

AMERICAN UNIVERSITY OF BEIRUT

TOWARDS SUSTAINABLE MANAGEMENT OF THE
MEDITERRANEAN FRUIT FLY (*Ceratitis capitata*) IN APPLE
ORCHARD IN NORTH OF LEBANON

by
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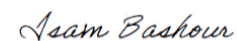
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ABSTRACT OF THE THESIS OF

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Major: Plant protection

Title: Towards sustainable management of the Mediterranean fruit fly (*Ceratit*
capitata) in apple orchard in Bane North of Lebanon.

The Mediterranean fruit fly (*Ceratit*
capitata) Wiedmann, is considered one of the most destructive fruit insect pests because of its wide distribution and host range which includes more than 350 host plants. During the past years, the medfly started causing serious damage on apple orchards in high elevation in Lebanon. Our project aims at elucidating the biological characteristics of the medfly at high altitude and the efficacy of the mass trapping as an alternative control method against the medfly. Three orchards of apple were selected in Bane area, North of Lebanon at three altitudinal gradients, 1100masl, 1200 masl and 1300 masl. The monitoring of the medfly in the region was conducted for five consecutive years 2015 to 2020 using McPhail traps, which were installed each year on May 1 and kept all season until no medfly was captured. Mass trapping experiments were conducted in 2017, 2018, 2019 and 2020 using TMA card consisting of a lure mixture of the synergistically acting attractants, ammonium acetate, trimethylamine chloride, and trimethylamine. Different trapping densities were done each year from one trap per tree to 3 traps per tree to one trap every 3-4 trees on Golden Delicious and Red Starking Delicious apple varieties. A comparison between two mass trapping baiting lures was also conducted on Gala apple variety in 2019.

Results showed variations in peaks of emergence over years on the three different altitudes. The flight patterns extended between 4 months and 5 months and a half depending on the years. There were no distinct peaks in most of the flight patterns indicating the continuous presence of the flies or the overlapping between generations. Mass trapping results in golden Delicious and Red Starking Delicious indicated a clear preference of the medfly towards the white colored apple varieties since the highest infection rates were recorded in this variety (98.54%). The red apple varieties infection rates ranged between 36.36% and 47.19% in the mass trapping techniques. Gala apple variety turned out to be the least infested since its infection rate did not exceed the 16.77% in the conventionally sprayed orchard and 8.96% in the TMA card mass trapping treated trees. Mass trapping did not prove to be an efficacious method of control of traditional apple varieties Golden Delicious and Red Starking Delicious since the infection rate of apples was similar to conventionally treated apples. In the early maturing apple variety Gala, mass trapping proved to be an efficient method since the rate of infection was reduced by half in comparison with the conventionally treated orchard.

Keywords: *Ceratit*
capitata, apple, mass trapping, flight pattern, feed attractant, lure

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ABBREVIATIONS

Technical terms:

MAT	Male annihilation technique
S.A.	Success attract
SIT	Sterile Male Technique
Masl	Meter above sea level

Statistic:

%	Percentage
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Units of measurement:

cm, mm, nm	centimeter, millimeter, nanometer
h, min	Hour, minute
g, mg	gram, milligram
l, ml, μ l	Liter, milliliter, micro liter
$^{\circ}$ C	Celsius degree

CHAPTER 1

INTRODUCTION

The Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann) (Diptera: Thephritidae) is considered one of the most destructive pests worldwide. Medfly is able to attack more than 300 species of plants and has the ability to adapt to a wide range of climatic zones and has an elevated invasive potential (Meats et al. 2007). The Medfly can also cause serious economic losses in orchards, which can be estimated over 2 Million dollars losses annually (Malavasi 2014).

Ceratitidis capitata is originated from the Sub-Saharan Africa but can be found in many tropical, sub-tropical and mild temperate regions worldwide (FAO/IAEA 2013, CABI 2017). They are quarantine restrictions in some countries related to fruit fly infected products (EPPO 2017, CDFA 2017) and eradication programs in new infected areas (CDFA 2003, Enkerlin et al. 2015). Global warming was also a factor in medfly geographic distribution in higher latitudes. This was mainly observed in Northern Italy in 2015(Zanoni et al. 2017), were it was recorded a new niche of the Medfly which was assessed to a climatic change and recorded in new geographic areas were Medfly infestation wasn't recorded before that date (De Meyer et al. 2008, Guitierrez 2011, Papadapoulos et al.2013, Szyniszewska et al. 2014 and Kaya et al. 2017.)

Several researches have agreed that the Mediterranean fruit fly can adapt to cold temperatures in high altitudes (Thomas *et al.*, 2016). Although it may appear as a pest on citrus, it is more a serious pest of some deciduous fruits, such as, pear, peach and apple. However, a study in the central mountain of Israel (Israely *et al.*, 2004) showed that the *Ceratitidis capitata* isn't overwintering in high altitudes. The population of *C.*

capitata is affected by environmental factors, the species and the host plants (Celedonio-Hurtado *et al.*, 1995). However some factors have been found as a key in the fruit fly activities and there is no environmental component that stands out as a determinant of fruit fly abundance of global importance (Bateman 1972).

Climate change is a change in the statistical distribution of weather patterns that lasts for an extended period of time (i.e., decades to millions of years). Climate change may refer to a change in average weather conditions, or in the time variation of weather around longer-term average conditions (i.e., more or fewer extreme weather events). The Mediterranean fruit fly (*Ceratitis capitata*) is one of the most serious pests that can attack around 300 different species of fruits and vegetables. This pest, like all other insects, is forced to adapt to climate changes in order to survive. Once the insect is out of its natural environment, it will adapt to new environment via three essential changes: first one is the local extinction; the second is the permanent colonization and the third is the physiological and behavioral responses (Begon *et al.*, 1990). Scientists and entomologists have agreed that the Med. fruit fly has adapted to all climates around the world and is present now in all continents except Antarctica (Siebert and Cooper 1995). According to (Israely *et al.* 2004) and (Israely and Oman 2005), *C.capitata* is not overwintering in mountains, but it is appearing there in the early summer coming mainly from the coast, since that pest has low probability of resistance to cold areas for overwintering.

In Lebanon, as for other Mediterranean basin (Lebanon, Syria, Jordan, Palestine territory and Israel) medfly attacks all soft skin fruits and some vegetables considered as hosts. Fruit fly damages occur starting April till November was population become

more abundant. From December till mid-March, which is winter season, Medfly population drop in some cases and economic damages remain negligible.

It has been estimated in the Mediterranean basin, if MMF control is ignored, economic losses would reach a total of M 579 \$ per year which is approximately 53% of the total annual revenue of fruit production in those countries (Enkerlin and Mumford 1996, Enkerlin 1997).

In order to study the development of the fruit fly in higher altitudes, such as overwintering and what environmental factors may affect its biological cycle, this study investigates the biological cycle of the medfly and its probability of overwintering in high altitude in Bane, North of Lebanon. Thus three altitudinal gradients will be explored and the main host tree will be apple because of its high infestation during the last years.

In the present research, we address the question of the probability that *C. capitata* has expanded its distribution in the mountains of Lebanon, specifically in the Bcharry area. Beside the overwintering study, we have conducted many experiments related to the mass trapping in apples orchards in order to study its efficiency and try to include it in the integrated pest management program against the Mediterranean fruit fly.

CHAPTER 2

LITTERATURE REVIEW

2.1 The apple (*Malus domestica*) sector in Lebanon

2.1.1 Generalities

The genus of apples, *Malus*, belongs to the subfamily Pomoideae of the Rosaceae family. Another important fruit tree, pear (*Pyrus*), belongs to the same subfamily. There are over 30 primary species of apple and most can be readily hybridized (Korban 1986, Way et al. 1991). The cultivated apple is likely the result of initial domestication followed by inter-specific hybridization (Harris et al. 2002). Its primary wild ancestor is *M. sieversii* whose range is centered at the border between western China and the former Soviet Union. Apples are the main forest tree there and display the full range of colors, forms and tastes found in domesticated apples across the world (Forsline et al. 1994, Hokanson et al. 1997). The domesticated apple has been referred to with the epithet *Malus domestica* (Korban and Skirvin 1984), although Mabberley et al. (2001) proposed that *Malus pumila* should properly refer to the domesticated apple and its presumed wild relative *M. sieversii*. Other species of *Malus* which contributed to the genetic background of the apple likely include: *M. orientalis* of Caucasia, *M. sylvestris* from Europe, *M. baccata* from Siberia, *M. mandshurica* from Manchuria, and *M. prunifolia* from China. It is likely that these species hybridized with domesticated apples as they were spread by humans (Harris et al. 2002).

It is the fourth most extensively produced deciduous fruit crop worldwide (FAO, 2010). The world production is 84630275 tons with 5051851ha planted all over the world, apples are cultivated for commercial use in 89 countries (FAO, 2014). In 2001,

China becomes the leader of the world's apple production followed by the United States of America and Turkey. Apple trees in particular, require cold winter during their dormancy period.

2.1.2 Climatic and Agronomic requirements of apples:

2.1.2.1 Temperature:

Temperature is the main factor related to dormancy, specifically low temperature. The evolution of the apple phenological stages during the vegetative phase, like the induction and flower differentiation, bud break, full bloom, fruit development, maturation and quality, are affected by climatological conditions mainly the temperature. Temperature shouldn't reach below - 15⁰C in Winter and not more than 37⁰C in Summer (Chadha and Awasthi, 2004). Apple tree can grow in high altitude between 1000m and 1500m. The fruit matures in late Summer or Autumn, apple skin can generally be red, yellow, pink, bicolor or green with different shape. Apples need 250 to 1500 hours of chilling 0 – 7 °C depending on the variety.

2.1.2.2 Soil:

The soil favorable pH varies from 5.5 to 6.5 and should be checked every three years. Soil drainage is the most important factor to be considered and aeration should be suitable for cultivation. Apple can grow in a wide range of soil type.

2.1.2.3 Irrigation:

Apple's need in water is high, about 800mm per year. Water quantity needed for the tree is high and important at the first period: It starts from bud break and finishes

when the tree fully develops the first groups of shoots, if the water availability is not adequate the fruit set will not happen.

The second period includes the main water usage stage. Water need is at the maximum because the foliar volume is huge and the heat causes the highest evapotranspiration of the whole growing season.

2.1.3 Lebanese varieties of apples:

The traditional apple varieties planted in Lebanon are introduced by Jesuit monks and from immigrants returning to the country after World War II. The dominant apple varieties are the Starking (60%) as well as Golden Delicious (40%) (Medawar, 2008). These cultivars have put Lebanon's name on the international market as apple producer. However, these varieties are no longer appreciated by international markets. Other strains are more recommended: Royal Gala, Gale Gala, Scarlet Spur, Super Chief, Braeburn, Granny Smith, Sun Fuji and Red Fuji. As we have already shown in the apple trees section, the apple called "Starking" is the most cultivated, where about 60% of the plantations, while the apple called "Golden" about 40% of the plantations. The Starking variety is by far the predominant among red apples accounting for about 60% of the area of red apples while the Scarlet Spur, Early red and Ace varieties account for minimal rates between 2% and 6% of the area planted with apples. red. Other red apples, which are of undefined varieties, account for about 31% of the total area of red apples.

The Golden variety represents approximately 56% of the area of yellow and green apples while other varieties such as Granny Smith, Sans Pareille, Gala and Early Gold each represent minimal rates not exceeding 4% for each. Likewise, there is a

category of other yellow apples corresponding to undefined varieties, which account for about 26% of the total area of yellow apples (Ministry of Agriculture, 2010).

2.1.4 Apple Production in Lebanon:

Lebanon had since 1841 adopted a production which was that of natural silk and this lasted until the thirties of the 20th century, when it was replaced by the production of apples. Lebanese farmers replaced mulberry trees with apples in the early 1940s, and they made a major effort to develop this production in the absence of any state intervention in their favor. “During the two decades of the 1950s and 1960s, the Lebanese agricultural horizon was marked by the cultivation of apples. This production covered more and more areas in the upper and central Mount Lebanon, and also spread to the Bekaa Valley and also to Jezzine. In the absence of competition in the markets of Arab countries during the fifties and sixties, officials did not find it necessary to set up a production system that respects fundamental natural factors to ensure better apple quality, and to benefit from the development of new varieties and modern production techniques, and this continued through the 1970s until and after the war. So the drainage crises started to worsen by the end of the sixties, which led to a reduction in the areas of apples to become about 5000 ha by the end of the seventies, and we note that they were close to 11,000 ha in the middle of the same 1970s (CREAL 2014).

Apple cultivation was characterized by phases of expansion and periods of decline. The evolution of the area under apple cultivation, production and export is subject to year-to-year variations due to climatic, economic and political causes.

According to statistics from the Ministry of Agriculture in 2007, fruit growing is the sector which occupies the largest agricultural area in Lebanon: 135,783 ha, which

forms 53.75% of the arable area and according to FAO-stat the cultivation of apple trees occupies an area of 13,500 ha as observed in the data relating to the regional distribution section of agriculture in the previous part. According to the report of the overall results of the census carried out in 2010 by the Ministry of Agriculture in collaboration with the FAO, it is found that the area planted with apple trees is of 124,246 dunums, in other words 12,424.6 ha, or about 87% of the total area of pome fruits. However, in 2018, the total area of harvested apple was 9,842 ha and the total production was 124,464 tonnes with an average yield of 126,463 hg/ha (FAOSTAT, 2018)

Different statistics can be found regarding the area of apple trees in Lebanon, which proves the evolution over the years. First we will present the regional distribution made by the Ministry of Agriculture in 1960, then that carried out by the FAO in 2004, and also that carried out by coordination of these two actors brought together for data displayed in 2010.

In the reports of the Ministry of Agriculture for the year 1960, the areas cultivated with apple trees were presented during the regional study for each region. The most productive regions were in decreasing order: the Lebanese mountain below 900 m; the Bekaa; the Lebanese mountain between 900 and 1200 m. The two most cultivated varieties including Starking and Golden, with a preference for Starking in highland orchards.

Then, the regional distribution of apple trees that was given by the FAO organization in 2004 showed that apple trees are mainly concentrated in North Lebanon with 45% of the apple tree area, followed by Mount Lebanon where this percentage is 35% .The apple tree constitutes the main fruit crop in the cazas of Jbeil, Bcharré where

the area of apple trees constitutes respectively 58% and 56% of the area of fruit trees in each of these cazas. This percentage is 48% in Batroun and 45% in Kesrouane.

The distribution of the area planted with apple trees by Mohafaza shows a co-dominance at the level of the Mohafazas of Mount Lebanon with 26%, of North Lebanon with 23%, and of Baalbeck-Hermel with 22%, followed by Aakkar with 13% and Béqaa 12%.

The apple crop is distributed for the year 2014 as follows: North: 29.03%; Mount Lebanon: 32.79%; Bekaa: 37.16%; South: 1.02% (CREAL, 2014).

It can be concluded that the percentage change in apple tree areas in the different regions of Lebanon from the year 1994 to 2014 as follows: Bekaa: + 61.9%; Mount Lebanon: - 3.3%; North: - 6.8%, with regard to the South region we have considered the evolution since 2000 (when this region started to plant this tree), and we found a decrease of -23.5% in comparison with 2014. Finally, for the whole of Lebanon, the areas of apple trees increased by 13.6% between 1994 and 2014.

In Lebanon, fruits constitute the largest share of agricultural production (26%), and apples constitute 23% of the total fruit production. Apples are grown on a surface of 13,500 ha producing 264,000 tons, of which only 10–15% according to the Lebanon Industry Value Chain Development (LIVCD) project of the United States Agency for International Development – USAID (2014) are Grade 1 apples and are sold with higher margins.

The apple sector is facing many difficulties which have had a direct impact on the national production of this fruit, which has experienced a decline since 1990 (Ministry of Agriculture). This drop in production is linked to several factors, including the varieties of apples in Lebanon in this section.

In Lebanon, fruits constitute the largest share of agricultural production (26%), and apples constitute 23% of the total fruit production. Only 10–15% of the total apple production according to the Lebanon Industry Value Chain Development (LIVCD) project of the United States Agency for International Development – USAID (2014) are Grade 1 apples and are sold with higher margins. The Lebanese agriculture sector lacks research in harvest and postharvest and technical expertise aiming to develop and recommend good practices for Lebanese farmers to reduce postharvest losses. Inappropriate postharvest practices, transport methods and below-standards cold storage conditions constrain competitiveness of Lebanese apples in export markets which led to a poor reputation of Lebanese produce in these markets. Furthermore, Lebanon does not produce a sufficient quantity of Grade 1 apples to meet domestic and export markets demand. Thus, regardless of high yields, Lebanese farmers are struggling to generate a good income at the end of the season (USAID, 2014). Besides, no research projects dealing with harvesting and postharvest handling practices in Lebanon have been achieved, which makes this work original with regional interest. A recent study by Abi Tarabay et al. (2018) demonstrated that the incidence of bruising is reduced from 93% in the non-treated control to 37% when good harvesting and handling practices are performed.

2.1.5 Pests of apple in Lebanon:

Although a large number of insects and diseases attack apple crops, some of them are very serious and need control measures. Insects and mites cause significant economic losses and can be divided into two groups: the direct insect pests that attack the fruits and the indirect insect pests that attack the leaves, trunk and other parts of the

tree. The most important representatives of the direct insect pests include the Mediterranean fruit fly (*Ceratitis capitata*), codling moth (*Cydia pomonella*). The indirect pests include the mites, leaf miners, aphids and stem borers. While the most important diseases and physiological disorders include scab, powdery mildew, canker and bitter pit. Postharvest diseases are also important and include blue mold, gray mold in addition the aforementioned insects and diseases. Of these pests, only the codling moth will be discussed since it causes an economic damage as the *Ceratitis capitata* which will be discussed in the next part.

