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Contributions to the knowledge of *Ceratitis capitata* Wied. (Diptera, Tephritidae) in Northern Italy II. Overwintering in Lombardy(*)

Abstract - During three winter seasons since the year 2000 until 2003, surveys have been held in order to study *Ceratitis capitata* overwintering potential in Lombardy (Northern Italy), an area classified outside the tephritid stable establishment limits. The surveys have been carried out on the field, over infested fruits and pupae, and in indoor environments, residential premises, with adults. The mortality rate was 100% for the larvae inside the fruits and for the pupae. The adults were able to overwinter in two winter seasons out of three. In 2000-01 about 45% of the specimens resulted to be still alive at the beginning of June, when the first ripe fruits appeared in the field. The last adults survived until the first half of August, after more than 260 days since the emergence. In 2002-03 survival rates at the beginning of June ranged from 0% to 85% and the last adults died in the first ten days of August, when they were more than 250 days old. The surviving females had laid fertile eggs from the beginning of June to August 1st in 2001 and to July 4th in 2003, from the 190th to the 250th day of life. These results together with some peculiar aspects of the Lombard fruit growing, show that in indoor environments the adults could overwinter, giving birth to new populations the following spring.

Riassunto - *Contributi alla conoscenza di* Ceratitis capitata *Wied. (Diptera, Tephritidae) in Italia Settentrionale. II. Possibilità di svernamento in Lombardia*

Nel corso di tre inverni, tra il 2000 e il 2003, sono state compiute indagini per verificare le capacità di svernamento di *Ceratitis capitata* Wied. in Lombardia, Italia Settentrionale, un'area considerata al di fuori dei limiti di insediamento stabile del tefritide. Le sperimentazioni sono state effettuate in pieno campo, con frutta infestata e pupe, e in ambienti protetti, locali di edifici residenziali, con adulti. La mortalità è risultata del 100% per le larve entro la frutta e per le pupe. Gli adulti sono riusciti a svernare due inverni su tre. Nel 2000-01 circa il 45% degli esemplari era vivo all'inizio di giugno, quando sono disponibili in campo i

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primi frutti in maturazione, e si sono registrati individui superstiti fino alla metà di agosto, ragion per cui gli adulti avevano oltre 260 giorni di vita. Nel 2002-03 la sopravvivenza a giugno è stata tra lo 0 e l'85% e gli ultimi individui sono morti nella prima decade di agosto, dopo oltre 250 giorni dallo sfarfallamento. Le femmine superstiti hanno deposto uova fertili in un periodo compreso tra l'inizio di giugno e il 4 luglio/1 agosto, tra i 190 e i 250 giorni di vita. Questi risultati, unitamente ad alcune caratteristiche della frutticoltura lombarda, indicano che gli adulti, in ambienti protetti, potrebbero svernare e dar luogo a nuove popolazioni la successiva primavera.

Key words: Medfly, Lombardy, Northern Italy, Overwintering, Adults, Indoor environment.

INTRODUCTION

The Mediterranean fruit fly, *Ceratitis capitata* Wied., due to its poliphagy and biotic potential, is one of the most dangerous pests for fruit cultivations. It spread from native African areas (Balachowski, 1950) invading all the tropical and subtropical areas of the world (Christenson & Foote, 1960; Harris, 1977; Fimiani, 1989; Liquido *et al.*, 1991). Even if Baas (1959) suggested that the Fruit fly is able to overwinter in Central Europe as well, the Northern areas of the Mediterranean region are still considered external to its stable establishment environment as it calls for definite thermic conditions (Delrio, 1986). The northern limit seems to coincide with the 41° North latitude (Fischer-Colbrie & Bush-Petersen, 1989; Delrio & Prota, 1977; Papadopoulos *et al.*, 1996). In these areas the density of population results heavily dependent on the seasonal climatic conditions, with possible temporary eradications (Ortu, 1995).

Nevertheless, in Lombardy for 15 years without interruption severe *C. capitata* infestations diffusely affected the fruit growing areas of the plain notably in the provinces of Brescia and Pavia. Moreover the presence of isolated populations that last for quite many years in little non professional orchards is ascertained (Romani, 1997; Rigamonti *et al.*, 2002; Süss, personal communication). This appears to be in contrast with many Authors' theories according to which in areas such as Tuscany and Southern France, regions that present climatic conditions definitely milder than Lombardy, the infestations have a temporary nature and originate from infested fruit imported from warmer Countries (Feron *et al.*, 1956; Feron & Guennelon, 1958; Bonnemaison, 1962; Caroli & Loni, 1991; Cayol & Causse, 1993). They maintain that in those environments infestations that last for more than a year depend on exceptional climatic conditions. Virtually, what happens in Lombardy can not either be explained with the natural migration of the tephritid from infested areas.

Considering that previously Grandi (1951) and Ferrari (1966) had already suggested the possibility that the Fruit fly overwinters in Northern Italy, we decided to investigate the possibility that the *C. capitata* could be able to survive in the unfavourable winter climate conditions that characterise the Lombardy Region.

