UNIVERSITÀ DEGLI STUDI DEL MOLISE

Department of Agricultural, Environmental and Food Sciences

PhD Course in:

AGRICULTURE TECHNOLOGY AND BIOTECHNOLOGY

(CURRICULUM: SUSTAINABLE PLANT PRODUCTION AND PROTECTION)

(CYCLE XXX)

Related scientific-disciplinary sector: AGR/11 (General and Applied Entomology)

PhD thesis

Study of the bio-ethology of *Ceratitis capitata* Wied. in Trentino and development of sustainable strategies for population control

Coordinator of the PhD Course: Prof. Giuseppe Maiorano
Supervisor: Prof. Antonio De Cristofaro
Co-Supervisor: Prof. Claudio Ioriatti

PhD Student: Sara Zanoni
153767

ACADEMIC YEAR 2016/2017

Index

SUMMARY

The Mediterranean fruit fly (Medfly), *Ceratitis capitata* Wiedemann (Diptera Tephritidae), is a ubiquitous pest in subtropical and tropical regions worldwide. The gravid female deposits eggs inside the fruit and the newly emerged larvae feed on fruit pulp, causing fruit decay. The mature larvae leave the fruit and pupate in the soil, where new adults emerge. *C. capitata* is a highly polyphagous, multivoltine pest species and it is one of the world's most economically important fruit pests. From its supposed origin in Africa, it has spread to a number of countries, including the Mediterranean, parts of South and Central America and Australia. It was first detected in southern Italy in 1863, from where it gradually spread northward and it now infests all major temperate fruit crops, including the apple, throughout the country. *C. capitata* is reported to complete up to seven generations per year in the most southerly regions and this number gradually decreases as the pest spreads northward.

The northern limit of its distribution has been indicated to be around the 41st parallel north and its presence above this is considered to be occasional, mainly due to infested fruit trading, as this species is reported to be unable to overwinter above this latitude.

The presence of *C. capitata* was first reported in a limited area of Trentino in 1990. After this initial report, it was not observed until 2010, when severe apple damage caused by *C. capitata* was reported in a much larger area. From this year on, Mediterranean fruit fly infestation regularly appeared in the warmest apple growing areas of Trentino, claiming the attention of local research institutes and demanding in-depth study of the bio-ethology of this Tephritid pest in relation to environmental conditions and the apple production system in this northern Italian region.

The main objectives of this project were:

- to select the most effective trap in order to implement an efficient monitoring plan;
- to evaluate the susceptibility of apple varieties to oviposition and larval development in relation to physical-chemical parameter values at harvest time;
- to monitor the flight activity of the fly at area-wide level;
- to validate Tassan's degree-day model (Tassan *et al*., 1982) for estimation of the length of life-cycles and number of potential Medfly generations in Trentino;
- to assess the survival of overwintering populations;
- to evaluate the efficacy of different insecticides in controlling Medfly fruit infestation.

Four types of differently baited commercial traps (chromotropic, pheromone and food attractant) were compared in an apple orchard. A Decis® trap baited with a food attractant and catching both males and females was shown to be the most suitable for monitoring pests.

In Trentino, adult Medfly flight starts during the first week of July, but the intensity (number of captures) remains at a very low level until August, when it begins to increase, peaking during September. After this, adult activity decreases in October and stops by the end of November. According to our observations, performed both in the open field and under controlled conditions, *C. capitata* overwinters at the larval stage in infested apples falling to the ground in orchards.

The application of Tassan's degree-day model based on temperature records provided a good estimate of first generation development (325.2 °DD from eggs to adult and 44 °DD for the adult preoviposition period) in the area where direct behavioural observations were carried out.

To evaluate apple susceptibility, qualitative parameters (e.g. hardness, acidity, sugar and starch) of the main apple varieties cultivated in Trentino (Gala, Red Delicious, Golden Delicious, Granny Smith, Kanzi, Morgen and Fuji) were measured and correlated with the oviposition preference and larval survival of two Medfly strains in laboratory and field tests. The results showed that susceptibility to *C. capitata* oviposition increased when fruits had a high sugar content and a low penetrating resistance, as in the case of Golden Delicious, Kanzi and Fuji. In contrast, Granny Smith, Red Delicious and Morgen showed low susceptibility, due to their lower sugar content and higher peel and pulp hardness.

As regards larval fitness, the results suggested that the tested varieties considerably affected various aspects of the biology of both immature and adult stages such as larval survival, duration of larval and adult developmental stage and size of the pupae. Golden Delicious, Gala, Kanzi and Fuji were the most favourable environments among the seven tested varieties. In contrast, Granny Smith, Red Delicious and Morgen were shown not to be favourable for larval and adult development.

Two Medfly strains, one from Trentino and one from Spain, were used to assess the efficacy of five commercial insecticides containing the following active ingredients: Etofenprox, Cyazypir, Beta-Cyfluthrin, Spinosad and Thiacloprid.

Sublethal doses of Spinosad and Beta-Cyfluthrin caused high mortality in Spanish strains. Moreover, Beta-Cyfluthrin, Etofenprox and Spinosad also reduced damage by females to apples at the recommended field rate. When used against the Trentino strain, Spinosad caused high adult mortality at the recommended field dose, while Cyazypyr and Etofenprox did not work sufficiently to prevent puncture, egg laying and larval development in fruit.

Recommended field rates of all the selected insecticide formulations were repellent for egg-laying females of both strains, so both oviposition and fruit damage were significantly reduced on treated ripening fruits.

The behavioural observations and experimental results obtained in this thesis allow a better understanding of the bio-ethology of *C. capitata* in a northern fruit growing region such as Trentino, providing fundamental information for advisors and growers to optimise the current pest management strategy. In the future, some of the results obtained in this study will be of great relevance for developing innovative and more sustainable control tactics.

RIASSUNTO

Ceratitis capitata Wiedemann (Dittero: Tefritide), chiamata anche mosca mediterranea o mosca della frutta, è un insetto estremamente polifago e polivoltino originario delle aree tropicali e subtropicali. Si presume originaria dell'Africa diffusa successivamente in numerosi areali tra cui il bacino del Mediterraneo, parte del centro-sud America e Australia.

Le femmine fecondate ovidepongono nell'epicarpo del frutto in via di maturazione e le larve, con la loro attività trofica, provocano il disfacimento della polpa causando una perdita commerciale del prodotto. Le larve a maturità fuoriescono dal frutto, si lasciano cadere a terra e si impupano nel suolo dove successivamente sfarfalleranno i nuovi adulti.

La sua presenza è stata registrata per la prima volta in Italia nel 1863 e successivamente si è gradualmente spostata verso nord colonizzando la maggior parte delle piante coltivate nelle zone a clima temperato.

Nelle regioni meridionali questo insetto è in grado di compiere fino a sette generazioni all'anno; questo numero decresce gradualmente mano a mano che la specie si trova a vivere in aree più a nord. In bibliografia, il limite settentrionale della sua distribuzione è indicato attorno al 41° parallelo. La sua presenza al di sopra di esso è imputabile ad infestazioni occasionali, data dal trasporto e commercializzazione di frutta infestata, in quanto è stata valutata incapace di superare l'inverno al di sopra di queste latitudini.

La presenza di *C. capitata* in Trentino è stata registrata per la prima volta nel 1990, ma solo a partire dal 2010 sono stati individuati numerosi danni su frutta in via di maturazione su vasta superficie coltivata a melo. A partire da questo momento la mosca mediterranea si è riproposta costantemente sul territorio trentino richiamando l'attenzione di ricercatori e istituzioni locali per svolgere studi di comportamento e biologia su questo Tefritide in relazione alle condizioni ambientali ed al sistema melicolo di produzione della regione Trentino (nord Italia).

I principali obiettivi di questo lavoro erano infatti:

- individuare la miglior tipologia di trappola in grado di implementare un efficacie piano di monitoraggio;
- valutare la sensibilità varietale alle ovideposizioni e allo sviluppo larvale in relazione al valore di alcuni parametri fisico-chimici delle mele al momento della maturità commerciale;
- monitorare l'attività di volo di questo insetto a livello locale;
- validare il modello gradi giorno di Tassan (Tassan *et al.*, 1982) per stimare la durata del ciclo vitale e il numero delle potenziali generazioni di mosca mediterranea in Trentino:
- valutare la capacità di svernamento della popolazione durante il periodo invernale;

• valutare l'efficacia di diversi insetticidi per il controllo di infestazioni localizzate di *C. capitata*;

Quattro tipi diversi di trappole commerciali, caricate con attrattivi diversi (cromotropiche, para-feromonale, attrattivi alimentari) sono state comparate in appezzamenti trentini di melo. Decis® Trap caricata con attrattivo alimentare, in grado di catturare sia maschi che femmine, è risultata essere la più adatta per il monitoraggio di questo insetto.

In Trentino, il volo degli adulti di *C. capitata* inizia durante la prima settimana di luglio ma l'intensità delle catture (numero di catture per trappola) rimane a livelli molto bassi fino ad agosto quando incrementa producendo un picco di catture durante il mese di settembre.

Nel mese di ottobre l'attività degli adulti decresce fermandosi completamente alla fine di novembre. Confermando le nostre osservazioni, eseguite in campo e in condizioni climatiche controllate, questo insetto riesce a superare l'inverno allo stadio di larva in mela caduta a terra in campo.

L'applicazione del modello gradi giorno di Tessan, basato sulle temperature registrate durante tutta la stagione, è in grado di fornire una buona stima sullo sviluppo della prima generazione (da uovo ad adulto 325,2 °DD e 44 °D per il periodo di pre-ovideposizione) nell'area in cui sono state fatte osservazioni dirette sul comportamento di questo insetto.

La suscettibilità varietale è stata valutata mettendo in relazione i parametri qualitativi (durezza, acidità, succosità e amido) delle principali varietà di mela coltivate in Trentino (Gala, Red Delicious, Golden Delicious, Granny Smith, Kanzi, Morgen and Fuji) con la preferenza di ovideposizione e la fitness larvale di due diversi ceppi di *C. capitata* in condizioni di laboratorio e di campo. I risultati ottenuti hanno dimostrato che la suscettibilità all'ovideposizione incrementa quando in frutti con un alto contento di zucchero e bassa resistenza alla penetrazione come nel caso delle varierà Gala, Golden Delicious, Kanzi and Fuji. Al contrario, le varietà Granny Smith, Red Delicious and Morgen hanno mostrato una bassa sensibilità per il basso contenuto zuccherino ed elevata durezza della buccia e polpa.

Per quanto riguarda la fitness larvale, i risultati suggeriscono che le varietà testate influenzano in modo considerevole vari aspetti della biologia degli stadi pre-immaginali e degli adulti come la sopravvivenza larvale nei frutti, la durata del ciclo di sviluppo (da uovo ad adulto) e la dimensione delle pupe. Golden Delicious, Gala, Kanzi and Fuji sono risultate essere le più favorevoli delle sette varietà messe in prova. Granny Smith, Red Delicious e Morgen invece, non hanno premesso un adeguato sviluppo delle larve e degli adulti.

Due diverse popolazioni di mosca mediterranea, una originaria del Trentino e una Spagnola (Girona) sono state utilizzate per testare l'efficacia di cinque insetticidi commerciali, ognuno

con un principio attivo diverso quali: Etofenprox, Cyazypir, Beta-Cyfluthryn, Spinosad and Thiacloprid.
Dagli studi di efficacia insetticidi è risultato che Spinosad e Beta-Cyfluthryn a dosi sub-letali causano elevata mortalità in adulti della popolazione spagnola. Inoltre, Beta-Cyfluthryn, Etofenprox e Spinosad, alla dose raccomandata di campo, riducono i danni da ovideposizione su frutta. Sulla popolazione trentina, alla dose raccomandata di campo Spinosad ha provocato una mortalità elevata negli adulti, mentre Cyazypyr ed Etofenprox non hanno funzionato a sufficienza per prevenire i danni di ovideposizione su frutta, sul numero di uova e sullo sviluppo larvale nei frutti.
Tutti i formulate insetticidi utilizzati alla dose di etichetta sono comunque risultati repellenti per l'ovideposizione da parte delle femmine in entrambe le popolazioni testate infatti i danni su frutta sono risultati significativamente ridotti su frutta trattata.

Le osservazioni comportamentali e i risultati sperimentali ottenuti nell'ambito di questo lavoro di tesi consentono di comprendere meglio la bio-etologia di C. capitata in una regione frutticola settentrionale come il Trentino. Queste informazioni sono fondamentali per consulenti tecnici e agricoltori al fine di ottimizzare le attuali strategie di gestione del fitofago. Nelle prospettive future, molti di questi risultati saranno di grande importanza per lo sviluppo di tattiche di controllo innovative e più sostenibili.

INTRODUCTION

The IPM concept

An increasing number of chemical methods for pest control were developed from the end of the Second World War, soon becoming widely used. The advent of synthetic insecticides gave rise to the so called “good period", during which these products were deemed to be able to solve all plant protection problems. This caused a disproportionate use of synthetic insecticides, underestimating their negative side-effects. Despite warnings from various quarters, and in particular from Stern *et al.* (1959), the use of these toxic substances grew exponentially until the 1970s, when the first ecological, toxicological and economic problems started.

It was indeed Stern *et al.* (1959) who created the “Integrated Control” term to precisely define the concept. This term was used to indicate the use of selective chemical products that do not harm the natural enemies present in the ecosystem. In this first concept, the economic injury level (EIL) and economic threshold (ET) were also described (Castle, 2009). In the early 1960s this term was implemented in Integrated Pest Management (IPM), which is “a comprehensive approach to pest control that uses combined means to reduce the status of pest to tolerable levels while maintaining a quality environment” (Alston, 2011).

The IPM approach was further incremented with other components aimed at improving the integration of pesticides and biological control. These components are: the recognition of ecosystem-level interactions between pests and their natural enemies, the methods of sampling and predicting pest occurrence, enhancing benefits of natural enemies through importation, augmentation or conservation and understanding the effects of pesticides on natural enemies (Jones *et al.*, 2009). The goals of the IPM programme are: the rational use of pesticides, sustaining agricultural or natural resources, reducing environmental contamination, the use of natural and biological control methods, minimising pesticide resistance, minimising pest resurgence and secondary pests, the reduction of pesticides residues on food, improving the safety of workers and last but not least, optimising profits (Diane, 2011).

Integrated production (IP) was indeed originally defined by the International Organisation for Biological and Integrated Control (IOBC) as “a farming system that produces high quality food and other products by using natural resources and regulating mechanisms to replace polluting inputs and to secure sustainable farming” (Boller *et al.*, 2004).

IPM is the main driving force of IP programmes, focusing on arthropod pests, pathogens and weed management.

Until recently, the application of IPM was a voluntary approach used by the most advanced and environmentally friendly fruit production systems. Since the approval of the EU Directive

128/2009, which established a framework for Community action to achieve the sustainable use of pesticides, the adoption of IPM in many European countries has become compulsory (Damos *et al.,* 2015).

European studies have demonstrated that the IPM system improves biodiversity and reduces pesticide use by approximately 20% in comparison with conventional farming (Damos *et al.*, 2015). Nowadays, the IPM system is considered the standard method in Europe to produce perennial crops (such as fruit, grapes and so on) (Freier *et al.,* 2009). Consumer demand for certified products has had a significant impact on current fruit production. Indeed, market studies have shown that European consumers are willing to pay for a reduction in pesticides, and apples benefit from a premium price when produced using the IPM process (Ehler, 2006).

IPM in Italy and the Trentino/South Tyrol Region

With more than 2 million tons of apples produced over a surface area of 52,000 ha (Dalpiaz, 2014), Italy is the fifth largest producer of apples in the world. Most apple cultivation takes place in northern regions, mainly in Trentino and South Tyrol and secondarily in Veneto, Emilia-Romagna, and Piedmont. In general, fruit production on this scale normally entails high use of pesticides (http://agri.istat.it), with major toxicological and environmental implications. Increasing awareness of the potential adverse effects of pesticides on pesticide applicators, agricultural workers and bystanders, as well as on the environment, has promoted the adoption of integrated control strategies aimed at reducing pesticide use and replacing the most dangerous compounds with more acceptable alternatives. In this context, Integrated Apple Production Guidelines have been implemented since 1991 in Trentino (Agnolin *et al.*, 2000). This voluntary approach to IPM implementation has been shown to result in an overall average improvement to the environmental impact of apple protection strategies that has been estimated to range from 23% to 24% (Ioriatti *et al.,* 2011). More recently, implementation of IPM became compulsory all over Europe after the approval of EU Directive 128/2009, which established a framework for Community action to achieve the sustainable use of pesticides (Ciampitti and Cavagna, 2014; Damos *et al.,* 2015).

Tortricid moths (Lepidoptera, Tortricidae) are the most important insect pests in apples, and historically these have been controlled with multiple insecticide applications. Economic losses associated with the codling moth *Cydia pomonella* (L.) on apple crops are also significant in Trentino, as indeed they are in other Italian regions, and these have been controlled with multiple insecticide applications. In addition to the ubiquitous codling moth, other species of tortricid moths appear occasionally and need specific control measures (Ioriatti and Lucchi, 2016). Beside tortricid moths, the rosy apple aphid *Dysaphis plantaginea* (Passerini) and the

woolly apple aphid *Eriosoma lanigerum* (Hausmann) (Hemiptera, Aphididae), as well as the apple proliferation phytoplasma vectors *Cacopsylla melanoneura* (Foerster) and *Cacopsylla picta* (Foerster) (Hemiptera, Psyllidae), require regular insecticide treatments. In recent years, a new pest, the Mediterranean fruit fly *Ceratitis capitata* (Wiedmann) (Diptera, Tephritidae), has appeared in apple orchards in Trentino. It was first detected in the early 1990s and since then its damage to apples has frequently been observed, mainly in the most southerly and warmest part of the region. Periodically, its presence has affected apple production heavily and required control with specific insecticide treatments, given that the pest attacks apples near harvest time, and chemical control results in increased insecticide residues on fruit, limiting market access.

Ceratitis capitata (Wiedmann)

The Tephritidae family includes about 4,000 species and 1,400 of these grow in maturing fruit, including many species of marketed fruit. These species are also commonly known as "fruit flies" because the larvae develop inside the ripening fruit. The particular characteristic of these species are the wings, which have different patterns with different dimensions and colours. The genus *Ceratitis* contains about 65 species, the majority of which are highly polyphagous (White *et al.*, 1992). White *et al.*, (1992) listed different synonyms that have identified *C. capitata* over the years: *Tephritis capitata* Wiedemann (1824), *Trypeta capitate* (Wiedemann) (1824), *Ceratitis citriperda* MacLeay (1829), *Ceratitis hispanica* De Brême (1842) and *Pardalaspis asparagi* Bezzi (1924).

The taxonomy for the Mediterranean fruit fly is:

Class: Insecta

Order: Diptera

Suborder: Brachycera

Infraorder: Muscomorpha (or Cyclorrhapha)

Family: Tephritidae

Subfamily: Dacinae

Tribe: Ceratitidini

Genus: *Ceratitis*

Subgenus: *Ceratitis*

Species: *Ceratitis capitata* (Wiedemann), 1824

C. capitata is also known as the "Mediterranean fruit fly", "Medfly" or "Fruit fly". Native to sub-Saharan Africa, *C. capitata* has spread to Mauritius, Reunion, Seychelles, North Africa, southern Europe, the Middle East, Western Australia and parts of Central, South and North

America (Figure 1). Details of its current geographical distribution are available in the EPPO Database Plant Quarantine Data Retrieval system (EPPO, 2011 and CAB-International, 1999). This fly attacks more than 300 different types of fruit, vegetables and nuts; host preference changes in different regions and includes apples (*Pyrus malus* L.), avocados (*Persea Americana* Mill.), citrus fruit (*Citrus* spp.), figs (*Ficus carica* L.), mangoes (*Mangifera indica* L.), peaches (*Prunus persica* L.) and pears (*Pyrus domestica* L.). Liquido *et al.* (1991) and Rigamonti (2005) report a long host list for *C. capitata*.

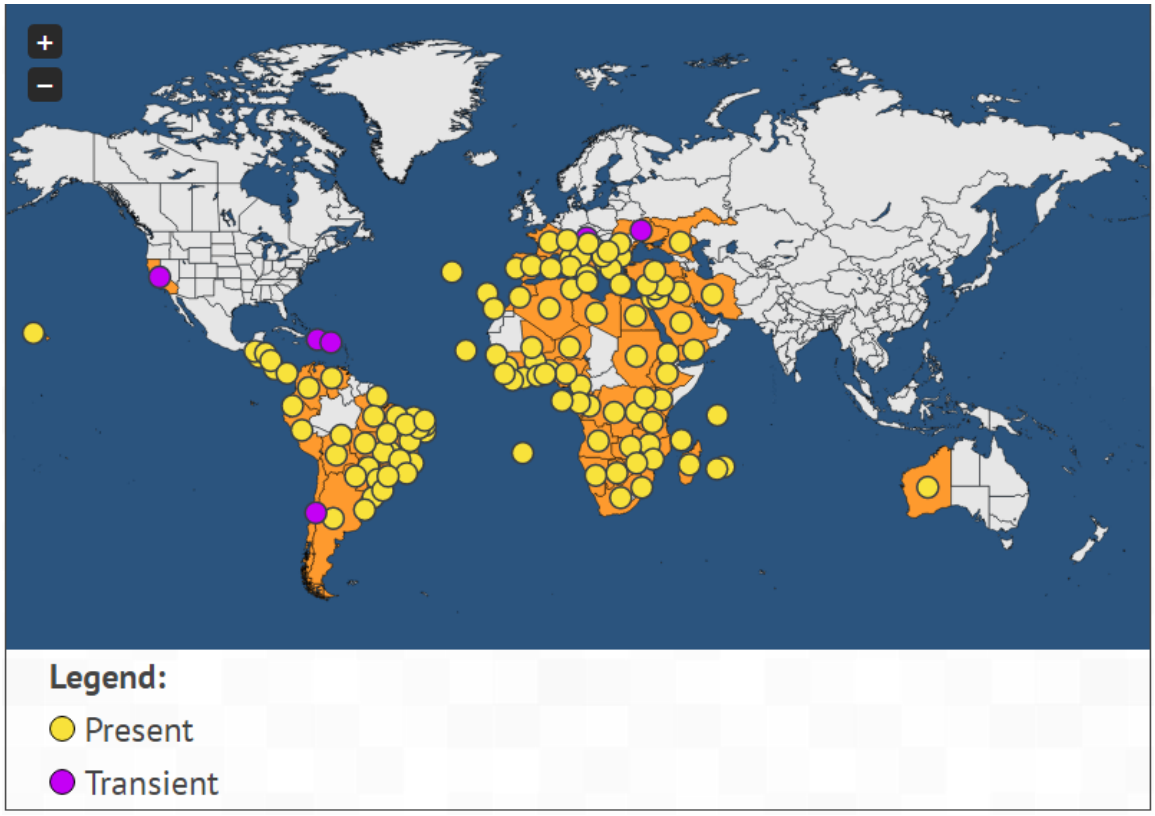


Figure 1: distribution of C. capitata in the world (https://gd.eppo.int/taxon/CERTCA/distribution)

Biology and bio-ethology

The Medfly is an insect with a complete metamorphosis or holometabolism, consisting of four stages: egg, larva, pupa and adult. The life cycle of the Medfly begins when the adult female lays from 1 to 10 eggs under the skin of fruit and vegetables. The newly emerged larvae feed on the fruit pulp and cause fruit decomposition. When the larva is mature, it emerges from the fruit and usually falls to the ground, where it stays until it pupates. The morphology of this insect is detailed in different works, such as those by Berg (1979) and White *et al.* (1992), and development of the different life stages is mainly correlated with temperature (Shoukry *et al.*, 1979; Duyck *et al.*, 2002; Grout *et al.*, 2007).

Adult: adults are from 4 to 5.5 cm long, with ochre-yellow head and iridescent eyes. The males can be differentiated from the females because in the front region of the head they have a pair of extended black antenna in the terminal section. The wings are 4 to 6 mm long, yellow and have a series of characteristic small brown spots. The thorax is shiny black with a number of cream-coloured stripes. They are black and white, with a yellow abdomen and yellow marks on the thorax, while the wings are banded with yellow (Bergsten *et al.*, 1999).

Under laboratory conditions, males live an average of 36 days at 25 ºC, while at the same temperature female longevity is 31 days, and longer when reared without males (67 days). The mating process begins with lekking behaviour, when male adults emit a pheromone and agitate their wings for 10-15 minutes. When a female approaches the male, she goes around him, he moves his head and flaps his wings, jumps on her and begins copulation (Field *et al.*, 2002). Copulation generally lasts 2-3 h (Whittier *et al.*, 1992). The courtship behaviour of the male was illustrated in Riceno *et al*., (1996). The pre-oviposition period lasts 4-6 days (Boller, 1985) and 826 eggs/female have been recorded for females reared with males and 248 eggs/female for those reared without them (Shoukry *et al.*, 1979).

Eggs: the eggs of the Medfly are 1 x 0.2 mm, curved, shiny white when recently deposited and later yellowish (Ros, 1988). The threshold of egg development occurs at 11ºC (Shoukry *et al.*, 1979). The eggs hatch after 48 hours (Boller, 1985). The viability of eggs placed at 9°C reduces from 98% to 48% after 48 hours and these eggs do not hatch after 6 days. The eggs do not hatch if they remain in dry conditions for 12 hours (Shoukry *et al.*, 1979).

Larvae: there are three different stages of larvae (L1, L2 and L3). The first larval instar is 2 mm and the third has an average length of 6.5-9 mm and a width of 1.2-1.5 mm (White *et al.*, 1992). The larval stage lasts 7-8 days (Boller, 1985). These apoda larvae destroy fruit pulp with their chewer buccal system (Remigio *et al.*, 2010). The zero point of larval development occurs at 5 ºC (Shoukry *et al.*, 1979).

Pupae: the pupa is cylindrical, 4–4.3 mm long and dark reddish-brown. At temperatures between 22 ºC and 30 ºC, this stage lasts 9-11 days (Boller, 1985). Other studies have demonstrated that a temperature of 35 ºC is fatal. At 60% R.H. the threshold of pupal development is 13 ºC (Shoukry *et al.*, 1979).

If the conditions are not favourable because of low temperatures, this stage can be extended by several days (Mavrikakis *et al.*, 2000). Medflies emerge from the ground, mate and complete the cycle.

Dispersion: *C. capitata* spreads with the transport of infested fruit and through adult flight. Although adults generally travel short distances, they have been reported to be airborne or to fly from 3.7 to 20 km (Fletcher, 1989; Díaz *et al.*, 2008). Although the wind is the main

determinant of long-distance dispersal (Aluja, 1993), the pest has the capacity to locate host fruit species and to continue its movement depending on the availability of fruit (Fletcher, 1989; Prokopy *et al.*, 1989; Barbosa *et al.*, 2000).

Overwintering study: the northern limit of distribution has been indicated at around the 41st parallel north and its presence above this is occasional (Rigamonti, 2002).

Thus the presence of *C. capitata* in orchards in this area can be explained with two hypotheses: 1) new introduction with infested fruit or trade and/or 2) the possibility of overwintering. The two different models and hypotheses are explained in detail by Carrey (1991).

Adults of this species are present throughout the year in several areas, including the southern (Fimiani, 1989) and eastern coast of Spain, particularly Tarragona and Valencia (Martínez-Ferrer *et al.*, 2007) and northern Greece (Papadopoulus *et al.*, 1996, 1998, 2002). In southern Italy the temperate climate allows the Mediterranean fruit fly to overwinter at an adult stage (Bateman, 1972).

In northern Italy, adults have been shown to overwinter in sheltered parts of indoor environments (Rigamonti *et al.*, 2002; Rigamonti, 2004a; 2004b). One of the most significant studies concerning the overwintering mechanism of the Mediterranean fruit fly in cold regions took place in northern Greece, close to the northernmost boundary of its distribution limit. Papadopoulos *et al.* (1996, 1998, 2002) suggested that the Medfly overwinters at the larval stage, mainly in late apple varieties, and that the adult emerges in spring and produces the first generation. These studies have reported overwintering larval survival to be lower than 40% (Papadopoulos *et al.* (1998).

On the other hand, Israely *et al*., (2004) did not support the hypothesis that the Mediterranean fruit fly overwinters either as a pupa or adult in cold winter areas. Thus migration from favourable areas nearby is the only remaining option. None of the infested apples produced adults at high altitude sites (700 m), whereas at lower sites (400 m), flies emerged from the tested fruits.

Studies of the population dynamics of the Mediterranean fruit fly have shown that the main factor affecting population is the abundance of fruit and higher winter temperatures. In contrast, the absence of host fruit for a long period (Israely *et al.*, 1997; Papadopoulos *et al*., 2001a; 2001b) and lower temperatures are the two main factors that can inhibit overwintering, reducing the population after the winter or delaying the presence of individuals during the season (Escudero-Colomar *et al.*, 2008; Trematerra *et al.*, 2008).

Abiotic factors significantly affect population fluctuations in the Mediterranean region (Papadopoulos *et al*., 1996; 1998; 2001a; 2001b). In a study of the geographical distribution of the Medfly in different areas using the CLIMEX program, the main limiting factor determining

survival in winter was found to be cold stress. This program does not exclude the lethal effect of low temperature (e.g. freezing), but extreme temperatures appeared to be less restrictive to distribution than the limitation imposed by the need for thermal accumulation in winter (Vera *et al.*, 2002). Indeed, the insect's survival depends on both temperature and the duration of exposure (Denlinger *et al.*, 1998).

The combination of factors such as dry and cold stress also play an important role (Vera *et al*., 2002) and the duration of low temperatures could be responsible for the low incidence of the pest in a particular area.

On the other hand, in subtropical areas, the abundance of *C. capitata* is mainly affected by rainfall and relative humidity (Harris *et al.*, 1987), while in Morocco both humidity and temperature affect the population fluctuations of this insect (Mazih and Debouzie, 1996). Moreover, Vergani (1961) argued that temperate winters followed by hot, humid summers ensure a short biological cycle and abundant proliferation.

Monitoring

The presence of adult insects in the field leads to a risk of damage to ripe fruit. For this reason it is important to monitor the presence of the fly during the season. Different traps and lures are available for monitoring infestation in the orchard. The most widespread traps are the delta trap or Jackson Trap (Biogard, CBC, Milan Italy; Bioplanet, Cesena, Italy) with a sticky bottom, yellow sticky Cromotrap (Isagro S.p.A., Adria, Italy) and McPhile traps or Decis® trap (Bayer, Milan, Italy) covered with Deltametrin. Traps can be baited with the para-pheromone Trimedlure or with a food attractant. The para-pheromone lasts for 30 days and is attractive only for males (Di Franco *et al*., 2013). As regards food attractants, there are many commercial baits with different composition and formulations on the market: Ferag® CC D TM Compacto (SEDQ, Barcelona, Spain) with ammonium acetate, diaminoalkane and trimethylamine in an single packet; Biolure Unipak (Suterra, LLC, Valencia, Spain) with 1,4-diamminobutano, ammonium acetate and trimethylamine hydrochloride in a single packet; Tripack MFL (Sumitomo Chemical, Kenogard, Barcelona, Spain) with ammonium acetate, trimethylamine hydrochloride and trimethylamine in three different packets. The duration of these food attractants is guaranteed for 120 days, attracting both males and females (Espanada, 2009).

Control

Chemical control. Control of the Medfly is still largely based on the application of chemical insecticides. Adults are targeted, because the eggs, larvae and pupae are protected under the skin, inside fruit and in the ground. Insecticides are applied according to a threshold based on

the capture of flies: one capture in two consecutive trap (trimedlure or food baited) controls (Gencat, 2009) or 3-4 adult/traps/week/ in a peach or apple (Golden Delicious) orchard (Pollini, 2009). Few insecticides are currently registered for *C. capitata* control and their efficacy for Mediterranean fruit fly adults is not completely known. Chemical treatments are mainly based on pyrethroids and organophosphate, but their use is limited, especially near harvesting, because of potential residues on fruit.

The only insecticides registered for use against *C. capitata* for pome fruit trees are shown in Table 1.1.

Table 1.1 Identification, safety period and uses of active ingredients authorised for use in pome fruit trees against the Medfly (IRAC, 2017).

Group and IRAC mode of action	Chemical subgroup	Active ingredient	Commercial name	Safety period (days)
1 Acetilcholine esterase inhibitor	1B Organo-phosphate	Chlopyrifos-methyl	Reldan Lo	21
3 Sodium channel modulators	3A Pyrethroids Pyrethrins	Etofenprox	Trebon up	7
		Deltamenthrin	Decis	7
		Beta-Cyfluthrin	Bayteroid 25 EC	7
		Pyrethrins (pyrethrum)	Asset/Piganic 1.4	2
4 Nicotinic acetylcholine receptor (nAchR)	4A Neonicotinoids	Acetamiprid	Epik SL	14

Spinosad, a combination of purified spinosyns of the actinomycete *Saccharopolyspora spinosa* Mertz & Yao, has recently become a valuable alternative to traditional insecticides for Medfly control on stone fruits (Thompson *et al*., 2000).

In field experiments, the effectiveness of this product for the control of *C. capitata* has been demonstrated (Burns *et al.*, 2001). Spinosad has fewer environmental effects and is less toxic to beneficial organisms than pyrethroids, organo-phosphates and neonicotinoids (Stark *et al.*, 2004).

Spinosad is also available as the toxic bait Spintor Fly (Dow AgroSciences). In addition to spinosad, the bait formulation contains other ingredients that make it attractive and persistent on the tree canopy. When sprayed onto vegetation, it attracts and kills Medfly adults (Boselli *et al*., 2013). Spintor Fly is not registered for control of *C. capitata* on the apple. The market offers several different systems that can reduce chemical insecticide treatments, have good selectivity on the beneficial organism, lower water usage and lower costs for distribution (Lux *et al.*, 2003; Espadas, 2009; Boselli *et al.*, 2013). An attract and kill system is a combination of attractants

and insecticides to lure and kill males and females of the Medfly and only one application is required for the entire cropping season (Zaidan Khalaf *et al.*, 2013). There are other attract and kill systems similar to Spintor Fly; Magnet Med® (Suterra, Europe Biocontrol S.L., Valencia, Spain) panel with deltamethrin and food attractant (50-70 panels/ha) (Caponero *et al*., 2014); Ceratrap (Bioiberica, Barcelona, Spain), distributed in 90-120 bottles/ha, contains a liquid attractant that lures flies and kills them inside (Boselli *et al*., 2013); Ceranock (Russell IPM, United Kingdom).

Tabilio *et al.* (2008) showed that NeemAzal-T/S (azadirachtin, oviposition repellent, CBC Europe s.r.l., Monza-Brianza, Italy), GF120 (Spinosad, for ingestion, DowAgrosiences) and Biophytoz L2 (rotenone, for contact and ingestion, Euphytor sarl., Vidauban, France) have been shown to be effective against the Mediterranean fruit fly in laboratory bioassays. The first two insecticide products mentioned above, admitted in organic production, are reported to be a good alternative to chemical insecticides, while the latter is not permitted in organic farming.

Another alternative to chemical control is kaolin clay, which is a non-toxic material. Its efficacy has been tested both in laboratory and field trials by exposing treated fruit to Medfly adults. The almost complete fruit protection (Michal *et al.*, 2004) is counterbalanced by the need to wash the fruit after harvesting (www.mensileagrisicilia.it/immagini/ciainserto.pdf).

Biological control. Biological control offers little prospect of success, also because this insect spends much time of its life cycle inside the fruit or in the soil. Despite the abundance of natural enemies, the biotic potential of this species is very high and only in particular contexts can the antagonists exercise effective control action. In the Mediterranean there are no real antagonists that can play a significant role in biological control. Their contribution to containing the pest is limited to the occasional predator. The parasitoids whose action has been reported are *Psyttalia (= Opius) concolor* (Slépligeti) (Hymenoptera, Braconidae) and *Pachycrepoideus vindemmiae* (Rondani) (Hymenoptera, Pteromalidae) in North Africa and the Middle East. The former species has also been used in biological control programmes in the Hawaiian Islands. This experience, started with introduction by Silvestri in 1914, represents one of the best results for biological control of the Medfly during the past 70 years (David *et al.*, 1996).

Some exotic parasitoids have been studied in an attempt to control the Medfly in Spain: the larval-pupal parasitoid *Diachasmimorpha tryoni* (Cameron), the egg-pupal parasitoid *Fopius arisanus* (Sonan) and the two larval endoparasitoids *Diachasmimorpha longicaudata* (Ashmead) and *P. concolor*, all of them Hymenoptera of the Braconidae family (Alonso-Muñoz *et al*., 2008; Beitia *et al.*, 2008). Further research on these species is needed to understand the

optimal conditions for guaranteeing success in Medfly control in the event of their release, (Jang *et al.*, 2000; Moretti *et al.*, 2003;Wang *et al*, 2003; Rousse *et al.*, 2009)

Few studies have evaluated the impact that predators have on *C. capitata* (Debouzie, 1989). Predators of pupae and larvae such as ants, carabid, staphylinid beetles and spiders have been cited (Allen *et al.*, 1990; Eskafi *et al.*, 1990; Galli *et al.*, 1996) as having great importance in regulating the abundance of the fruit fly population in the field (Bateman 1974; Wong *et al.*, 1984; Hogdson *et al.*, 1998).

Many Diptera species have been found to be susceptible to *Bacillus thuringiensis* (Berliner). These include mosquitoes, blackflies, chironomids, tipulids, muscids, sciarids and drosophilids (Alberola *et al.*, 1999; Charles, *et al.*, 2000), together with tephritid flies such as the olive fruit fly *Bactrocera oleae* (Gmelin) (Karamanlidou, *et al.*, 1991; Alberola *et al.*, 1999), the Mexican fruit fly *Anastrepha ludens* (Loew) (Robacker *et al.*, 1996; Toledo *et al.*, 1999) and the Mediterranean fruit fly (Gingrich, 1987).

The effectiveness of a bioinsecticide based on the fungus *Beauveria bassiana* (Balsamo-Crivelli) and *Beauveria brongniartii* (Saccardo) has been tested on the Medfly in the laboratory. The results showed that these two fungi caused respectively 97.4% and 85.6% mortality of *C. capitata* adults (Konstantopoulou *et al.*, 2005). Another study performed in a greenhouse demonstrated pre-pupal control efficiency of 66.6% (Almeida *et al.*, 2007).

The pathogenicity of several entomopathogenic nematodes against Medfly larvae and pupae has been studied. In particular, Tabilio *et al*. (2008) tested different species: *Heterorhabditis bacteriophora* (Poinar), *Steinernema feltiae* (Filipjev,) and *Steinernema carpocapsae* (Weiser). The tests were set up in the laboratory. *H. bacteriophora* and *S. carpocapsae* showed little effectiveness, while *S. feltiae* showed nearly 100% larval parasitisation. This result was not confirmed in semi-field tests, where a parasitisation percentage of around 56% was obtained.

Mass trapping. McPhile traps with food lure. This system initially adopted liquid protein bait or fermented sugar substances (McPhail, 1939), but in recent years new baits have been developed that are more effective and easier to manage. The first solid food-based synthetic attractants consisted of ammonium acetate and 1,4-diaminobutane (putrescine) (Epsky *et al.*, 1995). The food lures are the same mentioned above: one dispenser is Ferag® CC D TM (SEDQ, Barcelona, Spain), and putrescine, ammonium acetate and trimethylamine were formulated in one dispenser as BioLure® Med Fly (Suterra S.L.) (Navarro-Llopis *et al*., 2012; Penarrubia-Maria *et al.*, 2013).

Food baits developed for the Medfly attract both males and females and can reduce the number of mated females, so they are a useful tool in fruit fly control (Lux *et al.*, 2003). Using food-

based attractants in mass trapping in apple production has proved effective against higher and lower Medfly population levels (Escudero-Colomar *et al.*, 2005).

Attraction and sterilisation. Lufenuron bait is a phenyl-benzoylurea, a chitin synthesis inhibitor (Bachrouch *et al.,* 2008). Navarro-Llopis *et al*. (2004) showed that a method based on chemical sterilisation was able to reduce the Mediterranean fruit fly population. This method bases its effectiveness on the horizontal transmission of sterility, so by using the Medfly's capacity to find other Medflies we can sterilise a significant proportion of the population even when the bait has been not ingested (Casaña-Giner *et al.*, 1999). This product causes sterilisation of male and female Mediterranean fruit flies, avoiding the hatching of eggs inside the fruit, so the larvae do not emerge, thus reducing the subsequent generation. In Italy, Adress (Syngenta, Basel, Switzerland) is registered for use against *C. capitata* on citrus, apple and stone fruit, with a concentration of 24 dispensers/ha.

Sterile insect technique (SIT). The SIT is an eco-friendly method for the management of selected insect pests. It relies on mass rearing of insects of the target population, sterilising them with ionising radiation, followed by release of the sterile males in the target area, where they will mate with virgin wild females and transfer their sterile sperm, which results in unviable eggs (Knipling, 1955). Successive releases of sterile insects will gradually reduce the density of the target population to a very low, economically acceptable level, and in some cases, eradication may be achievable (Knipling, 1955). The SIT has been shown to be very effective for the suppression, containment, prevention or eradication of Mediterranean fruit fly populations (Hendrichs *et al.*, 2002).

VIENNA 8 is a genetic sexing strain (GSS) that has white pupae (wp) and a temperature sensitive lethal (tsl) mutation, the latter killing all female embryos when the eggs are exposed to high temperatures (34 °C). In this case, it is necessary to produce only male larval lines for their release in the field (Rempoulakis *et al.*, 2016).

After release, it is necessary to keep the situation under control (Vreysen *et al.,* 2005). The most commonly used marking system for Mediterranean fruit flies relies on dyeing of the pupae with fluorescent powder, which gets sequestered in the frontal suture of adults during their emergence from the puparium (Schroeder *et al.*, 1972, 1981). Use of the SIT system leads to a decline in the wild population, while the number of insects released remains constant through repeated releases, the proportion of infertile mates increases and the rate of suppression of the wild population gradually increases (dense-inverse dependence) until eventual eradication is achieved (Hendrichs *et al.,* 2009). For initial releases the FAO-IAEA has recommended ratios from 25-50:1 in preventive operations, to 100-150:1 in eradication actions (Enkerlin, 2007). In

Mexico, the MOSCAMED programme carries out releases to maintain populations of 1,500 to 5,000 sterile males per hectare, according to various criteria (Guzmán-Plazola, 2010).

Around 1,800 sterile males/ha were released every week in Valencian citrus groves (Generalitat-Valenciana, 2009). In 2003, 1,000 sterile males/ha were released each week in a commercial area in Tunisia and another 2,000 sterile males/ha in the surrounding "buffer" area to reduce the possibility of re-infestation (M'Saad Guerfali *et al.*, 2008).

