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Population dynamics and integrated control of the damson-hop aphid *Phorodon humuli* (Schrank) on hops in Spain

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Abstract

The hop aphid *Phorodon humuli* (Schrank) (Hemiptera: Aphididae) is a serious pest in most areas where hops are grown. A field trial was performed on a hop yard throughout 2002, 2003 and 2004 in León (Spain) in order to analyse the population development of *Phorodon humuli* and its natural enemies, as well as to determine the most effective integrated program of insecticide treatments. The basic population development pattern of *P. humuli* was similar in the three years: the population peaked between mid to late June, and then decreased in late June/early July, rising again and reaching another peak in mid-July, after which it began to decline, rising once more in late August; this last rise is characteristic of Spain and has not been recorded in the rest of Europe. The hop aphid's main natural enemy found on the leaves was *Coccinella septempunctata* (Coleoptera: Coccinellidae). The multiple regression analysis showed that aphids are positively related with the presence of beetle eggs and mean daily temperatures and negatively related with maximum daily temperature integral above 27°C in plots without insecticide treatment. The most effective program of insecticide (imidacloprid) treatments consisted of an initial treatment in June and a second treatment in the second half of July or at the beginning of August. However, a single treatment in June would be sufficient when in this last period the maximum daily temperatures were higher than 27°C for at least 15 days, avoiding in this way the harmful effects of imidacloprid on predators.

Additional key words: Aphididae; Coccinelidae; *Humulus lupulus*; high temperature; population dynamics; control; insecticide.

Introduction

The cones or flowers of the hop (*Humulus lupulus* L.) are of great importance to the brewing industry. This is because the lupulin they contain provides beer with bitterness and other characteristic organoleptic or sensorial properties (Neve, 1991). The quality of bitterness is measured by the quantity of lupulin's alpha-acids. At present, Spain is the seventh highest hop-producing country in the European Union. Most of the plantations are situated in the Province of León where 95% of the land cultivated for hops in Spain is located (The Barth Report, 2010/2011). The most common cultivar in León is Nugget (98%) compared with Magnum (1%) and Columbus and Perle (1%).

The hop aphid *Phorodon humuli* (Schrank) (Hemiptera: Aphididae) is a serious pest in most areas where hops are grown. *P. humuli* can inhibit growth and reduce the number of flowers. Production losses associated with large numbers of aphids are due to reductions in the dry weight of the crop (a yield loss of 44% was observed when the population rised to 4400 aphids m^{-2} in late June), rather than a reduction in alpha-acid content (Lorenzana, 2006). Aphid populations in hop cones seriously reduce their economic value because of arbitrary commercial criteria related to the presence of aphids in cones (Lorenzana *et al.*, 2010), and in some cases can lead to total loss (Campbell, 1978; Thomas *et al.*, 1983). If any aphids are noted inside cones in Spain there is a penalty of up to 10% of the dry weight (Lorenzana *et al.*, 2010). The extent of the

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damage caused by *P. humuli* has resulted in the use of aphicides. Imidacloprid [1-(6-chloro-3-pyridylmethyl)-N-nitroimidazolidin-2-ylidenamine], the first neonicotinoid insecticide, is particularly effective against sucking pests such as aphids (Zeng & Wang, 2010). As a result, most German hops are treated with this insecticide (Weichel & Nauen, 2003) and it has been widely used to control vegetable pests in other countries, such as China (Guan *et al.*, 2010). Imidacloprid is also the most important insecticide in hop cultivation in Spain (J. A. Magadán, *pers. comm.*).

Integrated pest management encourages conservation of beneficial organisms while decreasing insecticide use (Hoheisel & Fleischer, 2007). Coccinelids are known to be important in regulating *P. humuli* populations on hops, as it is shown in Campbell & Cone (1994) or Weissenberger *et al*. (1997). The integration of natural enemies activity and imidacloprid applications is an efficient approach to enhancing the whitefly control level in Hoseini & Pourmirza (2011) study.

Population development of *P. humuli* has been studied in different countries like the United States by Campbell & Cone (1994), Germany by Goller *et al*. (1997) and Benker (1997), Czech Republic by Goller *et al*. (1997) and Zeleny *et al*. (1981), England by Aveling (1981), Campbell (1978) and Barber *et al*. (2003), Poland by Solarska (*pers. comm.*) and in France by Trouve *et al*. (1997), but not in a detailed way in Spain. One way to increase selectivity of common pesticides might be to adapt them more carefully to aphid population dynamics (Niehoff & Poehling, 1995), which are particularly sensitive to temperature change (*e.g.* Parry *et al*., 2006; Zamani *et al*., 2006).

Although a considerable amount of literature is available on the effect of low temperatures on aphid mortality, there is very little information on the effects of high temperatures. High temperature is a key factor in the development of aphid populations in corn fields of the Northeastern Iberian Peninsula, playing an important role in the decrease of populations at the end of June and in the relative abundance of aphid species throughout the season (Asin & Pons, 2001).

The aim of this research was to study the seasonal dynamics of *P. humuli* populations and their natural predators in hop plants. In addition, as the plants were treated for aphids at different times of year, a second objective was to establish which program of insecticide treatments would be most efficient in controlling the aphid population in Spain.

Material and methods

Location and methodology of sampling

Two experiments were carried out in León, Spain, the first during 2002 and 2003, and the second in 2004. A garden planted with the hop cultivar Nugget (0.72 ha), consisting of 40 rows (3 m apart) each with 40 plants (1.5 m apart), was chosen for the study. This garden was situated at the University of León's experimental farm maintained by the School of Agricultural Engineering. The height of the wirework was 6 m with two strings per rootstock. Three hop bines were trained to each string.

