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RESEARCH ARTICLE

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Within-field distribution of the damson-hop aphid *Phorodon humuli* (Schrank) (Hemiptera: Aphididae) and natural enemies on hops in Spain

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Abstract

A field trial was performed in a hop yard throughout 2002, 2003 and 2004 in order to determine the within-field distribution of *Phorodon humuli* (Schrank) (Hemiptera: Aphididae) and its natural enemies. The distribution of *P. humuli* was directly affected by the position of the hop plants in the garden, with significantly higher concentrations of aphids (p=0.0122 in 2002 and p=0.0006 in 2003) observed along the edge. However, in 2004 the plants located on the marginal plots had similar populations to those on the more inner plots. This can be explained by a higher wind speed which made it more difficult to land on edge plants first. The hop aphid's main natural enemy was *Coccinella septempunctata* (Coleoptera: Coccinellidae), whose population was greatest where the aphids were most abundant with a significantly greater number of eggs (p=0.0230) and adults (p=0.0245) in 2003. Lacewing eggs were also frequently observed, with a significantly higher population (p=0.0221 in 2003 and p=0.0046 in 2004) where the aphid numbers were high. The number of winged aphids was greatest towards the margins of the garden in 2003. It is argued that the spatial distribution of the hop aphid and its natural enemies could be used to plan a sampling program and to estimate the population densities of these insects for use in integrated pest management programs.

Additional keywords: *Humulus lupulus*; spatial distribution; Coleoptera; Coccinelidae; winged aphids; integrated pest management.

Authors' contributions: Conceived and designed the experiments, and analyzed the data: AL, AHM and PAC. Performed the experiments, and wrote the paper: AL and PC. Obtaining funding: VS. Coordinating the research project: AL, AHM and VS.

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Introduction

Spain is the sixth largest hop-producing country in the European Union (The Barth Report, Hops, 2014/2015). Most of the plantations are located in the Province of León and occupied 524 ha in 2013. These, together with 8 ha in La Rioja, 5 ha in Palencia and 2 ha in Navarra, account for all of the land cultivated for hops (Humulus lupulus L.) in Spain (MAPAMA, 2014). The most common cultivar in León is 'Nugget' (90.2%) compared with 'Columbus' (8.7%), 'Magnum' (0.7%), 'Perle' (0.1%) and 'Summit' and 'Millenium' under field tests (0.3%).

The hop aphid, Phorodon humuli (Schrank), is a major pest on hops in the northern hemisphere (Weihrauch & Moreth, 2005). *P. humuli* can inhibit growth and reduce the number of flowers, which constitute an essential raw material for brewing beer. Aphid contamination of hop cones also seriously reduces their economic value because of arbitrary commercial criteria related to the presence of aphids in cones (Lorenzana *et al.*, 2010). *P. humuli* uses several common *Prunus* spp. as primary hosts (Eppler, 1986). The eggs hatch in early spring, and usually after two or three wingless generations winged emigrants appear and fly to hops, *Humulus lupulus L.,* the sole secondary host (Blackman & Eastop, 1984). On hops, *P. humuli* does not produce winged morphs capable of re-infesting other hops (Campbell, 1985), so the pattern and intensity of aphid infestation within hop plantations largely reflects the colonization, accumulation and secondary flight behaviour of aphids migrating from *Prunus* spp. (Campbell & Ridout, 1999).

[1]http://www.barthhaasgroup.com/images/mediacenter/downloads/pdfs/412/ barthreport2014-2015en.pdf

