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Spatial distribution of *Cydia fagiglandana* (Zeller) in an exploited holm oak (*Quercus ilex* L.) forest

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Abstract

Cydia fagiglandana (Zeller) (Lepidoptera: Tortricidae) is a carpophagous insect which affects the quality and viability of the fruits of beech, oak and chestnut trees. The majority of the works on this species in holm oak woods dealt with assessing its damages. No investigations have yet been performed on the spatial distribution of this pest, although this element is one of the key features to take in account in order to develop proper integrated management programs. The present study, performed during a four years period in an exploited holm oak forest area in Southern Spain, investigated the spatial distribution of *C. fagiglandana* integrating classic methods based on dispersion rates and distribution fitting together with the elaboration of population density maps based on GIS. Our results showed that the infestation rate progressively decreased from 0.339 in 2001 to 0.102 in 2004. Density maps for *C. fagiglandana* also show an heterogeneous and aggregated distribution of this insect and a spatio-temporal stability in this area. It is concluded that during the four years of the study inter-specific competition with the weevil *Curculio elephas* was the factor that most affected the spatial distribution of *C. fagiglandana* in the experimental plot.

Additional key words: carpophagous pests; dispersion rates; population density maps; Tortricidae.

Resumen

Distribución espacial de *Cydia fagiglandana* (Zeller) en una dehesa de encinas (*Quercus ilex* L.)

Cydia fagiglandana (Zeller) (Lepidoptera: Tortricidae) es un insecto carpófago que afecta a la calidad y viabilidad de los frutos de muchas frondosas. La mayoría de los trabajos sobre esta especie en los encinares tratan de la evaluación de sus daños. Hasta ahora, no se han realizado estudios sobre la distribución espacial de esta plaga, aunque este elemento es una de las características clave a tener en cuenta a fin de desarrollar programas de manejo integrado. En el presente trabajo, realizado durante un período de cuatro años en una dehesa de encinas en el sur de España, se ha estudiado la distribución espacial de *C. fagiglandana*, integrando métodos clásicos basados en los índices de dispersión y ajustes de distribución, junto con la elaboración de mapas de densidad de población basadas en GIS. Los resultados muestran que los índices de infestación disminuyen progresivamente del 0,339 en 2001 al 0,102 en 2004. Los diferentes mapas de densidad elaborados para *C. fagiglandana* muestran una distribución heterogénea y agregada, además de una estabilidad espacio-temporal en esta área. Se concluye que durante los cuatro años de estudio la competencia inter-específica con *C. elephas* fue el factor que más afectó a la distribución espacial de *C. fagiglandana* en la parcela experimental.

Palabras clave adicionales: carpófago; índices de dispersión; mapas de densidad poblacional; Tortricidae.

Introduction

The genus *Quercus* L. is widely spread all over the world and is commonly found in most of the forest ecosystems. Holm and cork oak are the most representative tree species in the Mediterranean forest, which is characterized by a climate with moderately wet winters and very hot and dry summers. In Europe,

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Abbreviations used: DI (dispersion index), GI (Green's aggregation index); GIS (geographic information system).

Spain has the largest extension of holm oak forests, with 2,835,839 ha (MARM, 2007). About 26% of the Spanish holm oak forests are located in Andalucía (Southern Spain) (Montoya, 1989), where they have been transformed in «dehesas», that are agro-forestal ecosystems integrating economic exploitation with the maintenance of environmental and ecological values (Scarascia-Mugnozza *et al.*, 2000; Plieninger *et al.*, 2003), in such a way that in 2002 an area of 425,000 ha of the dehesas in Sierra Morena (SW Spain) was declared Reserve of Biosphere by UNESCO. Moreover, the dehesas of Andalucía account for 37% (about 78,480 t yr^{-1}) of the total acorn production of Spain (Junta de Andalucía, 2003).