2.1.5.1 Codling Moth (*Cydia pomonella*):

Codling moth belongs to the family Tortricidae. This is one of the largest families of moths, with about 950 North American species. It includes a number of important tree fruit pests, e.g., codling moth, oriental fruit moth and several species of leaf rollers. These moths are small, usually gray or brown, and their wings have bands or mottled areas. The front wings are usually square tipped. While at rest, these moths hold their wings roof-like over the body. Codling moth originated in Asia Minor but has been a principal pest of apple and pear in North America for more than 200 years. With the exception of Japan and part of mainland Asia, it is found wherever apples are grown throughout the temperate regions of the world. Codling moth larvae bore deep into the fruit, making it unmarketable. If uncontrolled, codling moth can destroy most of the crop. By the first half of the 20th century, the codling moth was a major pest in all apple growing districts of North America. It was not until synthetic organic insecticides became available in the late 1940s that the codling moth could be maintained at very low levels in commercial orchards (Brunner, 1993).

2.1.5.2 Main hosts

Codling moth prefers apple but also attacks pear, large-fruited hawthorn and quince. In California, races of codling moth attack prune and walnut. Pears have some natural resistance to attack by codling moth when fruit are small because of their hardness, however, pears can become heavily infested in late summer as they mature. Infestations in stone fruits such as apricot and cherry are extremely rare and usually occur only where heavy infestations of apple or pear are nearby. (Brunner 1993)

2.1.5.3 Life stages:

Egg:

The codling moth egg is oval, flat and, when first laid, almost transparent. It is about 1/12-inch (2 mm) long. Eggs are laid individually on leaves or fruit and are very difficult to find, especially in a commercial orchard (Brunner, 1993).

Larva:

The newly hatched larva is only about 2 to 3 mm long. Its head is black, and the body is creamy white. The full grown larva is 12 to 20 mm long, has a brown or black head capsule and thoracic shield. The body is usually creamy white but turns slightly pink when mature. Unlike other caterpillar larvae that feed on the flesh of the fruit, such as oriental fruit moth and lesser apple worm, the codling moth larva burrows through the flesh and feeds primarily on seeds. When mature the codling moth larva exits the fruit and searches for a sheltered location on the tree or at the base of the tree and spins a cocoon (Brunner, 1993).

Pupa:

The codling moth pupa is brown and about 12 mm long. It resides inside a cocoon spun by the mature larva on the tree beneath bark scales or in a sheltered place at the base of the tree. (Brunner, 1993).

Adult:

The adult codling moth is about 12 mm long. At first glance, it seems a nondescript dull gray, but closer inspection shows the wings are crossed with fine alternating gray and light-colored bands. The wings are tipped by a patch of bronze-colored scales that reflect in sunlight. The moth holds its wings tent-like over its body when at rest (Brunner, 1993).

2.1.5.4 Life history:

The codling moth spends the winter as a mature larva in a cocoon. Larvae are found under loose bark scales on the tree, in litter at the base of the tree, in wood piles, on picking bins in the orchard or on farm buildings near packing sheds where culled apples might have been dumped. Overwintering larvae begin changing into pupae early in the spring prior to the opening of blossoms. The first adult moths begin to emerge around the time of full bloom of Red Delicious. Peak emergence is usually 17 to 21 days later, though this depends on temperature. Adults continue to emerge for 6 or 7 weeks. Moths are most active on warm evenings, but are inactive at temperatures below 18°C. Moths mate and begin laying eggs within a day of emerging. First generation eggs are laid primarily on leaves, although some may be found on fruit. Eggs require 8 to 14 days to incubate.

Newly hatched larvae find fruit and enter either at the calyx end or through the side. They bore through the skin and feed on the fruit flesh for a few days, then move towards the apple core where they feed on seeds and flesh surrounding the seeds. As they feed, they push excrement out of the apple through an entry hole, which is gradually enlarged and often serves as an exit hole. Larvae are fully grown in three to four weeks, at which time they leave the fruit in search of sheltered places to spin cocoons. In our region, most larvae pupate and, in two to three weeks, emerge as second-generation adults. However, a small percentage of first generation larvae enter diapause, a state of arrested development, and do not emerge as adults until the following spring. Second generation adults usually begin emerging in early July. Adult activity peaks in mid-July to early August and continues into early September. Second generation larvae are in the fruit from mid-July until late September. Mature larvae of the second generation leave fruit as early as mid-August in search of overwintering sites. It has become more common to have a third codling moth generation and, in exceptionally warm years, a partial fourth generation. Moths representing a third flight emerge in late August or early September and deposit eggs. While larvae of the third generation enter fruit, causing severe levels of crop loss in some instances, most do not complete development before winter conditions arrive or fruit is harvested (Brunner, 1993).

Damages:

Injury is caused when larvae feed on fruit. There are two types of damage: stings and deep entries. Stings are shallow entries where a larva burrows into the flesh and then dies or a larva briefly feeds at a location then abandons that site and moves to another location. On more mature fruit, apple or pear, a reddish colored ring often forms

around a new entry or sting. Deep entries occur when a larva bores through the flesh of the fruit, eventually arriving at the center of the apple or pear where it feeds primarily on seeds. Deep entries are often characterized by brown frass, or excrement, extruding from an enlarge entry hole or a new hole destined to as an exit for the mature larva. In pear, deep entries are most often noticed when frass appears at the calyx end of the fruit. Both types of damage make fruit unmarketable, but deep entries are a problem in stored fruit because bacteria and fungi associated with the entries enhances fruit rot (Brunner, 1993).

2.2 The Mediterranean fruit fly (*ceratitis capitata*)

2.2.1 Generalities

The Mediterranean fruit fly belongs to the family Tephritidae which comprises roughly 4300 species in almost 500 generations and of these insects; about 1,400 species are known to develop in ripening fruits. More than 250 species are known to attack fruits that are either grown commercially or harvested from the wild (Penarrubia Maria, 2010). Although they are commonly known as fruit flies, larval development can occur in other parts of the host plants, such as flowers and stems. The wings of these flies are covered with patterns of variable size globally distributed along the wing (White and Elson-Harris, 1992).

The genus *Ceratit* Macleay, 1829 is endemic to tropical Africa (also known as the Afrotropical Region, in the Southern Sahara) belongs to the tribe Ceratitidini and contains about 65- 95 species, the majority of which are highly polyphagous. The description of species has been revised over the last two decades (White and Elson-Harris, 1992, De Meyer 1996, 1998, 2000; De Meyer & Copeland 2001; De Meyer

& Freidberg 2006). Additional descriptions of species may raise this number to 100 (De Meyer *et al.*, 2016).

Ceratitis capitata is the most widespread member of the Tephritidae family (White and Elson-Harris, 1992), with a worldwide distribution and has been recorded in 132 countries so far and more than 400 plant species have been reported as host (Malacrino *et al.*, 2018).

Over time, medfly has had several different synonyms (White and Elson-Harris, 1992) (ITIS, 2010): *Tephritis capitata* Wiedemann (1824), *Trypeta capitata* (Wiedemann) (1824), *Ceratitis citriperda* MacLeay (1829), *Ceratitis hispanica* De Brême (1842) and *Pardalaspis asparagi* Bezzi (1924). Nowadays, the taxonomic hierarchy of medfly 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

2.2.2 Distribution in the world:

The Mediterranean fruit fly (*ceratitis capitata*) (Wiedemann 1824) is one of the most destructive insect pests of fruit trees in the world (Liquido *et al.*, 1994).

Worldwide, the larva is attacking over 400 species (Karpati, 1983; Penarrubia Maria, 2010; Malacrino *et al.*, 2018). The Mediterranean fruit fly is a cosmopolitan pest (Penarrubia Maria, 2010; Christenson and Foote, 1960). Originating in Sub-Sahara of Africa (Kourti *et al.*, 1992); it has successfully spread in all the continents during the 20th century (Harris, 1977) except Antarctica. It reached the 41° north in latitude (Fisher-Collobrie 1989). The Med fly has an ability to tolerate cooler climates better than most other species of tropical fruit flies and it ranked first among economically important fruit fly species. Laboratory and field works have studied Medfly resistance to low temperatures (Carante et Lemaitre, 1990). Although laboratory studies have showed that the fly is sensitive to low temperatures (Messenger et Flitters 1954) and some field studies showed the capacity of small survive of population even under subfreezing temperatures (Greenberg 1954).

The medfly is established in the western hemisphere in Argentina, Bermuda, Brazil, Costa-Rica and Uruguay. It occurs in Western Australia, in many countries in Europe and Africa and the Mediterranean area such as Lebanon, Palestine, Syria and Turkey (Diaz 2007).

2.2.3 Life cycle

The medfly has a complete metamorphosis (holometabolic) and passes through four stages of development: egg, larvae, pupa and adult. A female will lay 1 to 10 eggs in egg cavity of 1mm deep, and can lay around 22 eggs per day and a total number of

around 800 eggs during lifetime. Eggs are usually deposited under the skin of fruit that is just beginning to ripen, and the deposition is more often in areas where fruits are previously damaged. The threshold of egg development occurs at 11°C (Shoukry and Hafez, 1979). The egg stage lasts two days (Boller, 1985). In one spot, females may deposit 75 or more eggs. When the eggs hatch, larvae will start eating and remain close to each other till the full grown larvae. Recent studies indicated that the host plant influenced the percentage of average daily laying of female flies. In a non-choice test, the highest mean eggs was observed on persimmon fruits (6.95 eggs per female per day) whereas it was 0 on Valencia orange and 1.41 eggs per female per day on clementin Manadarin, In the choice test, the number of eggs was higher on peaches (4.74 eggs/female/day) than red apples (1.15 eggs/female/day) and golden smoothie apple (0.56 eggs/female/day) (Naserzadeh et al., 2019). They also reported an egg hatching percentage of 93.65% for clementin mandarin and 90.3% for red apple.

The first larva instar is 2 mm and the third has an average length of 6.5 - 9 mm and width 1.2 - 1.5 mm (White and Elson-Harris, 1992). These larvae destroy the pulp of the fruits with their chewing mouthparts (Domínguez, 2007). The zero point of larval development occurs at 5°C (Shoukry and Hafez, 1979) and the entire larva stage lasts 7 - 8 days (Boller, 1985). The host will affect the development time of the larval stage. For example the larval stage development in citrus takes around 14 to 26 days while it only takes 10 to 15 days in green peaches. Larvae leave the fruit in most of the times to pupate in soil. Naserzadeh et al. (2019) reported a total larval developmental time of 8.92 days for Valencia orange, 10.4 days for peaches, 10.68 days for clementine mandarin, and 12.04 days for persimmon, 14.84 days for golden apple smoothie and 24.08 days for red apple.

The cylindrical pupa is 4 to 4.3 mm long and dark reddish brown in color. Generally, the pupal stage occurs at temperatures between 22°C and 30°C, with 35°C being fatal. At 60% R.H. the threshold of pupal development is 13°C (Shoukry and Hafez, 1979) and this stage lasts 9 - 10 days (Boller, 1985). Other studies demonstrate that at 26°C the pupal stage lasts 10 - 11 days and if the conditions are not favorable because of the low temperatures, this stage can be extended by several days (Mavrikakis et al., 2000). Naserzadeh et al. (2019) reported a pupal developmental time of 7.76 days for peach, 8.28 days for red apple, 9.72 days for golden smoothie apple, 12.04 days for Valencia orange, 12.12 days for clementine mandarin and 13.84 days for persimmon.

The adult medflies will emerge in the morning and the body size is 4 mm and the wing length is 4.1 mm (De Meyer, 2000). They are black and white with a yellow abdomen and yellow marks on the thorax and their wings are banded with yellow (Bergsten et al., 1999). Both sexes can be easily separated from all other members of the family: the adult males have a black pointed expansion at the apex of the anterior pair of orbital setae and the females have a characteristic yellow wing pattern and the apical half of the scutellum is entirely black (White and Elson-Harris, 1992). Under laboratory conditions, males live an average of 36 days at 25°C, while at the same temperature female longevity is 31 days, and longer when reared without males (67 days).

The new emerged adults are not sexually mature. Males show their sexual activity four days after emergence and both sexes are active through the day. When the daily mean temperature is 25°C, most females are ready to mate 6 to 8 days after eclosion. Oviposition will take place four to five days after emergence during warm weather and from eight to ten days when temperature is 21°C (Boller, 1985).

Medfly adults are unable to resist cold in some Mediterranean areas (Papadopoulos *et al.*, 1996; Penarrubia-Maria *et al.*, 2012). The susceptibility to cold is affected by the microhabitat moisture, developmental temperature and desiccation (Danks, 2006). In Greece, adults could stay alive the whole winter season even though the temperatures were between 1°C and 4.5°C (Mavrikakis *et al.*, 2000). In the study conducted by Penarrubia-Maria *et al.* (2012), all adults died during winter where the minimum temperature were between -2°C and -8°C. the medfly has a temperature threshold between 12°C and 35°C (Vera *et al.*, 2002). Other studies conducted on the fruit fly *Dacus tryoni* showed that mortality was directly linked to the minimum temperatures and mortality rate increased at subzero temperatures (Fletcher, 1979).

The types of fruit largely affect the biological and reproductive characteristics of the medfly (Dias *et al.*, 2018; Naserzadeh *et al.*, 2019). However, the ratio of male to female individuals was not affected (Naserzadeh *et al.*, 2019). The factors that affect the biological characteristics are the thickness of fruit skin (Dias *et al.*, 2018, Manrakhan *et al.*, 2018), fruit color (Papadopoulos *et al.*, 2015), fruit size (Aluja *et al.*, 2018), the relative humidity and the quality of fruits (Falchi *et al.*, 2015).

2.2.4 Overwintering of the Mediterranean fruit fly:

Insects, like other organisms, are forced to deal with environmental variability in both time and space. Climatic variability is the main source of limitation, impeding organisms from further expansion away from their origin. Once an insect species finds itself out of its original habitat, three paths can be taken: 1) local extinction, 2) permanent colonization or 3) evolutionary, physiological and behavioral adaptation. E.g., migration and cold withstanding mechanisms (Begon *et al.* 1990). Hence,

dormancy and migration are alternative mechanism strategies for dealing with environmental variability (Levin 1992). The first escape is an escape from the original environment, second escape become the new environment by escape in space (Begon et al. 1986). In temperate zones, climatic conditions are the most important factors for instability in space and time (van Emden and Williams 1974). Therefore, the ability of tropical and subtropical multivoltine, nondiapausing insect species to colonize the temperate zone depends upon their ability to endure local cold weather by cold withdrawing or behavioral mechanisms. Developing a behavioral or physiological mechanism is believed to be a lengthy evolutionary process, which may eventually lead to sub speciation or formulation of a new species. Hence, one may conclude that most invading pests that successfully colonize new climate regions have been pre adapted for it.

Three hypotheses have been proposed to explain how the Mediterranean fruit fly survive winter in temperate climates. First, they can survive as adults (Papadopoulos et al. 1996, Katsoyannos et al. 1998, Papadopoulos et al. 1998, Israely et al. 2004, Peñarrubia-María et al. 2012, Ricalde et al. 2012). In Southern Greece, e.g. Mavrikakis et al. (2000) found that the Mediterranean fruit fly adults survived absolute minimum temperatures of 1 and 4.5 °C; though they were unable to survive in Northern Greece where mean absolute minimum temperature can fall to -5.9°C in winter (Papadopoulos et al. 2001). Bateman (1972) suggested that overwintering adults congregate in refuges that provide shelter and food such as citrus and may become active during the day to feed. Second, in areas with subfreezing temperatures where adults cannot survive, eggs and larvae overwinter in citrus (Katsoyannos et al. 1998, Mavrikakis et al. 2000, Sproul 2001) and apple (Papadopoulos et al. 1996), and as pupae in the soil (Papadopoulos et

al. 2010). In Northern Greece, Papadopoulos et al. (1996) found that the larvae remained inside apples left on the tree during winter. Third, annual reinvasion occurs from nearby favorable areas (Israely et al. 2004, Peñarrubia-María et al. 2012). Israely et al. (2004) hypothesized that the Mediterranean fruit fly does not overwinter in Central Mountain in Israel where temperature fall below 0°C, but reinvade from neighboring warmer locations. Broughton et al. (2015) predicted that in WA's mild winter in which temperature can briefly reach °C Mediterranean fruit fly is present in all life stages and adults are active and can oviposit if suitable host is present.

The Mediterranean fruit fly (*Ceratitidis capitata*), like other tropical and subtropical multivoltine, nondiapausing tephritids, is not known to possess either cold tolerance or diapausing ability (Christenson and Foote 1960; Greenberg 1960, Bateman 1972, 1976). Thus, its annual seasonal presence in areas with subfreezing winter temperatures remains unexplained. Laboratory and field work have studied Mediterranean fruit fly low-temperature survival (Carante and Lemaitre 1990). Although laboratory studies have concluded that the fly to be highly sensitive to low temperatures (Messenger and Flitters 1954), field studies have claimed that a small percentage of the population manages to survive the winter even subfreezing temperatures (Greenberg 1954).