In autumn, winged females (return migrants) and later winged males are produced. The return migrants produce wingless sexual females on primary hosts, which lay the overwintering eggs after mating (Campbell & Muir, 2005). Population development of this aphid has been studied in several countries. Specifically, within-field distribution have been studied by Campbell (1977) in England, Ilharco *et al*. (1979) in Portugal and Wright *et al.* (1990) in the USA but has not been studied in detail in Spain. A knowledge of the distribution of naturally-occurring enemies is fundamental to an integrated pest management system and this has not been studied here either. Campbell (1977) found that various features in plantations of hops such us bine density, bine height, plant position, hill type, string orientation and hop variety influenced the patterns of colonisation by migrant *P. humuli* and concluded that most of these probably reflected variation in local patterns of wind shelter within which aphids could manoeuvre and land (Campbell & Ridout, 1999). In addition, edge effects tend to be greatest where there are windbreaks, which are frequent in hop gardens in the UK. Field studies of aphid distributions have identified several forms of edge effects (Lewis, 1969), with the immigrants being observed first on the lea side of the edge of the crop (Dean & Luuring, 1970; Dean, 1973). By contrast, Ruggle & Holst (1995) studied a 60 m by 40 m area within a winter wheat field and concluded that aphids were concentrated in the centre of the field when population levels were high. Longley *et al*. (1997) studied the spatial and temporal distribution of aphids and parasitoids following insecticide application in winter wheat in the UK whilst Schotzko & Smith (1991) demonstrated that the host plant (winter wheat) itself may influence aphid distributions.

A quantitative knowledge of the distribution of arthropod pests and of their natural enemies is essential for an understanding of their interactions, as well as being a prerequisite for the development of reliable sampling plans for estimating and monitoring the pest and its natural enemy abundance (Onzo *et al*., 2005). A knowledge of spatial distribution of prey and predator is important in evaluating the system's persistence and the potential of natural enemies to reduce prey density (Stavrinides & Skirvin, 2003). The spatial distribution of an insect can be employed in investigating population dispersal behavior, establishing a precise sampling scheme and for sequential sampling (Margolis *et al*., 1984), binomial sampling (Binns & Bostanian, 1990), the study of population dynamics (Jarosik *et al*., 2003), detecting pest levels that justify control measures (Arnaldo & Torres, 2005) and assessing crop loss (Hughes, 1996).

The aim of this research was to study the within-field distribution of *P. humuli* populations (apterous and winged aphids) and their natural enemies in hop plants.

Material and methods

Location and methodology of sampling

The experimental site was in León, Spain, during 2002, 2003 and 2004. A garden planted with the hop cv. 'Nugget' (0.72 ha), consisting of 40 rows (3 m apart) each with 40 plants (1.5 m apart), was chosen for the study, situated at the University of León's experimental farm maintained by the School of Agricultural Engineering. The garden was surrounded on the north by a road and on the south by arable crops (sugar beet in 2002 and 2004, and barley in 2003). The eastern boundary was a small area of mixed woodland and the western boundary was a small area of mixed vineyards and fruit trees. There were no edges or other structures to reduce the wind. The height of the wirework was 6 m with two strings per rootstock. Three hop bines were trained to each string.

Five plots for each treatment, "marginal plots" and "inner plots", were established in the field. Each plot was made up of 18 plants in three adjacent rows of six plants per row. The area of each plot was 81 m² (9 m × 9 m). Three adjacent rows were left between both treatments (Fig. 1).

Only the three central plants were sampled in each experimental plot of eighteen plants. Counts were taken in the following manner: a wooden frame measuring 20 cm by 30 cm was placed on the surface of one of the bines 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 non-destructive sampling was carried out weekly, one week measuring aphid population on the left bine of the plant, and the following week on the right one.

The population density of *P. humuli* has habitually been expressed as the number of aphids per leaf, although other parameters can be used, such as the number of aphids per dm² of leaf (Campbell, 1978), or per m² of plant (Hermoso de Mendoza *et al*., 2001, for *Aphis gossypii* on clementines). This study expresses aphid population density by number of aphids per m² of hop bine in a two-dimensional approach, by extrapolating the frame area (6 dm^2). For each treatment, the mean was calculated first for the three plants in each plot and then for its five repetitions.