A randomized complete block design, with five treatments and three replicates (15 plots), was used. Each plot was made up of 18 plants in 3 adjacent rows of 6 plants per row. The area of each plot was 81 m^2 (9 m × 9 m). Treatments in this study were different aphid densities: in 2002 and 2003 by applying the same insecticide to plots at different times, and in 2004 using a combination of insecticides and aphid introductions (Table 1). Imidacloprid was used because it was the standard insecticide used by Spanish growers during the study period. It was sprayed using a back-pack sprayer in order to reduce contamination among treatments.

In order to prevent the treatment of one group affecting the results of neighbouring groups, in each group of eighteen plants, only the three central plants were sampled in each experimental plot. Counts were taken in the following manner: on the surface of one of the bines of the plants a wooden frame measuring 20 cm × 30 cm was randomly placed at heights of 2, 3.25, and 6 m from the ground. Within the area enclosed by this frame, counts were taken of the total number of leaves, the number of leaves with aphids, the total number of aphids and the average number of them per leaf attacked. The average of these counts for each repetition was included in the statistical analysis. Sampling was carried out weekly, one week measuring aphid population on the left bine of the plant, and the following week on the right.

Population density of *P. humuli* has often been expressed as the number of aphids per leaf, although other parameters can be used, such as the number of aphids per dm^2 of leaf surface (Campbell, 1978), or per m^2 of plant surface (used by Hermoso de Mendoza *et al.*, 2001, for *Aphis gossypii* on clementines). This

Table 1. Treatments in 2002, 2003 and 2004

Treatment	Years 2002 and 2003	Treatment	Year 2004
1. Untreated	Without treatments	1. Untreated	Without treatments and with an initial level Ipopulation of 584± 53 aphids m^{-2} in 25 June
2. Early	Imidacloprid[1] on 18 June (2002 and 2003)	2. Untreated level II	Without treatments and with an initial population of 1,262 ± 131 aphids m^{-2} in 25 June
3. Intermediate	Imidacloprid[1] on 25 July (2002) and 24 July (2003)	3. Untreated level III	Without treatments and with 937 ± 88 aphids m^{-2} in 25 June and with aphids introduced once in June and twice in July. Five leaves with aphids (150 aphids/leaf) were released on each bine at a height of between 2 and 3.25 m each time
4. Last	Imidacloprid[1] on 21 August (2002) and 20 August (2003)	4. Early	Imidacloprid[1] ground on 14 June
5. Monthly	Imidacloprid[1] with three repeated treatments on18 June, 25 July, 21 August (2002) and 18 June, 24 July, 20 August (2003)	5. Monthly	Imidacloprid[1] on14 June, 6 July and 23 August

[1] 0.008 L Imidacloprid /16 L water /243 m^2.

study expresses aphid population density by number of aphids per m^2 of hop bine. Studying the number of aphids on the leaves within a surface area of 6 dm^2 from the lower, middle and upper section of the plant provides much more complete and representative data than counting the number of aphids on a leaf chosen at random among the plants, which is how the majority of studies have been carried out (Lorenzana, 2006). In addition, it is important to emphasize that there is a relationship between aphid population density on leaves and cones (R^2 = 0.895) (Lorenzana *et al*., 2010).

Population development of aphids and natural enemies

Sampling in 2002 began on 21st June, and terminated on 6th September. In 2003, it began on 30th May for the groups 'Untreated" and 'Last", whilst for the other groups it began on 20th June, and sampling on all groups finished on 29th August. The arrival of winged aphids to hops during this year was also recorded, and that is why the sampling began earlier in the untreated group and in the group treated last. In 2004, it began on 25th June, and terminated on 3rd September.

For each year, natural enemies of *P. humuli* were sampled on the experimental plot during the same period as aphid sampling. In 2002, the number of natural enemies was not recorded, although field notes were taken of all fauna observed whilst sampling aphids, and unrecognised species were collected and taken back to the laboratory for identification. In 2003 and 2004, the number of natural enemies found on leaves was recorded at the same time as the *P. humuli* population was recorded. Both species name and total number found within the frame measuring unit were recorded. When species identification was not possible in the field, a sample was collected for later identification in the laboratory. Population development of the most abundant natural enemies was studied, using the same unit as for aphids, number of natural enemies per m^2 of hop bine surface area.

Population development of aphids and temperature

Maximum and mean temperatures were recorded with a local weather station located about 200 m from the hop plot in order to analyse the relationship between them and the population development of aphids.

In 2006 apterous *P. humuli* were reared in groups of six on plants of the Nugget cultivar in a controlled environment room. One experiment was made at a range of constant temperatures according to the temperature regime of the region during the hop crop cycle in 2002, 2003 and 2004. Mean temperature was around 19°C in June, 18.5°C in July and 18°C in August. The maximum temperature during these months exceeds 27°C some days in 2002 and 2004 and very frequently in 2003. The temperatures used in the experiment were 19 and 27°C. The experiment was conducted in climatic chambers with Gro-lux lights (2000 lx), 70% RH and a photoperiod of 16:8 (L:D) h. Plants of similar age and size with two pairs of fully expanded leaves were grown in 13.5 cm diameter plastic pots. Soil moisture was maintained and a balance liquid fertilizer was applied once a week. One clip-cage per plant enclosing a leaf area of 3.2 cm^2 was used. Two recently moulted adult aphids were transferred to each clip-cage. They were removed 24 h later and their offspring reduced to six per cage. Cages were examined daily and development time was recorded until the third generation. Cages were carefully transferred to newly expanded leaves weekly.

