Studies of Non-Chemical Strategies for Postharvest Management of Mediterranean Fruit Fly (*Ceratitis capitata*)

This thesis is presented for the degree of
Doctor of Philosophy of Murdoch University

by

FARHAN JASIM MOHAMMED AL-BEHADILI

College of Science, Health, Engineering and Education
Murdoch University
Perth, Western Australia
December 2020

Declaration

I declare that this thesis is my own account of my research and contains as its main content work, which has not previously been submitted for a degree at any tertiary education institution.

Signature: F. AL-BEHADILI Date: 20 /10/2020

Acknowledgment

"In the name of Allah, the Most Gracious and the Merciful."

O Allah bless Muhammad and Muhammad's household. My God uninterrupted flow of your graciousness and your flood bounty has amazed me from thank you and rendered me incapable of counting your praises. I never forgotten AL-imam AL-Hussein (عليه السلام), my mother, father and Martyrs and I gift this my humble effort to their immaculate souls.

I gratefully acknowledge the Iraqi Government, represented by the Ministry of Higher Education and Scientific Research, for providing the fantastic opportunity to complete my doctoral study in Australia. I greatly appreciate its financial support allowing me to focus solely on my research without any obstacles.

I also extend my sincere gratitude to my supervisors, Dr. Wei Xu, Professor Yonglin Ren, and Dr. Manjree Agarwal; each of you have been a fabulous supporter for me. I would like to thank you for supporting my research and assisting me in thinking like a scientist. Your advice and guidance regarding my Ph.D. study have been appreciated. I would also like to thank Professor Una Ryan as my advisory committee chair for assisting me with my Ph.D. study and providing valuable feedback. Many thanks are going to Professor Giles Hardy and Professor Michael Calver for their comments and suggestions. I also want to thank Dr. Vineeta Bilgi, Dr. Junxi Li, Dr. Penghao Wang, Miyuki Taniguchi James Newman and Sue Pan from the postharvest biosecurity and food safety laboratory for their helpful laboratory technical support. I am also hugely grateful to Dr. Sonya Broughton and Emma Mansfield from the Department of Primary Industries and Regional Development (DPIRD); for providing me with the fruit fly colony.

I am also very grateful to my friends Dr. Khalid Al-Hasi, Belinda Lycett, Dr. Arkan Mohammed, and Dr. Araz Sedqi Abdullah, for their support and encouragement. I would

thank all my friends in Iraq, especially my colleagues at the Agriculture College of Misan and Basrah Universities.

I would like to thank my colleague's Dr Mohammed Ibrahim, Ahmed Auda, Dr. Hassan Hadi, Dr. Qasim Hussein Ahmed, Basman Aljalely, Thamer Alshowili, Nadia Al-Saedi, Dr. Ihab Abdul-Kreem Alnajim, Dr. Khalid Mohammed, Dr. Zaid Al-habbar and Dr. Mohammed Yaseen. Your commitment, intelligence, persistence, and hopefulness were more appreciated than you could ever imagine.

I greatly appreciate the support that I have received from my dear wife, and I thank you for being so sympathetic with me through the hardest days of my life. Without your patience, prayers and support, I can't complete my Ph.D. study. I also cannot forget my beloved son Mosadaq, my affectionate brothers and sisters, my uncles and aunts, my relatives and neighbours.

Abstract

The Mediterranean fruit fly *Ceratitis capitata* (Medfly), is a highly polyphagous insect species with a host range of over 400 plant species. It is one of the most destructive horticultural insect pests which has resulted in huge pre and postharvest losses, costing billions of dollars every year for pest management. Quarantine and phytosanitary treatments are essential to ensure no live Medfly in imported and exported fruit, vegetable and hay. However, it is increasingly difficult to be clarified due to the ban of key pesticides, methyl bromide, an ozone-depleting substance, and chemical residues related food and consumer safety issues. Therefore, the non-chemical strategies, including the controlled atmosphere (CA), heat and low temperature and irradiation treatments have been applied for eradication of fruit flies in postharvest fruits. However, the response of Medfly to low-temperature and low-oxygen has not been extensively studied in the lab diet and blueberries.

My Ph.D. research has systematically evaluated low-temperature and low-oxygen treatments of Medfly on a lab diet and two blueberry cultivars. The results demonstrated that in the lab diet, both the 1st and 3rd larval instars were the most cold-tolerant stages. In blueberries, the 3rd larval instar was the most cold-tolerant stage. In the low-oxygen/high-nitrogen treatment, the 3rd larval instar was the most tolerant stage at 25°C. No significant differences in sex ratios of survived Medfly were observed between treated and non-treated Medfly populations after both low-temperature and low-oxygen treatments.

This study improved our understanding of Medfly responses to low-temperature and low-oxygen treatments. The results and knowledge will help refine current fruit fly postharvest management and develop more efficient and environmentally friendly control approaches, which will contribute to the integrated postharvest pest management strategies for Medfly.

List of publications

Al-Behadili, F. J.., Bilgi V., Li, J., Wang, P., Taniguchi, M., Agarwal, M., Ren, Y. and Xu, W. 2019. Cold Response of the Mediterranean Fruit Fly (*Ceratitis capitata*) on a Lab Diet. *Insects*, 10(2), p.48.

Al-Behadili, F.J., Agarwal, M., Xu, W. and Ren, Y. 2020. Cold responses of the Mediterranean fruit fly *ceratitis capitata* wiedemann (Diptera: Tephritidae) in Blueberry. *Insects*, 11(5), p.276.

Farhan J.M. Al-Behadili, Manjree Agarwal, Wei Xu, Yonglin Ren. 2020. Mediterranean fruit fly Ceratitis capitata (Diptera: Tephritidae) eggs and larvae responses to a low-oxygen/high-nitrogen atmosphere. *Insects.* In press

List of international conference presentations

Oral presentation:

19th International Conference on Entomology. The International Scientific Committee (ICE 2017), October 19-20, 2017, Paris, France.

Evaluation of Low Temperature (0°C) as A Treatment Tool for Eradication of Some Immature Stages of Mediterranean Fruit Fly (*Ceratitis capitata*) in Artificial Diet.

Abbreviation

E.E.	Early Egg
L.E.	Late Egg
1^{st}	First Larvae Instar
2^{nd}	Second Larvae Instar
3^{rd}	Third Larvae Instar
Probit	The cumulative distribution function of the standard normal distribution
Logit	The cumulative distribution function of the logistic distribution
h	hour
D	Day
CA	Controlled Atmosphere
DPIRD	Department of Primary Industries and Regional Development's
LT_{50}	Lethal time required to kill 50 % of experimental animal
LT_{90}	lethal time required to kill 90 % of experimental animal
LT_{95}	lethal time required to kill 95 % of experimental animal
LT_{99}	lethal time required to kill 99 % of experimental animal
L	Litter
Kg	Kilo gram
mL	Millilitre
g	Gram
HCL	Hydrochloric acid
O_2	Oxygen
N_2	Nitrogen
CO_2	Carbon dioxide
mm	Millimetre
m^3	Cubic meters
m	Meter
min	Minute
SE	Standard Error
C00-09	Name of a blueberry cultivar
C99-42	Name of a blueberry cultivar
RGB	RGB Colour model to measure colour density (Red, Grey and Black)
kg/cm^2	Kilo gram on cubic centimeter

Kg/cm	Kilo gram on centimeter
cm	centimeter
F	F-statistic is a value when run an ANOVA test or a regression analysis
P <	p-value is less than
p >	p-value is greater than
°C	Celsius Degree
RH	Relative Humidity
CL	Confidence Limits
D:N	darkness light cycle (Day/Night)

Table of Contents

List of Figures

List of Tables

List of Supplementary Materials

Chapter one

Literature review

1.1. Background

The demand for high-quality fruits and vegetables is growing globally. Fresh-product supply is a worldwide concern due to rapid human population expansion and the degradation of their quality after harvest (Kiaya, 2014). The current quality assurance system utilizes various tools and methods to control pest risks on fresh produce to protect food quality. However, improvement in adopted techniques, implementation of the best production and handling practice will ensure food safety of fresh produce in the supply chain (Jacxsens et al., 2010). One of the critical factors that contribute to the deterioration of the fruit quality after harvesting is insect pests and the side effects of some control methods, including change of climate, biological diversity, crop quality, and environmental pollution.

The most important side effects of control methods are the direct impact on consumer health (residues) and economic effects. Integrated pest management, especially postharvest treatments, regulates fruit quality and, eventually, market prices and access. Therefore, biosecurity controls to protect fresh products and maintain their quality from insect damages are vital to the sustainability of the food.

1.2. Fruit fly

Fruit flies are one of the world's most destructive horticultural pests, which pose risks to most commercial fruit and vegetable crops. They are about 2-14 mm long, possess bright red eyes, and stay around fruit trees (E.F.F., 2015; A.G., 2020). Usually, their wings are held outwards like blades during rest. Fruit flies are larger than vinegar flies, which also feed on fruits. Fruit flies lay eggs inside fruits, so hatched larvae can directly feed on the fruits. The Tephritidae family is one of the two fruit fly families, while the other is Drosophilidae, which includes the biological model organism *Drosophila melanogaster*. Nearly 5,000 described species of

tephritid fruit fly are categorised in almost 500 Tephritidae genera (Norrbom et al., 1999). Description, categorisation, and genetic analyses are constantly changing the taxonomy of this family. Many species of Tephritidae are a significant threat to the horticulture industry throughout the world, leading to economic loss in fruit and vegetable crops, and their quarantine implications (Badii et al., 2015).

1.2.1. Classification of Tephritid Fruit Fly

The taxonomic hierarchy of Tephritidae is as below (Esther, 2010):

Kingdom Animalia (Animals)

Phylum Arthropoda (Arthropods)

Subphylum Hexapoda (Hexapods)

Class Insecta (Insects)

Order Diptera (Flies)

Superfamily Tephritoidea

Family Tephritidae (Fruit Flies)

Tephritidae is one of the most destructive insect pest families (fruit flies) with around 5,000 species, in which nearly 200 species are considered as pests. Five representative Tephritidae fruit fly species are Mediterranean fruit fly or Medfly (*Ceratitis capitata*), Queensland fruit fly (*Bactrocera tryoni*), Melon fly (*Bactrocera cucubitae*), Oriental fruit fly (*Bactrocera dorsalis*) and Solonaceous fruit fly (*Bactrocera latifrons*) (Figure 1.1 A, B, C, D and E) (De Meyer and Freidberg, 2005).

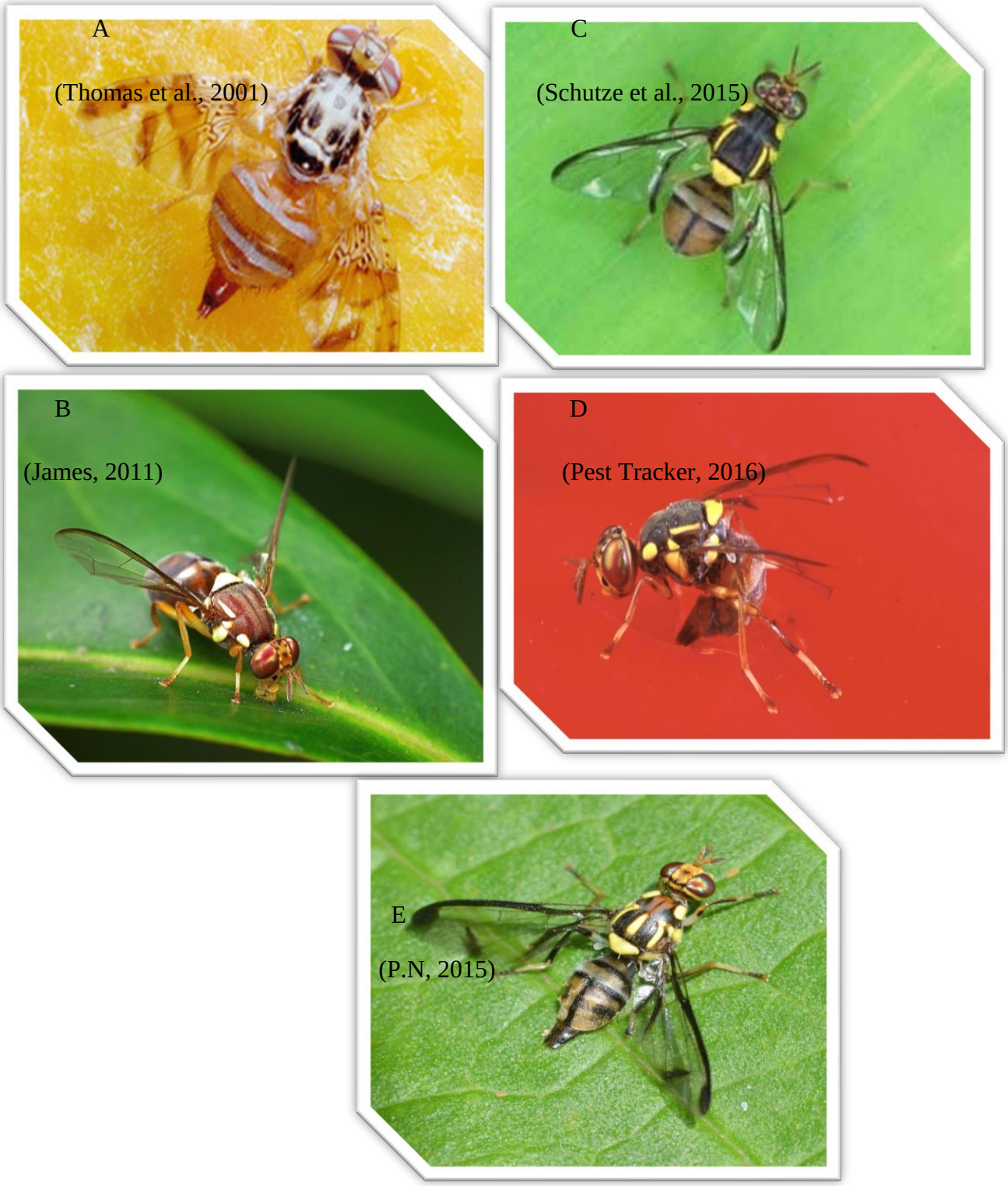


Figure 1.1. Five Tephritid fruit flies including (A) Mediterranean fruit fly (*C. capitata*), (B) Queensland fruit fly (*B. tryoni*), (C) Oriental fruit fly (*B. dorsalis*), (D) Solonaceous fruit fly (*B. latifrons*) and (E) Melon fly (*B. cucurbitae*).