Agronomical measures. The destruction of infested fruit or unsold fruit after harvest plays an important role in reducing insect populations in the field. It is possible to put the fruit in the soil at a depth of least 25 cm to prevent new adults from emerging (Chueca, 2007; Guzmán-Plazola, 2010; Chueca, *et al.,* 2013). It is also possible to destroy the fruit in the field and treat it with hydrated lime (Guzmán-Plazola, 2010).

Exclusion nets. The first tests on the effectiveness of coverage with mesh (Mesh antimosque, 1 x 2 mm) were carried out in a biodynamic peach orchard in Sicily, in southern Italy, by comparing the results with plots treated with pyrethrum or rotenone, or left untreated. In this test, the fruit under the net arrived at harvest without damage, in contrast with peach treatments, which showed more damaged fruit. This coverage involves substantial initial investment. Studies carried out by the Department of Arboreal Crops, at the University of Palermo, showed that peach orchard coverage for one month with this 1 x 2 mm mesh net does not significantly modify the physiology of the plant and the characteristics of the fruit (Progetto di sviluppo dell'agricoltura biologica in Sicilia, 2011).

Post-harvest treatment

Cold treatment. This consists in treatment of the fruit at low temperature, below the thermal tolerance limit of *C. capitata*. However, Mercado (2011) mentions that fruit stored at 0.56, 1.11 and 1.67 ºC for 18, 20 and 22 hours respectively can suffer from a decrease in its commercial value. Cold treatments keeping fruit at 1.1 ºC, 1.7ºC and 2.2 ºC for 14, 16 and 18 days respectively are currently being used to disinfest tangerines shipped from Spain to the United States and China (Palou *et al.*, 2007).

Heat treatment. To eliminate eggs, it is also possible to use heat treatment of the fruit. Queb-Gonzalez *et al*., (2014) showed that 100% of mortality of eggs is obtained with treatment at 44°C for 50-80 minutes. The larval stage is more tolerant to higher temperature, because the larvae create galleries inside the fruit pulp where they can shelter. For this reason, the temperature and the duration of exposure to heat depend on the type of fruit (Wang *et al.,* 2001).

Insecticidal atmosphere. Another treatment that can be exploited is so-called insecticidal atmosphere. Contrearas-Olive *et al.* (2011) showed that by treating citrus fruit with 95 % CO_2 at 1.5 °C for 3 days, 100 % mortality of the *C. capitata* population was obtained.

Ionising irradiation. This can be performed with X-rays or γ-rays (Hallman, 1999). Gamma irradiation has been shown to be a successful alternative disinfestation treatment for various fruit and vegetables (Torres-Rivera *et al.*, 2007; Hallman, 2008).

It is possible to use this method after packing and palletizing the product, but it also has limitations, as it does not guarantee high Medfly mortality, although individuals are later unable to completely develop and reproduce.

The minimum absorbed dose to prevent the emergence of adults of *C. capitata* is of 100 Gy. This irradiation treatment should not be applied to fruits and vegetables stored in modified atmospheres (ISPM, 2011).

Combination of several control methods

To guarantee adequate control of the Medfly in the field, it is possible to use a combination of different control systems. This is also interesting for IPM systems, allowing a reduction in insecticide treatment.

For example, it is possible to combine mass trapping with spinosad-based bait spray control. These two methods have also shown good compatibility with biological control and could be combined in an IPM system for *C. capitata* (McQuate *et al.*, 2005).

Another study (Piñero *et al.*, 2009) showed that spinosad-based protein bait sprays, distributed with foliar applications in combination with systematic field sanitation (destruction of infested fruit, removal of all fruit in the field after harvest and so on), proved to be successful in reducing the population level of female *B. dorsalis* and damaged fruit in papaya orchards.

Furthermore, Barry *et al.* (2003) demonstrated that protein-deficient Medfly adults (males without protein feeding in the diet) were more active and found the bait more often than those which were protein-fed. This study suggests that adding protein to the diet of male adults to be used in SIT would reduce their response to baits and consequently reduce their mortality. This would indicate the need for simultaneous use of spinosad bait sprays and the sterile insect technique.

The economic importance of *Ceratitis capitata* in Trentino orchards

The Medfly is a highly adaptive polyphagous tropical fruit fly (Papadopoulos *et al.*, 1996) and attacks more than 350 botanical species (Weems, 1981; Liquido *et al.*, 1991). The most vulnerable family is Rosaceae, which includes the main fruit species cultivated in the Trentino

area. Indeed, as mentioned above, the apple is the main crop cultivated. Damage by the Medfly is caused by mated females, which lay eggs on host fruit and damage the fruit skin with their ovipositor. The larvae eat the pulp, causing its decomposition (Fig. 2), and at the same time cause the entry of secondary pathogens, which destroy the fruit (Bergsten *et al.,* 1999).

The presence of *C. capitata* was reported for the first time in a limited area of Trentino in 1990. Then, after a period without observed fruit damage, it reappeared in 2010 and 2012, causing damage in many apple orchards in the southern area of Trentino and along the River Adige (Tait *et al*., 2012; Dallago, 2013). Rigamonti (2002) declared that the northern limit of its distribution was indicated around the 41st parallel north, but the presence of *C. capitata* in Trentino has been constant from 2010 until the present day. In most apple orchards (around 100 ha) control was carried out using the Magnet Med system (attract and kill) and insecticide treatments. In our case, the use of these methods for Mediterranean fruit fly control did not enable the correct economic damage threshold to be reached. For this reason it is necessary to carry out further study into this tephritid fly, in particular as regards its bio-ethology in the Trentino region.

Aims of the research

The general aim of this Ph.D. is to explore the bio-ethology of the Medfly (maximum possible number of generations in our region, susceptibility of different apple varieties and larval stage development in different apple varieties), the most suitable monitoring system and the best insecticide system for control. Overall, more research is needed on alternative *C. capitata* control tools, in order to develop more globally sustainable IPM strategies.

It is therefore necessary to have the different stages of the Medfly available in the laboratory. Indeed, in our laboratory we breed two different strains of insects to have the various stages of insect available whenever we need them for experiments.

Mass rearing. This is essential for practical application of the main control methods, including the sterile insect technique (SIT) (Steiner *et al.*, 1962). These breeding systems were indeed used and adapted to our needs. Marlow (1934) was able to obtain a complete cycle of *C. capitata* in laboratory conditions, therefore suggesting interesting possibilities for using this method on a large scale. In our laboratory two different insect strains were maintained, a wild strain (Trentino) and a laboratory strain (ISPRA). Both were reared in the same conditions.

The adults are placed inside a completely closed black plastic cage with an opening sealed with white gauze; they are fed with water and solid food (sugar and brewer's yeast, 10:1). The opening is positioned in front of an artificial light source. A gauze with a mesh of around 300 microns and direct light are necessary to guarantee adequate stimuli for oviposition (Cavalloro

et al., 1969). The eggs are laid on the gauze surface and then fall into the water. Indeed, under the cage there is a tray with water that allows the fallen eggs to remain hydrated. The eggs are then collected and positioned in the larval diet (hydrated alfalfa with water, sodium benzoate, citric acid, sugar and brewer's yeast) inside a plastic box (Manoukas, 2002). There are many works about the best "pabulum" for larval development (Cavalloro *et al*., 1969; Harvey *et al*., 1995; Kaspi *et al.*, 2002). Before the pupal stage, mature larvae emerge from the diet and pupate. The pupae are then collected and positioned inside a new adult cage. The adults stay in the rearing cage for three weeks. The mass rearing units are maintained at 25 ± 2°C, 65 ± 5% R.U. and 16:8H (L:D).

References

Agnolin, C.; Ioriatti, C.; Pontalti, M.; Venturelli, M.B. (2000). IFP experiences in Trentino, Italy. *Acta. Hort.* (ISHS) 525:45-50.

Alberola, T.; Aptosoglou, M.S.; Arsenakis, M.; Bel, Y.; Delrio, G.; Ellar, D.J. (1999). Insecticidal activity of strains of *Bacillus thuringiensis* on larvae and adults of *Bactrocera oleae* Gmelin (Dipt. Tephritidae). *J. Invertebr. Pathol.*, 74: 127-136.

Allen, W.R.; Hagley, E.A. (1990). Epigeal arthropods as predators of mature larvae and pupas of the apple maggot (Diptera: Tephritidae). *Environ. Entomol.* 19: 309-312.

Almeida, J.E.M.; Batista, A.; Oliveira, F.C.; Raga, A. (2007). Pathogenicity of the entomopathogenic fungi and nematode on medfly Ceratitis capitata (Wied.) (Diptera: Tephritidae). *BioAssay.*, 2: 1-7.

Alonso-Muñoz, A.; Arilla, J.; Torres, J.; Fernández, L.; García, E.; García-Marí, F. (2008). Influence of nearby fig trees on medfly captures of traps located in citrus orchards. *First Meeting of TEAM*, Palma de Mallorca pp. 53.

Alston, D. G. (2011). The Integrated Pest Management (IPM) Concept. Published by Utah State University Extension and Utah Plant Pest Diagnostic Laboratory. All Archived Publications. Paper 766: 1-2.

Aluja, M. (1993). The study of movement in Tephiriditid flies: Review of concepts recent advances. In: Aluja, M.; Liedo, P. [eds.], *Fruit flies. Biology and management.* Springer, Verlag, New York, pp. 105-113.

Bachrouch, O.; Mediouni-Ben, J.; Alimi, E.; Skillman, S.; Kabadou, T.; Kerber, E. (2008). Efficacy of the lufenuron bait station technique to control Mediterranean fruit fly (Medfly) *Ceratitis capitata* in citrus orchards in Northern Tunisia. *Tunisian J. plant protection.*, 3: 35-45.

Barbosa, S.; Mexic, A.; Pereyra, R. (2000). Dispersal and survival of sterile male (tsl strain). Mediterranean Fruit Flies. In: Ken-Hong-Tan [eds.], *Area wide control of fruit flies and other insect pest*. Penerbit Universiti Sains Malaysia Pinang, pp. 527.

Barry, J.D.; Vargas, R.I.; Miller, N.W.; Morse, J.G. (2003). Feeding and foraging of wild and sterile Mediterranean fruit flies (Diptera: Tephritidae) in the presence of spinosad bait. *J. Econ. Entomol.*, 96: 1405-1411.

Bateman, M.A. (1972). The ecology of fruit flies. *Annu. Rev. Entomol.*, 17: 493-518.

Bateman, M.A. (1974). Fruit flies. In: V.L. Delucchi (eds), *Studies in Biological Control*. IBP 9. Cambridge University Press, Cambridge. pp. 11-49.

Beitia, F.; Pérez-Hinarejos, M.; Garzón, E.; Santiago, S.; Falcó, V.; Castañera, P. (2008). Potential for biological control of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) by parasitoids in Spanish Mediterranean areas. *First Meeting of TEAM*, Palma de Mallorca,

Berg, G. H. (1979). Pictorial key to fruit fly larvae of the family Tephritidae. Organismo Internacional Regional de Sanidad Agropecuaria. San Salvador, El Salvador. pp. 36.

Bergsten, D.; Lance, D.; Stefan, M. (1999). Mediterranean fruit flies and their management in the U.S.A. The Royal Society of Chemistry 10: 207-212.

Boller, E.F. 1985. *Rhagoletis cerasi* and *Ceratitis capitata*. Handbook of Insect Rearing 2: 135-144.

Boller, E.F.; Avilla, J.; Joerg, E.; Malavolta, C.; Wijnands, F.G.; Esbjerg, P. (2004). Integrated Production: principles and technical guidelines (3rd edition). *IOBC/WPRS Bulletin*, at http://www.iobc.ch. 27(2).

Boselli, M.; Bariselli, M. (2013). Contro le mosche della frutta senza danneggiare l'ambiente. *Servizio fitosanitario Regione Emilia Romagna,* Agricoltura pp. 74-75.

Bulletin OEPP/EPPO. European and Mediterranean Plant Protection Organization PM 7/104 (1), 2011. *Ceratitis capitata. Bulletin* 41: 340-346.

Burns, R.E.; Harris, D.L.; Moreno, D.S.; Eger, J.E. (2001). Efficacy of spinosad bait sprays to control Mediterranean and Caribbean fruit flies (Diptera: Tephritidae) in commercial citrus in Florida. *Fla. Entomol.,* 84: 672-678.

CAB-International. (1999). Distribution maps of plant pests, *Ceratitis capitata*. Commonwealth institute of entomology. www.cabi.org/.

Caponero, A.; Lacertosa, G.; Quinto, G. R. (2014). MagnetMed, sistema efficacie contro la mosca della frutta. *L'Informatore Agrario* 26: 52-55.

Casaña-Giner, V.; Gandia-Balaguer, A.; Mengod-Puerta, C.; Primo-Millo, J.; Primo-Yufera, E. (1999). Insect growth regulators as chemosterilants for *Ceratitis capitata* (Diptera: Tephritidae). *J. Econ. Entomol.,* 92: 303-308.

Castle, J.S. (2009). Implementing principles of the integrated control concept 50 years later current challenges in IPM for arthropod pests. *Pest Manag. Sci.,* 65: 1263-1264.

Cavalloro, R.; Girolami, V. (1969). Miglioramento nell'allevamento in massa di *Ceratitis capitata* Wied. (Diptera:Trypetidae). Redia, LI: 315-327.

Charles, J. F.; LeRoux N.C. (2000). Mosquitocidal bacterial toxins: Diversity, mode of action and resistance phenomena. *Mem. Inst. Oswaldo Cruz.,* 95: 201-206.

Chueca, P. (2007). Mecanización de métodos de control de *Ceratitis capitata* (Wiedemann) en cítricos. Departamento de mecanización y tecnología agraria. Unversidad Politécnica de Valencia, Valencia, pp. 246.

Chueca, P.; Garcera, C.; Urbaneja, A.; Molto, E. (2013). A new mechanised cultural practice to reduce *Ceratitis capitata* Wied. populations in area-wide IPM. *Spanish J. Agric. Res.,* 11(4): 1129-1136.

Ciampitti, M; Cavagna, B. (2014). Developments in integrated pest management in Italy. *Phytopathologia Mediterranea,* 53(3): 379-384.

Contreras-Oliva, A.; Prerz-Gago, M.B.; Palou, L.; Rojas-Argudo, C. (2011). Effect of insecticidal atmosphere and low dose X-ray irradiation in combination with cold quarantine storage on bioactive compounds of clementine mandarins' cv. 'Clemenules'. *Int. J. Food Sci. Tecn.* 46: 612-619.

Dallago, G. (2013). La mosca mediterranea della frutta. Una presenza ormai costante. *Rapporto 2013 Centro di Trasferimento Tecnologico* pp.47-48.

Dalpiaz, A. (2014). Innovazione e organizzazione, le uniche risposte per uscire dalla crisi. *Frutticoltura,* 11: 2-6.

Damos, P.; Escudero-Colomar, A.L.; Ioriatti, C. (2015). Integrated fruit production and pest management in Europa: The apple case study. *Insects.* 6(3): 626-657.

Dati ISTAT. (2011). http://dati.istat.it/Index.aspx?DataSetCode=DCSP_COLTIVAZ.

David, H.; Headrick, H.; Goeden R.D. (1996). Issues Concerning the Eradication or Establishment and Biological Control of the Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), in California. *Biological Control,* Vol. 6(3): 412-421.

Debouzie, D. (1989). Biotic mortality factors in tephritid populations. In: A.S. Robinson and G. Hooper [eds], *World Crop Pests*, Vol. 3B, Fruit flies. Elsevier, New York. pp. 221-227.

Denlinger, D.L.; Lee, R.E. (1998). Physiology of cold sensitivity. Temperature sensitivity in insects and application in Integrated Pest Management, pp. 55-95.

Di Franco, F.; Audion, P.; Magnano San Lio, R.; Oppedisano, R.; Benfatto, D. 2013. Mosca mediterranea su agrumi: efficacia della cattura massale. L'Informatore Agrario 17:59-62.

Díaz, L.M.; Murúa, F.A.; Acosta, J.C; Escobar, J.M. (2008). Capacidad dispersiva de *Ceratitis capitata* (Diptera: Tephritidae) entre valles agrícolas en San Juan, Argentina. *Rev. Soc. Entomol. Argent*., 67: 155-161.

Duyck, P.E.; Quilici, S. (2002). Survival and development of different life stage of three *Ceratitis* spp. (Diptera: Thephritidae) reared at five constant temperatures. *Bull. Entomol. Res.*, Vol. 92: 461-469.

Ehler, L.E. (2006). Integrated pest management (IPM): Definition, historical development and implementation, and the other IPM. *Pest Manag. Sci.*, 62, 787–789.

Enkerlin, W. (2007). Guidance for packing, shipping, holding and release of sterile flies in area-wide fruit fly control programmers. Joint FAO/IAEA Programme of Nuclear Techniques in Food and Agriculture

Epsky, N. D.; Heath, R. R.; Guzman, A.; Meyer, W. L. (1995). Visual cue and chemical cue interactions in a dry trap with food-based synthetic attractant for Ceratitis capitata and Anastrepha ludens (Diptera: Tephritidae). *J. Environ. Entomol.*, 24: 1387-1395.

Escudero-Colomar, A.L.; Vilajeliu, M.; Batllori, L. (2005). Captura masiva para el control de la mosca mediterránea de la fruta (*Ceratitis capitata* (Wied.)) en manzano. *Phytoma España*, pp. 5.

Escudero-Colomar, A.; Vilajeliu, M.; Batllori, L. (2008). Seasonality in the occurrence of the Mediterranean fruit fly [*Ceratitis capitata* (Wied.)] in the northeast of Spain. J. Appl. Entomol. 132: 714-721.

Eskafi, F.M.; Kolbe M.M. (1990). Predation on larval and pupal Ceratitis capitata (Diptera: Tephritidae) by the ant *Solenopsis germinata* (Hymenoptera: Phormicidae) and other predators in Guatemala. *J. Environ. Entomol.*, 19: 148-153.

Espanada, A.L. (2009). Informe final de la campana realizada en el cultivo de uva de mesa y citricos para el control de mosca de la fruta (*Ceratitis capitata*) durante el ano 2008. *Agricola Vergel*, 1-6.

Field, S.A.; Kaspi, R.; Yuval, B. (2002). Why do calling medflies (Diptera: Tephritidae) cluster? Assessing the empirical evidence for models of medfly lek evolution. *Florida Entomol.*, 85: 63-72.

Fimiani, P. (1989). Mediterranean region. In: Fruit flies, their biology, natural enemies and control. *World Crop Pests*, pp. 39-50.

Fletcher, B. S. (1989) Ecology; movements of Tephritid fruit flies. In: Robinson, A.S. and Hooper, G. [eds.], *World crop pests* 3(B). Fruit flies; their biology, natural enemies and control. Elsevier, Amsterdam, Netherlands. pp. 209-219.

Freier, B.; Boller, E.F. (2009). Integrated pest management in Europe – history, policy, achievements and implementation. In: *Integrated pest management: dissemination and impact.* Vol. 2. [Ed.] by Peshin, R. Dhawan, A.K. Springer, Dordrecht, The Netherlands, 435–454.

Galli, J. C.; Rampazzo E. F. (1996). Enemigos naturales predadores de Anastrepha (Diptera, Tephritidae) capturados con trampas de gravedad de suelo en huertos de Psidium guajava L. Bol. San. Veg. Plagas 22: 297–300.

Gencat, (2009). Norma tècnica per a la producció integrada de fruita de llavor. www.produccionintegrada.cat.

Generalitat-Valenciana. (2009). Las actuaciones para el control de la mosca de la fruta reducen su incidencia en un 90% en el último año. http://www.agricultura.gva.es.

Gingrich, R.E. (1987). Demonstration of Bacillus thuringiensis as a potential control agent for the adult Mediterranean fruit fly, *Ceratitis capitata* (Wied.). *J. Appl. Entomol.,* 104: 378-385.

Grout, T.G.; Stoltz, K.C. (2007). Developmental rates at constant temperatures of three economically important Ceratitis spp. (Diptera: Thephritidae) from southern Africa. *J. Environ. Entomol.,* 36 (6): 1310-1317.

Guzmán-Plazola Remigio, A. (2010). Mosca del Mediterráneo *Ceratitis capitata* (Wiedemann). Colegio de postgraduados-direccion general de sanidad vedetal. *Ficha técnica*. Secretaria de agricoltura, ganadesia, desarollo rural, pesca y alimentacion, Mexico 1, 45.

Hallman, G.J. (2008). Potential increase in fruit fly (Diptera: Tephritidae) interceptions using ionizing irradiation phytosanitary treatments. *J. Econ. Entomol.,* 101: 716-719.

Hallman, G.J. (1999). Ionizing radiation quarantine treatments against Tephritid fruit flies. *Postharvest Biol. Technol.,* 16, 93-106.

Harris, E.J.; Lee C.Y. (1989). Development of *Ceratitis capitata* (Diptera: Tephritidae) in coffee in wet and dry habitats. *J. Environ. Entomol.,* 18: 1042-1049.

Harvey, T.; Chan, J.R.; Jang, E.B. (1995). Diet pH effects on mass rearing of Mediterranean fruit fly (Diptera: Tephritidae). *J. Econ. Entomol.,* 8(3): 569-573.

Hendrichs, J.; Robinson, A. (2009). Sterile Insect Technique. In: Resh, V.H. and Cardé, R.T.. *Encyclopedia of Insects* (2nd Ed). Academic Press-Elsevier. pp. 953-957.

Hendrichs, J.; Robinson, A.S.; Cayol, J.P.; Enkerlin, W. (2002). Medfly area wide sterile insect technique programs for prevention, suppression or eradication: the importance of mating behavior studies. *Florida Entomol.* 85: 1-13.

Hodgson, P.J.; Sivinski, J.; Quintero, G.; Aluja, M. (1998). Depth of pupation and survival of fruit fly (*Anastrepha* spp.: Tephritidae) pupae in a range of agricultural habitats. *J. Environ. Entomol.* 27: 1310-1314.

Ioriatti, C.; Agnello, A.M.; Martini, F.; Kovach, J. (2011). Evaluation of the environmental impact of apple pest control strategies using pesticide risk indicators. *Integr. Environ. Assess. Manag.*,7(4): 542-549.

IRAC. (2017). Insecticide Resistance Action Committee www.irac-online.org

ISPM. (2011). International standards for phytosanitary measures ISPM 28:2007 phytosanitary treatments for regulated pests PT 14: Irradiation treatment for *Ceratitis capitata*.

Israely, N.; Ritte, U.; Oman, S.D. (2004). Inability of Ceratitis capitata (Diptera: Tephritidae) to overwinter in the judean hills. *J. Econ. Entomol.* 97: 33-42.

Israely, N.; Yuval, B.; Kitron, U.; Nestel, D. (1997). Population fluctuations of adult Mediterranean fruit flies (Diptera: Tephritidae) in a Mediterranean heterogeneous agricultural region. *J. Environ. Entomol.,* 26: 1263-1269.

Jang, E.B.; Messing, R.H.; Klungness, L.M.; Carvalho, L.A. (2000). Flight tunnel responses of *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae) to olfactory and visual stimuli. *J. Insect Behavior,* 13: 525-538.

Jones, V.P.; Unruh, T.R.; Horton, D.R.; JMills, N.; Brunner, J.F.; Beersa, E.H.; Shearerd, P.W. (2009). Tree fruit IPM programs in the western United States: the challenge of enhancing biological control through intensive management. *Pest Manag. Sci.,* 65: 1305-1310

Karamanlidou, G.; Lambropoulos A.F.; Koliais, S.I.; Manousis, T.; Ellar, D.; Kastritsis, C. (1991). Toxicity of *Bacillus thuringiensis* to laboratory populations of the olive fruit fly (*Dacus oleae*). *Appl. Environ. Microbiol.,* 57: 2277-2282.

Kaspi, R.; Mossinsin, S.; Drezner, T.; Kamensky, B.; Yuval, B. (2002). Effect of larval diet on development rates and reproductive maturation of male and female Mediterranean Fruit flies. The royals Entomological Society, *Physiol. Entomol.,* 27: 29-38.

Knipling, E.F. (1955). Possibilities of insect control or eradication through the use of sexually sterile males. *J. Econ Entomol.,* 48: 459-469.

Konstantopoulou, M.A.; Mazomenos, B.E. (2005). Evaluation of *Beauveria bassiana* and B-*brongniartii* strains and four wild-type fungal species against adults of *Bactrocera oleae* and *Ceratitis capitata*. *Biocontrol,* 50: 293-305.

Liquido, N.J.; Shinoda, L.A.; Cunningham, R.T. (1991). Host plants of the Mediterranean fruit fly (Diptera: Tephritidae): an annotated world review. *Misc. Publ. Entomol. Soc. Am.,* 77: 1-52.

Lux, S.A.; Ekesi, S.; Dimbi, S.; Mohamed, S.; Billah, M. (2003). Mango-infesting fruit flies in Africa: perspectives and limitations of biological approaches to their management. pp. 277-293. In Neuenschwander P., Borgemeister C., and Langewald, J. [eds.], *Biological control in integrated pest management systems in Africa*. CABI, Wallingford, United Kingdom.

Maelzer, D.A. (1990). Fruit fly outbreaks in Adelaide, S.A., from 1948-1949 to 1986-1987. I. Demarcation, frequency and temporal patterns of outbreaks. *Aust. J. Zool.,* 38: 439-452.

Manoukas, A.G. (2002). Composition and efficiency of a larval diet containing alfalfa instead of yeast for rearing the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Proceedings of 6th *International Fruit Fly Symposium* 6-10 May 2002, Stellenbosch, South Africa pp. 405-407.

Marcado, S.E. (2011). Ionizing Radiation as quarantine treatments in Fruits. In: Vazquez M., Ramierz J.A. *Advantage in post-harvest treatments and fruit quality and safety* (pp. 19-30). Nueva York EE. UU. Nova Science Publisher, Inc.

Marlow R.H. (1934). An Artificial food medium for Mediterranean fruit fly (*Ceratitis capitata* Wird). *J. Econ.Entomol.,* Geneva 27(5): 1100.

Martínez-Ferrer, M.T.; Alonso, A.; Campos, J.M.; Fibla, J.M.; Garcia-Marí, F. (2007). Dinámica poblacional de la mosca de la fruta *Ceratitis Capitata* en tres zonas citrícolas mediterráneas. *Levante Agrícola,* 385: 1-7.

Mavrikakis; P.G.; Economopoulos, A.P.; Carey, J.R. (2000). Continuous winter reproduction and growth of the Mediterranean fruit fly (Diptera: Tephritidae) in Heraklion, Crete, southern Greece. *J. Environ. Entomol.,* 29: 1180-1187.

McPhail M. (1939). Protein lures for fruit flies. J. Econ. Entomol. 32: 758-761.

McQuate, G.T.; Sylva, C.D.; Jang, E.B. (2005). Mediterranean fruit fly (Diptera: Tephritidae) suppression in persimmon through bait sprays in adjacent coffee plantings. *J. Appl. Entomol.,* 129: 110-117.

Michal, M.; Amnon, E. (2004). Processed kaolin protects fruits from Mediterranean fruit fly infestations. *Crop Protection.,* 23: 47-51.

Moretti, R.; Calvitti, M. (2003). Mortality by parasitization in the association between the egg-pupal parasitoid *Fopius arisanus* and *Ceratitis capitata*. *Biocontrol,* 48: 275-291.

M'Saad Guerfali, M.; Loussaief, F. (2008). The Sterile Insect Technique for the control of *Ceratitis capitata* (Weideman) in oranges orchards in Beni Khaled, Tunisia. *First Meeting of TEAM,* Palma de Mallorca 7-8 April 2008, pp. 78.

Navarro-Llopis, V.; Sanchis-Cabanes, J.; Ayala, I.; Casana-Giner, V.; Primo-Yufera, E. Efficacy of lufenuron as chemosterilant against Ceratitis capitata in field trials. *Pest Manag. Sci*. 60: 914-920 (online: 2004) DOI: 10.1002/ps.902.

Navarro-Llpois, V.; Primo, J.; Vacas, S. (2012). Efficacy of attract-and-kill devices for the control of *Ceratitis capitata*. *Pest Manag. Sci.,* 69: 478-482.

Palou, L.; Del Río, M.A.; Jacas, J.A. (2007). Aplicación en poscosecha de cítricos de tratamientos cuarentenarios contra la mosca mediterránea de la fruta. *Levante Agrícola,* 385: 192-199.

Papadopoulos, N.T.; Carey, J.R.; Katsoyannos, B.I.; Kouloussis, N.A. (1996). Overwintering of the Mediterranean fruit fly (Diptera: Tephritidae) in northern Greece. *Ann. Entomol. Soc. Am.,* 89: 526-534.

Papadopoulos, N.T.; Katsoyannos, B.I.; Carey, J.R.; (1998). Temporal changes in the composition of the overwintering larval population of the Mediterranean fruit fly (Diptera: Tephritidae) in northern Greece. *Ann. Entomol. Soc. Am.,* 91: 430-434.

Papadopoulos, N.T.; Katsoyannos, B.I.; Carey, J.R.; Kouloussis, N.A. (2001a). Seasonal and annual occurrence of the Mediterranean fruit fly (Diptera: Tephritidae) in northern Greece. *Ann. Entomol. Soc. of Am.,* 94: 41-50.

Papadopoulos, N.T.; Katsoyannos, B.I.; Kouloussis, N.A.; Hendrichs, J.; Carey, J.R.; Heath, R.R. (2001b). Early detection and population monitoring of Ceratitis capitata (Diptera: Tephritidae) in a mixed-fruit orchard in northern Greece. *J. Econ. Entom.,* 94: 971-978.

Penarrubia-Maria, I.E.; Quilici, S.; Schmitt, C.; Escudero-Colomar, A.L. (2013). *Pest Mang. Sci.* 70: 448-453.

Piñero, J.C.; Mau, R.F.L.; Vargas, R.I. (2009). Managing Oriental Fruit Fly (Diptera: Tephritidae), with Spinosad-Based Protein Bait Sprays and Sanitation in Papaya Orchards in Hawaii. *J. Econ. Entom.,* 102: 1123-1132.

Pollini, A. (2009). Le ultime restrizioni rendono difficile la lotta della mosca Mediterranea. La difesa delle drupacee. *L'Informatore Agrario* 30: 17-18.

Progetto di sviluppo dell'agricoltura biologica in Sicilia. (2011). www.mensileagrisicilia.it/immagini/ciainserto.pdf

Prokopy, R.J.; Roetberg, B.D. (1989). Fruit Fly Foraging Behavior. In: Robinson A.S. and Hoper G. [eds.], *World Crop. Pest*. Elseiver Science, Amsterdam, 3(A): 293-306.

Provincia autonoma di Trento. (2014). https://www.ufficiostampa.provincia.tn.it.

Rempoulakis, P.; Taret G.; ul Haq, I.; Wornayporn V.; Ahmad, S., Sto Tomas, U.; Dammalage, T.; Gembinsky, K.; Franz, G.; Cáceres, C.; Vreysen, M.J.B. (2016). Evaluation of Quality Production Parameters and Mating Behavior of Novel Genetic Sexing Strains of the Mediterranean Fruit Fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Quality Evaluation of Medfly Genetic Sexing Strains. *PLOS ONE* | DOI:10.1371. pp. 1-14.

Riceno, R.D.B.; Ramos, D.; Eberhard, W.G. (1996). Courtship behavior of male *Ceratitis capitata* (Diptera:Tephritidae) in captivity. *Florida Entomol.,* 79(2): 130-143.

Rigamonti I. (2005). La *Ceratitis capitata* in Lombardia. Quaderni della ricerca n. 47 – Regione Lombardia.

Rigamonti, I. E. (2004a). Contributions to the knowledge of *Ceratitis capitata* Wied. (Diptera, Tephritidae) in Northern Italy I. Observations on the biology. *Bollettino di Zoologia Agraria e di Bachicoltura* 36: 89-100.

Rigamonti, I.E. (2004b). Contributions to the knowledge of *Ceratitis capitata* Wied. (Diptera, Tephritidae) in Northern Italy II. Overwintering in Lombardy. *Bollettino di Zoologia Agraria e di Bachicoltura* 36: 101-116.

Rigamonti, I.E.; Agosti, M.; Malacrida, A.R. (2002). Distribuzione e danni della Mosca Mediterranea della Frutta *Ceratitis capitata* Wied. (Diptera Tephritidae) in Lombardia (Italia settentrionale). *Atti XIX Congresso nazionale italiano di Entomologia*. Catania, 10-15 giugno 2002. pp. 579-585.

Robacker, D.C.; Martínez, A.J.; García, J.A.; Díaz, M.; Romero, C. (1996). Toxicity of *Bacillus thuringiensis* to Mexican fruit fly (Diptera: Tephritidae). *J. Econ. Entomol.,* 89: 104-110.

Romani, M. (1997). Biology, ethology and control of *Ceratitis capitata* in northern Italy. *L'Informatore Agrario* 53: 59-62.

Ros, J.P. (1988). La Mosca Mediterranea de la fruta, *Ceratitis capitata* Wied. Biología y métodos de control. Hojas Divulgadoras, Ministerio de Agricultura, Pesca y Alimentacion, España 8, 28.

Rousse, P.; Gourdon, F.; Roubaud, M.; Chiroleu, F.; Quilici, S. (2009). Biotic and abiotic factors affecting the flight activity of *Fopius arisanus*, an egg-pupal parasitoid of Fruit Fly pests. *J. Environ. Entomol.,* 38: 896-903.

Schroeder, W.J.; Cunningham, R.T.; Miyabara, R.Y.; Farias, G.J. (1972). A fluorescent compound for marking Tephritidae. *J. Econ. Entomol.,* 65: 1217-1218.

Schroeder, W.J.; Mitchell, W.C. (1981). Marking Tephritidae fruit fly adults in Hawaii for release-recovery studies. *Hawaiian Entomol. Soc. Proc.,* 23: 437-440.

Shoukry, A.; Hafez, M. (1979). Studies on the biology of the Mediterranean fruit fly *Ceratitis capitata*. *Entomol. Exp. Appl.,* 26: 33-39.

Silvestri, F. (1914). Report on an expedition to Africa in search of the natural enemies of fruit flies (Trupaneidae) with descriptions, observations and biological notes. Territory of Hawaii. *Board of Agriculture and Forestry Division of Entomology Bulletin* Vol. 3, 1–176.

Stark, J.D.; Vargas, R.; Miller, N. (2004). Toxicity of Spinosad in protein bait to three economically important Tephritid fruit fly species (Diptera: Tephritidae) and their parasitoids (Hymenoptera: Braconidae). *J. Econ. Entom.,* 97: 911-915.

Steiner, L.F.; Mitchell, W.C.; Baumhover, A.H. (1962). Progress of Fruit Fly control by irradiation sterilization in Hawaii and Mariana Island. *Int. J. Appl. Rad.,* 13: 427-434.

Stern, VM; Smith, R.F.; Van den Bosch, R.; Hagen, K.S. (1959). The integrated control concept. *Hilgardia,* 29: 81-101.

Tabilio, M.R.; De Salvador, F.R.; Mandatori, R.; Campus, L. (2008). Laboratory tests to evaluate some biological insecticide against *Ceratitis Capitata* (Wied.) VI Convegno Nazionale sulla peschicoltura Meridionale. Caserta 6-7 Marzo 2008.

Tabilio, M.R.; Mandatori R.; Quaranta M.; De Salvador F.R. (2008). A prospective of control of *Ceratitis Capitata* (Wiedemann) by the use of ento-mopathogenic nematodes. *Atti del VI Convegno Nazionale sulla peschicoltura meridionale*. pp.291-293.

Tait, P.; Pellegrini, F.; Zaffoni, M.; Morten, M. (2012). Il ritorno di *Ceratitis capitata* nei frutteti trentini. *Rapporto 2012 Centro di Trasferimento Tecnologico* pp. 31-34.

Thompson, G.D.; Dutton, R.; Sparks, T.C. (2000). Spinosad a case study: an example from a natural products discovery program. *Pest. Manag. Sci.*, 56: 696-702.

Toledo, J.; Liedo, P.; Williams, T.; Ibarra, J. (1999). Toxicity of *Bacillus thuringiensis* β-exotoxin to three species of fruit flies (Diptera: Tephritidae). *J. Econ. Entomol.*, 92: 1052-1056.

Torné, M. (2008). *Ceratitis capitata*: situación actual y perspectivas de futuro. Jornada tècnica: problemàtica actual en el control dels paràsits dels fruiters. Perspectives de futur, Lleida 26 Setembre, 2008, pp. 14.

Torres-Rivera, Z.; Hallman, G.J. (2007). Low-dose irradiation phytosanitary treatment against Mediterranean fruit fly (Diptera: Tephritidae). *Florida Entomol.*, 90: 342-346.

Trematerra, P.; Salvador, R.D.; Cesare, D.; Sciarretta, A. (2008). Spatio-temporal distribution of *Ceratitis capitata* trap catches and integrated pest management in an agricultural landscape of central Italy. *Giornate Fitopatologiche* 2008, Cervia (RA), 12-14 marzo 2008, 1: 167- 174.

http://www.trentinoagricoltura.it/TrentinoAgricoltura/Settori2/Ortofrutticoltura/Mela

Vera, M.T.; Rodriguez, R.; Segura, D.F.; Cladera, J.L.; Sutherst, R.W. (2002). Potential geographical distribution of the Mediterranean fruit fly, Ceratitis capitata (Diptera: Tephritidae), with emphasis on Argentina and Australia. J. Environ. Entomol. 31: 1009-1022.

Vergani, A. R. (1961). La mosca del Mediterráneo. Boletín de divulgación, INTA. Concordia.

Vreysen, M.J.B.; Dyck, V.A.; Hendrichs, J.; Robinson, A.S. (2005). Monitoring sterile and wild insects in area-wide integrated pest management programmes. In: Dyck V.A., Hendrichs J., Robinson A.S. [Eds]. *Sterile Insect Technique Principles and Practice in Area-Wide Integrated Pest Management*. Dordrecht, the Netherlands: Springer; pp. 325-362.

Wang, S.; Tang, J.; Cavalieri, R.P. (2001). Modeling fruit internal heating rates for hot air and hot water treatments. *Post-harvest Biol. Technol.*, 22: 257-270.

Wang, X. G.; Messing, R. H. (2003). Intra- and interspecific competition by *Fopius arisanus* and *Diachasmimorpha tryoni* (Hymenoptera: Braconidae), parasitoids of Tephritid fruit flies. *Biological Control.*, 27: 251-259.

Weems, H.V. (1981). Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Entomology Circular, Division of Plant Industry, Florida Department of Agriculture and Consumer Services, 12 pp.

White, I.M.; Elson-Harris, M.M. (1992). Fruit flies of economic significance: Their identification and bionomics. Wallingford, Oxon, CAB International.

Whittier, T.S.; Kaneshiro, K.Y.; Prescott, L.D. (1992). Mating behavior of Mediterranean fruit flies (Diptera: Tephritidae) in a natural environment. Ann. Entomol. Soc. Am. 85: 214-218.

Wong, T.T.Y.; McInnis, D.O.J.I; Nishimoto, A.K; Chang, V.C.S. (1984). Predation of the Mediterranean fruit fly (Diptera: Tephritidae) by the argentine ant (Hymenoptera: Formicidae) in Hawaii. *J. Econ. Entomol.*, 77: 1454-1458.

Zaidan Khalaf, M.; Fadhil Alrubeai, H.; Al-zaidi, S. (2013). Efficacy of Ceranock Attract and Kill System as a Control Method of Mediterranean Fruit Fly, *Ceratitis capitata* in Apricot Orchards in Central Iraq. *J. Agricul. Sci. Techn.* ISSN 1939-1250. pp. 732-736.

CHAPTER 1

The efficacy and sensitivity of different traps for monitoring *Ceratitis capitata* in apple orchards in the Trentino region

Abstract

Trapping experiments were conducted in 2016 during the period of flight activity of the Mediterranean fruit fly in Trentino orchards, in northern Italy, to test several traps with different food attractants.

The objective of this study was to compare the efficacy of some of the commercially available traps in order to make recommendations for effective monitoring throughout the season. The experimental design used was randomised distribution, with four type of traps and ten replications. Four different trap models were evaluated: the Decis® trap baited with food attractant (Decis® Trap), the Jackson trap baited with MagnetMad (Magnet Med), the Jackson trap baited with Trimedlure (CBC) and the yellow sticky trap with Trimedlure attractant (ISAGRO).

The results showed that the Decis® trap with food attractant was the most effective of the four tested devices. It effectively attracted males and females, providing useful information about population dynamics. Decis® traps were shown to be more effective than the other traps in catching *C. capitata* at the beginning of the season, when the population density is expected to be very low.

On the other hand, at the end of the season, when females migrate to other orchards to find ripe fruit, males were captured mainly in the yellow sticky trap baited with trimedlure.

Introduction

Monitoring with traps is a common and critical component in pest detection, delimitation, suppression and eradication programs worldwide (Apple, 1976; El-Sayed *et al.*, 2006; Goldshtein *et al.*, 2017). Over the years, many traps have been developed for detecting and monitoring populations of adult tephritid pests (Cunningham 1989, Economopoulos 1989). The first traps were baited with carbohydrates and fermenting fruit or sugar solutions, while subsequently traps baited with hydrolysed proteins were developed. Currently, synthetic chemicals and food-related chemicals are the primary substances used as bait in traps to monitor populations of the Mediterranean fruit fly (MFF), *Ceratitis capitata* (Wiedemann). These traps

include Trimedlure-baited traps, which are used worldwide for detecting and monitoring populations of *C. capitata* (Beroza *et al*., 1961). Trimedlure is a parapheromone (tetr-butyl-4-[and 5]-chloro-2-methylcyclohexane-1-carboxylate) that selectively attracts males (Nakagawa *et al*., 1970) (Beroza *et al*., 1961). These traps are often preferred because they are more specific for *C. capitata* and they attract flies over a greater distance (Delrio *et al*., 1983, White *et al*., 1992). Trimedlure dispensers are typically mounted in Jackson traps (Harris *et al*., 1971), which are triangular cardboard traps containing a sticky insert, or are attached to yellow panels coated with sticky material (Cunningham 1989). Many studies have shown that yellow is more attractive for the Mediterranean fruit fly than other colours when used to monitor the MFF population (Prokopy *et al*., 1976; Epsky *et al*., 1995).

Another type of trap is the McPhail trap baited with aqueous protein solution (Newell, 1936). Although these traps capture both male and female *C. capitata,* they have numerous disadvantages: bait solution may be spilled outside the trap during checks, checking of the insects trapped is time-consuming and difficult because the content of the trap needs to be filtered to separate the insects from the bait solution, while the flies in the solution deteriorate and lose parts that are important for identification. (Newell, 1936; Lucas *et al*., 2008).