Statistical analysis

Collected data in the field were transformed using the square root transformation $(X + 0.5)^{1/2}$, where X are the original data. This transformation is appropriate for insect data especially with zeroes present (Steel & Torrie, 1986). These square-root values were used in the analysis of variance. Pooled analysis of variance of measurements over time appropriate to randomized complete block design was performed using the general linear models (GLM) procedure. Analyses for aphid and natural enemy densities between treatments and between weeks were carried out. Mean comparisons were performed using the LSD test to examine differences ($p < 0.05$) among treatments or weeks. Linear regressions were performed between natural enemies (beetles, lacewing eggs and mummies) per m^2 of bine surface and the number of *P. humuli* per m^2 of bine surface. Stepwise multiple linear regression analysis was performed in order to better understand the relationship between aphids and temperatures and beetles. Aphid m^{-2} was the dependent variable, while temperatures (mean daily temperature (MeanT), maximum daily temperature integral above 27°C in the fortnight before the aphid count (MaxTemInt)) that were significant ($p < 0.05$) were included as independent variables. As there were experimental units treated and not treated with aphicide, the multiple regression analysis was conducted separately by untreated and treated experimental units using the PROC REG procedure of SAS. All analyses were performed using SAS software version 9.1.2 (SAS Institute Inc., 2004).

Results

Population development of aphids and insecticide control

In broad terms it could be said that the pattern of *P. humuli* population development for the years 2002 (Fig. 1a) and 2004 (Fig. 2) was similar. From the first day of sampling the population showed a decrease until the end of June – beginning of July, from when it then increased, and peaked in mid-July. It decreased again from mid-July onwards, until the end of August, when it once again began to increase. The initial quantity of aphids in 2004 was greater than in 2002, although the first drop in population was more marked in that year, and in July the peak in both years was similar. Maximum population for the year 2002 was reached in the July peak, whilst in 2004 maximum population was recorded at the outset of sampling towards the end of June.

Significant differences in aphid density between treatments for each week are shown in Fig. 1a (year 2002), Fig. 1b (year 2003) and Fig. 2 (year 2004) with capital letters. Population dynamics for each treatment are shown in the figures after the date of each aphicide application during the three years.

Statistical analysis of the different weeks studied during 2002 (Fig. 1a) of *P. humuli* population density (capital letters) showed that after treatment in June, the groups 'Untreated', 'Last' and 'Intermediate' had significantly greater populations than the other groups. After treatment in July, significantly greatest population was observed in the groups 'Untreated' and 'Last'. After the August treatment, 'Untreated' and 'Early' registered the significantly greatest populations. The differences between weeks for each treatment in 2002 (Fig. 1a, lower-case letters) show that the treatments 'Untreated' and 'Early' had the significantly greatest population the last week of sample (6th September). 'Intermediate' had no significant differences in its population after the day of its treatment, although population was greater the day after the aphicide treatment (26th July) and the last week of sample (6th September). 'Last' also had no significant differences in its population after the day of its treatment, although population was greater the last week of sample (6th September). 'Monthly' had no significant differences in its population throughout the whole sampling process.