1.2.2. Tephritid Fruit Fly

Tephritid fruit flies are mostly coloured and the subcostal vein is bending forward at a right angle (Gillott, 2005). The head is hemispherical and generally short. The face is vertical or retreating and the fron is wide. Ocelli and Ocellar bristles are clear. The post vertical bristles are parallel to divergent. Two to eight pairs of frontal bristles are present. Generally, many lower pairs bend inwards and at least one of the upper pairs bend towards the back. Interferential setulae are often absent or represented by one or two tiny setulae near the lunula. True vibrissae do not exist, but many genera have strong bristles close to the vibrissal angle. The wings are mostly yellow, brown, or dark-coloured with lighter markings or have black markings. The costa has a humeral and a subcostal break. The upper part of the subcostal is often unclear or transparent. The geniculated (CuA2) vein is convex. The tibiae lacks a dorsal preapical bristle. The ovipositor is present in the female.

The larvae have only the anterior and posterior pairs of spiracles, so they are called amphipneustic. The colour of the body differs from white to yellowish or brown. Sometimes pale-colored species have a black posterior end. The anterior part of the body is tapering. The larvae sometimes have teeth along the ventral margin of the two mandibles. There are several transverse oral ridges or short laminae posteriorly directed in the antenna-maxillary lobes at each side of the mandibles. The prothoracic spiracles are not elongated. Also, it has three slots containing up to 50 arranged transversely in 1-3 groups or irregular arrangment. Any anal spiracle (posterior spiracle) does not have a peritreme and each has three spiracle slots in the mature larvae case. These are more or less collimated, horizontal and often carry branched spiracle hairs in four tufts (Smith, 1989).

1.2.3. Mediterranean fruit fly (Medfly)

Medfly (*Ceratitis capitata*) belongs to Tephritidae family, Genus Ceratitis and Species: *capitata.* It has many synonyms names (White et al., 1992) such as *Ceratitis capitata (*Wiedemann), *Trypeta capitata* (Wiedemann, 1824), *Tephritis capitata* (Wiedemann, 1824), *Ceratitis citriperda* Macleay, *Ceratitis hispanica* De Breme and *Pardalaspis asparagi* Bezzi.

Medfly originated in sub-Saharan Africa and then spread throughout the Mediterranean region, Europe, the Middle East, Western Australia and the South and Central America.

1.3. Life cycle and stages

1.3.1. Eggs

Female Medfly lays eggs individually or in groups into the fruits or vegetables. The egg is very slender, curved, about 1.01mm long, smooth and shiny white (Figure 1.2) (Rini, 2003).

Figure 1.2. Eggs of the Medfly, *C. capitata* (Wiedemann) (Thomas et al., 2001). Photograph by Jeffery Lotz, Florida Department of Agriculture and Consumer Services-Division of Plant Industry; www.forestryimages.org.

1.3.2. Larvae

Medfly larvae are elongate, cream-coloured, and cylindrical maggot-shaped. Their anterior ends are narrow and somewhat recurved ventrally with anterior mouth hooks and flattened caudal end. The first instar larva is around 1.06 mm or less, and its body is transparent. The second larval instar is slightly transparent with clear food in the intestine. The fully grown third larval instar is 6.35 to 8.46 mm long, completely opaque and appears non-transparent white or the colour of food that it feeds upon (Figure 1.3). The Medfly larvae can be differentiated from other fruit fly larvae by the front, or thoracic and spiracles that bear small, finger-like tubules between 7 to 11 in number.

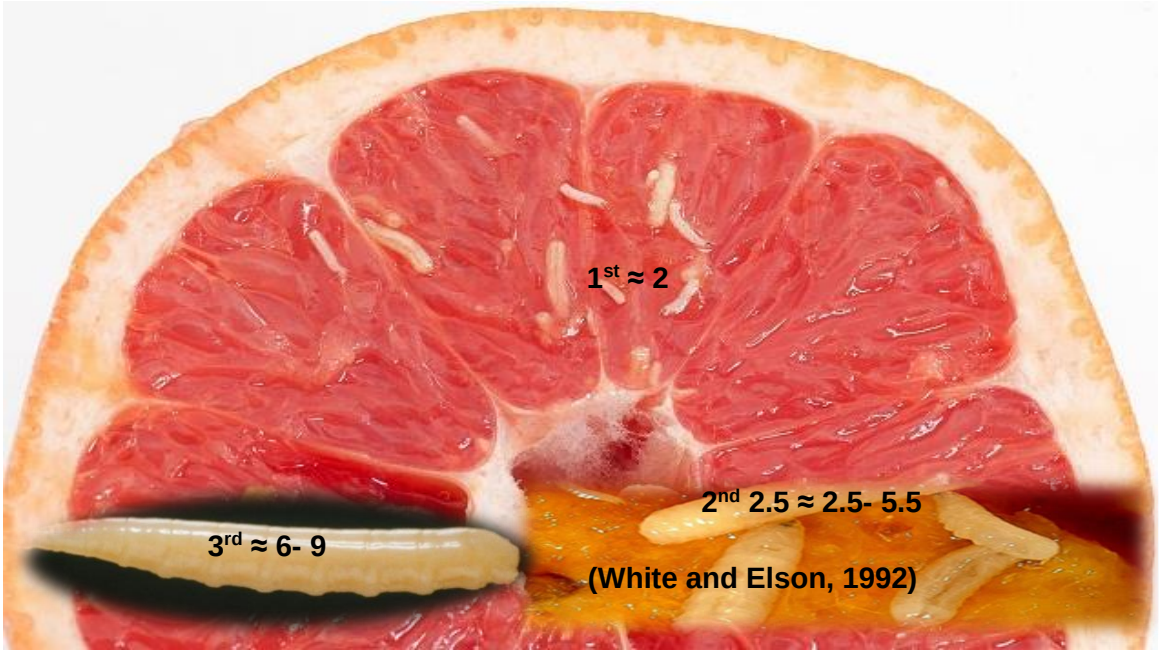


Figure 1.3. Larvae of the Medfly, *Ceratitis capitata* (Wiedemann). (Thomas et al., 2001). Jeffery Lotz, Florida Department of Agriculture and Consumer Services-Division of Plant Industry; www.forestryimages.org.

1.3.3. Pupae

Medfly pupae are cylindrical, nearly 3.17 mm long, and dark reddish-brown. Pupae usually develop in the soil and 25.4-50.8 mm under the surface (Figure 1.4) (Rini, 2003).

Figure 1.4. Pupae of the Medfly, *C. capitata* (Wiedemann) (Thomas et al., 2001). Photograph by Jeffery Lotz, Florida Department of Agriculture and Consumer Services-Division of Plant Industry; www.forestryimages.org.

1.3.4. Adult

Medfly adults are 4.23-5.08 mm long (Back and Pemberton, 1915). The body colour is yellowish with a brown hue, particularly the abdomen, legs, and some of the markings on the wings. The oval shaped abdomen is wrapped on the upper surface with fine, sprinkled black bristles, and has two narrow, crosswise, light coloured belts on the basal half. The female can be differentiated via its long ovipositor at the end of the abdomen (Figure 1.5). When fully extended, the ovipositor (the egg laying tube) is about six times as long as its greatest width (Figure 1.5). The apical surface of the thorax is convex and the ground color is creamy-white to yellow, marbled with black blotches. The lighter areas are wrapped with very fine bristles, and many black bristles grow from the darker areas of the thorax.

The wings, mostly grasped in a curvature site on live flies, are broad, transparent and vitreous with black, brown and brownish yellow markings with tints. There is a complete, brown-yellow band across the middle of each wing. The extreme base is blotched with brownish yellow, with the rest of the basal area marked with black lines of the radiating wing veins, with dark spots between them. The male head bears two long, black bristles with broadly flattened, slightly diamond-shaped tips, arising between the eyes close to the antennae. The eyes are reddish-purple.

Figure 1.5. The differences between adult females have ovipositor and male of the Medfly, *C. capitata* (Thomas et al., 2001).

The time required for the Medfly to accomplish its life cycle during ideal summer weather conditions is 21-30 days (Figure 1.6). A female Medfly lays 1-10 eggs in a 1 mm deep egg hole and sometimes lays 22 eggs per day. Generally, during her lifetime, one female adult can lay about 300 eggs. Female Medflies can form eggs continually during her adult life; therefore, the total number of eggs found in the reproductive organs is not an indication of the total number of eggs an individual female can produce. Females mostly die soon after stopping oviposition (Rini, 2003).

Eggs are deposited beneath the skin of ripened fruits, often in a part with damage in the skin . Multiple females can use the same deposition cavity with 75 or more eggs clustered in one spot. When the eggs hatch, the larvae immediately start eating, and tunnels are formed inside the fruits. A hard or semi-ripe fruit is better than a fully ripened fruit for oviposition because ripe fruit is juicier, and hence ovipositing in ripened fruits may be related to a high deathrate of young larvae and eggs.

Medfly females do not oviposit when the temperature is below 16°C except when exposed to sunlight for several hours. The development of eggs, larvae, and pupae will stop at 10°C. In warm weather, the eggs will hatch through 1.5-3 days. Lower temperatures significantly increase the period of egg development. Pupae carry the species through unfavourable conditions, such as lack of food, water, and temperature extremes. There are three instars for the larvae stage (Christenson, 1960).

When the average temperature is between 25.0-26.1°C, it may take 6-10 days for Medflies to complete larval stage (Figure 1.6). The conditions of the fruit usually influence lengths of the larval stage. It appears to be longer in citrus fruits (especially limes and lemons) than other fruits. In a ripe lemon, larvae need 14-26 days to reach maturity while 10-15 days in a green peach (Thomas et al., 2001)

After daybreak, 3rd instar larvae leave the fruit and jump to the soil, where they develop to pupate under the soil. When the mean temperature ranges from 24.4-26.1°C, the minimum

duration of the pupae stage is 6-13 days. This duration possibly increases to at least 19 days when the daily mean temperature drops to about 21.7°C (Back and Pemberton, 1915).

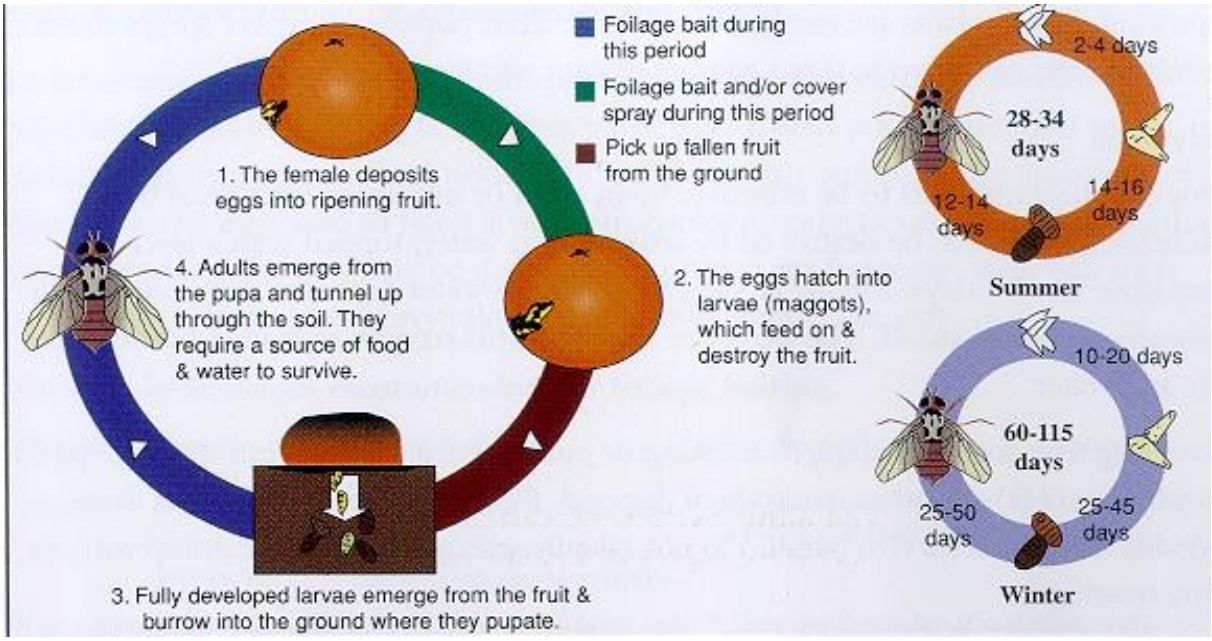


Figure 1.6. The life cycle of the Medfly, *Ceratitis capitata* (Wiedemann) (Broughton and De Lima, 2002).

Most adults emerge early in the morning in warm weather, while during cold weather, adults emerge more sporadically. Adults can fly a short distance. For example, they may fly a mile or more away with the assistance of the wind. Mating can happen at any time during the day. Newly emerged adults are not sexually mature, so males mostly show sexual activity four days after emergence (Thomas et al., 2001). Male and female adults are sexually active during the day. Most females are ready to mate from 6 to 8 days after emergence when the daily mean temperature ranges from 24.4-25.6°C (Thomas et al., 2001).

Oviposition may occur 4-5 days after emergence through highly warm weather, but not until 10 days after emergence when temperatures average from 20 to 22.2°C (Back and Pemberton, 1915). Between 2 and 4 days after emergence, adults die in a large number if they do not get food. Approximately 50% of the flies may die throughout the first two months after emergence. When favourable food (fruit, honeydew, or plant sap), water, and cool

temperatures are provided, some adults may survive up to a year or more (Thomas et al., 2001).