Within-field distribution of aphids and natural enemies

Within-field distribution of *P. humuli* was studied, and the mean number of aphids per m² of hop bine surface for each replicate in the "marginal plots" and "inner plots" was calculated. The weekly sampling in 2002 began on 21 June

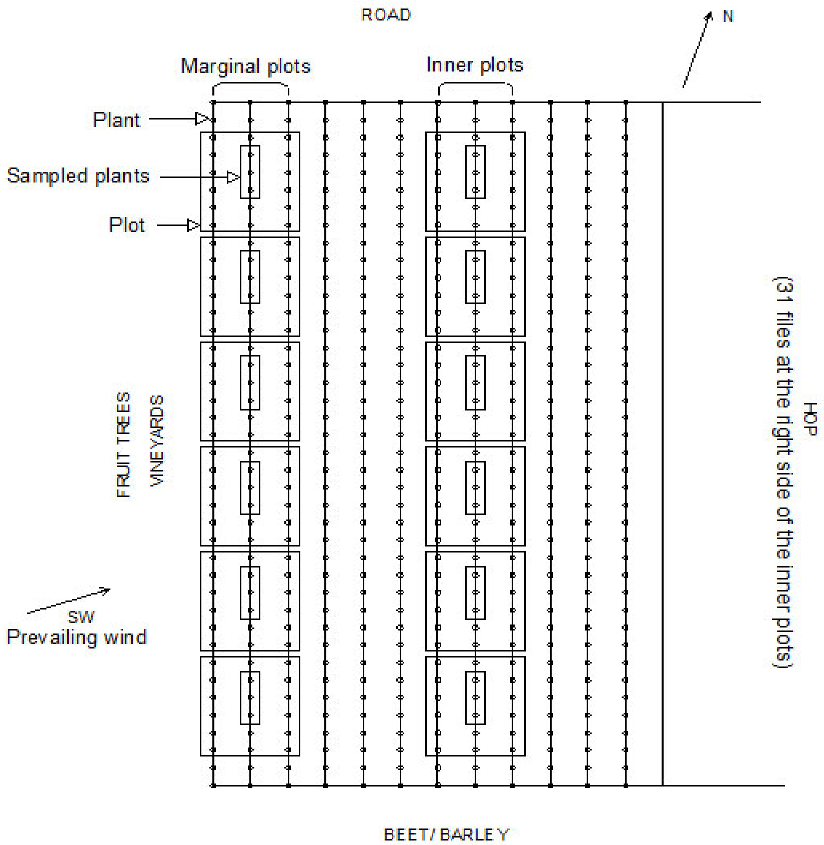

Figure 1. Diagram of sampling in the hop yard during 2002, 2003 and 2004.

and terminated on 6 September, and included just apterae. In 2003, it began on 30 May and finished on 29 August, and included both apterous and alate forms. In 2004, it began on 25 June and terminated on 3 September, and included just apterae. We started sampling when plants had reached their full height (6 m) in 2002 and 2004, whereas sampling begun before in 2003 in order to record the alatae aphid, although this was not the aim of this study.

In the same way, the distribution and frequency of the natural enemies (ladybird beetles (egg clutches, larvae and adults) and lacewings eggs) was studied in 2003 and 2004, giving the number of natural enemies per m^2 of hop bine.

In 2003, within-field distribution for winged aphids was studied in the same way as for total aphids.

Meteorological data 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 distribution of aphids.

Statistical analysis

The field data were transformed using the square-root transformation $(X + 0.5)^{½}$, where X is the original data. This transformation is appropriate for insect data especially when zeroes are present (Steel & Torrie, 1986). These square-root values were used in the analysis of variance.

Analysis of variance was performed using the general linear models (GLM) procedure. Analyses for aphid and natural enemy density for each week and for their total number between marginal and inner plots were carried out. Mean comparisons were performed using the LSD test to examine differences ($p<0.05$). Linear regressions were performed between aphids and beetles on the marginal and on the inner plots. All analyses were performed using SAS software version 9.1.2 (SAS Institute Inc., 2004).