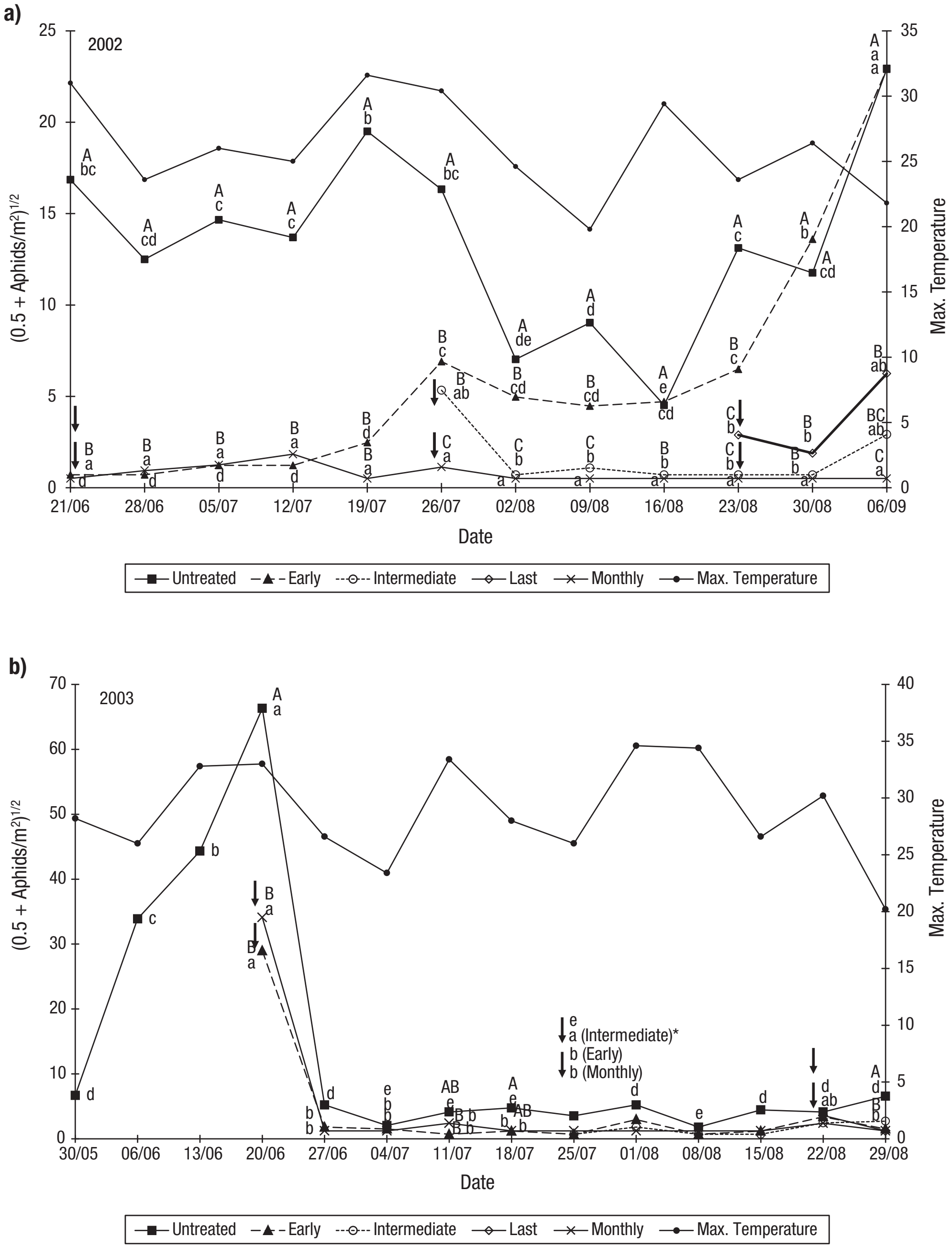

Figure 1. Population development of *P. humuli* and maximum temperatures in the five groups of treatments ('Untreated', 'Early', 'Intermediate', 'Last' and 'Monthly') during the year 2002 (a) and 2003 (b). Mean comparisons between treatments (at the same date) are shown with capital letters (means followed by the same letter are not significantly different). Letters are not shown if there are no significant differences at that date. Mean comparisons between weeks (for each group) are shown with lower-case letters (means followed by the same letter are not significantly different). Population dynamics appear after the date of their treatment. Arrows (↓) indicate date of insecticide treatment (18th June for 'Early' and 'Monthly' treatments, 25th July for 'Intermediate' and 'Monthly' treatments and 21st August for 'Last' and 'Monthly' treatments in 2002, and 18th June for 'Early' and 'Monthly' treatments, 24th July for 'July' and 'Monthly' treatments and 20th August for 'Last' and 'Monthly' treatments in 2003). * Lower-case letters for 'Intermediate', 'Early' and 'Monthly' between 25th July and 29th August and for this reason they are not repeated between these dates.

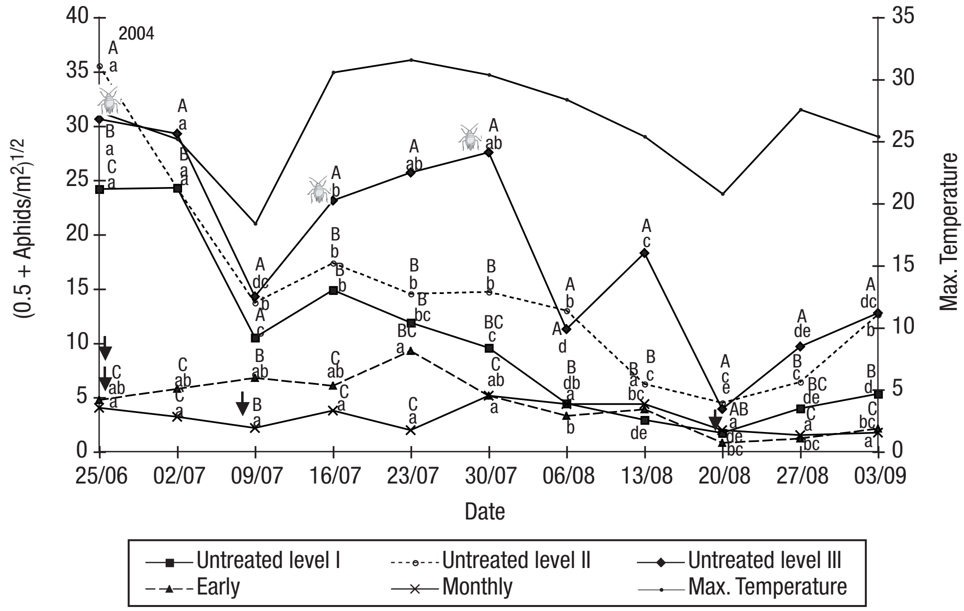

Figure 2. Population development of *P. humuli* and maximum temperatures in the five groups of treatments ('Untreated level I', 'Untreated level II', 'Untreated level III', 'Early' and 'Monthly') during the year 2004. Mean comparisons between treatments (at the same date) are shown with capital letters (means followed by the same letter are not significantly different). Letters are not shown if there are no significant differences at that date. Mean comparisons between weeks (for each group) are shown with lower-case letters (means followed by the same letter are not significantly different). Treatments 'Early' and 'Monthly' appear after the date of their treatment. Aphid sketch indicates date of introduction of aphids. Arrows (↓) indicate date of insecticide treatment (14th June for 'Early' and 'Monthly' treatments, 6th July for 'Monthly' treatment and 23rd August for 'Monthly' treatment).

Mean comparisons between treatments for the year 2004 (Fig. 2, capital letters) showed that the untreated groups reached a significantly greater population than the treated groups during most weeks. If differences between weeks are observed (Fig. 2, lower-case letters) it is remarkable that treatments without insecticides had the significantly greatest population the first week of sample (25th June), although there were no differences with the second week (2nd July) in the case of treatment 'Untreated level I', and with the second (2nd July), fifth (23rd July) and sixth (30th July) weeks in the case of treatment 'Untreated level III'. 'Early' treatment had the significantly greatest population the 23rd July, although without differences with weeks 25th June to 16th July, 30th July and 13rd August. 'Monthly' treatment had no significant differences in its population during all sampling.