One of the most significant studies concerning the Mediterranean fruit fly overwintering mechanism in cold region took place in Northern Greece, close to the northernmost boundary of its distribution limit. Papadopoulos et al. (1996, 1998, and 2002) suggested that the Medfly overwinters mainly in late apple, and they predicted that adults would emerge from March to May accompanied by a simultaneous infestation of early hosts. To support this hypothesis, traps were hanged intensively to

capture overwintering flies and detect summer infestations. The first adult male captured and detected from mid June 1994 and early July of 1995 and at the end of June of 1998 (Papadopoulos et al. 2000). The first infested host was detected in September 1998 (Papadopoulos et al. 2001). These findings did not support the hypothesis of “over wintering”

2.2.5 Hosts and damages

The Mediterranean fruit fly attacks more than 400 fruits, vegetables and nuts (Liquido et al. 1991; Penarrubia Maria, 2010; Malacrino et al., 2018). Thin-skinned and ripen fruits are preferred. Host preferences vary in different regions. For example, in Lebanon, this fly is attacking citrus family, stone and pome fruits. *C. capitata* have the ability to recognize migrate to and exploit fruit parts of supreme nutritional value (Zucololto 1987, 1991). Attacked fruits will often have puncture marks made by the female’s ovipositor. Sometimes there may be some tissue decay or secondary rot around these marks and some fruits with high concentration of sugar such as peach, there will be some exudates sugar globules visible around the ovipositor puncture (White and Elson-Harris, 1992). Rotting will cause decomposition on the surface and cause a decomposition of plant tissue by invading microorganism (Diez, 2007).

The damages caused by the Mediterranean fruit fly in some countries are serious to the point of impossibility of commercial production (Follett et al., 2019). In California alone, the financial loss is about \$1.1 billion. In Europe, the damage exceeds 70% in Tangerine production (Nasezadeh et al., 2019).

2.3. Management of Mediterranean fruit fly:

2.3.1 Mechanical and monitoring control

2.3.1.1 Fruit bagging

Fruit bagging was an alternant technique in plant protection, in order to exclude pests, reduce chemical residues and bird damage and improve fruit quality (Bentley and Viveros 1992, Kitgawa *et al.* 1992, Hofman *et al.* 1997, Joyce *et al.* 1997, Tyas *et al.* 1998, Amarante *et al.* 2002, Xu *et al.* 2010 Leite *et al.* 2014, Sharma *et al.* 2014); it has been applied all over the world, in this technique fruits or fruit branches are bagged on the tree for a specific period.

The simple action of wrapping individual fruits has proven effective at preventing fruit infestation by fruit flies. Wrapping materials can be newspaper, paper bags or polyethene sleeves in this case of long/thin fruits (Sarker *et al.* 2009). This system also provides physical protection from mechanical injuries (scars and scratches) and in some case reduces fungal spots on the fruits.

Bagging the fruit is a technique applied after fruit picking whereby eggs and larvae are destroyed and is considered as the most effective mechanical control. The objective of this technique is to destroy pre-imaginal stages of the fruit fly founded in the infected fruit. This technique requires a high temperature to destroy eggs and larvae. This method is applied in black bags without holes and demands labors to pick the infected fruits and put them in the black bags and expose them to the sun for 48 hours in order to destroy eggs and larvae. That kind of techniques can reduce the parasitic pressure in field and increase the efficiency of the bait (GF-120) when the population of the fruit fly is high. But, this technique has one disadvantage which is the high number of labor that requires (IITA-CIRAD 2009).

Bagging, a physical protection technique, not only protects fruit from pests and diseases but also effects the quality of the produce by changing microenvironment of fruit during development (Son and Lee, 2008). Bagging of different fruits during development can reduce the chances of physical damage; improve color at harvest (Byers and Carbaugh, 1995; Muchui *et al.*, 2010) and yields high quality fruit (Kitagawa *et al.*, 1992). Several countries have adopted this technique to control the damage caused by fruit fly. The pre harvest bagging reduces agrochemical residual effects, prevents sunburn, decreases the mechanical damage and controls the insect pest damage in the fruits (Amarante *et al.*, 2002a). In Taiwan this practice is regularly used to protect different fruits (mango, passion fruit and guava) from oriental fruit fly *Bactrocera dorsalis* (Lee, 1988).

This technique was reported as a successful control measure against the fruit fly for different types of cucurbits (Fang 1982). It is extensively used in Asia in apple commercial orchards because it's labor intensive, it is only recommended in the United States of America on small scale orchards (Grasswitz and Fimbres 2013) or in high value markets (Bentley and Viveros 1992).

2.3.1.2 Handpicking

The use of human's hands to remove harmful insects is a common action applied by farmers. It's also classified as the most direct and quickest way to remove clearly visible pests on hosts. However, this method has it disadvantages especially that require high number of labors, and the damages can't be noticeable all time for the farmers (Sarwar *et al.*, 2015).

2.3.1.3 Insect vacuum

The use of vacuum to remove insects is growing up in popularity especially among commercial producers. These tools contain cartridge with sticky gel to trap insects sucked up by the vacuum machine. Vacuums can also remove the larvae stages. This method can be applied on leaves but caution is needed to protect leaves from diseases (Sarwar 2015).

2.3.1.4 Water pressure Sprays

A forceful stream of water can sometimes dislodge insects such as adult flies from fruits foliage and stems. This practice should be repeated since many of the adult flies' returns to hosts. This technique should only be applied on sturdy plants to avoid damages. This method is also a problem by itself since the huge usage of water and by increasing that use; it can cause some roots problems. Therefore, the use of water pressure sprays should only be applied in early morning. (Sarwar 2015).

2.3.1.5 Mechanical Control Trapping

It includes other trapping system. Sticky fly papers are a kind of traps. Ultraviolet light traps are other tools, and used in commercial localities. These traps should be well located in target areas. This type of traps should be placed where it cannot be seen from the outside of the farm and be placed 2.5-3m above the floor. This kind of traps should be changed once per year but it can help a little bit since the flies are more attracted to host's odor to the ultra violet light. (Sarwar 2015)

2.3.1.6 Field sanitation

The cultural control is related to the field sanitation. Field sanitation is performed by the destruction of all infested fruits. The infested fruits should be buried 1m under the soil with the addition of sufficient lure to kill the larvae. The weekly fruit harvest also can reduce food sources due to the fact that large population of medflies may develop when keeping quantities of ripening fruits on trees.

2.3.1.7 Chemical Control:

Although sterile insect (SIT), mass trapping and mating disruption technologies have been employed extensively to manage tephritide fruit flies, use of chemicals has been a principal tool in many control practices worldwide (Roessler 1989). Use of baits and cover sprays remains the most common strategies.

It is from the moment when the fruit is ripe that, fruit will be sensitive to any *C. capitata* attacks. Currently, organophosphate (e.g. chlorpyrifos-methyl) and pyrethroids (e.g. lambda-cyhalothrin), and Spinosad mixed with the protein baits are applied in medfly treatment.

As insecticides been applied near harvest in order to control the pest, there's a high risk of a pesticide residue in fruits at harvest that has negative effects on the fruit market itself and it can result in secondary outbreaks of other pests, such as Tetranychidae mites (Gerson and Cohen 1989). Up to eight sprays can be applied during one season (Chueca, 2007). However, chemical treatment may not guarantee a fruit protection from the medfly.

Malathion bait sprays are used to help eradicate Mediterranean fruit fly invasions, but their use have been controversial because of human health concerns

(Flessel et al. 1993, California department of Food and Agriculture 1994, Marty et al. 1994) and harmful for beneficial insect like bees and natural enemies (Troetschler 1983, Ehler and Endicott 1984, Hoy and Dahlsten 1984, Cohen et al. 1987, Daane et al. 1990, Hoelmer and Dahlsten 1993, Messing et al. 1995).

Resistance to malathion has been identified in Spanish field population of Mediterranean fruit fly (Magaña et al., 2007). Results showed that field treated with malathion, Mediterranean fruit fly were more resistant than none treated field, and hundred times tolerant in population susceptibility comparison.

For example, in Tunisia, most of the currently applied methods are frequently based on the use of organophosphate, insecticide especially Malathion mixed with based protein-baits. (Barhouch et al., 2010). Malathion aerial sprays are very common, and it's only carried by the ministry of agriculture mainly between September and November with an average of 3 sprays. Besides many growers will add some ground-malathion treatments. (Khder et al., 2012). Resistance to Malathion was reported in *C. capitata* populations in Spain. These insecticides were found to have negative impacts on beneficial insects (Michaud, 2003) and can result in secondary outbreaks of other pests (Gerson et al. 1989).

The overuse of organophosphate insecticides has been implicated in the secondary pest outbreaks, negative effects on beneficial insects, environmental contamination and it affects human's health (Carson 1962, Hoy and Dahlsten, 1984 ; Emden and Peakall, 1966). According to a study made in fields, many *C. capitata* females depending on the physiological stage have avoided to enter the malathion trap (Vargas et al., 2001) since malathion can kill the insects by contact, vapor action or a

stomach poison (Matsumura, 1975). Consequently, the replacement for the compounds was sought.

After intensive use of malathion and organophosphate insecticides in pest control, resistance mediated by alternations in the Ache has been selected in many insects species (Oakeshott et al., 2005).

Finding malathion replacements has been identified as a primary concern for agencies such as California's fruit fly programs (Dowell 1995, Buchinger 1996). Two possible malathion replacement; spinosad and the photoactive dye phloxine B, are used under consideration.

Spinosad, an insecticide derived from the metabolites of the soil bacterium, which may reduce the insecticides residues in fruits is currently used and is somehow replacing the malathion (Chueca et al. 2007). This insecticide has a better environment profile and less toxic to natural enemies compared to malathion (Urbenja et al., 2004; Williams et al., 2003; Sparks et al., 2001) and it has showed a good control of different Tephritid pests worldwide (Burns et al., 2001; Profsky et al., 2003) as for Lepidoptera (Adàn et al., 1996, King and Hennessey 1996, Sparks et al., 1998). A study in Tunisian orchards, showed that spinosad bait has a double efficiency comparing to malathion, and that spinosad is more efficient on *C. capitata* (Braham et al., 2007) and *Bractocera dorsalis* (Vargas et al., 2003). Spinosad was found to be a good alternant to Malathion to control *C. capitata* and *C. rosa* (Manrakhan et al., 2013). In addition, spinosad can be used due to its ecological profile (Thomas et al., 2005). No insecticides residues are found after the use of spinosad (GIF, 2014) and it has a low toxicity to honey bees and several natural enemies (Stark et al., 2004; Urbaneja et al., 2009; Vargas et al., 2001). Spinosad is recommended on citrus in order to control *C. capitata*

and it can be applied on well managed prickly pears and figs orchards (Miranda et al., 2004).

Kaolin is also a potential alternative pest management method that's safe and reduces environmental impacts. Kaolin, a white non-porous, chemically inert, non-swelling, low-abrasive, and fine grained aluminum-silicate mineral that can easily be dispersed in water (Glenn and Purteka, 2005). Kaolin is used to protect plants from insects and pests as well from heat sunburn and stress (Glenn et al., 1999; Glenn and Purteka, 2005; Melgarejo et al.2004; Wand et al., 2006).

The formulated WP of Kaolin product was able to show the effective against insects and diseases (Glenn et al., 1999; Knight et al., 2000; Saour and Makee, 2003; Mazor and Erez, 2004; Saour, 2005) in particular on pear psylla, *Cacopsylla pyricola* (Forester) and *C.pyri* (L.) (Puterka et al., 2000; Pasqualini et al., 2002), olive fruit fly, *Bactrocera oleae* (Gmelin) (Saour and Mackee, 2003) and *Ceratitidis capitata* on nectarine, apple and persimmon (Mazor and Ertez, 2004).

Phloxine B is a photoactive dye effective against a variety of insects (Heitz, 1995). When an insect ingests the dye and it's exposed to light, the dye will cause a death for the insect. Phloxine B has no contact toxicity against fruit flies and it's considered to have a little impact on beneficial insects (Dowell, 1997).

In Spain, Mediterranean fruit fly, has been traditionally managed by pesticides applications against flies (Urbaneja et al., 2009) since it's the only stage that can chemically be monitored. Chemical control is still widely used on citrus against *C. capitata* (Urbaneja et al., 2011), although its use poses serious problems, not only the negative effect that affect natural enemies (Ehler and Endicott, 1984; Urbaneja et al., 2004) or it will generate resistance problems (Magana et al. 2007), but also the presence

of pesticides in the fruit can make them unmarketable (Fernandez et al., 2001; Ortelli et al., 2005).

2.3.1.8. Biological control:

Between 1947 and 1952, Hawaii has introduced thirty-two species and varieties of natural enemies to control fruit flies. However, only three species “*Opius Longicaudatus* var. *malaianesis* Fullaway”, “*O. vandebooschii* Fullaway” and “*O. oophilus* Fullaway” have become established. These parasites are primarily effective on the Mediterranean fruit fly in cultivated crops (Diez, 2007).

In 2007, Diez introduced a number of parasites into Hawaii to control Medfly. The most important were the braconid wasps, *Opius humilis* and *Diachamsa tryoni*. Later, parasites of the Oriental fruit fly, *Dacus dorsalis* (Hendel) were found destroying the Mediterranean fruit fly. They are *Biosteres oophilus*, *B. vandenboschi*, and *B. longicaudata* listed in order of effectiveness (Diez, 2007).

2.3.1.8.1 Bioesteres oophilus

The Mediterranean fruit fly, *Ceratitidis capitata* (Wiedemann), and the oriental fruit fly, *Dacus dorsalis* (Hendel), became established in Hawaii in 1910 and 1944, respectively. Many entomophagous insect species have been introduced since then to suppress the population of the 2 pests (Back & Pemberton, 1918; Bess, 1953; Bess et al., 1961). Since 1950 the egg larval parasite, *Biosteres oophilus* (Fullaway) (= *Opius oophilus*), has been reported to be the dominant parasite of *D. dorsalis* and *C. capitata* in Hawaii (Van den Bosch & Haramoto, 1951; Bess et al., 1961). Haramoto & Bess (1970) reported that during 1951-70 parasitism averaged 60 % in host samples from guava fruits (*Psidium guajava* L.) and as high

as 100 % from most samples of coffee (*Coffea arabica* L.) berries. The effectiveness of this parasite in Hawaii has stimulated considerable effort to introduce it into other areas for the control of fruit flies. Efforts to mass rear *B. oophilus*, which is an arrhenotokous species, were made by Haramoto (1953), Finney (1953), Snowball et al., (1962), and Chong (1962), but they were not successful because of the high percentage of male produced. Haramoto (1953) reported that when this parasite was reared from field-collected guava and papaya fruits, a ratio of 1.4:1 was obtained, but when reared in the laboratory, usually only male were produced probably due to a lack of mating in captivity.

2.3.1.9 The insect sterile technique (“SIT”)

The Sterile Insect Technique (SIT) is amongst the most non-disruptive pest control methods. Unlike some other biologically-based methods it is species specific, does not release exotic agents into new environments and does not even introduce new genetic material into existing populations as the released organisms are not self-replicating. However, the SIT is only effective when integrated on an area wide basis, addressing the total population of the pest, irrespective of its distribution. There has been considerable progress in the development and integrated application of the SIT against the Mediterranean fruit fly (medfly), *Ceratitidis capitata*, as reflected by operational programs for prevention, suppression and eradication of this pest. There is however, considerable scope for improving the efficiency of medfly SIT, an indispensable requirement for increased involvement of the private sector in any future application. One way to achieve this has been the development of genetic sexing strains, making it possible to release only sterile males. Another is improving sterile male

performance through a better understanding of the sexual behavior of this insect. Unlike other insects for which the SIT has been successfully applied, medfly has a complex lek based mating system in which the females exert the mate choice selecting among aggregated and displaying wild and sterile males.

The application of SIT against medfly focused initially on the concept of eradication, following the successful example of the screwworm, *Cochliomyia hominivorax*, which over the last fifty years has been eradicated from the U.S., Mexico and recently also from all of Central America and most of Panama (Wyss, 2000). A number of medfly SIT eradication programs have eliminated populations of this species, succeeding in the establishment of medfly free regions or whole countries. The first large SIT program against medfly was initiated in southern Mexico in 1977, with the construction of a 500 million sterile fly mass rearing facility in Tapachula. The aim of the Moscamed program was to prevent the spread of medfly, which had become established in Central America, into Mexico and the U.S.A. Establishment of medfly in Mexico would have threatened a multi-million fruit and vegetable export trade with the U.S.A. The program succeeded in 1982 in eradicating medfly from areas it had already infested in southern Mexico (Hendrichs et al., 1983) and since then a sterile fly barrier has been maintained from southern Belize through Guatemala to southern Mexico to assure the fly-free status of Mexico, U.S.A. and a large part of Guatemala (Vil-laseñor et al., 2000).

Even though various workers had studied various aspects of medfly behavior in the pre-SIT era (Féron 1962), it was the implementation of SIT that stimulated most research into medfly sexual behavior. Concerns about whether mass-reared, or even any laboratory-reared, medfly strains were exhibiting wild-like characters in terms of sexual

behavior and competitiveness motivated research as early as the 1970s. Pilot SIT activities against medfly in Central America, Hawaii, Israel and elsewhere resulted in assessments of the mating competitiveness of irradiated and non-irradiated medflies (Causse, 1970; Holbrook & Fujimoto, 1970; Fried 1971; Rössler 1975), and the publication of a collection of quality control tests for fruit flies in general (Boller & Chambers 1977), which included various tests relevant to medfly mating behavior.

