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Seasonal and annual trends in field populations of Mediterranean fruit fly, *Ceratitis capitata,* in Mediterranean citrus groves: comparison of two geographic areas in eastern Spain

M. T. Martínez-Ferrer[1], C. Navarro[2], J. M. Campos[1], C. Marzal[2], J. M. Fibla[1], L. Bargues[2] and F. Garcia-Marí*

[1] *IRTA Amposta. Ctra. de Balada, km 1. 43870 Amposta (Tarragona). Spain*
[2] *Institut Agroforestal Mediterrani. Universitat Politècnica de València. Camí de Vera, 14. 46022 València. Spain*

Abstract

Seasonal and annual trends in *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) populations were analyzed to determine the factors that influence population fluctuations in the field. Adult flies were monitored along 2003-07 in two citrus areas in eastern Spain with similar climate, Valencia and Tarragona. Adults were present throughout the study period, even in winter. The initial annual population increase was related to previous winter and spring temperatures. Captures started to increase in April-May and usually reached a peak in July. This peak corresponded to the maximum capture period in Valencia, but not in Tarragona, where there was usually a second peak in autumn, with capture levels similar to the first peak. Gravid females were found throughout the year, even in overwintering populations of medfly. The availability of other host fruit species in the vicinity of the citrus groves may explain the differences in annual abundance and distribution of captures between the two areas studied.

Additional key words: medfly, population dynamics, temperature, Tephritidae.

Resumen

Tendencias estacionales y anuales en las poblaciones de campo de la mosca mediterránea de la fruta, *Ceratitis capitata,* en cítricos del Mediterráneo: comparación de dos áreas geográficas en el este de España

Se analizó la evolución estacional de las poblaciones de *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) para determinar los factores que influencian sus fluctuaciones poblacionales en campo. Se muestreó la población de adultos entre 2003 y 2007 en dos áreas citrícolas del este de España con clima similar, Valencia y Tarragona. Se encontraron adultos a lo largo de todo el periodo de estudio, incluso en invierno. El primer incremento anual de la población estuvo relacionado con las temperaturas previas del invierno y la primavera. Las capturas comenzaron a incrementarse en abril-mayo y generalmente alcanzaron un máximo en julio. Este máximo correspondió al máximo periodo de capturas en Valencia, pero no en Tarragona, donde hubo generalmente un segundo máximo en otoño, con niveles de captura similares al primer máximo. Se encontraron hembras grávidas a lo largo de todo el año, incluso en las poblaciones invernantes de mosca de la fruta. La disponibilidad de otras especies de frutales hospedantes cercanas a las parcelas de cítricos puede explicar las diferencias en abundancia anual y distribución estacional de capturas entre las dos áreas estudiadas.

Palabras clave adicionales: Tephritidae, mosca de la fruta, dinámica poblacional, temperatura.

Introduction

The Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is one of the most serious pests affecting cultivated plants in the world. It is highly polyphagous and attacks more than 300 different fruit species (Christenson and Foote, 1960; Liquido *et al*., 1991). Its life strategy includes changes of host species throughout the year, because larvae develop inside fruits only when they are mature, adults survive a long time in the field, and they disperse rapidly when no mature fruits are available in a particular area (Fletcher, 1989). *Ceratitis capitata* is a species of Afrotropical origin which has adapted to the climatic conditions of the Mediterranean basin,

* Corresponding author: fgarciam@eaf.upv.es
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where it has been present for many years causing great damage to citrus and other fruits (Delrío, 1986; Fimiani, 1989; Franco *et al*., 2006).

Citrus are cultivated all along the Mediterranean coast, where climate is characterized by seasonal changes in average temperature, with a growing season from spring to fall, in which medflies proliferate, and a cool season in winter, in which field populations and damage are normally very low. *Ceratitis capitata* populations barely survive winter as larvae (Papadopoulos *et al*., 2001), or are unable to survive (Israely *et al*., 2004), towards the northern limit of its Mediterranean distribution. However, winter survival of medfly is greater in coastal areas, where citrus fruits are grown (Mavrikakis *et al*., 2000). During this cool period of the year, when average temperatures fall below 14-15°C, medfly populations survive in reduced numbers as either eggs or larvae inside fruits, as pupae in the soil, or as adults. Medfly populations usually increase in the growing season, from May to July, and then fluctuate until the end of autumn, when decreasing temperatures reduce the population of flying adults (Bodenheimer, 1951; Fimiani, 1989; Del Pino, 2000).