In these environments, used for cattle grazing and for Iberian pigs fattening, acorns, which are mainly eaten by the pigs, represent a food source also for small rodents and for several carpophagous insects (Leiva and Fernández-Alés, 2005). The acorn moths (*e.g. Cydia*) and weevils in the genus *Curculio* are economically important pests causing damage to fruits of oaks (genera *Quercus* L.) and chestnuts (*Castanea sativa* Miller). These insects are the most serious pests of oak and chestnut fruits in Europe; damage is caused by larvae feeding on mature acorns and nuts (Branco *et al.*, 2001; Yu *et al.*, 2003; Leiva and Fernández-Alés, 2005). In Andalucía, *Cydia fagiglandana* (Zeller) (Lepidoptera, Tortricidae) and *Curculio elephas* Gyllenhal (Coleoptera, Curculionidae) are considered the most important pests of holm oak acorns (Soria *et al.,* 1996, 2005; Jiménez, 2003), as their larvae develop inside the acorns causing premature drops, destruction of the cotyledons and a reduction in weight and size (Vázquez *et al.*, 1990; Soria *et al.*, 1996, 1999a,b; Branco *et al.*, 2001; Yu *et al.*, 2003).

C. fagiglandana has a univoltine cycle with five larval stages. In the holm oak forests of Southern Spain, adults emerge from late May to the end of October and egg-laying occurs from late June to late October (Jiménez *et al.*, 2006). Usually one egg is laid inside the new shoot leaves near the acorns. Larvae appear in acorns from middle July to late December and have an endophytic development inside the acorns lasting about 30-40 days. The mature larvae exit the acorns to overwinter in the ground or in bark cracks, protected by a cocoon. Diapause period begins in late September or early October after the exit from acorns and pupation occurs during May to September (Jiménez *et al.*, 2006).

C. fagiglandana is especially known for its damage to chestnuts and most of the studies on monitoring and control methods for this lepidopteran have been performed in chestnut groves (Rotundo and Rotundo, 1986; Angeli *et al.,* 1997). Starting in 1990, investigations on the detrimental effects of *C. fagiglandana* on acorn production and quality were performed in Spain (Vázquez *et al.*, 1990; Soria *et al.*, 1996, 1999a,b). A study on the spatial distribution of this moth was undertaken for the first time by Jiménez *et al.* (2006).

The spatial distribution of an insect within an ecosystem is usually analyzed using dispersion rates and fitting them to statistical distributions (*i.e.* Poisson or negative binomial) to detect if the population presents a random, aggregated or regular distribution (Iwao and Kuno, 1968; Cadahia, 1977; Taylor, 1984). On the other hand, it is possible to build detailed spatial distribution maps of a species over the topography of an area, thanks to the use of geographic location systems by satellite and geographic information systems (GIS) (Arbogast *et al.*, 2000; Ribes-Dasi *et al.*, 2001; Ramírez-Dávila *et al.,* 2005).

The study of the spatial distribution of pest species within an ecosystem is one of the key features to develop programs for integrated pest management (Brenner *et al.*, 1998; Blom and Fleischer, 2001; Sciarretta and Trematerra, 2006). The application of geo-statistical techniques was therefore undertaken to study the distribution of *C. fagiglandana* in a controlled area, to acquire a better knowledge on where and when to reduce the population of this pest in ecosystems with a precarious ecological equilibrium, such as «dehesas».

The purpose of this study was to analyze the spatial and temporal pattern of the infestation of *C. fagiglandana* in an exploited holm oak forest area during a four years period, with the aim of obtaining information useful to implement a sustainable management strategy of this forest pest.

Material and methods

Study site

Monitoring of *C. fagiglandana* was performed in a holm oak «dehesa» managed for cattle grazing and Iberian pigs fattening located in Castilblanco de los Arroyos, 47 km north of Seville (Spain) (UTM 30S 235517: 4182360), in the Sierra Morena Natural Park. Climate is typically Mediterranean with hot-dry summer and cool-rainy winter. Annual average temperature is 15.8°C and average precipitation is approximately