Further refinements have come with behavioral studies in the open field to validate the med-fly behaviors observed on field-caged host trees (Hendrichs & Hendrichs 1990; Hendrichs et al. 1991; Whittier et al. 1992). Other studies addressed many other aspects including the various effects of mass rearing (Calkins et al., 1994; Calkins et al., 1996), male size (Orozco & Lopez, 1993), nutritional status (Blay & Yuval, 1997), mating induced changes in female behavior (Jang, 1995; Jang et al., 1998), strain differences (Liedo et al., 1996), and behavioral incompatibility between wild and mass reared flies (McInnis et al., 1996). The conclusion from all these studies has been that although sterile mass reared medflies do join and compete within leeks, achieve a portion of mating with wild females, and transfer sperm and induce female refractoriness and sterility in off spring, they are clearly less competitive than their wild counterparts. These behavioral changes appear not to be caused by mating incompatibility among different medfly populations (Cayol, 2000a), but rather by mass-rearing conditions, the irradiation process and the years a strain is held in colonization (Cayol, 2000b).

2.3.1.10 The Mass trapping technique as a control measure

The mass trapping system attempts to provide a successful pest control, and it can prevent the pest problems early. This technique can reduce as much as possible the foraging adult medfly population in the area, by hanging on trees, traps baited with lure and toxicants to catch the flies.

Several studies have proved the efficiency of this method (Agunloye 1987; Ros et al., 2002; McQuante et al., 2005) for several fruit trees species. Recent studies demonstrate that the McPhail pheromone trap baited with Biolure (three-component food-type attractant) is highly efficacious in capturing adult fruit flies (Gazit et al., 1998; Katsoyannos et al., 1999b; Katsoyannos and Papadopoulos 2004). The combination of traps also provided satisfactory results in stone fruits orchards (Batlorri et al., 2005). Indeed, mass trapping technique proved to be a powerful method to control *C. capitata* and it has increased in the Mediterranean countries and has been used as a control method (Navvaro-Llopis et al., 2008).

The mass trapping however is being used in large areas of the Mediterranean basin to control also the olive fruit fly, *Bactrocera olea* (Gmelin) (Delerio, 1989; Broumas et al., 2002).

These technique and lure-and-kill methods are now being used worldwide with good results (Cunningham et al. 1978, Agunloye 1987, McQuate et al. 2005).

Cohen and Yuval (2000) pointed out that the perimeter trapping strategy results to avoid fruit fly damages in medium to large orchards depends on the efficacy of traps and lures.

Many studies showed the efficacy of trapping using female targeted attractants that depend on the type of traps. Gazit et al. (1998) indicated that trap type modifies the

proportion of females' capture. Navvaro-Llopsi et al. (2008) reported that the best way to capture the highest numbers of females is by using traps. The dispenser attractants consisted of ammonium acetate and the trimedlure which were the best for insect catches (Navvaro-Llopsi et al., 2008). This appears to support the results of Toth et al (2007) who also reported that the best results in capturing *C. capitata* was with baits lacking putrescine.

Katsoyannos and Papadopoulos (2004) demonstrated that yellow sphere traps baited with female attractant "biolure" were 30 and 12 times more attractive for females and males, respectively, than unbaited traps. Moreover, field trials showed, that traps with three component attractant captured the same numbers of females as the McPhail-type traps baited with an aqueous solution of protein hydrolysate Nulure and Borax as preservative (Epsky et al., 1999; Katsoyannos et al., 1999 a,b).

Various degree of crop protection were achieved under low and high *Ceratitidis capitata* population densities in most fruit growing countries of the Mediterranean region (Navvaro-Llopsi et al. 2008, Jemâa et al.2010; Martinez-Ferrer et al.2012; Hafsi et al.2015b). The efficacy of attract and kill strategy depends on a number of factors which may occur in the female choice especially on the quality of food attractant (Epsky and Heath 1998, Broumas et al. 2002). A synthetic food based female attractant consisting of putrescine, ammonium acetate and thrimethylamine (Epsky and Heath 1998, Heath et al. 2004, 2009) was developed as replacement to aqueous protein hydrolysate in order to increase the number of captured *C.capitata* females and decrease the number of captured beneficial insects (Katsoyannos 1994; Heath et al. 1997).

Broughton de Lima (2002) reported that the synthetic female attractants is recommended as a replacement of the protein hydrolysate lure and may also replace the

McPhail traps in monitoring of populations and for the detection of *C. capitata*. On the other hand, Toth et al. (2004) suggested that the best way of monitoring and detection of the medfly is to use both male and female baited traps separately.

Regarding trap types, Mediouni et al. (2010) have agreed with Miranda et al. (2001) to avoid the use of Tephri-traps for mass trapping. In contrast Navvaro-Lopsis et al. (2008) reported that Tephri-traps should only be avoided for female mass trapping because it captures fewer females than all other kind of traps and according to the same study, females capture is always the main target.

In Tunisia and in the counties where the Medfly cause serious damage, synthetic female attractant in mass trapping and bait station techniques have proved to be more efficient than chemical treatment in reducing population level and fruit damage at harvest in orchards with minimum adverse effects on none targeted arthropods (Bouagga et al. 2014, Jemâa et al. 2010, Hafsi et al. 2015b, 2016). In previous studies, mass trapping using Ceratrap® and Tripack ® food attractant was found to be more effective than insecticides bait sprays against *C. capitata* in citrus orchards in Tunisia (Jemâa et al.2010, Hafsi et al. 2015b, 2016). Ceranock® bait station was considered as effective as conventional applications of insecticides, if not better, against *C. capitata* (Bouagga et al. 2014). It was especially noted that Ceranock® and AAL&K® bait station devices were not effective than chemical treatment using organophosphate by low density of the Medfly population (Hafsi et al., 2016).

Currently, >30000 ha of citrus groves in Spain are being treated with mass trapping, and surface treatment increase year by year. Initial field trials showed a good efficacy this technique using a density of 50 traps per ha during the 3 mo before harvest.

2.4. Monitoring of the Mediterranean fruit fly:

Chemical signals play an important role in any ecosystem; it has an influence on the physical, ecological and behavior of the insect in a variable ways, including plant-insect interaction, insect-insect interaction and insect-microbe interaction (Tan *et al.*, 2014). Olfaction sense is a key for most of flying insects mainly in their adult stage and have a high developed olfaction sensory. Chemical signals that denote odour attractiveness are usually related to behavior activities such as sexual communication, host location, food searching, host sustainability assessment, etc. Insects are involved in great odours environment that rule their lives aspects (Schoonhoven, *et al.* 2005).

Attractant might be defined as substance or blend of substances that exerts in an exposed insect of behavior of movement toward the source of such substance. In general, it's the opposite of repellence (Debboun, *et al.* 2014). The attraction of certain product is related to the chemical significance that expresses the target insect when it detects the odour. This is the case of tephritid flies, a food attractant denoted movement by a hungry insect toward the source of attraction (Epsky, *et al.* 2014). Sex pheromone is attractive only for specific potential couple for copulation purposes and it's a suitable host for insects that are ready to oviposit (Yuval and Hendrichs 2000).

Attractants have been largely used in control programs against fruit flies (Epsky N., *et al.*, 2014). It's an important tool in ethological control, were insects are captured, eliminate and economic losses are reduced. Monitoring and trapping system using attractants are used also in early detection programs in some countries to monitor early fruit flies outbreaks in order to control the life cycle (Tan, *et al.* 2014).

Early detection of a medfly infestation is crucial for a successful control because it allows prompting an implementing program and management activities, such as

delimitation, fruit stripping, protein bait spraying and the sterile male technique against small population (Hendrichs *et al.* 1995, Dowell *et al.* 1999)

Medfly detection currently relies on two types of traps:

Food based traps which are mimic and a suitable source. Early control strategies of tephritid trapping used products such fermenting, sugar baits, yeast and sub-products of liquor industry such brewery yeast and related, other (Gurney, 1925, Epsky *et al.* 2014). Synthetic products are protein based lures have been used as attractants in monitoring programs (CABI 2014). These attractants include protein hydrolyzed from soybean, maize, whey and others and torula yeast used as components or blended baits for attraction of both males and females in mass trapping programs, or combined with insecticides attract-and-kill systems (Benelli, Daane *et al.* 2014). Proteinaceous lures are highly attractive for females searching for food and also oviposition hosts (Placido-Silva *et al.* 2005).

Food attractants active compounds are mainly ammonia usually the key compound for attraction (Mazor, 2009) and can be used in monitoring populations of fruit flies and mass trapping of integrated control (Hafez and Ezzat, 1967, Saafan, 2001 and Mohammad 2002). Other compounds are also used for attraction like ammonium acetate and putrescine (Health, *et al.* 1997). Although food attractant are a powerful tool for capturing target fruit flies, two disadvantages remain: 1) weakly attractive, capture both sexes but females mainly (Epsky *et al.* 2014) and 2) attracts beneficial insects like bees and wasps and lacewing.

Food attractants used in traps, involves different designs of traps in order to allow insects capture and avoid it escape, avoid the capture of beneficial insects and hold and preserve the liquid as much as it can be (Tan, *et al.* 2014). McPhail trap is the most

common trap for food based attractants traps; an invigilated glass bottle baited with a aqueous protein hydrolysate (McPhail, 1939). Those baits are usually deployed in McPhail traps (Newell, 1936) which has been tested and found the most effective for the *C. capitata* capture (Roessler 1989). Jackson traps used to be the monitoring trap for fruit flies populations (El-Minshawy et al. 1999; Hashem et al. 2004 and Ghanim 2009). Some variants of traps were developed, and eventually the “Tephritrap” remains the most successful trap in catching fruit flies with liquid baits. The design includes the same inverted funnel principle with the addition of four small circular windows in every direction in the middle part, funnel like (Miranda, et al. 2001). These windows allow better release of attractants and entrance of flies.

Male Lures: Trimedlure (TML) was described over 50 years and since then; TML has been used as a male lure in early season detection programs (Beroza et al. 1961, Miranda et al. 2001). Commercial TML is a mixture of 90-95% transisomers (McGovern et al. 1986). So far, TML has been used as a male lure in monitoring and mass killing programs of *C. capitata* along with chemo sterilization (Navvaro-Llopis et al. 2004, 2007, Israely and Oman 2005). Male lures as their name indicated attracts only males (Jang and Light 1996) but Nakagawa (1970) reported an exception on this theory. The lure is mostly used as trimedlure which was replaced earlier with other attractants such as seed oil (Streiner et al. 1957) and siglure (Streiner et al. 1961).

Aqueous solutions of the corn hydrolysate lure and sodium tetraborate dehydrate (Borax) are currently used for female targeted detection monitoring programs (Gilbert et al. 1984). During this period, a number of compounds were found that are potent lures for male *C. capitata* (Cunningham 1989b). This culminated is the development of trimedlure; trimedlure baited traps are used worldwide for detecting and

monitoring population of *C. capitata*. Trimedlure dispensers are typically placed in Jackson traps (Hariss et al. 1971), which are triangular cardboard that are coated with sticky material (Cunningham 1989a.). Trimedlure is efficient in male attraction, but weakly for female attraction (Nakagawa et al. 1970). Male annihilator approaches via deployment of large numbers of male attractant traps have not been successful for control of *C. capitata* when used without other control methods (Cunningham 1989a).

CHAPTER 3

MATERIAL AND METHODS

3.1 Study sites

This study was conducted at several locations representing fruit production areas in Bane, North of Lebanon. In particular, it was carried out in “Al-Hareem” (altitude 1100 masl), “Al-Nsoob” (altitude 1200 masl) and “Al-Chawyi” (altitude 1300 masl). Although all of these areas have a typical Mediterranean climate, however, certain differences exist due to the altitudinal gradient, particularly between “Al-Hareem” and “Al-Chawyi”. Bane area has a hot dry summer (34°C, summer 2016), high precipitation in winter (400mm), and temperatures occasionally fall below freezing.

A wide variety of fruit and forest trees grow in these areas, including stone fruits, pome fruits, figs (*Ficus carica* L.), oaks (*Quercus* Spp.), Cedars (*Cedrus libani*) and grapes (*Vitis vinifera*). The fruiting season in Bane starts in June and ends in November, starting with apricots (*Prunus armeniaca* L.), peaches (*Prunus persica* L.) and plums (*Prunus domestica* L.) in addition to cherries (*Prunus avium*) that matures first, followed by pears (*Pyrus communis* L.), apples (*Malus domestica*), quince (*Cydonia oblonga* MILL) and oriental persimmon *Diospyros kaki* that matures at the end of the season. In Bane, apple orchards occupy more than 85% of the cultivated land, while the remainder is planted mostly with cherry, peach, pear and few other fruit trees.

3.2 Monitoring of the Medfly:

The monitoring of the fruit fly has been carried out in all the three areas using the McPhail trap (Figure 1) in order to determine the flight activity and have an idea about the population density in all the three areas.

3.2.1 Trapping method

In each of the study areas, one McPhail trap was installed every year for consecutive years 2015, 2016, 2017, 2018, 2019 and 2020 in three fruit tree orchards in the areas of Al-Hareem, Al-Nsoob and Al-Chawyi . The traps were suspended at a height of 1.5-2 m from the ground and baited with attractant (TMA card) dispensers (Figure 2) which were replaced every three weeks. The traps were checked weekly, the number of the trapped flies was recorded and then the McPhail trap was emptied and the sticky inserts replaced whenever necessary. All orchards have a size of approximately one dunum.

The orchards consisted of stone fruit trees and pome fruit trees; however, and in order to have a maximal catching efficiency, the traps were removed from the stone fruit trees part of the orchards during the beginning of the month of August and hanged in the pome fruit part of the orchard until no adults was captured. This applied for the Al-Nsoob and Al-Hareem orchards. In “Al-Chawyi” orchard, the different pome and stone fruit trees were mixed together; the traps were left unchanged during the entire season.



Figure 1: Mcphail Trap used in the Monitoring study.



Figure 2: *Ceratitits capitata* attractant (TMA) card used as a dispenser in the McPhail Trap

Meteorological data provided by the Lebanese Agriculture Research Institute (LARI) for the different years covered during the monitoring period. The station was located in Bsharry and is the only nearest station to the experimental plot (about 10 km). The difference in altitude between the location of the meteorological station and Bane is about 300 meters from the highest altitude and therefore, a two degrees' difference is

recorded. The mean monthly temperature ranged between 15.21 °C and 22.1 °C from June to September, and dropped to 13.17 °C and 15.6 °C in October and 8.92 in November of the year 2015. In 2016, the temperature ranged between 14.61°C and 21.2°C from June to September and dropped to 11.65°C and 14.61°C in October and 8.28°C and 13.44°C. In 2017, the temperatures recorded for the months June to September were of 13.51°C and 23.2°C and in October the temperatures were of 11.65°C and 14.54°C and in November the temperatures were between 8.28 °C and 13.44 °C. In 2018, The temperatures of the months June to September were of 13.51 °C and 23.2 °C and 8.92 °C and 12.17 °C in October, and 4.29 °C and 11.31 °C in November. In 2019, the recorded temperatures were 16.05°C and 22.18°C in the months of June to September, and 15.99°C and 18.64°C in October and 6.55°C and 14.0°C in December. In 2020, the temperatures were 17.69°C and 25.41°C in the period of June to September, and 17.26°C and 20.43 °C in October and 9.7°C and 10.8 °C.

3.2.2 Experimental setup:

Ceratitis capitata adults were monitored using the McPhail trap baited with TMA Card (Susbin SA, Argentine), baited with in a dispenser loaded with the synergistically acting attractants, ammonium acetate (2.2 g), trimethylamine chloride (8.58 g), and trimethylamine (11 g). To capture the flies, the lower part of the traps was not filled with any solvent. Traps were checked on weekly basis and if other insects of the carnivorous group or a lizard accidentally dropped inside, they were removed immediately.

The traps were installed in 2015 in May; however, no medfly adult was trapped inside until the June and therefore the traps in subsequent years were installed in June and in some years we noticed that the first catches did not occur until July.

The installation of the traps in the three locations started on May 1st and ended on July 30th in the orchards Al-Nsoob and AL-Hareem where the traps were removed from the stone orchard part and placed in the pome fruit orchard part. The traps were then kept until no adult is anymore trapped. In some the 0 catches were reached in early November and in other years in late November.

In Al-Chawyi location, the trap was installed on May 1st and kept until no adult was caught by the trap for two consecutive years. The numbers of trapped adults were recorded in order to compare the flight activity of the Med Fly for the consecutive years from 2015 to 2020.

3.3. Efficacy of Mass trapping as a control method

3.3.1 Site location

In order to compare the efficiency of using Mass trapping as an alternative to chemical insecticide, four experiments were designed in Bane village, in “Al Chaghoury” orchard for year 2017 and “Al-Ksara” orchard located at 1250 masl for three consecutive years 2018, 2019 and 2020.

3.3.2 Mass trapping Scenario Concept

The trap used in all experiments is a McPhail trap; the trap (Figure 1) is a new version of the conventional McPhail trap, a transparent glass pear shape container. This trap was first used with a liquid food bait, based on hydrolyzed protein. This new

version of McPhail trap consists of two plastic pieces shaped invaginated container. The separation of the upper and lower part allows to use the trap as a liquid bait trap and a dry bait trap where the dispensers can be hanged on the upper part. The yellow coloration of the bottom part is intentional since most Tephritidae flies are attracted by the yellow color. This trap is low in cost compared to the glass trap and more economical to use and is less labor intensive than the conventional traps, particularly in loading the lures.