In 2003 (Fig. 1b) there is a peak towards the end of June. A slight increase in the middle of July and a further slight population increase towards the end of August also occurred. In this year, the abundance of aphids is markedly different from those of the years 2002 and 2004. The peak in June was much higher and the decrease at the end of June to the beginning of July was so marked that the population practically disappeared, with the following two peaks showing a very low aphid population in comparison to the June peak. Mean comparisons between treatments for the year 2003 (Fig. 1b, capital letters) showed that on the 20th June the groups 'Untreated', 'Last' and 'Intermediate' had significantly greater populations than the other groups. If differences between weeks are observed (Fig. 1b, lower-case letters) it would be possible to emphasize that treatments 'Untreated', 'Early' and 'Monthly' had the significantly greatest population on 20th June. 'Intermediate' and 'Last' had no significant differences in its population after the day of its treatment.

Natural enemies and aphids

The natural enemies encountered on leaves during sampling in 2003 and 2004 are shown in Table 2.

Table 2. Natural enemies on hop leaves during the years 2003 and 2004

	Species	Abundance[1]
Year 2003		
Coleoptera	*Coccinella septempunctata* Linnaeus, 1758	++++
	Propylea quatuordecempunctata (Linnaeus, 1758)	++
	Adalia decempunctata (Linnaeus, 1758)	+
	Adalia bipunctata (Linnaeus, 1758)	++
Neuroptera	No identified larvae	+
	No identified eggs	++++
Thysanoptera	*Aeolothrips* sp.	+
Parasitoids	Mummies: no identified parasitoid	+++
Year 2004		
Coleoptera	*Coccinella septempunctata*	++
	Propylea quatuordecempunctata	++
	Adalia decempunctata	++
	Adalia bipunctata	++
Neuroptera	No identified larvae	+
	No identified eggs	+++
Thysanoptera	*Aeolothrips* sp.	++

[1] +: scarce (< 5 records in 2 years); ++: regular (< 20 records in 2 years); +++: frequent (10-20 records every year); ++++: very frequent (> 20 records every year).

Coccinelids and eggs of neuroptera were the most abundant natural enemies found on leaves during both years. *Coccinella septempunctata* was the most common species in 2003, while this species was found in the same frequency than the rest of coccinelid species in 2004. *Aeolothrips* sp. was also registered in both years, while mummies were found only in 2003.

Mean comparisons between weeks for beetle density in the group 'Untreated' in 2003 (Fig. 3a, lower-case letters) showed that larvae and eggs had the significantly greatest population on the 20th June, the same as aphids, although without differences with the week 13rd June for the egg population. Beetle adults had the significantly greatest population on 13rd June. Statistical analysis of the different weeks studied during 2003 of beetle larvae population density showed that after treatment in June, the groups 'Untreated', 'Last' and 'Intermediate' had significantly greater populations than the rest of the groups. The group 'Untreated' had significantly greater adult beetle populations than the rest of the groups on 4th July. Regression analysis showed a positive correlation between aphids and beetles in 2003 (Fig. 4).

Mean comparisons between weeks for beetle density in the group 'Untreated level III' in 2004 (Fig. 3b, lower-case letters) showed that adults, larvae and eggs had the significantly greatest population on 2nd July, although without differences with weeks 25th June, 23rd July and 30th July in the case of beetle larvae, and with the week on 25th June in the case of beetle eggs. The significantly greatest population for aphids was reached 25th June, without differences with the 2nd July. Statistical analysis of the different weeks studied during 2004 of beetle larvae and egg population density showed that after treatment in June, the groups without treatments had significantly greater populations than the other groups. Regression analysis showed the positive correlation between aphids and beetles in 2004 in the same way as 2003, although this year the greatest correlation was obtained between aphids and beetle larvae (R^2 = 0.8333).

Population of mummies reached the significantly highest level on 20th June in 2003 (without differences with the population during the 15th August), the same as aphids. Otherwise, lacewing eggs population did not reach the significantly greatest population at the same time as aphids in 2003 and 2004. Statistical analysis of the different weeks studied during 2003 of lacewing eggs population density showed that after treatment in July, the groups 'Untreated', and 'Last' had significantly greater populations than the rest of