The fluctuations of *C. capitata* populations differ from year to year in the same area, and also between different areas in the same year, in Mediterranean citrus groves. In some areas, the adult population peaks at the end of spring or at the beginning of summer, as observed in Israel (Rivnay, 1950) and Crete (Michelakis, 1992), whereas in other areas populations grow throughout the summer and reach maximum levels in fall, as in Sardinia and Sicily (Italy) (Delrío, 1986; Benfatto *et al*., 1987; Ortu *et al.,* 2005). Several factors are considered directly or indirectly responsible for these variations in *C. capitata* populations: climate, abundance and temporal sequence of suitable host fruits throughout the year, and crop sanitation practices (Harris *et al*., 1993; Katsoyannos *et al*., 1998).

Eastern Spain has a heterogeneous fruit growing area which extends all along the coast of Iberian Peninsula, from north to south. This area includes a wide range of citrus varieties, which coexist with other kinds of fruits. *Ceratitis capitata* mostly overwinters in citrus groves, where damage is initially produced at the end of spring in late-maturing varieties like Valencia oranges. Between June and August there are no suitable host fruits in citrus groves, since all citrus fruits are unripe in this three-month period. The most important damage to citrus fruits is produced between September and November, when satsuma (*Citrus unshiu* Markovitch) and clementine (*Citrus reticulata* Blanco) mandarins reach maturity and suffer heavy attacks (Gómez Clemente and Planes, 1952; Martínez-Ferrer *et al*., 2006). Traditional control methods for reducing medfly populations and damage in citrus groves rely on the use of chemical sprays applied to fruits near harvest. Problems of toxicity, residues left on fruits and impact on beneficial arthropods arise as a consequence of this strategy. Alternative strategies for reducing or suppressing medfly populations include releasing sterile males and mass-trapping techniques. These new strategies require a thorough knowledge of seasonal changes and of the factors that influence medfly population dynamics. This paper analyzes and compares seasonal and annual trends in *C. capitata* populations in two citrus producing areas of eastern Spain with similar climatic conditions in an attempt to determine the factors that influence the fluctuations observed in the field.

Material and methods

The study was conducted in two citrus growing areas in eastern Spain, Tarragona and Valencia, over a five-year period, from 2003 to 2007. The Tarragona area was located in the southern part of Tarragona province, and the Valencia area was in the center of Valencia province.

Environments of citrus groves in Tarragona and Valencia

The Tarragona citrus area (40.23° N, 0.34° E) was located towards the north of the Spanish citrus belt and has an area of approximately 10,000 ha. This citrus growing area is largely located along the banks of the Ebro river and up to 20 km to the south of it. It is at low altitude (< 200 m) and located within 30 km of the Mediterranean Sea. Various varieties of clementines account for more than 60% of this area's citrus production. These varieties are very sensitive to attacks from *C. capitata*. In this area, it is common to find some isolated fruit trees, grown for personal consumption, either in the citrus groves or near to them. Figs (*Ficus carica* L.) and jujubes (*Ziziphus jujuba* Mill.) are the most common fruits, but on occasion, loquats (*Eriobotrya japonica* Thumb.), apricots (*Prunus armeniaca* L.), peaches (*Prunus persica* L.) and Indian figs (*Opuntia ficus-indica* L.) can also be found.

The area selected in Valencia (39.14°C N, 0.28° W) was located 200 km to the south of the Tarragona area and the citrus groves were within 30 km of the sea and at low altitude (<200 m). This area included the coastal plain extending 5 km to the north and 30 km to the south of the city of Valencia, with a total citrus growing area of around 15,000 ha. The main citrus species grown in this area include sweet oranges [*Citrus sinensis* (L.) Osbeck] and clementines. Scattered fruit trees for personal consumption are also very common. These include peaches, apricots, loquats, persimmons (*Diospyros kaki* L.), plums (*Prunus domestica* L.) and figs. There are also many regular plantations of fruit trees, mostly of early-producing varieties of peaches and apricots, which mature between May and July. The presence of these regular plantations of fruit trees marks an important difference between the two areas compared in this study.