MATERIALS AND METHODS

Experimental sites and climate

Trials were carried out during the winter seasons 2000-2001, 2001-2002, 2002-2003 in a private house in Meda, a town located in the Milan hinterland, and in a fruit growing farm placed in Brescia. These sites are both located in the Po Plain at roughly 45° 45' North latitude. The climate, as it is everywhere in the Lombard plain, has to be considered as a transitional one between the Mediterranean climate and the European continental one. It is characterised by cold winters with mean temperatures that during the months of December and January range from 0° and 5°C, and warm summers with mean monthly temperatures in July and August ranging from 20° to 25°C.

The material used in the tests came from different Lombard farms located in the provinces of Brescia, Milan and Pavia, that presented mixed orchard with a predominance of peach, *Prunus persica L.* or apple, *Malus domestica L.* and some secondary groves such as apricot, *Prunus armeniaca L.*; pear *Pyrus communis L.*; plum, P*runus domestica L.*; lotus *Diospyros cachi L.* and kiwifruit, *Actinidia chinesis* Planchon. The fruiting period begins in June and ends in October.

Some of the trials stages were performed in the laboratory of the Institute of Agricultural Entomology of the Milan University. In several occasions the fruits and the pupae have been kept in climatic chambers at temperatures of 20 ±2 or 26 ±2°C and 65 ±5% R.H. Adults coming from infested fruits harvested in Brescia fields and kept at temperatures of 26 ±2°C, 65 ±5% R.H. and 16:8 hours L:D photo-period were used in order to cause artificial fruits infestation. In some cases F1 individuals descendant from wild adults were also used.

Temperatures data related to field trials were provided by a meteorological station located in Brescia, whereas those related to studies performed in Meda were recorded directly in the field. Ten days mean temperatures recorded during the winter semesters are shown in Table 1.

Overwintering of larvae

Trials performed during the winter seasons 2001-02 and 2002-03 had the purpose to study the overwintering capacity of larvae within apple fruits.

Every year during the month of October, about 220 Golden Delicious apples were collected in farms located in Brescia and Broni (a town in the province of Pavia). In the year 2002 part of the fruits, 80 in all, were moved to laboratory and put for a few hours in cages with wild adults in order to increase the number of the larvae, rather scanty the previous year. Further, the apples were preserved at 26°C for 7-10 days and then transferred in the experimental site, after an acclimatisation period of 7 days at a temperature of 15-18°C. The fruits were placed in plastic containers (30 by 40 cm base, 10 cm high) on a 3 cm deep sand layer that was used in order to absorb any liquid dripping from the rotting fruits and to allow larvae pupation. The containers were placed under a porch, in order to preserve them from unfavourable weather condi-

Table 1 - Ten days mean temperatures for the six months winter period in Meda, porch, and in Brescia. Mean temperatures (minimum - maximum).

Meda - Porch		Ten days	Brescia		
2001-02	2002-03		2000-01	2001-02	2002-03
10.9 (8.5 – 13.2)	10.6 (8.2 – 12.9)	November I		9.5 (4.6 – 14.5)	9.4 (4.3 – 14.4)
8.4 (6.1 – 10.6)	11.1 (9.9 – 12.4)	November II	9.4 (5.8 – 12.9)	7.4 (2.7 – 12.1)	11.3 (7.9 – 14.8)
5.7 (3.0 – 8.4)	10.8 (9.5 – 12.1)	November III	7.7 (3.0 – 12.5)	4.8 (-0.1 – 9.6)	12.4 (9.0 – 15.8)
4.7 (1.9 – 7.6)	7.6 (6.1 – 9.2)	December I	9.0 (6.8 – 11.3)	5.0 (0.3 – 9.7)	5.9 (2.8 – 9.0)
-0.8 (-3.8 – 2.2)	5.7 (4.3 – 7.0)	December II	6.1 (2.1 – 10.0)	-0.4 (-5.9 – 5.1)	4.2 (1.2 – 7.2)
2.0 (-0.8 – 4.8)	7.7 (6.4 – 9.0)	December III	2.4 (-1.5 – 6.4)	1.2 (-5.0 – 7.3)	6.5 (4.1 – 9.0)
0.3 (-3.1 – 3.7)	4.8 (3.4 – 6.2)	January I	2.5 (-3.3 – 8.3)	0.3 (-7.1 – 7.7)	3.4 (1.0 – 5.9)
0.4 (-2.9 – 3.6)	3.9 (1.8 – 5.9)	January II	3.4 (-2.3 – 9.2)	0.7 (-6.3 – 7.7)	2.1 (-2.9 – 7.1)
3.8 (1.9 – 5.7)	5.2 (2.7 – 7.8)	January III	2.1 (-3.1 – 7.2)	4.6 (0.7 – 8.6)	4.5 (-1.3 – 10.4)
5.2 (3.0 – 7.5)	4.6 (1.7 – 7.5)	February I	6.9 (1.9 – 11.9)	6.3 (1.6 – 11.1)	2.3 (-3.7 – 8.2)
6.2 (3.6 – 8.8)	2.4 (-0.1 – 4.8)	February II	6.0 (1.0 – 11.9)	6.5 (2.6 – 10.5)	1.3 (-4.6 – 7.2)
6.4 (3.3 – 9.5)	4.4 (1.1 – 7.7)	February III	6.8 (1.4 – 12.1)	6.8 (1.7 – 11.9)	4.2 (-3.9 – 12.2)
9.5 (7.4 – 11.7)	8.8 (6.1 – 11.4)	March I	9.3 (2.9 – 15.8)	10.6 (5.1 – 16.1)	9.3 (2.9 – 15.6)
11.8 (8.8 – 14.8)	8.9 (6.0 – 11.8)	March II	11.3 (5.4 – 17.0)	12.4 (5.8 – 19.1)	9.8 (3.4 – 16.1)
12.1 (7.8 – 16.3)	10.7 (7.5 – 14.0)	March III	10.7 (5.7 – 15.6)	10.6 (2.7 – 18.4)	11.0 (3.6 – 18.4)
10.7 (8.6 – 12.8)	10.0 (7.1 – 12.8)	April I	11.9 (7.7 – 16.0)	11.5 (6.2 – 16.8)	7.8 (2.6 – 13.1)
11.8 (9.8 – 13.9)	12.8 (10.1 – 15.6)	April II	14.3 (10.3 – 18.4)	12.1 (7.6 – 16.6)	12.9 (7.0 – 18.8)
15.8 (12.6 – 19.1)	16.2 (13.4 – 18.9)	April III	17.8 (11.9 – 23.7)	16.4 (10.3 – 22.5)	16.6 (10.1 – 23.1)