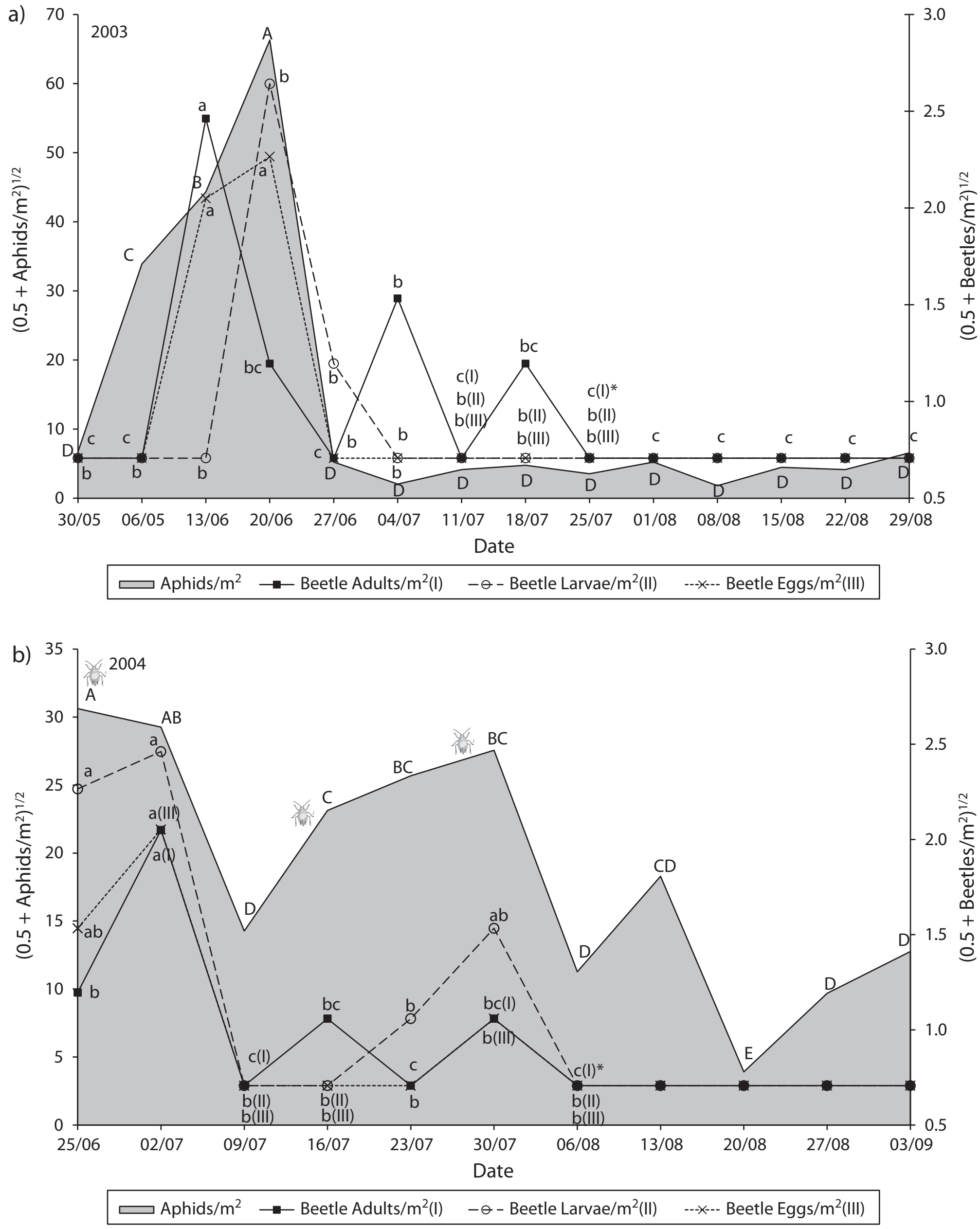

Figure 3. Population development of *P. humuli* and beetles (adults, larvae and eggs) in the treatment 'Untreated' during the year 2003 (a) and in the treatment 'Untreated level III' during the year 2004 (b). Mean comparison between weeks are shown with capital letters for aphids and with lowercase letters for beetles (means followed by the same letter are not significantly different). * Same letters between 25th July and 29th August (a) and between 6th August and 3rd September (b) and for this reason they are not repeated between these dates. Aphid sketch indicates date of introduction of aphids (b).

the groups. In the case of 2004 the group 'Untreated level III' had significantly greater populations than the rest of the groups on 16th July, 20th and 27th August. There was no significant correlation between aphids and mummies or lacewing eggs.

Temperature and aphids

Mean generation time of *P. humuli* on Nugget cultivar hop plants at 19°C in the laboratory was 10.3 ± 0.1 days. No generation was completed at 27°C,

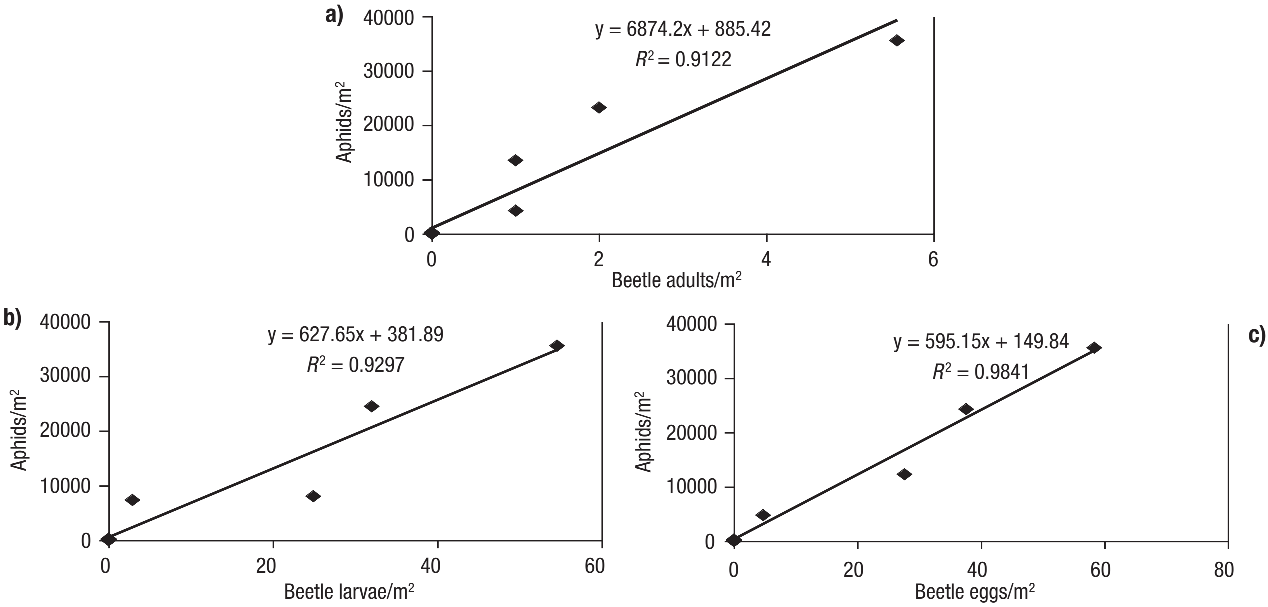

Figure 4. Correlation of the mean of the sum for each treatment between aphids and beetles in 2003: (a) *y* is the number of aphids per m² and *x* is the number of beetle adults per m²; (b) *y* is the number of aphids per m² and *x* is the number of beetle larvae per m²; (c) *y* is the number of aphids per m² and *x* is the number of beetle eggs per m².

at which temperature all nymphs died from 14 ± 1.5 days from the beginning of the experiment.