The climate in the two areas sampled is typically Mediterranean, with average monthly minimum and maximum temperatures of 20 and 30°C, respectively, during the summer months, and of 5 and 15°C in winter (Fig. 1). Minimum recorded winter temperatures between December and March dropped below 0°C for only 2 to 10 days per year during the five-year study period. Maximum daily temperatures recorded during the five-year study period were between 36 and 38°C. Meteorological data were obtained from representative stations in towns located to the north and south, and in the middle of each study area (in Amposta, Aldover and Alcanar in Tarragona, and in Benifaió, Carlet, Carcaixent, Moncada, Picassent and Sagunt in Valencia).

Groves and monitoring

In the Tarragona area, 25 groves were selected in 2003 and 2004, and five in 2005 to 2007. The number of groves was reduced in 2005-2007 because the initial number of groves (25) was too labor-intensive and we realized from our data that five groves selected in representative localities sufficed to obtain population trends adequate for our study. All selected plots were commercial clementine groves. In the Valencia area, seven groves were selected for the whole 2003-2007 period, with three producing clementines, two producing Fortune hybrids (Clementine × Mandarin 'Dancy') and two producing sweet oranges. We considered the overall population trend of *C. capitata* populations obtained with these group of groves as representative of the citrus area (of around 15,000 ha) where they were located. All groves were commercial plantations in full production and were representative of the crops grown in the area. Their surface area ranged from 0.5 ha to 2 ha. Cultural practices were standard for the area and included regular chemical spraying to control aphids, diaspidids and mites, and occasional applications of bait sprays for medfly.

C. capitata adults were monitored using Tephri-trap (Kenogard) traps baited with male-specific parapheromone trimedlure (Aralure™, Agrisense) or female-targeting attractants: ammonium acetate, putrescine and trimethylamine (Tripack™, Kenogard). DDVP (Econex) was used as a toxicant in all traps. Trimedlure plugs were changed every 90 days and female-targeting attractants and DDVP were replaced every 45 days.

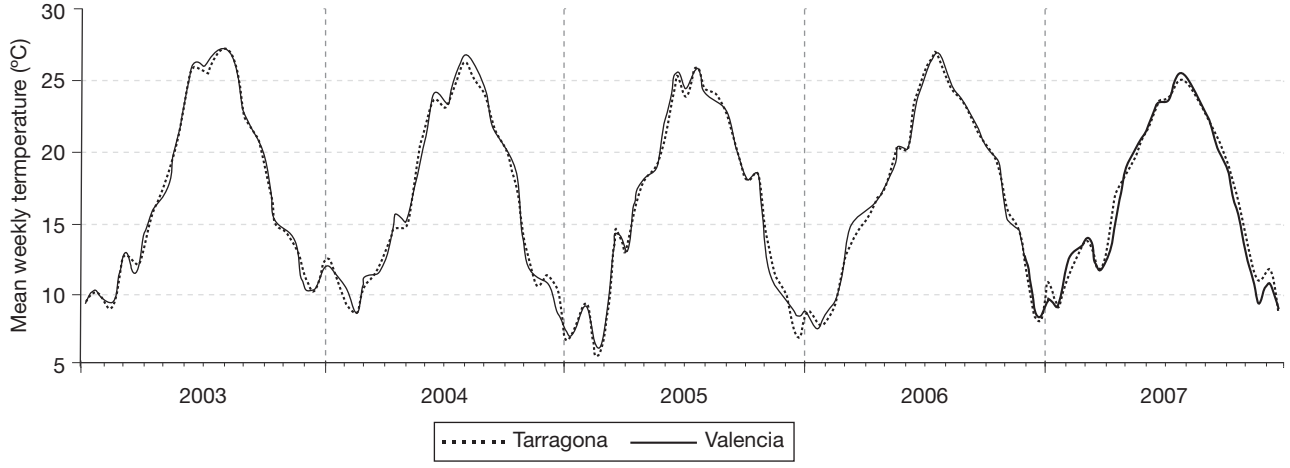

Figure 1. Mean weekly temperatures in the two areas studied, Tarragona and Valencia, between 2003 and 2007. Average values for three weather stations in Tarragona and six in Valencia.