In our experiment the lures that were used are female and male attractants composed of three different components in one dispenser (Figure 2). Based on the experiments conducted by Manukis *et al.* (2015); we have decided to increase the density of traps to determine the optimal trap density. Manukis *et al.*, (2015) found that a 65% probability of capture was obtained at 7 m distance.

3.3.3 Experimental setup 1

For the first trial in 2017, three McPhail traps (Figure 3) were hanged on one apple tree replicated three times on the “MUFTI” apple variety. The trees were separated from each other by 100m to ensure that no interference of odors is present between the traps. The traps were installed on the 15th of July 2017 until the 20th of August 2017, which is the picking time of this apple summer variety in “Al-Chaghoury” area (altitude 1350m). TMA in traps were changed every two weeks in order to ensure that the TMA dispenser is still releasing the same amount of volatiles.

For the 2018 trial, one McPhail trap (Figure 4) was installed on one apple tree replicated on the whole orchard that contains 49 apples trees (25 Golden Delicious, 21 Starking Delicious and 3 Royal Gala). The traps were installed on the 15th of July 2018

until 15th of September, which is the picking time of these apple varieties in “Al-Ksara” area (altitude 1250 masl). TMA in traps were changed every two weeks in order to ensure that the TMA dispenser is still releasing the same amount of volatiles.

For the 2019 trial, one McPhail trap (Figure 4) was installed on one apple tree replicated on the whole orchard that contains 49 apples trees (25 Golden Delicious, 21 Starking Delicious and 3 Royal Gala). The traps were installed on the 15th of July 2019 until 15th of September, which is the picking time of these apple varieties in “Al-Ksara” area (altitude 1250 masl). TMA in traps were changed every two weeks in order to ensure that the TMA dispenser is still releasing the same amount of volatiles.

For 2020 trial, one Mcphail trap (Figure 5) was installed for every 4 trees, replicated on the whole orchard. Traps were installed on the 16th of July 2020 until 18th of September, which is the picking time of these apple varieties in “Al-Ksara” area altitude (altitude 1250 masl). TMA traps were changed every two weeks in order to ensure that the TMA dispenser is still releasing the same amount of volatiles.



Figure 3: McPhail traps hanged on apple trees for mass trapping- Trial 2017



Figure 4: McPhail Traps hanged on apple trees for mass trapping- trial 2018 and 2019



Figure 5: McPhail traps hanged on apple trees for mass trapping- trial 2020

At the end of every season, apples on the trees were collected and counted and they were checked for fruit fly damage. The number of infected fruits was recorded then the rate of infection was calculated following this formula:

$$\text{Infected fruits (in percentage): } \frac{\textit{infected fruits}}{\textit{total number of fruits}} \times 100$$

The result of this formula was compared with the results we got from three trees that were subject to a conventional spraying during the whole cycle of the apple fruit development; from flowering stage until maturity.

3.3.4 Experimental setup 2

The efficiency of mass trapping using two different lures was conducted in 2019 on Gala apple variety in Bane. The experimental plan was conceived in an orchard of 122 apples and the experiment was conducted on 27 apple trees as in represented in Figure 6.



Figure 6: Experimental set-up of the experiment conducted on apple Gala variety in Bane 2019

The first one is a lure that is female attractants and composed of three different components in one dispenser. TMA Card (Susbin SA, Argentine), consist of a dispenser loaded with the synergistically acting attractants, ammonium acetate (2.2 g), trimethylamine chloride (8.58 g), and trimethylamine (11 g). To capture the flies, the lower part of the traps was filled with water. Traps were checked on weekly basis and if other insects of the carnivorous group or a lizard accidentally dropped inside, they were removed immediately.

The second food attractant used in our model is the insecticidal bait Anamed WB, a new product to improve management of fruit flies, distributed by the Lebanese Ministry of Agriculture and manufactured by ISCA Technologies Inc., USA. Anamed is

a non-toxic and bio-degradable emulsion containing oils, waxes, and hydrolyzed protein (6%), and an insecticide abamectin (0.5%). It is a widely used fruit fly attractant and feeding stimulant. It's used as an effective attract-and-kill formulation targeted specifically at fruit flies attracted to hydrolyzed protein type attractants (Anamed^R).

The agricultural practices adopted in this field were the same as of the previous year and the pruning of the trees was conducted in November 2018. The soil was ploughed two times; one ploughing was conducted in November 2018 and a second ploughing in April 2019. The orchard was irrigated through a drip irrigation system. The field control trees were sprayed with Thiametoxam and lambda cyhalothrin starting March 2019 and until August 14, 2019.

3.3.4.1 Installation of McPhail traps

The installation of traps was done on June 16, 2019. The traps were hanged on the trees about 1.8 m above ground and in the sunniest part of the tree. Trees with the same treatment were side by side in the same row (Figure 6). The McPhail traps with the TMA card were replaced every 6 weeks. (Figure 7).



Figure 7: McPhail traps baited with TMA card lure

Traps baited with Anamed were cleaned each time the product dried and new dose of Anamed equivalent to 20 ml, was added. The installation time for both lures was from June 19, 2019 until September , 2019 at the time of harvest.

3.3.4.2 Data collection

The effectiveness of trapping techniques was recorded weekly by counting the number of Mediterranean fruit flies in the traps in both treatments (Figure 8).

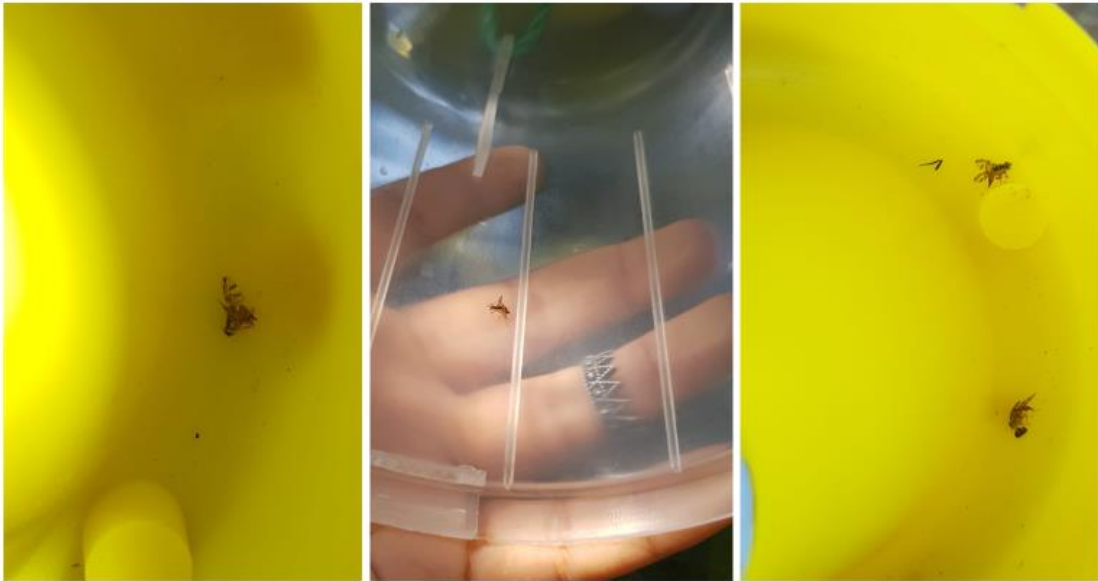


Figure 8: *Ceratitits capitata* captured in the trap.

The number of fruits that fell under the trees in the three treatments was also recorded and calculated each week. All fruits were dissected and the presence or absence of fruit flies was recorded. At harvest, the fruits presenting oviposition symptoms and medfly damages were counted and the total number of fruits for each tree was counted. Fruits with holes or spots on their outer skin were cut to determine if the cause was *C. capitata* or the codling moth, *Cydia pomonella*.

3.4 Statistical Analysis

The data on the number of trapped adults of *C. capitata* was plotted in order to generate flight curves and determine peaks of emergence which will consequently lead to the number of generations observed during the years 2015 and 2016 in all the three localities of Bane.

In the first set of experiments, Anova one way was used to determine the efficiency of Mass trapping against conventional spraying by comparing the percentages of infections in the two different treatments.

In the second experimental setup, the number of trapped Mediterranean fruit flies was compared between the two treatment using *t-test*. The number of infected fruits as well as the yield per tree was analyzed using an analysis of variance (ANOVA) to assess the effect of both baiting methods and positive control. Means were separated using Tukey HSD following a significant effect of treatment ($P < 0.05$).

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Flight emergence, number of generations and population densities of *Ceratitis capitata*

The results obtained over the 6 years of continuous monitoring of *C. capitata* in the apple orchards showed similarities and dissimilarities over the years in the three altitudinal different apple orchards.

For the 1100 masl altitude, the total number of catches for the six years between 2015 and 2020 were respectively 392, 785, 912, 318, 201 and 151 in 2015, 2016, 2017, 2018, 2019, and 2020 respectively (Figures 9-14). In 2015, four peaks of emergence were observed, the first in August 23, September 6th, September 20th and October 1st. However, these 4 peaks, taking into consideration that the lifecycle of the medfly is 28-32 days in summer may actually corresponds to 3 peaks with different intervals of emergence and corresponding to 3 generations (Figure 9). In 2016, at the same altitude seven peaks of emergence were recorded; June 30th, July 14th, August 8th, August 29th, September 19th, October 8th and October 29th. In reality, it is difficult to determine the number of generations but there is at least one generation more than the previous year and it is the first generation emerging in June and July (Figure 10). In 2017, Number of peaks of emergence is four; 23 July, 5-19 September, 17 October and 14 November indicating the presence of 4 generations (Figure 11). In 2018, the low population of medfly made it difficult to differentiate between the peaks since the populations was not showing a lot of variability (Figure 12). In 2019, the number of peaks, particularly from the first catch in June and until then of august was very difficult to be determined

because of the low population recorded at this altitude (Figure 13). In 2020, the low number of *C. capitata* also made it difficult to discern between the peaks; however, three generations might be present with one generation appearing at the end of July another one at the end of August and the third one in October through November (Figure 14).

For the 1200 masl altitude, the total number of catches for the six years were 761, 552, 633, 958, 200 and 481 in 2015, 2016, 2017, 2018, 2019, and 2020, respectively. In 2015, one peak was distinct from all the other peak and was on August 9. The other peaks were not distinct as the population range was more or less constant between 16 August and 18 October (Figure 9). In 2016, at the same altitude six peaks of emergence were recorded; June 30th, July 14th, August 8th, August 29th, September 12 and October 8th (Figure 10). However, these peaks may only correspond to 4 generations if we compare them to the duration of the life cycle in the summer season with one generation in June-July, the second generation in August, the third in September and the last one in October (Figure 10). In 2017, Number of peaks of emergence has increased to six; 23 July, 5 September, 19 September, 3 October, 24 October and 14 November (Figure 11). The number of generations may not be equivalent to the number of peaks and according to the duration of the lifecycle the number might be equal to 3 and a fourth generation may be present in November. In 2018, number of peaks of emergence has decreased to five; 24 June, 29 July, 26 August, 30 September and 21 October (Figure 12). These peaks may indicate the presence of 5 different generations; however, care should be taken since the emergence period for each generation is long and thus might be affected by the temperatures. In 2019, the number of peaks is hardly distinctive since the population is too low this year (Figure

13). In 2020, the number of peaks is five, 24 July, 15 August, 29 August, September 26, and 24 October (Figure 14). Noting that a small peak of only 4 adults were recorded on July 3rd, taking into consideration the lifecycle of the medfly, the peaks correspond to 4 generations.

For the 1300 masl altitude, the total number of catches for the six years between 2015 and 2020 were respectively 43, 309, 246, 168, 187 and 420. In 2015, although the population was low (43 adults for the total season), four peaks of emergence were observed the first in August 9, September 13th, September 28th and October 18th (Figure 9). These peaks represent a maximum of 3 generations if we consider the lifecycle duration of the medfly. In 2016, at the same altitude four peaks of emergence were recorded; July 14th, August 8th, September 19th and October 8th (Figure 10). These peaks represent more two generations rather than 3 generations, with the first generation emergence period taking place between July 14 and August 8 and the second generation between 12 September and October 8 (Figure 10). In 2017, the number of peaks of emergence was five, on 9 and 23 July, 8 August, 12 September and 3 October (Figure 11). These peaks correspond to a maximum of 3 generations (Figure 11). In 2018, the number of peaks of emergence was almost 6, however, only two distinctive peaks on June 24 and Jul 22 are fairly well separated and the rest of the peak probably represent one generation and they extend from September 9 to October 21 (Figure 12). In 2020, the number of peaks were distinct in August 15, September 26 and October 24 indicating the presence of 3 generations occurring in August, September and October (Figure 14).

These results indicate that there's a huge difference between the catches over the years on different altitudes.