1.4. Damage

Medfly causes serious economic damage because it can breed multiple generations per year and has developed resistance to chemical pesticides (Hill et al., 1988). Therefore, Medfly is a serious threat to international trade and the horticulture industry. The loss of productivity and thousands of tons of fruits damage caused by fruit flies lead to millions of dollars of loss per season (NSW, 2012). To control fruit flies, farmers spray chemical insecticides, which are not environmentally friendly and are also expensive (Vargas et al., 2015).

The average annual value of fruit fly susceptible Australian Horticulture is $4.8 billion, with roughly 25% traded interstate. From 2003-2008, the Australian industry and government invested more than $128 million in the management of fruit flies (Abdalla et al., 2012). The financial impact of a fruit fly incursion to the New Zealand kiwifruit industry is estimated to cost between 2 and 430 million US dollars per year (KVH, 2014).

In Western Australia (WA), thin-skinned stone fruit (apricots, nectarines, and peaches), mangoes, persimmon, apples, pears and mandarins are particularly susceptible to Medfly. It has been considered that without control, Medflies would infest all sensitive fruits such as apricots, nectarines, peaches and mandarins and, to a lesser extent, apples and pears.

Adult female fruit flies lay their eggs in the pulp of ripening fruits. As soon as the eggs hatch, the maggots start to feed within the fruits, which will ripen early (Figure 1.7). Fruit skin may seem sound or display twinge marks; however, beneath the skin, the pulp becomes mushy, soft and brown with wriggling white maggots (Figure 1.7). Fruit flies pose a severe economic threat to commercial fruit and vegetable growers (Muthuthantri, 2013). Maggots feeding inside the fruit will destroy the pulp of the fruit, so the fungi and bacteria will enter and cause rotting of the fruit and degrade the quality of vegetables and fruits (Figure 1.7). Moreover, huge numbers of rotting fruits on the ground can make an undesirable mess, especially in landscaped situations (Vayssières et al., 2009). Fruit fly damages fruits in three ways. Firstly,

the ovipositor of fruit fly may leave indentations, which are undesirable marks for customers. Secondly, some small fruits (like olives) that are probed by the fruit fly's ovipositor may abort and fall from the tree, reducing yield. Third, the value of the fruits is destroyed due to the maggot infestation (Daane and Johnson, 2010).

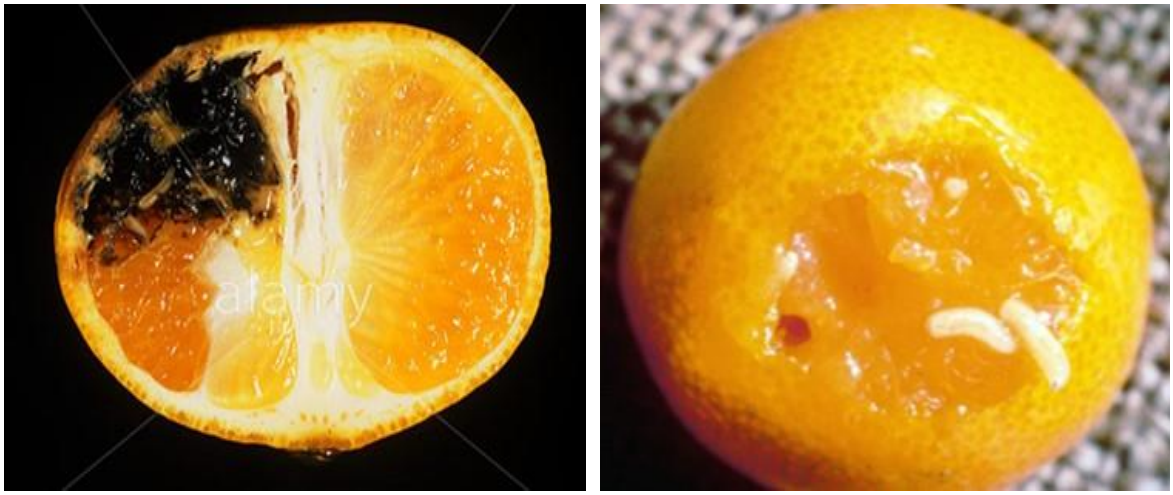


Figure 1.7. Damage of Medfly *C. capitata* (Cattlin, 2010).

1.5. Distribution

Medfly originates from Africa and spread in many countries in Africa: Algeria, Angola, Berin, Botswana, Burkina Faso, Burundi, Cameroon, Cape Verde, Congo, Congo Democratic Republic, Cote d'Ivoire, Egypt, Ethiopia, Gabon, Ghana, Guinea, Kenya, Liberia, Libya, Madagascar, Malwai, Mali, Maritius, Morocco, Mozambique, Niger, Nigeria, Reunion, Sao Tome and Principe, Senegal, Seychelles, Sierra Leone, South Africa, St. Helena, Sudan, Tanzania, Togo, Tunisia, Uganda, Zimba (Thomas et al., 2001). Medfly has spread and established in Europe such as Albania, Azores, Balearic Islands, Canary Islands, Corsica, Croatia, France, Greece, Italy, Madeira Islands, Portugal, southern Russia, Sardinia, Sicily, Slovenia, Spain, and Yugoslavia; in the Middle East and Asia like Cyprus, Jordan, Lebanon, Saudi Arabia, Syria, Turkey, and Yemen; in South American including Argentina, Brazil, Colombia, Ecuador, Paraguay, Peru, Uruguay and Venezuela. Medfly infestations were

found in the United States, including Hawaii, Florida, Texas and California (Dawson et al., 1998; Papachristos et al., 2008). Australian distribution of Medfly is currently restricted to Western Australia (Dominiak and Mapson, 2017). The global Distribution of Medfly, as shown in Figure 1.8.

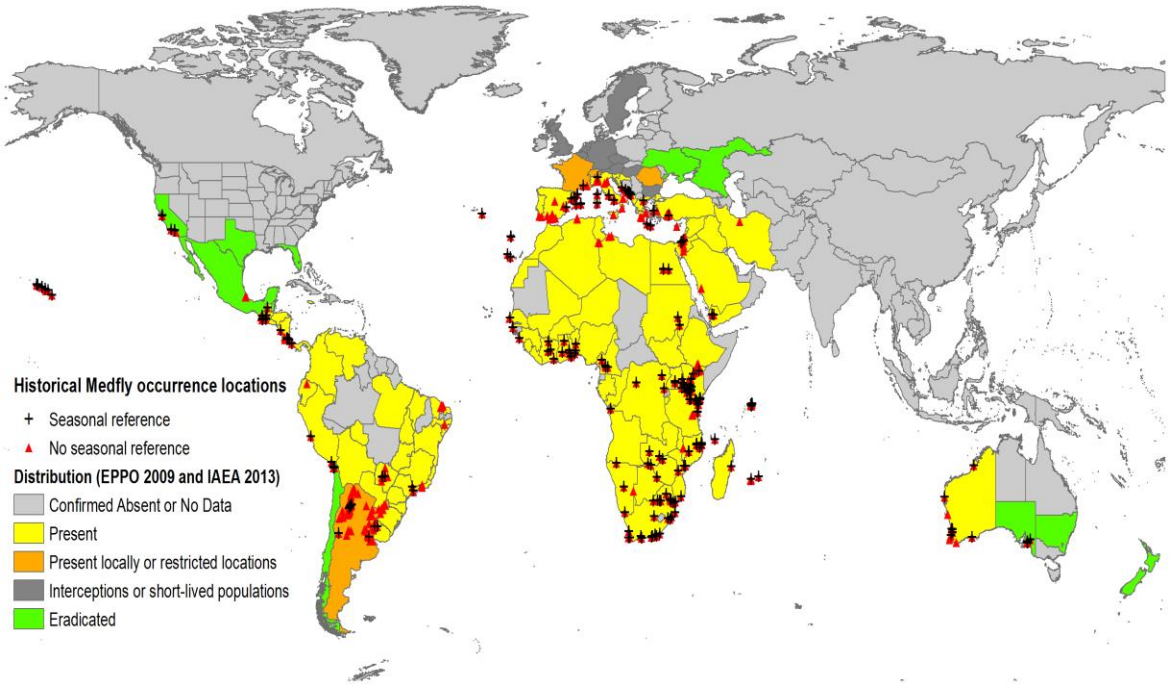


Figure 1.8. Updated Global Distribution Map of Medfly (Szyniszewska and Tatem, 2014).

1.6. Host Preference

Medfly has been recorded from over 400 fruit, vegetable and nut plant species (Capinera, 2020). For example, several cucurbits species, including watermelon and musk melons, have been recorded as hosts of Medfly. The main hosts include citrus, stone fruits, pome fruits, peppers, tomatoes, and figs. Host plants also include avocado, apricots, nectarines, peaches, mandarins, persimmon, strawberry, cherry, grape, banana, bittermelon, carambola, coffee, guava, mango, papaya, peppers and blueberry.

Australian blueberry export to Japan was quite successful from 1998 to 2011. However, in 2010, the Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF) conducted a

review of the status of blueberries as a host of fruit flies. It determined that blueberries are a host to both Medfly and Queensland fruit fly (Qfly), *B. tryoni* (Froggatt). Qfly is endemic to eastern Australia, and Medfly has a limited distribution in Western Australia. Consequently, exports to Japan were suspended in 2011 (Brazelton, 2013).

1.7. Movement and mating

1.7.1. Movement

The spatiotemporal dispersion patterns of *C. capitata* population probably take into consideration the addition of individual adult fly foraging behaviours. Flies need essential resources such as food, mates, and places to lay eggs (Prokopy et al., 1994). Flies are known to change their foraging behaviour in response to the differences in the spatial, temporal, and seasonal allocation of food and other resources (Hendrichs et al., 1991).

Iwahashi (1972) found that the oriental fruit fly moves from island to island frequently. Nine of 3000 signed males, released at Haha Jima, ca. 50 km from Chichi Jima, were taken by three traps set on Chichi Jima. The movement of this fly between islands was considered a case of failure of an elimination program of this species. Medfly was reported in New South Wales and Victoria in the years 1941 and 1953, respectively (Dominiak and Daniels, 2012). Tasmania has not recorded any incursions of Medfly since 1920 and the Northern Territory exterminated the last attack in 1994. In contrast, Qfly is usually found in parts of Queensland, New South Wales, Victoria, and the Northern Territory.

In Australia, Medfly is currently allocated only in Western Australia. However, occasional detections happen in South Australia and the Northern Territory Medfly has previously existed on the eastern coast of mainland Australia. However, it is considered to have been replaced by Queensland fruit fly (*B. tryoni* Froggatt, 'Qfly') (Dominiak and Daniels, 2012). Medfly and Qfly were transported between states in a range of infested produce before quarantine controls were developed (May, 1963). Subsequently, both Qfly and Medfly co-existed in areas of NSW and Victoria for some time.

1.7.2. Mating

The mating affects the number of progenies produced by females. For example, females with multiple mating's of *C. capitata*, on average, had greater longevity and higher productivity than one-time maters (Whittier and Shelly, 1993). In a study on Medfly, *C. capitata* (Wiedemann), Whittier & Kaneshiro (1991) discovered that females that mated with virgin males resulted in more offspring than females that mated with non-virgin males. Interestingly, a decline in fertility was evident even when the non-virgin males were allowed redemption period after the first mating. Lux et al., (2002) found that age can affect the mating of *C. caiptata*. The optimal age for in situ flies ranged about 7 to 13 days while, in the laboratory, it was from 3 to 5 days. The parentage from mating in the laboratory was 72%, while the in situ 67% of the mating fruit fly was successful.

1.7.3. Dispersion and migration

The Medfly *C. capitata* is an economically important horticulture pest in Western Australia and several countries. It appears to have the potential to spread in many countries around the world through infested fruits (White and Elson-Harries, 1994). A better understanding of the dispersal of insect pests can assist in improving their management. In particular, the success of increasingly popular environmentally sound control practices, such as pheromone-based mating disruption and the release of sterile insects, hinge on dispersal performance. Fruit insects compete with humans for food, and the resulting crop losses remain severe, despite intense agrochemical input. Protecting yields from pests is one of the pillars of programs aimed at securing the food demands of the growing world population (Mazzi and Dorn, 2012). Brenner et al. (1998) studied spatial dispersion patterns of insects, which are based on the variance-mean relationship of samples without looking at the spatial location of those samples. However, the sample observation might be interdependent, so other strategies may be more appropriate for analysing spatial dispersion patterns. These methods can provide a more direct measure of spatial dependence than dispersion indices. (Liebhold et al., 1991; Nestel and Klein,1995).

C. capitata can be seen in large population density in agricultural areas where high quantities of fruits provide lots of food. This species is widespread and may exist anywhere from sea level to mountainous regions. Its habitat may be affected by other fruit fly species. When first introduced to Hawaii, Medflies were discovered in the lowlands. However, since the subsequent introduction of the oriental fruit fly (*B. dorsalis*) in 1945, Medflies were only found at larger elevations. (Copeland, et al., 2002; McPheron and Steck, 1996).

Israely et al. (2005) studied the large-scale spatial-temporal distribution pattern of Medflies to further understand whether long-term dispersal may change the maintenance in an isolated, desolate and arid environment. The results showed that flies migrated at the lowest 50 km, probably over a single generation. This long-distance migration has far-reaching importance for the maintenance of isolated populations and should be taken into consideration for future study and control practices of *C. capitata.*

1.8. Management of fruit fly

Fruit fly management includes pre-harvest and postharvest treatments. Some pre-harvest treatments include chemical methods, biological control, sterile insect technique (SIT) and attractive traps (Table 1. 1). Postharvest treatments include fumigation, irradiation and Controlled Atmosphere /Temperature Treatment System) (CATTS).

1.8.1. Pre-harvest fruit treatments

There are five major approaches available to achieve for pre-harvest pest management: chemical control (synthetic and naturally derived), biological control (predators, parasitoids, pathogens and other bioagents), cultural control (including cover crops and genetically resistant plants), physical control and human factors (legal restrictions) (Vincent et al., 2003). The cultural, physical and biological measures are traditional approaches, being followed by farmers since ages. Cultural control is the practice of modifying the growing environment to reduce the prevalence of unwanted pests. Examples include changing soil pH or fertility levels, irrigation practices, amount of sunlight, temperature, or the use of beneficialorganisim. This control method depends on breaking the reproductive cycle of the

pests, so the farm sanitation and crop hygiene targeted are the main factors (Badii et al., 2015; Hill, 1990). 1.1 The advantages and disadvantages are listed in Table 1.1.