Temperature, natural enemies and aphids

The combination of beetle eggs, mean daily temperature (MeanT) and maximum daily temperature integral above 27°C in the fortnight before the aphid count (MaxTemInt) explained 91% of aphid m^{-2} variation in untreated plots, with beetle eggs alone explaining 51% of the variation (Table 3). Beetle eggs and MeanT had a positive regression coefficient whereas MaxTemInt had a negative regression coefficient. No variables remained in the regression model in treated plots.

Discussion

Population development of aphids

The population development for *P. humuli* obtained in this study will now be compared with that described in other countries for hop plants where aphid pesticide treatment was not given. Population dynamic in 2002 (Fig. 1a), with the maximum peak in mid-July, coincides with that found by Campbell & Cone (1994) in the USA in 1992. This dynamic is also very similar to that found in Germany in 1992 by Goller *et al*. (1997) and in the Czech Republic (Hrdŷ, 1980, quoted in Goller *et al*., 1997), except that the increase of population at the end of August was not found probably due to temperatures registered in these countries at this time. In studies carried out in England by Aveling (1981) in 1975, in Germany by Benker (1997) in 1996, and in Poland by Solarska (*pers. comm.*) in 2004, the maximum peak of aphid population was also reached in mid-July, with no peak in either June or August. Following analysis of population development over 2002 in our study the conclusion is that two aphicide treatments are necessary to control the population during this year: the first in June and the second in mid-July (or beginning of August) in order to prevent the final population increase. The final population increase could be the most damaging if the population reaches very high levels.

Regarding the population dynamics obtained in this study of the year 2004 (Fig. 2), with maximum population peak at the end of June or beginning of July, the same results appear in the work carried out in England by Campbell (1978) for the years 1971 and 1972 (these plants were treated with an aphicide at the end of June) and in the study carried out by Barber *et al*. (2003) on a series of hop plant varieties in 1997 and 1998 in the same country, except that the population increase at the end of August was not observed. It is worth pointing out that in the study of Barber *et al*. (2003) this

Table 3. Stepwise multiple linear regression results for aphids m^{-2} as a function of temperatures and beetles in treated and untreated plots (2003 and 2004)

Dependent variable	Insecticide treatment	Intercept	Independent variable	b coefficient	Pr < F[a]	R^2
Aphids m^{-2}	Untreated	12.69	Beetle eggs m^{-2}	11.24	0.02	0.51
			MeanT[b]	1.43	0.02	0.37
			MaxTemInt[c]	–14.79	0.05	0.03
Aphids m^{-2}	Treated	–9.45	—	—	—	—

[a] F: test statistic used to reject or fail to reject the null hypothesis; Pr: probability of obtaining the F test statistic, assuming that the null hypothesis is true. [b] MeanT: mean daily temperature. [c] MaxTemInt: maximum daily temperature integral above 27°C in the fortnight before the aphid count.

slight population increase was observed in one of the varieties studied in 1998.

The population dynamics described for 2003 (Fig. 1b), with a maximum population peak at the end of June followed by a decrease and practical disappearance of the population for the remainder of the period of cultivation, coincides with that obtained in the Czech Republic by Zeleny *et al*. (1981) in the years 1969, 1973 and 1979, in Germany by Benker (1997) in the years 1994 and 1995, in France by Trouve *et al*. (1997) and in England by Barber *et al*. (2003) in certain hop plant varieties for the years 1998 and 1999. In view of the population development over 2003 in our study it would be possible to conclude that the aphid population could probably be controlled with just one treatment in June during this year.

Factors affecting population development of aphids

The multiple regression analysis performed in order to better understand the relationship between aphids and temperatures and beetles (Table 3) suggests that higher concentrations of aphids are related to the presence of beetle eggs and high mean temperatures in untreated plots. MaxTemInt coefficient (parameter estimate) is –14.79, so, for every unit increase in MaxTemInt a 14.79 units decrease in aphids m^{-2}. Aphid population was not correlated neither with the temperatures nor beetles in treated plots, probably due to negative effect of imidacloprid on aphids and beetles (AiZhi *et al*., 1999).

The population development obtained in the year 2002 (Fig. 1a) coincided exactly with those described by Campbell & Cone (1994) in the USA for the year 1992. This study shows the important role of natural predators, even to the point of explaining aphid population fluctuations exclusively in terms of predators. Coccinelids are probably considered the main natural enemies of *P. humuli* worldwide, as Tsvetkov (1962), Campbell (1973), Campbell & Cone (1994) or Weissenberger *et al*. (1997) emphasized in their studies. Coccinelid population increased at the same time as *P. humuli* population during June and nearly all July in Campbell & Cone (1994) study. Adults and larvae of Neuroptera as well as parasitoids could also be important natural enemies of aphids, although *Aeolothrips* sp. would probably prefer an alternative host.

In the case of this study, the first population decrease between the end of June and beginning of July could be associated more with elevated temperatures (highest temperatures above 30°C for several days) than with natural predators, given the low number of them found. Later, temperatures fell, which produced a population peak in mid-July. The following decrease in population could once again be attributed more to high temperatures than to predators, although when the temperatures fell again, the population did not recover. Towards the end of August, the total absence of predators and lower temperatures provided a more favourable habitat for aphids.