Traps were placed on trees in each grove at a height of 1.5-2 m above the ground. They were positioned facing south, in a shaded part of the canopy, and spaced ~50 m apart to prevent interference.

Four traps per grove were applied in Tarragona (two of trimedlure and two of Tripack) in 2003 and 2004, and seven (one of trimedlure and six of Tripack) from 2005 to 2007. In the Valencia area, two traps were placed per grove (one of trimedlure and one of Tripack) in 2003 and 2004, and one of trimedlure in the period 2005 to 2007. In winter and early spring (January to May), the number of traps per grove in Valencia was increased to six (three of trimedlure and three of Tripack) in 2004 and 2005, and to three of trimedlure in 2006 and 2007.

The traps were checked once a week or once a fortnight, with all of the captured *C. capitata* adults being counted and separated according to sex. Adults were removed after each visit. Captured adult females, up to a maximum of 20 per trap, were taken to the laboratory, where their ovarian development stage was determined under a stereoscopic microscope. To do this, their abdomens were dissected and females were divided into three different categories, according to their ovarian development: (1) non-gravid females, without developed ovaries; (2) females with partly developed ovaries, with some ovarioles that were not fully developed; and (3) gravid females, with all of their ovarioles fully developed.

Total fly captures per trap and year were compared with a factorial ANOVA, taking site and year as the main factors. Only data for trimedlure traps were included in this analysis. Comparisons of captures using trimedlure and Tripack attractants were analyzed with a one-way ANOVA. Before analysis, yearly capture values were log10 transformed to correct for departures from normality. Two separate one-way ANOVAs were used to examine the influence of site and type of attractant on the date of the first population peak. Simple linear correlation was used to examine the relationship between the Julian date (day of the year) of the first population peak in each year and temperatures during the previous winter and spring. The average daily temperature between January and June was used as an estimator of temperatures during winter and spring. Statistical analyses were performed using the SAS statistical software (SAS Inst., 2002-2003).

Results and discussion

Seasonal abundance of adult medfly throughout the year in Valencia and Tarragona

Captures of medfly adults generally differed between traps in the same grove and between traps in different groves in the same geographical area, because local factors acting at short distance from a trap can have a major influence on the number of flies captured. Proximity to mature infested fruits and local climatic conditions, such as temperature and exposure to direct sunshine or wind, can produce dramatic changes in the number of captures. Annual fly captures per trap ranged from a minimum of 31 to a maximum of 10,604 when the attractant used was trimedlure, and from 11 to 2,057 with Tripack (Table 1). Differences in the average number of captures between the two areas (Tarragona and Valencia) depended on the year considered with captures considerably higher in Valencia in 2003 and 2004 and in Tarragona between 2005 and 2007 (for trimedlure data, year: $F = 1.13$, $df = 4, 130$, $P = 0.3451$; area: $F = 0.11$, $df = 1, 130$, $P = 0.7436$; interaction between year and area: $F = 6.32$, $df = 4, 130$, $P < 0.0001$).

Table 1. Number of *Ceratitis capitata* adults (mean ± SEM) captured per trap, per year in the Tarragona and Valencia areas

Year	Attractant[a]			
	Trimedlure		Tripack	
	Tarragona	Valencia	Tarragona	Valencia
2003	1,002 ± 167 (31-3,501)	4,616 ± 811 (978-10,604)	487 ± 90 (15-1,896)	1,574 ± 283 (1,031-1,984)
2004	792 ± 137 (45-2,498)	2,641 ± 701 (630-4,860)	268 ± 56 (11-1,181)	1,201 ± 290 (253-2,057)
2005	3,209 ± 676 (1,659-4,486)	1,357 ± 236 (88-4,156)	743 ± 206 (276-1,140)	—
2006	3,034 ± 566 (985-4,956)	1,708 ± 310 (199-5,970)	778 ± 53 (612-908)	—
2007	2,789 ± 1,185 (1,192-8,074)	1,822 ± 297 (622-6,635)	902 ± 274 (290-1,892)	—

[a] In brackets, minimum and maximum number of flies captured per trap and year.