Results

Natural enemies

Coccinelids and eggs of Neuroptera were the most abundant natural enemies found on leaves during 2003 and 2004. *Coccinella septempunctata* was the most common species in 2003 (>20 records) and this species was as frequent as the other coccinelid species in 2004 (<20 records), i.e. *Propylea quatuordecempunctata* (Linnaeus, 1758), *Adalia decempunctata* (Linnaeus, 1758) and *Adalia bipiunctata* (Linnaeus, 1758). A large number of lacewing eggs (>20 records in 2003 and 10-

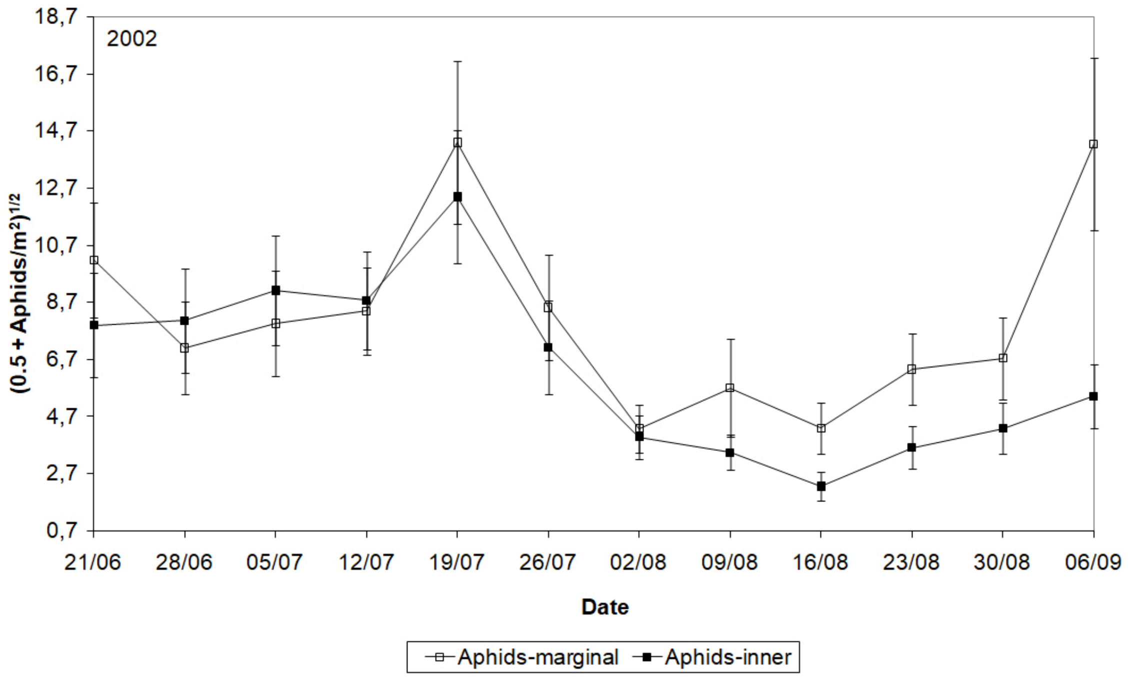

Figure 2. Within-field distribution of *P. humuli* (aphids on the marginal plots and aphids on the inner plots) on hops in 2002.

20 records in 2004) and *Aeolothrips* sp. (<5 records in 2003 and <20 records in 2004) were also registered while parasitized aphids were found only in 2003 (10-20 records) (Lorenzana *et al.*, 2013).

Within-field distribution of aphids and natural enemies

In 2002, the marginal plots contained the highest characteristic population peaks of mid July and early September (Fig. 2). The final cumulative aphid density was significantly greater in the marginal plots than on the inner plots (F=3.30; DF=1; *p*=0.0122) (Fig. 3). In 2003, the peak aphid population on the marginal plots was also significantly greater than those on the inner plots (20 June) (F=18.14; DF=1; *p*=0.0237) and also in the following week (F=24.86; DF=1; *p*=0.0155) (Fig. 4). In 2003, the final cumulative aphid density was significantly greater in the marginal plots than in the inner plots, as in 2002 (F=34.74; DF=1; *p*=0.0006) (Fig. 3). Unlike the previous two years, the highest population peaks of mid July and early September in 2004 are reached on the inner plots (Fig. 5). The final cumulative aphid density in the two treatments was not significantly different (Fig. 3).