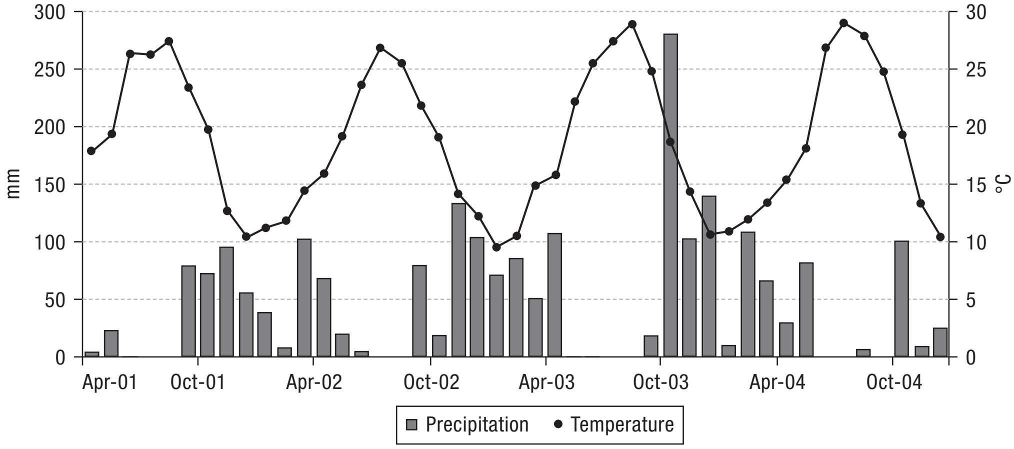

Figure 1. Monthly mean temperatures and precipitation recorded from 2001 to 2004 in the weather station of Guillena (Sevilla), the closest to the study area.

796.7 mm yr^{-1}. Average monthly temperature and precipitation recorded in the weather station closest to the study site during the sampling period are shown in Figure 1.

The experimental plot was a rhomboidal shape area of about 2 ha, with 104 randomly distributed holm oaks. In the plot, tree perimeters varied from 52 cm to 139 cm, with an average value of 89.5 cm. In 2001, the experimental plot was delimited by a metallic fence, to prevent access to the livestock (cattle and pigs).

Sampling method

All holm oaks in the plot were marked (104 trees), referenced with a high precision GPS and measured in perimeter at breast height. Twenty five fruits per tree were picked from the ground once a year, from 2001 to 2004. Acorns were collected in the area of projection of the crown of each tree. Sampling period was late November or early December when most of the fruits of the holm oak are on the ground. The harvest was performed randomly under the crown projection area. Collected acorns were dissected and analyzed in the laboratory. The number of the larvae, alive or dead, and the number of the exit holes made by the lepidopteran (each hole corresponds to an individual) were recorded. Moreover, considering that in this area acorns were infested also by *C. elephas* (no other carpophagous species were detected), also the number of fruits occupied by both pests was recorded. Population rates were calculated as infestation rate (proportion of infested acorns) and larval density (average number of larvae per acorn). Larvae obtained from the fruits were identified according to Villagrán *et al.* (2000).

Statistical analyses of data (infestation rate and larval density) were performed by means of non parametric tests Kruskal-Wallis and Spearman correlation. The level of significance was set at $p < 0.05$.

Spatial distribution analysis

The spatial distribution of *C. fagiglandana* larvae was studied using classic statistical methods and developing maps of population density. The classic methods were the Poisson and negative binomial distributions and the dispersion rates, DI (dispersion index: $DI = S^2/\mu$, S^2 = variance, μ = mean) and GI (Green's aggregation index: $GI = (DI - 1)/(n - 1)$, n = number of samples). These methods depend on the size of the sampling unit and on the relationship between the mean and the variance (Sawyer, 1989; Hurlbert, 1990). Xlstat 2006.3 software (Addinsoft SARL, USA) was used to fit the statistic distributions, using as estimation methods for the Poisson's distribution and maximum likelihood for the negative binomial. The negative binomial model, frequently used to fit aggregated populations is defined by two parameters: the mean m and the exponent k, which come from the equation $(q-p)^{-k}$, where $p = m/k$ and $q - p = 1$. Aggregation increases when k tends to 0 (Fisher *et al.*, 1943).