Heath *et al*., (1996) developed a synthetic food-based lure (ammonium acetate and putrescine), used in a dry insect trap and able to capture both males and females (Heath *et al*., 1995, Epsky *et al*., 1995).

While it is known that trap design, including colour and shape, is a critical factor in effectively catching fruit flies (Epsky *et al*., 1995, Vargas *et al*., 1997), there is no published information on the performance of these different trapping systems throughout the season and their sensitivity in detecting small populations, such as those present early in the season in temperate areas (Papadopoulos *et al*., 2001). The objective of this study was to compare the efficacy of some of the commercially available traps in order to make recommendations for effective monitoring throughout the season.

Material and methods

The area of this study is located in southern Trentino, near Lake Garda (45°35’25.08’’N and 10°51’30.82’’E at 369 m above sea level). This is an area with a high population density of this pest. Different species of plant (peaches, apples, apricots and pears) are cultivated in the orchards. Four different types of traps with different lure were tested.

Were tested two different traps with food attractant:

1) Decis® trap (Bayer CropScience S.r.l., Milan, Italy) with 0.015 g deltamethrin/trap situated at the top, baited with Unpack® Biolure (ammonium acetate 29.8 %, trimethylamine 12.4 %

and putrescine 0.2 %) (Suterra, Europe Biocontrol S.L., Valencia, Spain). This trap is a plastic device made up of a yellow base and a transparent lid with a hanger, and a bottom part with four 2.5 cm diameter holes) (Figure 1.1 c.). To simplify, we called this trap the Decis trap.

2) Jackson trap with MagnetMed. This trap is a prism-shaped trap with sticky bottom loaded with a MagnetMed® panel; this is a paper envelope attract-and-kill device impregnated with deltamethrin (0.010 g) that contains two membrane dispensers, with trimethylamine and ammonium acetate as attractants (Suterra, Europe Biocontrol S.L., Valencia, Spain) (Figure 1.1 b.). To simplify, we called this trap Magnet Med.

Two types of traps baited with Trimedlure:

1) Yellow sticky traps with Trimedlure (Isagro, Sumitomo Chemical Italia, Milan). This is made up of two sticky yellow panels in the form of a cross with a hanger (Figure 1.1 d.). To simplify we called this trap ISAGRO.

2) Jackson trap with Trimedlure (Biogard, CBC, Europe). This trap is a prism-shaped trap, made with waxed cardboard and containing a rectangular waxed cardboard insert covered with a sticky material that catches the flies once they land on it (Figure 1.1 a.). The trap contains a small polymer pellet where the attractant was placed. To simplify we called this trap CBC.

Each trap type was replicated 10 times and they were installed 15 m apart with completely randomised distribution.

The traps were installed in the tree canopy, 1.60 m from the ground, on 29 June 2016 and checked weekly to count males and females caught and to clean the sticky bottom by removing all the insects captured. The Trimedlure attractant was changed after 35 days, while the food attractant (Suterra), whose efficacy is guaranteed for 120 days, was changed only once during the season.

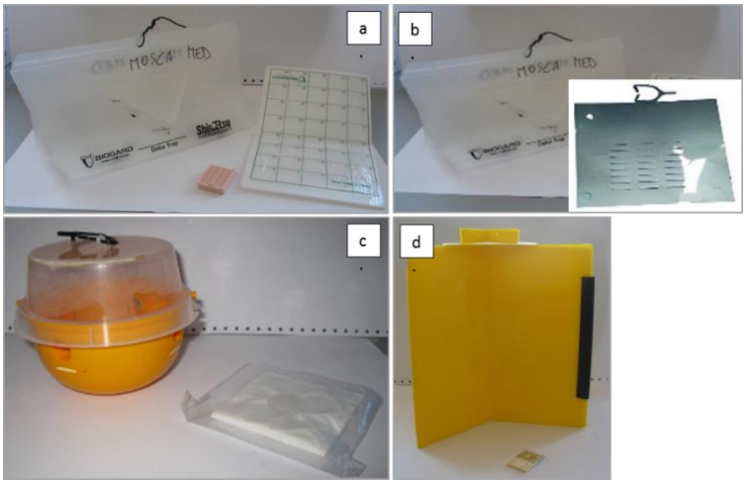


Figure 1.1 Types of traps installed in the field to evaluate the best system for C.capitata monitoring. A) Jackson trap baited with Trimedlure (Biogard , CBC); B) Jackson trap baited with MagnetMed® (Suterra); C) Decis® trap (Bayer) with Unipack biolure (Suterra); D) Yellow sticky trap with Trimedlure (Isagro)

Statistical analysis. To analyse capture data during the season, total, male and female captures were analysed with one-way ANOVA (± SD) for different traps at different times (during the season). JMP®Pro 12.0.1 (2015) was used to analyse the data. Capture data was transformed with the squared root of the capture + 0.5 to fit with ANOVA requirements for the normality of data. Significant differences were analysed using the least significant difference Student's t-test when necessary.

Results

During the 2016 season, in the area where the trial was set up *C. capitata* was shown to be constantly present. Indeed, females of *C. capitata* started flight on 7 July 2016 and stopped on 18 November 2017.

Decis® traps with Unipak biolure captured 380 (± 384.59) adults in total (male and female), a significantly higher number of flies than all the other types of traps. The total numbers of flies caught in Jackson trap CBC with Trimedlure (128.80 ± 116.76), Isagro (101.3 ± 116.35) and the Jackson trap with MagnetMed® (17.9 ± 17.16) were not statistically different (ANOVA: F= 5.58, P < 0.01) (Figure 1.2).

The numbers of males captured in CBC (127.4 ± 116.32), ISAGRO (93.8 ± 111.48 and Decis® Traps (112 ± 113.62) were not statistically different, while all three traps were more effective in catching male flies than Magnet Med (11 ± 13.19) (ANOVA: F=2.78, P=0.05) (Figure 1.3).

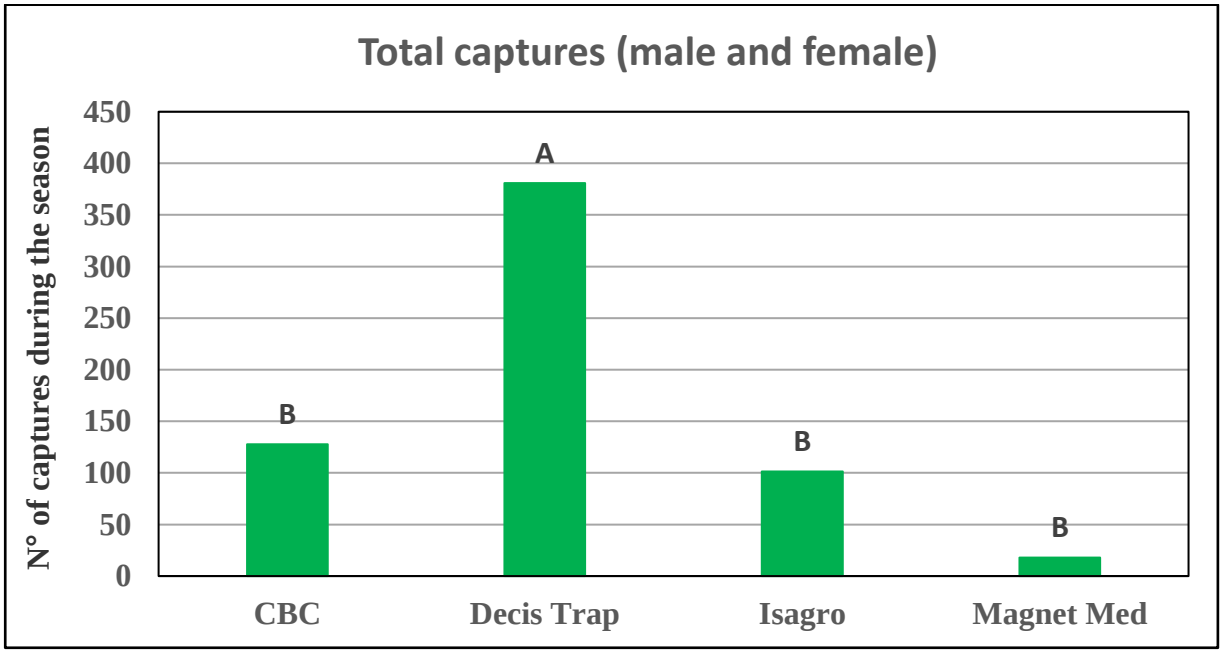


Figure 1.2 Difference in total captures (male and female) during the 2016 season in the four different traps. Different letters indicate significant statistical differences between traps (ANOVA F=5.58, P < 0.01).

The numbers of females captured in Decis® Traps (268 ± 271.29) was statistically different from the other traps. CBC (0.4 ± 0.70), ISAGRO (7.5 ± 7.79) and Magnet Med (7.01 ± 6.23) showed lower female captures (ANOVA: F=9.39, P < 0.01) (Figure 1.3).

The traps were compared during the season at different weekly control times for total, male and female captures.

Total captures in the four types of traps did not differ until 9 August. From 17 August to 5 October, total catches in Decis® traps were significantly higher than in the other traps (ANOVA: 17/8: 18.9 ± 21.19 F=14.61, P<0.01; 26/8: 36.1 ± 39.91, F=12.90, P<0.01; 2/9: 66.8 ± 77.42, F=18.20,P<0.01; 13/9: 76.8 ± 80.11, F=20.24, P<0.01; 19/9: F=5.80, P<0.01; 27/9: 52.5 ± 55.35. F=20.66, P<0.01; 5/10: 40.4 ± 42.92, F=14.26, P<0.01) (Table 1.1).

After that, the number of total adult captures declined and CBC, ISAGRO and Decis® Traps were not statistically different, while all three traps were more effective in catching flies at the end of season than Magnet Med (ANOVA: 13/10 F=5.04, P<0.01; 20/10 F=3.78, P<0.01; 27/10 F=6.06, P<0.01; 4/11 F=3.99, P<0.01; 18/11 f=6.01 P<0.01).

Decis® traps caught some females soon after installation on July 7 and continued to catch small numbers of females over the following two weeks until July 17 (ANOVA: F = 2.25, P = 0.1).From July 20 to October 4, a significantly higher number of females were consistently caught in Decis® traps than in the other three type of traps (ANOVA P < 0.01) (Figure 1.4 and Table 1.2). Male flight started after female flight on 20 July. The numbers of males captured during the season in ISAGRO, CBC and Decis® traps were not statistically different, while all three traps were more effective in catching male flies than Magnet Med (Table 1.2).

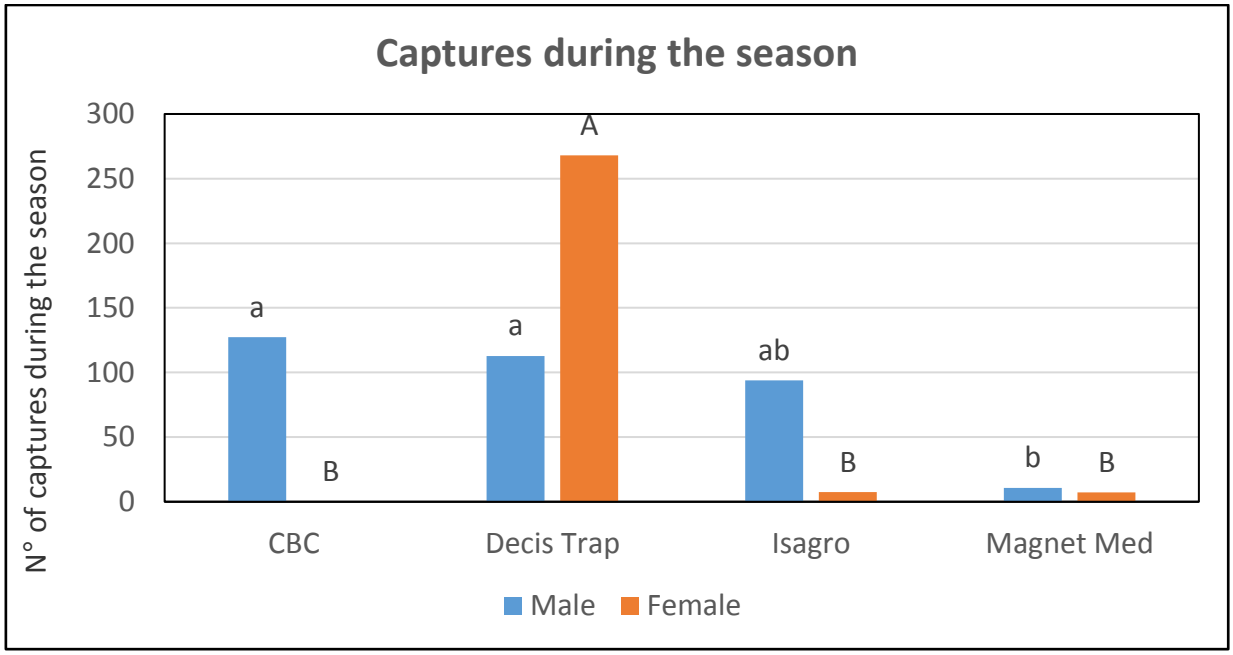


Figure 1.3: Difference in male and female captures during the 2016 season in the four different traps. Different letters indicate statistical differences between traps for males (ANOVA F=2.78, P < 0.05) and significant differences between traps for females (ANOVA F=9.39, P < 0.01).

Table 1.1 Differences in total captures in traps during the 2016 season at Riva del Garda. Statistical analysis was carried out using capture data, transformed to fit in with ANOVA requirements for the normality of data

	CBC	DECIS TRAP	ISAGRO	MAGNET MED	P
7/7	0 ± 0 a	0.2 ± 0.42 a	0 ± 0 a	0 ± 0 a	0.099
14/7	0 ± 0 a	0.2 ± 0.42 a	0 ± 0 a	0 ± 0 a	0.099
20/7	0 ± 0 a	0.6 ± 1.26 a	0.3 ± 0.48 a	0 ± 0 a	0.115
29/7	2.1 ± 2.92 a	5.1 ± 8.40 a	1.8 ± 3.16 a	0.2 ± 0.63 a	0.156
3/8	2.6 ± 4.22 a	5.3 ± 7.47 a	1.7 ± 3.43 a	0 ± 0 a	0.067
9/8	3.9 ± 6.06 ab	10.7 ± 15.25 a	5 ± 7.53 a	0 ± 0 b	0.025
17/8	4.4 ± 6.19 b	18.9 ± 21.19 a	5.5 ± 7.18 b	0.5 ± 0.97 b	0.020
26/8	12 ± 15.64 b	36.1 ± 39.91 a	5.6 ± 10.20 b	0.4 ± 0.97 b	0.001
2/9	19.1 ± 23.87 b	66.8 ± 77.42 a	6.2 ± 11.16 b	0.8 ± 1.14 b	0.000
13/9	15.1 ± 17.30 b	76.8 ± 80.11 a	1.5 ± 2.27 b	5.1 ± 10.67 b	0.000
19/9	16.2 ± 23.48 ab	33.5 ± 34.97 a	11.2 ± 13.20 bc	1.6 ± 1.58 c	0.002
27/9	18.5 ± 24.41 b	52.5 ± 55.35 a	13.8 ± 15.80 b	3.8 ± 3.46 b	0.003
5/10	10.3 ± 11.78 b	40.4 ± 42.92 a	10.7 ± 12.61 b	2 ± 2.40 b	0.004
13/10	6.1 ± 5.45 ab	14.0 ± 11.87 a	11.6 ± 15.01 a	0.7 ± 1.06 b	0.005
20/10	9.1 ± 11.66 a	6.3 ± 4.42 a	10.8 ± 10.82 a	1.6 ± 3.10 b	0.019
27/10	2.9 ± 3.03 a	4.7 ± 2.75 a	4.5 ± 4.48 a	0.4 ± 0.70 b	0.002
4/11	5.3 ± 5.48 a	8.1 ± 8.25 a	9.5 ± 10.71 a	0.7 ± 0.82 b	0.015
18/11	0.2 ± 0.42 b	0.6 ± 0.70 a	1.6 ± 1.51 a	0.1 ± 0.32 b	0.002

Table 1.2 Differences in female captures in traps during the 2016 season at Riva del Garda. Statistical analysis was carried out using capture data , transformed to fit in with ANOVA requirements for the normality of data

DATA	CBC	DECIS TRAP	ISAGRO	MAGNET MED	P
7/7	0 ± 0 a	0.2 ± 0.42 a	0 ± 0 a	0 ± 0 a	0.099
14/7	0 ± 0 a	0.2 ± 0.42 a	0 ± 0 a	0 ± 0 a	0.099
20/7	0 ± 0 b	0.5 ± 0.97 a	0 ± 0 b	0 ± 0 b	0.039
29/7	0.1 ± 0.32 b	4.8 ± 7.91 a	0 ± 0 b	0.2 ± 0.63 b	0.008
3/8	0 ± 0 b	3.2 ± 4.61 a	0.2 ± 0.63 b	0 ± 0 b	0.004
9/8	0 ± 0 b	9.2 ± 12.81 a	0 ± 0 b	0 ± 0 b	0.000
17/8	0 ± 0 b	13.5 ± 14.40 a	0.8 ± 1.32 b	0.5 ± 0.97 b	0.000
26/8	0.1 ± 0.32 b	27.9 ± 33.34 a	1.7 ± 3.53 b	0.2 ± 0.42 b	0.000
2/9	0 ± 0 b	58 ± 67.84 a	1.1 ± 2.60 b	0 ± 0 b	0.000
13/9	0 ± 0 b	52.9 ± 55.37 a	0.7 ± 0.95 b	1.7 ± 3.33 b	0.000
19/9	0 ± 0 b	21.1 ± 24.66 a	0 ± 0 b	0.6 ±0.84 b	0.000
27/9	0.2 ± 0.63 b	33.8 ± 33.11 a	0.2 ± 0.63 b	2.2 ± 2.57 b	0.000
5/10	0 ± 0 b	25.3 ±23.41 a	2 ± 6.32 b	0.6 ± 1.35 b	0.000
13/10	0 ± 0 b	7.1 ± 5.95 a	0.2 ± 0.63 b	0 ± 0 b	0.000
20/10	0 ± 0 b	3.5 ± 2.72 a	0.4 ± 0.96 b	0 ± 0 b	0.000
27/10	0 ± 0 b	2.7 ± 1.70 a	0 ± 0 b	0.4 ± 0.70 b	0.000
4/11	0 ± 0 b	4 ± 3.33 a	0.2 ± 0.63 b	0.6 ± 0.70 b	0.000
18/11	0 ± 0 a	0.1 ± 0.32 a	0 ± 0 a	0.1 ± 0.32 a	0.578

Table 1.3 Differences in male captures in traps during the 2016 season at Riva del Garda. Statistical analysis was carried out using capture data, transformed to fit in with ANOVA requirements for the normality of data

DATA	CBC	DECIS TRAP	ISAGRO	MAGNET MED	P
20/7	0 ± 0 a	0.1 ± 0.32 a	0.3 ± 0.48 a	0 ± 0 a	0.084
29/7	2 ± 2.87 a	0.3 ± 0.67 a	1.8 ± 3.16 a	0 ± 0 a	0.058
3/8	2.6 ± 4.22 a	2.1 ± 3.73 a	1.5 ± 3.41 a	0 ± 0 a	0.174
9/8	3.9 ± 6.06 a	1.5 ± 3.10 ab	5 ± 7.53 a	0 ± 0 b	0.034
17/8	4.4 ± 6.19 a	5.4 ± 6.93 a	4.7 ± 6.40 a	0 ± 0 b	0.046
26/8	11.9 ± 15.73 a	8.2 ± 7.84 a	3.9 ± 7.13 ab	0.2 ± 0.63 b	0.024
2/9	19.1 ± 23.87 a	8.8 ± 9,74 ab	5.1 ± 10.32 b	0.8 ± 1.14 b	0.026
13/9	15.1 ± 17.30 ab	23.9 ± 25.35 a	0.8 ± 1.75 b	3.4 ± 10.75 b	0.013
19/9	16.2 ± 23.48 a	12.4 ± 11.30 a	11.2 ± 13.20 a	1 ± 1.25 b	0.020
27/9	18.3 ± 24.01 a	18.7 ± 22.40 a	13.6 ± 15.88 a	1.6 ± 2.41 b	0.033
5/10	10.3 ± 11.78 a	15.1 ± 20.02 a	8.7 ± 11.63 a	1.4 ± 1.78 a	0.122
13/10	6.1 ± 5.45 a	6.9 ± 6.38 a	11.4 ± 14.77 a	0.7 ± 1.06 b	0.023
20/10	9.1 ± 11.66 ab	2.8 ± 2.57 bc	10.4 ± 10.74 a	1.6 ± 3.10 c	0.007
27/10	2.9 ± 3.03 a	2 ± 2.45 a	4.5 ± 4.48 a	0 ± 0 b	0.002
4/11	5.3 ± 5.48 a		9.3 ± 10.80 a	0.1 ± 0.32 b	0.004
18/11	0.2 ± 0.42 b	0.5 ± 0.71 b	1.6 ± 1.51 a	0 ± 0 b	0.000

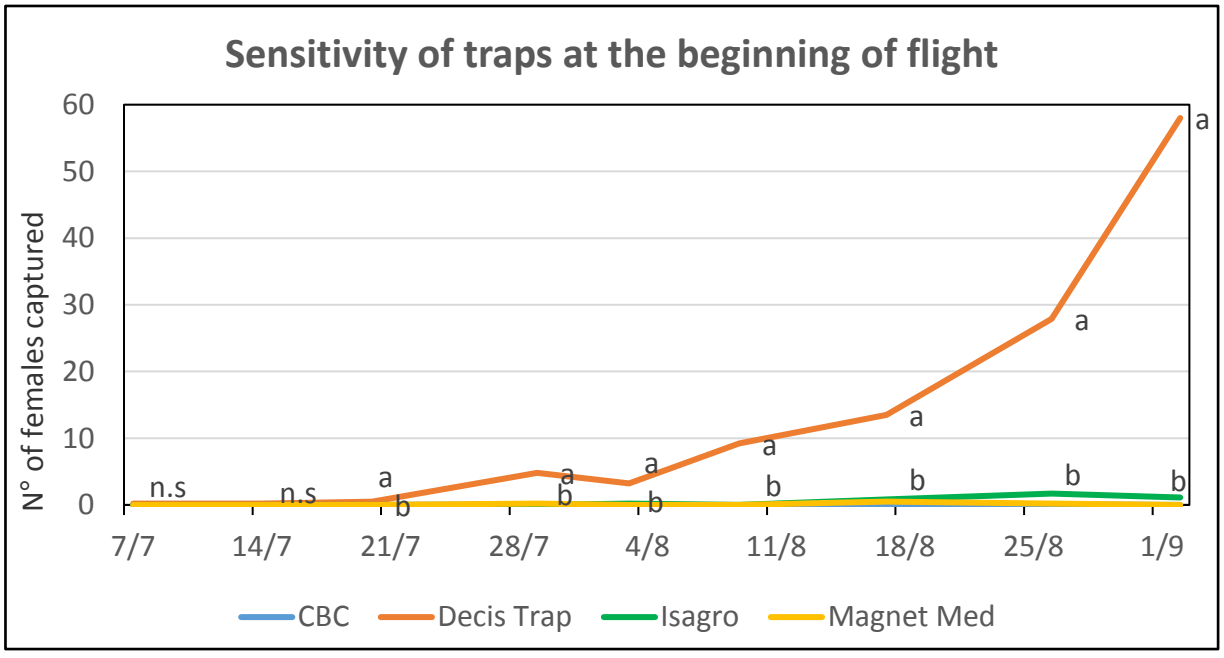


Figure 1.4 Graph showing the sensitivity of traps at the beginning of the adult flight season. The graph illustrates that the Decis® trap showed statistical differences compared to other traps for female captures starting from 20 July 2016

Discussion and conclusion

In our study, the Decis® trap was the most effective of the four tested devices. It effectively attracted males and females, providing useful information about population dynamics. Decis®

traps were shown to be more effective than other traps in catching MFFs at the beginning of the season, when the population density is expected to be very low.

In particular, this type of trap baited with food lure showed higher captures of females. Female flies tend to be more active in searching for proteinaceous sources because protein is critical for egg maturation (Hendrichs *et al.*, 1991). Indeed, Bakri *et al.*, 1998 and Cohen *et al.*, 2000 showed that high rates of mature *C. capitata* were captured with dry lures. In contrast, Heath *et al.*, (1995) reported a high prevalence of mated *C. capitata* females in McPhail traps (which are similar to the Decis® trap) baited with liquid lures, but more unmated females in traps containing Biolure dry lures. Moreover, mated females are more likely to be attracted to traps that mimic the shape, size, and colour of natural fruit when searching for mates and oviposition sites (Nakagawa *et al*., 1978, Economopoulos 1989). Indeed, the Decis® trap was more similar than McPhile traps in terms of colour, size and shape, and for this reason females are more attracted to this type of trap than the others.

This particular trap, with Deltamethrin on the top, baited with the food attractant Unipak® Biolure, is typically used in mass trapping systems to reduce the population and fruit damage in the field (Tlemsani *et al*., 2015; Penarrubia-Maria *et al*., 2014;).

Other studies have also showed that this type of trap baited with dry food attractant was the best for *Anastrepha ludes* (Loew) monitoring (Lasa, 2014). The small number of holes in this trap may also increase the retention time of traps and thereby increase the probability that trapped flies receive a lethal dose of toxicant (Epsky *et al.*, 1995).

Lasa's (2014) results suggest that the shape and position of entry holes would seem to be more important in fly retention than the diameter of the hole.

The other advantage of traps baited with dry food is that control in the field and recognition of males and females is easier than in traps baited with liquid lure (Lucas *et al.*, 2008). Moreover, liquid bait needs to be replaced every few days to avoid variations in the pH content of the bait protein, which strongly reduces its attractiveness (Epsky *et al.*, 1993)

At the end of the season, when females migrate to other orchards to find ripe fruit, males were mainly captured in the yellow sticky trap baited with trimedlure (Isagro).

Indeed, several studies have shown that male Mediterranean fruit flies are attracted to the synthetic compound trimedlure. Despite the common use of this parapheromone in control programmes, the underlying basis of male attraction remains unknown (Beroza *et al.*, 1961; Manoukis, 2016; Hendrichs *et al.*, 1989; Shelly *et al.*, 1996).

On the other hand, it was demonstrated that the Jackson sticky trap baited with MagnetMed® was not a good trap for monitoring. In this case, it is possible that the MagnetMed® panel

obstructed the Jackson trap opening and prevented flies from entering or occupied more space inside the traps, giving adults a way to escape or not to get stuck to the sticky bottom.

In conclusion, this study showed that the best system for male and female monitoring was the Decis® trap with Unipak® Biolure food lure (Suterra, Europe Biocontrol S.L.), both due to trap sensitivity in terms of female captures at the beginning of the season, and the number of individuals captured during the season.

References

Bakri, A.; Hadis, H.; Epsky, N.D.; Heath, R.R.; Hendrichs, J. (1998). Female *Ceratitis capitata* (Diptera: Tephritidae) capture in a dry trap baited with food-based synthetic attractant in an argan forest in Morocco. Part I: low population field test. *Can. Entomol.,* 130: 349 -356.

Beroza, M.; Green, N.; Gertler, S.I.; Steiner, L.F.; Miyashita, D.H. (1961). Insect attractants. New attractants for the Mediterranean fruit fly. *J. Agric. Food Chem.,* 9: 361-365.

Cohen, H.; Yuval, B. (2000). Perimeter trapping strategy to reduce Mediterranean fruit fly (Diptera: Tephritidae) damage on different host species in Israel. *J. Econ. Entomol.,* 93:21-725

Cunningham, R. T. (1989). Population detection. In A. S. Robinson and G. Hooper [eds.], *World Crop Pests*, Volume 3B, Fruit flies, their biology, natural enemies and control. Elsevier Science Publishers B. V., Amsterdam. pp. 169-173.

Delrio, G.; Zu¨mreoglu, A. (1983). Attractability range and capture efficiency of medfly traps pp. 445-450. In Cavalloro, R. [ed.], *Fruit flies of economic importance*. Proceedings, CEC/IOBC International Symposium, Athens, November 1982. A. A. Balkema, Rotterdam.

Goldshtein, E.; Cohen, Y.; Hetzroni, A.; Gazit, Y.; Timar, D.; Rosenfeld, L.; Grinshpon, Y.; Hoffman, A.; Mizrach, A. (2017). Development of an automatic monitoring trap for Mediterranean fruit fly (*Ceratitis capitata*) to optimize control applications frequency q computers and electronics in Agriculture 139: 115–125.

Economopoulos, A.P. (1989). Use of traps based on color and/or shape, pp. 315-327. In Robinson, A. S. and Hooper, G. [eds.], *World Crop Pests*, Volume 3B, Fruit flies, their biology, natural enemies and control. Elsevier Science Publishers B. V., Amsterdam. 447 pp.

Epsky, N.D.; Heath, R.; Guzman, A.; Meyer, W.L. (1995). Visual cue and chemical interactions in a dry trap with food-based synthetic attractants for *Ceratitis capitata* and *Anastrepha ludens* (Diptera: Tephritidae). *Environ. Entomol.,* 24: 1387-1395.

Epsky, N.; Heath, R.; Sivinski, J.; Calkins, C.; Baranowsi, R.; Fritz, A. (1993). Evaluation of protein bait formulation for the Caribbean Fruit Fly (Diptera: Tephritidae). *Florida Entomol.,* 76: 626-635.

Harris, E.J.; Nakagawa, S.; Urago, T. (1971). Sticky traps for detection and survey of three tephritids. *J. Econ. Entomol.,* 64: 62-65.

Heath, R.T.; Epsky, N.D.; Dueben, B.D.; Meyer, W.L. (1996). System to Monitor and suppress *Ceratitis capitata* (Diptera: Tephritidae) populations. *Florida Entomol.,* 79 (2): 144-153.

Heath, R.T.; Epsky, N.D.; Guzman, A.; Dueben, B.D.; Manukian, A.; Meyer, W.L. (1995). Development of a dry plastic insect trap with food-based synthetic attractant for the Mediterranean and Mexican fruit flies (Diptera: Tephritidae). *J. Econ. Entomol.,* 88: 1307-1315.

Hendrichs, J.; Katsoyannos, B.I.; Papaj, D.R.; Prokopy, R.J. (1991). Sex differences in movement between natural feeding and mating sites and tradeoffs between food consumption, mating success and predator evasion in Mediterranean fruit flies (Diptera: Tephritidae). *Oecologia.,* 86: 223-231.

Holler, T.C.; Peebles, M.; Young, A.; Whiteman, L.; Olson, S.; Sivinski, J. (2009). Efficacy of the Suterra Biolure Individual Female Fruit Fly Attractant Packages vs. the Unipak Version. *Florida Entomol.,* 92 (4): 667-669. Florida Entomological Society.

Lasa, R.; Olinda, E.; Zquez, V.; Ortega, R.; Acosta E. (2014). Efficacy of commercial traps and food odor attractants for Mass Trapping of *Anastrepha ludens* (Diptera: Tephritidae). Ecology and Behavior. *J. Econ. Entomol.,* 107: 198–205.

Lucas, A.; Hermosilla, A. (2008). Eficacia de Ceratrap y otros atrayentes y mosqueros, en el control de mosca de la fruta (*Ceratitis capitata*) en uva de mesa. *Agrícola Vergel,* 319: 304-310.

Nakagawa, S.; Farias, G.J.; Steiner, L. F. (1970). Response of female Mediterranean fruit flies to male lures in the relative absence of males. *J. Econ. Entomol.,* 63: 227-229.

Nakagawa, S.; Prokopy, R. J.; Wong, T.T.Y.; Ziegler, J. R.; Mitchell, S. M.; Urago, T.; Harris, E.J. (1978). Visual orientation of *Ceratitis capitata* flies to fruit fly models. *Entomol. Exp. Appl.,* 24: 193-198.

Newell, W. (1936). Progress report on the Key West (Florida) fruit fly eradication project. *J. Econ. Entomol.,* 29: 116-120.

Papadopoulus, N.T.; Katsoyannos, B.I.; Kouloussis, N.A.; Hendrichs, J.; Carey, J.R.; Heath, R.R. (2001). Early Detection and Population Monitoring of *Ceratitis capitata* (Diptera: Tephritidae) in a Mixed-Fruit Orchard in Northern Greece. Entomological Society of America. *J. Econ. Entomol.,* 94 (4): 971-978.

Penarrubia-Maria, I.E.; Quilici, S.; Schmitt, C.; Escudero-Colomar, L.A. (2014). Evaluation of candidate systems for mass trapping against *Ceratitis* spp. on La Reunion Island. *Pest Manag. Sci.,* 70 (3): 448-453.

Prokopy, R. J.; Economopoulos, A.P. (1976). Color responses of *Ceratitis capitata* flies. *J. Appl. Entomol.,* 80: 434–437.

Tlemsani, M.; Kheder, S.B. (2015). Comparison of four trapping systems for the control of the medfly *Ceratitis capitata*. Tunisian. *J. Plant Prot.,* 10: 131-140.

Vargas, R.I., Prokopy, R.J.; Duan, J.J.; Albrecht, C.; Lì., Q.X. (1997). Captures of wild Mediterranean and oriental fruit flies (Diptera: Tephritidae) in Jackson and McPhail traps baited with coffee juice. *J. Econ. Entomol.,* 90: 165-169.

White, I.M.; Elson-Harris, M.M. (1992). *Fruit flies of economic significance: their identification and bionomics.* CAB, Wallingford, UK.

Apple, J.L.; Smith, R.F. (1976). *Integrated pest management*. New York: Plenum, pp.200;

El-Sayed, A.M.; Suckling, D.M.; Wearing, C.H.; Byers, J.A. (2006). Potential of mass trapping for long-term pest management and eradication of invasive species. *J. Econ. Entomol.,* 99: 1550–1564.

Manoukis N. C. (2016). To Catch a Fly: Landing and Capture of *Ceratitis capitata* in a Jackson Trap with and without an Insecticide. *PLoS One*. 11(2): 1-10.

Hendrichs, J.; Reyes, J.; Alluja, M. (1989). Behaviour of female and male Mediterranean fruit flies, *Ceratitis capitata* in and around Jackson traps placed on fruiting host trees. *Int. J. Trop. Insect Sci.,* 10: 285–294.

Shelly, T.E.; Whittier, T.S.; Villalobos, E.M. (1996). Trimedlure affects mating success and mate attraction in male Mediterranean fruit flies. *Entomol. Exp. Appl.,* 78: 181–185.

CHAPTER 2

Susceptibility of selected apple cultivars to the Mediterranean fruit fly

Abstract

Ceratitis capitata (Wiedemann), the Mediterranean fruit fly, is one of the key pests affecting deciduous fruit orchards along Mediterranean coasts. Because of global warming, *C. capitata* is gradually spreading north and is becoming a major pest of apples. The susceptibility of the main varieties grown in the region is the cornerstone in management of the pest. This study will show the preliminary results of field and laboratory no-choice tests conducted at FEM in order to determine the apple cultivar preference of the Medfly. The seven main varieties of apples (Gala, Red Delicious, Golden Delicious, Granny Smith, Kanzi, Morgen and Fuji) were tested.

The results demonstrate that *C. capitata* lays its eggs on all apple cultivars, in both field and laboratory conditions. Granny Smith, Red delicious and Morgen showed the lowest susceptibility in laboratory conditions (0.75, 1.55 and 2 oviposition holes/fruit), with significant differences in comparison to Golden Delicious, Kanzi and Fuji (3.27, 3.31 and 3.1 oviposition holes/fruit), which were shown to be the most susceptible to Medfly attack in the laboratory. On the other hand, only slight and not statistically significant differences emerged from the field trials.

In relation to physical-chemical characteristics, the apple cultivars showing the lowest susceptibility (Granny Smith, Red delicious and Morgen) had harder peel and pulp and a lower sugar content in comparison to more susceptible ones (Golden Delicious, Fuji and Kanzi). Gala, which generally escapes Medfly attack in the orchard, was susceptible to Medfly oviposition both in the field and laboratory bioassays

Keywords: Medfly, *Ceratitis capitata*, Trentino, laboratory bioassay, field test

Introduction

The Mediterranean fruit fly (MFF), *Ceratitis capitata* (Wiedemann), is one of the main insect pests worldwide, attacking more than 300 different host fruit, including several cultivated species of high commercial value (Liquido *et al.,* 1990; Papadopolus *et al.,* 2001; De Meyer *et al.,* 2002).

In Italy this pest, which is extremely polyphagous, develops on many ripening fruits, including the apple. Its presence in Italy was reported for the first time in 1863. The biology of this fruit

fly is now largely known, although most of the studies have been carried out in the Mediterranean area (Shoukry & Hafez, 1979; Varga *et al.,* 1984; Papadopoulos *et al.*, 1996, 1998, 2001, 2002). Its range has been expanding, partly due to climate change, and the insect has now colonised most of Italy. The northern limit of distribution was indicated around the 41st parallel north and there were only occasional reports of its presence above this, mainly due to trading of infested fruit. Indeed, this species cannot survive in these areas in a stable manner (Rigamonti, 2002; Papadopoulos *et al*., 1996).

Trentino (northern Italy), one of the most important apple production areas worldwide, is located above the 46th parallel. The presence of *C. capitata* was first reported in a limited area of this region in 1990. After this initial report no further damage was reported till 2010, when the Medfly reappeared, and in subsequent years it has been found permanently in the more southerly part of the region.

The Medfly reaches a high population level in the autumn and causes serious damage to apples (*Malus sylvestris* Mill.), which mature at this time (Papadopoulus *et al.*, 2001). From early August until the end of October, many apple varieties progressively ripen and become a susceptible host for *C. capitata*. Understanding the susceptibility of the main varieties grown in the region is the cornerstone in management of the pest. The susceptibility of different fruit cultivars to different levels of fruit fly infestation has been reported in a few studies (Papachristos *et al.*, 2013; Prokopy, 1973, Tabilio *et al.*, 2013). Significant variability in Medfly field infestation in relation to different apple varieties has been reported in northern Greece (Papadopoulos *et al.*, 1996, 2001), but specific studies on the susceptibility of different apple varieties to oviposition of this pest under laboratory or field conditions have not yet been performed.

The aim of this study was to evaluate apple cultivar susceptibility to oviposition by the Medfly. For this purpose, a no-choice test was performed both in the laboratory and in the field. The seven main varieties of apples (Gala, Red Delicious, Golden Delicious, Granny Smith, Kanzi, Morgen and Fuji) were tested. The oviposition preference for the studied apple varieties was investigated in relation to the evolution of certain physical and chemical parameters that change during the ripening process.

Materials and methods

Insects. Two insect strains were used to perform the no-choice laboratory tests; a long term mass-reared strain called J.R.C. ISPRA, estimated to have completed 30 generations on artificial medium, and a second strain collected from infested apples in the field in 2016 and called SMA2016. Both the strains were reared on an artificial diet (alfalfa desiccate, sugar,

water, citric acid and sodium benzoate for larval development, and a mixture of yeast hydrolysate, sugar and water for adults) and were maintained in a climatic chamber with a photoperiod of 16:8 (L:D) hours, 25 ± 1°C and 60 ± 5 % R.U. at FEM's Entomological Laboratory.

No-choice tests. Seven apple cultivars were used, namely Gala, Red Delicious, Golden Delicious, Granny Smith, Kanzi, Morgan and Fuji, which are the three main varieties cultivated in the area where the MMF is present. The apples were collected at commercial harvest time and individually inspected to exclude pre-infestation. (Papachristos *et al.*, 2013); Follett *et al.*, 2011).

In the laboratory bioassays, each piece of fruit was placed in a cage (30 cm long and with a diameter of 18 cm) with five mature females (i.e. mated 10-day-old females) and one male of *C. capitata.* Water and sugar were administered to ensure adequate survival and oviposition on the apple (Figure 1.1).

The cages were kept in a climatic chamber with constant climatic conditions: 25 ± 1 °C temperature, 60 ± 5% R.H. and photoperiod of 16:8 (L:D) for 48 h.

For the field tests, the individually hung apples were caged with organza tissue and infested with five mature females (i.e. mated 10-day-old females) and one male of *C. capitata.* Water and sugar were administered. Cages were removed after 48 hours and the apples were brought into the laboratory to check oviposition.

In both tests, cultivar susceptibility was evaluated by counting the number of oviposition holes per apple after 48 h of exposure to the insects. A sample of damaged apples was dissected to count the number of eggs per hole under a stereomicroscope (Figure 2.2). Laboratory tests were carried out with both fly strains.

The number of holes/apple was related to certain physical-chemical characteristics of the different apple varieties: peel and pulp hardness (force measured using a penetrometer) and soluble solid content (measured using a refractometer, I.R. %). Soluble solid content (Brix°) and firmness (kg/cm^2) were assessed using Pimprenelle (an automatic laboratory system), by placing the apples with the pedicel placed horizontally, and carrying out lateral measurements at the centre of the apple. Peel and pulp hardness were measured using respectively 6 and 11-mm diameter blunted needle probes. We used 25 apples for each apple variety.

Figure 2.1 Different apple varieties inside the cage with insects, water and sugar ab libitum

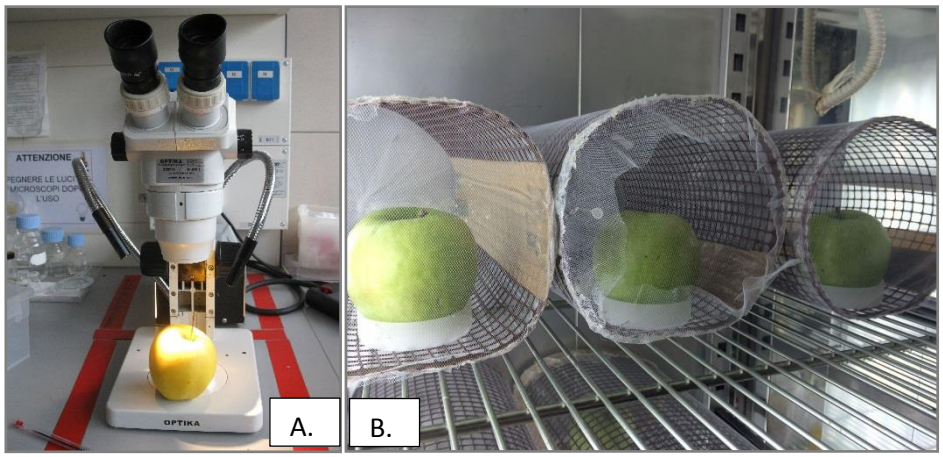


Figure 2.2 A) Control of oviposition holes on the apple under a stereomicroscope. (B) Apples in a climatic cell during the experiment.