We believe that in the year 2003 (Fig. 1b), high temperatures throughout the period of cultivation were mainly responsible for the population development described. In mid-June, the elevated temperatures could have provoked the abrupt population decrease, whilst the repetition of these temperatures in the following months prevented the population from recovering. It was only when the temperatures fell at the end of August that the aphid population was able to increase slightly. Natural enemies could have contributed to the population decrease in mid-June, although the number of adults, larvae and eggs beetles (Fig. 3a), or lacewing eggs and mummies found was not very high. The beetle eggs found between the beginning and middle of June were the source of the larvae peak a week later. Aphid numbers continued to fall after predator numbers peaked because the daily consumption rate of larvae for hop aphids is positively correlated with larval instar (Campbell & Cone, 1994). The beetle eggs found in the latter half of June were the source of the larvae found at the end of this month, although their number was very low probably due to temperatures higher than 30°C.

In 2004, the population of aphids fell between the end of June and beginning of July, the same as in the two previous years (Fig. 2). The population of adults, larvae and eggs beetles (Fig. 3b) reached its maximum peak at the same time as aphids in the majority of groups, just before this fall. Peak abundance of coccinelids coincided with the peak of *P. humuli* in July in the study of Campbell & Cone (1994). Besides the influence of natural enemies, aphids in the present study could have survived high temperatures for an extended time, which would have contributed to the fall in aphid population. Beetle larvae numbers also declined, but the daily consumption rate is positively correlated with instar (Campbell & Cone, 1994). Following a slight drop in temperature, degrees rose again between the beginning and middle of July, and the population began to recover slightly from this date onwards. This fact leads to the conclusion that it is not only temperature which affects aphid population development. Larvae completed their larval development and pupated, allowing the aphid population to recover. Later high temperatures could have caused the mid-July peak to be minor, as well as the presence of some natural enemies observed at this time in some of the treatments. The peak which occurred in the group 'Untreated level III' was much more marked than in any of the other groups due to aphids having been introduced twice. This peak coincided with a new increase in the number of larvae. Aphid population decreased later, as well as larvae population, although the final instars larvae eat many more aphids than the first ones. Aphid population recovered slightly when these larvae pupated. Population development went on to display the same pattern as in the previous two years, and the slight rise in population which occurred at the end of August was explained in the same way as for the year 2002.

Lacewing eggs were found regularly with the highest numbers at the end of the sample. This predator does not seem to be efficient in controlling the hop aphid, as in Zeleny *et al.* (1981) and Trouve (1995) studies.

Following the analysis of those factors which could have affected population development of *P. humuli* throughout the three years of sampling, it is possible to conclude that in 2002 and 2004, the temperature had a certain effect, although the influence of natural enemies should also be taken into account. High temperature was not the primary reason soybean aphid populations remain low in Missouri (USA), as some speculate, being more likely that resident predators are responsible (Meihls *et al.*, 2010). In 2003, high temperatures meant that in a very short time the population practically disappeared. Temperature is one of the main factors that affect development rate, fecundity and lifespan of aphids (Aalbersberg *et al.*, 1987). The optimum constant temperature for *P. hunuli* is near 18°C-20°C and suffers from heat stress as temperature increase (C. A. M. Campbell, *pers. comm.*). Campbell (1983) reared aphids on plants of the cvs. Tolhurst, Fuggle and Northern Brewer at different temperatures in a controlled environment room. Mean generation times at temperatures fluctuating between 15° and 20°C (10.7 ± 0.1, 10.4 ± 0.1 and 10.5 ± 0.1 days respectively in the three varieties) were similar to those found in cv. Nugget in this study at 19 (10.3 ± 0.1 days). Development was faster at 25°C than at 20°C, although size, net fecundity and life expectancy were reduced. Thus Campbell (1983) established that 25°C may be near the upper thermal threshold for *P. humuli*. Thanks to the results obtained in this study we can establish an upper thermal threshold of less than 27°C for this aphid. Although insects are not subjected to constant temperatures in nature, controlled laboratory studies can provide a valuable insight into the population dynamics of aphids (Satar *et al.*, 2005). In this way, the results obtained in our experiment confirm the explanation of the phases of the hop aphid population dynamics in Spain. The upper optimal temperature for *Acyrthosiphon pisum* on pea was 23.1°C in Morgan *et al.* (2001) study, where the highest temperature used (26.7°C) had a deleterious effect on aphid development. High temperature is a key factor in the development of aphid populations in corn fields of Northeastern Iberian Peninsula, where the mortality of *Sitobion avenae* (Fabricius) nymphs reached 100% at 30°C and that of *Metopolophium dirhodum* (Walker) reached 100% at 27.5°C (Asin & Pons, 2001).

In conclusion, it is important to emphasize the basic population development pattern of *P. humuli*: the population peaked between mid to late June, and then decreased in late June/early July, rising again and reaching another peak in mid-July, after which it began to decline, rising once more in late August. This increase in the population of aphids in August is characteristic of Spain and was different from the studies reported in the rest of Europe. High temperatures had a negative effect on the aphid population. In addition, it would be necessary to take into account the influence of natural enemies on the aphid population. Following analysis of population development over three years of study, it was concluded that most effective treatments with aphicides were: an initial treatment in June, when natural predators have not yet appeared on the hop plants, and a second treatment in mid-July (or beginning of August) in order to prevent the final population increase, although with this treatment, any possible beetles which might have reappeared at the end of August would be eliminated. When in mid-July (or beginning of August) temperatures are higher than 27°C for at least 15 days, a single treatment applied in June would be sufficient, avoiding in this way the harmful effects of imidacloprid on predators.

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