Four larval density maps, one per year, were obtained using the inverse distance method for interpolation of density date, with Surfer 7 software (Golden software, Golden, CO, USA). In order to study the temporal

Table 1. Summary data for *Cydia fagiglandana* in holm oaks during four years of sampling. (Infestation rate = proportion of infested acorns; larval density = number of larvae per acorns; mixed infestation = proportion of acorns affected by *C. fagiglandana* and *C. elephas*)

	2001	**2002**	**2003**	**2004**
Infestation rate of *C. fagiglandana*	0.339 ± 0.017	0.259 ± 0.015	0.119 ± 0.009	0.102 ± 0.010
Larval density of *C. fagiglandana*	0.340 ± 0.017	0.266 ± 0.015	0.119 ± 0.009	0.105 ± 0.010
Infestation rate of *C. elephas*[1]	0.320 ± 0.010	0.574 ± 0.011	0.393 ± 0.010	0.502 ± 0.011
Larval density of *C. elephas*[1]	0.436 ± 0.015	0.809 ± 0.020	0.555 ± 0.016	0.819 ± 0.023
Mixed infestation	0.045 ± 0.004	0.055 ± 0.005	0.018 ± 0.002	0.033 ± 0.004

[1] From Jiménez *et al.* (2008).

evolution of the population of *C. fagiglandana* in the area, the above-mentioned parameters were used to perform two by two comparisons of successive years, using the modification of Cramér-Von Mises' statistic test (Ψ) proposed by Syrjala (1966), which is independent from the population size because population density data are normalized by dividing each observed density by the sum of all population densities observed for the target species.

Results

Infestation rate

During the study period, the percentage of infestation of *C. fagiglandana* ranged between 10.2% (in 2004) to 33.9% (2001) and *C. elephas* from 32% (2001) to 57.4% (2002). Nevertheless, very few fruits were colonized by both insects (Table 1) and a negative correlation (Table 2) was detected all years between the larval densities per fruit of the two carpophagous insects. Significant differences were detected in the infestation rate and in the larval density of *C. fagiglandana* during the period of sampling (infestation rate, H = 134.720, $p < 0.0001$; larval density, H = 133.611, $p < 0.0001$): both rates progressively decreased from 2001 to 2004 (approximately about 24%) (Table 1). The values of infestation and larval density were very similar indicating that in most cases there was only one larva per acorn. A negative correlation occurred in all years between the larval densities of both species (Table 2). Considering the relationship between the larval density of *C. fagiglandana* and the fruit density for each tree, a significant positive correlation was detected only for the year 2001, when the density of the lepidopteran was the highest (Table 2).

The average acorn crop production from the *Q. ilex* forest in the study area was 25.49 ± 5.819 seeds m^{-2} in 2001, 23.22 ± 4.905 seeds m^{-2} in 2002, 21.5 ± 4.239 seeds m^{-2} in 2003 and 14.39 ± 2.595 seeds m^{-2} in 2004, with no significant differences among years (Kruskal-Wallis H = 7.477, $p = 0.058$).

Spatial distribution: classic methods

All the results obtained using different statistical methods (Table 3) indicated that distribution of *C. fagiglandana* larvae was aggregated during the four years. DI values were always higher than 1, increasing over the years as the infestation rates decreased. Considering the GI, values always higher than 0 indicated also a slightly and increasing aggregated

Table 2. Spearman correlation values obtained for the different years of sampling between a) larval density of *C. fagiglandana* and that of *C. elephas in fruits;* b) between larval density of *C. fagiglandana* and fruit density per tree

Spearman correlation	**2001**		**2002**		**2003**		**2004**	
	r	***p***	**r**	***p***	**r**	***p***	**r**	***p***
a) Larval density *C. fagigalandana*/ Larval densitiy *C. elephas* in fruits	–0.292	0.01	–0.421	0.01	–0.178	0.01	–0.122	0.01
b) Larval density *C. fagiglandana*/ Fruit density per tree	0.431	0.01	0.009	0.931	–0.194	0.06	–0.10	0.381

$p = 0.01$ indicate a correlation between variables.