The reason for this shift could be related with seasonal differences in climate between years, which affect medfly population differently, and with differences in the period of fruit maturity between the two areas sampled. The average number of flies captured per trap and year was almost three times greater when trimedlure was used as attractant (1,610 ± 217) than with Tripack (593 ± 66) ($F = 110.5$; df = 1, 70; $P < 0.0001$).

A characteristic population pattern over the year was observed in each area (Tarragona and Valencia) during the five years of the study when all the traps in a given area were considered together (Fig. 2). This population trend was similar for the two types of attractants used, trimedlure and Tripack. Captures were very low from January to May and showed a substantial increase in June. Adult captures peaked generally in July. The date of this first population peak occurred at a similar time in both trimedlure and Tripack traps ($F = 0.43$; df = 1, 10; $P = 0.5272$) and in the Tarragona and Valencia areas ($F = 0.40$; df = 1, 10; $P = 0.5416$). However, it changed from year to year and was closely related to previous winter and spring temperature ($r^2 = 0.7879$; $F = 55.73$; df = 1, 15; $P < 0.0001$). The lower the average daily temperature between January and June, the later this peak occurred. The year 2005 was the coldest of the five years (8.7°C in Tarragona and 8.9°C in Valencia) and medfly populations increased one month later than usual in both areas. The warmest temperatures were observed in 2007 (11.7°C and 11.8°C in Tarragona and Valencia, respectively) and medfly populations increased earlier in this year.

There was usually a decline in adult captures in August, which was more or less marked according to the grove and year. Later, there was a new increase in adult captures in Tarragona between September and November, with captures reaching similar levels to those of the first peak. This pattern was not observed in Valencia, where the population never returned to the level of the first peak of July and captures generally remained low until November. This result confirms previous observations in Valencia region, which show a first population peak in July much higher than the population level observed in autumn (Alonso Muñoz and Garcia Marí, 2003). Adult population generally