tions. Every week the sand was sieved in order to collect the pupae and the fully-grown larvae. They were further transferred, according to the number, in plastic jars 12 cm high and with a diameter of 12 cm, or inside plastic test tubes 12 cm high and with a diameter of 3 cm, fitted out with holes in the base in order to avoid water stagnation. The pupae were placed over a 5 cm sand layer and covered with a 5 cm layer of sand, while the larvae were put over a 10 cm sand layer and left free to pupate. Further, the jars and the test tubes were closed with nylon tulle fixed with a rubber band and planted in the soil. A 150 cm high roofing open on all sides protected the area in which the containers have been placed. The containers were periodically checked to ascertain the adults emergence. In April the containers were moved to a climatic chamber at 26°C in order to subject them to a final inspection. Every week, starting from the second half of November, a prearranged number of fruits (10 fruits in the season 2001-02 and 6 until December 15th, 11 fruits later in the season 2002-03) were dissected in order to ascertain any larvae survival; those who were found alive were inoculated in non infested apples. These fruits were put together with the others and controlled every week in addition to the dissected fruit.

Furthermore surveys have been carried out in the field in order to estimate the number of residual fruits and in order to ascertain the presence of alive larvae. These investigations were held in two farms located in the province of Brescia where a substantial number of fruits weren't harvested in consequence of hailstorms or of cultivation drawbacks. The first one was monitored in the year 2001. Located in Brescia,

it had a surface of 0.25 ha and was planted with about 250 plants, 75% of which Golden Delicious variety and Royal Gala variety the remaining. The second farm was located in Calcinate and the apple trees here covered a surface of 2 ha planted with about 3300 plants Golden Delicious, Royal Gala, Red Delicious and Granny Smith varieties. At the beginning of December 2001 and at the end of November 2002 the first estimate of the quantity of fruits present on the trees and on the ground was done checking one tenth of the plants. In January and in February the same evaluation was repeated. Moreover 20 fruits taken from the plant and 20 taken from the ground were collected and then subjected to an incubation period of 4 weeks at 26°C.

Overwintering of pupae

In this case trials have been performed during the years 2000-01, 2001-2002, 2002-03. Fully-grown larvae (wild specimens coming from naturally infested apples or their F1 offsprings) were used in order to avoid interference with the pupation process. The fruits had been held either in laboratory at a temperature of 20°C or in the field.

During the first and the second winter the larvae pupated in the field in a farm located in Brescia. In order to avoid the dispersion of the larvae and to preserve them from predators the larvae were placed in plexiglas cylindrical cases (50 cm high and 30 cm diameter, planted in the soil for 25 cm and with the upper part closed with nylon tulle in order to prevent the escape of the adults). 200 larvae shared in four cases in groups of 50 individuals were used in the year 2000, one hundred pupated on September 30th and the same number on October 13th, whilst 240 specimens were placed in the field on October 12th 2001. They were split in 4 cases in groups of 60 individuals. Starting from the end of March, every two weeks, the probable adults emergence was monitored. Moreover, at the end of March and April the pupae present in one of the cases were recovered, whereas the control of the remaining two cylinders took place at the second half of June. The recovered pupae were placed at 26°C and thus preserved for about one month.

In the year 2002, the trial was performed in a different way in Meda. The larvae were put in 40 test tubes and in 4 plastic jars, previously described, filled up with a 10 cm sand layer in groups of 10 and of 50 larvae each respectively. The containers were closed with nylon tulle fixed with a rubber band and planted in the soil under the roofing. Weekly, starting from December 3rd two test tubes were recovered and transferred to a climatic chamber at 20°C and there kept until the possible emergence of the adults. Whereas, the jars were recovered the next spring, one every two weeks starting from the end of March, and treated the same way. Eventually, in June the containers' residual material was checked.

Overwintering of adults

Trials over the adults were carried out in Meda. In this case the individuals were placed in plastic cages (30 by 30 by 30 cm) Bugdorm-1 type[1], and equipped with

[1] Produced by Mega View Science Education Services Co. Ltd., Taichung - Taiwan.

water and food, a mixture of sugar and yeast autolysate in a 10:1 ratio. The cages were placed in non-heated premises.