Table 3. Dispersion rates values and statistic distributions fittings for *Cydia fagiglandana* larvae

Years	DI	GI	Poisson	Negative binomial	*k*
2001	1.790	0.0097	$p > 0.05$	$p < 0.05$	9.690
2002	1.982	0.0114	$p > 0.05$	$p < 0.05$	6.272
2003	1.978	0.0104	$p > 0.05$	$p < 0.05$	2.596
2004	2.015	0.0131	$p > 0.05$	$p < 0.05$	2.122

DI: dispersion index. GI: Green's aggregation index. *k:* negative binomial dispersion parameter

distribution of *C. fagiglandana* larvae during the study period. The same trend arises observing the *k* parameter, which is inversely proportional to the aggregation pattern. The *k* value was the lowest during 2004, showing a higher aggregated distribution during the last year of the study.

Spatial distribution: distribution maps

Distribution maps for *C. fagiglandana* obtained for each year of sampling (Fig. 2) indicated that the insect presented an heterogeneous distribution in the experimental plot during the four study years. The insect colonization occurred in form of different aggregation centres, where trees and areas with low insect density were assorted with others at higher density, this picture being spatially different every year. Comparison of distribution maps by means of Cramér-Von Mises' test (Ψ) (Table 4) did not detect significant differences in the four years of the studied period except between 2001 and 2003, suggesting a spatio-temporal stability of *C. fagiglandana* larvae population.

Discussion

C. fagiglandana is one of the most important carpophagous insect in holm and cork oak forests in the south of the Iberian Peninsula (Soria *et al*., 1999a,b; Villagrán *et al*., 2000). The present study showed that during the study period, the holm oaks had differents infestation rates of *C. fagiglandana*, decreasing overtime from 33.9% to 10.2%.

Table 4. Comparison of distribution maps with Cramér-von Mises' bivariable test

Years	Ψ	*p*
2001 *vs*. 2002	0.078	0.672
2001 *vs*. 2003	0.464	0.030
2001 *vs*. 2004	0.096	0.940
2002 *vs*. 2003	0.277	0.122
2002 *vs*. 2004	0.113	0.888
2003 *vs*. 2004	0.361	0.160

Ψ: statistic value. *p*: probability.

An important role in the change of acorn infestation rates observed during the four years of sampling must have been played by the setting of a metallic fence in 2001 to prevent the entrance of the Iberian pigs in the study area. The absence of acorn foraging by pigs in the plot likely allowed a higher number of larvae of *C. elephas* and *C. fagiglandana* to complete their development leading to the observed population increase of this weevil (Jiménez, 2003), however it was observed that the population of *C. fagiglandana* decreased overtime (Table 1). Although both carpophagous insects begin to colonize the acorns at the same time, by mid September (Jiménez *et al*., 2005a, 2006), the number of fruits with mixed attacks was very low and a significant negative correlation appeared between the larval densities of the moth and the coleopteran during the four years of sampling (Table 2). These results indicate repulsion between the two insect species and are in agreement with findings from Debouzie (1984) and Jiménez *et al*. (2005b). According to Soria *et al*. (1999a) and Jiménez *et al.* (2006) the larvae of *C. fagiglandana* are able to select intact fruits, rejecting the acorns already colonized by other larvae, whereas *C. elephas* females are unable to discriminate fruits hosting other larvae (Desouhant *et al*., 1998). In chestnuts infested by both insects, Debouzie *et al.* (1996) observed that there was no direct competence for food between moth and weevil larvae, therefore when these two carpophagous species are inside an acorn both are able to complete larval development within it. However, it is important considering that the oviposition of *C. elephas* causes premature falling of acorns (Soria *et al.*, 2005; Bonal and Muñoz, 2008), thus reducing the number of acorn that are available for attack to moth larvae and this may be have also contributed to the