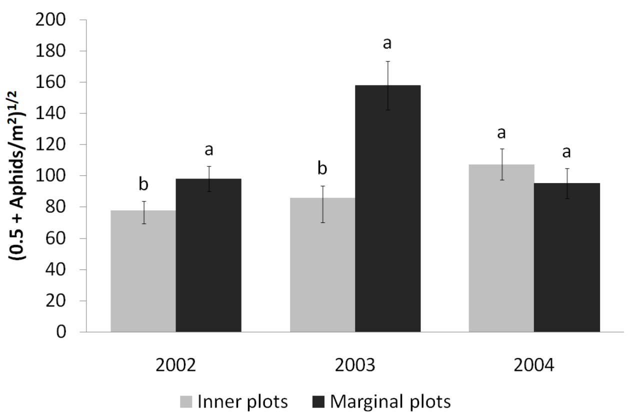

Figure 3. Within-field distribution of *P. humuli* on hops in 2002, 2003 and 2004. Columns represent the final cumulative aphid density on the marginal plots and on the inner plots. Different letters above the histogram bars indicate significant differences between groups ($p<0.05$, Tukey's test).

With regard to the natural enemies, in 2003, the final cumulative ladybird beetle (egg, larva and adult) and lacewing egg density was greatest on the marginal plots (Fig. 6). Beetle adults (F=1.12; DF=1; *p*=0.0245) and eggs (F=8.42; DF=1; *p*=0.0230), as well as lacewing eggs (F=6.16; DF=1; *p*=0.0221) were significantly more abundant in the marginal plots (Fig. 6) whereas, in 2004, the final cumulative ladybird beetle (egg, larva and adult) density was somewhat higher on the inner plots as was the number of lacewing eggs (F=14; DF=1; *p*=0.0046) (Fig. 6). Statistical analysis for each week over both years indicated that there were no significant differences between the natural predator populations between the marginal and inner plots, except for 3 September,

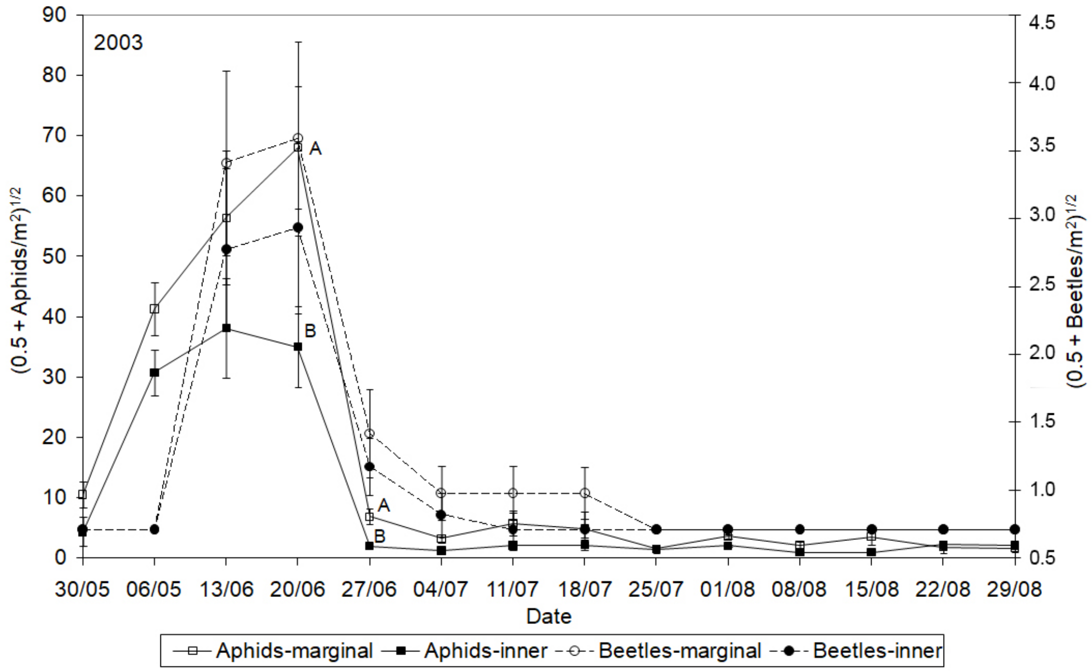

Figure 4. Within-field distribution of *P. humuli* (aphids on the marginal plots and aphids on the inner plots) and ladybirds beetles (beetles on the marginal plots and beetles on the inner plots) on hops in 2003. Mean comparisons significantly different between sides for aphids (at the same date) are shown with capital letters (p<0.05)

2004, when lacewing eggs were significantly more abundant in the marginal than in the inner plots (F=11.89; DF=1; p=0.0040) (Figs. 4–5).