Biological control is one of several strategies of integrated pest management which used to control pests to avoid economic damage on crop plants, in farming, or recreation areas. Natural enemies of insect pests, also known as biological control agents, include predators, parasitoids, and pathogens (Eilenberg, 2006). Mostly biological control agents have no adverse effect on human health or environment and is self-sustaining (Vincent et al., 2009; Bale et al., 2008). However, biological control agents are expensive, slow, never eradicate a pest (Vincent et al., 2009).

The entomopathogenic fungi and their extracts, such as *Metarhizium anisopliae, Paecilomyces fumosoroseus, Beauveria bassiana*, *Verticillium lecanii* and *Aspergillus ochraceus* were used to control Medfly, *M. anisopliae* was the most pathogenic fungi to control *C. capitata* adult (Castillo et al., 2000). Other entomopathogen is nematodes, for instance, *Heterorhabditis bacteriophora*, *Heterorhabditis zealandicaand* and *Steinernema khoisanae* which were used to control pupariating larvae, pupae and adults of *C. capitata* (Malan and Manrakhan, 2009).

The wasps *Diachasmimorpha longicaudata*, *D. kraussii, D. tryoni, Fopius ceratitivorus, F. arisanus, Psyttalia humilis, Dirhinus giffardii, Coptera silvestrii, Aceratoneuromyia indica, Pachycrepoideus vindemmiae* and *Tetrastichus giffardii, Muscidifurax raptor* were successfully attacking the adults of *C. capitata* (Kroder and Messing, 2010). Pheromone, such as spiroacetal and parapheromone, such as trimedlure and ceralure are both considered a biological method to control *C. capitata* (Navarro et al., 2011)

Though there is a controversy of using chemicals due to environmental pollution, it is a fact that this is the common method that is widely accepted for effective control of pests. The reporting of pesticide uses, and market data is patchy and irregular, it is generally clear that the use of synthetic pesticides in agriculture has grown steadily, and now amounts to 3.5 billion kg of pesticide per year (Pretty and Zareen, 2015). China's consumption of pesticides

is 146 million tons in 2006, and globally, the top ten pesticide consuming countries are China, the USA, Argentina, Thailand, Brazil, Italy, France, Canada, Japan, and India (Sharma et al., 2019). Integrated Pest Management (IPM) is most likely to be misunderstood as pest management without chemicals, which use chemicals as one of the components.

Chemical control is highly effective, provides quick results of insect mortality, and can be used in various climatic conditions. There is a wide range available for a selection of chemicals, and this method is economical, too (IPM, 2009). The insecticides that have been used most frequently in *C. capitata* control include dimethoate, fenthion, naled, malathion, fenitrothion, formothion, and methomyl (Vontas et al., 2011). Disadvantages include the repeated application of chemicals, non-target species like natural enemies of insects are affected, resurgence of minor pest, food residue and direct hazard to the applicator, and continuous application of chemicals develops resistance in insects (IPM, 2009).

Physical control uses physical actions to remove pests of plant hosts completely. The two principal physical control methods are fruit stripping and host elimination (Vargas et al., 2015). It is a method of getting rid of insects, birds, and small rodents by removing, attacking, or setting up barriers that will prevent further destruction of plants. These methods are primarily used for crop growth.

There are advantages of physical methods for pest control; for example, the development of resistance in pests is highly improbable as physical methods require the implementation of simple actions. It does not alter the quality of the controlled products and can be regarded as rather safe in terms of workers' safety. Physical control methods leave no residues. (Reppchen et al., 2010). The modern patterns of pest control include biotechnological approach such as SIT, mass trapping, use of para pheromones and use of food baits (Dyck et al., 2006, Lamichhane et al., 2016, Gupta, 1960, Lehane, 2005). For physical control of *C. capitata* to be effective, it is fundamental for growers (both home garden and commercial) to dispose of fly-infested or unwanted fruit, including fruit left on the tree, whether infested or unifested. There are many physical destruction methods to control *C. capitata.* For instance, boiling

infested fruit, solarising by placing fruit in plastic bags, preferably black rubbish bags so that the heat from the sun kills the eggs and larvae, freezing for at least 24 hours, mulching to a pulp consistency and burial to a depth of at least 1 metre. (Arevalo-Vigne, 2017)

Table 1.1. Comparison of Biological, Chemical, Cultural and Physical control for Medfly (Vincent et al., 2009).

Characteristic	Method			
	Chemical	**Biological**	**Cultural**	**Physical**
Residual action (residues & persistence)	Yes (variable)	Yes, if reproduction occurs	Negligible	Negligible
Possibility of combining with another method	Yes (sometimes difficult with biological methods)	No	Yes	Yes
Application	Active	Active	Active & passive	Active & passive
Application to field crops	High level	Low level	High	Moderate level
Application to crops with a high-profit margin per hectare	High	Moderate to high	Moderate to low	Moderate to high
Safety for crop	Moderate to high (phytotoxicity)	High	High	High (passive) Moderate (active)
Labor requirements	Low	High (inundate biological control) Low (classical biological control)	Low	Medium to high
Work rate (hectares treated per hour)	High	Variable	High	High (passive) Low (active)
Site of action	The photosynthetic system, endocrine & nervous systems	Pest body	Systems allowing abiotic/biotic stress	Systems allowing abiotic/ biotic stress
Environmental or toxicological implications, safety	High & costly	Low to moderate (e.g., virus)	Low	Low (exception: electromagnetic radiation)
Geographic impact	Drift, run-off, evaporation, food chain	Colonization of no target habitats by parasitoids or predators	Restricted to area treated	Restricted to area treated (exception: electromagnetic radiation)
Energy requirements	High for production	Low	Low	High (active) Low (passive)
Machinery required	Ground or aerial sprayer	Little or none	General farm equipment	Many types of equipment, few machines are suited to more than one purpose

1.8.2. Postharvest fruit treatments

Sometimes the farm products that carry a significant hazard of harbouring pests, such as *C. capitata* that cannot be controlled in the field may still be marketable with disinfestation treatment during postharvest processing (Heather and Hallman, 2008). Although all life stages of the *C. capitata* are targeted with disinfestation measures, evidence exists to show that the response of some life stages such as eggs and larvae to physical treatments varies with the insect age (Corcoran, 1993). Moss and Jang (1991) reported that the mortality of Medfly eggs subjected to hot water immersion was also dependent on age. Since the withdrawal of Ethylene Dibromide due to its carcinogenicity (EPPO/CABI, 1997g), many alternative disinfestation techniques have been developed. For example, hot air and hot water treatments, use of radiation and cold storage to control Medfly in some fruits such as mango, apple stone fruits (McPheron & Steck, 1996). Without postharvest treatment exports of fruit and vegetable crops to lucrative markets abroad are limited due to quarantine restrictions. Therefore, effective postharvest quarantine treatments that are not harmful to either the product or consumer in contact with or consuming the fruits must be applied to the export commodities. The available quarantine treatment technologies, as alternatives to toxic fumigation include, heat treatment to increase the temperature of host fruits above thermal limits of the fruit fly, for example, eradication of Medfly in the mango (Nascimento et al., 1992), cold treatment to decrease the temperature of host fruits below the thermal limits of the fruit fly, for instance, eradication of Medfly in the citrus spp., (De Lima et al., 2007), also, irradiation with gamma rays from a Cobalt-60 or Caesium-137 source to kill the developing flies, such as control of the Medfly in the papayas fruit (Follett and Armstrong, 2004).

1.8.2.1. Fumigation

Usually, chemicals in gaseous form are used to destroy pests under a cover or shelter. Fumigations associated with fruit fly management are normally conducted where commodities are gathered or stored (USDA, MRP and APHIS, 2003). Fumigation with methyl bromide, a broad spectrum fast-acting fumigant, has been widely used to control pests since 1930s (Bell et al., 1996). The insecticidal properties of ethylene dibromide (EDB) were

reported by Neifert et al. in 1925. EDB has become important as an insecticidal fumigant because of its destructive ability to tephritid fruit flies in fruits (Viel and Catelot-Goldman, 1957). Although EDB is a fumigant of considerable utility, it has a high boiling point and is absorbed by many materials (food and containers), into which it does not penetrate well. It is thus more limited in usefulness than some of the more volatile fumigants. It has, however, found extensive use in soil fumigation. It is also effective as an ingredient in very low proportions of dips to control fruit flies in fruit (Wolfen Barger, 1962; Burditt et al., 1963).

Methyl bromide fumigation was applied to infested stone fruits thus proving its effectiveness against target pests. This fumigation treatment could be performed either pre-shipment or on-arrival as a quarantine measure. Methyl bromide treatment schedules for insects are provided in the Food and Agriculture Organization manual of fumigation control (FAO updated) and the USDA Treatment Manual, and some evidence is available on the effectiveness of methyl bromide specifically on pests of stone fruits (Armstrong et al., 2014). Methyl bromide fumigation was evaluated as a quarantine treatment for the walnut husk fly, *Rhagoletis completa* on peaches by Yokoyama et al. (1992). 40 g/m^3 of methyl bromide for 2 h at 21°C, 100% mortality of eggs and larvae, however, late 3rd instar larvae were least susceptible. Complete mortality of 2203 late 3rd instar larvae was attained in a large-scale test of a proposed quarantine treatment for peaches packed in 2-tray shipping containers for export to New Zealand. Organic bromide residues were <0.001 ppm after seven days in storage at 2.5°C. Inorganic bromide residues were below the U.S. tolerance level of 20 ppm. Except for one variety, fumigation did not significantly influence fruit firmness, soluble solids, or titratable acids. Reduction in fruit and stem quality was more associated with temperature than with methyl bromide concentration. Fumigation with methyl bromide as a biosecurity treatment negatively affects fruit and stem appearance (Retamales et al., 2003). Fumigation retards fruit softening and increases the severity of mealiness and internal breakdown in nectarines stored for more than three weeks (Harman et al., 1990). Since the use of methyl bromide is being phased out, alternative treatments are being investigated; for example, a new non-phytotoxic formulation of ethyl formate in carbon dioxide.

1.8.2.2. Cold Treatment

Cold treatment involves the refrigeration of produce over an extended period, according to treatment schedules established in the Plant Protection and Quarantine (PPQ) Treatment Manual (USDA, APHIS). Cold treatment is used to kill fruit flies in regulated articles as a prerequisite for the movement of those articles out of quarantined areas. Cold treatment is preferable to fumigation for commodities that are known to be damaged by methyl bromide. Cold treatment may also be combined with methyl bromide fumigation as an authorised regulatory treatment for some commodities (USDA, MRP and APHIS, 2003). Weems Jr and Fasulo (2002) suggested that the apple maggot, *Rhagoletis pomonella*, in fruit may be killed by placing the fruits in cold storage at 0°C for 40 days, and disinfestation by cold storage at 0°C for 40 days is approved by some regulatory agencies (Hallman 2004). The Canadian Food Inspection Agency requires that imported fruit has been continuously maintained at a maximum temperature of 0.6°C for a minimum of 42 days (CFIA, 2008).

Typically, the harvest of a given cultivar covers about ten days (Curtis et al., 1992). The fruit is harvested primarily into field crates or individual buckets and cooled to 20°C immediately after harvest (pre-cooling). Various cooling methods are used, such as running through chilled water, holding in a cold holding room, or forced-air cooling, in which the sides and tops of the bins are covered and large fans pull cold air through the bins (AG-PB-BA, 2008). Packed product and packaging are to be protected from pest contamination during and after packing, during storage and movement between locations. Packaged fruit should be placed in cold storage at a temperature of approximately 0°C as soon as possible.

Cold disinfestation treatment is used as a control in various fruits and has the advantage of being applied in several ways. The treatment can be carried out in the exporting country, in transit, in the importing country, or through a combination of these options. The most frequently used temperature range is between 0 and 3°C (Mangan & Hallman, 1998). Temperatures above 3ºC might not kill all insects associated with the commodity and temperatures below 0ºC may harm the commodity and affect its market value. Cold treatment

is an effective quarantine measure for fruit flies on a wide range of fruits (De Lima et al., 2007; Heather et al. 1996; Paull 1994). Low-temperature storage, between 0 and 2°C for up to 22 days, is accepted as a treatment for many fruits and vegetables infested with Medfly and Queensland fruit fly for entry into the United States (Burditt & Balock, 1985). Cold treatments at 1- 3°C for 14 days for Australian stone fruit were shown to be highly effective as quarantine treatments against Queensland fruit fly (De lema et al., 2007). In the case of tephritid fruit flies, susceptibility to cold in eggs of the caribebean fruit fly, *Anastrepha suspensa* (Loew), decreased with age (Benschoter & Witherell, 1984).

It has been shown that cold treatment of commodities can be an effective disinfestation technique for obscuring mealybug (*Pseudococcus viburni*), which is believed to be one of the most cold-tolerant insects (Hoy and Whiting, 1997). The lethal treatment for 99 per cent of the population ranged from 16 days at 0°C for first instars to 77 days at 4°C for adult females.

1.8.2.3. Heat treatments

Heat treatments are used as a quarantine treatment against fruit flies in chilling sensitive fruit crops. They are expensive, technically difficult and can damage the product. Heat treatments have generally been focused on chilling sensitive, high-value fruit crops such as mangoes, avocados and papaya. Treatments have also been developed for vegetable crops, including zucchini, squash, and tomatoes.