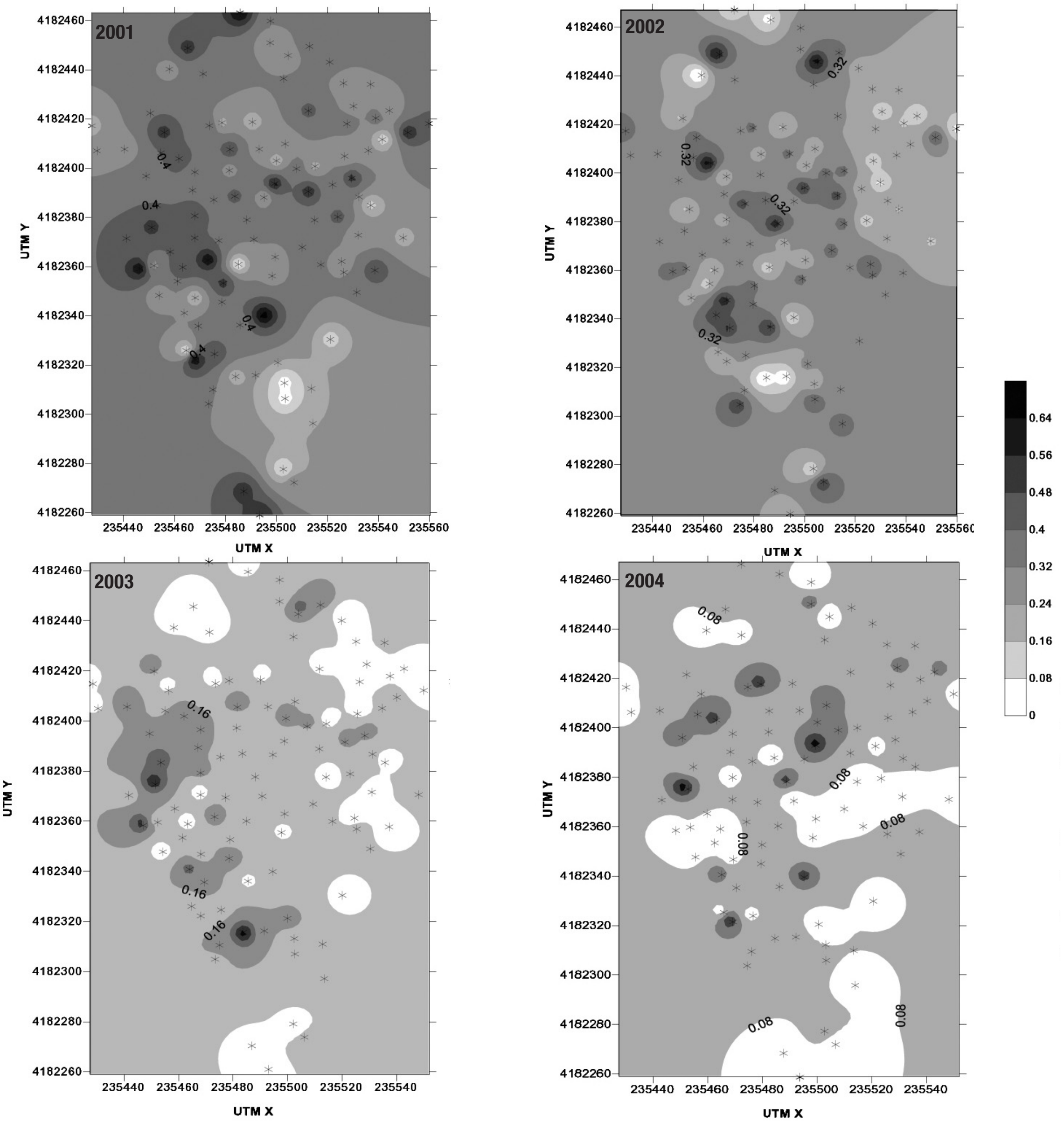

Figure 2. Larval density maps of *Cydia fagiglandana* from 2001 to 2004.

apparent progressive displacement of *C. fagiglandana* by *C. elephas* in the study area.

On the other side, values of the distribution indexes obtained during the years of the study indicate that the moth distribution in the acorns was uniform (Jiménez *et al*., 2006) and that a negative discrimination of the acorns attacked by conspecificis also ocurred, as suggested by the results (Table 1) and the observation that less than 2% of infested fruits hosted two *C. fagiglandana* larvae (Jiménez *et al*., 2006; Torres-Vila *et al*., 2006).