Regression analyses showed a positive correlation between aphids and ladybird beetles in the marginal plots in both 2003 and 2004 (y = 0.0451x + 0.6655 and R^2 = 0.958 in 2003; y = 0.0518x + 0.5054 and R^2 = 0.846 in 2004) and in the inner plots (y = 0.06x + 0.6459 and R^2 = 0.961 in 2003; y = 0.1145x + 0.1401 and R^2 = 0.89 in 2004).

With regard to within-field distribution of winged aphids in 2003, they were most abundant in the marginal

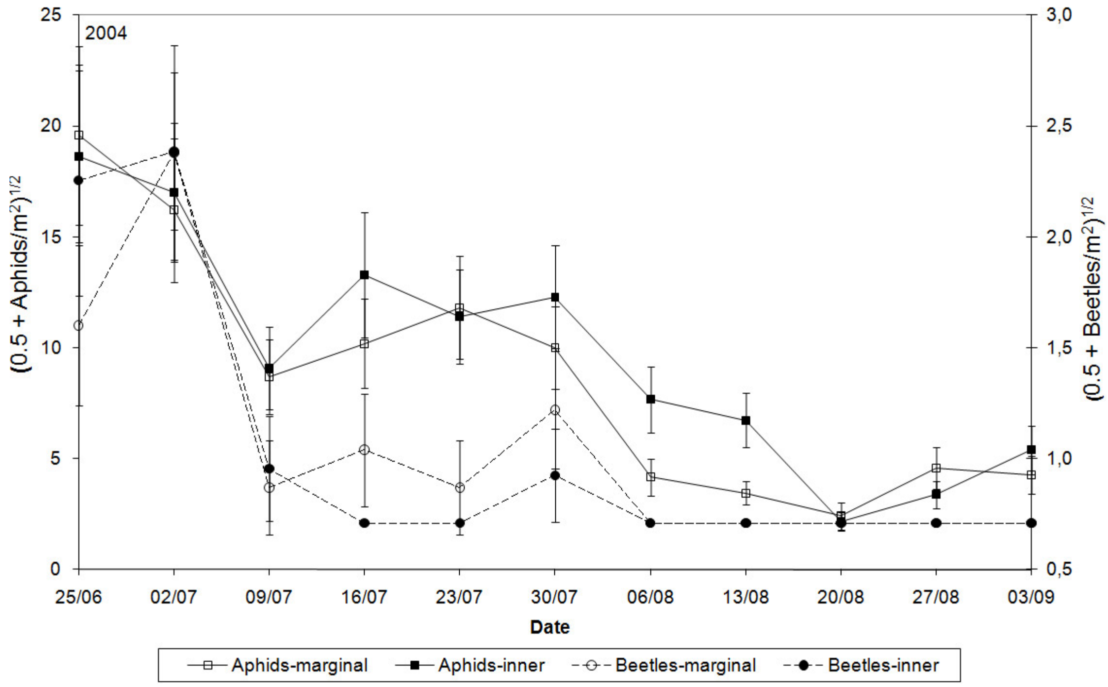

Figure 5. Within-field distribution of *P. humuli* (aphids on the marginal plots and aphids on the inner plots) and ladybird beetles (beetles on the marginal plots and beetles on the inner plots) on hops in 2004.