The specimens came from natural infested fruit taken in Vaprio d'Adda, in the province of Milan, and in Brescia. They were preserved in laboratory at a temperature of 20°C. In the year 2001 specimens coming from Palermo were added, given by the S.EN.FI.MI.ZO. Department and obtained by feijoa they served as a mean of comparison. In the year 2002 the fruits from Vaprio, part of the fruits coming from Brescia and the pupae so obtained were preserved at 26°C until the achievement of the adults. These were further moved in the premises in which the trials were carried out. The remaining fruits coming from Brescia and the relative pupae were instead preserved at a temperature of 20°C. During the three years 239, 309 and 338 adults respectively were used. The number and the relative features of the individuals used during the three years have been reported in Table 2. The cages were daily controlled in order to survey the number of dead individuals and, when necessary, to add water and food.

Starting from the beginning of June the cages that still presented alive adults were moved under the porch and periodically, more or less in weekly intervals, peaches and apricots were introduced in order to test the fertility of the females. The fruits were inserted in the morning and taken back in the afternoon and so left to incubate under the porch until the pupation of the probable descendants.

RESULTS

Overwintering of larvae

Table 3 shows the number of larvae and pupae recovered from the sand during the trials carried out in Meda in 2001-2002 and 2002-2003, whereas in Table 4 you

Table 2 - Features of the adults used for the overwintering trials.

Year	Origin	Adults n°	Emergence	Year	Origin	Adults n°	Emergence
2000-01	Brescia	70	9 – 25 XI	2001-02	Palermo	66	19 – 20 X
2000-01	Vaprio d'Adda	169	17 – 30 XI	2002-03	Brescia 1 [a]	216	19 – 24 X
2001-02	Brescia	90	27 - 30 X	2002-03	Brescia 2 [b]	32	20 – 26 XI
2001-02	Vaprio d'Adda 1	79	18 – 19 VIII	2002-03	Vaprio d'Adda [a]	90	7 – 15 X
2001-02	Vaprio d'Adda 2	74	21 X – 6 XI				

a = fruits and pupae preserved at 26 °C
b = fruits and pupae preserved at 20 °C

Table 3 - Fully grown larvae and pupae collected sieving the sand during the overwintering trial performed on larvae inside the fruits, Meda 2001-02 and 2002-03

2001-02		2002-03			
Date	Larvae / Pupae	Date	Larvae / Pupae	Date	Larvae / Pupae
4 XI	12 / 51	3 XI	6 / 28	1 XII	4 / 5
11 XI	2 / 7	10 XI	3 / 11	8 XII	0 / 2
18 XI	2 / 4	17 XI	2 / 5	15 XII	0 / 1
25 XI	0 / 4	24 XI	1 / 9		

Table 4 - Overwintering trial performed on larvae inside the fruits. Outcomes of the fruit dissections, Meda 2001-02 e 2002-03.

2001-02						2002-03					
	Larvae			Larvae			Larvae			Larvae	
Date	Alive	Dead	Date	Alive	Dead	Date	Alive	Dead	Date	Alive	Dead
25 XI	34 / -	30 / -	3 II	0 / -	6 / -	17 XI	15 / -	5 / -	26 I	0 / 1	30 / 0
2 XII	7 / 34	6 / 0	10 II	0 / -	24 / -	24 XI	13 / 15	0 / 0	2 II	0 / 0	97 / 1
9 XII	5 /34	6 / 7	17 II	0 / -	1 / -	1 XII	2 / 28	0 / 0	9 II	0 / -	37 / -
16 XII	3 / 16	20 / 23	24 II	0 / -	15 / -	8 XII	1 / 30	0 / 0	16 II	0 / -	31 / -
23 XII	0 / 4	21 / 15	3 III	0 / -	11 / -	15 XII	8 / 29	0 /2	23 II	0 / -	25 / -
30 XII	0 / 2	12 / 2	10 III	0 / -	4 / -	22 XII	5 / 26	49 /11	2 III	0 / -	59 / -
6 I	0 / 2	14 / 0	17 III	0 / -	5 / -	29 XII	2 / 10	141 / 21	9 III	0 / -	46 / -
13 I	0 / 0	6 / 2	24 III	0 / -	4 / -	5 I	0 / 4	36 / 8	16 III	0 / -	27 / -
20 I	0 / -	2 / -	31 III	0 / -	3 / -	12 I	0 / 3	76 / 1	30 III	0 / -	38 / -
27 I	0 / -	5 / -				19 I	0 / 1	159 / 2	6 IV	0 / -	34 / -

N° of larvae: after weekly dissection / after previous checks.

Table 5 - Estimated number of residual fruits in Brescia farms during the winter seasons 2001-02 and 2002-03.

Date	On the plant	Sound on the ground	Rotten on the ground	Total	Data	On the plant	Sound on the ground	Rotten on the ground	Total
2001-02					2002-03				
6 XII	1200	800	600	2600	26 XI	1600	6000	1000	8600
10 I	200	100	200	500	8 I	300	300	600	1200
14 II	50	0	100	150	19 II	40	0	60	100

can see the data relative to the fruits dissections. No individual survived to give birth to adults in spring. In the initial stages, until the last days of November 2001 and at the beginning of December 2002 a quite good quantity of fully-grown larvae came out from the fruits and quite all pupated. Then, because of the temperature fall, the larvae suspended their activity and remained inside the fruits, included those that during the dissections had proved to be fully-grown. From the pupae no adults emerged and during the spring check the puparia resulted entire, but all the individuals had died. In most of the cases where the identification of the puparium content was possible, a pupa or an adult fully developed was found, notably if the individuals had been able to pupate at the beginning of the trials. Nevertheless, in many cases the metamorphosis was not achieved.