Statistical analysis. Differences in physical and chemical parameter measurements were assessed using one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference test to statistically separate the means (SPSS Base ver. 15.0 software). Statistical differences in the number of oviposition holes in the tested apple varieties were assessed using ANOVA (and Tukey's test for mean separation) and when Levene's test showed significance, using non-parametric Kruskal-Wallis analysis followed by Mann-Whitney U-tests for pairwise comparison (software SPSS Base ver. 15.0).

Principal component analysis (PCA) was carried out to obtain the relative value for the contribution of each of the respective parameters regarding susceptibility to *C. capitata* egg laying. The main parameters for PCA were peel hardness, pulp hardness and soluble solid content. Samples were grouped by variety and categorised at three infestation severity levels (IR) (no infestation= 0 holes/apple; medium = 1 hole/apple; high > 1 hole/apple) (Statistica 13.1 Dell^{TM} software).

Pairwise correlation (Pearson product moment correlations) was conducted to measure the correlation between each parameter and the infestation rate. All statistical analysis was conducted using Statistica software (Statistica 13.1 Dell^{TM} software).

The statistical differences between the number of oviposition punctures made by wild and laboratory strains were determined with the chi-square test (Statistica 13.1 Dell^{TM} software).

Statistical differences in the number of eggs per oviposition puncture in the tested varieties were analysed with the non-parametric Kruskal-Wallis test, because Levene's test shows significance (Statistica 13.1 Dell^{TM} software).

Results

The results shown in Table 2.1 demonstrate that *C. capitata* lays eggs on all apple cultivars under both field and laboratory conditions. In the no-choice trials, the seven varieties showed statistically significant differences in the number of oviposition holes/apple. Under laboratory conditions, Granny Smith, Red Delicious and Morgan showed the lowest number of oviposition holes per fruit (0.75 ± 0.75, 1.55 ± 1.21 and 2 ± 1.61 respectively) with no significant differences between them. Granny Smith had significantly different results to three other varieties: Golden Delicious, Kanzi and Fuji (3.27 ± 3.27, 3.31 ± 1.75, 3.1 ± 1.76, oviposition holes/fruit). In field conditions, only Golden Delicious showed differences with Granny Smith (2 ± 1.1 oviposition holes per apple vs 0.25 ± 0.45). The other varieties in this condition showed similar susceptibility.

No significant differences were found between the laboratory strain and wild strain in terms of oviposition preferences on the three tested varieties (Table 2.2).

Table 2.1 Oviposition holes on the different apple varieties. Means in columns followed by the same letter are not significantly different (Mann-Whitney U-test).

Variety	Laboratory holes/apple	Field holes/apple
Gala	3.38 ± 1.80 a	1.92 ± 1.38 ab
Red Delicious	1.55 ± 1.21 bc	1 ± 1 bc
Golden Delicious	3.27 ± 1.56 ab	2 ± 1.1 a
Granny Smith	0.75 ± 0.75 c	0.25 ± 0.45 c
Kanzi	3.31 ± 1.75 ab	1.31 ± 1.32 ab
Morgan	2 ± 1.61 bc	0.45 ± 0.69 bc
Fuji	3.1 ± 1.76 ab	1.55 ± 0.93 ab
χ^2	29.112	25.489
df	6	6
P	0.0001	0.0001

Table 2.2 Oviposition punctures made by the two different strains on three of the tested apple varieties. Marked differences in the T-test are significant when p < 0.05 for the two different insect strains

Strain	Gala	Golden Delicious	Granny Smith
Laboratory	3.38 ± 1.80	3.27 ± 1.56	0.75 ± 0.75
Wild	3.69 ± 1.32	3.45 ± 1.57	1 ± 1.04
P	0.625224	0.74909	0.515235

Table 2.3 Eggs per oviposition puncture for different varieties in field conditions. Marked differences in the Mann-Whitney U-test are significant when p < 0.05 for the different varieties. Means in columns followed by the same letter are not significantly different

Varieties	Eggs/Puncture
Gala	7.825 ± 7.36 bc
Red Delicious	68.13 ± 29.33 a
Golden Delicious	28.67 ± 11.32 abc
Granny Smith	22 ± 19.97 abc
Kanzi	5.02 ± 3.82 c
Morgan	12.33 ± 6.03 abc
Fuji	33.48 ± 16.54 ab
H (chi^2)	30.80
P	< 0.0001

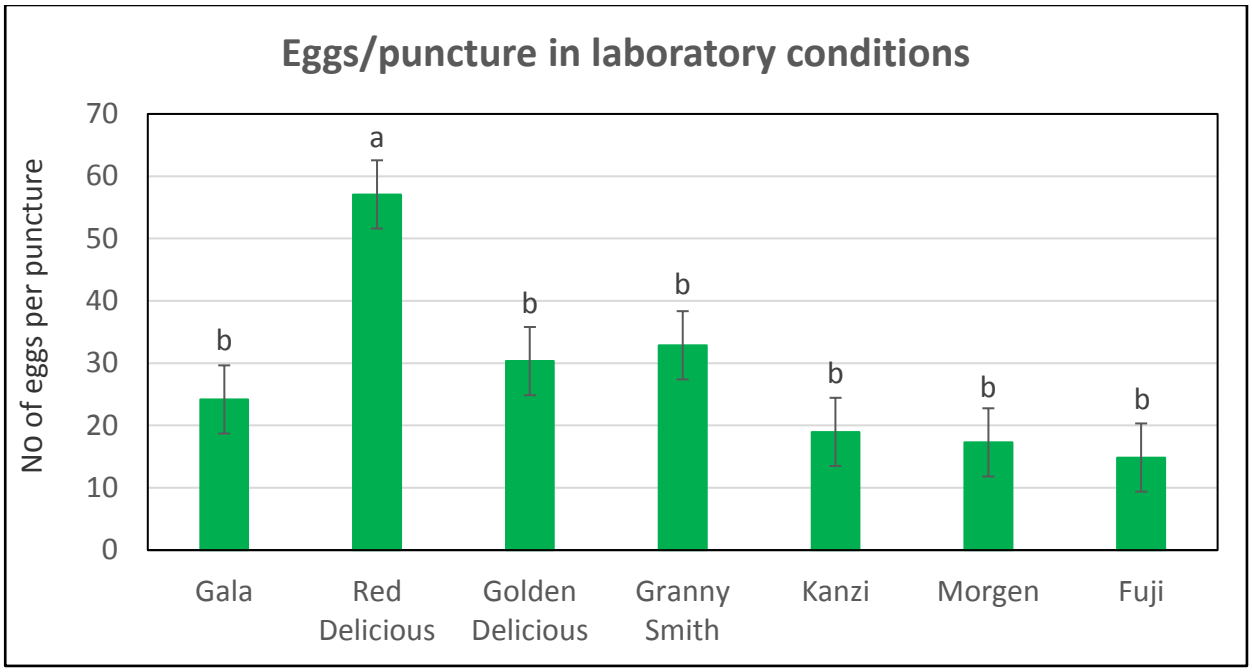


Figure 2.3 Eggs per oviposition puncture for different varieties in laboratory conditions. Marked differences in Tukey's test are significant when p < 0.05 in the different varieties. The same letters are not significantly different.

In laboratory conditions the Red Delicious variety showed statistically significant differences with the other varieties in terms of the number of eggs per oviposition puncture (ANOVA, F = 12.6, P < 0.0001, df = 17.66). Indeed, the Red Delicious variety had 57.08 (± 15.04) eggs per oviposition puncture (Figure 2.3).

On the other hand, only slight and not statistically significant differences emerged from the field trials (Table 2.3). In particular, Red Delicious showed no statistical differences with Gala and Kanzi (Kruskal-Wallis test, H = 30.88, P < 0.0001).

Table 2.4 Physical and chemical parameters measured in the tested apple varieties. Means in columns followed by the same letter are not significantly different (Tukey's HSD, P<0.05).

Variety	°Brix	Pulp hardness Kg/cm^2	Peel hardness Kg/cm^2
Gala	11.5 ± 0,56 a	7.808 ± 1.11 bcd	2.6292 ± 0.46 bc
Red Delicious	10.682 ± 1.09 a	8.018 ± 0.56cd	3.1527 ± 0.20 d
Golden Delicious	14.582 ± 0.84 c	5.409 ± 0.43 a	1.0664 ± 0.34 a
Granny Smith	10.925 ± 0.22 a	8.292 ± 0.59 d	2.7892 ± 0.32 cd
Kanzi	13.662 ± 0.49 b	7.092 ± 0.37 bc	2.6785 ± 0.25 bc
Morgan	12.891 ± 0.96 b	9.373 ± 1.43 e	3.1555 ± 0.38 d
Fuji	15.118 ± 0.59 c	6.927 ± 0.66 b	2.3655 ± 0.22 b
F	69.014	25.749	52.567
P	0.000	0.000	0.000

Table 2.5 Pearson product correlation coefficients for apple parameter means (m). Values in bold indicate significant differences in the level of P < 0.05.

Parameters	m	SD	Sugar	Peel hardness	Pulp hardness	IR lab	IR field
Sugar	12.73	1.78	1	**-0.53**	**-0.54**	**0.36**	**0.23**
Pulp hardness	7.56	1.38	**-0.53**	1.00	**0.59**	**-0.33**	**-0.40**
Peel hardness	2.56	0.72	**-0.54**	**0.59**	1.00	-0.30	-0.19
IR lab	2.50	1.78	**0.36**	**-0.33**	**-0.30**	1.00	0.17
IR field	1.22	1.196	**0.23**	**-0.40**	-0.19	0.17	1.00

In relation to physical-chemical characteristics (Table 2.4), Fuji and Golden Delicious were the varieties with the highest sugar content (15.12 ± 0.59 and 14.58 ± 0.84 Brix° respectively), statistically different from Kanzi (13.662 ± 0.49) and Morgan (12.891 ± 0.96), which were also significantly different from Gala (11.5 ± 0.56), Granny Smith (10.925 ± 0.22) and Red Delicious (10.682 ± 1.09). Golden Delicious was also the variety with the lowest pulp hardness (5.409 ± 0.43 Kg/cm2), statistically different from all the other varieties. Fuji (6.927 ± 0.66), Kanzi (7.092 ± 0.37) and Gala (7.808 ± 1.11) showed intermediate values, while Red Delicious (8.018 ± 0.56), Granny Smith (8.292 ± 0.59) and Morgan (9.373 ± 1.43) were the three varieties with the highest resistance to pulp penetration. Peel hardness values essentially reproduced the same gradient, while Golden Delicious showed the lowest penetration resistance (1.07 ± 0.34) and Morgan the highest (3.16 ± 0.38).

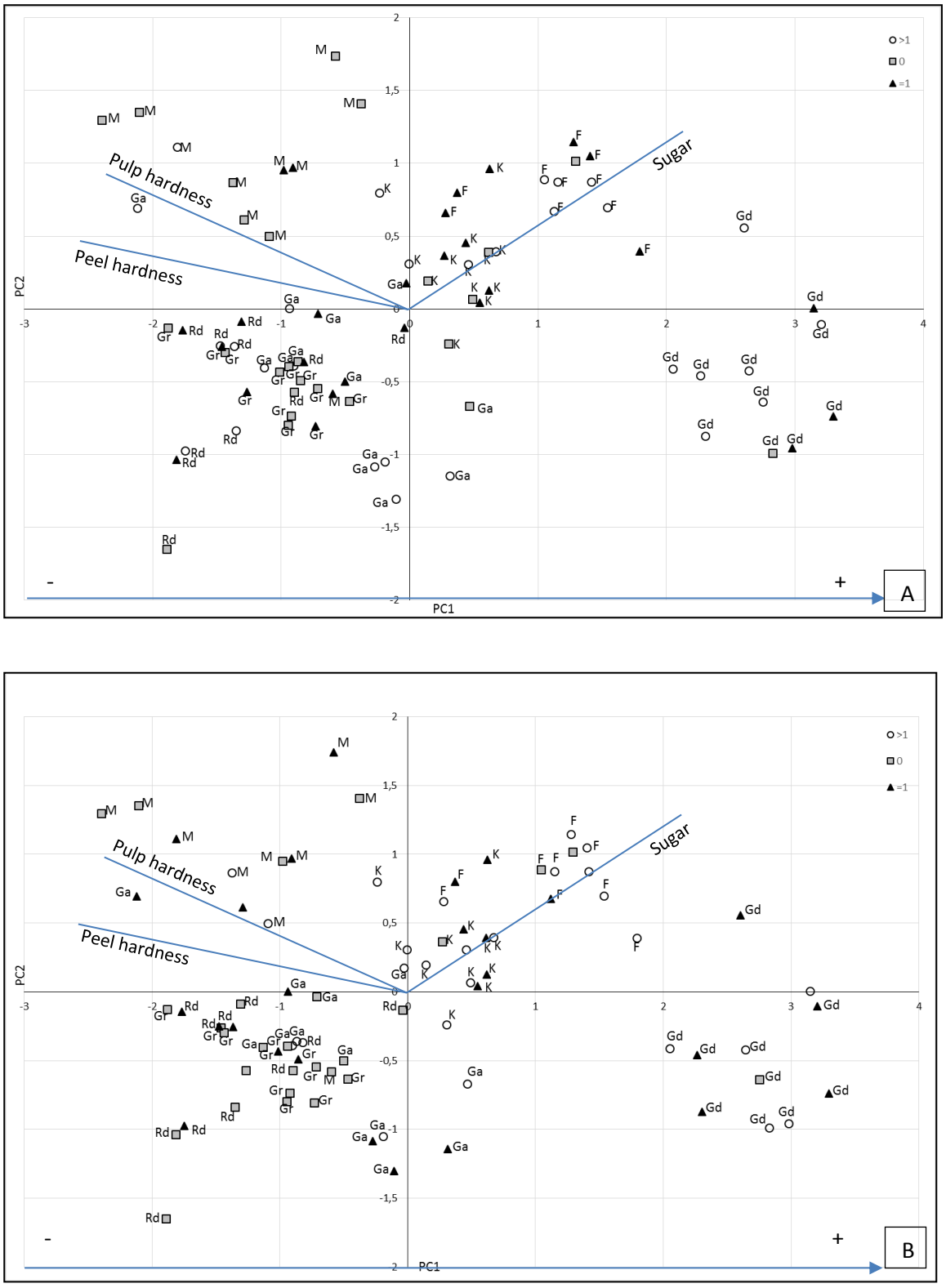


Figure 2.4 Principal component analysis based on three quality parameters; sugar content, and penetrating resistance of peel and pulp. Twenty-five apples were observed in the field (A) and in laboratory conditions (B), to assess the infestation level (0, no damage; =1, one oviposition hole per apple; >1, more than one oviposition hole per apple) in seven apple varieties (Ga, Gala; Rd, Red Delicious; Gd, Golden Delicious, Gr, Granny Smith; K, Kanzi; M, Morgan; F, Fuji). Correlation with principal components gave PC1 70.61% and PC2 18.47%).

PCA of the values shown in Figure 2.4 showed that the sugar content, peel and pulp hardness of the apple varieties were significantly correlated with *C. capitata* oviposition in both field and laboratory conditions.

More than 60% of infested fruit was plotted in the positive area of PC1 (in both conditions), while non-infested Granny Smith, Red Delicious and Morgan fruit, was plotted on the negative side of PC1. On the other hand, more than 77% of non-infested fruit was plotted in the negative area of PC1. Indeed, the correlation matrix revealed a significant correlation ($P < 0.05$) between all the parameters (sugar, peel and pulp hardness) for laboratory susceptibility. For field susceptibility, the correlation matrix revealed a significant correlation ($P < 0.05$) between sugar and pulp hardness parameters. Therefore, in relation to the PC1 factor for the seven varieties at commercial harvest, high penetration resistance values appeared to be correlated with a low infestation level, while a higher percentage of sugar was correlated with a high infestation level.

As regards the number of eggs for the different varieties, Red Delicious was shown to be the variety with the largest number of eggs per oviposition puncture, both in laboratory (57.08 ± 15.03) and field conditions (68.13 ± 31.35).

As regards the difference between the wild and ISPRA strains, no difference in oviposition susceptibility was shown in laboratory conditions for three different apple varieties (Gala $P=0.63$, Golden Delicious $P=0.75$ and Granny Smith $P= 0.52$).

Discussion and conclusion

The results demonstrate that *C. capitata* lays eggs on all apple cultivars in both natural and laboratory conditions.

The two different strains did not show a different number of holes per apple for three different varieties of apples, therefore the aggressiveness of the two populations was identical. For this reason, the results for varietal susceptibility were the same for both wild and laboratory insects. Processing of visual, chemical and mechanical-sensory environmental information also helps the insect to locate host plants for oviposition (Schoonhoven *et al.*, 2005).

Our no-choice observations showed that susceptibility to *C. capitata* oviposition increased when the fruit had a higher sugar content and lower penetration resistance, as in the case of Golden Delicious, Kanzi and Fuji. On the other hand, Granny Smith, Red Delicious and Morgan showed lower susceptibility than the aforementioned varieties, both in field and laboratory conditions, because of their lower sugar content and higher penetration resistance.

When considering PC1, the first principal component of PCA analysis, it appears clear that a distinct threshold exists between uninfested (negative vector) and infested (positive vector) apples.

The penetration resistance and quantity of sugar are considered to be important factors that influence the susceptibility of fruit (Ioriatti *et al.,* 2015; Papadopoulos *et al.,* 2002).

In addition, in nature, a positive correlation between oviposition preference and larval performance on different plants suggests that holometabolous phytophagus insects have the ability to choose the host plant on which their offspring develop more fully and quickly; this is also affected by the characteristics of plants, including their substance and nutritional value (Via, 1986, Joachim-Bravo *et al.,* 2001).

Red Delicious had a higher number of eggs per oviposition puncture than the other varieties (in laboratory conditions). This suggests that other factors not considered in this experiment (such as the colour or odour) can be more attractive for females for oviposition, but the higher peel and pulp hardness reduced the number of oviposition punctures per apple. Indeed, further study confirmed that the female used the same hole to reduce the cost of oviposition. Levinson *et al.*, 2003 indeed observed that before laying her eggs, the gravid Medfly female examines the fruit with her proboscis, tarsi, and probably olfactory sensilla. Females prefer a depressed or injured site on the fruit surface (Papaj *et al.,* 1989).

In this work it was seen that the principal varieties cultivated in Trentino – namely Golden Delicious, Fuji and Kanzi - are more susceptible to *C. capitata* oviposition. The Gala variety, which generally escapes Medfly attack in Trentino orchards, was susceptible to oviposition in both field and laboratory bioassays, though its physical and chemical parameter values would classify the variety as one of the least susceptible. Other factors not considered in this study, such as fruit olfactory stimuli or colour, could also play a role, by increasing susceptibility to Medfly oviposition. Indeed, Levinson *et al*. (2003) have suggested that Medfly oviposition is favoured by high fruit humidity and appropriate tactile and optical stimuli, and the odour of fruit plays a secondary role (Fletcher and Prokopy 1991; Katsoyannos 1989a; Katsoyannos 1989b; Hernandez *et al.,* 1996; Ioannou 2005; Papadopoulos *et al.,* 2006; Tabilio *et al.,* 2013). These varieties therefore need adequate control and careful management to reduce product damage.

References

De Meyer, M.; Copeland, R.S.; Lux, S.A.; Mansell, M.; Quilici, S.; Wharton, R.A.; White, I.M.; Zenz, N. (2002). Annotated checklist of host plants for Afrotropical fruit flies (Diptera: Tephritidae) of the genus *Ceratitis*. *Documentation zoologiques.,* 27. Royal Museum for Central Africa, Tervuren, Belgium.

Fletcher, B.S.; Prokopy, R.J. (1991). Host location and oviposition in tephritid fruit flies, pp. 140-171. In Bailey, W. J. and Ridsdill-Smith, J. [eds.] *Reproductive behavior of insects*. Chapmann & Hall.

Follett, P.A.; Zee, F.T.; Hamasaki, R.T.; Hummer, K.; Nakamotoj, S.T. (2011). Susceptibility of low-Chill Blueberry cultivars to Mediterranean Fruit Fly, Oriental Fruit Fly, and Melon Fly (Diptera: Tephritidae). *Econ. Entomol.*, 104 (2): 566-570.

Hernandez, M.M.; Sanz, I.; Adelantado, M.; Ballach, S.; Primo, E. (1996). Electroantennogram activity from antennae of *Ceratitis capitata* (Wied.) to frech orange airborne volatiles. *J. chem. Ecol.*, 22: 1607-1619.

Ioannou, C.S. (2005). Effect of olfactory compounds from orange on the oviposition and other biological parameters of the Mediterranean fruit fly *Ceratitis capitata* (Diptera: Tephritidae). Masters, Aristotle University of Thessaloniki, *Master thesis*.

Ioriatti, C.; Walton, V.; Dalton, D.; Anfora, G.; Grassi, A.; Maistri, S.; Mazzoni, V. (2015). Drosophila suzukii (Diptera: Drosophilidae) and its potential impact to Wine Grapes during harvest in two cool climate wine grape production regions. *J. Econ. Entom.,* 108 (3): 1148-1155.

Joachim-Bravo, I.S; Fernandes Odair, A.; De Bortoli, S.A.; Zucoloto, F. (2001). Oviposition behavior of *Ceratitis capitata* Wiedmann (Diptera: Tephritidae): Association between oviposition preference and larval performance in individual females. *Neotrop. Entomol.*, 30: 559-564.

Katsoyannos, B.I. (1989a). Response to shape, size and colour, pp. 307-324, In Robinson, A.S. and Hooper, G. [eds.] *World Crop Pests*, Vol. 3a.

Katsoyannos, B.I. (1989b). Field response of Mediterranean fruit fly to sphere of different color patterns and to yellow crossed panels, pp. 393-400, In Cavalloro, R. [ed.] *Fruit flies of economic importance*, Vol. 87.

Levinson, H.; Levinson, A.; Osterried, E. (2003). Orange-derived stimuli regulation oviposition in the Mediterranean Fruit fly. *J. Appl. Entomol.* 127: 269-275.

Liquido, N.J.; Cunningham, R.T.; Nakagawa, S. (1990). Host plants of Mediterranean fruit-fly (Diptera: Tephritidae) on the island of Hawaii (1949–1985 Survey). *J. Econ.Entomol.,* 83: 1863-1878.

Papachristos, D.P.; Papadopoulos, N.T.; Maglaras, E.; Michaelakis, A.; Antonatos, S.A. (2013). Susceptibility of kiwifruit (*Actinidia* spp.) cultivars to *Ceratitis capitata* (Diptera: Tephritidae) infestation. *J. Appl. Entomol.,* 138: 433-440.

Papadopoulos, N.T.; Carey, J.R., Katsoyannos, B.I.; Kouloussis, N.A. (1996). Overwintering of *Ceratitis capitata* (Diptera: Tephritidae) in northern Greece. *Ann. Entomol. Soc. Am.,* 89: 526-534.

Papadopoulos, N.T.; Katsoyannos, B.I.; Carey J.R.; Kouloussis, N.A. (2001). Seasonal and annual occurrence of the Mediterranean fruit fly (Diptera: Tephritidae) in northern Greece. *Ann. Entomol. Soc. Am.,* 94: 41-50.

Papadopoulos,; Katsoyannos, B.I.; Carey, J.R. (2002). Demographic Parameters of the Mediterranean fruit fly (Diptera: Tephritidae) Reared in Apples. *Ann. Entomol. Soc. Am.*, 95 (5): 564-569.

Papadopoulos, N.T.; Katsoyannos, B.I.; Carey, J.R. (1998). Temporal changes in the composition of the overwintering larvae population of the Mediterranean fruit fly (Diptera: Tephritidae) in northern Greece. *Ann. Entomol. Soc. Am.,* 91: 430-434.

Papadopoulos, N.T.; Koulossis, N.A. Katsoyannos, B.I. (2006). Effect of plant chemicals on the behavior of the Mediterranean fruit fly. Fruit fly of economic importance: from basic to applied knowledge. Proceedings of the 7th *International Symposium on Fruit flies of economic importance*, 97-106.

Papaj, D.R.; Katsoyannos, B.I.; Hendrichs, J. (1989). Use of fruit wounds in oviposition by Mediterranean fruit flies. *Entomol. Exp. Appl.,* 52: 203-209.

Prokopy, R.J.; Moericke, V.; Bush, G.L. (1973). Attraction of Apple Maggot Flies to Odor of Apples. *Environ. Entomol.,* 2(5): 743-750.

Rigamonti, I. (2002). La *Ceratitis capitata* in Lombardia; Quaderni della ricerca.

Schoonhoven, L.M.; Van Loon, J.J.A.; Dicke, M. (2005). *Insect-Plant Biology*. 2nd ed. Oxford University Press.

Tabilio, M.R.; Fiorini, D.; Marcantoni, E.; Materazzi, S.; Delfini, M.; De Salvador, F.R.; Musmeci, S. (2013). Impact of the Mediterranean fruit fly (Medfly) *Ceratitis capitata* on different peach cultivars: The possible role of peach volatile compounds. *Food Chem.,* 140: 375-381.

Via, S. (1986). Genetic covariance between oviposition preference and larval performance an insect herbivore. *Evolution,* 40: 778-785.

CHAPTER 3

Development of *Ceratitis capitata* (Diptera: Tephritidae) in seven apple varieties under laboratory conditions

Abstract

This work aimed to study the development of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedmann) (Dipthera Tephritidae), in seven different apple varieties (Gala, Golden Delicious, Red Delicious, Granny Smith, Kanzi, and Fuji) under laboratory conditions.

Full development of the larvae was recorded in higher percentages in Gala, Golden Delicious, Kanzi and Fuji apples (76%, 88%, 84% and 80% respectively). Granny Smith, Red Delicious and Morgen showed a significantly lower percentage of larvae which completed their development successfully (8%, 4% and 12% respectively).

The varieties with the highest number of emerged pupae were Gala, with 5.58 ± 2.93 pupae per apple, Golden Delicious with 2.90 ± 1.60 pupae per apple, Kanzi with 3.04 ± 1.96 and Fuji with 2.20 ± 1.32 pupae per apple.

Development from egg to pupa inside the fruit was measured in the different varieties. Gala and Golden Delicious permitted significantly faster larval development (24.95 ± 6.19 days and 20.82 ± 4.07 days, respectively) compared to Kanzi and Fuji (25.81 ± 3.53 days and 23.60 ± 2.80 days, respectively).

Furthermore, the development from egg to adult was significantly longer in Gala (38.38 ± 5.58 days) than in Fuji (32.12 ± 2.75), while in Golden Delicious it took 36.00 ± 4.75 days and in Kanzi 37.58 ± 4.13 days.

At the same time, the fitness of emerged pupae, expressed as the size of the pupae, was evaluated in the different varieties. Pupae perimeters showed significant differences in the different varieties. Larvae developed in the Morgen variety generated pupae with a shorter perimeter compared to the other varieties (22.32 ± 0.74 mm).

Analysis of the physical-chemical characteristics of fruit showed that pulp firmness was higher in Granny Smith, Red Delicious and Morgen, acidity was higher in Granny Smith and the quantity of sugar was lower in Red Delicious and Granny Smith compared to the other varieties.

In conclusion, these results indicate that Gala, Golden Delicious, Kanzi and Fuji are the most suitable varieties for both larval development and pupae dimension. As regards the chemical-physical characteristics of fruit, pulp firmness, acidity and sugar content seem to be the most

important factors in explaining differences in the development of *Ceratitis capitata* in the seven varieties.

Introduction

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is the most polyphagous of all pestiferous fruit flies (Aluja *et al.*, 2000), causing extensive fruit loss worldwide. Females lay their eggs in ripening fruit and the resulting products cannot be sold. (Eskafi, 1988; Katsoyannos *et al.*, 1998; Mavrikakis *et al.*, 2000; Papadopoulos *et al*., 2001). Earlier studies have addressed the effects of various hosts, including some citrus species, on Medfly population parameters (Carey, 1984; Krainacker *et al.*, 1987). Recently, Papachristos *et al*., (2008) demonstrated differential developmental and survival rates of immature *C. capitata* when newly hatched larvae were placed in either the peel or the flesh of the fruit. The development and survival rates of immature insects, as well as the survival and fecundity of adults of this pest have been studied under constant conditions in the laboratory, by using both a laboratory-reared population (Shoukry *et al.*, 1979; Vargas *et al.*, 1984, 1996, 1997; Carey *et al.*, 1986; Krainacker *et al.*, 1987, 1989; Cavalloro *et al.*, 1969), and wild strains generally originating from the tropics (Harris *et al.*, 1989; Vargas *et al.*, 1989, Harris *et al.*, 1991). There are few studies dealing with the behaviour of wild populations originating from the Mediterranean basin (Rivnay 1950, Carey 1984).

Moreover, despite the abundance of studies carried out on the bio-ethology of this insect both in laboratory and field conditions, there is little information about its development inside the fruit and in particular in infested apples. Observation of the duration of the larval stage, larval mortality and the fitness of adults of *C. capitata* in pome fruit showed that different apple varieties demonstrated significant variability in terms of Medfly field infestation (Papadopoulus *et al.*, 1996, 2001; 2002a).

Apples are one of the most important crops in northern Italy and in particular in Trentino. The presence of *C. capitata* was first reported in a limited area of Trentino (northern Italy) in 1990. After that, no damage was reported till 2010, when the Medfly reappeared, and it has been found permanently in subsequent years in the southern part of the region.

Determining the host status of a crop or cultivar for an emerging pest species is of paramount importance in assessing any associated risk (Aluja *et al.*, 2004; Aluja *et al.*, 2008). Determination of host status is a complex procedure encompassing many aspects of the biology of the fruit fly, involving physiological and behavioural host finding mechanisms and utilisation in relation to the physiological parameters of the host fruit (Aluja *et al.*, 2008).

This study aimed to investigate some of the fitness parameters of *C. capitata* when reared on seven of the main apple varieties cultivated in Trentino (Italy). The impact of feeding on different apple cultivars on the fitness of *C. capitata* was addressed by examining larval survival, development time from newly hatched larva to adult, the number of pupae obtained, the size of the pupae and the number of adults emerging.

Material and methods

Insects. A colony of *C. capitata* was established using larvae-infested apples collected in different orchards in Trentino (TN), Italy in 2016. This wild population was reared in laboratory conditions and maintained on an artificial diet (Cavalloro *et al.*, 1969) in a climatic chamber with a photoperiod of 16:8 (L:D) hours, 25 ± 1 °C and 60 ± 5 % R.U. at the Foundation Edmund Mach (FEM, San Michele all'Adige, TN, Italy, (200 m a.s.l., 46° 12' N, 11° 8' E).

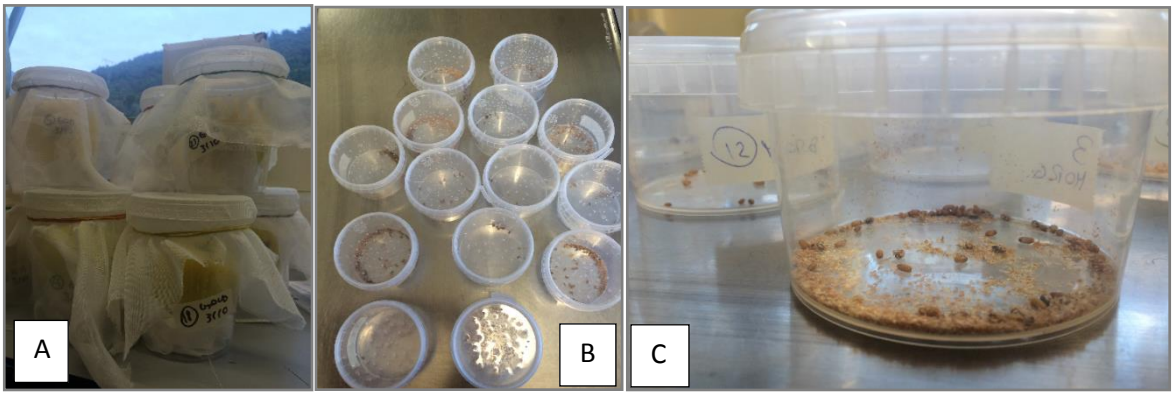


Figure 3.1 Experimental material: A. Apples placed in individual containers with absorbent paper; B. Pupae emerging from the apples and placed in different containers until adults emerged; C. Close-up of pupae in a plastic container

Apples. Seven apple cultivars - Gala, Red Delicious, Golden Delicious, Granny Smith, Kanzi, Morgen and Fuji – were collected at commercial harvest time in nearby orchards. Before use, all the fruit was inspected to exclude infestation. Samples of fruit (N = 7 with three repetitions) were used to measure certain physical and chemical characteristics. The firmness of the pulp (kg/cm2) was measured with a penetrometer in the central area of apples with an 11 mm diameter cap. Soluble solid content (°Brix), quantity of juice (% weight/weigh) and acidity (mg malic acid/ 100 g) were measured with an automatic laboratory system ("Pimprenelle", Setop, Giraud, France). The quantity of starch in the apples was analysed with Lugol's solution (potassium iodide). This method involves dipping the apples (cut transversally across the middle section of the fruit) in Lugol's solution. The starch present in the fruit reacts with the iodine in the solution, causing blue-violet pulp colouring. The colour intensity is directly proportional to the starch content, which is quantified with a colorimetric scale from 1.0 to 5.0, where 1.0 corresponds to a completely dark fruit, rich in starch and unripe fruit and 5.0 is the maximum value with starch completely degraded in sugar.

Larval development time. Newly emerged larvae were obtained from eggs placed in a Petri dish with agar (0.8%) to guarantee adequate humidity (Figure 3.2 A). The Petri dishes were kept in a climatic chamber (16:8 L:D, 25 ± 1 °C, 60 ± 5 % R.U) for 48 hours. Ten newly emerged larvae were gently introduced into small holes made with an entomological pin on the surface of the fruit with a fine brush (Papadopoulus *et al.*, 2002b). 25 apples were used for each variety (*i.e*. 250 larvae).

After larval inoculation, each apple was placed in a plastic box, on absorbent paper, and covered with gauze to guarantee adequate ventilation (Figure 3.1). The pupae obtained from each individual apple and the related larval development time were recorded daily. The size of the pupae was measured under a stereomicroscope with the Leica programme. For this purpose, the pupa was put on its side and the length and width at the median site were measured (Figure 3.2 B). The measurements were used to calculate the ellipsoid perimeters with the formula: $2*\pi* \sqrt{((length/2)^2 + (height/2)^2)} / 2$

After that, the pupae were placed in a climatic chamber in the same conditions until the adults emerged, and the time when the first adult emerged was recorded.

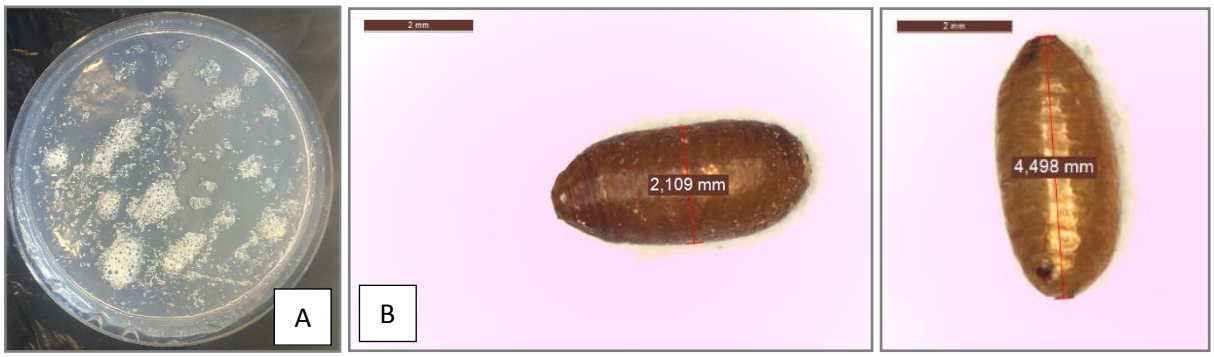


Figure 3.2 A) Petri dish with agar, where the eggs were positioned for the newly emerged larvae. B) Example of pupae measurements

Statistical analysis. Statistical differences were assessed with ANOVA (and Tukey's test for mean separation) and when Levene's test showed significance, non-parametric Kruskal-Wallis analysis was used, followed by Mann-Whitney U-tests for pairwise comparison or chi-square tests (SPSS Base software, ver. 15.0).

The data, as a percentage of apples permitting larval survival for each variety, were assessed with the chi-square test, followed by Ryan's multiple comparison test on proportions. Software used: Statistica v.13, STATSOFT.

The differences in the number of pupae that developed fully and the number of adults emerging for the seven apple cultivars were analysed with the non-parametric Kruskal-Wallis test for equal means. Only data for varieties with at least ten replications were analysed.

The development time from eggs to pupae, and from eggs to adults, was analysed with the Kruskal-Wallis test and median separation was tested with the Mann-Whitney U test. Only data for varieties with at least ten replications were analysed.

Pupa dimension data (ellipsoid perimeters) were analysed with ANOVA, followed by Tukey's honestly significant difference test to statistically separate means ($P<0.05$).

Physical-chemical parameters (sugar, acidity, quantity of juice and starch) were analysed with the Kruskal-Wallis test and Mann-Witney test for the separation of medians, while pulp hardness was analysed with ANOVA, followed by Tukey's test for mean separation.

Principal component analysis (PCA) was carried out to provide a relative value for the contribution of each of the respective parameters, to identify apple varieties in relation to the physical-chemical characteristics. PCA adopted the principal parameters of pulp hardness, sugar, starch, acidity and quantity of juice. Past 3.11 software (2016) was used for statistical analysis.

Results

The percentage of fruit (N=25) allowing the development of at least one pupa of the 10 larvae used to infest each apple of the seven varieties is shown in Figure 3.3. A significantly higher percentage of Golden Delicious (88%), Kanzi (84%), Fuji (80%) and Gala (74%) apple varieties allowed the development of at least one larva, in comparison to Red Delicious (4%), Granny Smith (8%) and Morgen (12%) ($P=0.000$).

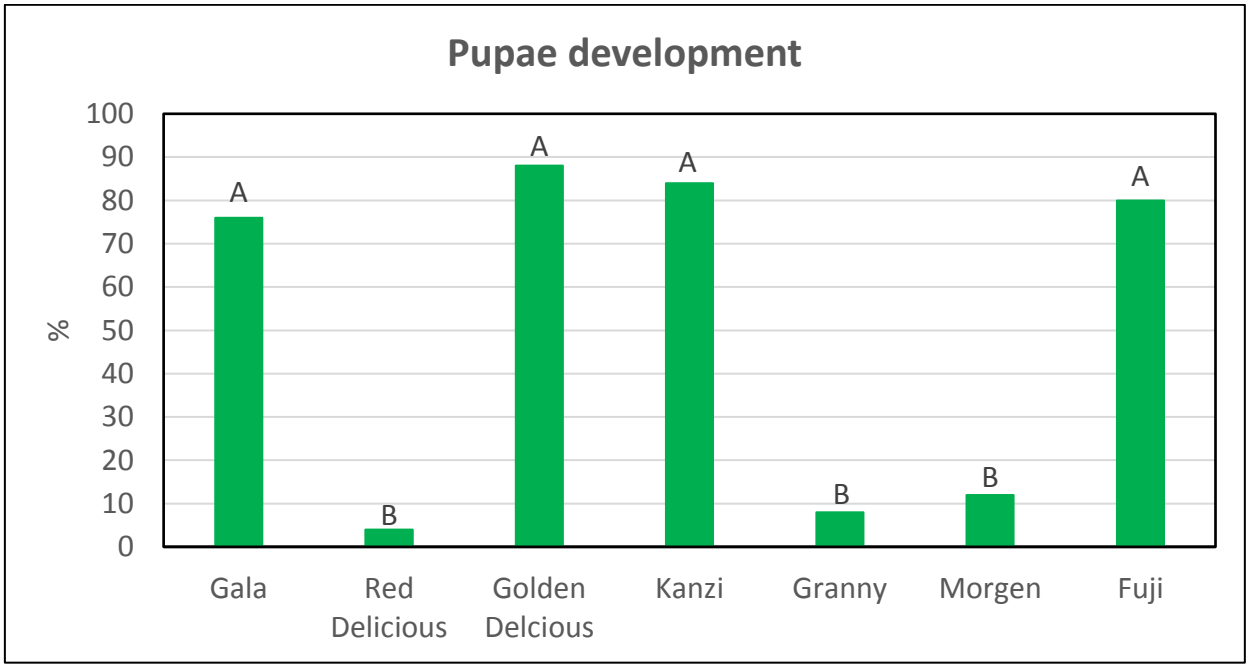


Figure 3.3 Percentage of fruit allowing the development of at least one pupa from the ten larvae used to infest each apple of the seven tested varieties. Chi square tests P=0.000, df=6.

Gala showed the lowest percentage of larval mortality (57 %; Chi squared and Ryan's test P=0.000), significantly different from Golden Delicious and Kanzi (74%). Larval mortality on Fuji (82%) was not significantly different from the previous varieties, but also not different from Morgen (88%). The highest percentage of larval mortality was recorded on Red Delicious, Granny Smith and Morgen with 98%, 96% and 88% respectively.

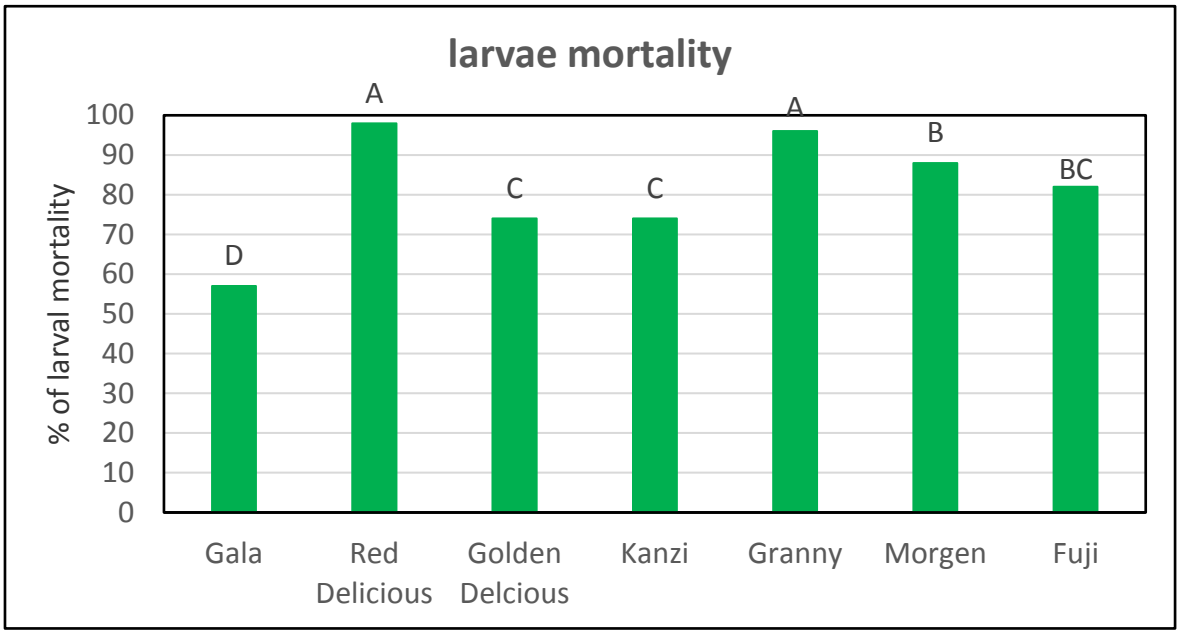


Figure 3.4 Graph of larval mortality (%) for the different varieties. Chi square tests P=0.000, df=6

Only in four varieties - Gala, Golden Delicious, Kanzi and Fuji - did the larvae accomplish their development in at least 10 apples, making it possible to test the statistical differences between varieties (Figure 3.4). In Morgen only eight apples permitted the complete larval development of twenty pupae. None of the larvae developed to the pupae stage in Red Delicious and Granny Smith (Figure 3.5). The highest number of pupae/apple emerged on Gala (5.58 ± 2.93 pupae/apple), which was statistically different (Kruskall-Wallis test H Chi^2= 16.06; P=0.0009) from the number of pupae obtained in the other three varieties: Golden Delicious (2.90 ± 1.60), Kanzi (3.04 ± 1.96) and Fuji (2.20 ± 1.32) Figure 3.6.