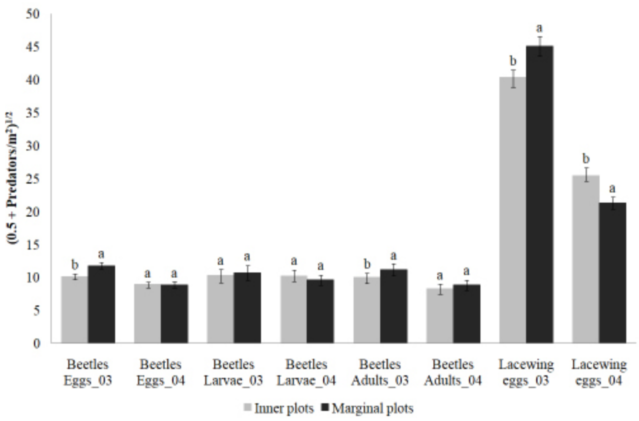

Figure 6. Within-field distribution of natural enemies of *P. humuli* on hops in 2003 and 2004: beetles adults, beetles larvae, beetles eggs and lacewing eggs. Columns represent the final cumulative natural enemy density on the marginal plots and on the inner plots. Different letters above the histogram bars indicate significant differences between groups (p<0.05, Tukey's test).

plots since the beginning of sampling (30 May) until the last alatae aphid was found (18 July), apart from on the 4 July when one alatae aphid was found in the inner plots. The final cumulative winged aphid density (spring migration) was significantly greater in the marginal plots than in the inner plots in 2003 (F=4.13; DF=1; p=0.0446) (Fig. 7).

During the period May-July (spring migration from *Prunus* spp.) the prevailing wind direction was between 200º and 340º, with a greater number of days between 200º and 250º (south-west). It was registered a greater number of days with rain and wind in 2004 than in 2002 and 2003 in the same period. Wind speed did not exceed 36 km/h between May and July in 2002 and 2003, whereas wind speed exceeded 36 km/h on ten days and 50 km/h on two days in 2004. In the same period, there was seven days with storms in 2002, five days with storms in 2003 and twelve days with storms in 2004.

Discussion

Within-field distribution of hop aphids and their natural enemies

The results obtained in 2002 and 2003 are similar to those obtained for hop aphids in other countries. Thus, Campbell (1977) in England, Ilharco *et al.* (1979) in Portugal and Wright et al. (1990) in the USA stated that the position of the plots relative to the margin influenced distribution of *P. humuli*, with more aphids to be found on the plants at the edge and on those plants close to them. If, in addition, there are wind breaks, these would have had a marked effect on the settling of the alatae, causing significant greater colonization within the more marginal rows. The fewer aphids in the marginal plots than the inner plots in 2004 might have been caused by the wind, which could have made it difficult for them to settle on the plants. When wind speed is low, aphids tend to accumulate around the edges of the plantation (as happened in the previous years); in this way, aphids could direct their flight in the slower moving air on the lea side of the hedge. Wind speed was not upon 36 km/h between the spring migration of *P. humuli* in 2002 and 2003, so aphids could fly even against the wind to land in the shelter of the plants. However, wind speed was higher in 2004, so aphids had difficulty in settling on any plants. This was confirmed by Campbell (1977) in his trials in hop gardens in England. Furthermore, the greater number of days with rain, wind and storms during the spring migration of aphids in 2004 damaged mainly the plants around the edge, making them in an unfavorable habitat for the aphids.

Similar within-field distributions have been reported for other aphid species such as *Aphis gossypii* in *Capsicum annuum* (Rahman *et al*., 2010), where aphid populations formed larger patches towards the edge of the chilli fields than in the centre, and *Rhopalosiphum padi* in cereal crops (Parry *et al.*, 2006) where the highest aphid densities were found on the field edge where the wind played a significant role in the distribution of individuals.

Edge effects may be due to a number of factors. The most important may be the effect field edges have on the deposition of small insects; this could enhance aphid immigration within the margins (Winder *et al*., 1999). Small flying insects such as aphids generally accumulate on the lea side of wind breaks (Lewis, 1966, 1969, 1970; Lewis & Stephenson, 1966). Evans & Allen-Williams (1993) argued that the edge of any crop ecosystem is where herbivores, including aphids, first locate abundant food sources, using either visual or olfactory cues. Dean & Luuring (1970) concluded that aggregation was higher at field margins because more airborn aphids become established there.