During the dissections alive larvae were observed until January 6th in 2002 and until January 25th in 2003. In 2001-2002 the last survived larvae had resisted to intense cold periods that had lasted for more than one week, whereas the next year the last surviving larva died before the coldest stage of the winter had begun. That year this stage coincided with the month of February.

Field trials showed the same results. From the fruits collected during the months of January and February no individual was obtained. The data referring to the presence

Table 6 - Adults obtained in the overwintering trial performed with pupae, Meda, 2002-03.

Recovery day	Adults	Recovery day	Adults	Recovery day	Adults	Recovery day	Adults
3 XII	5	7 I	0	11 II	0	25 III	0
10 XII	6	14 I	0	18 II	0	8 IV	0
17 XII	4	21 I	0	25 II	0	22 IV	0
24 XII	0	28 I	0	4 III	0	6 V	0
31 XII	0	4 II	0	11 III	0		

of fruits in the field have been entered in Table 5. They prove an early degradation of the fruits that were already exhausted in the depths of winter.

Overwintering of pupae

No individual survived to give birth to adults in spring in tests carried out during the year 2000-2001 and 2001-2002. During the two first spring controls performed in 2001, 42 pupae were recovered. They proved in all the cases a fill-in depth in the soil lower than 10cm with more than 90% of the puparia concentrated in the first 5cm. In June some dozens of pupae were recovered. Quite no one resulted to be entire, and in most cases there were only remains. In spring 2002, during the months of March and April about the half of the pupae that had been used were recovered, 32 and 26 respectively. Quite all were found at a depth of less than 5cm and nearly all presented quite good preservation conditions. During the last control, 28 pupae were recovered together with various remains, they were all within a depth of 5cm and almost all the puparia proved to be damaged.

2002-03 test results have been reported in Table 6. Adults were obtained only by the test tubes that were recovered on December 3rd, 10th and 17th. Hence it is clear that the pupae had lost their vitality already at the end of December i.e. while the temperatures were still mild and the minimum were not yet below 0°C. Considering that no adults has been obtained for about 3 months, the recuperation of the test tubes were stopped at March 11th. From the jars no adult emergence was recorded too. During the final check in June 2003, 90% of the specimens used were recovered; 95% of the individuals had regularly pupated and all the pupae were at a depth between 0 cm and 5 cm and 70% of them was found in the first 2 cm of depth.

Overwintering of adults

In the season 2000-01 the test begun on December 1st, the winter season was extremely mild and the temperature in the experimental site never fell under 10°C. In December-February period, mean temperatures ranged between 12 and 16°C and progressively rose starting from the beginning of March. In these conditions the adults shown extraordinary liveability (Fig. 1). On June 1st, a date in which in the fields appeared the first ripe fruits, 106 individuals were still alive, 76 of which came from Vaprio and 30 from Brescia, 44.4%; 45.0%; and 42.9% of the initial specimens, respectively. On June 20th the survived specimens (68 from Vaprio and 24 from Brescia)

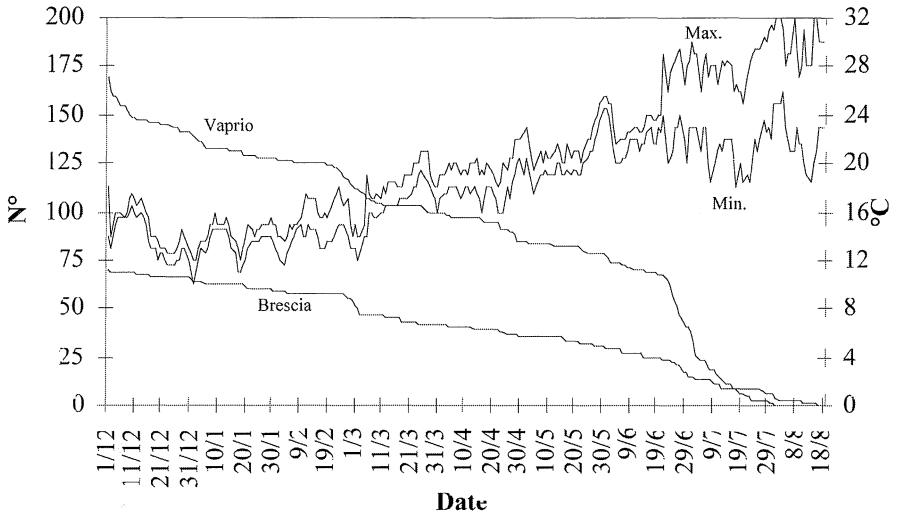

Fig. 1 - Maximum and minimum daily temperatures trend and adults survival trend in winter season 2000-01.

were placed under the porch. Starting from this moment the mortality rate increased, notably among Vaprio d'Adda adults, even if the last specimen died after about 45 – 60 days, on August 1st the one coming from Vaprio and on August 16th the one coming from Brescia. They were at least 244 and 263 days old, respectively. Starting from June 6th until August 10th for 8 times fruits were introduced in the cages. Vaprio d'Adda adults laid fertile eggs in all the five introductions performed in the period between

Table 7 - Roundup data concerning some biological parameters related to adults used in overwintering trials.