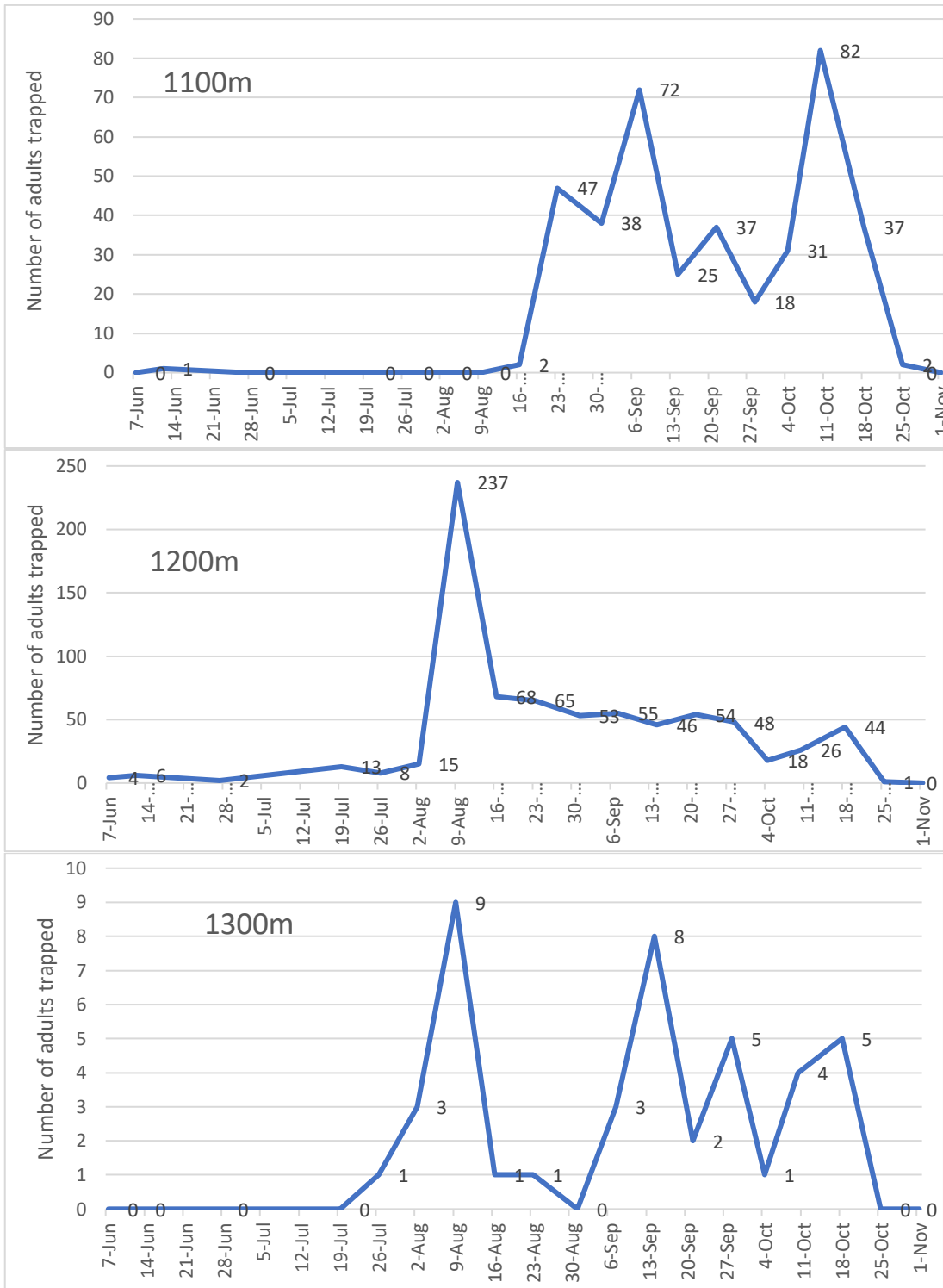


Figure 9: Medfly catches during 2015 in the areas of Bane, North of Lebanon

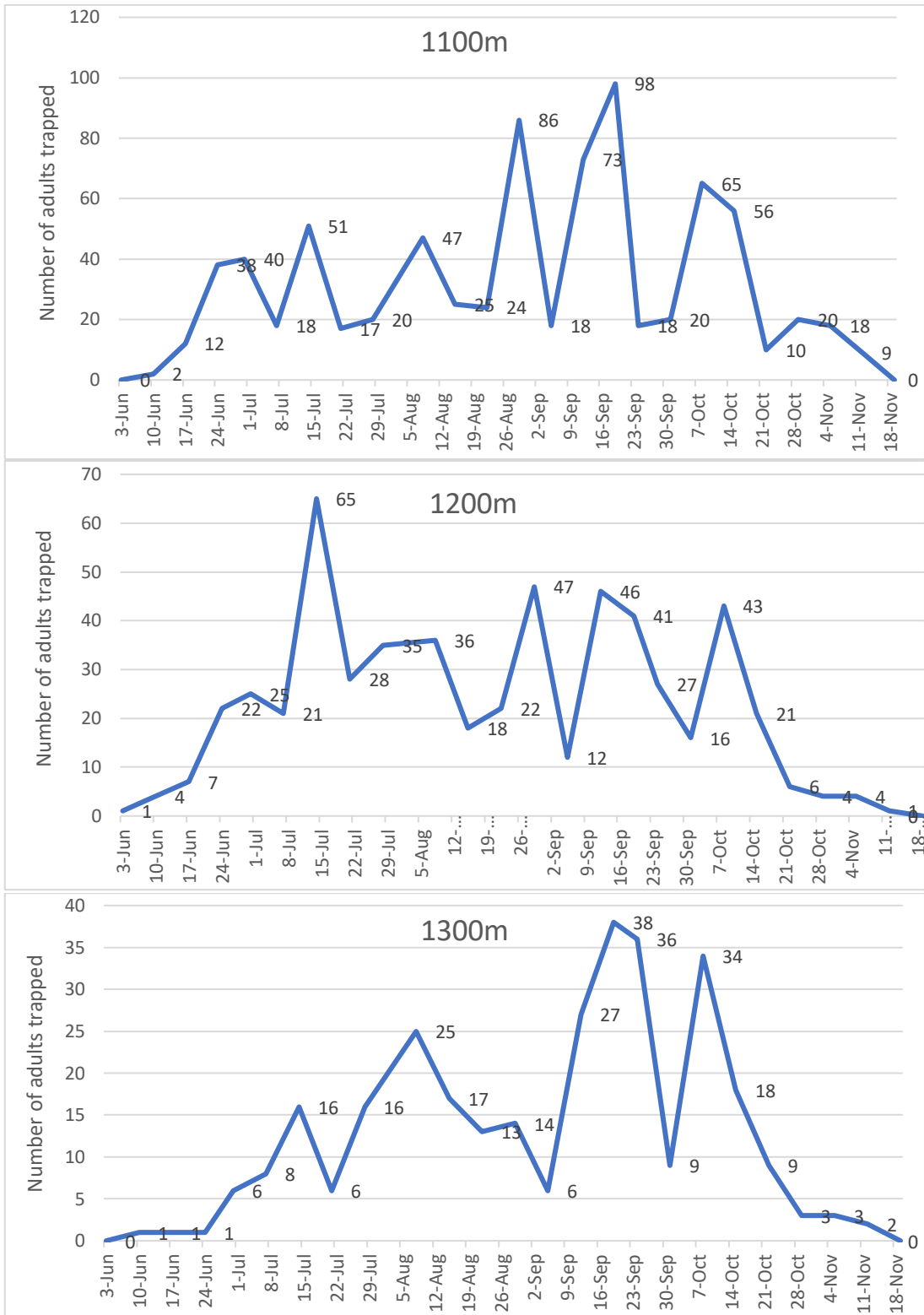


Figure 10: Medfly catches during 2016 in the three areas of Bane, North of Lebanon

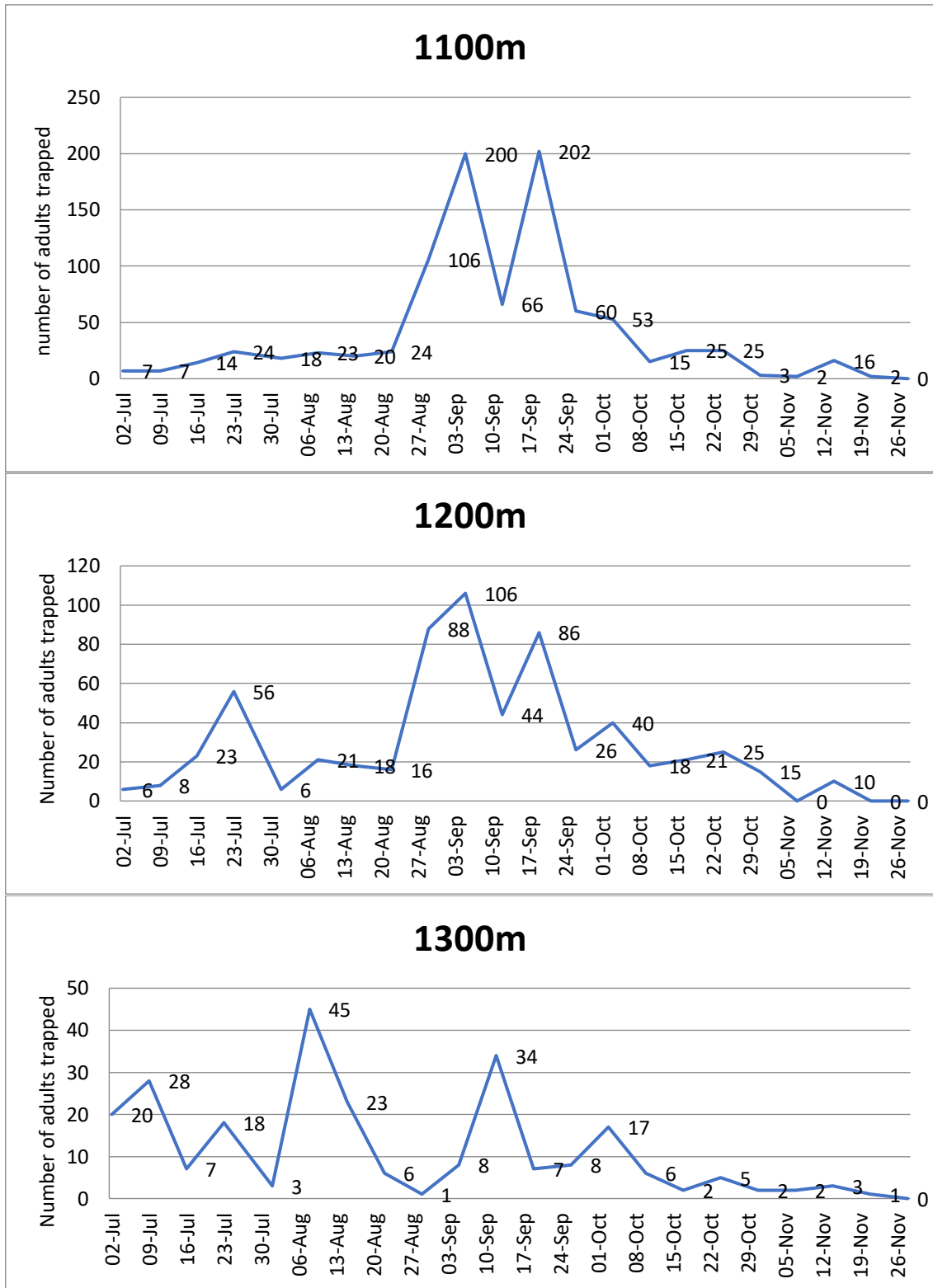


Figure 11: Medfly catches during 2017 in the three areas of Bane, North of Lebanon

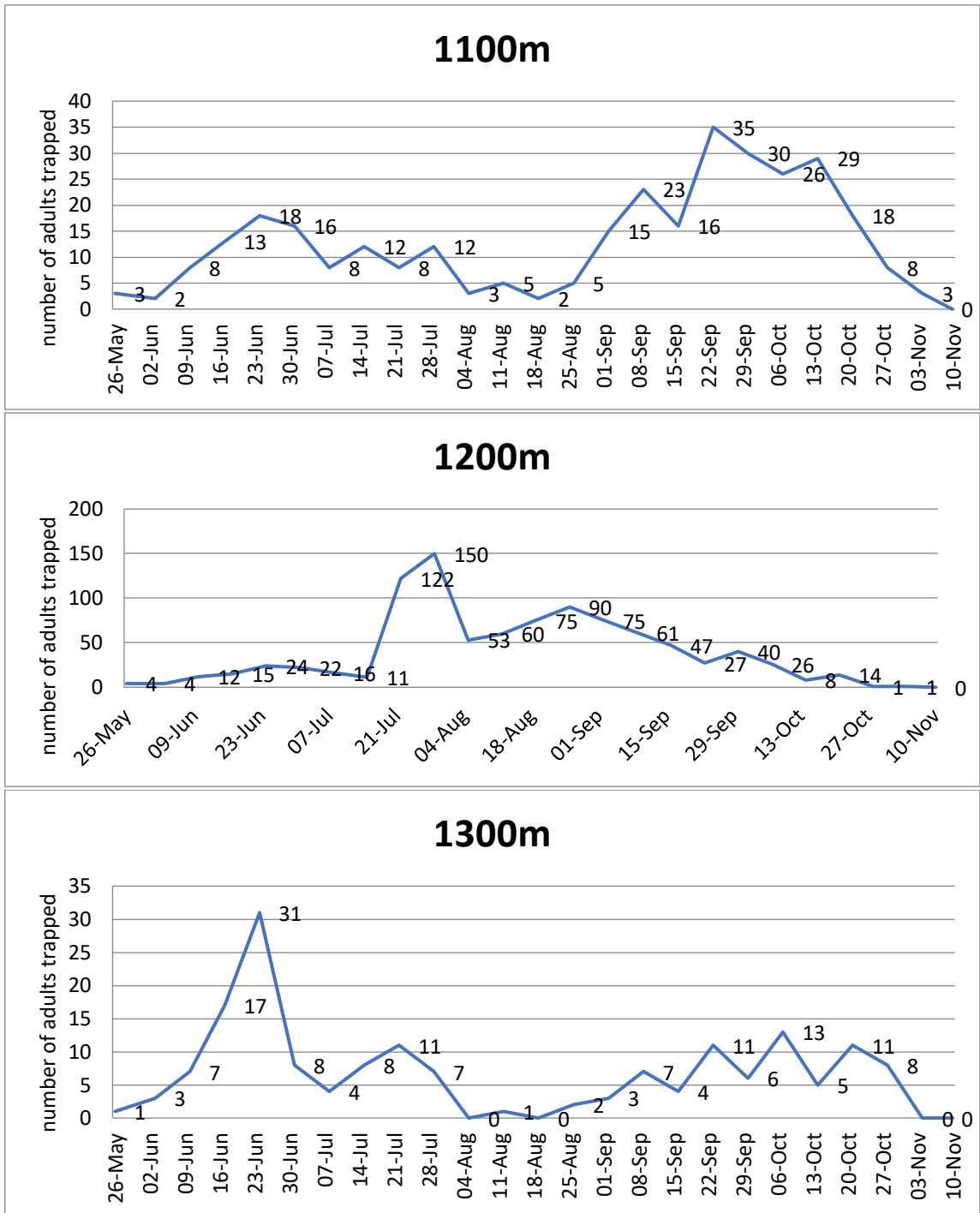


Figure 12: Medfly catches during 2018 in the three areas of Bane, North of Lebanon

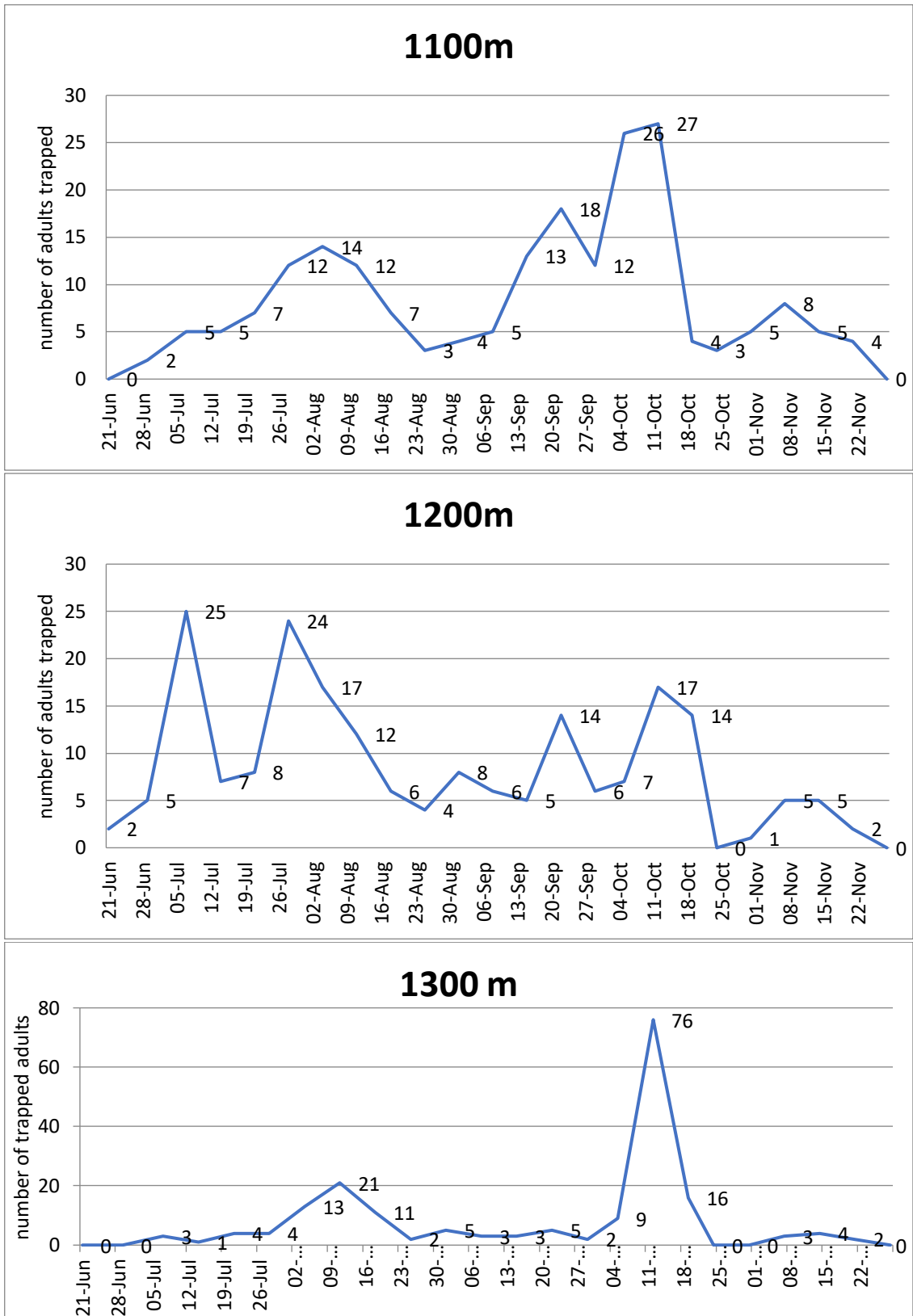


Figure 13: Medfly catches during 2019 in the three areas of Bane, North of Lebanon.

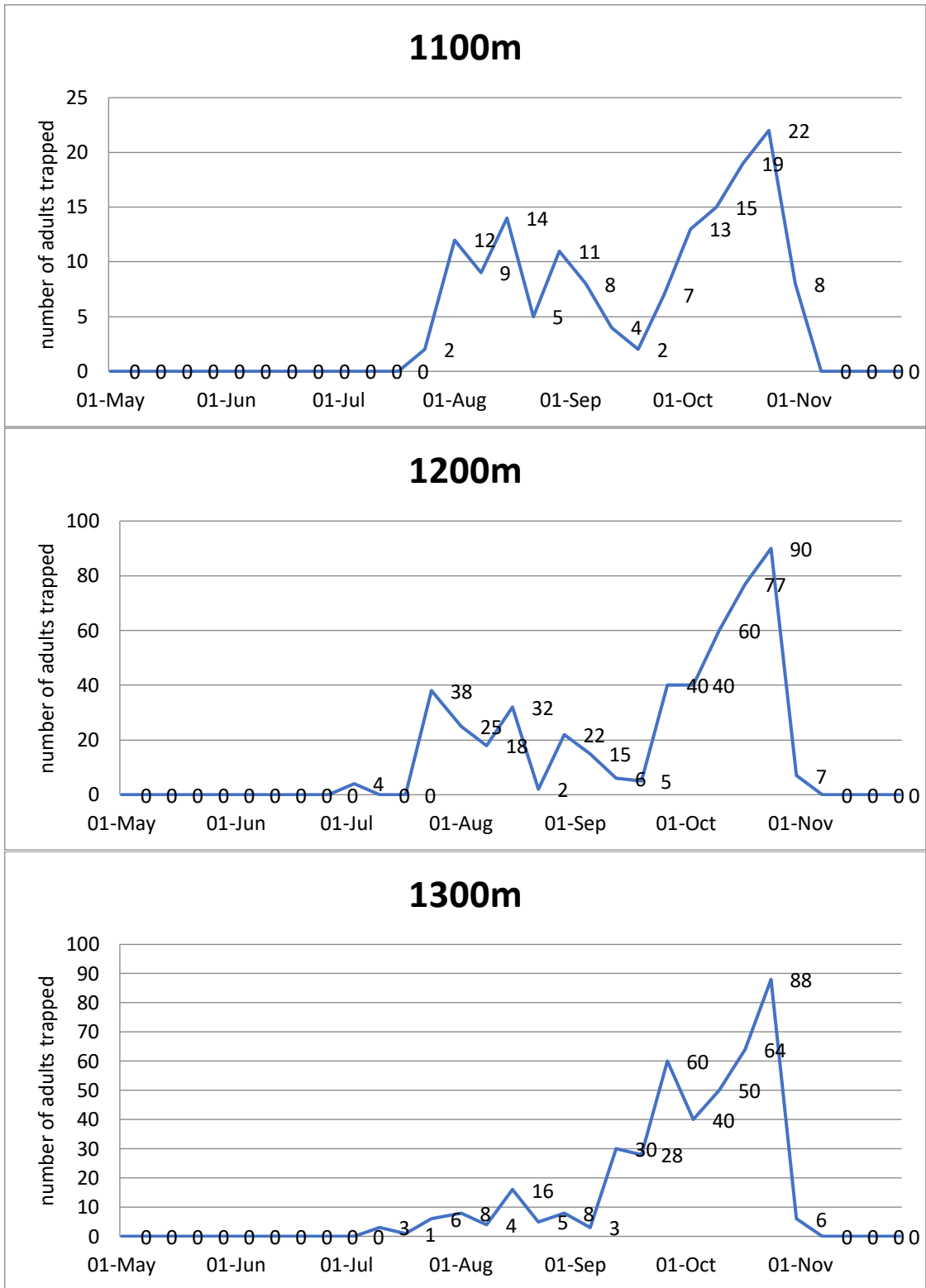


Figure 14: Medfly catches during 2020 in the three areas of Bane, North of Lebanon

When plotted against the temperatures, we noticed that each peak of emergence coincided with a peak in the temperature or after the temperature peak by one week. After October 25, 2015, when the mean temperatures were of 13.77°C, no flight activity was recorded in the three areas of Bane (Figure 15). It should be noted that starting of the week of July 19, 2015; medflies population was always present indicating an overlapping of generations on the three different altitudes (Figure 15).