Figure 3.5: Fruit with damage but without larval development and emerging pupae

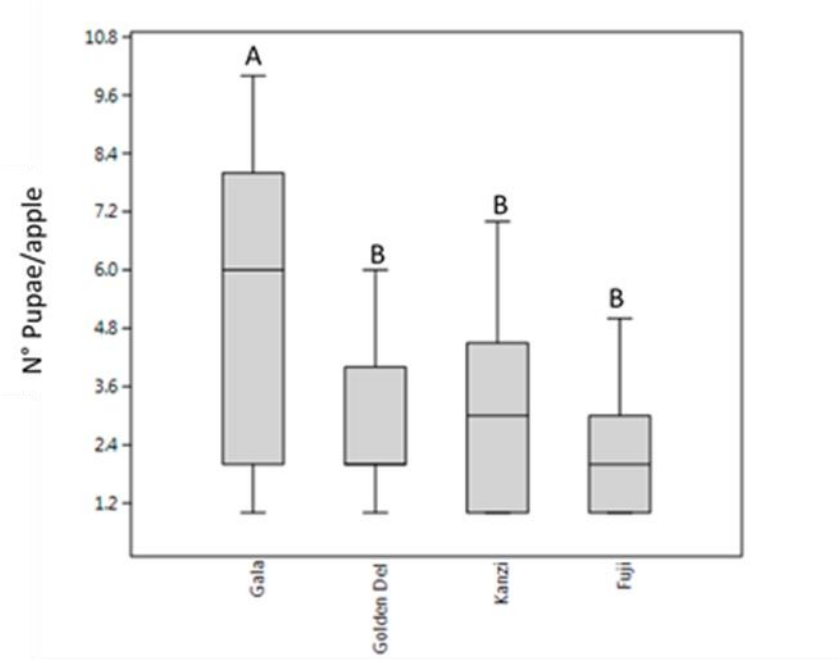


Figure 3.6 Number of pupae developing in the different varieties. Chi square tests H χ^2=16.05; p<0.01 Median and interquartile range (IQR, 25th-75th percentile).

The highest number of adults per apple emerged in the Gala variety (4.06 ± 2.40 adults/fruit), although this was not significantly different from the number of adults obtained in Golden Delicious (2.50 ± 1.40) and Kenzi (2.20 ± 1.44) (Kruskall-Wallis test H Chi^2= 10; p=0.0131). The lowest number of adults emerged in Fuji (1.64 ± 0.86), which was shown to be significantly less suitable for Medfly development than Gala (Figure 3.7).

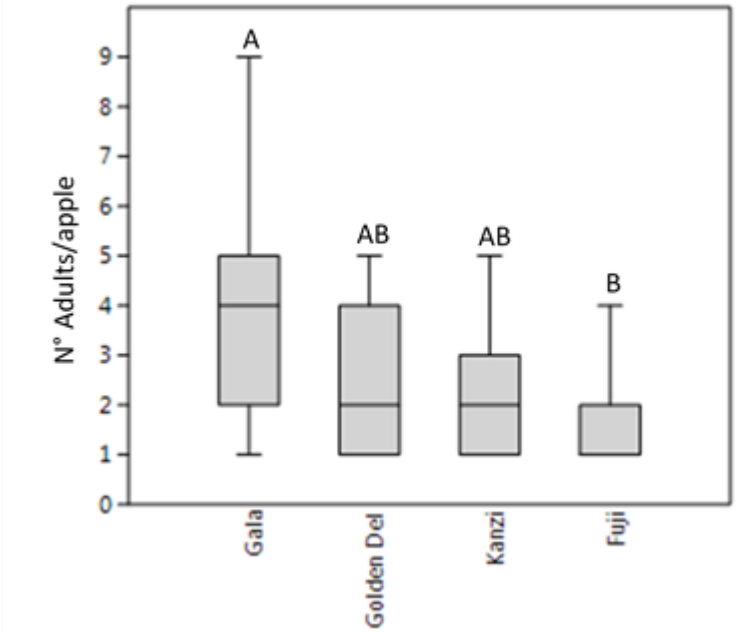


Figure 3.7 Number of adults emerging in the 4 apple varieties. Chi square tests H χ^2=10; p<0.01 Median and interquartile range (IQR, 25th-75th percentile).

Newly emerged larva developed to the pupa stage significantly faster in Golden Delicious (20.82 ± 4.07 days) than in Kanzi (25.81 ± 3.53). Gala (24.95 ± 6.19 days) and Fuji (23.60 ±

2.80) did not show any statistical differences from the previously mentioned varieties (Figure 3.8) (Kruskal-Wallis H Chi^2= 17.23; P=0.00055).

The development time from newly emerged larva to adult (Figure 3.9) was significantly faster in Fuji (32.12 ± 2.75 than in Gala (38.38 ± 5.58) and Kenzi (37.58 ± 4.13), while the development time in Golden Delicious (36 ± 4.75) was not statistically different from either Fuji or Gala and Kenzi. (Kruskal-Wallis H Chi^2= 16.06; P=0.0010).

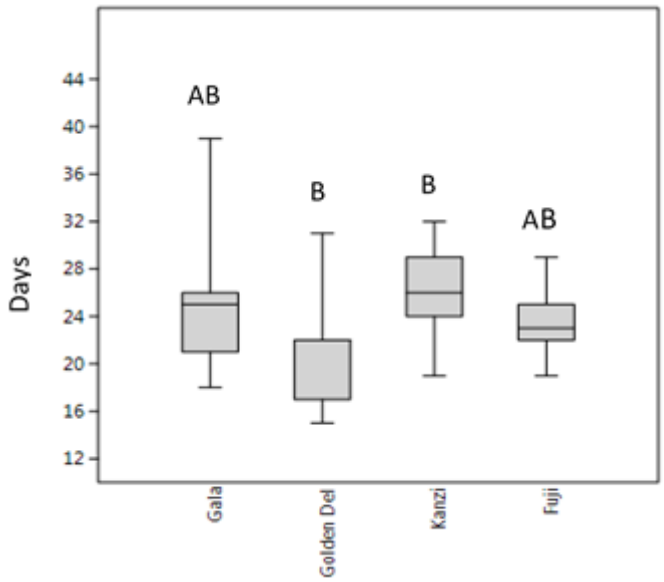


Figure 3.8 Development time (days from newly emerged larva to pupa in 4 apple varieties. Median and interquartile range (IQR, 25th-75th percentile). Different letters mean statistically significant differences between the median values (Kruskal-Wallis test H χ^2=17.23; P<0.01).

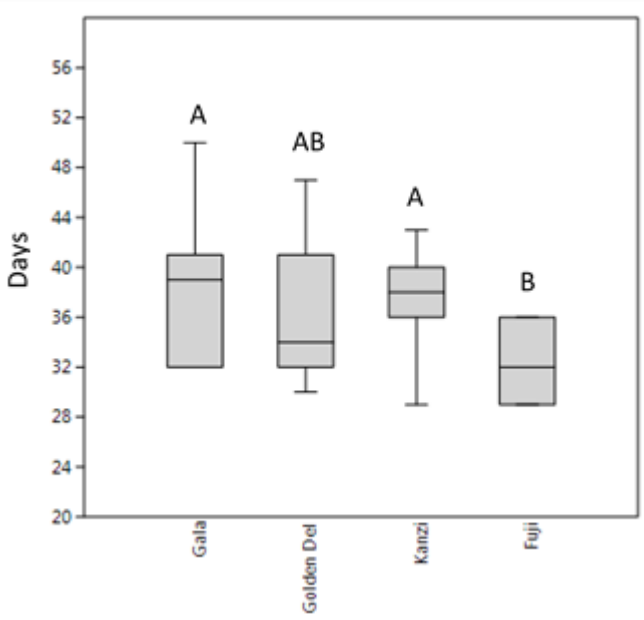


Figure 3.9 Development time (days from newly emerged larva to adult in 4 apple varieties. Median and interquartile range (IQR, 25th-75th percentile). Different letters mean statistically significant differences between median values (Kruskal-Wallis test H χ^2=16.06; P<0.01.

The size of the pupae obtained in the apple varieties is shown in Figure 3.10. The ellipsoid perimeters of the pupa obtained in the tested apple varieties were statistically different (ANOVA F=12.92; P=0.000). The largest pupae were obtained in Golden Delicious (24.42 ± 1.03 mm), Gala (24.01 ± 0.98 mm) and Kanzi (23.43 ± 0.88 mm). The size of the pupae was not significantly different in Gala, Kanzi and Fuji (23.5 ± 1.23). Larvae developing inside eight Morgan apples generated twenty pupae with a perimeter shorter than the other varieties (22.32 ± 0.75 mm).

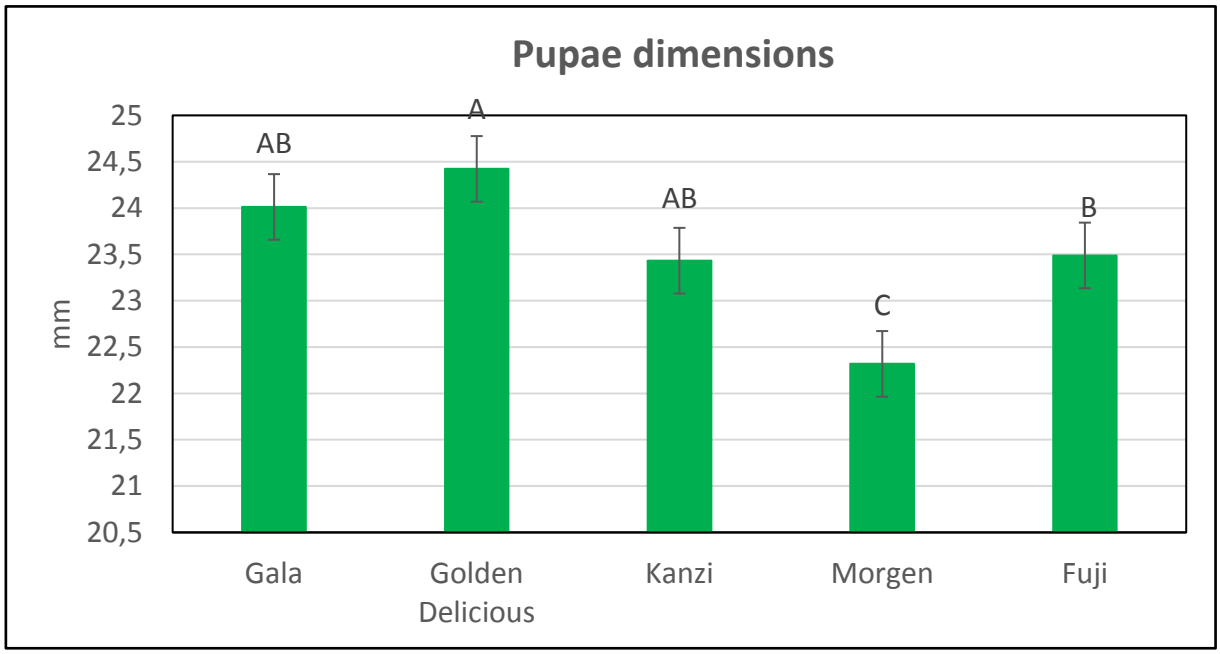


Figure 3.10 Average size (+/- SD) of pupae obtained in the five apple varieties. Different letters mean statistically significant differences between median values (ANOVA test, F=12.92; P<0.01).

The values of the physical-chemical parameters are shown in Table 3.1. Pulp firmness was significant higher in Granny Smith, Red Delicious and Morgan than in Golden Delicious, Kanzi and Fuji (Tukey's test, P<0.05). Fuji was sweeter than the other varieties, while Red Delicious had the lowest sugar content. Granny Smith and Red Delicious had a smaller quantity of starch than the other varieties. Fuji was also the variety with the highest quantity of juice, while Kanzi was the least juicy. Granny Smith was the variety with the highest acidity value, while Gala and Red Delicious had the lowest values.

Table 3.1 Physical-chemical parameter data for each apple variety. Sugar (°Brix), starch (colorimetric scale 1:5), juice (%) and acidity (mg malic acid/100g) were analysed with the Kruskall-Wallis and Mann-Whitney tests. Pulp hardness was analysed with the ANOVA test and Tukey's test for mean separation.

	Gala	Red Delicious	Golden Delicious	Kanzi	Granny Smith	Morgen	Fuji	p
Sugar	11.22±0.54 D	9.78±1.044 F	12.47±0.67 B	13.00±0.47 C	10.81±0.48 E	12.54±0.98 B	14.15±1.29 A	>0.000
Starch	2.75±0.48 B	2.09±0.17 C	3.20±0.60 B	4.17±0.35 A	2.05±0.11 C	3.58±0.79 AB	3.93±0.47 A	>0.000
Juice	8.5±1.05 C	10.3±1.15 B	10.4±0.08 AB	7.47±0.73 D	8.67±0.84 BC	9.2±0.22 BC	12.87±1.92 A	>0.000
Acidity	2.87±0.13 E	2.8±0.25 E	3.7±0.08 C	4.43±0.17 B	8.53±0.46 A	3.1±0.17 D	3.46±0.34 C	>0.000
Firmness	7.74±0.79 A	7.26±0.82 A	6.62±0.59 B	6.49±0.53 B	7.57±0.52 A	8.16±0.54 A	6.66±0.71 B	>0.000

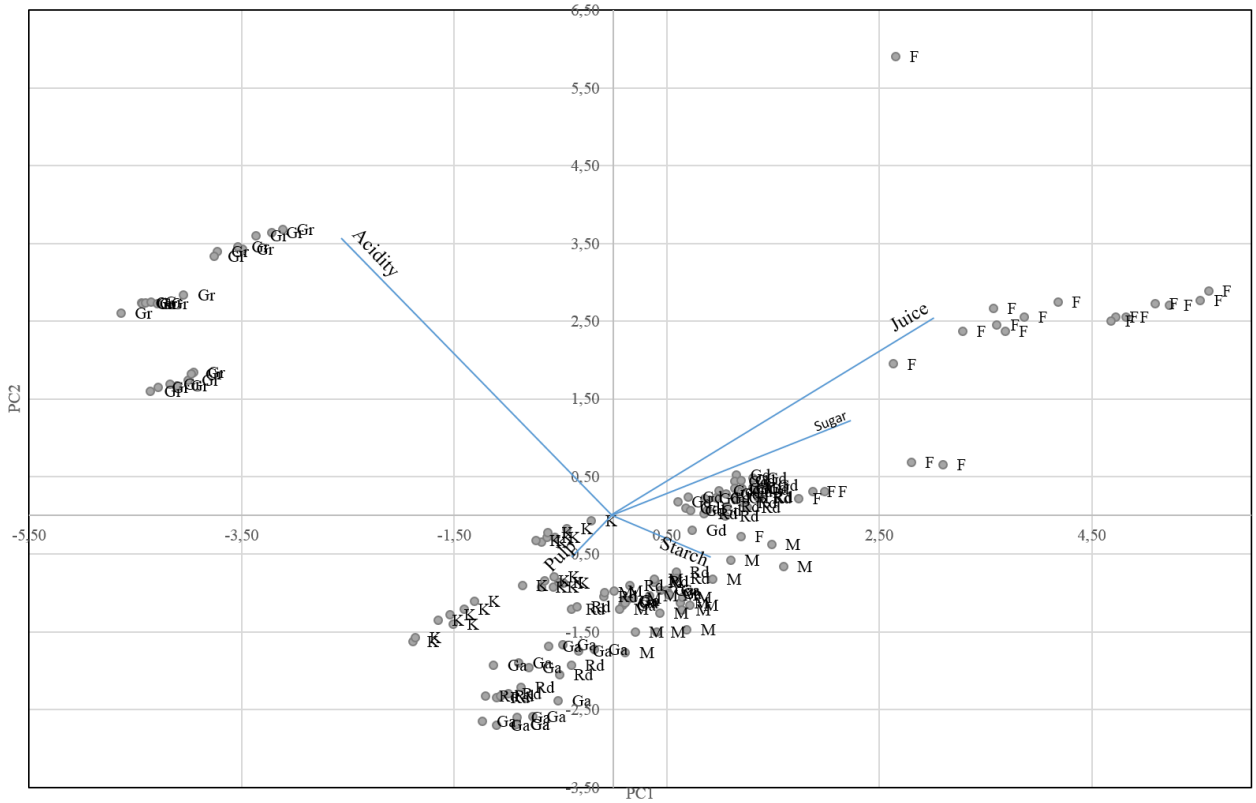


Figure 3.11 PCA analysis considering the two principal components - PC1 (53.106%) and PC2 (22.684%) – which represent 75.79% of sample variance. The graph shows the location of the different varieties in relation to the physical-chemical characteristics (pulp hardness, starch, sugar, juice and acidity).

The first two components of PCA, PC1 and PC2, accounted for 53.1% and 22.7% of variance respectively (Figure 3.11). This figure shows the distribution of the different varieties in distinct areas representing the specific physical-chemical characteristics. Indeed, Granny Smith is located in the area where acidity is higher than the other varieties. Gala, Red Delicious and Morgan are localated in the section where pulp firmness is greater. At the same time, Golden Delicious and Fuji are located in the section where sugar is higher and there is less pulp hardness than the other varieties.

Discussion and conclusion

The Mediterranean fruit fly has a high level of ethological plasticity, and is able to develop in different host fruit species (Carey 1984, Krainacker *et al.*, 1987).

To understand the wide geographical distribution of this species and the ample range of host fruit that can be affected by its feeding activity, it could be useful to examine how its life history traits change in response to different environments (including host species) (Papadopoulos *et al.*, 2002a).

Our results suggest that even within the same fruit species, different fruit varieties may considerably affect various aspects of the biology of both immature and adult Medflies. The

different apple varieties greatly influenced biological traits such as larval survival, duration of larval and adult development time and pupae dimension.

Papadopoulus *et al.* (2002b) showed that *C. capitata* reared on apples (Golden Delicious) under laboratory conditions did not experience adequate development and for this reason he concluded that the apple was not a favourable host.

The result of this study show that Golden Delicious, Gala, Kanzi and Fuji are the most favourable varieties of the seven tested. Granny Smith, together with Red Delicious and Morgan, were the least favourable apple varieties for larval survival and development until the emergence of adults. These results are in accordance with the findings of Papadopoulus *et al.*, (2002a).The results are also consistent with Papadopoulus *et al.*, (2001), who reported that Golden Delicious was more heavily infested than Granny Smith and Red Delicious in the field. A low percentage of apples allowing complete pupae development in Red Delicious, Granny Smith and Morgan was also reported by Zucoloto (1993).

Pulp firmness seemed to be the most important factor in determining immature development and survival. Indeed, a high level of pulp hardness is considered to be a critical factor in young larvae development.

Other studies showed that low soluble solid content and high acidity values affect development time and pupal weight and can cause low larval and pupal survival rates (Vargas *et al.*, 1984; Papadopoulus *et al.*, 2002b; Papacristos *et al.*, 2008).

Several authors have reported variations in life history parameters in *C. capitata* according to the host fruit (Carey, 1984; Krainacker *et al.*, 1987; Zucoloto, 1987; Kaspi *et al.*, 2002.). Our work showed that Golden Delicious, Gala, Kanzi, and Fuji produced the largest pupae (calculated by pupae perimeter). In contrast, the pupae emerging from the Morgan variety were the smallest.

It has been reported that the larger the pupae, the larger are the emerging adults, these being more competitive during mating and having a greater dispersion capacity and fertility (Sharp *et al.*, 1983; Krainacker *et al.*, 1989).

Our results show that the varieties with the highest sugar content, the lowest pulp firmness and the lowest acidity allowed more rapid development to pupae and adults. The same varieties produced pupae that were bigger and a higher rate of insects completing their development, in accordance with Rivnay (1950), Navarro *et al.*, (2011) and Papadopoulos *et al.* (2002).

In conclusion, this work shows that Gala, Golden Delicious, Kanzi and Fuji are the varieties most suitable for *C. capitata* larval development. This suggests that these apple varieties contribute more than other varieties to growth of the Medfly population during the season. Moreover, late ripening varieties suitable for Medfly infestation, such as Golden Delicious and

Fuji, are reported to allow larval development inside the fruit during the winter in northern Greece and Spain (Papadopoulus *et al.*, 1998, 1996, 2001; Escudero *et al.*, 2008). This is considered to demonstrate that the Medfly is able to overwinter in apples at the larval stage. Granny Smith, Red Delicious and Morgan are the three varieties that did not allow adequate larval and adult development and reduced the possibility of completing a new generation.

References

Aluja, M.; Diaz-Fleischer, F.; Arredondo, J. (2004). Non-host status of commercial *Persea americana* 'Hass' to *Anastrepha ludens*, *Anastrepha obliqua*, *Anastrepha serpentina* and *Anastrepha striata* (Diptera: Tephritidae) in Mexico. *J. Econ. Entomol.,* 97: 293-309.

Aluja, M.; Mangan, R.L. (2008) Fruit fly (Diptera: Tephritidae) host status determination: critical conceptual. Methodological and regulatory considerations. *Ann. Rev. Entomol.,* 53: 473–502.

Aluja, M.; Norrbom, A. (2000). Fruit Flies (Diptera: Tephritidae): *Phylogeny and Evolution of Behavior*. Boca Raton, USA. CRC Press. p. 968.

Carey. R.J. (1984). Host-specific demographic studies on the Mediterranean fruit fly *Ceratitis capitata*. *Ecol. Entomol.,* 9: 261-270.

Cavalloro. R.; Girolami. V. (1969). Miglioramento nell'allevamento in massa di *Ceratitis capitata* Wied. (Diptera: Trypetidae). Redia. LI: 315-327.

Escudero-Colomar, A.L.; Vilajeliu, M.; Batllori, L. (2008). Seasonality in the occurrence of the Mediterranean fruit fly *Ceratitis capitata* (Wied.) in the northeast of Spain. *J. App. Entom.,* 132: 714-721.

Eskafi, F.M. (1988) Infestation of citrus by *Anastrepha* spp. and *Ceratitis capitata* (Diptera: Tephritidae) in high coastal plains of Guatemala. *Environ. Entomol.,* 17: 52-58.

Harris, E.J.; Carey, R.J. (1989). Laboratory studies of the Mediterranean fruit fly (Dipteta: Tephritidae) in coffee. *Environ. Entomol.,* 18: 103-110.

Harris, E.J.; Carey, R.J.; Krainacker, D.A.; Lee, C.Y.L. (1991). Life history of *Ceratitis capitata* (Dipteta: Tephritidae) reared from mock orange in Hawaii. *Environ. Entomol.,* 18: 1048-1052.

Kaspi, R.S.; Mossinson, T.; Drezner, B.; Kamensky, B.; Yuval, B. (2002). Effects of larval diet on development rates and reproductive maturation of male and female Mediterranean fruit fly. *Physiol. Entomol.* 27: 29-38.

Katsoyannos, B.I.; Kouloussis, N.A.; Carey, J.R. (1998) Seasonal and annual occurrence of Mediterranean fruit flies (Diptera: Tephritidae) on Chios island. Greece: Differences between two neighboring citrus orchards. *Ann. Entomol. Soc. Am.,* 91: 43-51.

Krainacker, D.A.; Carey, J.R.; Vargas, R.I. (1987) Effect of larval host on life history traits of the Mediterranean fruit fly, *Ceratitis capitata*. *Oecologia,* 73: 583–590.

Krainicker, D.A.; Carey, J.R.; Vargas, R.I. (1989). Size-specific survival and fecundity for laboratory strains of two tephritids (Diptera: Tephritidae) species: implications for mass rearing. *J. Econ. Entomol.,* 82: 104-108.

Mavrikakis, P.G.; Economopoulos, A.P.; Carey, J.R. (2000) Continuous winter reproduction of the Mediterranean fruit fly (Diptera: Tephritidae) in Heraklion, Crete, Southern Greece. *Environ. Entomol.,* 29: 1180-1187.

Navarro-Campos, C.; Martinez-Ferrer, M.T.; Campos, J.M.; Fibla, J.M.; Alcaide, J.; Bargues, L.; Marzal, C.; Garcia-Mari, F. (2011). The influence of host fruit and temperaure on the body size of adults *Ceratitis capitata* (Diptera: Tephritidae) under laboratory and field condition. *J. Environ. Entomol.,* 40 (4): 932-938.

Papachristos, D.P., Papadopoulos, N.T., Nanos, G.D. (2008). Survival and development of immature stages of the Mediterranean fruit fly (Diptera: Tephritidae) in citrus fruit. *J. Econ. Entomol.,* 101: 866-872.

Papadopoulos, N.T.; Carey, J.R.; Katsoyannos, B.I.; Kouloussis, N.A. (1996). Overwintering of the Mediterranean fruit fly (Diptera: Tephritidae) in northern Greece. *Ann. Entomol. Soc. Am.,* 89: 526-534.

Papadopoulos, N.T.; Katsoyannos, B.I. (2002b). Development of Ceratitis capitate (Diptera: Tephritidae) in tree apple varieties in the laboratory. Proceedongs of *6th International Fruit Fly Symposium* in South Africa. pp.19-22.

Papadopoulos, N.T.; Katsoyannos, B.I.; Carey, J.R. (2002a). Demographic parameters of Mediterranean fruit fly (Diptera: Tephritidae) reared in apples. *Ann. Entomol. Soc. Am.,* 95: 564-569.

Papadopoulos, N.T.; Katsoyannos, B.I.; Carey, J.R.; Koulousis, N.A. (2001) Seasonal and annual occurrence of the Mediteranean fruit fly (Diptera: Tephritidae) in northen Greece. *Ann. Entomol. Soc. Am.,* 94: 41-50.

Papadopoulos, N.T.; Katsoyannos, B.I; Carey, J.R. (1998). Temporal changes in the composition of the overwintering larval population of the Mediterranean fruit fly (Diptera: Tephritidae) in the northern Greece. *Ann. Entomol. Soc. Am.,* 91: 430-434.

Rivany, E. (1950). The Mediterranean fruit fly in Israel. *Bull. Entomol. Res.,* 41: 321-341.

Ryan, T.A. (1960). Significance tests for multiple comparison of proportions, variances, and other statistics. *Psychol. Bull.,* 57: 318-328.

Sharp, J.L.; Boller, E.F.; Chambers, D.L. (1983). Selection for flight propensity of laboratory and wild strains of *Anastrepha suspense* and *Ceratitis capitata* (Diptera: Tephritidae). *J. Econ. Entoml.,* 76: 302-305.

Shoukry, A.; Hafez, M. (1979) Studies on the biology of the Mediterranean fruit fly *Ceratitis capitata*. *Entomol. Exp. Appl.,* 26: 33-39.

Vargas, R.I.; Carey, J.R. (1989). Comparison of demographic parameters for wild and laboratory reared Mediterranean fruit fly (Dipteta: Tephritidae). *Ann. Entomol. Soc. Am.* 82: 55-59.

Vargas, R.I.; Miyashi, D.; Nishida, T. (1984). Life history and demographic parameters of the laboratory-reared tephritids (Diptera: Tephritids). *Ann. Entomol. Soc. Am.,* 77: 651-656.

Vargas, R.I.; Walsh, E.B.; Armstrong, W.; Kanehisa, D.T. (1996). Survival and development of immature stages of four Hawaiian fruit fly (Dipteta: Tephritidae) at five constant temperatures. *Ann. Entomol. Soc. Am.,* 89: 64-69.

Vargas, R.I.; Walsh, E.B.; Kanehisa, D.T.; Armstrong, W. (1997). Demography of four Hawaiian fruit fly (Dipteta: Tephritidae) reared at five constant temperatures. *Ann. Entomol. Soc. Am.,* 90: 162-168.

Zucoloto, F.S. (1993). Acceptability of different Brazilian fruits to *Ceratitis capitata* (Diptera: Tephritidae) and fly performance on each species. *Brazilian J. Med. Biol. Res.,* 26: 291-298.

CHAPTER 4

Bio-ethological observation of *Ceratitis capitata* (Diptera: Tephritidae) in the Trentino region

Abstract

The Mediterranean Fruit fly (MFF), *Ceratitis capitata* (Wiedemann, 1824), is considered to be one of the most important fruit pests, as it attacks several cultivated species of high commercial value. Its phenology and population dynamics have been studied extensively in their native areas, but little in the temperate and colder areas recently covered by its geographical distribution.

In Trentino this pest was reported for the first time in 1990. It then reappeared in 2010 on a much broader scale, also reaching the northern part of the province.

This study investigates the population dynamics of the MFF in Trentino. The primary goals were to monitor the presence and seasonality of the MFF in this area, establishing the importance of different host fruits for population development and determining the maximum number of generations potentially occurring in Trentino.

This work is the first study of the bio-ethology of the MFF in an alpine context. In particular, the results showed that the MFF is a polyvoltine species and that generations can also overlap in this northern region. Oviposition starts as early as July, when the first females appear in the orchards. *C. capitata* completed three generations: a first generation in summer ripening fruit (peach), a second generation in early apple varieties and a third generation in later apple varieties. 2016 temperature data were used to validate the Tassan degree-day model, which used a development threshold of 9.7°C; according to our observations, the tested model could be used to describe the first generation life cycle, from eggs too adult, quite accurately.

Introduction

The Mediterranean fruit fly is one of the most important pests affecting several fruit crops of high commercial value (Mitchell and Saul, 1990; Liquido *et al.*, 1991). It can adapt to different climates and is widespread in most parts of the world (Hagen *et al.*, 1981; Fischer-Colbrie *et al.*, 1989; Kourti *et al.*, 1992; White *et al.*, 1992). Indeed, its high level of fecundity, polyphagy and polyvoltinism are the biological features that allow it to cause severe damage in a host plant range that includes more than >350 hosts (De Meyer *et al.*, 2002; Liquido *et al.*, 1991; Wemes, 1981; Rigamonti, 2004).

Most of the studies on the population dynamics of *C. capitata* have been carried out in tropical regions (Harris *et al.,* 1986, 1987, 1989, 1991, 1993; Eskafi *et al.,* 1990; Liquido *et al.,* 1990) and report that the abundance of the fly is influenced by the humidity rate and host availability. Few studies have been conducted in temperate areas such as the southern Greece (Harris, 1975; Papadopulus *et al.,* 1997; Israely *et al.,* 1997; Katsoyannos *et al.,* 1998). In these areas, host fruit is available all year round and the main factor regulating populations is the relatively low winter temperature. Indeed, in colder temperate areas of Europe, low winter temperature profoundly affects the phenology and population dynamics of *C. capitata*. In addition, unusually low temperatures in early spring following a mild winter may result in dramatically high mortality of the overwintering population (Katsoyannos *et al.,* 1998). As a consequence, in temperate areas the number of generations is lower than in the tropics (3-4 generations per year and 5 to 10 generations per year respectively, Rigamonti, 2004).

In Greece the number of adults caught in early summer is very low, increasing and giving rise to the following generations during the summer, when hosts are available and the temperature becomes favourable. In this area it is estimated that *C. capitata* arrives at four or five overlapping generations (Papadopoulos *et al.,* 1996; 1998; 2001a; 2001b.).

Other studies have shown that population development is closely associated with the host fruit species cultivated in the area. The rate of infestation and oviposition and the suitability of fruit for development, combined with the total biomass of fruit of each host species is of considerable importance (Vergas *et al.,* 1983; Katsoyannos *et al.,* 1998).

Papadopoulus *et al.,* (2001) showed that apricots maturing early in the season are very important for population development because they are the principal host on which adults from the overwintering generation oviposit first. Other important hosts during the season are peaches and figs, and later, in the autumn, pears and apples.

C. capitata phenology and population dynamics have been studied extensively in the native areas, but less in temperate and colder geographical areas which it has spread to recently (Papadopoulos *et al.,* 2001a.). In Trentino, in northern Italy, this pest was reported for the first time in 1990. It then reappeared in 2010 in a much more widespread manner, also reaching the northern part of the province (Dallago, 2013; Tait *et al.,* 2012).

This study aims to investigate the population dynamics of the MFF in Trentino, an area close to the northern limits of its distribution in Europe. The primary goals were to monitor the presence and seasonality of the MFF in this area, establishing the importance of different host fruit for population development. Furthermore, we aimed to determine the maximum number of generations that this pest can complete in Trentino orchards and to validate the Tassan degree-day model (Tassan *et al.,* 1982).

Material and methods

Pest monitoring. This study was conducted in the 2016 season in an area near Riva del Garda, in the southern part of the Trentino region, where the presence of the pest has been repeatedly detected at high levels in the last few years. The study was carried out in a 3 ha orchard where different host fruit crops are present: apricots (*Prunus armeniaca*, L.), peaches (*Prunus persicae*, L.), different apple varieties (*Malus sylvestris*, Mill.) such as Elstar, Golden Delicious, Jonagold, Florina and Fuji, and pears (*Pyrus communis* L.).

Observations of population dynamics were carried out in an untreated part of the orchard using monitoring traps and fruit sampling. When necessary, insecticide treatments against other pests, such us the codling moth (*Cydia pomonella* L.) or aphids, were applied to surrounding trees, but these treatments did not cause interference with Medfly development and dynamics.

Male and female adult flight was monitored with Decis® traps (Bayer Cropscience S.r.l., Milan, Italy) with 0.015 g deltamethrin/trap at the top, and baited with Unpack® Biolure (ammonium acetate 29.8 %, trimethylamine 12.4 % and putrescine 0.2 %) (Suterra, Europe Biocontrol LLC.). This trap is a plastic device made up of a yellow base and transparent lid with hanger and a bottom part with four holes (2.5 cm diameter) (Figure 4.1 A).

The 10 traps were installed on 15 June 2016, at a height of 1.5 m above the ground in a shaded part of the canopy of the host tree and were controlled weekly from June to the end of November (Figure 4.1 B). The bait was changed once during the season, because its life is guaranteed for 120 days. Traps were checked weekly during the season and the number of males and females was counted under a stereomicroscope. Moreover, the numbers of females with eggs in the abdomen and without eggs were determined. Weekly fruit sampling was performed to assess the percentage of damage. Fruit with damage was checked to determine larval stage development. The number of checked fruits ranged from 200 to 1000, according to the fruit tree species.

Fruit infestation. When the percentage of females with eggs in the abdomen was 100% and the first fruit damage was detected in orchards, newly emerged "wild strain" larvae were used to artificially infest the ripe fruit species present at that time in the field. The wild strain of insects comes from a colony established from insects collected in an apple orchard in 2015 and kept on an artificial diet in the laboratory (at FEM's Entomological Laboratory) in constant conditions (25 ± 1°C and 60 ± 5% R.U) (Cavalloro *et al.*, 1969). The eggs were placed in a Petri dish with agar at 0.8% to maintain humidity, and after 48h the new larvae emerged. The larvae were then collected with a small brush, and placed inside artificial holes made on the fruit with a pin. 12 fruits per species were infested for each infestation time, and each fruit received 10 newly emerged larvae.

The individual infested fruit was put in a box with paper on the bottom and the box was covered with gauze to guarantee adequate ventilation. The artificially infested fruits were placed in the field in natural conditions until the emergence of pupae and adults. The artificially infested fruits were checked weekly to monitor Medfly development and detect the emergence of pupae and adults in the box. When the adults emerged from this first infestation, a second artificial fruit infestation was initiated using the fruit of the host species ripe in the field at that time (Figure 4.5). The same procedure was repeated until no ripe fruit was available in the orchards, in order to establish the maximum number of generations that *C. capitata* can potentially complete in Trentino. Data collected in this trial were used to validate a Tassan degree-day model (Tassan *et al.*, 1982), which was expected to estimate the length of the life-cycles and the number of potential Medfly generations.

To run the model, we used the maximum and minimum daily temperatures provided by meteorological stations in Riva del Garda during the 2016 season and the minimum temperature threshold of 9.7 °C. Table 4.1 shows the predicted degree-day sum which is needed to achieve a certain development stage of *C. capitata* according to the Tassan degree-day model.

Table 4.1 Tassan degree-day model

	From eggs to pupae	From pupae to adult	Pre-oviposition period
DD (°D)	142.8	182.4	44.2
Total	325.2		44.2

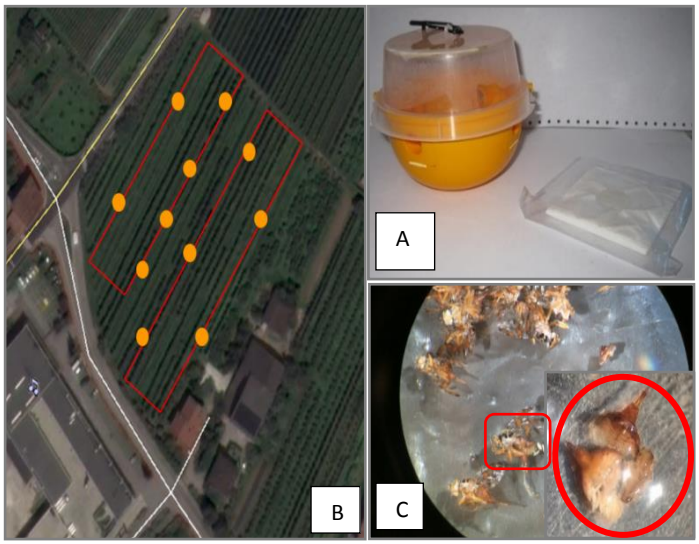


Figure 4.1 Trap distribution in the study area (A); Decis® Traps used for population monitoring (B); Checks on females under a stereomicroscope to check eggs inside the abdomen (C).

Results

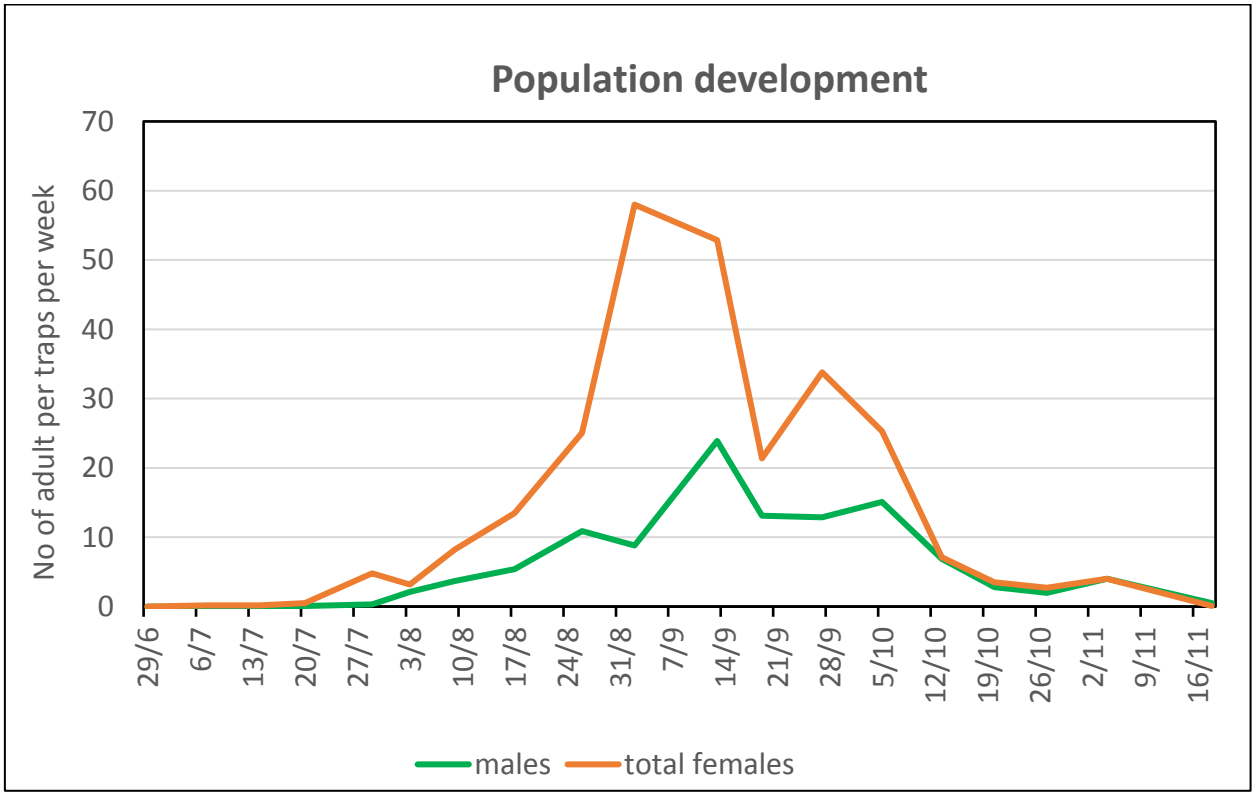


Figure 4.2 Seasonal pattern for C. capitata adult captures in Decis® traps in the Riva del Garda area (Trentino) in 2016

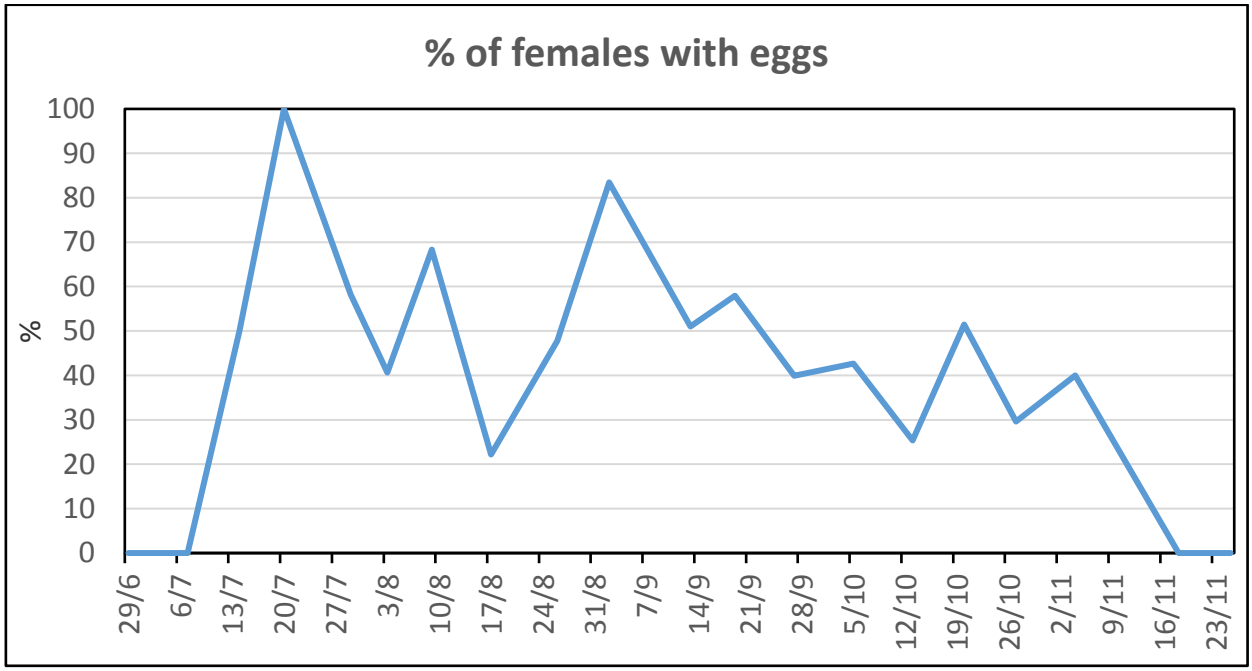


Figure 4.3 Checking of females, evaluation of females with eggs in the abdomen.