Weather conditions are among the most important factors affecting insect population dynamics. In the study area, whereas temperature trend was similar during 4 years (Fig. 1), the exceptionally high precipitation recorded during October 2003 might have caused a decrease of the population of *C. elephas* as the water excess tended to accumulate in some parts of the plot (mainly in the central-left area) likely causing the drowning of the curculionid larvae and pupae in the ground, thus resulting in a lower percentage of emerged adults and therefore lower larval densities in the following year (Menu, 1993; Jiménez *et al.*, 2006). This fact may also have contributed to the observed population reduction of *C. fagiglandana* that year, as heavy rain prevent the moth from flying and washes off the eggs and neonate larvae (Ozols, 1950; Gharekhani, 2009).

The distribution detected for *C. fagiglandana* may have also been affected by the host selection behaviour operated by the larva. Factors that might affect directly the selection process by the larvae are the fruit quality and quantity or the masting of the host plant (there are holm oaks that do not produce fruits every year) (Kelly, 1994; Herrera *et al.*, 1998). In particular, fruit quality is a key factor in fruit selection by carpophagous insects. Although adults of *C. fagiglandana* show a very long flight period, from June to October, no larvae can be detected inside the fruits before the beginning of October, when the fruits are more mature (Jiménez *et al.*, 2006). Such a preference for mature acorns is shared with *C. elephas* females when ovipositing (Debouzie *et al.*, 2002; Soria *et al.*, 2005), and this may increase the interespecific competition for the food resources among the moth larvae. On the other hand, during the sampling period no differences were detected in the acorn production by the holm oaks in the experimental plot, therefore it seems unlikely that a reduction in the produced acorns might have affected the distribution of *C. fagiglandana* larvae.