1.8.2.3.1. Vapor Heat Treatment

Vapour heat, the oldest of the three methods of quarantine temperature treatment, consists of heating the host fruits by moving hot air saturated with water vapour over the fruit surface. Vapour heat treatment (VHT) is a high humidity air treatment. When mango is at dew point temperature or lower temperature, the air will condense on the fruit surface, and the condensate will conduct heat energy from the surface into the centre of fruit flesh. Heat is transferred from the air into the commodity by condensation of the water vapour (heat of condensation) on the relatively cooler fruit surface (Armstrong and Mangan, 2007). Fruit

may be heated over a time to a target temperature, which may be the end of the heat treatment, or fruit may be held for a specific time (holding time) that is required to kill the insect pests. It usually takes 3 to 4 hours for complete treatment. In 1913, VHT was one of the essential methods to control the Mexican fruit fly in Mexico (Tang, 2007). Vapour heat treatment is used for mangoes exported from Australia, Thailand, Philippines, and Taiwan, particularly for the Japanese market. An established VHT, though not commercially implemented, for 'Manila' mangoes from Mexico is still on the approved list but requires a 6-hour hold time at a core temperature of 43.3ºC.

VHT involves fruit being heated in humid air, at greater than 90% relative humidity, to temperatures possibly lethal for insects but non-injurious to the fruit (Jacobi et al., 1993). The most frequently used temperature range for VHT is between 43 and 49°C (Mangan & Hallman, 1998). VHT differs from high temperature since forced air in that moisture accumulates on the surface of the fruit. The water droplets transfer heat more efficiently than air, allowing the fruits to heat quickly. There are no efficacy data for the vapour heat treatment of surface pests on stone fruit such as scales and mealybugs. Hansen et al. (1992) carried out experiments on removing surface pests from cut flowers in Hawaii. They determined the efficacy of VHT for scales, mealy bugs, thrips, and aphids after 2 hours at 45.2°C. These experiments suggested that this temperature and timeframe killed all adult and nymphal stages of these groups and could, therefore, be an appropriate treatment to eliminate these organisms from stone fruits (Hansen et al., 1992). However, VHT may also increase physical injury to the fruits. Differences between varieties and maturity may influence fruit heat sensitivity, as well as the capacity for long term storage (Jacobi et al., 1993). These factors must be considered when researching VHT for commercial and quarantine application.

1.8.2.3.2. Hot Water Treatment

Hot water immersion is an efficient treatment to disinfest some fruits of fruit flies (Mitcham and Yahia, 2009). The USDA Animal and Plant Health Inspection Service (APHIS) approved

the hot water immersion quarantine treatment for Tephritidae fruit flies in mangoes in 1987. Moss and Jang (1991) reported that the mortality of Medfly eggs subjected to hot water immersion was dependant on age. Hot water treatments have been used by growers in several countries as quarantine treatments for mango and papaya fruits. Large commercial hot water treatment facilities are routinely used to treat mangoes with hot water immersion at a temperature of 46.1 to 46.5ºC for 65 to 110 minutes, depending on the fruit weight. According to USDA APHIS, fruit up to 500g are treated for 75 minutes, fruits weighing 501 to 700g are treated for 90 minutes, and mangoes 701 to 900g (only approved for Mexico and Central America) are treated for 110 minutes. For flat, elongated varieties (Frances, Ataulfo, Manila), fruits up to 375 grams are heated for 65 minutes, and fruits weighing 375 to 570 grams are heated for 75 minutes. There are specific requirements for the water temperature during the first few minutes of treatment, and the hot water system must be certified each year before it is first used. There are approximately 75 hot water treatment facilities in Mexico, five in Ecuador, six in Guatemala, 11 in Peru, and 10 in Brazil (Mitcham and Yahia, 2009). Hydro-cooling is now allowed immediately following the hot water treatment if 10 minutes is added to the heat treatment time, or fruit may be hydro cooled after a waiting period of at least 30 minutes at ambient temperature. The hydro-cooler water must be no colder than 21.1ºC, according to APHIS (Mitcham and Yahia, 2009).

1.8.2.3.3. Forced Hot-Air Heating Treatment (FHAT)

Forced hot-air, also known as high-temperature forced air, is a modification of the VHT developed by Armstrong et al. (1989) to kill Medfly, melon fly and oriental fruit fly eggs and larvae in papaya. It is essentially the same as VHT except that the fruit surfaces are dry during forced hot-air treatment. Improvements in temperature and moisture monitoring, and air delivery have advanced forced hot-air treatments (Hallman and Armstrong, 1994) for the commodities previously treated with vapour heat and being developed for new commodities (Tang, 2007). Forced hot air treatment appears to be as effective in controlling internal pests

as vapour heat, and provides better fruit quality (Armstrong et al., 1996), becoming the treatment of choice for many fruits previously treated with vapor heat. The fruit skin temperature remains cooler during forced hot air treatments than during VHT while the tissue just beneath the skin heats to lethal temperatures because of the occurrence of evaporative cooling on the fruit surface during forced hot air treatment at a lower relative humidity (Shellie and Mangan, 2000). Forced hot air is the second most common method of quarantine heat treatment and has been used in the Cook Islands and Fiji, and more recently, is expanding to the Pacific Basin and Pacific Rim. It is regularly used to treat papayas in Hawaii for shipment to the U.S. mainland with good success. In Mexico, there are four forced hot air units in Michoacán, Nuevo, Leon, and Yucatan that were all designed by the same individual specifically to treat citrus (mostly grapefruits) and utilize steam heat. In Hawaii, fruits are treated in large field bins with mesh bottoms (Mitcham and Yahia, 2009).

1.8.2.4. Irradiation Treatment

Irradiation treatment is a method that has been used to sterilize or kill certain species of fruit flies. Irradiation treatments must be conducted in an approved facility and the treatments are conducted in accordance with strict safety guidelines. The irradiation equipment releases radiation to the treated commodity, but it does not retain any radioactivity from the exposure. However, some commodities are not compatible with irradiation treatment and would tend to be destroyed if such treatments were employed. Irradiation treatment would not be used much as a control method because the facilities would be lacking in most quarantine areas and effective treatments that do not damage the regulated articles have not been developed for most of the commodities (USDA, MRP and APHIS, 2001).

The use of ionizing treatments such as gamma and x-rays (irradiation) is gaining popularity as a quarantine treatment. Irradiation is an efficient, non-residue, broad-spectrum disinfestation treatment that has been recognised for its quarantine potential in fresh produce. It is a low dose application that is tolerated well by most fresh commodities (Biosecurity New Zealand 2009b). The major commercial uses of ionising radiation for fruit and vegetables

include the inhibition of sprouting (potatoes and onions) and the extension of shelf-life in strawberries (Frazier et al., 2006). Although irradiation can prolong the shelf life of foods where microbial spoilage is the limiting factor, fruits and vegetables generally do not retain satisfactory quality at the irradiation doses required (Lacroix & Vigneault, 2007). Loss of firmness, colour changes, and increased internal breakdown are evident in both apricots and peaches at irradiation doses above 600 Gy (Drake & Neven, 1998). Irradiation can be used with stone fruits as a quarantine treatment at 300 Gy or less with little loss of quality (Drake and Neven, 1998). For example, doses of 57 Gy and 92 Gy are recommended for quarantine disinfestation of host fruits of apple maggot (Hallman 2004) and plum curculio (Hallman, 2003). Follett (2006) showed that irradiation treatment with a minimum absorbed dose of 150 Gy provides quarantine security to control white peach scale (*Pseudaulacaspis pentagona*) on exported papaya, as well as other commodities. Based on existing data, Corcoran and Waddell (2003) state the following recommended doses for the listed arthropod pests (Table 1.2). Irradiation can work by rendering pests sterile rather than killing them. The irradiation treatment must ensure the pests are unable to reproduce. The guidelines suggest the pests should be unable to emerge or escape the commodity unless they can be practically distinguished from non-irradiated pests (FAO AND IPPC, 2016). Advantages and disadvantages of irradiation comparison with Hot Water, Forced Hot Air and High-Temperature CA treatment are shown in Table 1.3.

Table 1.2. Recommended irradiation treatment doses for contains Arthropod groups.

Arthropod Pest Group	Recommended Treatment Dose (Gy)	Author(s)
Tephritidae (fruit flies)	150 (non-emergence eggs, larvae)	Bustos et al. 1991, Gould and Hallman 2004
Hemiptera (bugs, scales, mealybugs)	250 (sterility)	Hara et al. 2002, Follett 2006
Lepidoptera (moths, butterflies)	250 (non-emergence- eggs, larvae)	Follett & Lower 2000
Coleoptera (beetles)	250 (sterility)	Tilton et al. 1966, Todoriki et al. 2006
Acari (mites)	350 (sterility)	Lester and Petry, 1995; Jadue et al.

Table 1.3. Advantages and disadvantages of treatment options for disinfestation of mango fruit (Mitcham and Yahia, 2009).

Method	Advantages	Disadvantages
Hot Water	➢ Easy and facilities available ➢ Less capital cost for new facilities ➢ Many years of experience, system optimized ➢ Provides decay control	➢ Narrow tolerance to prevent injury ➢ Need to cool the fruit after treatment ➢ potential food safety risk from the immersion of hot fruit in cool
Forced Hot Air	➢ Reduced potential for fruit injury ➢ Some decay control	➢ Few good facilities currently available ➢ Longer treatment than hot water ➢ More complex to operate ➢ Need to cool the fruit after treatment ➢ potential food safety risk
High-Temperature (CA)	➢ Less potential for fruit damage than forced-hot air alone ➢ Shorter treatment time than forced hot air - Some decay control	➢ Additional research needed to prove efficacy against fruit fly pests over hot air alone and confirm fruit tolerance ➢ More complex to operate ➢ Facilities would need to be built ➢ More parameters to measure and that may be out of range leading to treatment failure Longer treatment than hot water Need to cool the fruit after treatment ➢ potential food safety risk from the immersion of hot fruit in cool water
Irradiation	➢ Relatively short treatment time ➢ Fruit are not heated during treatment; no extra cooling required ➢ No added food safety risk	➢ A limited number of facilities -Potential for fruit damage, especially at higher doses ➢ A chance for consumer resistance

1.9. Controlled atmosphere (CA)

Controlled atmosphere (CA) technology involves the use of inert gases for controlling the storage and structural insect pests (Donahaye, 2000). Reduced oxygen (O_2) and elevated carbon dioxide (CO_2) atmospheres have been known for many years to be effective in killing various insect pests but were generally applied at ambient or lower temperatures (Mitcham et al., 2001). At elevated temperatures, kill time is faster. Along with the forced hot-air, nitrogen is used to replace oxygen, and carbon dioxide is added. The mechanism of control is to increase the respiratory demand of the insects with heat treatment while at the same time

modifying the atmosphere, both of which contribute to the death of the insect. Treatment times with high-temperature CA can be one-half that with heat treatments alone (Donahaye, 2000).

The different types of controlled atmosphere storage depend mainly on the methods or degrees of control of the gases. Some researchers prefer to use the terms “static controlled atmosphere storage” and “flushed controlled atmosphere storage” to define the two most commonly used systems. “Static” is referred to when the product generates the atmosphere and “flushed” is where the atmosphere is supplied from a flowing gas stream, which purges the store continuously. Systems may be designed such that flushing is done initially to reduce the O_2 content then either injecting CO_2 or allowing it to build up through respiration, and then the maintenance of this atmosphere by ventilation and scrubbing (Yahia, 2009).

The benefits of CA include, environmentally-friendly postharvest mitigation treatment, high efficiency in the control all stages of pests, mostly does not affect plant production rates, a considerable decrease in fruit respiration rate, an extension in storage life, reduced potential for the development of skin diseases like “scald’’ on the fruits and at the same time increased longevity of the fruits and prolonged storage time and in a reliable gas-tight storage system (Yahia, 2009).

One of the disadvantages of CA is that instead of improving quality of some fruits, it affects the normal post-harvest changes leading to reduction in fruit quality. Additionally, special equipment may be required, and treatments are expensive and time-consuming (Dewey, 1984).

1.9.1. Fruit storage under CA

The storability of fruits is strictly related to their respiration rate, which is an expression of metabolic activity. Aerobic respiration requires O_2 with release of CO_2 and heat. More than 95% of the energy released is lost as heat. If the temperature reduction, due to a modified atmosphere, leads to a reduction in respiration rate, and therefore to an increase in storage life in fruits with critical respiration (Yahia, 2009).

The selection of the most suitable atmosphere depends on cultivars, stage of maturity, environmental and cultivation parameters. Therefore, there is no atmosphere suitable for all produces, specific recommendations and cautions must be determined for each crop over the range of storage temperature and periods. Some examples of CA atmospheres are given in Table 1.4.

Table 1.4. Controlled atmosphere conditions for some fruit species (Gormley, T.R., 1985)

Species	**Temperature °C**	**RH (%)**	**O_2 (%)**	**CO_2 (%)**	**Time**
Avocado (1)	7/12	90	2-3	3-10	2 months
Cherry (2)	0	95	3-10	10-12	30 days
Kiwi (3)	0	98	2	4-5	7 months
Nectarine (4)	-0.5/0	95	2	5	50 days
Peach (4)	-0.5/0	95	2	4-5	40 days
Plum (5)	0	95	2	5	45 days

1. In avocado, CA reduces chilling injury and delays softening
2. Pre-cooling is necessary
3. Kiwi is damaged by high CO_2 and low O_2. Small amounts of ethylene must be eliminated for a long storage life
4. Pre-cooling is necessary; also, rapid attainment of a CA is useful
5. Different varieties behave differently in CA storage; some varieties are susceptible to internal breakdown.

1.9.2. Controlled Atmospheres (CA) or Temperature Treatment System) (TTS)

The Controlled Atmosphere/Temperature Treatment System) (CATTS) is a system of high temperature forced air combined with a modified atmosphere consisting of low oxygen and high carbon dioxide. The CATTS treatments for apples, sweet cherries, peaches, and nectarines are to date the only modified atmosphere treatments that are approved for quarantine. This treatment combines the stress of heat with that of atmospheric stress, referred to as a modified atmosphere (MA) or controlled atmosphere (CA), due to reduced oxygen and/or elevated carbon dioxide concentrations (Neven and Mitcham, 1996). Treatments with CA in combination with forced hot air have been tested for control of Mexican Fruit Fly and West Indian Fruit Fly in 'Manila' mangoes (Yahia and Ortega, 2000; Ortega and Yahia, 2000). 'Manila' mangoes tolerated treatment with 0% O_2 and 50% CO_2 at temperatures <44ºC and 50% RH for 160 min (Ortega and Yahia, 2000), but damage occurred at 44ºC and increased with rise in temperature. However, treatment at <44ºC was not fully effective in controlling the two fruit fly pests (Yahia and Ortega, 2000). High-temperature CA treatments were approved in 2008 by USDA APHIS for export of U.S. nectarines, sweet cherries and apples to control codling moth (*Cydia pomonella*), oriental fruit moth (*Grapholita molesta*) and western cherry fruit fly (*Rhagoletis indifferens*) (Neven & Rehfield-Ray, 2006). Table 1.5. shows the time of exposure, temperature, and gases castration to control some pests on different.