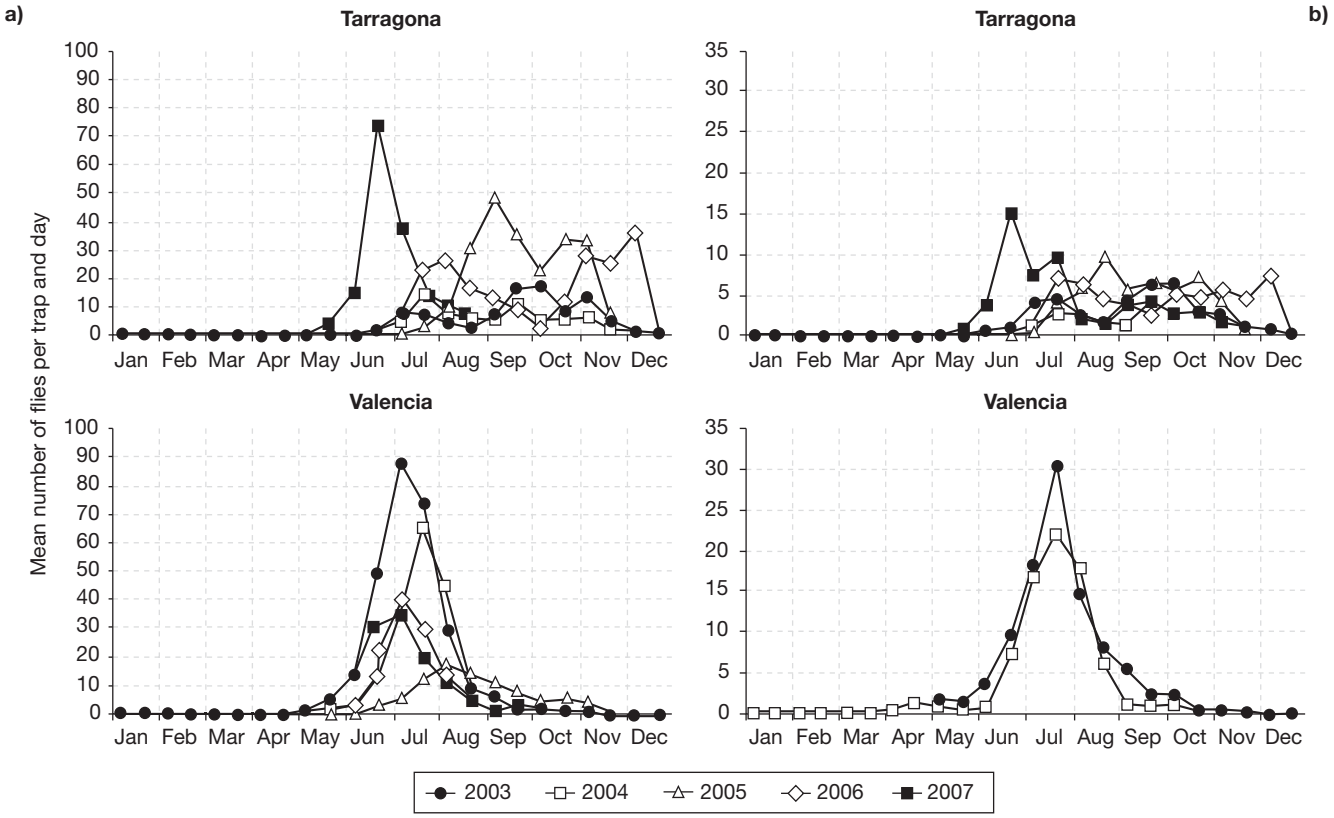

Figure 2. Seasonal *C. capitata* adult captures between 2003 and 2007 in citrus orchards in Tarragona and Valencia. a) Using trimedlure as attractant. b) Using Tripack as attractant.

began to decline in November, presumably due to the rapid descent in temperatures.

The annual trend in medfly abundance also varied between the citrus groves sampled. Some groves sampled in Tarragona showed a population peak in summer, others in autumn, and some others in both summer and autumn. These different patterns were not related to geographic locality, but rather to the presence of mature host fruits in the vicinity of the groves sampled. The summer peaks were probably related to loquats, apricots or varieties of peach maturing in that season, whereas the autumn peaks were probably related to nearby fruits like figs, Indian figs or jujube, and also to early-maturing citrus varieties (Martínez-Ferrer *et al*., 2006). Fig fluid represents an important source of exogenous nitrogen for adult *C. capitata* (Hendrichs and Hendrichs, 1990). The fecundity and longevity of flies feeding on figs has been shown to be relatively high (Hendrichs *et al*., 1991). Furthermore, *C. capitata* larval development time on fig fruits is relatively fast and larval survival rates are moderate to high (Rivnay, 1950; Carey, 1984). The presence of these fruits together with jujube trees in the Tarragona zone would therefore provide an exceptional potential breeding site for generations that could later attack clementine varieties in September.

The differences in abundance pattern observed between the two areas is worthy of comment, since their climatic conditions were very similar (Fig. 1). All the traps in this study were placed inside citrus groves which contained mature fruits suitable for medfly only from October until January or February of the following year, depending on the citrus species or variety. The high levels of insect captures in July, especially in the Valencia area, clearly showed that the flies in citrus groves in this period must have come from other types of fruits in the vicinity of these citrus groves.

The flight pattern of medfly in cooler areas, located towards the northern limits of its distribution, differs from that of warmer areas as the ones we have studied. The initial increase in population after winter occurs usually in August in these cooler areas, as in the mountainous areas of Israel (Israely *et al.,* 1997), northern Greece (Papadopoulos *et al*., 2001) and north-eastern Spain (Escudero *et al.,* 2008). However, this first annual increase starts earlier, in June or July, in warmer areas, as Granada (southern Spain) (Ros *et al*., 1979), Sicily (Benfatto *et al*., 1987), Sardinia (Ortu *et al.,* 2005) and in our study. The population trend throughout the year varies in these warmer areas when different localities or countries are compared. Thus, population peaks do not occur in the same period. Whereas in Valencia, in our study, the maximum annual peak occurred in July, in Granada this peak was observed at the beginning of August (Ros *et al*., 1979), in Sardinia it occurred in October (Ortu *et al*., 2005), and in Sicily in November (Benfatto *et al*., 1987). Bimodal and plurimodal distributions, like the one found in Tarragona, have also been reported. In Crete, adult populations exhibit one peak during the June-August period and another in September (Michelakis, 1992). On the island of Chios (Greece), the population peaks in August and November (Katsoyannos *et al*., 1998). In coastal parts of Israel, flies are continuously caught from May until mid-December, showing three waves of population, in May, July and August-September (Israely *et al*., 1997).