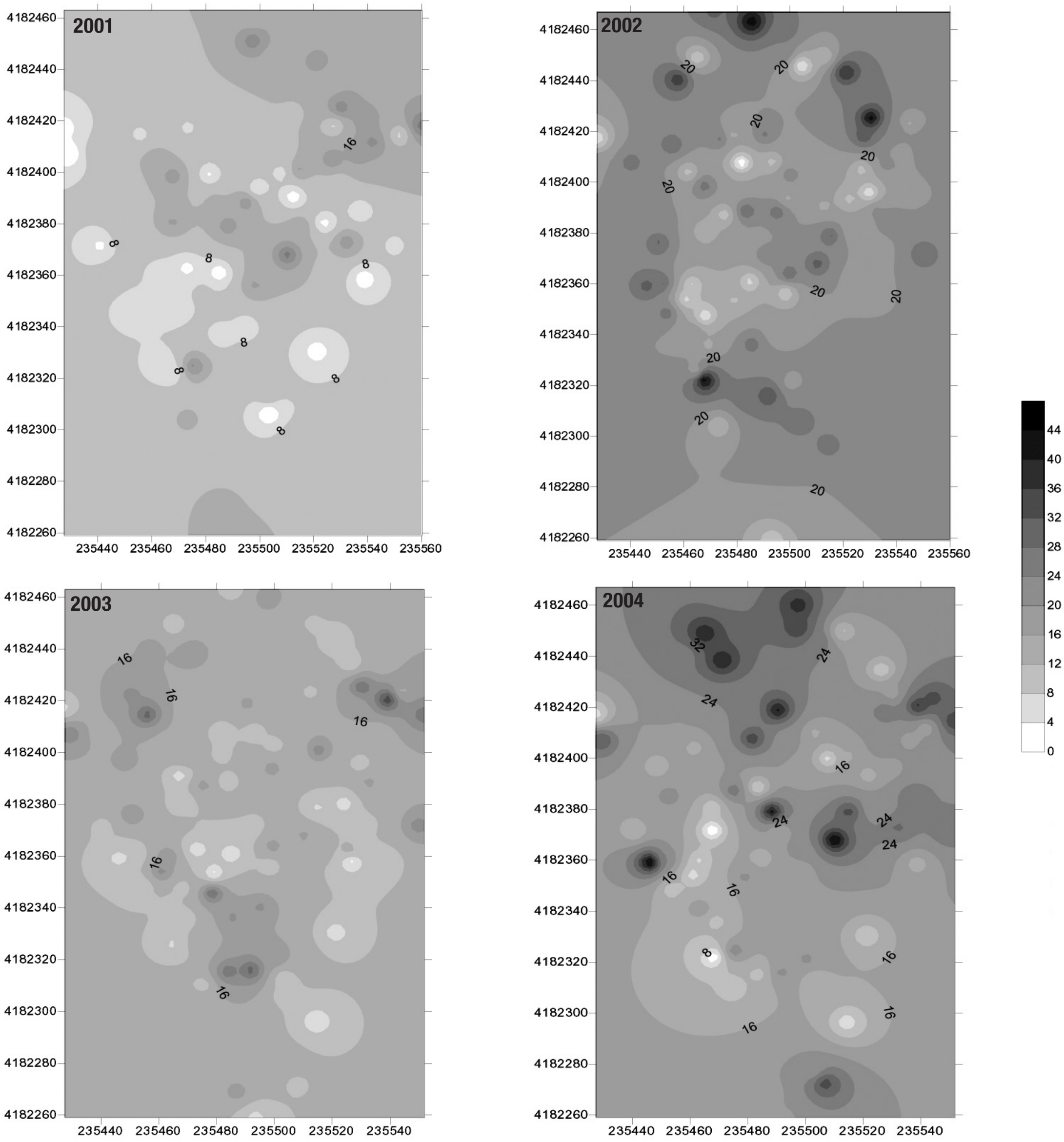

Figure 3. Percentage of larval density maps of *Curculio elephas* from 2001 to 2004. Axes = UTM coordinates (from Jiménez *et al.*, 2008).

From the results on dispersion rates and statistic distributions it emerged that during the four years of the study the spatial distribution of *C. fagiglandana* in the sampled plot was aggregated, although in a low degree. Moreover, fitting the data to the negative binomial distribution for all the considered years further confirmed the aggregated distribution pattern of this insect. It is interesting to highlight that as the infestation rates decreased, the spatial distribution indexes showed values indicating increasing aggregation, which is apparently incongruous with the fact that *C. fagiglandana* negatively discriminates the already colonized acorns, and should therefore present a uniform distribution (Jiménez *et al.*, 2006).

The maps of *C. fagiglandana* larval density show an aggregated and heterogeneous distribution inside the plot. As it is shown in the maps, decline of larval density coincided with a reduction of the centers of aggregation, and this is due to the fact that both variables are directly related (Jumean *et al.*, 2009). As a matter of fact, a comparison of the distribution maps for *C. fagiglandana* with the those for the curculionid in the same area and period (Jiménez *et al.*, 2008; Fig. 3), shows that the aggregation centers of the moth are localized in the areas which were not occupied by the weevil. This may be observed in the year 2003 (Fig. 2), when it was detected a displacement of the aggregation centers of *C. fagiglandana* in the central area of the plot towards the left, to the area where recorded flooding likely killed most of the underground *C. elephas* larvae.

The use of the distribution maps and correlated statistics allowed detecting a spatio-temporal stability for *C. fagiglandana*, more evident during the first two years when the moth density was higher, but nevertheless maintained during the whole study period. This indicates that, although the spatial structure of the population of this moth can change over the years, its stability remains unchanged.

Among all the above-mentioned factors, we conclude that inter-specific competition was the one that most affected the spatial distribution of *C. fagiglandana* in the experimental plot. During the four years of the study, the presence of the weevil caused a reduction of acorns available to the moth, due to two facts: 1) a negative discrimination of the acorns attacked by *C. elephas* and 2) the premature fall of acorns colonized by weevil. Therefore, infestation rates by *C. fagiglandana* were reduced and its spatial distribution was more aggregated on those areas and trees with a lower presence of the weevil.

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