Table 1.5. The CA parameters for controlling fruit flies of different fruits.

Fruit	**Fruit fly (Tephirid)**	**Time, high temperature with O_2 and CO_2**	**Author(s)**
mandarins	*C. capitata*	20 hours at 1.5°C with 5% O_2, 95% CO_2	(Alonso et al., 2005)
Sweet cherry	*Rhagoletis indifferens*	45 min at 45°C with 1% O_2, 15% CO_2	(Neven and Rehfield, 2006)
Apples	*Rhagoletis pomonella*	3-12 h, 30°C, with 1-2% O_2	(Hulasare et al., 2013)
Papayas	*Bactrocera cucurbitae*	42-48 h, 25°C, with 1-2% O_2, 6.5-21% CO_2	(Follett et al., 2013)
Mango	*Bactrocera dorsalis*	48h, 26°C, with 6-9 % O_2, 8-27% CO_2	(Srimartpirom et al., 2018)

Mitcham and Yahia (2009) demonstrated that high-temperature CA treatments could be a successful option for fruit fly control in mango fruit, assuming the treatment will eventually be approved for fruit imported into the U.S. It is not clear that the shorter treatment time or the improved fruit quality would be enough to warrant the extra expense of the equipment, but this treatment option appears worthy of further consideration. This is especially true since a large investment has not yet been made in forced-hot air treatment facilities. Insecticidal CA treatments could also be investigated at room temperature (20-25ºC) as insects might be controlled within a period of 48 to 72 hours.

1.9.3. Effects of CA on Insects

Insects can tolerate low levels of oxygen for prolonged periods. CA (<0.5% O_2 + >50% CO_2) at different temperatures (from 20–55ºC) and RH was tested on the sensitivity/tolerance of several fruits including different cultivars of mango, avocado, guava and papaya, and on the

mortality of different stages of fruit flies. Eggs are generally most susceptible to CA treatment than larvae (Yahia, 2006). The lowest level of tolerance to O_2 was around 1% concentration. Ke and Kader (1989) concluded that 1% concentration of O_2 is necessary to kill insects in two to several days in fruits and vegetable. The time required for low oxygen to completely kill insects in fresh fruit depends on temperature, O_2 concentration, relative humidity, species and stage of development of the insect (Ke and Kader, 1989).

Elevated CO_2, O_2 and N_2 levels cause spiracles to open, resulting in insect death from water loss. Above 10% of CO_2 spiracles remain permanently open. Toxic effects are entirely through the tracheae, not the haemolymph; CO_2 has direct toxic effects on the nervous system. In some cases, CO_2 can acidify the haemolymph leading to membrane failure in some tissues (Nicolas and Sillans, 1989). Elevated, but sublethal CO_2 levels, for prolonged periods can have deleterious effects on insect development, growth, and reproduction (Nicolas and Sillans 1989; White et al., 1995). Atmospheres containing about 60% CO_2 are rapidly killing an insect.

Atmospheres with 60% CO_2 and 8% O_2 are very effective at killing internal seed-feeding insects, while low O_2 atmospheres are more rapid in killing external-feeding insects (Calderon and Barkai-Golan, 1990). High CO_2 levels, even with 20% O_2, rapidly kill insects because of CO_2 toxicity. CO_2 levels must be at 40% for 17 days, 60% for 11 days, 80% for 8.5 days at temperatures above 20°C, or 70% declining to 35% in 15 days at 20°C (Annis, 1987). Higher temperatures accelerate CO_2 toxicity as insect metabolism is elevated. Even low levels of CO_2 (7.5-19.2%) for prolonged periods sharply increase immature and adult mortality (White et al., 1995).

Insect mortality increases more rapidly as temperatures rise and their metabolism speeds up. Cool temperatures slow rates of mortality while lower relative humidity (RH) hasten toxic effects, notably in high CO_2 atmospheres because of desiccation of insects (Fields, 1994).

Mortality of insects under low pressures is caused mainly by the low partial pressure of O_2, resulting in hypoxia (Navarro and Calderon, 1979). The partial pressure of oxygen has a

decisive effect on insect mortality, while no significant function could be attributed to the low pressure itself. At 50 mm Hg, partial pressure of O_2 is equivalent to 1.4% O_2, this being similar to the target O_2 concentration under a modified atmosphere obtained by N_2 flushing.

Benschoter (1987) assessed the response of Caribbean fruit fly eggs and larvae invitro to modified atmospheres of 20, 50, or 80 kPa CO_2 and 10, 20 or 50 kPa O_2 at 10°C and 15.6°C. Increased mortality generally coincided with the highest carbon dioxide concentration, regardless of O_2 concentration. At the lowest CO_2 concentration, however, increased mortality coincided with lower O_2 levels. Mortality was somewhat increased at 15.6°C, the higher of the two temperatures. Complete mortality of 150 insects tested occurred in 7 days with some of the treatment combinations. Prange and Lidster (1992) achieved up to 90% mortality of blueberry maggot (*Rhagoletis mendax*), after 48 h in various levels of CO_2 at 21°C. Mortality reached a peak at about 70 kPa CO_2 and then declined until less mortality was achieved at 100 kPa CO_2 than at 50 kPa CO_2. Complete mortality of apple maggot, *Rhagoletis pomonella*, larvae in apples was accomplished in 14 days at 10°C in atmospheres with 15 or 19 kPa CO_2 and the balance nitrogen (Agnello et al., 2002). It was found that the combination of low oxygen, high carbon dioxide environment (1 kPa O_2, 15 kPa CO_2) effectively controlled apple maggot in a 12°C/h heat treatment of apples to a chamber temperature of 44°C or 46°C. These MA levels also controlled western cherry fruit fly (*Rhagoletis indifferens*) in sweet cherries using a flow-through treatment to 45°C and 47°C using heating rates of 264°C/h and 146°C/h, respectively. Treatments under regular atmospheres were not effective in controlling these pests (Agnello et al., 2002). Nitrogen controlled atmosphere (N_2-CA) applications started from the late 1960s, which were carried out to study low-oxygen grain storage technology using either hermetic storage or the introduction of nitrogen into the grain mass. Benschoter et al. (1981) explored the effects of modified atmospheres on a tephritid fruit fly, the Caribbean fruit fly (*Anastrepha suspense*). Complete control of green peach aphid, western flower thrips, sweetpotato whitefly, and two spotted spider mites was achieved with18 hours using >99% N_2 without damage to begonia seedlings. Lettuce aphid, black widow spiders and western flower thrips were successfully

controlled on head lettuce, table grapes and broccoli, respectively, without unfavourable impact on product quality. Vine mealybug was successfully eradicated without reducing the vitality of grape benchgrafts (Liu, 2010)

Valentin and Preusser (1990) noticed that exposure time to obtain a certain level of mortality of the pest under a CA system is closely linked with temperature. The difference of N_2 concentrations and pest's species affected the lethal time for complete control as in Table 1.6. The lethal effects of nitrogen were linked to temperature, moisture content, and a nitrogen level.

Table 1.6. Different nitrogen concentrations affect the lethal time of fresh products pests.

Pests	**control of eggs, pupae and larvae (LT_{99})**		**Author(s)**
	N_2 level = 90%~95%	**95%~99%**	
Drosophila melanogaster	10.6 (day)	-	(Peck and Maddrell, 2005.)
Frankliniella occidentalis	-	4 (day)	(Liu, 2008)
Latrodectus hesperus	-	1 (day)	(Liu et al., 2008)
Anastrepha ludens	3.5 (hours)	3.5 (hours)	(Shellie et al., 1997)

1.10. integrated pest management

Integrated control of fruit flies requires a mix of monitoring (traps and lures), excellent crop hygiene and host weed control, mass trap killing, baiting sprays and periodically a cover spray that is as soft as possible on beneficial's. As with all IPM protocols, best results are achieved when practised in tandem with an area-wide program.

1.11. Research gap and aim of the study

The long-term goal is to develop more environment friendly, cost-effective and efficient postharvest strategy to control Medfly. Aspects of biosecurity and phytosanitation, quarantine, trade and economic impacts are currently suffering from threats of insect pests. Therefore, new approaches (safe biological and ecological postharvest tools; effective and reliable quarantine methods; suitable, easy and available trade and cost-effective treatments) are urgently needed to its confrontation. To obtain such strategies, fundamental studies on postharvest treatments should be implemented. Medfly responses to the cold treatment and low-oxygen treatment are the keys of understanding how these two treatments can kill Medfly, one of the most horticultural destructive pests. Hence, evaluation of low temperature, low oxygen as phytosanitary treatment tools for eradication of Medfly is an urgent requirement to refine Medfly control strategies. This thesis provides new information about *C. capitata* response to cold treatment and low-oxygen treatment. The project was conducted on the most harmful stages of *C. capitata*, which are early eggs (<6 h), late eggs (>42 h), first instar, second instar and third instar larvae. The study objectives include:

- Cold Response of the Mediterranean Fruit Fly (*C. capitata*) on a Lab Diet.
- Mediterranean fruit fly *Ceratitis capitata* (Diptera: Tephritidae) eggs and larvae responses to the low-oxygen/high-nitrogen atmosphere.
- Cold Responses of the Mediterranean Fruit Fly *C. capitata* Wiedemann (Diptera: Tephritidae) in Blueberry.

Statement of Contribution

Title of Paper	Cold Response of the Mediterranean Fruit Fly (*Ceratitis capitata*) on a Lab Diet
Publication Status	published
Publication Details	Insects Journal, 2019, 10(2), 48
Principal Author	
Name of Principal Author (Candidate)	Farhan J.M. Al-Behadili
Contribution to the Paper	Methodology, Collected Data, Writing- Original Draft Preparation, Conceptualization and Validation
Overall Percentage (100%)	77 %
Signature	Date 28/ 07 /2020

Co-Author Contributions

By signing the statement of contribution, each author certifies that:

- The candidate's stated contribution to the publication is accurate (as detailed above).
- Permission is granted for the candidate to include the publication in the thesis
- The sum of all co-author contributions is equal to 100% less the candidate's stated Contribution

Name of Co-Author	Vineeta Bilgi
Contribution to the Paper	Data curation
Overall Percentage (100%)	2 %
Signature	Date 16/ 09 /2020

Name of Co-Author	Junxi Li
Contribution to the Paper	Methodology
Overall Percentage (100%)	2 %
Signature	Date 28/ 07 /2020

Name of Co-Author	Penghao Wang
Contribution to the Paper	Statistical analyses
Overall Percentage (100%)	2 %
Signature	Date 28/ 07 /2020

Name of Co-Author	Miyuki Taniguchi
Contribution to the Paper	Resources
Overall Percentage (100%)	2 %
Signature	Date 28/ 07 /2020

Name of Co-Author	Manjree Agarwal
Contribution to the Paper	Supervision
Overall Percentage (100%)	5 %
Signature	Date 28 7 /2020

Name of Co-Author	Yonglin Ren
Contribution to the Paper	Supervision, Funding acquisition
Overall Percentage (100%)	5 %
Signature	Date 28 / 07 /2020

Name of Co-Author	Wei Xu
Contribution to the Paper	Supervision, Funding acquisition, Validation, Conceptualization
Overall Percentage (100%)	5 %
Signature	Date 03 / 08 /2020

Chapter two

Cold Response of the Mediterranean Fruit Fly (*Ceratitis capitata*) on a Lab Diet

2.1. Abstract

Cold treatment at 0.0°C with different exposure durations (0–12 days) was applied to the Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) fed on a lab diet. The examined developmental stages were early eggs (<6 h), late eggs (>42 h), first instar, second instar and third instar larvae. Pupation, adult emergence and sex ratios of survived flies were investigated to study the *C. capitata* responses to this low-temperature treatment. Our results showed that exposure time at low temperature has a clear effect on pupation and adult emergence. Based on pupation ratios, the first and third instar are the most cold tolerant stages, with LT_{99} = 7.3 for both of them. Cold tolerance at both stages are very close and no significant differences were detected. There were no significant differences in *C. capitata* sex ratios among different stages after treatment. This study improves our understanding of *C. capitata* responses to cold treatment, which may assist in the improvement of the current treatment strategies to control this destructive horticulture pest species.

2.2. Introduction

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) is one of the most destructive and invasive insect pests for horticulture biosecurity, global trade and world-wide phytosanitary (White and Elson, 1992.). *C. capitata* originated from sub-Saharan Africa and then spread throughout the Mediterranean region, Europe, the Middle East, Western Australia and both South and Central America (De Meyer et al., 2010).

C. capitata has been recorded feeding on over 300 fruit, vegetable and nut plant species. The main hosts include citrus, stone fruits, pome fruits, peppers, tomatoes and figs (Woods et al., 2005). Plant hosts also include avocadoes, apricots, persimmons, strawberries, grapes, bananas, bitter melons, carambolas, coffees, guavas, peppers, papayas and blueberries. After mating, one female adult *C. capitata* can lay as many as 800 eggs in her lifetime (Thomas et al., 2001). Usually a *C. capitata* attack happens when the majority of the production costs

have already been expended causing huge horticultural industry losses. Therefore, *C. capitata* is regarded as one of the most destructive horticulture pest species. Furthermore, in recent years, the growing international trade of plant products increased the risk of introducing fruit flies across countries. Strict government policies were quickly made to mitigate these risks and minimize the damages. For example, pre-harvest actions including spraying, monitoring and inspections (Broughton and De Lima, 2002), with postharvest treatments such as fumigation, irradiation, heat or cold are required to control fly species (Dohino et al., 2017). Nevertheless, the current global trade of fresh farm products is suffering from the damages by various fruit fly species, prompting the need to develop more effective ways of fruit fly control.