In 2016, the last trapping was on November 12, 2016 which corresponded to a temperature of 13.44°C (Figure 16). Most of the peak of emergence of the medflies corresponded with a peak of temperature or followed the peak of temperatures by one week (Figure 16). It should be noted that starting the week of June 10th 2016, medflies populations was always present indicating an overlapping of generations on the three different altitudes (Figure 16).

In 2017, the last trapping was on November 21, 2017 which corresponded to a temperature of 4.23°C (Figure 17). Most of the peak of emergence of the medflies corresponded with a peak of temperature or followed the peak of temperatures by one week (Figure 17). It should be noted that starting the week of July 2nd 2017, medflies populations was always present indicating an overlapping of generations on the three different altitudes (Figure 17).

In 2018, the last trapping was on November 4, 2018 which corresponded to a temperature of 13.44°C. Most of the peak of emergence of the medflies corresponded with a peak of temperature or followed the peak of temperatures by one week. It should be noted that starting the week of May 26th 2018, medflies populations was always present indicating an overlapping of generations on the three different altitudes.

In 2019, the last trapping was on November 21, 2019 which corresponded to a temperature of 7.92°C (Figure 18). Most of the peak of emergence of the medflies corresponded with a peak of temperature or followed the peak of temperatures by one week (Figure 18). It should be noted that starting the week of June 29th 2019, medflies populations was always present indicating an overlapping of generations on the three different altitudes (Figure 18).

In 2020, the last trapping was on October 31, 2020, which corresponded to a temperature of 10.08°C (Figure 19). Most of the peak of emergence of the medflies corresponded with a peak of temperature or followed the peak of temperatures by one week (Figure 19). It should be noted that starting the week of July 24th 2020, medflies populations was always present indicating an overlapping of generations on the three different altitudes (Figure 19).

Between 2015 and 2020, years of our study, cycle of the Medflies on apples was not the same; however, in 2015 the cycle was for 149 days (4 months, 29 days). In 2016, the cycle extended to reach 169 days (5 months, 19 days) and decreased in 2017 (4 months, 22 days). In 2018 the cycle re-extended to 162 days (5 months, 12 days) and reduced in 2019 to 153 days (5 months, 3 days), this number continue to shrink and reached 120 days (4 months) in 2020.

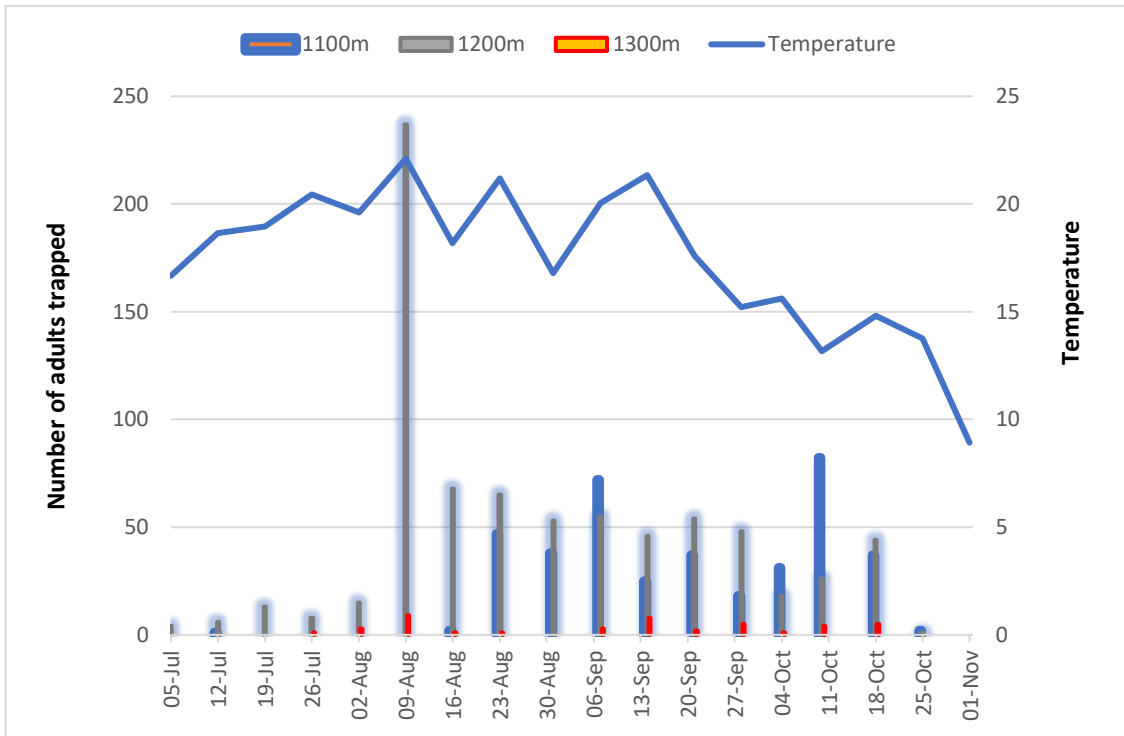


Figure 15: The evolution of the flies catches in relation to time and temperature in 2015

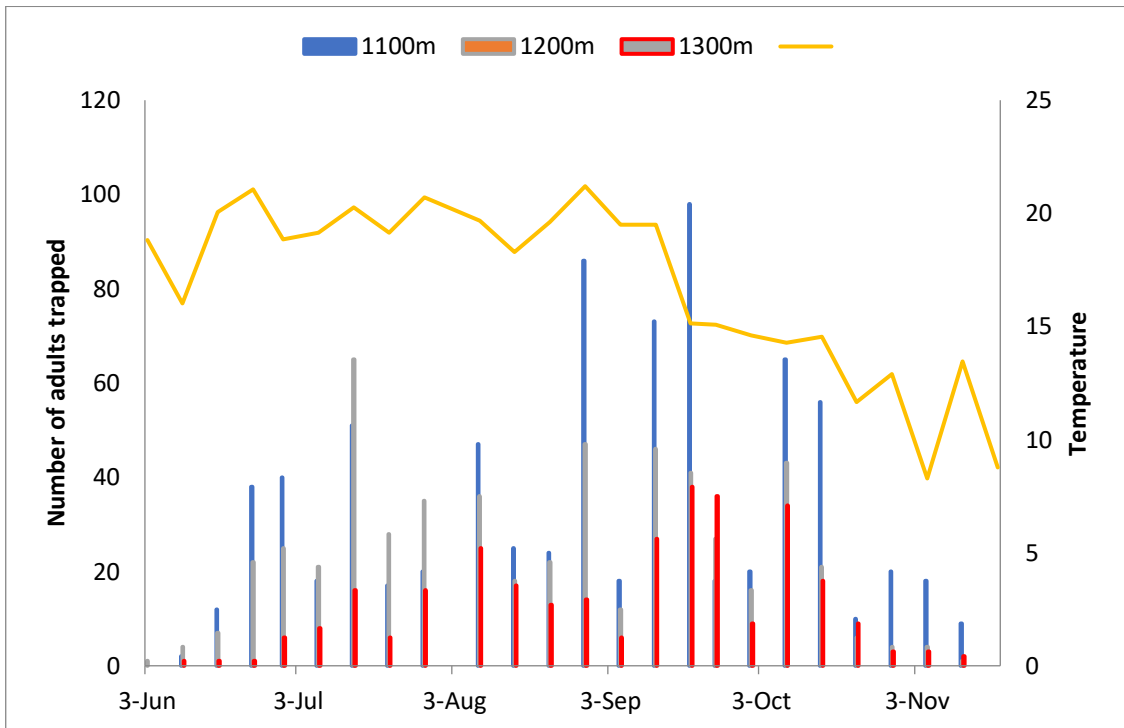


Figure 16: the evolution of the flies catches in relation to time and temperature in 2016

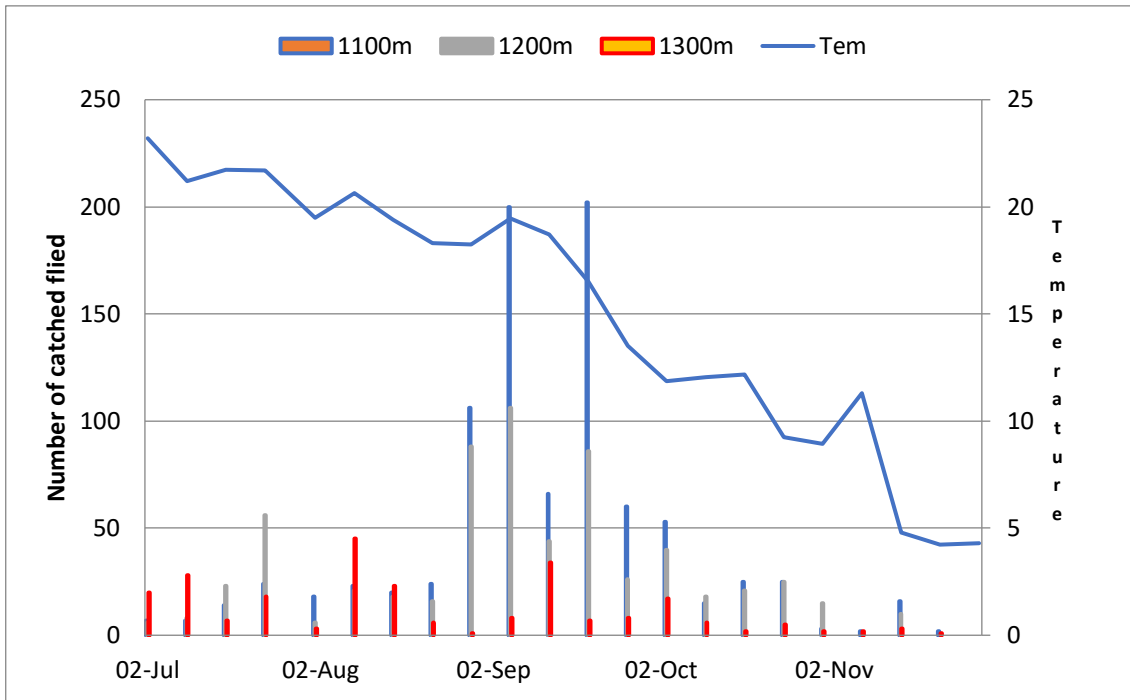


Figure 17: The evolution of the flies catches in relation to time and temperature in 2017

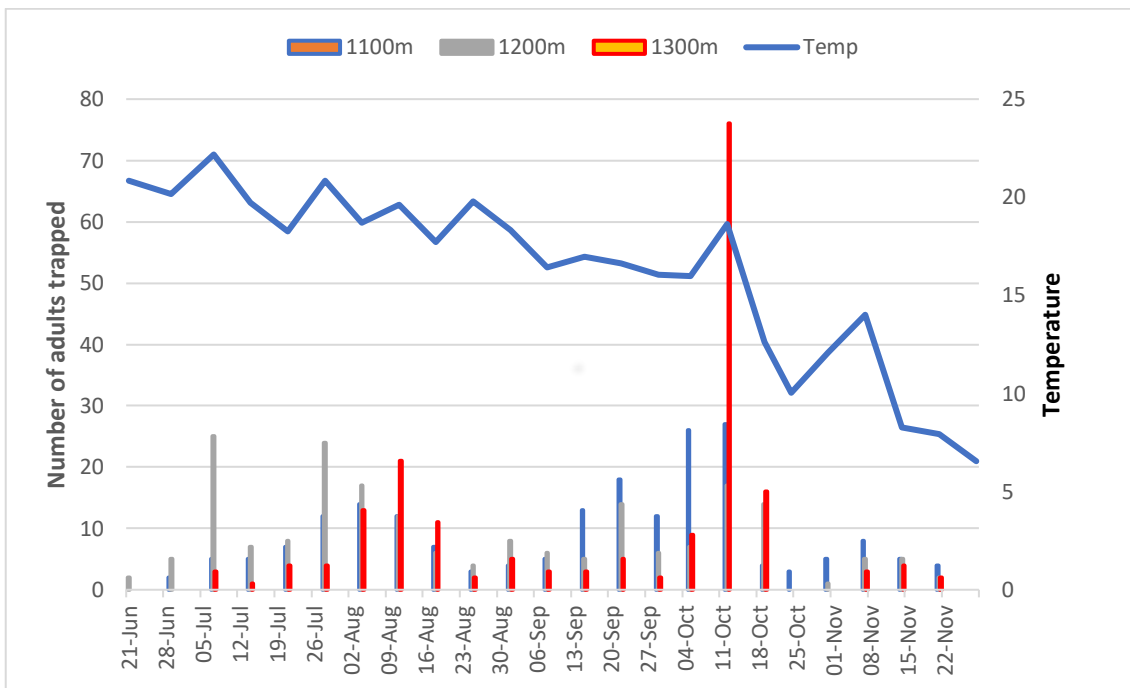


Figure 18: The evolution of the flies catches in relation to time and temperature in 2019

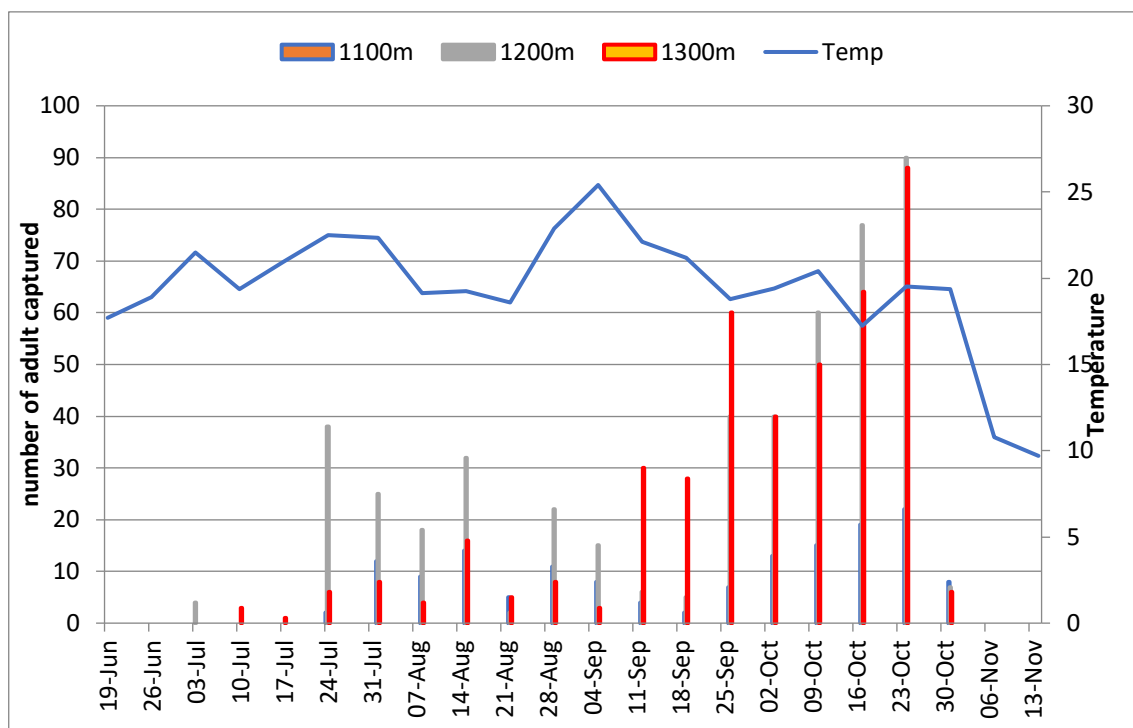


Figure 19: The evolution of the flies catches in relation to time and temperature in 2020

The medflies in the apple orchard of Bane are overwintering during winter although subzero temperature occurs. This was proved by the fact that during the five years the populations of medflies were always present with different densities. Like other tropical origin fruit flies, the Mediterranean fruit fly does not possess a cold-withstanding mechanism (Christenson and Foote 1960, Greenberg 1960, Bateman 1972, Carey 1984), yet it thrives in the summer in the region where subzero temperature occurs in the winter. Three alternatives have been explained for the summer populations in cold areas: 1) overwintering through adults (Messenger and Flitters 1954, Bateman 1972, Carante and Lemaitre, 1990); 2) overwintering through preimagos (Papadopoulos et al. 1996, 1998; Israely et al. 1997, Katsoyannos et al. 1998) and 3) summer migration from “nearby favorable areas” (Messenger and Flitters, 1954).