Origin	Initial n° of adults	Survival at June 1st total / females	Mean life days	Maximum life in days	
				Absolute value	Egg laying females
2000-01					
Brescia	70	30 / 18	160.9	264 – 280	249 – 265
Vaprio d'Adda	169	76 / 46	143.7	244 – 257	222 – 235
2001-02					
Brescia	90	-	83.9	178 – 181	-
Vaprio d'Adda 1	79	-	123.9	219 – 220	-
Vaprio d'Adda 2	74	-	40.3	108 – 124	-
Palermo	66	-	84.6	195 – 196	-
2002-03					
Brescia 1	216	-	70.3	195 – 200	-
Brescia 2	32	27 / 15	203.7	255 – 261	220 – 226
Vaprio	90	-	55.3	123 – 131	-

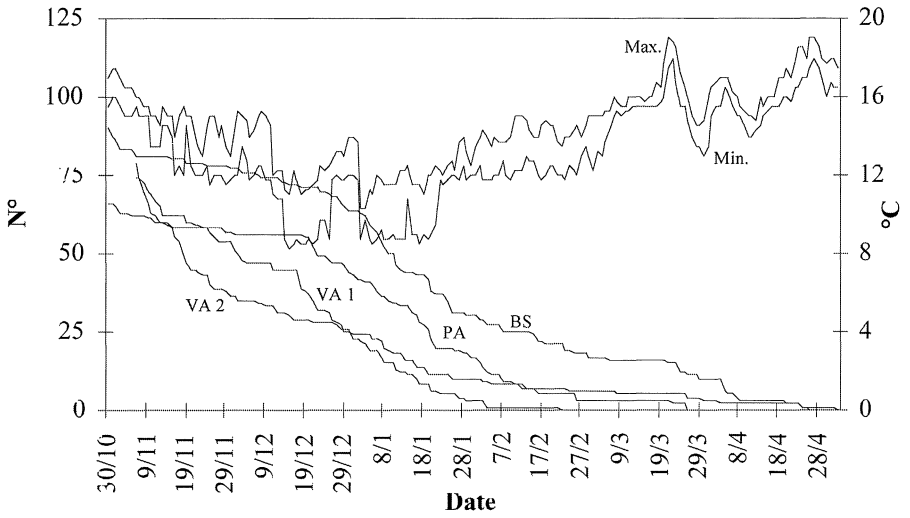

Fig. 2 - Maximum and minimum daily temperatures trend and adults survival trend in winter season 2001-02.
BS = Brescia; PA = Palermo; VA 1 = Vaprio d'Adda 1; VA 2 = Vaprio d'Adda 2.

June 6th and July 10th except that of July 1st. Those coming from Brescia on the other hand, laid fertile eggs in all the seven introductions performed between June 6th and August 1st. The last ovipositions were obtained from females at least 222 (Vaprio) and 249 (Brescia) days old (Table 7).

In 2001 trails begun on October 30th for adults coming from Brescia and Palermo and on November 6th for the two groups coming from Vaprio d'Adda (Fig. 2). No individual survived until the next summer. After a mild November, temperatures underwent a marked drop. The minimum temperatures during December and January remained long under 10°C attaining in more than one occasion the coldest extreme of 8.5°C. Maximum temperatures on the other hand, varied from 10 to 13°C. A rise was recorded starting from the end of January, even if only at the beginning of March the average temperatures exceeded 14°C. Adults survival has shown a trend related to those of the temperatures. In the first stage, until the beginning of December, the mortality was quite low, it was little higher than 15% for the strains coming from Brescia and Palermo, the "Vaprio 2" adults excepted, as this group showed a mortality of 60%. The data related to the group "Vaprio 1", with a mortality of more than 40%, even if quite high, are to be considered normal. These specimens in fact were more than 100 days old, had emerged in mid August and had been kept on the open air until the beginning of the trial. In the coldest winter period, between December 13th and January 22nd, a high mortality was recorded. At the end of this period the number of the survival specimens varied from the 6 adults of the "Vaprio 2" to the 37 of the Brescia group. Afterwards, two periods with relatively high mortality were recorded: first from

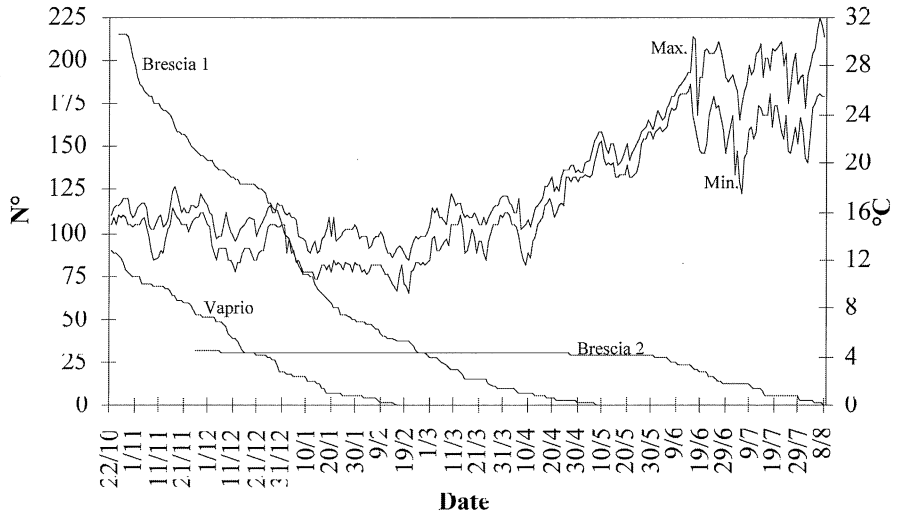

Fig. 3 - Maximum and minimum daily temperatures trend and adults survival trend in winter season 2002-03.