The available quarantine treatment technologies mainly consist of chemical (e.g., fumigation) and non-chemical treatments (e.g., cold, heat and irradiation). In recent years, with the discontinuation of several chemical products (e.g., Fenthion and Dimethoate), it has become even more urgent to rely on non-chemical postharvest control technologies to control fruit flies (Badii et al., 2015). Cold treatment is becoming an increasingly popular postharvest treatment to avoid chemical residues, mitigations or mortality of the pest population, as well as increasing the strength of the fruits, and prolonging storage time (Sproul, 1976; Burditt and Balock, 1985; Jessup et al., 1993). It can also be applied to fruit at multiple stages, for example, after packing and 'in transit' during lengthy transport by sea, as well as co-treatment with other postharvest treatments such as irradiation (Follett and Snook, 2013).

Optimal cold treatment conditions rely on the commodities, and most previous studies on *C. capitata* cold treatment reflect this, performing on the flies while they are within the fruits (Sproul, 1976; Burditt and Balock, 1985; Jessup et al., 1993; Jessup et al., 1998; De Lima et al., 2011; Grout et al., 2011; Ware et al., 2012; Gazit et al., 2014). On the other hand, because fruits vary in sizes, nutrients, compositions, and phytochemical profile, there were large differences in the efficacy of heat transfer and development of life stages of flies using similar cold treatment regime. For example, a previous study on the *C. capitata* cold tolerance in dates and mandarins at 1.11°C showed that *C capitata* is more sensitive to cold treatment in date fruits than in mandarins (Gazit et al., 2014). The identification of the most cold tolerant development stage is crucial to determine the thoroughness of the treatment; however, there were also discrepancies on which *C. capitata* developmental stage was the most cold tolerant

in previous reports. Hallman et al. showed that 3rd instar is the most cold tolerant stage (Hallman et al., 2011). We, therefore, find it compelling to conduct research on cold tolerance of *C. capitata* reared on a lab diet. The result will provide a fundamental baseline for comparison with data obtained from those conducted within fruits and will be essential to establish a cold response model, considering fruit sizes, compositions, nutrients and other variables. The overall objective of this study was to evaluate and understand *C. capitata* responses to low temperature. Based on these understandings, our long-term goal is to optimize current postharvest treatments and develop more environmentally friendly, cost-effective and efficient treatments for controlling *C. capitata*.

2.3. Materials and Methods

2.3.1. Insect Culture

C. capitata used in this study was originally established in 2015 from a laboratory colony maintained at the Department of Primary Industries and Regional Development's (DPIRD) in Western Australia, which has been periodically supplemented with the introduction of wild flies. Mature females lay eggs through the mesh (cloth sidewalls of the cages), which were collected and transported to the artificial rearing medium which consists of 1 kg ground dehydrated carrot, 300 g torula yeast, 4.5 L hot tap water, 36 mL HCL, 30 g nipagin and 500 mL boiling tap water (Tanaka et al., 1969). After 13–16 days, pupae were collected and transferred into the adult breeding cages. The emerged adult flies were reared on the yeast hydrolysate, crystalline sugar and water. Rearing conditions were 26.0±1.0°C, 60–65% room humidity, and darkness light cycle of 16:8 h (De Lima et al., 2011). Early eggs (<6 h), late eggs (>42 h), 1st instar, 2nd instar and 3rd instar larvae were used in this study.

2.3.2. Cold Treatment Rooms

The cold room is a prefabricated unit with walls and ceilings of 100 mm expanded polystyrene. Joints and base of rooms are sealed with silicon sealant under aluminium covering extrusions. The floor is concrete; the door is 1500 mm wide × 1900 mm high × 100 mm thick with expanded polystyrene for insulation. The room dimensions are 4.33 m wide × 3.77 m length × 2.00 m high (Follett and Snook, 2013).

The refrigeration for the cold room is supplied by two × Patton (Model CCH 250, Patton, Auckland, New Zealand) air cooled Condensing Unit with R22 refrigerant + 2 × Patton BL 38

Induced Draught Evaporator with a refrigeration capacity of 5090 Watts at 0°C. The temperature of the room is controlled through a surface-mounted electronic thermostat (DIXEL, Roma, Italy) having a temperature range from −50 to +110°C with a probe installed in the return air path. Up to 4× defrost cycles can occur per 24 h if required. Two fans in the room (300 mm 5 blade propeller types) circulate air across the evaporator at an air flow rate of approximately 960 L/s measured at various points in the room.

To maintain a consistent temperature and to avoid temperature changes when opening the door of cold rooms, tested flies were placed in a chamber (1.2 m × 0.8 m × 0.6 m = 0.576 m^3) located within the cold rooms. The temperatures inside the chamber were recorded every 30 min by Applent Multi-channel Temperature recorder (AT4508-128, Made-in-China.com, Nanjing-Jiangsu, China). The sensors were set inside the chamber at different levels. All sensors were calibrated in ice water (0°C) before and after the treatment.

2.3.3. Cold Treatment Tests

The carrot medium was taken from a fridge approximately 24 h before usage and was placed in sterile 90 mm plastic petri dishes at room temperature. The petri dishes were covered with lids and set aside on a lab bench until infestation with the insect of various stages. Approximately, 50 g of carrot medium was added per petri dish with a clean spoon, making sure that the medium was not aggregated or in contact with the lid. This ensured that larvae if hatched, were able to move freely through the medium and were not stuck to the lid. The lids from the control petri dishes were removed when larvae were first observed during incubation; the same number of days were then applied for treatment petri dishes when removing the lids.

Freshly laid *C. capitata* eggs were collected over a period of 1 h prior to the trial and allowed to settle in a clean glass beaker containing 100 mL double distilled water. To count the number of eggs (in this study 100), droplets of water with suspending eggs were placed on a sterile petri dish using a transfer pipet. Eggs inside each droplet were counted using a manual cell counter, carefully picked up using a transfer pipet and placed onto the carrot medium. Once the petri dishes were ready with the eggs, they were placed in a tray and transported to the cold rooms, where they were carefully placed in stacks of six replicate per exposure time inside the acrylic box.

One hundred eggs or larvae were collected, counted and transferred onto one petri dish with the carrot diet. Six replicates were prepared to be exposed from 0 to 12 days at 0.0°C (6 petri dishes ×100 = 600 egg or larvae each day). After treatment, the 6 petri dishes (replicates) containing eggs or larvae were retrieved at regular intervals of 24 h; then, each petri dish was placed in a 750 mL clear disposable container containing a layer of sand and kept in an incubation chamber at 26.0±1.0°C; 60–65% RH. Pupation and adult emerging over approximately 4 weeks were recorded. Untreated controls were kept in the same conditions (at 26.0±1.0°C; 60–65% RH) for pupae counting and adult emerging. In this study, the total number of each *C. capitata* stage experimented was 7800 (7200 for treatments + 600 for control); the final number of egg and larvae tested in the whole experiment was 39,000.

A piece of mesh cloth was put on top of the container, and the lid was then affixed. The lids were prepared to have six holes, ensuring air circulation when placed in the incubator. Control petri dishes were checked regularly, and on day 8, when instars were visible, the lid was removed and the remaining configuration was left as described. The lids of the petri dishes in treatment groups were removed after being placed in the incubator as follows: after 7–8 days for early eggs trial; after 5–6 days for late eggs; after 3–4 days for first instar larvae; and after 1–2 days for second instar larvae. Third instar larvae had no lids from the start. During each trial, the temperatures were recorded at 30 min intervals in each cold room, at the carrot diet, and in the air of the chamber. The sand was renewed three times over six weeks to collect pupae. Pupae, emerged adults and the sex ratios were recorded and analyzed.

To study the effects of cold treatment on eggs, mortality was recorded by comparing a total number of pupae or adults produced, and eggs that were incubated at 26.0°C were used as a control. This comparison provided mortality, and eggs were claimed “live” if pupae or adults were produced after treatment. This procedure included sieving through sand containing pupae, followed by counting the total number of pupae. Sieving was carried out using a metal mesh tray (1.6 mm) that allowed sand particles to pass through but retained pupae. Sieving was carried out as soon as pupae were first seen, with this process repeated three times until there were no more pupae found in the sand. Pupae were carefully placed on a clean surface, and any large sand particles that remained were separated using a glass slide. Pupae were then counted and placed in a sterile petri dish placed on a laboratory bench at room temperature. Additional

observations on the number of emerged flies were considered. This provided information on the viability of pupae and whether they were able to develop into adults.

To study if the cold treatment affected the sex development of the emerged adults, we compared the sex ratios of the treated eggs and larvae. We collected all the survival adults after treatments from five developmental stages and calculated the percentage of female.

2.3.4. Statistical Analysis

The mortality rate of the insect under cold treatment was statistically estimated following the Median lethal time method (LT). The 90% and 99% mortality (LT_{90} and LT_{99}) were estimated by using the selected models. We evaluated four different models separately for pupae and adults on every stage, including eggs, 1st instar, 2nd instar and 3rd instar. The best fitting model was selected for estimating the LT_{90} and LT_{99}. The LT estimated under generalized linear model with both a probit and a logit link function on cold treatment days, which are the most used dose–response model where in our scenario days of treatment was considered as dose. The model can be written as:

$$\eta = \beta\_0 + \beta\_1 x \quad (1)$$

Where η is the response or proportion mortality, x is the dose, β0 is the intercept and β1 is the coefficient of the dose. The four evaluated models include (1) probit model on log transformed treatment days; (2) logit model on log transformed treatment days; (3) probit model on treatment days without log transformation; and (4) direct logit model on treatment days. The best model was selected based on three different criteria, including exploratory analysis, Bayesian information criterion (BIC) value and regression residues. The best fitting model following these criteria for each insect stage was finally selected to estimate LT_{90} and LT_{99}. This demonstrates that we are using the best fitting model for LT estimation. A 95% confidence interval was also reported. R statistical environment (version 3.3.2, R Foundation for Statistical Computing, Vienna, Austria, URL http://www.R-project.org/) with the base library was used to estimate the LT and confidence interval, a ggplot2 package was used for generating plots.

An ANOVA single factor test was used to compare sex ratios of adult insects emerging from different stages after cold treatment.

2.4. Results

In this project, a total of ~39,000 *C. capitata* eggs or larvae were used for cold treatment. Exactly 7800 eggs/larvae were used for each stage (early eggs, late eggs, 1st instar, 2nd instar and 3rd instar larvae). The duration of treatment ranged from 0 to 12 days at 0.0°C to reach 100% mortality (Table 2.1). During the cold treatment, probes were used to monitor the temperature in the cold room, and the results showed that the temperature was stable at 0.0 ± 0.2°C, from the start to the end of the experiment.

The effects of cold treatment on *C. capitata* are shown in Table 2.1 and Figure 2.1 Pupation and adult emergence ratios were used to calculate the mortality rates. By using pupation, if egg/larvae could not develop to pupae after treatment, this was defined as "death". Similarly, by using adult emergence, if egg/larvae could not develop to an adult after treatment, this was defined as "death". Our results showed that the five developmental stages (early egg, late egg, 1st instar, 2nd instar and 3rd instar) differed in their cold tolerance at 0.0°C. By using the recovered pupation (Table 2.1 and Figure 2.S1), 1st instar larvae were the most cold-tolerate stage, as it took nine days at 0.0°C in the lab diet to reach zero pupae (100% mortality). The 3rd instar is the second most cold-tolerate stage, where it took seven days at 0.0°C to reach 0 pupae. Early eggs, late eggs and 2nd instar need six days to reach zero pupae after treatment. Interestingly, using the emerged adults to study the mortality achieved slightly different results (Table 2.1). For example, early eggs and 2nd instar all needed six days to achieve zero adults (100% mortality) from 600 eggs/larvae. Late eggs and 3rd instar both needed five days to achieve zero emerged adults, thus suggesting late eggs have a greater susceptibility to cold than early eggs. The 1st instar larvae stage is the most cold-tolerate stage, as it took the most days (9 days) to reach 100% mortality.

The pupation or adult emergence ratios from the non-treated (control) eggs/larvae varied (Figure 2.S1, Figure 2.1 A, B). The results for larvae stages (1st, 2nd and 3rd instar) are very similar (89–99% pupation and 77–89% adult emergence). The early eggs in control showed 69.5% pupation and 66.6% adult emergence, while the late eggs showed only 46.8% pupation and 32.3% adult emergence. The control eggs (including both early and late eggs) showed

much lower pupation/adult emergence ratios than the control larvae (1^{st}, 2^{nd} and 3^{rd} instar), which may be due to the low hatching ratios from eggs to larvae (Table 2.1).

Table 2.1. Pupation and emerged adult ratios of early eggs (< 6 h), late eggs (>42 h), 1st instar, 2nd instar and 3rd instar larvae of *C. capitata* fed on lab diet when subjected to various days of cold treatment at 0.0°C. (n = 6 and SE: standard error).