These variations in population patterns are probably the result of differences in the availability of mature fruits in these areas. *Ceratitis capitata* moves from one fruit species to another according to their maturity periods (Gómez Clemente and Planes, 1952). Israely *et al.* (1997) observed that flies are captured at different times following the ripening of different host fruits. They also observe that population peaks are related to the presence of mature fruits in either the same grove or in nearby groves. The high capture levels observed in our study in the Valencia area in July were presumably related to the presence of regular plantations, or isolated trees, of early-maturing fruits like apricots, nectarines and peaches, which were scattered throughout the citrus growing area. The second population peak observed in autumn was higher in Tarragona due probably to the higher abundance in this area of scattered fruits which reach maturity at the end of the summer, especially fig and jujube trees.

Female ovarian development throughout the year

We found the three physiological female stages considered (non-gravid females, females with partly developed ovaries, and gravid females) present throughout the year. The mean percentages of non-gravid females were very similar in Tarragona (59.5 ± 3.6%) and Valencia (60.6 ± 2.7%). Some general trends were observed when the ovarian development of *C. capitata* females captured in the citrus groves were analyzed as a monthly average of all captures in the groves sampled

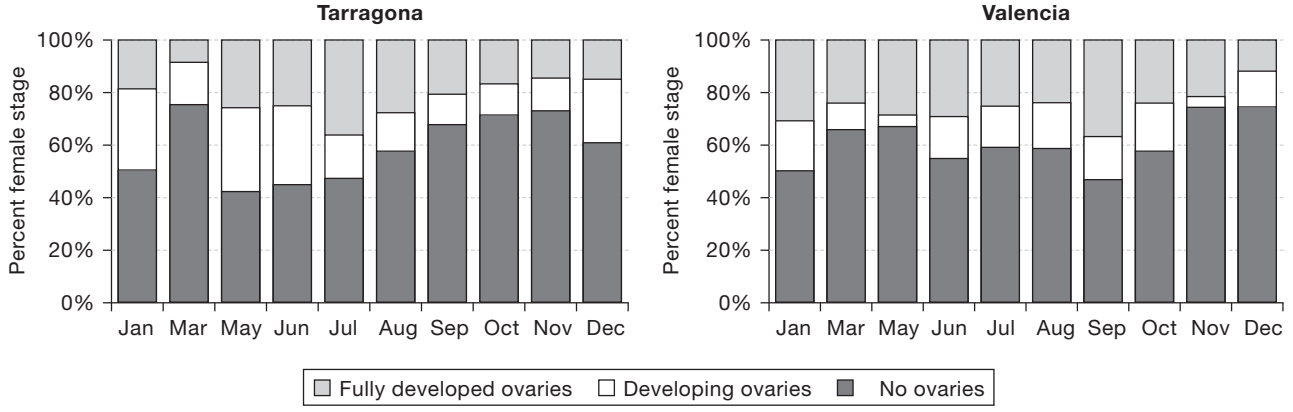

Figure 3. Monthly percentage of different ovarian development stages of *C. capitata* adult females captured in citrus groves. Average for all the groves sampled in the Tarragona and Valencia areas.

in each of the two areas, Valencia and Tarragona (Fig. 3). Non-gravid females were proportionately more abundant in the cooler months (October through March) and gravid ones during the warmer months (May-September). The average physiological age of females captured in citrus groves was, thus, higher in late spring and summer and lower in autumn and winter. The fact that we found gravid females throughout the year indicates that there was no diapause, or lack of reproduction, in overwintering populations of medfly.