The results of the different experiments in the present work are consistent and do support the hypothesis that the Mediterranean fruit fly overwinter as preimagos or as

an adult in cold areas. Our results coincide with previous studies suggesting that temperature is the most important factor affecting the Medfly activity and life history (Hill et al., 1998; Jessup et al., 1993, Vargas et al., 1996, 1997) followed by the effect of precipitation (Back and Pemberton, 1918, Gjullin, 1931, Rivnay, 1950, Messenger and Flitters, 1954).

The general seasonal trend in Bane indicates that Mediterranean fruit fly populations start to increase in late spring (May-June), peak in summer (July-August) and early autumn (September-October) then decline during late autumn (November). Similar trend in the Mediterranean fruit fly abundance was previously reported in the cooler pome fruit growing areas of Valencia and Tarragona Spain (Martínez-Ferrer et al., 2010).

The seasonality pattern of medfly population in Bane seems to be influenced by temperature and host availability. With regard to the latter, medfly abundance appears to be closely associated with the seasonal maturation of the most suitable host fruits in the area which are apricots, peaches, plums, cherries, apples and pears. Early in the season (May till July), the fly breeds in cherries, apricot plums and peach fruits. In late August and September, a large population builds up owing the availability of mature apple fruits that provide an important source of nutrients for *C. capitata* (Hendrichs and Hendrichs 1990). Therefore, the fecundity and longevity of flies feeding on apple fruit will be relatively high. Later in the season, the population peaks in response to the availability of late apples varieties (granny smith) and oriental persimmon. In fact, host fruit phenology and availability have been shown to exert a great influence on the phenology and abundance of the medfly (Harris et al., 1993).

Information on *C. capitata* abundance was also gathered from host fruits collected from traps. Based on infections and huge number of captured flies, Propky et al. (1994) have suggested that Medfly have a huge wide host range but females have preferences for certain host fruits.

In our case, two hypothesis may be formulated, the first linked to the fact that medfly has become acclimatized to cold temperatures which somehow explain its presence to high altitudes for five consecutive years in large numbers. The second hypothesis is related to the warm effect which has contributed to its presence at these high altitudes.

by 23-28 days and the life span exceeds in 3 months in the field while De Lima Our study shows that male and female Mediterranean fruit fly were trapped for 7 months, though the existence of adult populations in winter was attributed to increased longevity rather than any significant emergence of new populations. Papadapoulos et al. (2004) found that adult Mediterranean fruit fly experience senescence related mortality (2008) estimated Medfly adult longevity can range from 6 months at 18°C to 6 weeks at 30°C. A report by Escudero-Colmar et al. (2008) shows that high degree of survival of all life stages of the Medfly stages between 15°C and 30°C temperatures. Medfly adult longevity may vary from 10 days at 38°C to 100 days at 16-19.5°C (Rivnay et al., 1950). Our field experiment indicate that adult longevity lengthens at cooler temperature, with Medfly adults between 142 days and 168 days from 2015 to 2020 when exposed winter temperatures close to 0°C compared to 23-28 days for adults maintained at 25°C.

4.1 Efficacy of Mass trapping as a control method

4.1.1 Efficacy of TMA card as a mass trapping technique

Over the four years of the mass trapping experiments with TMA card, the rate of infected fruits with the Mediterranean fruit fly has varied. For the 2017 experiment, the white apple variety “Al-Mufti” showed that infected fruits were 75 fruits out of 175 which with a rate of 42.86% (Table 1). In 2017, three McPahil traps were hanged on three separate trees in the apple orchard. The results obtained from the fruits collected from a conventionally sprayed trees belonging to the “Al Mufti” variety were 41.49% infected fruits.

Table 1: Results of mass trapping on "Al Mufti" white apple variety in 2017

Results of Mass Trapping 2017				
	Infected	Non Infected	Total	Percentage of infected fruits
Mass trapping	75	100	175	42.86%
Positive control (Conventionally sprayed orchard)	78	110	188	41.49%

The 2018, 2019 and 2020 years' experiments were on a mixed orchard of the two main traditional Lebanese commercial varieties: Golden Delicious and Starking Delicious. The results with the rate of infection over the years between the different varieties are shown in Tables 2,3 and 4.

Table 2: Results of mass trapping on Golden Delicious and Starking Delicious varieties in 2018

Results of Mass Trapping 2018					
Treatment	Variety	Infected	Non Infected	Total	% of infected fruits
TMA card Control	Golden Delicious	111	85	196	56.63
		96	86	182	47.25
TMA card Control	Starking Delicious	36	63	99	36.36
		65	95	160	40.62

Table 3: Results of mass trapping on Golden Delicious and Starking Delicious varieties in 2019

Results of Mass Trapping 2019					
Treatment	Variety	Infected	Non Infected	Total	% of infected fruits
TMA card Control	Golden Delicious	135	2	137	98.54
		120	42	162	74.07
TMA card Control	Starking Delicious	59	82	141	41.84
		25	76	101	24.75

Table 4 Results of mass trapping on Golden Delicious and Starking Delicious varieties in 2020

Results of Mass Trapping 2020					
Treatment	Variety	Infected	Non Infected	Total	% of infected fruits
TMA card	Golden Delicious	62	31	93	66.67
		72	32	104	69.23
Control	Starking Delicious	42	47	89	47.19
		32	48	80	40.0

The infection rate has varied over the years of the experiment, however, in all the years the white apple varieties were more susceptible than the red apple variety (Tables 2, 3 and 4). There was not distinct separation from the percentage of infection in the conventionally treated apple indicating either a similar effect of both control strategy or an ineffective control of both.

The infection rate of Golden Delicious was 56.63% in the year 2018, and increased to 98.54% in 2019 and decreased in 2020 to 66.67%. The same trend was noticed in the conventionally treated apple trees where the infection rates were 47.25%, 74.07%, and 69.23% for the years 2018, 2019, and 2020. These results indicate that mass trapping using TMA card on Golden delicious variety did not help in decreasing the infestation.

For the Starking Delicious variety, infection rate in 2018 was 36.36%, increased in 2019 to 41.84% continue increasing in 2020 till 47.19%. The conventionally treated Red Starking Delicious variety recorded infections rates of 40.62%, 24.75%, and 40.0 % in the years 2018, 2019 and 2020, respectively.

4.1.1.2 Comparison between two different lures

Our weekly field observations allowed us to demonstrate that the medfly is only present in the orchard from the week of August 7 (Figure 20). So, even with the presence of fruits, the Mediterranean fruit fly arrived when the apple fruit was ripe. As a result of the attacks, the harvest started during the week of August 14, 2019.

In our experiment conducted in 2019 on Gala apple variety we found that the number of fallen fruits coincided with the first flies captured in the McPhail traps (Figure 18), indicating that the fruit drop is due to the attack of *C. capitata* or the codling moth. The fallen fruits were cut to confirm the presence of insects. The first fruit falls started during the week of August 7, 2019.

The results of fallen fruits showed a significant effect of the TMA card treatment in comparison with the control as well as with the treatment using the attract and kill technique, Anamed (Table 5).

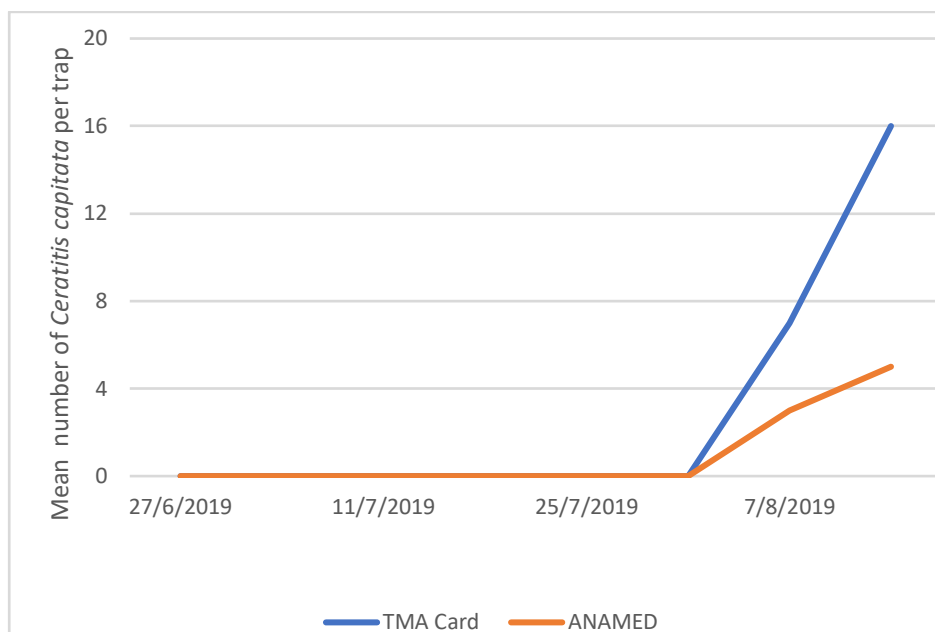


Figure 20 Mean number of *Ceratitits capitata* caught in the Gala apple orchard in Bane in 2019

Table 5 Effect of different trapping techniques on the number of fallen fruits of Gala apple variety in Bane orchard.

	Mean of fallen fruits \pm SD		
	7 August 2019	14 August 2019	20 August 2019
<i>Anamed</i> (n=9)	2.22 \pm 1.30 b*	3.67 \pm 1.5 b	13.0 \pm 8.83
<i>TMA Card</i> (n=9)	0.22 \pm 0.44 a	0.88 \pm 1.05 a	7.11 \pm 6.07
<i>Témoin</i> (n=9)	2.33 \pm 1.22 b	6.56 \pm 3.24 c	11.88 \pm 6.62

*Means followed by different letters in the same column are significant at $P < 0.05$.

Apple fruit drop was minimal in the TMA card treatment during the week of August 7, 2019 ($f=11.25$; $df=2$; $P=0.0004$) and August 14, 2019 ($F=15.61$; $df=2$; $P < 0.0001$). However, in the week of August 20, the number of fruits that fell under the trees was lower in the trees baited with TMA card compared to other treatments (Table

5) but no significant difference was found between the treatments ($F=1.64$; $df=2$; $P>0.05$).

The percentage of infested fruits was calculated by counting the cumulative number of fallen fruit is and infested fruits at time of harvest while counting the number of healthy fruits produced by each tree under the different treatment. The results showed a significant separation between the trees baited with TMA card and the control ones (Table 6).

The difference between the percentage of infested fruits between the Anamed and the positive control (treated with thiametoxam and lambda cyhalothrin) was not significant and the two percentages were very close. However, this difference is not statistically different between the trees treated with TMA and those treated with Anamed (Table 6) ($F=4.18$; $df=2$; $P=0.02<0.05$).

Table 6: Effect of different mass-trapping techniques on the yield of Gala apple variety in Bane orchard.

	Mean number of fruits/tree \pm SD	Mean number of infested fruits \pm SD	Infestation % \pm SD*
<i>Anamed</i> (<i>n=9</i>)	115.56 \pm 22.42	18.89 \pm 8.94	16.17 \pm 6.25 ab
<i>TMA Card</i> (<i>n=9</i>)	105.56 \pm 34.68	8.22 \pm 6.12	8.96 \pm 6.99 a
<i>Control</i> (<i>n=9</i>)	130.0 \pm 37.03	21.25 \pm 8.59	16.77 \pm 5.45 b

*Infestation % followed by the same letters are not statistically different at $P<0.05$.

The results obtained confirmed the hypothesis that TMA card as well as the Anamed insecticide used in the attract and kill technique are efficient as control method. The TMA card seemed better suited since the lowest infestation was recorded in the trees baited with TMA card. The positive control has also reduced the infestation since the infestation did not exceed 20%. In this study, the attractiveness of Ammonium acetate + trimethylamine + trimethylamine hydrochloride and the protein hydrolysate coupled with abamectin was demonstrated.

In our experimental model, we opted to use a high density of traps per hectare. The density of traps per hectare is dependent on several factors such as the host tree, topography, climate and physiology of the insect. Often a density of 50 traps / ha or 1 trap / 50 m² is used for massive trapping of the *C. capitata*. Other studies use 80 traps / ha (Leza et al., 2008). Recent researches recommend the use of a single trap per three trees (Munoz and Mari, 2013) while other recommend the use of one trap per tree (Epsky et al., 2013).

Mass trapping is currently being used over larger areas in the Mediterranean regions to control the Medfly (Delrio 1989, Broumas et al. 2002). Cohen and Yuval (2000) pointed out that the perimeter strategy has obtained satisfied results to avoid fruit fly actions in medium to large orchards and his strategy depends on the efficiency of traps and lures.

Target pest population density and isolation play a key role in the success of mass trapping with particularity high success in isolated areas (El Sayed et al. 2006). The presence of maturing fruits, especially plums and peaches in nearby fields, has been reported as increasing of *C. capitata* populations. (Israely et al. 1997, Alemany et al. 2004, Campos et al. 2007).

Modeling indicates that the efficiency of mass trapping increases as target population density decreases (Barclay and Li 1991), making it an effective method for early fruit varieties. The mid varieties reach maturity by mid-September and beginning of October, when adult medflies population are high and mass trapping is less effective. This is in line with our mass trapping experiments where effective control was obtained with the apple early variety Gala whereas the control was limited in late maturing variety as the golden delicious and the red Starking delicious varieties.

When analyzing overall infected fruits for the four trials per dunom, higher trap density did not decrease rate of infected fruits as for mass trapping experiment on apples was not efficient; this result supports the hypothesis of Mediouni-Ben Jemâa *et al.* 2010 that showed that mass trapping did not reduced significantly rate of infected fruits in comparison to Lufenuron and Malathion treated orchards.

Currently, in Lebanon the cost of mass trapping including labor, traps, and attractants amounts to 500 USD/dunom. To reduce this cost, it is essential to have a dispenser lifespan of >3 mo, because this allows for placing only one trap and dispenser per year. *C. capitata* population can therefore be reduced 1-2 mo before the beginning of fruit damage until the end of harvest, and this would make unnecessary the attractant replacement, halving the cost of mass trapping.

Insecticide applications (almost 3 applications in apples orchards) cost 12 USD/dunum per application with any contact insecticide.

Although mass trapping avoids insecticide residues in fruits and reduces affectation of non-target organisms, but economically speaking the use of chemical for treatment against medfly is still cheaper than the use of chemicals.

CHAPTER 5

SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

Ceratitis capitata (Wiedemann) is considered to be one of the major insect fruit pests because of its high capability to damage the production, its global distribution and its wide range of hosts. Recently, this insect pest has been reported to cause considerable damage on pome fruits, particularly apple varieties.

In our project, we demonstrated that the medfly is able to develop and sustain itself on high altitudes which indicate that this insect has developed adaptations towards harsh climate conditions. Results have shown that over the years between 2015 and 2020, witnessed a high population of the medfly which resulted in an increase number of generations at the altitude of the three different altitudes 1100 masl, 1200 masl and 1300 masl.

Mass trapping using the TMA card as a method of control in the two traditional apple varieties Golden Delicious and Red Starking Delicious demonstrated that mass trapping on these two apples varieties is not efficient. The rates of infection in the Golden Delicious trees were 56.63%, 98.54% and 66.67% in the years 2018, 2019, and 2020 indicating a very low efficacy of mass trapping. The results in the Red Starking Delicious apple variety were 36.36%, 41.84% and 47.10% for the year 2018, 2019 and 2002 indicating that the red variety is relatively less attacked by the medfly than the Golden Delicious apple variety. When compared to the traditionally sprayed orchards, the rates of infection recorded was not different. However, on the early maturing apple variety, Gala, the use of mass trapping has allowed us to obtain promising results in its use alone without any conventional spraying. The percentages of infestations in the TMA card baited trees were less than the conventionally treated trees with thiametoxam and lambda cyhalothrin. In addition, the field observations confirmed the absence of the medfly in the Gala apple orchard from June until the first week of August. Thus, the application of insecticides targeting the Mediterranean fruit fly is not necessary until August.

There is a lack of overwintering studies of medfly in the Mediterranean countries as it is important to find out the conditions of overwintering of the different

stages of development. Our study support the fact that the stages of larvae and pupae of medfly and maybe adults survived the natural conditions of late autumn and winter in the high altitude of North Lebanon. Adults continued to emerge until early November and then stopped emergence, so it was not possible to prove that adults found in the following year came from fruits infested in winter. Climatic conditions including daily temperature appeared to be involved in the number of generations observed each year.

This study has shown that the medfly is present in high altitude and future studies should be targeting the population of high altitude to determine the biological characteristics of this strain. Choice tests should be carried out on all the varieties of pome fruits and alternative methods of control should be sought of since the McPhail trap did not show its superiority in combating the medfly on the Red Starking Delicious and the Golden Delicious varieties.

More studies on the overwintering stages are necessary to conduct at the same altitudes and higher altitudes to determine if the medfly has evolved some adaptations mechanism to whist hand prolonged periods off cold.

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