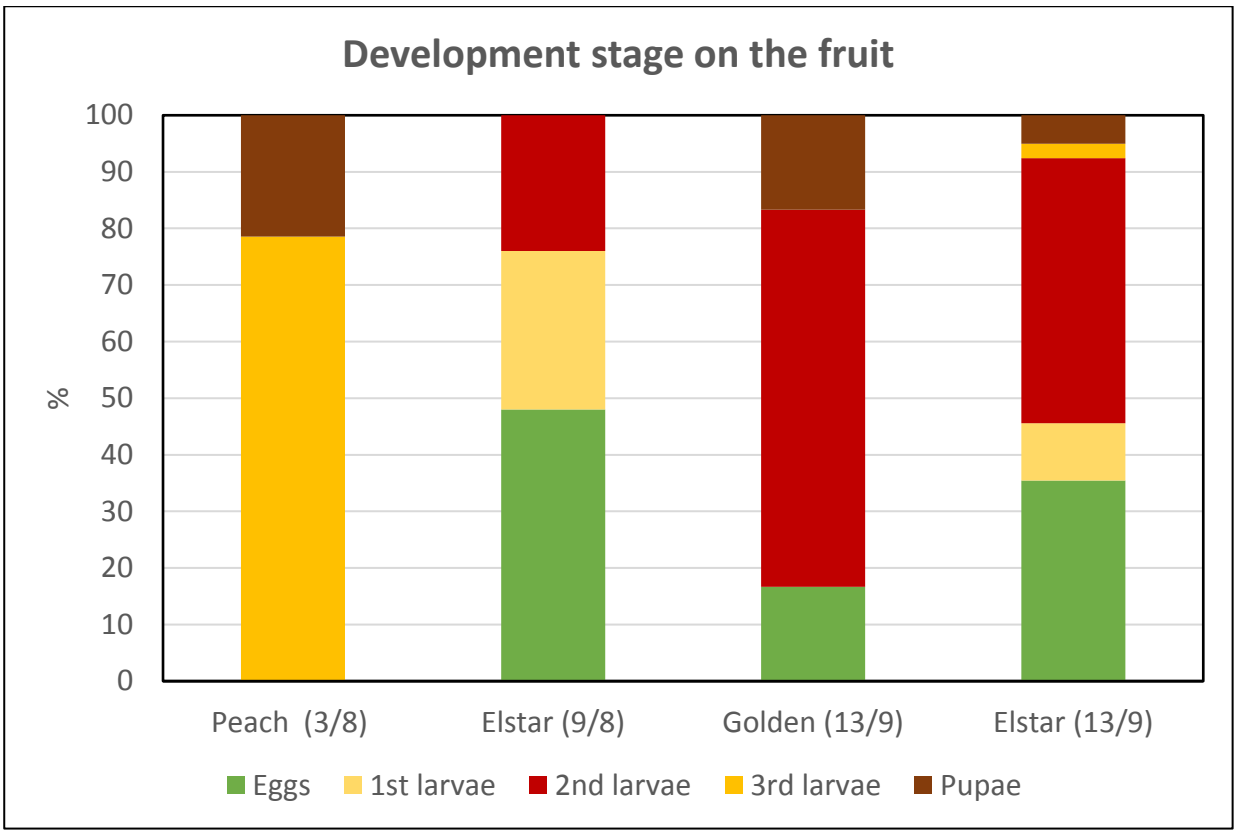


Figure 4.4 Seasonal trends for infestation of the most important C. capitata hosts in the Riva del Garda area (Trentino) in 2016. Percentage of different developmental insect stages in infested apples.

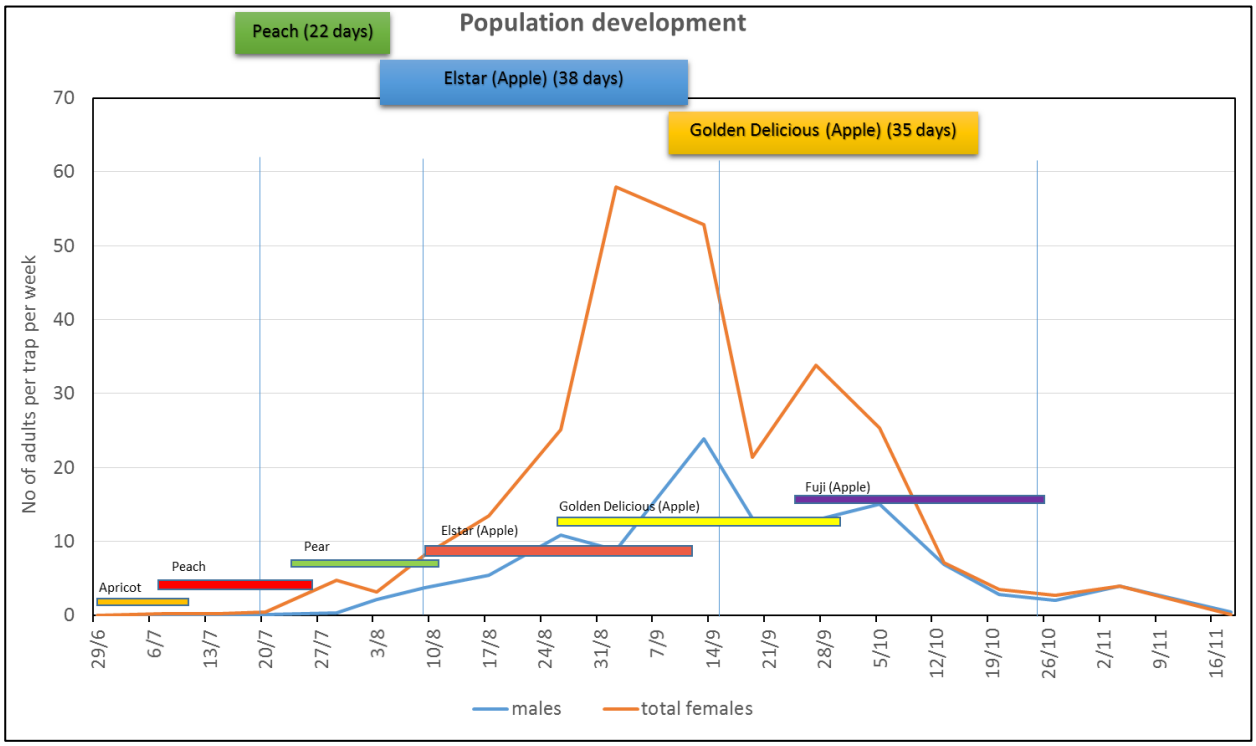


Figure 4.5 C. capitata phenology in the study area (Riva del Garda, Trento) in northern Italy. Horizontal bars indicate the ripening period of the most important fruit crops in this area. The vertical bars represent the developmental stage on different host fruits by 100% of females with eggs in the abdomen, in the early season (peaches), to the last adult emerging on Golden Delicious apples.

Table 4.2 Infestation data for various fruit crops collected during the 2016 season in the Riva del Garda area

Date	Host species	Total fruit collected (N°)	N° of fruits infested	Infested sample %	Developmental stage (N° of fruit)				
					Eggs	1st larva	2nd larva	3rd larva	Pupae
07-July	Apricot	1000	0	0	0	0	0	0	0
14-July	Peach	1000	0	0	0	0	0	0	0
20-July	Peach	1000	0	0	0	0	0	0	0
03-Aug	Peach	870	14	1.6	0	0	0	11	3
03-Aug	Pear	1000	0	0	0	0	0	0	0
09-Aug	Pear	300	0	0	0	0	0	0	0
09-Aug	Elstar (Apple)	1000	25	2.5	12	7	6	0	0
09-Aug	Golden Delicious (Apple)	1000	0	0	0	0	0	0	0
17-Aug	Elstar (Apple)	1000	30	3	0	9	21	0	0
17-Aug	Golden Delicious (Apple)	1000	0	0	0	0	0	0	0
26-Aug	Elstar (Apple)	500	20	4	0	7	8	3	2
26-Aug	Jonagold (Apple)	500	0	0	0	0	0	0	0
26-Aug	Florina (Apple)	500	0	0	0	0	0	0	0
26-Aug	Golden Delicious (Apple)	500	1	0.2	0	1	0	0	0
02-Sep	Elstar (Apple)	1000	52	5,2	0	20	15	10	7
02-Sep	Jonagold (Apple)	500	0	0	0	0	0	0	0
02-Sep	Florina (Apple)	500	0	0	0	0	0	0	0
02-Sep	Golden Delicious (Apple)	500	2	0.4	0	0	2	0	0
13-Sep	Golden Delicious (Apple)	1200	12	2,4	2	0	8	0	2
13-Sep	Elstar (Apple)	500	73	14.6	28	8	37	2	4
13-Sep	Jonagold (Apple)	1100	10	1	8	0	2	0	0
13-Sep	Florina (Apple)	1000	0	0	0	0	0	0	0
19-Sep	Jonagold (Apple)	500	4	0,8	1	1	2	0	0
19-Sep	Golden Delicious (Apple)	1000	6	0,6	3	1	2	0	0
19-Sep	Florina (Apple)	1000	0	0	0	0	0	0	0
27-Sep	Jonagold (Apple)	900	9	1	3	0	6	0	0
27-Sep	Golden Delicious (Apple)	716	5	0,7	2	3	0	0	0
05-Oct	Fuji (Apple)	316	0	0	0	0	0	0	0
05-Oct	Elstar (Apple)	200	135	67.5	14	25	32	46	18
13-Oct	Fuji (Apple)	300	0	0	0	0	0	0	0
20-Oct	Fuji (Apple)	210	0	0	0	0	0	0	0
27-Oct	Fuji (Apple)	500	0	0	0	0	0	0	0

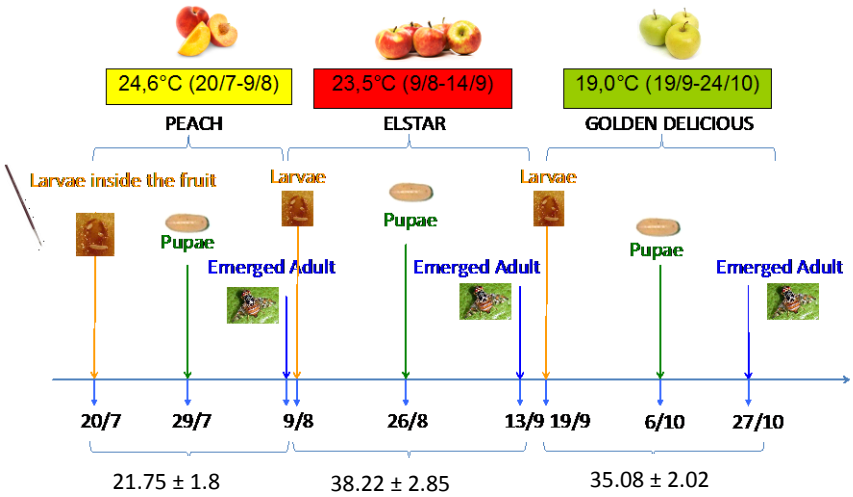
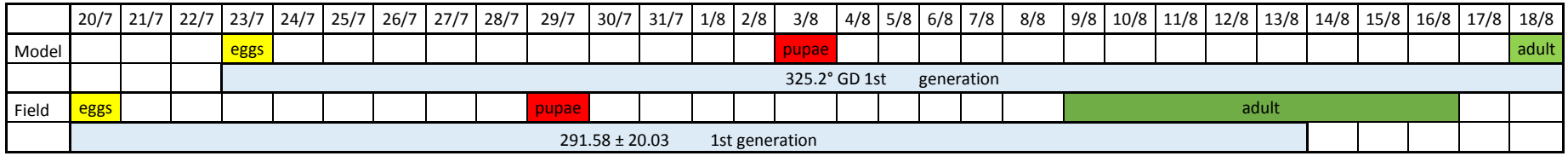


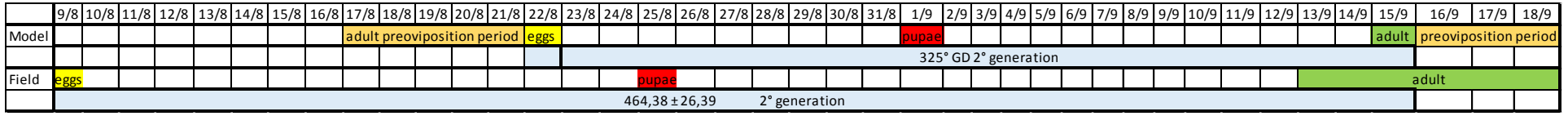
Figure 4.6 Method and development times for artificial fruit infestation

1st generation



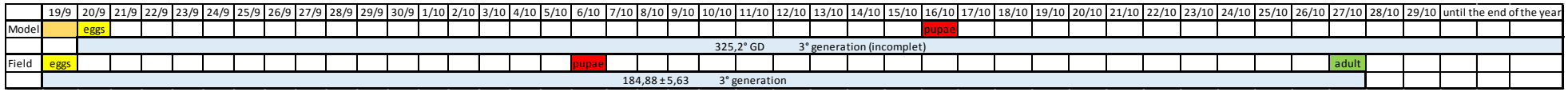
	Eggs	Pupae	Adult	Eggs_Adult
Model	0	142.8	182.4	325.2
Field	0	127.8 ± 0	163.78 ± 20	291.58 ± 20.03
Δ DD	0	15	18.22	33.22

2nd generation



	Eggs	Pupae	Adult	Eggs_Adult
Model	0	142.8	182.4	325.2
Field	0	284.18 ± 28.9	180.2 ± 25	464.38 ± 26.39
Δ DD	0	-141.38	1.8	-139.58

3rd generation



	Eggs	Pupae	Adult	Eggs_Adult
Model	0	142,8	182.4	325.2
Field	0	134.9 ± 4.5	49.98 ± 5.05	184.88 ± 5.63
Δ DD	0	7.9	132.02	140.32

Figure 4.7 Generation graph with respective table providing the average degree-day data (± SD) in the life cycle with the Tassan model

The first female capture was recorded on 7 July 2016 and the first male capture was two weeks later on 20 July 2016 (Figure 4.2). Weekly control of females' abdomens showed that 50% and 100% of females with eggs were recorded on 14 July and 20 July respectively (Figure 4.3).
Overall, the adult population was low in June, July, October and November, moderate in the first week of August and at the end of September, and much higher in August and September (Figure 4.2).
When the percentage of females with eggs reached 100% and the first infested fruit was detected in the fiel (Figure 4.3), newly emerged "wild strain" larvae were used to artificially infest ripe fruit species present at that time in the field. As the first ripening fruit present in the field when 100% of females were gravid was the peach, we infested peaches with "wild strain" larvae. In the peach, the Medfly took 22 ± 1.8 days to complete its development. When adults emerged from artificially infested peaches, on 9 August 2016, Elstar apples (which were ripe) were infested. In this case, *C. capitata* completed its cycle in 38 ± 2.85 days. When this second batch of adults emerged, on 19 September 2016, Golden Delicious fruit were infested and the insect took 35 ± 2.02 days to complete its development from newly emerged larvae to adults. No further artificial infestations were possible at this time in the season because all the fruit in the field had been harvested.
According to the weekly fruit sampling carried out during the season on the different species present in the field (Figure 4.2), the first infested fruits were detected on peach trees on 3 August, when 78% of damaged fruit contained larvae at the third developmental stage and 22% contained pupae.
This suggests that the first generation of Medfly developed entirely on peaches and that larval development was completed by early August. The following week, on 9 August, 2.5% of Elstar apples sampled were infested; as 76% of the damaged fruit contained eggs and the first larval stage, we can infer that *C. capitata* began a new generation on Elstar at this point (Figure 4.4). From this time on, the percentage of fruit damage in Elstar increased; on 13 September we detected 15.8% of damaged fruit and for the most part they contained eggs and the 2nd larval stage. At the same time, 2.4% of Golden Delicious apples were also infested and in this case 67% again contained the 2nd larval stage. No infested Fuji fruit was found.
The Medfly larvae used to artificially infest peaches at the beginning of the season took 291.58° DD (± 20.03) to complete their life-cycle to the adult stage, 33.22° DD less than the Tassan degree day-model (Tassan *et al.*, 1982) estimated would be needed from egg to adult. This discrepancy corresponds to about 3 days.
The second generation, developing on the apple, needed 464.38° D (± 26.39) to develop from egg to adult, meaning about seven days more than expected according to the degree-day model.

As regards the third generation, larvae used to infest apples took 184.88° D (± 5.63) to reach the adult stage at the end of October. According to the Tassan model, the third generation would have been stopped its development at the pupae stage and no adult would have emerged (Figure 4.7).

Discussion and conclusion

The results of this study demonstrate a clear and distinct seasonal evolution of the *C. capitata* population in southern Trentino (Italy) during the 2016 season.

Adult activity started in the first week of July. The population density was very low during June and July and increased in August. Decis® traps baited with food attractant identified a population peak in September. After that, adult activity decreased in October and November.

The population dynamics are similar to those reported in other studies conducted in Spain and northern Greece (Escudero *et al.,* 2008; Papadopoulos *et al.,* 2001).

At the beginning of the season, they did not find any infestation in apricots, probably because the population density was very low due to the small number of adults emerging from overwintering (Papadopoulus *et al.,* 1996). The presence of the Mediterranean fruit fly in summer caused damage in early susceptible host fruits, such as the peach. These early ripening fruits are very important for the build-up of population density because they constitute a suitable host available for oviposition by adults coming from the overwintering generation. (if overwintering at the larval stage inside the apples, Papadopoulus *et al.,* 1996; 1998).

This was confirmed by peach sampling in the field in the first week of August, which detected mature larvae and pupae inside the checked fruit, and also by the results for artificially infested fruit, from which adults emerged at the same time, 22 days after infestation. Adults from this first generation were able to find early apple varieties (such us Elstar) at ripening time. The Medfly oviposited the second generation in Elstar apples, the eggs developing into adults which could later oviposit on Golden Delicious apples to complete the third generation.

When the first generation developed inside the peach, it took 291.58° D (from egg to adult), 3 days less than expected according to the Tassan degree-days model. We could say that the model provides a good prediction of development time for the first generation. This was not true for the following generation, for which the model predicted development of the second generation one week earlier and provided incorrect information about the improbability of developing a third generation. Indeed, according to the Tassan degree-days model, the last generation would have stopped at the pupal stage and would not have been able to complete its life-cycle in the field where the observations were carried out. The life-cycle estimated by the model and the larval development observed in the field with artificially infested fruits

overlapped during the first generation in the 2016 season, but this not was the case for the following generations. This could be due to the different food sources available to the larvae. Other studies have reported that different food sources made different contributions to Medfly population growth (Papadopoulos *et al.,* 2002; Navarro-Campos *et al.,* 2011).

In conclusion, we estimate that *C. capitata* can complete a maximum of three generations in Trentino region.

This is in accordance with Papadopoulos *et al.*, (2001), who conducted observations in northern Greece, in an area considered near the northern European limit of Mediterranean fruit fly distribution (Fischer-Colbrie and Busch-Petersen, 1989). He estimated that the MFF is able to develop four to five generations, of which two or three complete generations. Other studies carried out in northern Italy (Lombardia) have reported that *Ceratitis capitata* are able to complete three-four generations per year (Rigamonti, 2004). Despite not being a favourable host for larval development (Carey 1984, Krainacker *et al.*, 1987, Zucoloto 1993, Papadopoulus 1990), the apple is reported to be crucial for overwintering of the pest. Inside apples the larvae are more resistant to unfavourable winter conditions (Papadopoulus *et al*., 1996; 1998; Escudero *et al*., 2008). The role of apples in relation to the overwintering ability of *C. capitata* should be investigated in more depth with additional studies.

References

Carey, R.J. (1984). Host-specific demographic studies of the Mediterranean fruit fly *Ceratitis capitata*. *Ecol. Entomol*., 9: 261-270.

Cavalloro. R.; Girolami. V. (1969). Miglioramento nell'allevamento in massa di *Ceratitis capitata* Wied. (Diptera: Trypetidae). Redia. LI: 315-327.

Dallago, G. (2013). La mosca mediterranea della frutta: una presenza ormai costante. *Rapporto 2013 Centro di Trasferimento Tecnologico* pp. 47-48.

De Meyer, M.; Copeland, R.S.; Wharton, R.A.; McPheron, B.A. (2002). On the geographic origin of the Medfly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). Pages 45-53. In: Proceedings of the *International Fruit Fly Symposium*. (Barnes B.N., Editor) May 6- 10, 2002, Stellenbosch, South Africa.

Escudero-Colomar, L.A.; Vilajeliu, M.; Batllori, L. (2008). Seasonality in the occurrence of the Mediterranean fruit fly (*C. capitata*) (Wied.) in the north-east of Spain. *J. Appl. Entomol.*, 132: 714-721.

Eskafi, F. M.; Kolbe, M.E. (1990). Infestation pattern of commonly cultivated, edible fruit species by Ceratitis capitata and *Anastrepha* spp. (Diptera: Tephritidae) in Guatemala and their relationship to environmental factors. *Environ. Entomol.*, 19: 1371-1380.

Fischer-Colbrie, P.; Busch-Petersen, E. (1989). Temperate Europe and West Africa, pp. 91-99. In Robinson, A.S. and Hooper, G. *Fruit flies: their biology, natural enemies and control.* Vol. 3A. Elsevier, Amsterdam.

Hagen, K.S.; William, W.; Tassan, R.L. (1981). Mediterranean fruit fly. The worst may be yet to come. *Calif. Agric.* March-April: 5-7.

Harris, E.J.; Ford-Livene, C.; Lee, C.Y.L. (1989). Population monitoring of Tephritidae fruit flies by stratified sampling on the island of Oahu, Hawaii, pp. 342-352. In Vijaysegaran, S. and Ibrahim, A. G. [eds.], Fruit Flies in the Tropics: Proceedings of *the First International Symposium*, Kuala Lumpur, 14-16 March 1988. Malaysian Agricultural Research and Development Institute, Kuala Lumpur.

Harris, E.J.; Lee, C.Y.L. (1986). Seasonal and annual occurrence of Mediterranean fruit flies (Diptera; Tephritidae) in Makaha and Waianae Valleys, Oahu, Hawaii. *Envir. Entomol.* 15: 507-512.

Harris, E.J.; Lee, C.Y.L. (1987). Seasonal and annual distribution of Mediterranean fruit fly (Diptera; Tephritidae) in Honolulu and suburban areas of Oahu, Hawaii. *Envir. Entomol.*, 15: 507-512.

Harris, E.J.; Lee, C.Y.L. (1989). Development of Ceratitis capitata (Diptera; Tephritidae) in coffee in wet and dry areas. *Envir. Entomol.*, 18: 1042-1049.

Harris, E.J. (1975). Biotic factors influencing population trends of the Mediterranean fruit fly; *Ceratitis capitata*, (Wiedemann), in selected host habitat in Tunisia. Ph.D. dissertation, University of Hawaii, Hawaii.

Harris, E.J.; Olalquiaga. (1991). Occurrence and distribution patterns of Mediterranean fruit fly (Diptera: Tephritidae) in desert areas in Chile and Peru. *Environ. Entomol.*, 20: 174-178.

Harris, E.J.; Vargas, R.I.; Gilmore J.E. (1993). Seasonality and occurrence distribution of Mediterranean fruit fly (Diptera: Tephritidae) in upland and lowland areas on Kauai, Hawaii. *Environ. Entomol.*, 22: 404-410.

Israely, N.; Yuval, B.; Kitron, U.; Nestel, D. (1997). Population fluctuations of adult Mediterranean fruit flies (Diptera: Tephritidae) in a Mediterranean heterogeneous agricultural region. *Environ. Entomol.,* 26: 1263-1269.

Katsoyannos, B.I.; Koulossis, N.A.; Carey, J.R. (1998). Seasonal and annual occurrence of Mediterranean fruit fly (Diptera: Tephritidae) on Chios island: differences between two neighboring citrus orchard. *Ann. Entomol. Soc. Am*., 91: 43-51.

Kourti, A.; Loukas, M.; Sourdis, J. (1992). Dispersion pattern on the medfly from its geographic center of the origin and genetics relationships of the medfly with two close relatives. *Entomol. Exp. Appl.* 63: 63-69.

Krainacker, D.A.; Carey, J.R.; Vargas, J. (1987). Effect of larval host on life history traits of the Mediterranean fruit fly, *Ceratitis capitata*. *Oecologia* (Berl.) 73: 583-590.

Liquido, N.J., Shinoda, L.A., Cunningham R.T. (1991). Host plants of Mediterranean fruit fly: an annotated world review. *Ann. Entomol. Soc. Am*., 77: 1-52.

Liquido, N.J.; Cunningham, R.T.; Nakagawa, S. (1990). Host plant of Mediterranean fruit fly (Diptera: Thepritidae) on the island of Hawaii (1949-1985 Survey). *J. Econ. Entomol.,* 83: 1863-1878.

Mitchell, A.C.; Saul, S.H. (1990). Current control methods for the Mediterranean fruit fly, Ceratitis capitata, and their applications in the USA. *Rev. Agric. Entomol.,* 78: 923-940.

Navarro-Campos, C.; Martinez-Ferrer, M.T.; Campos, J.M.; Fibla, J.M; Alcaide, J.; Bargues, L.; Marzal, C.; Garcia-Marì, F. (2011). The influence of host fruit and temperature on the body size of adult Ceratitis capitata (Diptera: Tephritidae) under laboratory and field conditions. Enviromental entomology pp. 931-938.

Papadopoulos, N. T.; Carey, J. R.; Katsoyannos, B. I.; Kouloussis, N. A. 1996. Overwintering of the Mediterranean fruit fly (Diptera: Tephritidae) in northern Greece. *Ann. Entomol. Soc. Am.,* 89, 526-534.

Papadopoulos, N. T.; Katsoyannos, B. I.; Carey, J. R.; 1998. Temporal changes in the composition of the overwintering larval population of the Mediterranean fruit fly (Diptera: Tephritidae) in northern Greece. *Ann. Entomol. Soc. Am.,* 91, 430-434.

Papadopoulos, N. T.; Katsoyannos, B. I.; Carey, J. R.; Kouloussis, N. A. 2001a. Seasonal and annual occurrence of the Mediterranean fruit fly (Diptera: Tephritidae) in northern Greece. *Ann. Entomol. Soc. Am.*, 94, 41-50.

Papadopoulos, N. T.; Katsoyannos, B. I.; Kouloussis, N. A.; Hendrichs, J.; Carey, J. R.; Heath, R.R. 2001b. Early detection and population monitoring of Ceratitis capitata (Diptera: Tephritidae) in a mixed-fruit orchard in northern Greece. *J. Econ. Entomol.*, 94, 971-978.

Papadopoulus, N. T. 1999. Study on the biology and ecology of the Mediterranean fruit fly Ceratitis capitata (Diptera: Tephritidae) in northern Greece. Ph.D. dissertation, Aristotle University of Thessaloniki, Thessaloniki, Greece.

Papadopoulus, N. T.; Katsoyannos, B. I. 2002. Development of *Ceratitis capitata* (Diptera: Tephritidae) in three apples varieties in the laboratory. Proceeding of 6th International Fruit Symposium. Pp. 19-22.

Rigamonti I. E. 2004. Contribution of knowledge of *Ceratitis capitata* Wied. (Diptera, Thepritidae) in Northern Italy. Observation on the biology. *Boll. Zool. Agr. Bachic. Ser.* II 36,89-100.

Tait, P.; Pellegrini, F.; Zaffoni, M.; Morten, M. 2012. Il ritorno della *Ceratitis capitata* nei frutteti trentini. Rapporto 2012 Centro di Trasferimento Tecnologico, pp. 31-34, Fondazione Edmund Mach.

Tassan, R. L.; Hagen, K. S.; Cheng, A.; Palmer, T. K.; Feliciano, G.; Bough, T. L. 1982. Mediterranean fruit fly life cycle estimations for the California eradication program. pp. 564-570 In Cavalloro R [ed.], Fruit Flies of Economic Importance. Proceedings of the CEC/IOBC International Symposium, Athens. Balkema, Rotterdam, The Netherlands.

Vergas, R. I.; Harris, J.; Nishida, T. 1983. Distribution and seasonal occurrence of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) on the island of Kauai in the Hawaii islands. *Environ. Entomol.* 12: 303-310.

Weems, H. V. Jr. 1981. Mediterranean fruit fly, *Ceratitis capitata* (Wiedman) (Diptera; Tephritidae). Entomology Circular No.230. Florida department of agriculture and consumer service, division of Plant Industry, Gainesville, FL, USA 1-12.

White, I. M.; Elson-Harris, M. M. 1992. Fruit flies of economic significance: Their identification and bionomics. Wallingford, Oxon, CAB International.

Zucoloto, F. S. 1993. Acceptability of different Brazilian fruits to *Ceratitis capitata* (Diptera: Tephritidae) and fly performance on each species. *Brazilian J. Med. Biol. Res.*, 26: 291-298.

CHAPTER 5

Survival of wild larvae and overwintering of pupa and adult stages of *Ceratitis capitata* under natural winter conditions in the Trentino region

Abstract

Overwintering by the Mediterranean fruit fly (Medfly) *Ceratitis capitata* (Wiedmann) at the northern limit of its geographical distribution has not yet been reported. The aim of this work was to study which developmental stages are able to survive in winter in the Trentino area, located in northern Italy (46th parallel north).

The experiments were carried out both in the field, where larvae and pupae were exposed to natural winter conditions, and in a shelter (warehouse), where adults are expected to be exposed to less extreme temperatures.

The Medfly was shown to be unable to overwinter at the pupal stage in the soil. The larvae inside fruit slowed down their development rate during the winter and were able to pupate, but not to give birth to adults. Adults of *C. capitata* kept in a sheltered environment were unable to survive the winter, even though they slowed down their activity compared to those kept in a climate chamber at 25 °C. The slowdown in activity allowed the adults in the warehouse to live longer than the control.

Introduction

The African *Ceratitis capitata* population do not have a diapause, but populations living in areas far from optimal conditions can adapt to winter conditions by suspending metabolic activity. This insect is indeed able to survive in different developmental stages in relation to the different winter conditions (Rigamonti, 2005).

There are two different hypotheses that can explain the presence of new populations of the Mediterranean fruit fly in spring. One is annual re-infestation from imported infested fruit in the area through trade (Romani, 1997; Israely *et al.*, 2004). This hypothesis has been mentioned for different areas including the Balearic Islands (Miranda *et al.*, 2001), the central mountains of Israel (Israely *et al.*, 2004; Israely *et al.*, 2005), and southern France (Cayol and Causse, 1993). The second theory is the ability of *C. capitata* to overwinter in temperate climates at low temperatures (Carey *et al.*, 1991), as already hypothesized for Medfly populations in central Italy (Sciarretta *et al.*, 2009).

The Medfly has been observed to overwinter as larvae inside fruit even at below zero temperatures in northern Greece (Papadopoulus *et al.*, 1996) and inside late-ripening varieties of orange in north-eastern Spain (Martinez-Ferrer *et al.*, 2006).

In southern Italy, the pest seems able to overwinter as adults (Fiamini, 1989) and larvae inside infested *Citrus* spp. fruits (Cirio *et al.*, 1972). According to Bass (1959), the fly overwinters in the pupal or adult stage in some countries in Central Europe, such as Germany, where it has been introduced and causes occasional damage in fruit when the climatic conditions are favourable. In northern Italy, adults have been shown to overwinter in sheltered parts of indoor environments (Rigamonti *et al.*, 2002; Rigamonti, 2004).

This ability to withstand low temperatures is probably an effect of the strong selective pressure on the small percentage of insects that can survive in these conditions (Papadopoulus, 1996).

On the other hand, Medfly distribution appears ultimately to be restricted by the severity of the winter, and climatic factors can limit or regulate the population dynamics of the species. In particular, the combination of different factors such as drought, temperature and duration of exposure could explain low pest incidence (Vera *et al.*, 2002).

In a study on the geographical distribution of the Medfly in different areas using the CLIMEX program, the main factor limiting Medfly survival in winter was found to be cold stress. In particular, even though the program did not exclude the lethal effect of low temperature, extreme temperatures appeared to be less restrictive to distribution than the limitation imposed by the need for thermal accumulation in winter (Vera *et al.*, 2002).

The most temperature-sensitive stage is the pupa, at which stage temperature can directly affect survival, or play an indirect role, reducing adult activity due to the thermal stress suffered in the previous stage (Segura *et al.*, 2004).

Papadopoulos *et al.* (1996) demonstrated that apples are an important host fruit that lasts through the winter in a condition good enough to provide a suitable refuge for larvae, favour slow growth and protect from natural enemies.

The aim of this study was to investigate which stages (larva, pupa and adult) of the Medfly are able to overwinter in northern Italy in natural conditions. Three different experiments were designed for this purpose: (a) assessment of the survival rate of pupae emerging after the larval stage inside apples in winter conditions; (b) estimation of the survival rate of pupae, and (c) of adults of *C. capitata* reared on infested apples in natural conditions and placed in winter conditions during the 2016 season.

Material and methods

Insects. The different insect stages were obtained from naturally infested apples kept in natural climatic conditions in a 3 m high plastic tunnel without lateral walls while being sheltered from the rain. Infested apples were collected in the field in autumn and stored in Plexiglas cages (100 x 100 x 100 cm) (Figure 5.1). The apples, placed on metal grids, were put in plastic trays containing sand to allow pupation of mature larval stages emerging from the fruit and to hold exudate dripping from rotting fruit.

Figure 5.1 Naturally infested apples placed in external conditions and covered by cages. The cages were placed in the plastic tunnel without lateral walls.

Overwintering of pupae. Every week from October to November pupae were collected from the sand, placed in a box and kept in natural conditions.

On 30 November 2016 the pupae were placed in perforated plastic containers to ensure adequate aeration, avoiding the entrance of ants or other insects. The pupae were covered with sand in the containers box to maintain suitable humidity. These small plastic boxes were placed 5 cm under the soil in the field and covered with a plastic tarpaulin to protect the insects from rain and snow (Figure 5.2). At the end of the experiment, 5 boxes with 40 pupae each were set up, for a total of 200 pupae. Simultaneously, the same experiment with the same number of pupae was set up in controlled conditions at 1 ± 5°C.

The threshold for Medfly pupal development is reported to be 11.2 ºC (Duyck and Quilici, 2002), and in this experiment, meteorological data were used to calculate the number of hours during which the temperature was below this threshold, called "cold hours below 11.2 ºC" (DAR, 2010).

Every month, one of the boxes with 40 pupae from both field and laboratory conditions was transferred to a climatic chamber at 25 °C to check adult emergence. They were kept there until 8 May, when the last inspection took place. During the experiment, the temperature (°C) and relative humidity (% R.H.) were recorded hourly with a data-logger using a sensor in the soil at a depth of 5 cm (Theodor Friedrichs Combilog 1020 datalogger with a Decagon 10HS soil moisture sensor and a Pt100 Nesa soil temperature sensor).

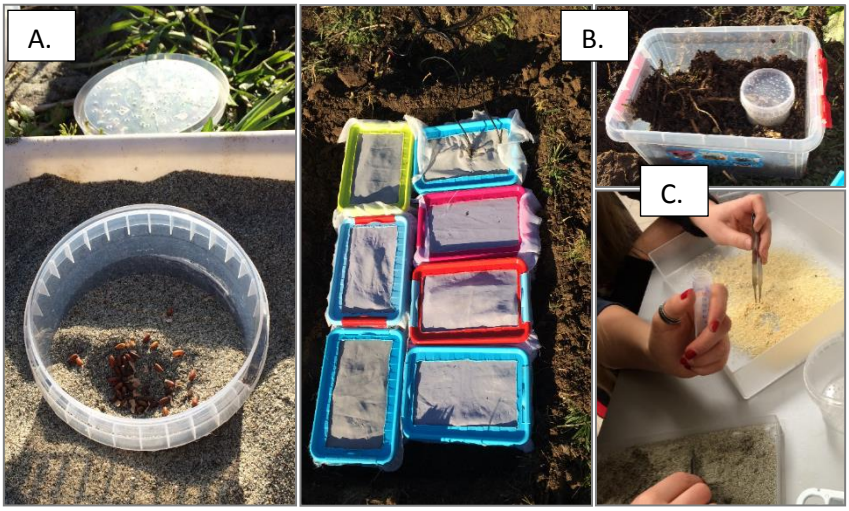


Figure 5.2 Overwintering experiment with pupae: A) Pupae from naturally infested apples; B) Trial set-up; C) Checking of pupae mortality.

Overwintering of larvae inside the fruit. To study the ability of larvae to overwinter in infested fruit falling from trees, 165 infested "Golden Delicious" apples were collected on 8 November 2016 in the orchard and placed on metal grids in three Plexiglas cages (100 x 100 x 100 cm). (N=55 with three repetitions). The metal grids were placed on a layer of sand to allow pupation of mature larval instars leaving the fruit and to collect exudate dripping from rotting fruit.

The infested apples were kept in natural conditions until 16 June 2017, when the sand was examined to check the pupae.

The temperature (°C) was monitored hourly from the beginning of the experiment to the last adult's death with a Marconi SPY data-logger and Sirius storage software. As a control, 25 apples were placed in a climatic chamber at 25 °C on 8 November 2016. Each apple used for the control was placed in a plastic box with cellulose absorbent paper and covered with gauze. The number of pupae was checked and counted daily.

Figure 5.3 A) Naturally infested apples after the winter; B)Sand containing pupae during checking; C) Checking for the presence of pupae

Overwintering of adults. Adults emerging from naturally infested fruit were kept in natural conditions in a cage. On 1 November 2016, 160 five to ten-day-old adults were divided into four groups of 40 adults (20 males and 20 females) and put into four cages (15 x 15 x 15 cm) closed with gauze and provided with water and food (sugar and yeast hydrolysate, 4:1) *ad libitum*. The cages were positioned inside a warehouse to simulate one of the potential locations where adults can survive naturally during the winter. One cage was positioned in a climatic chamber with constant conditions (25 ± 1°C) as a control. Mortality was recorded weekly from 1 November until all the insects died, and the dead adults were counted and removed. The temperature (°C) was monitored hourly from the beginning until the death of the last adult, using a Marconi SPY data-logger and Sirius storage software.

Statistical analysis. The mean and maximum number of days for which adults survived was calculated. Survival analysis was carried out using the Kaplan-Meier methodology, i.e. by obtaining estimates of mean survival and the standard error. Comparison of survival curves was carried out using statistics comparing the equality of survival distribution (Chi-squared with a significance level of 0.05, used with Log-rank evidence), and comparing the different treatments two by two. Thus, the hypotheses were:

H_0: both survival distributions are equal;

H_1: the two survival distributions are different.

This statistical analysis was carried out using Past v3.17 software.

Results

Overwintering of pupae. Analysis of climatic data showed that, at five centimeteres below the surface. The soil conditions during the pupae trial showed temperatures below 0 °C from 30 December (-0.32 ± 0.60 °C) to 2 February (-0.16 ± 0.70 °C), associated with several days with low humidity (from 12 to 23 January, ranging from a minimum of 2.55% R.H. to a maximum of 6.98% R.H.) (Figure 5.4).

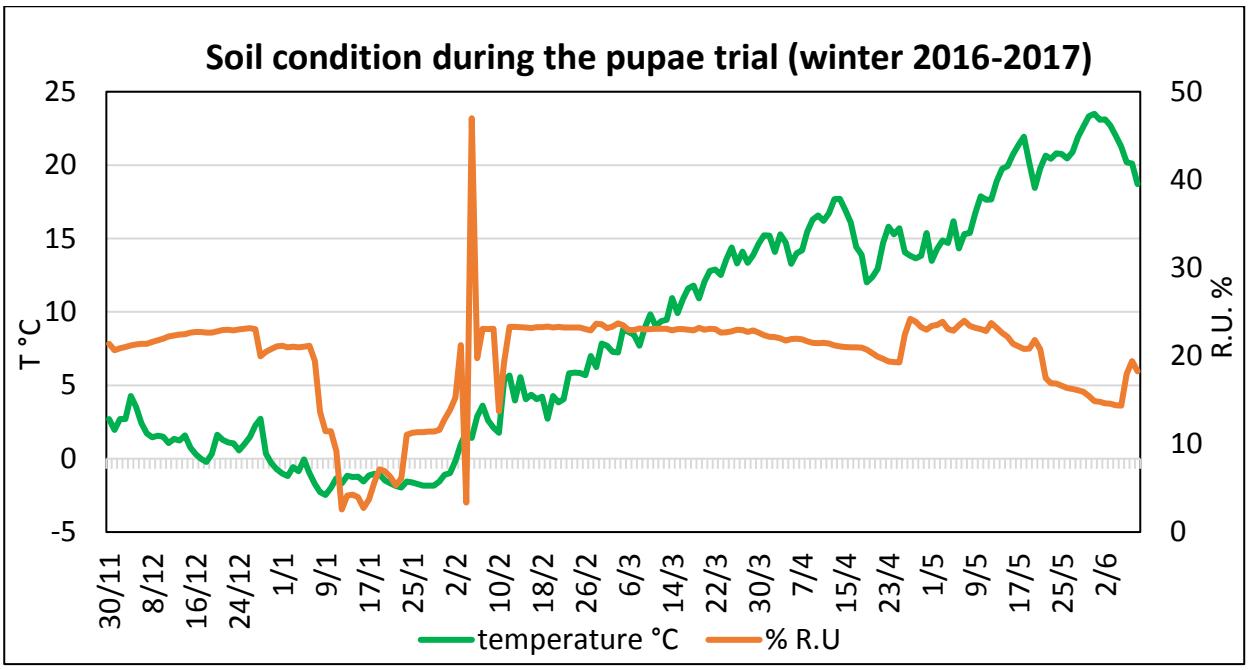


Figure 5.4 Soil conditions (temperature and humidity) during the pupae trial (winter 2016-2017).