Site-specific management (*i.e*., targeted insecticide applications) is appropriate for insects that aggregate during their initial colonization; targeted applications can direct control, improve cropping economics, reduce exposure to animals and the environment, and provide refuge to natural enemies (Weisz *et al.*, 1995, 1996). Site specific management is currently recommended for the green peach aphid, *Myzus persicae* (Sulzer), along potato field edges in the USA (Suranyi *et al*., 1999, Carroll *et al*., 2009) and for the grain aphid, *Sitobion avenae* F. in the UK (Winder *et al.*, 1999). However, Nault *et al*. (2004) suggest that the soybean aphid

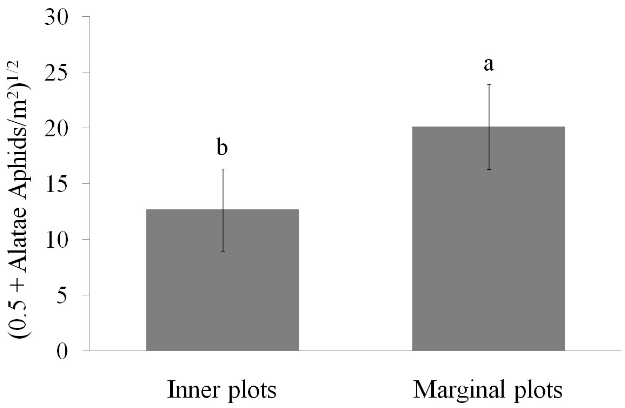

Figure 7. Within-field distribution of winged aphids on hops in 2003. Columns represent the final cumulative winged aphid density (spring migration) on the marginal plots and on the inner plots. Different letters above the histogram bars indicate significant differences between groups (p<0.05, Tukey's test).

(*Aphis glycines Matsumura*), pea aphid (*Acyrthosiphon pisum* (Harris)), corn leaf aphid (*Rhopalosiphum maidis* (Fitch)) and yellow clover aphid (*Therioaphis trifolii* (Monell)) all disperse randomly in snap bean, *Phaseolus vulgaris* L., in the USA. Differences in alatae spring colonization may be due to the overwintering potential of the aphid species and proximity to secondary hosts in the spring (Hodgson *et al.*, 2005). Landing behavior appears to vary among aphid species and cropping systems; therefore, a single management tactic is not appropriate for all aphids. The colonization behavior of *P. humuli* provides a window of opportunity for targeting aerial sprays of insecticides to field margins to control early colonizing aphids in hop gardens. This would provide direct benefits through lower production and application costs and a reduction in worker and environmental exposure. Reductions in the dry weight of the crop associated with large numbers of aphids could also be avoided (a yield loss of 44% was observed when the population rose to 4400 *P. humuli*/m² in late June in Spain) (Lorenzana, 2006). The effectiveness of targeted applications would dependent upon availability of timely information on local *P. humuli* presence and movement into hop field, taking into account that, if there were strong winds at that time, the aphids could not land at the edges of the plots.

Naturally enemy populations may be positively related to landscapes with many field margins (Landis *et al.*, 2000; Sutherland & Samu, 2000). Lady beetles appear to be able to effectively reduce hop aphid populations because they coincide spatially and temporally. These two-dimensional distributions of *P. humuli* and predatory lady beetles may also be helpful in developing accurate sampling techniques for forecasting the presence of *P. humuli* and its interaction with natural enemies.

In relation to within-field distribution of winged aphids, it has never been closely studied in Spain. As was to be expected, the total number of winged aphids was significantly greater on the marginal than on the inner plots.

In conclusion, it is important to emphasize that the position of plants within the yard influences the distribution of *P. humuli*, with more aphids occurring on the edges than inside. The number of winged aphids was also higher along the margins. The initial distribution of the aphid along field borders may make it possible to reduce insecticide usage by directing initial control efforts on field borders. However, in years with high winds, in fields without windbreaks, the aphid population may be similar within hop fields as in the marginal plots. The total number of ladybird beetles (eggs, larvae and adults) was greatest where the aphid population was most abundant. These results clearly indicate that the ladybird beetle population is an important factor to consider within an integrated management program for hop aphids, although some increase in the natural build-up of these natural enemies would be necessary in order to exploit them for control purposes.

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