table S2 (*continued*)

YEAR											
2019						2020					
County	Field	Larvae/5 net-sweeps		Eggs/5 net-sweeps		County	Field	Larvae/stem		Eggs/stem	
		UNCUT	**CUT**	**UNCUT**	**CUT**			**UNCUT**	**CUT**	**UNCUT**	**CUT**
	21	0.33 ± 0.19	0.25 ± 0.18								
	22	0.00 ± 0.00	0.00 ± 0.00				20	0.01 ± 0.01	0.00 ± 0.00		

Table S3

Cumulative monthly rainfall (l/m^2), from January to April, in 2019 and 2020 in the five counties in the study region (Urgell, Segrià, Baja Cinca, Monegros and Zaragoza Central). Source: AEMET.

County	2019					2020				
	Jan.	Feb.	Mar.	Apr.	Sum	Jan.	Feb.	Mar.	Apr.	Sum
Urgell	14.1	9.7	0.5	42.5	**66.8**	86.6	0.8	29.5	122.8	**239.7**
Segrià	12.1	3.2	0.6	28.7	**44.6**	85.4	0.6	35.8	75.2	**197.0**
Baja Cinca	12.2	7.8	3.4	55.9	**79.3**	101.1	2.2	74.7	66.8	**244.8**
Monegros	11.1	5.9	1.6	26.7	**45.3**	98.9	1.2	41.8	40.0	**181.9**
Zaragoza C.	20.2	2.1	13.6	33.6	**57.5**	62.8	0.6	71.2	35.4	**169.4**

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