There were 2,516 cold hours (equivalent to 105 days or 3.5 months) below the threshold for pupae development (11.2 ºC) at a depth of 5 cm below the surface during the 2016-2017 winter season (Figure 5.5).

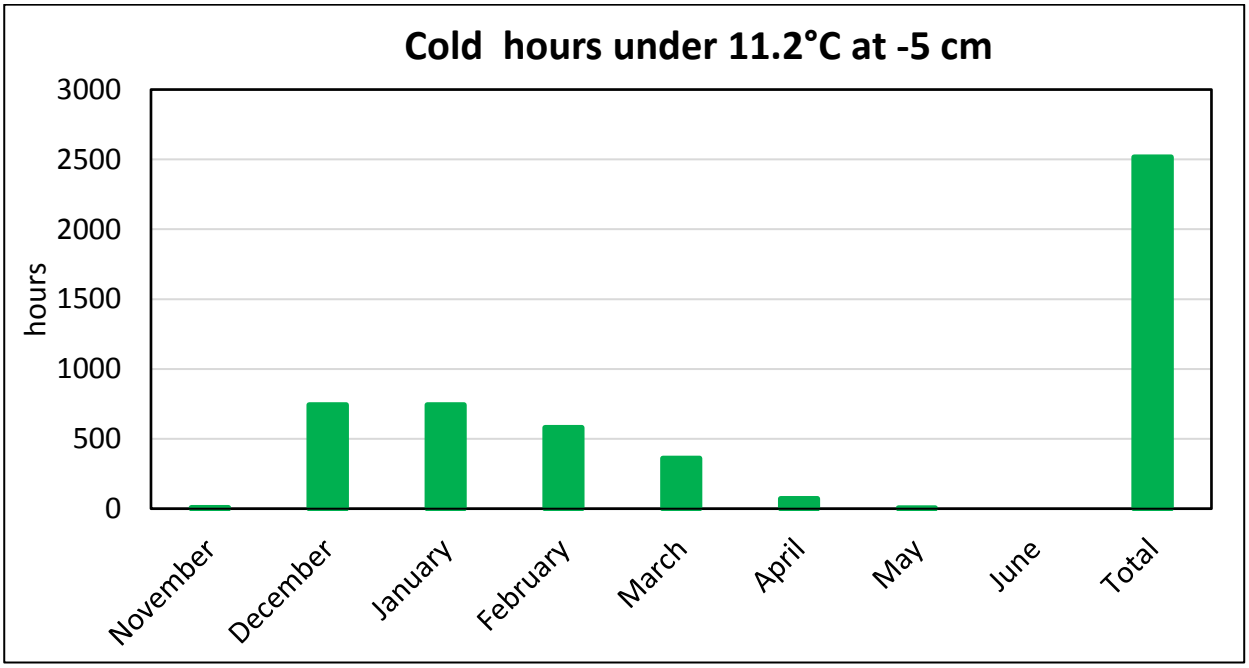


Figure 5.5 Cold hours under 11.2°C at -5 cm in the Trentino area during the pupae trial

There were 891 cold hours (below 0 °C) at a depth of 5 cm below the ground (equivalent to 37 days or 1.2 months) during the 2016-2017 winter season (Figure 5.6).

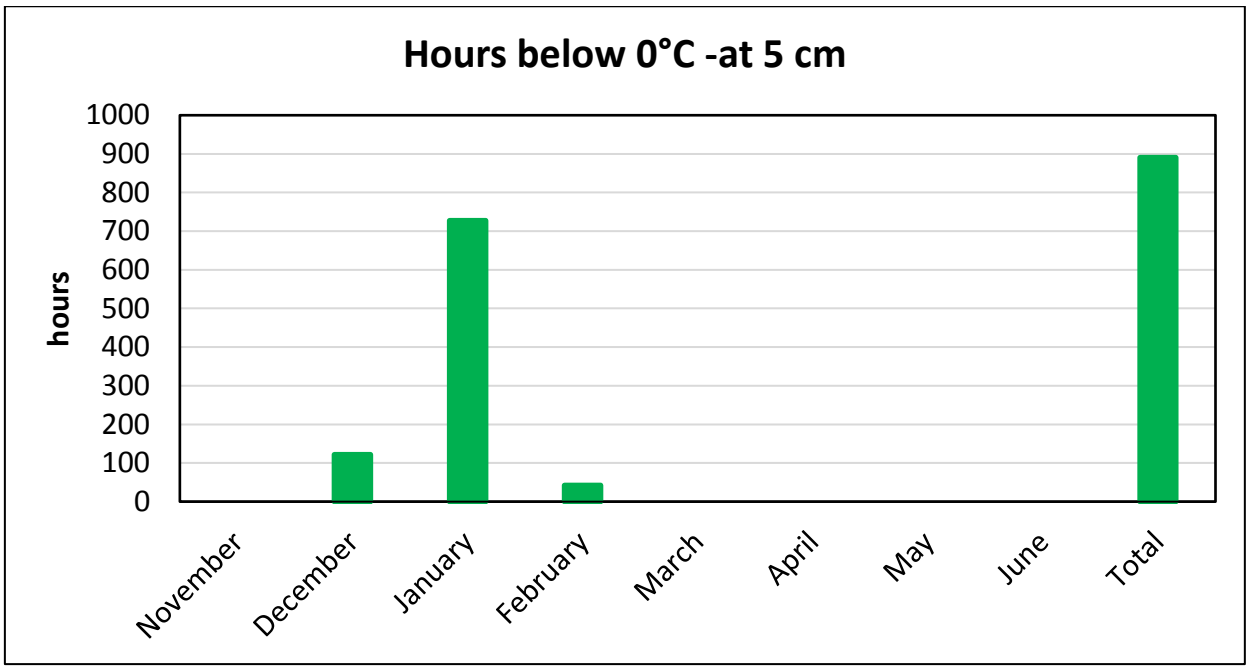


Figure 5.6 Cold hours at -5 cm- below 0 °C in the Trentino area during the pupae trial

No adults emerged from pupae during the experiment. After the eight months of study during the 2016-2017winter season, pupal survival in natural conditions was zero.

Overwintering of larvae in fruit. The 165 naturally infested fruits showed a mean number of 1.17 ± 0.41 (S.D.) oviposition punctures/apple.

The temperature was lower than 0 °C from 4 December to 29 January 2016, with a minimum temperature on 22 December (-5.32 °C) (Figure 5.7).

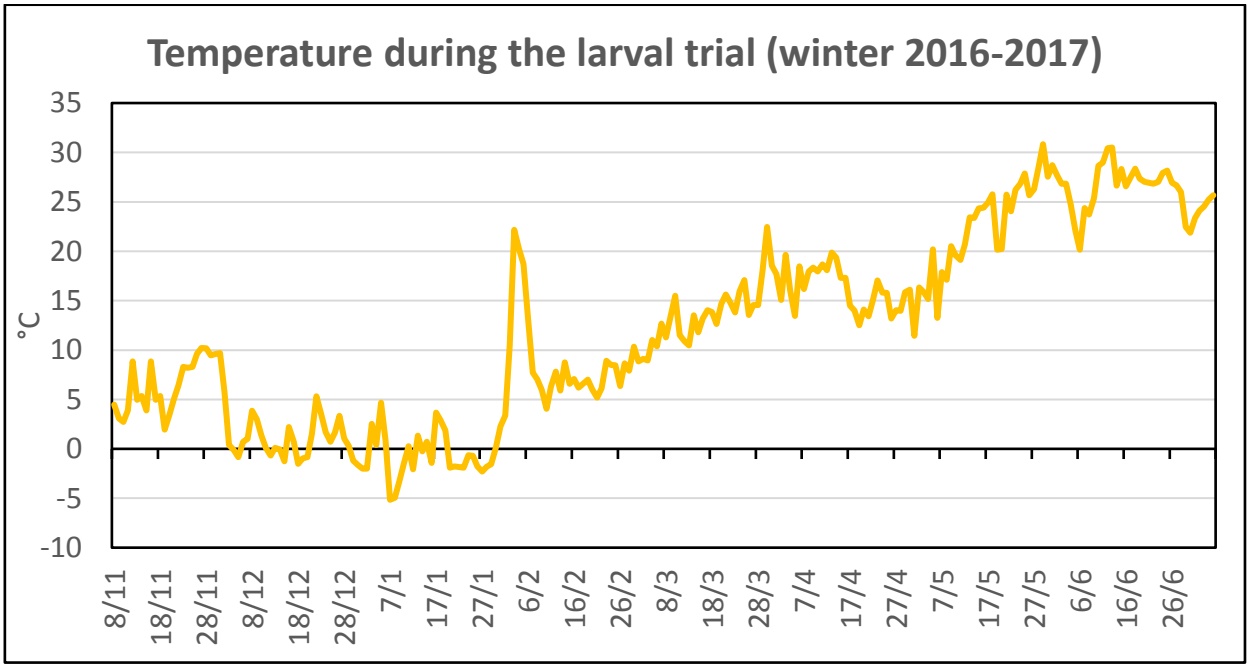


Figure 5.7 Temperature during the larval trial (winter 2016-2017).

There were 2,873 cold hours (equivalent to 119 days or 3.9 months) below the threshold for larvae development (10.2 ºC) during the 2016-2017 winter season (Figure 5.8).

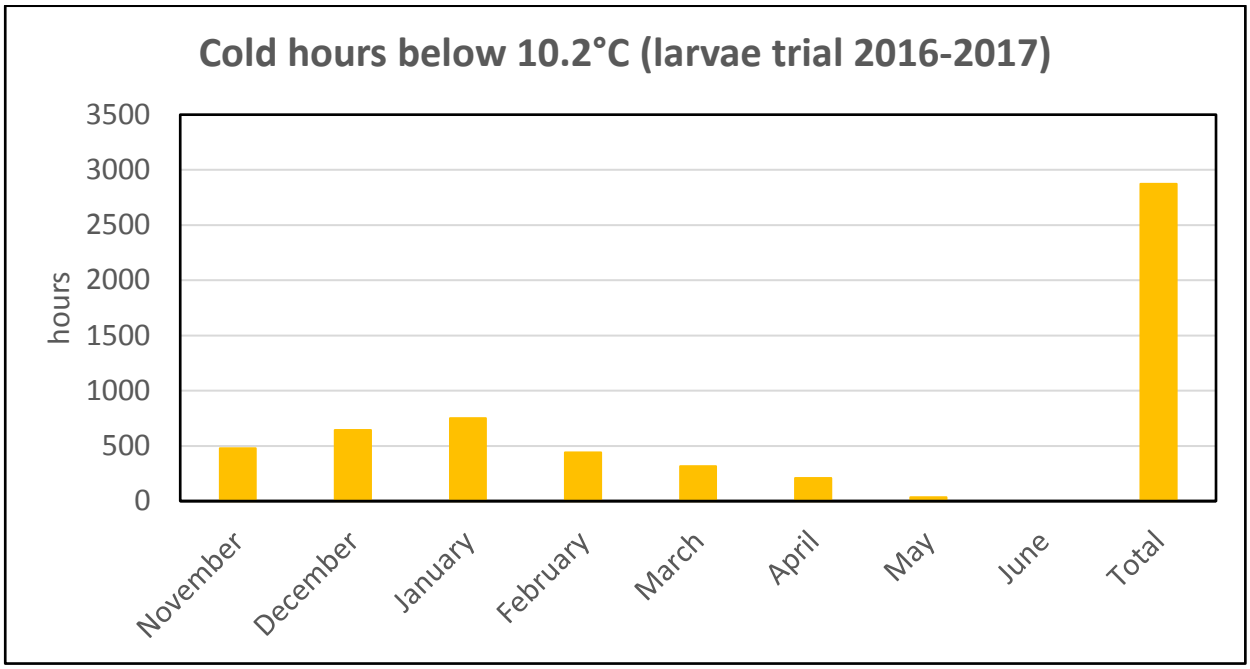


Figure 5.8 Cold hours below 10.2°C (larvae trial 2016-2017).

On inspection on 16 June 2017, 11 ± 2.65 (mean ± S.D.) pupae were found in the sand, but the average percentage of adult emergence from these pupae, developed in Golden Delicious apples in field conditions, was zero.

Overwintering of adults. The trial suggested that *C. capitata* is unable to survive the winter at the adult stage in warehouse conditions.

Adults subjected to critical conditions remained immobile inside the cages, resting on the mesh walls, while adults inside the chamber (control) were more active. In warehouse conditions, adult flies survived an average period ranging from 28 to 31.5 days over the whole study period (started in November), while in climatic chamber conditions they survived an average of 14 days. The maximum survival period in natural conditions was 56 days, while it was 21 days in controlled conditions (Table 5.1).

Table 5.1 Number of individuals, average survival period and maximum survival for Mediterranean fruit fly adults exposed to warehouse conditions during the 2016-2017 winter season.

Replications	N° of adults	Survival average ± S.D	Maximum days of survival
1	40	31.5 ± 17.15	56
2	40	31.5 ± 17.16	56
3	40	28 ± 15.12	49
4	40	28 ± 15.12	49
Control	40	14 ± 7	21

Data concerning the cumulative survival rates over the study period were plotted in order to ascertain the likelihood of survival. Individuals belonging to the control survived less well than the specimens in the warehouse (Figure 5.9). The Log-rank test (with 95% confidence interval) indicated significant differences between the survival of individuals in the control and those placed in the four cages during the 2016-2017 winter season. Furthermore, there were significant differences between replicates 4 and 3, and between replicates 4 and 2.

During the experiment, the temperature in the warehouse never dropped below zero, but there were 791 hours (corresponding to 33 days) below 9.2 °C.

Table 5.2 Pairwise comparison of survival averages, chi squared statistics and significance (Log Rank test, P < 0.05).

	Rep 1		Rep 2		Rep 3		Rep 4		Control	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Rep 1	-	-	0.46	0.50	0.53	0.46	2.83	0.09	4.08	0.04
Rep 2	0.46	0.50	-	-	0.001	0.97	5.77	0.02	6.63	0.01
Rep 3	0.53	0.46	0.001	0.97	-	-	6.08	0.01	16.36	< 0.000
Rep 4	2.83	0.09	5.77	0.02	6.08	0.01	-	-	4.44	0.03
Control	4.08	0.04	6.63	0.01	16.36	< 0.000	4.44	0.03	-	-

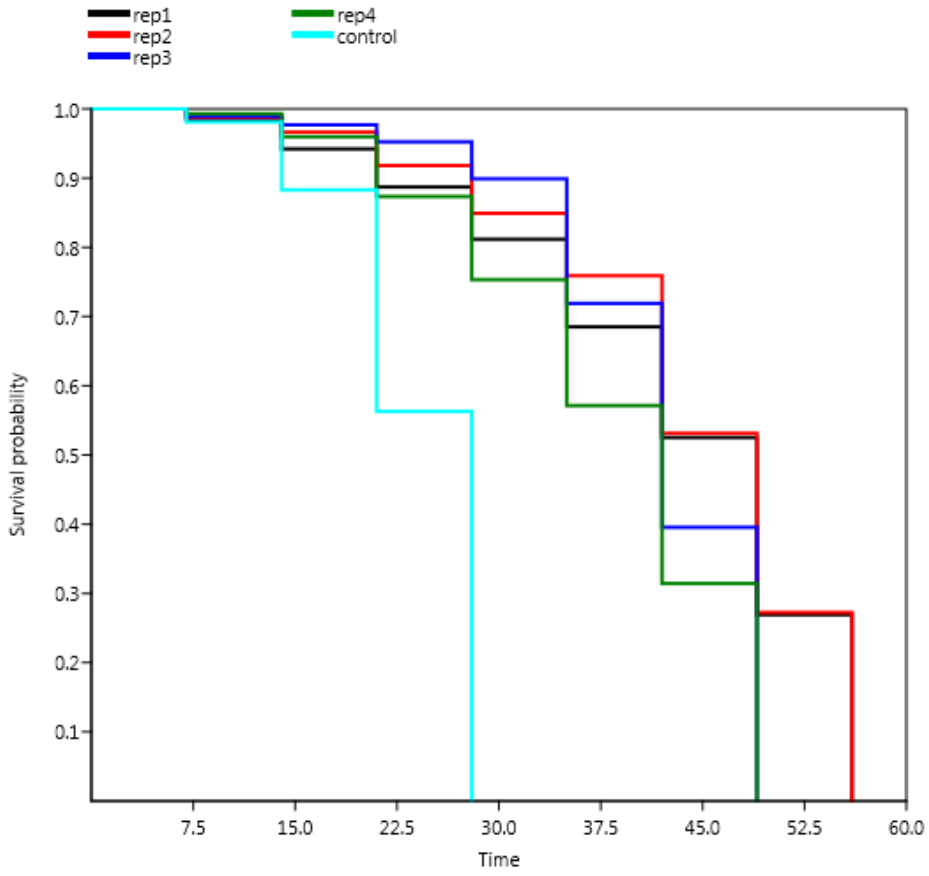


Figure 5.9 Survival of the four replicates kept under natural conditions in comparison with the control kept in the climatic chamber

Discussion and conclusion

Overwintering of pupae. In the overwintering trial conducted in the Trentino area, no adults emerged from the pupae in winter conditions during the 2016-2017 season.

Our results support previous studies carried out in northern Italy by Romani (1997), who reported that no adults emerged from pupae kept at 5-15 cm below the soil surface, and by Rigamonti (2004), who stated that pupae were unable to survive in natural winter conditions.

After leaving the fruit, the larvae fall to the ground to pupate and in this situation soil humidity has a strong and direct effect on pupal development of the Medfly (Eskafi and Fernandez, 1990). It has been shown that this species is also relatively tolerant to desiccation (Shoukry and Hafez, 1979; Duyck *et al.*, 2006).

The soil structure influences adult emergence after winter (Romani 1997). Indeed, water loss during the pupal period, associated with low soil temperatures and low water-retaining capacity during the dry season, can cause pupal mortality (Eskafi and Fernandez 1990).

Specifically, in our case the experiment was carried out in sandy-loam texture soil with a high sand content that has very low water-holding capacity, and the pupae were located in the upper five centimetres. A winter period with low temperatures associated with several days of low humidity could have increased the mortality of some pupae.

In the 2016-2017 winter, over 2,500 cold hours below the threshold for pupae (11.2°C) were recorded in the area. This was long enough to prevent their development at soil level; indeed, Feron and Guennelon (1958) showed that *C. capitata* pupae died when the soil temperature remained at 2 °C for more than eight days.

The soil temperature recorded in this study was below 0 °C for more than one month (from 30 December to 2 February). There were 891 cold hours below 0 °C, more than those recorded in other studies in the Girona area (around 300 h in 2004 and 2005, c. 150 in 2006 and 2007) (Escudero-Colomar *et al.*, 2008). The differences observed in the two areas confirmed the inability of pupae to survive winter conditions in Trentino.

Overwintering of larvae. Rigamonti (2004) showed that pre-adult development of *C. capitata* depends on temperature, but also on the host fruit and even on the cultivar. Other research has showed that low temperatures increase the developmental period of immature stages (Segura *et al.*, 2004). Moreover, Rigamonti (2004) demonstrated that larvae inside apples died when the temperature remained under 0 °C for more than one month; other studies performed in Israel confirmed that Medflies overwintering in later apple varieties slowed down their development rate during the winter (Israely *et al.*, 1997). Indeed, the slow development rate of Medfly larvae in apples and the good condition of fallen fruit over the winter allowed larvae which had hatched

in autumn to survive the winter months. An extended stay inside the fruit guarantees better conditions for larvae than soil does for pupae (Papadopoulos *et al.*, 1996).

The larval development threshold is 10.2 °C (Duyck and Quilici, 2002) and in our case we had more than 3.9 months with temperatures lower than 10.2 °C. Our results suggest that it is possible to obtain a small number of pupae in spring, but no adult emerged from them.

Other studies conducted in Greece showed that the Medfly overwinters in apples as first- and second-instar larva (with slowed-down development lasting up to several months) without lethal consequences, whereas mature larvae have a tendency to abandon the fruit and pupate. All other stages (grown larvae, pupae, adults and eggs) died during the winter (Papadopoulos *et al.*, 1996; 1998).

Low temperatures during the winter have been shown to cause a dramatic decrease in the overwintering population in Mediterranean areas (Papadopoulos *et al.*, 1996). In addition, the increase of immature stages associated with low temperatures could affect adult population levels (Katsoyannos *et al.*, 1998; Papadopoulos *et al.*, 2001a; Segura *et al.*, 2004). Monthly minimum absolute temperatures recorded in the experimental area remained below 0 °C from December 2016 to January 2017 in the Trentino area and this could have influenced adult emergence in spring.

This work, in accordance with Papadopoulus *et al.* (2001), Rigamonti (2004) and Escudero-Colomar *et al*. (2008), demonstrates the importance of collecting and destroying non-commercial fruit in the field before winter, thus reducing Medfly population levels in the following spring.

Overwintering of adults. Many insect species spend the cold winter periods in quiescence, dormancy or diapause, which changes in relation to the species and situation (Meats, 1989; Danks, 2006).

In some studies, a small number of Medflies were shown to be able to survive and to be active during the winter in some southern Mediterranean areas such as Valencia (Del Pino, 2000), southern Greece (Mavrikakis *et al.*, 2000) and southern Italy (Rigamonti, 2004).

On the other hand, adult Medflies were unable to overwinter in the field in natural winter conditions in the Girona area (Penarrubia-Maria *et al.*, 2012).

The significant difference found in the survival experiment between the control and the four replications demonstrated that the winter conditions in the warehouse influenced the mortality of *C. capitata* adults. Specifically, in our experiment the temperatures in the warehouse never dropped below 0 °C, but there were 791 hours (corresponding to 33 days) in which temperatures were below the adult threshold (9.2 °C) (Duyck and Quilici, 2002). The duration of exposure

to low temperatures caused a slowdown in adult activity compared to the control in the climate chamber at 25 °C.

The reduction in movement also caused a reduction in feeding and hydration activity (Romani, 1997), and this could cause their death during that time. Taking into account these observations, the hypothesis that the adult Medfly can survive the winter in warehouse conditions in Trentino area must be rejected.

References

Baas, (1959). The Mediterranean fruit fly *Ceratitis capitata* Wied. in Central Europe (Part 2) *Höfchen-Briefe*, 12: 113-140.

Carey, J.R. (1991). Establishment of the Mediterranean Fruit Fly in California. *Science*, 253: 1369-1373.

Cayol, J.P.; Causse, R. (1993). Mediterranean fruit fly *Ceratitis capitata* Wiedemann (Dipt. Trypetidae) back in Southern France. *J. Appl. Entomol.,* 116: 94-100.

Cirio, U.; De Murats, I.; Gozzo, S.; Enkerlin D. (1972). Preliminary ecological observation of *Ceratitis capitata* Wied. On the island of Procida with an attempt to control the species using the steril male technique. *Boll. Lab. Entomol. Agrar. Filippo Silvestri.,* 30: 175-188.

Danks, H.V. (2006). Insect adaptations to cold and changing environments. *Canadian Entomologist*, 138: 1-23.

DAR (2010) Agrometeorologia. http://ruralcat.net

Del Pino, A. (2000). Efecto de factores abióticos y edáficos naturales en el ciclo biológico de *Ceratitis capitata* (Wiedemann), Diptera: Tephritidae. *Ph.D. Thesis*. Dep. Producción Vegetal. ETSIA. Universidad Politécnica de Valencia. 217 pp.

Denlinger, D.L.; Lee, R.E. (1998). Physiology of heat sensitivity. In: Hallman G.J., Denlinger D.L., [Eds]. Temperature Sensitivity in Insects and Application in Integrated Pest Management. Boulder, CO: *Westview Press*, pp. 7–53.

Duyck, P.F.; David, P.; Quilici, S. (2006). Climatic niche partitioning following successive invasions by fruit flies in La Reunion. *J. Animal Ecol.,* 75: 518-526.

Duyck, P.F.; Quilici, S. (2002). Survival and development of different life stages of three *Ceratitis* spp. (Diptera: Tephritidae) reared at five constant temperatures. *Bull. Entomol. Res.,* 92: 461-469.

Escudero-Colomar, L.A.; Vilajeliu, M.; Batllori, L. (2008). Seasonality in the occurrence of the Mediterranean fruit fly (*Ceratitis capitata* Wied.) in the north-east of Spain. *J. Appl. Entomol.* 132: 714-721.

Eskafi, F.M.; Fernández, A. (1990). Larval-pupal mortality of Mediterranean Fruit Fly from interaction of soil, moisture, and temperature. *J. Environ. Entomol.* 19: 1666-1670.

Feron, M.; Guennelon, G. (1958). La Mouche des fruits dans la Vallée du Rhône. Jornées fruitières et Maraîchères d'Avignon. Avignon, France 24-25 January. pp. 6.

Fimiani, P. (1989). Mediterranean region. In: Fruit flies, their biology, natural enemies and control. *World Crop Pests*, pp. 39-50.

Israely, N., Yuval, B., Kitron, U., Nestel, D., (1997). Population fluctuations of adult Mediterranean fruit flies (Diptera: Tephritidae) in a Mediterranean heterogeneous agricultural region. *J. Environ. Entomol.,* 26: 1263-1269.

Israely, N.; Ritte, U.; Oman, S.D. (2004). Inability of *Ceratitis capitata* (Diptera: Tephritidae) to overwinter in the Judean hills. *J. Econ. Entomol.,* 97: 33-42.

Israely, N.; Ziv, Y.; Oman, S.D. (2005). Spatiotemporal distribution patterns of Mediterranean fruit fly (Diptera: Tephritidae) in the central region of Israel. *Ann. Entomol. Soc. Am.,* 98: 77-84.

Katsoyannos, B.I.; Kouloussis, N.A.; Carey, J.R. (1998). Seasonal and annual occurrence of Mediterranean fruit flies (Diptera: Tephritidae) on Chios Island, Greece: Differences between two neighboring citrus orchards. *Ann. Entomol. Soc. Am.,* 91: 43-51.

Martínez-Ferrer, M.T.; Campos, J.M.; Fibla, J.M. (2006). Population dynamics of *Ceratitis capitata* on citrus in northeast Spain: the influence of adjacent host fruit trees. *Bull. OILB/SROP*, 29: 77-84.

Mavrikakis, P.G.; Economopoulos, A.P.; Carey, J.R. (2000). Continuous winter reproduction and growth of the Mediterranean fruit fly (Diptera: Tephritidae) in Heraklion, Crete, southern Greece. *J. Environ. Entomol.*, 29: 1180-1187.

Meats, A. (1989). Acclimation, activity levels and survival. In: Fruit flies, their biology, natural enemies and control. *World Crop Pests*, pp. 231-239.

Miranda, M.A.; Alonso, R.; Alemany, A. (2001). Field evaluation of Medfly (Dipt. Tephritidae) female attractants in a Mediterranean agrosystem (Balearic Islands, Spain). *J. Appl. Entomol.*, 125: 333-339.

Papadopoulos, N.T., Katsoyannos, B.I., Carey, J.R. (1998). Temporal changes in the composition of the overwintering larval population of the Mediterranean fruit fly (Diptera: Tephritidae) in northern Greece. *Ann. Entomol. Soc. Am.*, 91: 430-434.

Papadopoulos, N.T.; Carey, J.R.; Katsoyannos, B.I.; Kouloussis, N.A. (1996). Overwintering of the Mediterranean fruit fly (Diptera: Tephritidae) in northern Greece. Ann. Entomol. Soc. Am. 89: 526-534.

Papadopoulos, N.T.; Katsoyannos, B.I.; Carey, J.R.; Kouloussis, N.A. (2001). Seasonal and annual occurrence of the Mediterranean fruit fly (Diptera: Tephritidae) in northern Greece. *Ann. Entomol. Soc. Am.*, 94: 41-50.

Penarrubia-Maria, E.; Avilla, J.; Escudero-Colomar, A.L. (2012). Survival of wild adults of *Ceratitis capitata* (Wiedmann) under natural winter conditions in North East Spain. Psyche., pp. 1-6.

Rigamonti I.E. (2005). La *Ceratitis capitata* in Lombardia. Quaderni della ricerca n. 47. Regione Lombardia.

Rigamonti, I.E. (2004). Contributions to the knowledge of *Ceratitis capitata* Wied. (Diptera, Tephritidae) in Northern Italy II. Overwintering in Lombardy. *Boll. Zool. Agraria e di Bachicoltura*, 36: 101-116.

Rigamonti, I.E.; Agosti, M.; Malacrida, A.R. (2002). Distribuzione e danni della Mosca Mediterranea della Frutta *Ceratitis capitata* Wied. (Diptera Tephritidae) in Lombardia (Italia settentrionale). *Atti XIX Congresso nazionale italiano di Entomologia*. Catania, 10-15 giugno 2002, pp. 579-585.

Romani, M. (1997). Biology, ethology and control of *Ceratitis capitata* in northern Italy. *L'Informatore Agrario*, 53: 59-62.

Sciarretta, A.; Cesare, D.; De Salvador, R.; Tabilio, M.R.; Trematerra, P. (2009). Spatio-temporal distribution of *Ceratitis capitata* trap catches in an agricultural landscape. Pheromones and other Semiochemicals, *Bull. IOBC/wprs.*, 41: 123-129.

Segura, D.F.; Vera, M.T.; Cladera, J.L. (2004). Fluctuación estacional en la infestación de diversos hospedadores por la mosca del Mediterráneo, *Ceratitis capitata* (Diptera: Tephritidae), en la provincia de Buenos Aires. *Ecologia Austral.*, 14: 3-17.

Shoukry, A.; Hafez, M. (1979). Studies on the biology of the Mediterranean fruit fly *Ceratitis capitata*. *J. Entomol. Exp. Appl.*, 26: 33-39.

Vera, M.T.; Rodriguez, R.; Segura, D.F.; Cladera, J.L.; Sutherst, R.W. (2002). Potential geographical distribution of the Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), with emphasis on Argentina and Australia. *J. Environ. Entomol.*, 31: 1009-1022.

Mobility

During my Ph.D I had the opportunity to spend six months abroad. The next chapter (Chapter 6) deals with the results obtained during research activities conducted during my attendance at an international host institute.

From March to September 2017, I worked at the Department of Sustainable Plant Protection of IRTA (Research and Technology Food and Agriculture) in Lleida (Spain). The short project "Laboratory bioassay of insecticides against *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) on apples" was carried out under the supervision of Dr. Adriana Lucia Escudero-Colomar.

CHAPTER 6

Laboratory bioassay of insecticides against *Ceratitis capitata* (Wied.) (Diptera: Tephritidae) in apples

Abstract

Laboratory bioassays were conducted to test different active ingredients that could be used in the case of high or localised infestation against two different *C. capitata* strains (from Italy and Spain). The main goal of this research was to compare the efficacy of different insecticides, including Etofenprox, Cyazypyr, Beta-Cyfluthrin, Spinosad and Thiacloprid, in preventing fruit damage by the Mediterranean fruit fly. All these insecticides, except Cyazypyr, are registered in Italy for use on the apple. In addition to Cyzypyr, Etofenprox is also not registered for use on the apple in Spain.

Spinosad caused the highest adult mortality when used against the Italian strain, while Cyazypyr and Etofenprox did not protect fruit against damage by the Italian strain.

When the Spanish strain of *C. capitata* was used, Spinosad caused the highest mortality at 40h when applied at the field dose; although its efficacy was initially similar to that of the other products, significant differences emerged after 17h.

Spinosad caused higher female mortality at sub lethal (0.1x) and field doses compared to the other products. At the field dose, Thiacloprid and Cyazypyr caused lower female and male mortality than the other products. Cyazypyr, Spinosad, Etofenprox and Beta-Cyfluthrin significantly reduced female oviposition damage.

The choice tests showed that treatment with the different insecticides at the field dose had a repellent effect for the two strains and reduced oviposition damage on ripening fruit.

Introduction

The Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann) (Diptera Tephritidae), is a ubiquitous pest in subtropical and tropical regions worldwide. It is a highly polyphagous and multivoltine pest species. *C. capitata* is one of the world's most economically important fruit pests due to its ability to feed on at least 250 different fruit hosts (Stewart *et al.,* 1999; Liquido *et al.*, 1990). In Trentino (northern Italy), this pest has been known to be present since the 1990s, but economic damage to apples has only been reported since 2012 (Dallago, 2013; Tait *et al.,* 2012). From the beginning, control programmes against *C. capitata* have been carried out in

this area using conventional insecticides and they are still the main control method for suppression of the Medfly.

Chemical control of these pests can be targeted at adults. Alternatively, or in addition, it is possible to target young larvae using cytotropic products. In Integrated Pest Management (IPM), insecticides are applied when the economic threshold of one capture in two consecutive monitoring trap services is verified (Gencat, 2009). Other authors suggest applying the insecticide when 3-4 adults/trap/week are caught in peach orchards or Golden Delicious apple orchards (Pollini, 2009). Due to the large numbers of insecticides that have been withdrawn from the market, finding new and effective active ingredients able to control infestation has become of paramount importance for protecting apples from *C. capitata* attacks (Caruso *et al.*, 2014).

Currently pest control is based mainly on organophospates and pyrethroids, but, due to their relative long pre-harvest interval (PHI), their use is limited close to harvesting, when the risk of infestation is the highest. Organophosphate insecticides are highly toxic for adult flies and have a relatively extended period of residual toxicity on apple fruit and foliage in the field, but they are very harmful for beneficial insects. Beside organophosphates and pyrethroids, very few other active ingredients are registered for use against the Mediterranean fruit fly and new entries are not expected in the near future because there are no candidates in the pipeline (Torné, 2008). In order to promote the use of the few more selective alternatives currently available, this study aims to fill in the gaps in knowledge about their efficacy against *C. capitata* in apples. The study was carried out with two *C. capitata* strains with a different insecticide exposure history. Efficacy bioassays were conducted in laboratory conditions using recognised standard methods (Reissig, 2003).

Material and methods

Bioassays were designed to evaluate the mortality of males and females and oviposition damage by gravid females of *C. capitata* during relatively short-term (40h) exposure to dry residues of different insecticides on apples.

This type of trial mimics the interaction between flies and insecticide when the insect is in treated apple orchards in summer. It is important that insecticide residues on apples are able to kill females within a short period or inhibit their ability to oviposit in order to reduce damage in the field.

Small (≈ 60 mm diameter) "Golden Delicious" apples were taken from cold storage and warmed to room temperature for 24 h. The apples were individually dipped in 500 ml of aqueous solution of formulated insecticide in 1000 ml beakers for 20 seconds. The solution was

continuously stirred with a magnetic stirrer to prevent the insecticide from settling during the treatment.

The insecticides tested in the laboratory bioassay included Etofenprox (Trebon up, 30%, Sipcam Italia S.p.a., Milan), Cyazypyr (Exirel 10%, Du Pont De Nemours Italiana s.r.l., Milano), Beta-Cyfluthrin (Baiteroid, 2.6%, Adama Italia s.r.l.), Spinosad (Laser, 44.2%, Dow AgroScience Italia s.r.l., Milan), Thiacloprid (Calypso, 40.4%, Bayer Corporation). After immersion, the apples were placed under a chemical hood to dry for 1.5-2h (Figure 6.1). Each insecticide was tested at six different concentrations (10x, 2.5x, x, 0.1x, 0.01x and 0.001x), including the recommended field concentration (x). Untreated apples dipped in distilled water and allowed to dry under a chemical hood were used as a control.

Individual apples were then placed in the centre of the bottom lid of a clear plastic container (15 cm diameter x 10 cm height). Adequate ventilation was assured by closing the container with a gauze positioned at the top and secured with an elastic band. Two gravid females and two males of *C. capitata*, ≈10 d old, were introduced into each box. Dry food (sugar and brewer's yeast, 9:1) was placed on a small Petri dish inside the plastic container. Moreover, the enclosed flies were provided with water by placing soaked cotton with water on a second small Petri dish (Figure 6.2).

The bioassay was carried out with two different insect strains:

1) A wild strain from Trentino (Italy), collected from infested apples in the field in 2016 and reared on artificial medium (Cavalloro and Girolami, 1969) for about 15 generations since that time.

2) A wild strain from Girona (Spain), collected from infested fruit in the field in 2016 and reared on apple and mango fruit for about 30 generations since that time.

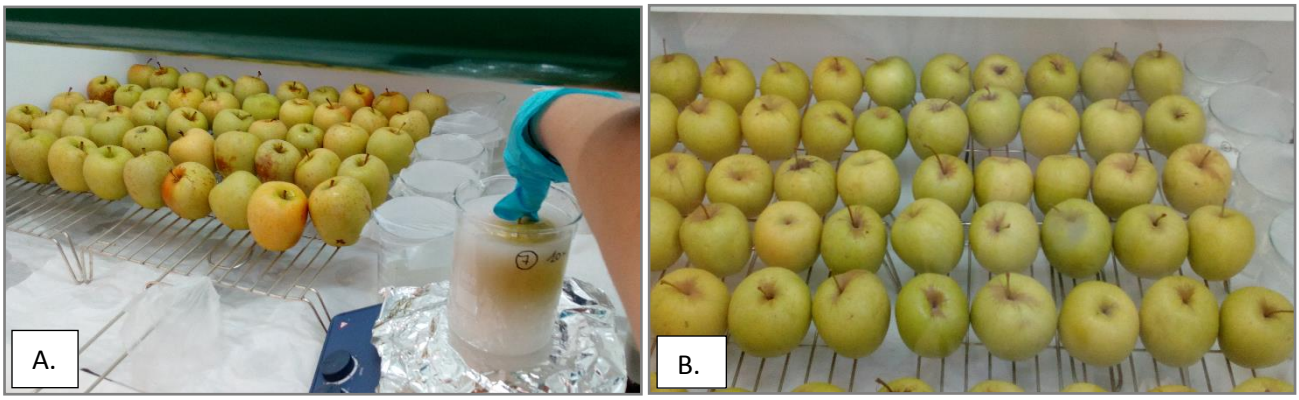


Figure 6.1 A) Apples individually dipped in the insecticide solution; B) Apples placed under a chemical hood to dry

Mortality was determined after 1h, 4h, 17h, 24h and 40h, and the adults were classified as dead if they were not able to walk. After 40h, the number of oviposition punctures on the apples was

counted and the apples were dissected under a microscope (Figure 6.3 A), to determine how many eggs had been laid. All the eggs laid in a single apple were removed and placed in a separate Petri dish with agar (0.8%) to guarantee adequate conditions for larval emergence. After 48h, the number of emerging larvae in each Petri dish (i.e. individual apple) was checked (Figure 6.3 B).

All dosages of each compound were repeated three times by treating 10 apples (i.e. 20 females and 20 males) for each replication, so that 120 insects were exposed to each dosage of insecticide.

To evaluate oviposition repellence caused by the insecticide, a choice test was set up where residues of two different concentrations of insecticides, the recommended field dose (x) and a hundredth of it (0.01x), were compared with an untreated control.

A treated apple was placed inside a transparent plastic tube together with an untreated one (Figure 6.3 C). Two gravid females about 10 days old were released inside the tube and provided with water and sugar. The two ends of the tube were closed with gauze to guarantee adequate ventilation. Ten replications for each concentration were performed.

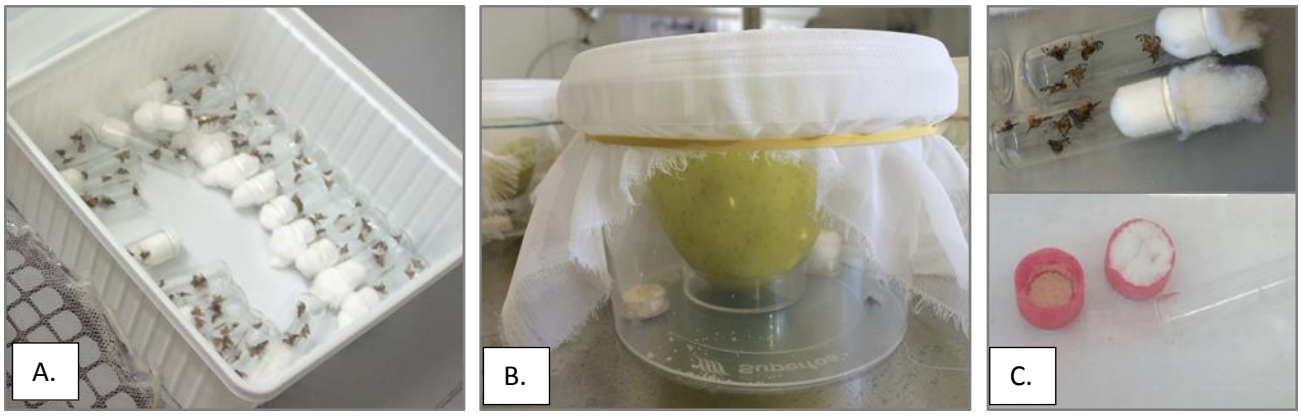


Figure 6.2 Two females and two males (A) were placed inside the transparent plastic container with the treated apple (B); water and food were provided during the bioassay (C).

Statistical analysis. The results were analysed with SASS Enterprise Guide 7.1 software. The mortality of insects recorded for each treatment was corrected according to Abbot (1925), using untreated control mortality. Corrected mortality data percentages were transformed by the arcsine of the square root of the percentage, divided by 100 to fit ANOVA requirements for the normality of data. As the bioassay was designed to review the same sample at five different times, a mixed model procedure was used to analyse results from repeated measurements.

The results for oviposition punctures, the number of eggs and the number of larvae were analysed with a generalised linear model (GLM) procedure to evaluate the statistical significance of the factors: insecticide products and doses, as well as their interaction. Tukey's test was used for mean separation.

To evaluate data from the choice tests, the number of punctures was analysed and Student's Test ($P < 0.05$) was carried out to assess the statistical significance of the difference between treatments (treated versus untreated apples).