January 23rd until February 8th and this seemed likely to be the consequence of the previous period; second from March 20th until April 11th to coincide with dramatic temperature variations. The death of the last specimen of each group has been recorded on February 22nd (Vaprio 2), on March 26th (Vaprio 1), on April 26th (Brescia) and on May 3rd (Palermo); i.e. before ripe fruits had appeared in the field. Thus, the maximum survival were between 108 days for "Vaprio 2" group and 219 days for the "Vaprio 1" group (Table 7).

The outcome of the test carried out in the winter 2002-03 proved not to be uniform among the groups involved. The trial begun on October 25th for the adults coming from Vaprio and "Brescia 1" and one month later, on November 26th, for the "Brescia 2" group. Vaprio and "Brescia 1" adults, which were kept at 26°C until the beginning of the test, proved to be weakly resistant and to have high mortality rate even in the first stages (Fig. 3). Yet they had been characterised by average temperatures, varying around 14°C, thus not particularly low. On December 31st the mortality was 79% for the Vaprio group and 51% for "Brescia 1". A high mortality was observed also during the coldest period of the winter, i.e. between January and February with average temperatures that fluctuated around 12°C. The last adult of the Vaprio group died on February 15th, after 123 days from the end of the adults emergence (Table 7). At the end of February the mortality among the "Brescia 1" group was 87%. The last specimen died on May 7th 195 days after the end of the emergence (Table 7), about 1 month before the infestable fruits had appeared in the field. It is possible that the mortality trend was influenced, at least in the first stage, by the fact that the individuals had not been

acclimatised and had subsequently suffered from the sudden change of temperature from the laboratory to the experimental premises.

"Brescia 2" trend was completely different (Fig. 3). Up to the end of April only one death had occurred. Further, a slight increase of the mortality rate was observed to coincide with a warm period that had led mean temperatures up to 27°C. Nevertheless, on June 1st, 27 adults were still alive. On June 15th the 22 survived specimens were moved outside. The adults which were already nearly 7 months old, were able to keep a high vitality even in the new conditions that is to say, with very high average temperatures ranging around 26°C and with quite elevated and sudden changes of temperatures. The last specimen died on August 8th after a 255 days life. In this case also, between June 4th and August 4th, for 9 times, some fruits were inserted in the cage and the survived females had laid fertile eggs for the first 5 times, i.e. until July 4th when the adults were at least 222 days old (Table 7).

DISCUSSION

The pupa is the Mediterranean fruit fly most resistant stage both at high and, with the adults, at low temperatures; and in regions near the northern limits of its distribution the Mediterranean fruit fly overwinters at the pupal stage (Delrio, 1986). Nevertheless pupae were the less resistant stage among those used in these overwintering trials. On the other hand, still according to Delrio (1986), pupae survive only a few hours when they are exposed to temperatures of -4° to -6°C and the lower threshold is 11°C (Crovetti *et al.*, 1986). These limits proved to be insufficient to guarantee the overwintering in areas such as Lombardy where regularly, during the winter season, more or less prolonged periods in which the soil is frozen are recorded. In order to overcome this drawback the development of cold resistance adaptations or the evolution of special behaviours, notably the possibility to perform pupation deeper in the soil are necessary. The first event is denied by Christenson & Foote (1960) whereas the second is unknown in literature, neither has emerged from these trials as the results have shown that all the larvae fill-in capacity did not exceed 5cm. Hence, we are able to maintain that the pupae are not able to overwinter in the areas of the Po Plains in Northern Italy, neither during the most favourable years.

Rather controversial is the case of the larvae inside the fruits. Like the pupae, they are responsible for the Fruit fly overwintering in areas such as the Campania district in Italy and central Greece (Cirio *et. al.*, 1972; Zervas *et. al.*, 1995). Papadopoulos *et. al.* (1996) have shown that overwintering takes place in this terms in apples even in regions particularly close to the Northern limits of the Fruit fly distribution, such as the area surrounding Thessaloniki (Northern Greece), that presents climatic features fairly similar to those present in Lombardy. These Authors maintain that the stay inside the fruit guarantees to the larvae better conditions than the soil for the pupae. This would allow the larvae to resist to temperatures that frequently fall below 0°C and once the spring has come, to pupate or to successfully continue their development.