In Tarragona, the proportion of non-gravid females reached its lowest level in May-July. Citrus groves do not have any ripe fruit susceptible to *C. capitata* infestation during this period. Flies captured at this time must therefore have come from other fruits that were rather scarce in this area and distant from the traps located in the citrus groves. As a result, the captured females were older. The proportion of non-gravid females increased from August onwards, reaching a peak in November. In September and October, the females captured in traps would have probably developed in nearby mature fig and jujube fruits, which are abundant in the Tarragona area. In October and November some species or varieties of citrus are already fully mature, so captured females were younger as they had developed in citrus fruits located closer to the traps. The lower proportion of non-gravid females observed in December and January could be related to the increase in the pre-oviposition period and delay in sexual maturity in adults which occurs at low ambient temperatures (Vargas *et al.,* 1984; Duyck and Quilici, 2002).

In Valencia, the observed trend for the physiological stages of medfly females was similar to that of Tarragona. However, we also observed differences possibly related to the different availability of mature fruits in the proximity of the traps. The maximum proportion of non-gravid females was observed in November and December, when citrus fruits were fully mature and the flies captured came from fruits located in the vicinity of the traps. Between May and July, the proportion of non-gravid females captured was higher than in Tarragona, perhaps because regular plantations of spring and/or early-summer maturing fruits, such as apricots and peaches, are more abundant in Valencia.

Adult captures in winter

During the cool period of the year (from November to May) the number of flies captured in traps in the field was much lower than in the rest of the year. It is usually considered that no adults fly during this period. In Sardinia, adults were only reported as present from July until December (Ortu *et al*., 2005). On the island of Chios, no captures were reported between February and May (Katsoyannos *et al*., 1998). In Israel, no *C. capitata* adult males were caught between January and April (Israely *et al.,* 1997).

However, our results demonstrate that adults were present during the cooler period of the year in both Tarragona and Valencia, although sometimes at very low levels. We found adult populations when we placed a greater number of traps during these months (Table 2). The same situation was found by Mavrikakis *et al.* (2000), which registered continuous growth and reproduction of *C. capitata* throughout the winter in citrus plantations in Crete (southern Greece). Adult captures progressively declined between November and January, while February to April was the period with fewest adult flights. The initial annual increase varied from

Table 2. Number of *Ceratitis capitata* adults captured in traps in winter in the Valencia and Tarragona groves

Period	2004		2005		2006		2007	
	Valencia (42)[a]	Tarragona (92)	Valencia (42)	Tarragona (0)	Valencia (21)	Tarragona (35)	Valencia (21)	Tarragona (35)
1-15 Jan	74	49	55	—	128	4	218	314
16-31 Jan	40	36	22	—	113	4	444	135
1-15 Feb	14	8	16	—	23	0	33	4
16-28 Feb	50	16	2	—	4	0	25	34
1-15 Mar	112	3	3	—	0	0	59	15
16-31 Mar	11	1	1	—	5	0	27	5
1-15 Apr	101	2	0	—	3	0	26	8
16-30 Apr	178	3	1	—	3	0	57	26
1-15 May	421	40	1	—	23	0	139	153
16-31 May	328	72	5	—	232	0	964	528

[a] In brackets, number of traps.

the second half of April to the second half of May, depending on the year and the area. Considering the period of lower temperatures from November to April and the temperature requirements of *C. capitata* (Vargas *et al.*, 1984; Duyck and Quilici, 2002), there would probably be only one generation throughout this six-month period. Temperatures were high enough to allow population development and adult flights in April (see Fig. 1). However, captures were almost zero in this month because most of the overwintering adults had already died and the flies of the first annual generation were still developing either as larvae inside fruits or as pupae in the soil.

In conclusion, the development and reproduction of *C. capitata* populations continue throughout winter in the citrus groves of eastern Spain, though at low population levels. Air temperature is probably the most important reason for the initial increase in population in spring and for its subsequent decline in autumn, whereas the presence of other fruits in the vicinity of citrus groves seems to be the main factor responsible for the overall trends observed in *C. capitata* populations in citrus groves.

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