Figure 6.3 A) The control under the stereomicroscope; B) Agar Petri dishes for the eggs; C) Material for choice tests

Results

For the Girona strains, statistically significant differences were found at the highest interaction level between the Product*Dose*Sex factors (ANOVA: $F=1.85$, $P < 0.01$).

At the sub-lethal dose (0.01x), all the insecticides caused the same male mortality, but Beta-Cyfluthrin (6.57 ± 7.91%) showed statistically higher female mortality than Etofenprox (1.33 ± 3.46%). At the sub-lethal dose of 0.1x, Spinosad showed the same female mortality as Beta-Cyfluthrin, with statistical differences from the other products (19.75 ± 23.53%, Tukey's test $P < 0.05$) (Table 6.1).

Spinosad killed significantly more females than all the other insecticides at the field dose (54.78 ± 40.33%, Tukey test $P < 0.05$). It also killed significantly more males than all the other products, except Beta-Cyfluthrin (50.85 ± 41.69% and 30.25 ± 26.50% respectively, Tukey's test $P < 0.05$) (Table 6.2).

Statistically significant differences were found after performing the mixed model procedure in the Girona strain at the highest possible interaction level between the Product*Dose*Time factors (ANOVA: $F=3.06$, $P < 0.01$). Tukey's test ($P < 0.05$) for means separation was conducted for all pairs of combinations. All the products tested at the field dose showed a similar efficacy at the beginning of trials, while significant differences emerged from 17h on. Spinosad was the most efficient product (81.33 ± 14.72% of mortality at 40h), with statistical differences from Beta-Cyfluthrin, the second best (42.56 ± 17.02%) (Figure 6.4). Beta-Cyfluthrin showed the same adult mortality as Etofenprox, with significant differences from Thiacloprid and Cyazypyr, which were the least effective (5.94 ± 8.11% and 10.97 ± 13.92%, respectively).

At the 2.5x dose, Spinosad was the most effective insecticide (79.40 ± 16.03 % mortality) after 17 hours and it showed significantly higher mortality than the other products at all the subsequent checking times. It was the only insecticide causing 100% mortality after 40h (Table 6.3).

A the 10x dose (Table 6.4), Beta-Cyfluthrin was the most effective product in the first 4h after exposure, then Spinosad became the most toxic insecticide and was the only one that caused 100% mortality after 40h. The mortality of Beta-Cyfluthrin did not differ statistically from that caused by Spinosad after 24h and 40h exposure. The mortality caused by Etofenprox did not differ from that of Beta-Cyfluthrin starting from 17h (51.78 ± 23.81% and 32.27 ± 13.56%, respectively), while it was significantly higher than Cyazypyr and Thiacloprid (6.66 ± 9.87% and 9.26 ± 16.27%, respectively; Table 6.4) at the last three checking times.

Beta-Cyfluthrin, Spinosad, Etofenprox and Cyazypyr reduced female damage by the Girona strain at the recommended field dose, as regards the number of oviposition punctures (ANOVA, $F=9.92$, $P < 0.000$; Table 6.5), eggs (ANOVA, $F= 10.45$, $P < 0.0001$; Table 6.6) and larvae (ANOVA, $F= 10.37$, $P < 0.000$; Table 6.7) inside the apples. Thiacloprid had the worst performance, with 0.53 ± 0.95 punctures per apple, 12.03 ± 19.40 eggs per apple and 10.4 ± 17.16 larvae per apple (Tukey's test, $P <0.05$).

For the Trentino strain, the highest interaction level (Product*Dose*Time) was not significant (ANOVA, $F=1.18$, $P=0.136$) (Figure 6.5).

The main statistically significant differences were found at the Product*Dose interaction level (ANOVA, $F=9.89$, $P <0.0001$) (Table 6.8). In this case, Spinosad caused a higher percentage of adult mortality than the other products at the field dose, at 2.5x and at 10x (29.14 ± 30.02%, 24.44 ± 26.12% and 47.85 ± 37.06%, respectively).

Beta-Cyfluthrin, Spinosad and Thiacloprid reduced female damage by the Trentino strain at the recommended field dose, as regards the number of oviposition punctures (ANOVA, $F=11.90$,

P < 0.0001; Table 6.9), eggs (ANOVA, F= 11.38, P < 0.0001; Table 6.10) and larvae (ANOVA, F= 10.84, P < 0.0001; Table 6.11) inside apples. Cyazypyr gave the worst performance with 0.97 ± 1.1 punctures per apple, without statistical differences from Etofenprox (0.77 ± 1.36); the same result was observed for eggs (with 16.87 ± 26.78 for Cyazypyr and 10.93 ± 18.84 for Etofenprox) and larvae (13.4 ± 21.88 and 8.33 ± 12.79, respectively) per apple.

As regards the choice test for both strains (Girona and Trentino), statistical differences were observed for different insecticides in apples treated at the recommended field dose and non-treated apples (Table 6.12). At the sub-lethal dose (0.01x), in the Trentino strain, only Beta-Cyfluthrin showed nearly significant differences between treated and non-treated apples. In the Girona strain no statistical differences were observed at the sub-lethal dose (0.01x) between treated and non-treated apples (Table 6.13).

Table 6.1 Percentage of male and female mortality (mean ± S.D.) for the Girona insect strain at the sub lethal dose. Means with the same letter are not significantly different (Tukey's test, P<0.05).

Dose	SEX	SPINOSAD	BETA-CYFLUTRIN	ETOFENPROX	CYAZYPYR	THIACLOPRID
0.01	Male	6.28 ± 11.22 a	6.07 ± 9.80 a	10.08 ± 12.84 a	6.82 ± 14.50 a	2.56 ± 5.57 a
	Female	4.86 ± 8.84 ab	6.57 ± 7.91 a	1.33 ± 3.46 b	3.77 ± 8.40 ab	2.85 ± 6.86 ab
0.1	Male	18.98 ± 22.99 a	15.91 ± 20.69 a	7.98 ± 12.04 ab	9.85 ± 17.91 ab	2.35 ± 13,78 b
	Female	19.75 ± 23.53 a	8.15 ± 12.44 ab	3.66 ± 9.27 b	6.00 ± 9.49 b	7.61 ± 4.82 b

Table 6.2 Percentage of male and female mortality (mean ± S.D.) for the Girona insect strain at the recommended field dose. Means with the same letter are not significantly different (Tukey's test, P<0.05).

SEX	SPINOSAD	BETA-CYFLUTRIN	ETOFENPROX	CYAZYPYR	THIACLOPRID
Male	50.85 ± 41.69 a	30.25 ± 26.50 ab	17.73 ± 15.36 b	4.49 ± 8.80 c	1.69 ± 5.80 c
Female	54.78 ± 40.33 a	23.96 ± 30 b	22.19 ± 21.53 b	6.51 ± 12.83 c	2.24 ± .63 c

Table 6.3 Percentage of mortality (mean ± S.D.) for the Girona insect strain at the 2.5x dose. Means with the same letter are not significantly different (Tukey's test, P<0.05).

Time	SPINOSAD	BETA-CYFLUTHRIN	ETOFENPROX	CYAZYPYR	THIACLOPRID	P
1	2.5 ± 4.52 a	8.33 ± 11.93 a	4.17 ± 9.00 a	3.33 ± 4.92 a	0 ± 0 a	>0.05
4	10.83 ± 9.96 abc	25.46 ± 16.63 a	20 ± 15.95 ab	4.17 ± 5.15 cb	3.33 ± 4.92 c	<0.05
17	79.40 ± 16.03 a	43.47 ± 19.66 b	29.56 ± 10.18 b	12.15 ± 12.41 c	5.46 ± 5.71 c	<0.05
24	96.57 ± 6.62 a	53.33 ± 17.92 b	37.64 ± 15.09 b	20.93 ± 18.26 c	9.33 ± 9.43 c	<0.05
40	100 ± 0 a	55.54 ± 23.94 b	38.50 ± 21.05 bc	28.45 ± 22.55bc	20.50 ± 13.62 c	<0.05

Table 6.4 Percentage of mortality (mean ± S.D.) for the Girona insect strain at the 10x dose. Means with the same letter are not significantly different (Tukey's test, P<0.05).

Time	SPINOSAD	BETA-CYFLUTHRIN	ETOFENPROX	CYAZYPYR	THIACLPRID	p
1	1.67 ± 3.89 b	13.33 ± 15.57 a	0.83 ± 2.89 b	0.83 ± 2.89 b	0 ± 0 b	>0.05
4	6.67 ± 6.51 b	33.70 ± 16.72 a	11.76 ± 11.92 b	4.17 ± 6.69 b	7.5 ± 13.57 b	<0.05
17	92.62 ± 10.76 a	51.78 ± 23.81 b	32.27 ± 13.56 b	6.66 ± 9.84 c	9.26 ± 16.97 c	<0.05
24	98.96 ± 3.61 a	64.44 ± 23.76 ab	50.51 ± 20.87 b	20.60 ± 14.71 c	11.57 ± 15.58 c	<0.05
40	100 ± 0 a	71.25 ± 23.95ab	57.97 ± 24.05 b	20.64 ± 18.45 c	26.64 ± 29.69 c	<0.05

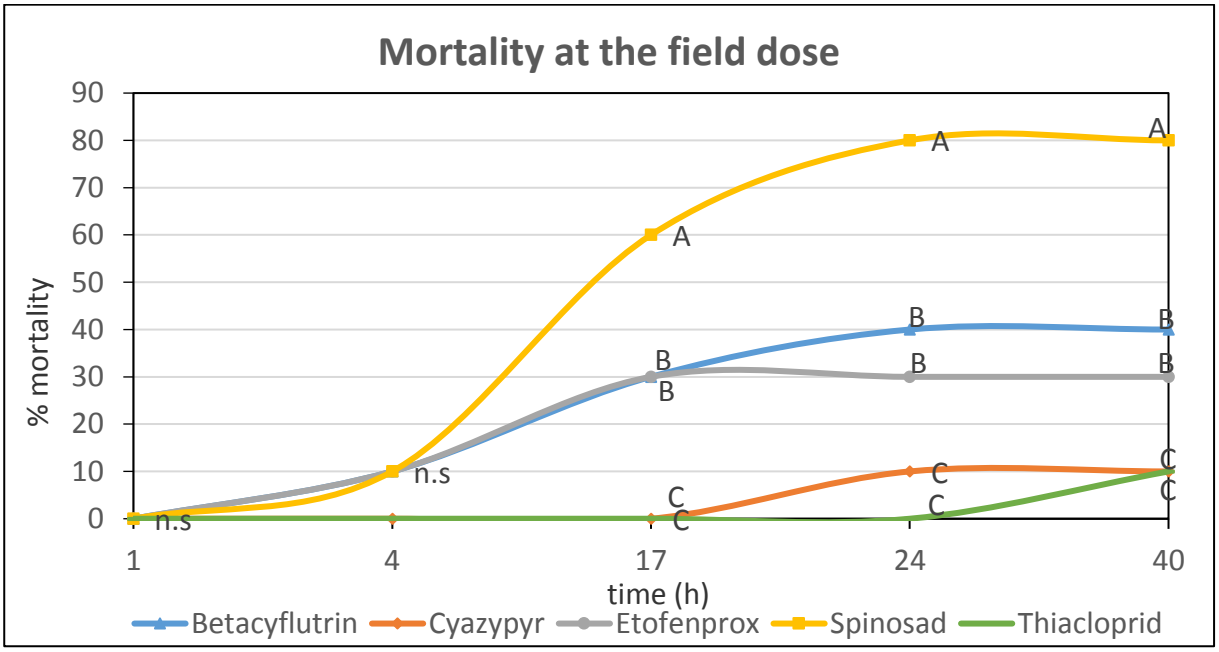


Figure 6.4 Adult mortality (%) at the field dose for the Girona strain at different timings. Means with the same letter are not significantly different (Tukey's test, P<0.05)

Table 6.5 Number of eggs per apple for the different insecticides when applied at the field dose, for the Girona strain. Means with the same letter are not significantly different (Tukey's test, P<0.05)

	SPINOSAD	BETA-CYFLUTHRIN	ETOFENPROX	CYAZYPYR	THIACLOPRID
Control	1.43 ± 1.10 a	1.43 ± 1.13 a	1.1 ± 0.96 a	1.57 ± 1.59 a	1.17 ± 0.74 a
Field dose (x)	0.16 ± 0.46 b	0 ± 0 b	0.03 ± 0.18 b	0.2 ± 0.48 b	0.53 ± 0.78 a

Table 6.6 Number of eggs per apple for the different insecticides when applied at the field dose, for the Girona strain. Means with the same letter are not significantly different (Tukey's test, P<0.05)

	SPINOSAD	BETA-CYFLUTHRIN	ETOFENPROX	CYAZYPYR	THIACLOPRID
Control	39.57 ± 30.24 a	22.27 ± 19.38 a	27.83 ± 32.46 a	30.4 ± 30.11 a	30.6 ± 26.46 a
Field dose (x)	2.5 ± 6.96 b	0 ± 0 b	0.43 ± 2.37 b	2.7 ± 6.61 b	12.03 ± 19.40 a

Table 6.7 Number of larvae per apple for the different insecticides when applied at the field dose, for the Girona strain. Means with the same letter are not significantly different (Tukey's test, P<0.05).

	SPINOSAD	BETA-CYFLUTHRIN	ETOFENPROX	CYAZYPYR	THIACLOPRID
Control	35.93 ± 27.14 a	19.83 ± 17.59 a	26.86 ± 31.63 a	28.66 ± 28.69 a	28.10 ± 24.27 a
Field dose (x)	2.13 ± 5.88 b	0 ± 0 b	0.4 ± 2.19 b	2.67 ± 6.55 b	10.4 ± 17.16 a

Table 6.8 Percentage of mortality (mean ± S.D.) for the different insecticides at different doses. Means with the same letter are not significantly different (Tukey's test, P<0.05).

Dose	SPINOSAD	BETA-CYFLUTHRIN	ETOFENPROX	CYAZYPYR	THIACLOPRID
0.001	4 ± 8.27 b	0.33 ± 1.81 c	1.83 ± 4.69 bc	8.17 ± 9.66 a	3.01 ± 7.47 bc
0.01	2.01 ± 5.79 a	4.37 ± 9.17 a	1.67 ± 3.76 a	2.17 ± 4.55 a	4.34 ± 8.90 a
0.1	4.34 ± 8.91 b	9.90 ± 15.79 a	1.83 ± 3.90 b	3.16 ± 7.24 b	4.01 ± 8.70 ab
1	29.14 ± 30.02 a	8.72 ± 13.93 b	9.01 ± 12.60 b	14.53 ± 16.67 b	4.51 ± 9.49 b
2.5	24.44 ± 26.12 a	11.89 ± 15.65 b	11.51 ± 14.13 b	10.68 ± 14.49 b	6.52 ± 12.34 b
10	47.85 ± 37.06 a	15.22 ± 17.69 bc	20.18 ± 16.73 b	10.51 ± 10.50 c	14.53 ± 17.44 bc
P	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05

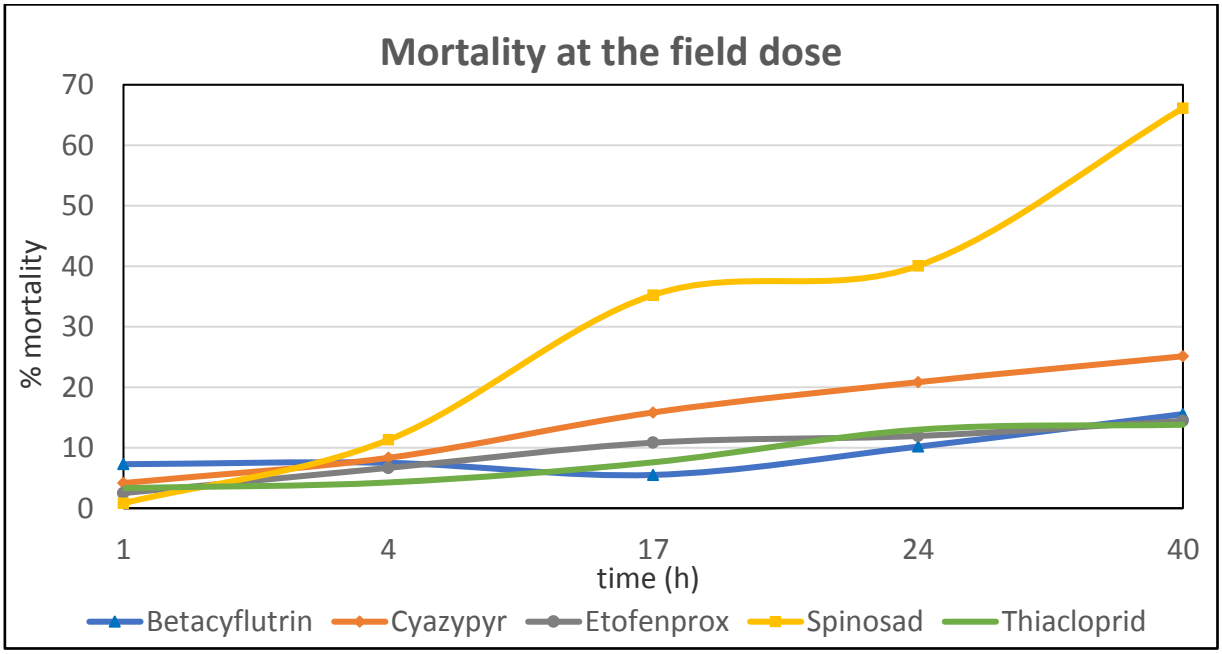


Figure 6.5 Adult mortality (%) at the field dose for the Trentino strain at different timings (ANOVA, F=1.18, P=0.136).

Table 6.9 Number of punctures per apple for the different insecticides when applied at the field dose, for the Trentino strain. Means with the same letter are not significantly different (Tukey's test, P<0.05).

	SPINOSAD	BETA-CYFLUTHRIN	ETOFENPROX	CYAZYPYR	THIACLOPRID
Control	1.03 ± 1.51 b	1.13 ± 1.33 ab	1.96 ± 1.52 a	1.9 ± 1.40 a	0.83 ± 0.83 b
Field dose (x)	0.4 ± 0.77 bc	0.03 ± 0.18 c	0.76 ±1.35 ab	0.97 ± 1.09 a	0.1 ± 0.31 bc

Table 6.10 Number of eggs per apple for the different insecticides when applied at the field dose, for the Trentino strain. Means with the same letter are not significantly different (Tukey's test, P<0.05).

	SPINOSAD	BETA-CYFLUTHRIN	ETOFENPROX	CYAZYPYR	THIACLOPRID
Control	11.9 ± 14.38 c	17.9 ± 27.81 bc	26.17 ± 23.98 ab	36.4 ± 29.87 a	11.13 ± 14.38 c
Field dose (x)	2.57 ± 5.39 bc	0 ± 0 c	10.93 ± 18.84 ab	16.87 ± 26.78 a	1.66 ± 6.25 c

Table 6.11 Number of larvae per apple for the different insecticides when applied at the field dose, for the Trentino strain. Means with the same letter are not significantly different (Tukey's test, P<0.05).

	SPINOSAD	BETA-CYFLUTHRIN	ETOFENPROX	CYAZYPYR	THIACLOPRID
Control	6.33 ± 9.35 b	16.3 ± 24.98 ab	21.43 ± 18.60 a	27.26 ± 25.64 a	8.36 ± 9.68 b
Field dose (x)	0,87 ± 1.76 bc	0 ± 0 c	8.33 ± 12.79 ab	13.4 ± 21.87 a	1.5 ± 5.88 c

Table 6.12 Number of punctures per apple (mean ± S.D.) for the two different insect strains in the choice test (non-treated vs treated at the recommended field dose). Numbers in bold indicate statistical differences from the mean, Student's t test, P<0.05.

	Girona			Trentino		
Product	T (x)	NT (x)	P	T (x)	NT (x)	P
SPINOSAD	0 ± 0	0.1 ± 0.3	**0.03**	0.4 ± 0.5	0.5 ± 0.7	0.72
BETA-CYFLUTHRIN	0.2 ± 0.6	0.5 ± 0.7	0.33	0 ± 0	0.4 ± 0.5	**0.02**
ETOFENPROX	0.1 ± 0.3	1.1 ± 1.4	**0.03**	0 ± 0	0.7 ± 0.7	**0.004**
CYAZYPYR	0 ± 0	0.6 ± 0.5	**0.02**	0 ± 0	0.6 ± 0.5	**0.002**
THIACLOPRID	0.2 ± 0.4	0.8 ± 0.6	**0.02**	0.3 ± 0.5	0.9 ± 0.6	**0.02**

Table 6.13 Number of punctures per apple (mean ± S.D.) for the two different insect strains in the choice test (non-treated vs treated at the sub lethal field dose (0.01x). Numbers in bold indicate statistical differences from the mean, Student's t test, P<0.05.

	Girona			Trentino		
Product	T (0.01x)	NT (0.01x)	P	T (0.01x)	NT (0.01x)	P
SPINOSAD	0.4 ± 0.7	1.1 ± 1.1	0.11	1.1 ± 0.9	1.5 ± 1.4	0.46
BETA-CYFLUTHRIN	0.6 ± 0.7	1.1 ± 0.9	0.21	0.3 ± 0.48	1.2 ± 1.3	**0.06**
ETOFENPROX	0.7 ± 0.6	0.8 ± 1.0	0.8	0.6 ± 0.7	0.7 ± 0.7	0.75
CYAZYPYR	0.4 ± 0.7	1.1 ± 1.1	0.11	0.4 ± 0.5	0.9 ± 1.3	0.27
THIACLOPRID	0.4 ± 0.5	0.8 ± 0.9	0.25	0.6 ± 0.7	0.8 ± 1.0	0.62

Discussion and conclusion

Our results showed that Spinosad was more effective than the other products, confirming the results of McQuate *et al*., (2005), El-Aw *et al.*, (2008) and Ahmad *et al.*, (2010), which indicated a high susceptibility of *C. capitata* to this product. Other studies have reported that Spinosad causes higher mortality to adults of fruit fly species (such as *Rhagoletis indifferens* Curran) than Thiacloprid, and that only Spinosad was able to prevent oviposition (Yee, 2006).

Moreover, Xin-Geng *et al.*, (2006) reported that Spinosad has recently become a primary tool for area-wide suppression or eradication of tephritid fruit flies.

Reissig (2003) and Wiese *et al.*, (2002) showed that Thiacloprid, used in the field, was a good substitute for organophosphate insecticides to control apple maggots (*Rhagoletis pomonella*, Walsh.), despite the fact that this product was not particularly toxic to flies in laboratory bioassays. Indeed, in our experiments, Thiacloprid was the worst product as regards adult mortality, but reduced oviposition damage when applied to the Trentino strain.

Beta-Cyfluthrin and Etofenprox are pyrethroids and are classified in the same IRAC class as Deltamethrin (IRAC, 2012). There are few studies on the activity of these substances against *C. capitata*, but data available on Deltamethrin show that it causes high mortality of fruit flies (Raga and Sato, 2005). In our experiments, Beta-Cyfluthrin and Etofenprox reduced adult populations at the recommended field dose and reduced oviposition damage for the Girona strain. Different results were obtained for the Trentino strain, because only Beta-Cyfluthrin reduced oviposition damage.

Despite several studies conducted on different Diptera, little information is available about the efficacy of Cyazypyr against *C. capitata* in apples. This active ingredient belongs to the anthranilic diamides group, insecticides acting on the ryanodine receptors of muscle cells, and intoxication of insects with this insecticide results in rapid muscle paralysis (Lahm *et al*., 2007). It is possible that females, exposed to Cyazypyr, experience ovipositor dysfunction, thus delaying egg deposition. Anthranilic diamides have been shown to be active against several species of *Rhagoletis* (Teixeira *et al.*, 2009). Zhang *et al*. (2014) also proposed the use of anthranilic diamides insecticides to control fruit flies and his findings are in agreement with the results we obtained with the Girona strain, as Cyazypyr reduced oviposition damage on the fruit.

According to the results of this study, for the Girona strain it is possible to recommend Spinosad and Beta-Cyfluthrin (7 days PHI) to control adults and Etofenprox (7 days PHI) to reduce oviposition damage on fruit at the field dose. Thiacloprid and Cyazypyr cannot be recommended to control these pests because they showed very low efficacy in the trials. Furthermore, Thiacloprid had a 14-day PHI, too long to protect fruit properly, while Cyazypyr has not yet been registered for use on apples against this target insect.

As regards the Trentino strain, it is possible to recommend Spinosad for control of adults; in contrast, Cyazypyr and Etofenprox were unable to protect fruit against fly damage in our experiments.

With both insect strains, the choice test showed that females (both at the field and sub-lethal doses) preferred to lay eggs in non-treated apples. The different insecticides showed a repellent effect and reduced oviposition damage on ripening fruit.

In conclusion, Spinosad can be used to control adults of both strains. It is a new and highly promising insecticide with efficacy against a wide range of insects (Shono *et al.,* 2003). In addition, the contact toxicity of Spinosad is very low for both vertebrates and invertebrates, and the active ingredient must be consumed in order to cause toxicity. Therefore, this active ingredient is also considered acceptable for use in organic agriculture (Organic Materials Review Institute [OMRI], 2002).

References

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. *J. Econ. Entomol*. 18: 265-267.

Ahmad, S.F.; Sohail, A.; Khan, R.R.; Nadeem, M.K. (2010). Evaluation of insecticides resistance in two strains of fruit fly, *Bactrocera zonata* (Saunders) (Tephritidae: Diptera), with fruit dip method. *Pak. Entomol.,* Vol. 32, No.2.

Caruso, S.; Tosi, L.; Lopolito, P.; Capela, A.; Belotto, D.; Romani, M.; Guastamacchia, F.; Querzola, P.; Capriotti, M. (2014). Valutazione dell'efficacia di acetamiprid (Epik®) nel contenimento della mosca della frutta e della mosca del ciliegio. *Atti delle giornate Fitopatologiche,* 1: 99-106.

Cavalloro, R.; Girolami, V. (1969). Miglioramento nell'allevamento in massa di *Ceratitis capitata* Wied. (Diptera:Trypetidae). Redia, LI: 315-327.

Dallago, G. (2013). La mosca mediterranea della frutta. Una presenza ormai costante. *Rapporto 2013 Centro di Trasferimento Tecnologico* pp.47-48.

El-Aw, M.A.M.; Draz, K.A.A.; Hashem, A.G.; El-Gendy, I.R. (2008). Mortality comparison among Spinosad, Actara, Malathion, and Methomyl containing baits against peach Fruit Fly, *Bactrocera zonata* Saunders (Diptera: Tephritidae) under laboratory conditions. *J. Appl. Sci. Res.*, 4 (2): 216-223.

Gencat, (2009). Norma tècnica per a la producció integrada de fruita de llavor. www.produccciointegrada.cat.

IRAC. MoA Classification Scheme, Version 7.2. IRAC International MoA Working Group. Koren, B.; Yawetz, A; Perry, A.S. (1984). Biochemical properties characterizing the development of tolerance to Malathion in *Ceratitis capitata* Wiedmann (Dieptera: Tephritidae) *J. Econ. Entomol.*, 77 (4): 864-867.

Lahm, G.P.; Stevenson, T.M.; Selby, T.P.; Freudenberger, J.H.; Cordova, D.; Flexner, L. (2007). Rynaxypyr®: a new insecticidal anthranilic diamide that acts as a potent and selective ryanodine receptor activator. *Bioorg. Med. Chem. Lett.,* 17: 6274–6279.

Liquido, N.J.; Cunningham, R.T.; Nakagawa, S. (1990). Host plants of Mediterranean fruit fly (Diptera: Tephritidae) on the Island of Hawaii (1949–1985 Survey). *J. Econ. Entomol.,* 83: 1863–1878.

McQuate, G.T.; Sylva, C.D.; Jang, E.B. (2005). Mediterranean fruit fly (Diptera: Tephritidae) suppression in persimmon through bait sprays in adjacent coffee plantings. *J. Appl. Entomol.*, 129 (2): 377-385.

Organic Materials Review Institute [OMRI], 2002. Spinosad. Plant Protection and Quarantine. Aphis. usda. Gov/index.

Pollini, A. (2009). Le ultime restrizioni rendono difficile la lotta della mosca Mediterranea. La difesa delle drupacee. *L'Informatore Agrario,* 30: 17-18.

Raga, A.; Sato M.E. (2005). Effect of tracer bait against *Ceratitis capitata* (Wied.) and *Anastrepha fraterculus* (Wied.) (Diptera: Tephritidae) in laboratory. *Neotrop. Entomol.,* 34: 815–822.

Raga, A.; Sato, M.E. (2006). Time-mortality for fruit flies (Diptera: Tephritidae) expoused to insecticies in laboratory. *Arq. Inst. Biol.*, São Paulo, 73 (1): 73-77.

Reissig H.W. (2003). Field and laboratory tests of new insecticides against the Apple Maggot, *Rhagoletis pomonella* (Walsh) (Diptera: Tephritidae). Entomological Society of America. *J. Econ. Entomol.,* 96 (5): 1463-1472.

Shono, T.; Scott, J.G. (2003). Spinosad resistance in the housefly, *Musca domestica* is due to a recessive factor on autosome. *Pestic. Biochem. Physiol.,* 75 (1-2): 1-7.

Stewart, T.P.; Johanson, D.S. (1999). The SPS agreement of the World Trade Organization and plant pest infestation: a case study of the 1997 Mediterranean fruit fly outbreak in Florida. *Am. Univ. Int. Law Rev.,* 14: 1107–1127.

Tait, P.; Pellegrini, F.; Zaffoni, M.; Morten, M. (2012). Il ritorno di *Ceratitis capitata* nei frutteti trentini. *Rapporto 2012 Centro di Trasferimento Tecnologico* pp. 31-34.

Teixeira, L.A.F.; Gut, L.J.; Wise, J.C.; Isaacs, R. (2009). Lethal and sub-lethal effects of chlorantroniliprole on three species of *Rhagoletis* fruit flies (Diptera: Tephritidae). *Pest Manag. Sci.,* 65: 137–143.

Torné, M. (2008). *Ceratitis capitata*: situación actual y perspectivas de futuro. Jornada tècnica: problemàtica actual en el control dels paràsits dels fruiters. Perspectives de futur, Lleida 26 Setembre, p. 14.

Wiese, J.C.; Gut L.J. (2002). Control of apple maggot. *J. Arthropod Manag. Tests,* 25: 43-44.

Wood, R.J.; Harrys, D.J. (1989). Genetics: *Ceratitis capitata* artificial and natural selection. In Robinson, A.S.; Hooper, G. Fruit flies: *their biology, natural enemies and control*. Amsterdam. 3B: 19-31.

Xin-Geng, W.; Russell, H.M. (2006). Feeding and attraction of non-target flies to spinosad-based fruit fly bait. *Pest Manag. Sci.,* 62 (7): 933-939.

Yee, W.L.; Alston, D.G. (2006). Effects of Spinosad, Spinosad Bait, and Chloronicotinyl insecticides on mortality and control of adult and larval western Cherry Fruit Fly (Diptera: Tephritidae). *J. Econ. Entomol.,* 99 (5): 1722-1732.

Zhang, R.; Jang, E.B.*;* He, S.; Chen, J. (2015). Lethal and sub-lethal effects of Cyantranilprole on *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). *Pest Manag. Sci.,* 71: 250–256.

GENERAL DISCUSSION AND CONCLUSION

Ceratitis capitata, also known as the Mediterranean fruit fly (MFF), is one of the main pests of commercial fruit throughout the world and causes the highest commercial losses in fruit production in the Mediterranean area. The apple is one of hundreds of fruit crops recognised as a host of the Medfly. In Trentino, *C. capitata* was reported to infest the apple for the first time in 1990, but only since 2012 has its presence become constant.

Knowledge about pest biology is the foundation for development of its management in a newly invaded area. According to our studies, *C. capitata* was able to develop three generations/year in the environmental conditions in the Trentino region. Adult flight started during the first week of July, but the intensity remained at a very low level until August, when it began to increase, peaking during September. Adult activity then decreased in October and stopped completely by the end of November. Our population dynamics are similar to those reported in other studies conducted in Spain and northern Greece (Escudero *et al.*, 2008; Papadopoulos *et al.*, 2001). In Greece too, no infestation was found in the apricot at the beginning of the season, probably because the population density was very low due to the small number of adults emerging from overwintering (Papadopoulus *et al.*, 1996). Moreover, the presence of the Mediterranean fruit fly in summer caused damage to early susceptible host fruit, such as the peach. These early ripening fruits are very important for the build-up of population density because they constitute a suitable host available for oviposition of adults deriving from the overwintering generation (if overwintering occurs at the larval stage inside apples; Papadopoulus *et al.*, 1996; 1998).

Trentino represents the northern limit of MFF distribution in Italy. Early detection of its population development is essential to set up effective control measures. Knowledge of the overwintering behaviour of the insect is another critical point for the implementation of integrated pest management strategies. Our results showed that adults are unable to overwinter in warehouse conditions or at the pupal stage in soil. On the other hand, we confirmed that the MFF is able to overwinter at the larval stage inside fruit, where it completes its development and pupates in spring. Our results support the previous studies carried out in northern Italy by Romani (1997). Moreover, Rigamonti (2004) demonstrated that larvae inside apples die when the temperature remains under 0 °C for more than one month. Other studies performed in Israel have confirmed that the Medfly, overwintering in later apple varieties, slows down its development rate during winter conditions (Israely *et al.*, 1997). As a consequence, sanitation by collecting and destroying fruit lying on the ground or left on the trees over winter is

considered a tactic of primary importance for the direct control of the MFF, as it contributes to reducing the pest population that could start summer infestation.

Further investigation and information about overwintering is necessary to confirm the results obtained.

Pest monitoring is necessary to understand the spatial and temporal distribution of the MFF and assess the effectiveness of the pest control measures chosen. Indeed, monitoring systems with traps are a common and critical component in pest detection, delimitation, suppression and eradication programmes worldwide. There is no published information on the performance of the different trapping systems throughout the season and their sensitivity in detecting small populations, such as those present in the early season in temperate areas. This is important information for predicting future infestation levels and deciding the best strategy for population control. We compared some of the commercially available trapping devices and found that the Decis® trap with Unipak® Biolure food lure (Suterra, Europe Biocontrol S.L.) performed best in monitoring the fly in Trentino apple orchards. This trap was attractive for both males and females from the beginning of the season and caught a consistently high number of flies throughout the summer. Our findings are in agreement with Hendrichs *et al.,* (1991) Bakri *et al.,* (1998) and Cohen *et al.,* (2000).

Studying female oviposition preferences for apple varieties provides further important information for the development of an adequate control strategy. According to our results, Gala, Kanzi, Fuji and Golden Delicious are much more susceptible to MFF oviposition than Granny Smith, Morgan and Red Delicious. Our results are in accordance with Ioriatti *et al.,* (2015) and Papadopoulos *et al.,* (2002), who reported that susceptibility to Dipteran oviposition increased when fruit had a higher sugar content and lower penetrating resistance. Moreover, the most susceptible apple varieties were also found to be the most suitable for larval development. In contrast, adults did not emerge from Granny Smith, Morgan and Red Delicious. These results are in accordance with Papadopoulus *et al.*, (2002a) and Papadopoulus *et al.*, (2001), who reported that Golden Delicious was more infested than Granny Smith and Red Delicious in the field. Zucoloto (1993) confirmed our results that Red Delicious, Granny Smith and Morgan allow lower pupae development.

This information is very useful when managing multivarietal orchards, because it makes it possible to adopt the most appropriate control strategy according to the risk assessment. Moreover, the choice of apple variety should be taken into consideration when the orchard is located in an area with a high population density.

For successful pest control management it is very important to use varied tactics, by combining different control methods depending on the operational context. In addition to sanitation

measures, mass trapping and insecticides could be adopted in an integrated approach. In the case of high or localised infestation, the use of insecticide is suggested to reduce both population and fruit damage. Of the insecticide formulations available to control the MFF in apple orchards and tested in this work, Spinosad was shown to be more effective than the other products tested. Our results confirm the observations of McQuate *et al*., (2005), El-Aw *et al.,* (2008) and Ahmad *et al.*, (2010), who reported the high susceptibility of *C. capitata* to this product. Xin-Geng *et al.,* (2006) reported that Spinosad, a new and highly promising insecticide with efficacy against a wide range of insects (Shono *et al.,* 2003), had also recently become a primary tool for area-wide suppression or eradication of tephritid fruit flies. Because of its low contact toxicity, Spinosad is selective for non-target vertebrates and invertebrates and is admitted for use in organic apple production.

Thus the research conducted in the last three years, ranging from pest monitoring and insect bio-ethology to insecticide efficacy, provides a scientific background representing a useful tool for creating collaboration between research and agriculture in the context of the development of sustainable strategies for the control of *C. capitata* in apples.

References

Ahmad, S.F.; Sohail, A.; Khan, R.R.; Nadeem, M.K. (2010). Evaluation of insecticides resistance in two strains of fruit fly, *Bactrocera zonata* (Saunders) (Tephritidae: Diptera), with fruit dip method. *Pak. Entomol.,* Vol. 32, No.2.

Bakri, A.; Hadis, H.; Epsky, N.D.; Heath, R.R.; Hendrichs, J. (1998). Female *Ceratitis capitata* (Diptera: Tephritidae) capture in a dry trap baited with food-based synthetic attractant in an argan forest in Morocco. Part I: low population field test. *Can. Entomol.,* 130: 349 -356.

Cohen, H.; Yuval, B. (2000). Perimeter trapping strategy to reduce Mediterranean fruit fly (Diptera: Tephritidae) damage on different host species in Israel. *J. Econ. Entomol.,* 93:21-725

El-Aw, M.A.M.; Draz, K.A.A.; Hashem, A.G.; El-Gendy, I.R. (2008). Mortality comparison among Spinosad, Actara, Malathion, and Methomyl containing baits against peach Fruit Fly, *Bactrocera zonata* Saunders (Diptera: Tephritidae) under laboratory conditions. *J. Appl. Sci. Res.,* 4 (2): 216-223.

Escudero-Colomar, A.; Vilajeliu, M.; Batllori, L. (2008). Seasonality in the occurrence of the Mediterranean fruit fly [*Ceratitis capitata* (Wied.)] in the northeast of Spain. *J. Appl. Entomol*. 132: 714-721.

Hendrichs, J.; Katsoyannos, B.I.; Papaj, D.R.; Prokopy, R.J. (1991). Sex differences in movement between natural feeding and mating sites and tradeoffs between food consumption, mating success and predator evasion in Mediterranean fruit flies (Diptera: Tephritidae). *Oecologia.,* 86: 223-231.

Ioriatti, C.; Walton, V.; Dalton, D.; Anfora, G.; Grassi, A.; Maistri, S.; Mazzoni, V. (2015). Drosophila suzukii (Diptera: Drosophilidae) and its potential impact to Wine Grapes during harvest in two cool climate wine grape production regions. *J. Econ. Entom.,* 108 (3): 1148-1155.

Israely, N.; Yuval, B.; Kitron, U.; Nestel, D. (1997). Population fluctuations of adult Mediterranean fruit flies (Diptera: Tephritidae) in a Mediterranean heterogeneous agricultural region. *J. Environ. Entomol.,* 26: 1263-1269.

McQuate, G.T.; Sylva, C.D.; Jang, E.B. (2005). Mediterranean fruit fly (Diptera: Tephritidae) suppression in persimmon through bait sprays in adjacent coffee plantings. *J. Appl. Entomol.,* 129: 110-117.

Papadopoulos, N.T.; Carey, J.R., Katsoyannos, B.I.; Kouloussis, N.A. (1996). Overwintering of *Ceratitis capitata* (Diptera: Tephritidae) in northern Greece. *Ann. Entomol. Soc. Am.,* 89: 526-534.

Papadopoulos N.T.; Katsoyannos, B.I.; Carey, J.R. (2002). Demographic Parameters of the Mediterranean fruit fly (Diptera: Tephritidae) Reared in Apples. *Ann. Entomol. Soc. Am.*, 95 (5): 564-569.

Papadopoulos, N.T.; Katsoyannos, B.I.; Carey, J.R. (1998). Temporal changes in the composition of the overwintering larvae population of the Mediterranean fruit fly (Diptera: Tephritidae) in northern Greece. *Ann. Entomol. Soc. Am.,* 91: 430-434.

Papadopoulus, N.T.; Katsoyannos, B.I.; Kouloussis, N.A.; Hendrichs, J.; Carey, J.R.; Heath, R.R. (2001). Early Detection and Population Monitoring of *Ceratitis capitata* (Diptera: Tephritidae) in a Mixed-Fruit Orchard in Northern Greece. Entomological Society of America. *J. Econ. Entomol.,* 94 (4): 971-978.

Rigamonti, I. E. (2004). Contributions to the knowledge of *Ceratitis capitata* Wied. (Diptera, Tephritidae) in Northern Italy I. Observations on the biology. *Bollettino di Zoologia Agraria e di Bachicoltura* 36: 89-100.

Romani, M. (1997). Biology, ethology and control of *Ceratitis capitata* in northern Italy. *L'Informatore Agrario* 53: 59-62.

Shono, T.; Scott, J.G. (2003). Spinosad resistance in the housefly, *Musca domestica* is due to a recessive factor on autosome. *Pestic. Biochem. Physiol.*, 75 (1-2): 1-7.

Xin-Geng, W.; Russell, H.M. (2006). Feeding and attraction of non-target flies to spinosad-based fruit fly bait. *Pest Manag. Sci.*, 62 (7): 933-939.

Zucoloto, F.S. (1993). Acceptability of different Brazilian fruits to *Ceratitis capitata* (Diptera: Tephritidae) and fly performance on each species. *Brazilian J. Med. Biol. Res.*, 26: 291-298.

Acknowledgments

This work was carried out with the Association of Fruit and Vegetable Producers in Trentino (APOT).

I would like to thank my colleagues in the Orchard Trials and Cold-storage Working Group (Foundation Edmund Mach) for their contribution to the analysis of apples' physical-chemical characteristics.

I would like to express my gratitude to my Ph.D supervisor Dr. Claudio Ioriatti, who allowed me to spend these three years at the Fondazione Edmund Mach.

Thanks are due to Dr. Valerio Mazzoni for his assistance and guidance on statistical analysis.

My thanks also go to Dr. Federico Pedrazzoli for assistance with writing the paper and help with English.

I thank Dr. Mario Baldessari for his support, enthusiasm and guidance.

I thank Dr. Gino Angeli for his precious advice.

I thank Dr. Adriana Lucia Escudero-Colomar and everybody at IRTA in Lleida for their warm welcome and for having made me feel at home.

Finally, my special thanks go to my wonderful FAMILY, who never abandon me, and to my boyfriend Michele for his support and love.