According to Papadopoulos *et. al.* (1998) first and second age larvae have the highest likelihood to stop their development without lethal consequences even for several months, whereas the older ones show a clear tendency to abandon the fruits to pupate and only a minimum percentage remains protected in the apples. In the course of these surveys instead, dissections and controls in the field have shown that no larva is able to survive after the end of January and that they die already in the first heavy period of intense cold. As during every winter season in Lombardy minimum temperatures remain considerably under 0°C even for more than one month in succession (Table 1), it is virtually impossible that the larvae can manage to survive. Even in the occasion of particularly mild winter seasons it remains that in the orchards in February all the apples are by that time completely degraded and that in these conditions a resumption of the development cycle is nearly impossible. In this sense we can maintain that even this overwinter modality, on the field, results to be precluded to the Mediterranean fruit fly in Lombardy or, at least, that it can assume an absolutely subordinate value that can manifest only in presence of peculiar field situations and only in case of exceptionally mild climatic conditions.

Only adults that were preserved in a protected indoor environment overwintered successfully. During two out of three winter seasons, with mean temperatures that had rarely fallen under 12°C, a high adults percentage managed to survive until June, a period of the year during which, in Lombardy, begin the fruiting period, the first maturing fruits being early peaches and apricots. The adults longevity even if dramatically variable in terms of groups and years, proved to be exceptional. In several cases it went over 250 days in maximum value, and approached or even exceeded 200 days in average terms (Table 7). Even during the winter season 2001-2002, the coldest winter ever had in the last 15 years, some adults emerged in full summer survived even longer than 200 days. All these aspects reveal to be quite significant as in the course of their imaginal life the most long-lived specimens have had to cope with dramatically variable thermic conditions; i.e. winter periods characterised by mean temperatures approaching 10°C and minimum temperatures of 8.5°C and summer periods in which mean temperatures went often above 25°C and maximum reached 32°C (Fig. 1, 2, 3). Another exceptional aspect to be underlined is the capacity to lay eggs. The females proved to be able to successfully reproduce 190-250 days after the emergence (Table 7). These biological features of the *C. capitata* anyway, are already known. In literature maximum survivals of about 230 days, in variable conditions, and of 315 days or even 566 days in optimum conditions, i.e. around 15°C, are reported, whereas the maximum length of the imaginal life at field condition is estimated to be more or less one year with an ability to lay eggs until 200 days of adult life (Back & Pemberton, 1918; Bodenheimer, 1951; Christenson & Foote, 1960).

In order to support the hypothesis of indoor overwinter it is important to underline the fact that in Lombardy the most infested fruit growing areas are located inside the urban areas, see for example the Brescia area that presents orchards mixed with residential areas. The same is true for non-professional orchards in which isolated and stable populations are present (Rigamonti *et al.*, 2002). Even other features, related to

the relatively poor specialisation of the fruit farms and the insufficient skilfulness of the non-professionals can make easier the Medfly settlement in indoor environments. The farms present mixed orchards, with fruiting period from June to October, this clearly makes easier the tephritid multiplication, even thanks to the fact that often the fruit growers do not use specific pest management measures. Moreover the soil tends to be not tilled, this also allows the survival of quite all the pupae. The fruits are normally sold directly in the farm, and quite often late fruits, notably apples and lotus, are usually preserved not in conditioned cells but in premises that act as warehouses. In this way, the larvae present in the infested fruits that arrive to the warehouses can continue their development giving birth to adults even in later stages than if they were in the field (Rigamonti *et al.*, 2002). In such environments however, the overwintering can not take place as larvae inside the fruits as they are sold well before the end of the winter season. These factors seem to be even accentuated in non-professional or semi-professional orchards, where the poor skilfulness of the owners as well as the fault of technical devices can on one hand foster the appearance of serious infestations of even 100% out of the whole late fruits (Rigamonti *et al.*, 2002); on the other hand they can retard the destruction of the infested material due to a default of identification of the early symptoms prolonging their preservation inside warehouses that usually are premises located inside the owner house.

Surely the real conditions are clearly more severe than those experimentally reproduced during the trials, this because of the improbable availability of food and water, the presence of parasites and the natural tendency of the Medfly to resume its activity, concurrently with even short periods of mild temperatures, leaving their shelter. Nevertheless, Fruit fly adults show a strong stress resistance. Carante & Lemaitre (1990) have shown that 10% of the adults that had been kept at a temperature of 10°C without food and water had managed to survive for more than 19 days. In all probability, only an extremely reduced percentage is able to fully complete the overwinter, but if one consider that during fall the populations can easily outnumber the 10 thousands specimen per hectare, and still, that the initial ones during spring have been estimated with even 4 – 8 adults per hectare (Cirio *et al.*, 1972; Delrio, 1986) we can deduct that it is likely possible that such event could occur at least in some "optimal" areas from which the flies are able then to gradually widespread in the neighbouring zones.

CONCLUSIONS

This survey allows us to conclude that with normal winter climate conditions in Lombardy the Mediterranean fruit fly is not able to overwinter in the field, at larva stages inside the fruits, or at pupa stage in the soil. The adults instead, prove in many cases to be able to overwinter denoting exceptional survival aptitudes if preserved in indoor environments, i.e. residential premises, and in presence of temperatures that do not fall under 10 - 12°C for prolonged periods. In the presence of these conditions the adults are likely to survive more than 250 days. Moreover, the survived females

proved to be able to lay fertile eggs until the beginning of August, assuring in this way new populations for the spring. These data, together with some other peculiar features of the Lombard fruit-growing that tend to foster the settlements of adults inside the premises, makes the adults the possible candidates for the preservation of stable colonisation of *C. capitata* in Lombardy.

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