Treatment		Early Egg		Late Egg		1st Instar		2nd Instar		3rd Instar	
Days	**Insect Numbers**	**Pupation (%) (Mean ± SE)**	**Emerged Adult (%) (Mean ± SE)**	**Pupation (%) (Mean ± SE)**	**Emerged Adult (%) (Mean ± SE)**	**Pupation (%) (Mean ± SE)**	**Emerged Adult (%) (Mean ± SE)**	**Pupation (%) (Mean ± SE)**	**Emerged Adult (%) (Mean ± SE)**	**Pupation (%) (Mean ± SE)**	**Emerged Adult (%) (Mean ± SE)**
0	600	69.5 (4.2)	66.6 (5.7)	46.8 (3.6)	32.3 (3.0)	89.6 (4.1)	77.3 (3.5)	98.5 (1.1)	89.3 (2.0)	98.3 (0.6)	81.6 (6.9)
1	600	40.5 (3.6)	35.5 (3.8)	39.6 (2.4)	32.1(2.3)	79.1 (6.9)	65.5 (8.7)	83.5 (1.9)	69.0 (4.6)	81.6 (4.9)	57.5 (8.1)
2	600	19.5 (2.2)	14.0 (1.9)	16.8 (1.4)	14.1 (1.5)	40.5 (7.5)	30.6 (5.8)	54.5 (2.9)	33.3 (3.0)	60.8 (9.1)	36.8 (8.1)
3	600	12.0 (3.7)	9.7 (4.0)	7.3 (0.5)	6.1 (0.3)	16.0 (1.7)	11.0 (1.0)	12.5 (2.6)	8.1 (2.5)	48.6 (5.0)	18.5 (2.9)
4	600	3.8 (1.6)	2.6 (1.0)	1.3 (0.4)	1.3 (0.4)	8.3 (0.8)	5.3 (1.2)	1.6 (0.6)	1.3 (0.5)	25.3 (8.3)	2.6 (0.84)
5	600	0.3 (0.21)	0.2 (0.2)	0.2 (0.2)	0.0	3.1 (1.3)	1.6 (0.6)	0.8 (0.3)	0.3 (0.2)	6.6 (2.9)	0.0
6	600	0.0	0.0	0.0	0.0	2.8 (1.3)	0.3 (0.3)	0.0	0.0	0.3 (0.2)	0.0
7	600	0.0	0.0	0.0	0.0	0.7 (0.7)	0.3 (0.3)	0.0	0.0	0.0	0.0
8	600	0.0	0.0	0.0	0.0	0.3 (0.3)	0.2 (0.2)	0.0	0.0	0.0	0.0
9	600	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10	600	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11	600	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12	600	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

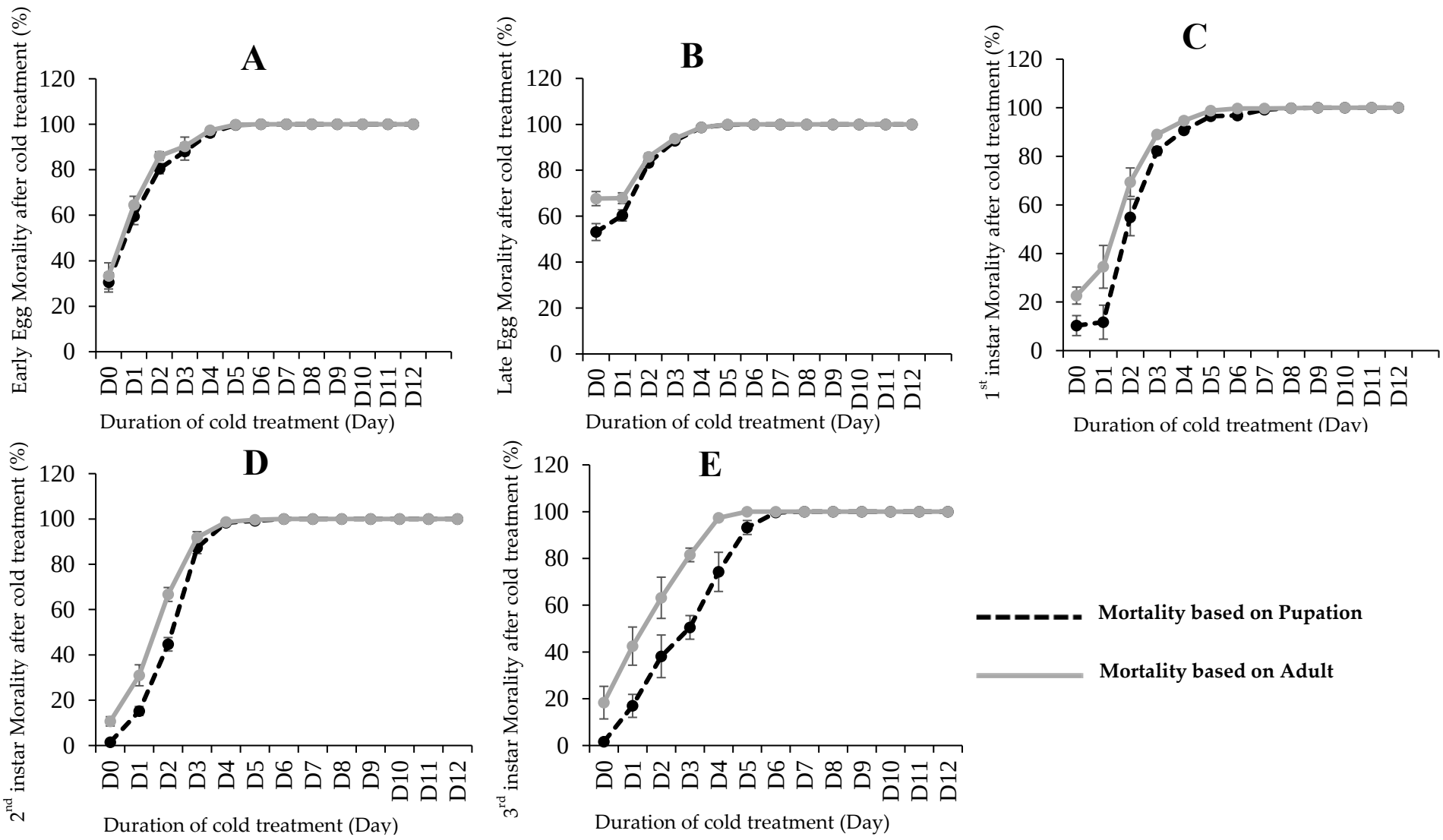


Figure 2.1. The mortality (%) calculated based on pupation and emerged adults at different immature stages including: early eggs (A), late eggs (B), 1st instar (C), 2nd instar (D) and 3rd instar (E) larvae of *C. capitata* fed on lab diet when subjected to various days of cold treatment at 0.0°C. Error bar means standard error.

We then further compared the pupation ratio and adult emergence ratio at each treated fly developmental stage (Figure 2.1). Our results showed that most mortality occurred before the pupation stage, as most insects that survived pupation would also emerge to adults. However, slight post-pupation mortality rates were observed in the early egg (Figure 2.1A) and late egg (Figure 2.1B). While the post-pupation mortality in 1st instar and 2nd were higher than eggs. In the 3rd instar treatment, post-pupation mortality rates were the highest (Figure 2.1E), suggesting a considerable proportion of recovered pupae from this stage did not successfully develop to adults.

As mentioned, four different models were selected separately for pupations and emerged adults on every stage, and the best fitting model was selected for estimating the LT_{90} and LT_{99} (Table 2.S1). The four models include: log on days with probit, log on days with logit, no-log on days with probit, and no-log on days with logit (Table 2.S1). The results showed that no-log models are better than log models in this study (Table 2.S1). When using pupation as the end point for mortality analysis, no-log on days with probit model was selected for the early egg, late egg and 3rd instar while no-log on days with logit was selected for 1st and 2nd instar larvae. When using emerged adults as the end point for mortality analysis, no-log on days with probit model was selected for the late egg while no-log on days with logit was selected for the rest stages. We modelled the duration of cold treatment to induce 90 and 99% mortality at five immature stages of *C. capitata* fed on a lab diet (Table 2.2). Using the recovered pupation as the end point for mortality modeling, the results showed that the 3rd instar and 1st instar are the most cold tolerant stages, with LT_{99} = 7.3 days for both of instars. Interestingly, based on the emerged adult ratios, LT_{99} was 5.7, 5.7, 6.4, 5.4, and 6.1 days for early eggs, late eggs, 1st instar, 2nd instar and 3rd instar, respectively. The 1st instar (LT_{99} = 6.4) is superior to 3rd instar (LT_{99} = 6.1). However, the statistical analysis by using SPSS software showed that there are no significant differences between 1st and 3rd instars in either pupation or emerged adults as endpoints.

Table 2.2. Cold treatment duration to induce 90 and 99% mortality based on pupation and emerged adults of five *C. capitata* developmental stages fed on lab diet. The models were selected based on the result in Table 2.S1.

Mortality	*Developmental*	*Pupae recovery as end point*			*Adults recovery as end point*		
	Stage	Treatment	95% Confidence Limits		Treatment	95% Confidence Limits	
(LT)		(day)	Lower	Upper	(day)	Lower	Upper
90	Early eggs	3.94	3.81	4.07	3.54	3.42	3.68
	Late eggs	3.58	3.45	3.73	3.34	3.2	3.5
	1st instar	5.25	5.14	5.38	4.27	4.15	4.4
	2nd instar	4.19	4.1	4.29	3.88	3.78	3.99
	3rd instar	5.72	5.6	5.84	4.27	4.15	4.4
99	Early eggs	5.74	5.54	6.01	5.71	5.47	6.03
	Late eggs	5.57	5.35	5.89	5.7	5.43	6.07
	1st instar	7.33	7.14	7.58	6.4	6.19	6.69
	2nd instar	5.48	5.34	5.68	5.41	5.24	5.63
	3rd instar	7.36	7.2	7.57	6.14	5.94	6.39

In terms of the sex ratio studies, female adults (%) developed from treated early eggs, late eggs, 1st, 2nd and 3rd instar were 51.7, 48.8, 52.2, 51.8 and 48.4, respectively. The ANOVA single factor test (Table 2.S2) showed that there are no significant differences between each stage. Overall, cold treatment at 0.0 °C on *C. capitata* does not affect sex ratio.

2.5. Discussion

Cold treatment is a common method for eradicating fruit flies in fresh fruits and other products. It has been studied, analysed and incorporated into quarantine regulations (Dohino et al., 2017; Sproul, 1976; Burditt and Balock, 1985; Jessup et al., 1993; Follett and Snook, 2013) and oversea countries (Badii et al., 2015; Jessup et al., 1998; De Lima et al., 2011; Grout et al., 2011).

In this study, we chose to study *C. capitata* cold response from the lab diet but not fruit, due to the variation in sizes, materials, nutrients and chemicals, resulting in significant differences in fly physiology and development. Secondly, some fruit may not be favorable hosts for fruit

flies and so might be associated with the production of weak flies, resulting in the differences in the mortality rates. Furthermore, there are different infestation methods for various fruit. For example, by using the natural infestation, how many eggs were laid into a single fruit is hard to clarify. By using artificial infestation (e.g., injecting eggs or larvae into fruits), methods may affect fruit quality, fruit fly development and may even cause bacterial or fungi contamination. All of these can result in very different results in cold treatment experiments. Therefore, here we chose to use lab diets, as the high homogeneity of the diet in terms of ingredients, quantity, size and dimensions of experimental units make the results more reliable and reducing of experimental error. This way, we could be clear about how many eggs/larvae were used in the analysis and avoid the fruit/infestation issues. Cold treatment analysis of *C. capitata* on a lab diet has previously been performed (Ware et al., 2012), but not in a comparative way on all five different stages as we did here.

To evaluate cold treatment effects on *C. capitata*, the first question is how to define mortality. Eggs or larvae may survive from the cold treatment but fail in pupation or adult emergence due to unknown effects. On the other hand, some of them succeed in adult emergence but fail in sex development and reproduction, so they are not biologically "alive" flies. Therefore, here we used the recovered pupae or adults as standards to help define our mortality here. If one egg or larva fails in developing to pupae or adult after treatment, it is a "dead" fly.

Our bioassays (Figure 2.1 and Table 2.1) showed that the 1st instar is the most cold tolerate stage. However, modelling analysis LT_{99} results showed the 1st and 3rd instar are equal stages in tolerance of cold treatment based on pupation. Based on the emerged adults, LT_{99} results showed that there are no significant differences between 1st and 3rd instars, in spite of 1st instar LT_{99} = 6.4 being superior to 3rd instar, because the range of the confidence interval range is 0.5 day. All these results suggest both 1st instar and 3rd instar are among the most cold tolerant stages, on which more attention should be paid in our postharvest treatment. The previous studies are not consistent concerning the most cold tolerant stage of *C. capitata*. There are several major reviews and seven annexes of International Standards for Phytosanitary Measures (ISPM) that deal specifically with the cold tolerance of this species. Grout et al. (2012) concluded that the most cold tolerant stage was the 2nd instar based on a commodity group research report from South Africa. Hallman et al. showed that the 3rd instar is the most cold tolerant stage (Ware et al., 2012). A study compared tolerance of eggs, a

mixture of 1st and 2nd instars, and mostly 3rd instars to 1.5±0.5°C in oranges and found both larval groups to be very similar, with the younger instars showing a very slight advantage in survival (Hill, 1990). Another study found that the 2nd instar was the most tolerant to 1.0±0.2°C in two cultivars of lemon (Burditt and Balock, 1985). The third study found that the 2nd instar was more tolerant than the 3rd in five types of citrus fruits at 2 and 3°C (Hallman et al., 2011). There are plentiful differences in the experiment set up, including, but not limited to fruits, infestation methods, temperatures, fruit sizes, materials and nutrient. Therefore, it is not surprising that the detected most cold tolerate stage is different. It is also likely that different population of fruit flies vary in relative tolerance of the different states to cold treatment. It was reported previously that geographically isolated populations of Medfly differ in reproductive patterns, survival, developmental rates and intrinsic rates of increase (De Lima et al., 2007), suggesting their different tolerance to low temperatures.

In this study, we compared early eggs (< 6 h) and late eggs (>42 h) in the cold treatment, because the embryo development stage may affect their biology, physiology and cold tolerance. For the fruit picked up and transferred to the cold storage, it is likely to collect some early eggs, which were laid just before the fruit picking, and late eggs, which have been laid for a period of time. The egg is a fast-developing stage, so the age difference can be a significant issue that has been ignored in previous research. Here, our results showed that early eggs are a bit more tolerant than late eggs.

The exposure durations required to achieve complete mortality of *C. capitata* immature stages have been reported in various fruits. For example, at 2°C, 18 days were required to control *C. capitata* in mandarins and oranges, compared to 16 days in lemons; whereas at 3°C, 20 days were required in mandarins and oranges, and 18 days were required in lemons (Diamantidis et al., 2011). At 0.5–1.5°C, 16 days were needed to control *C. capitata* in mandarins (De Lima, 1998). End point is an important factor affecting the lethal time. In this paper, when pupae and adults are used as end points respectively, the lethal time is obviously different. Our study showed that nine days on a carrot diet was enough to achieve 100% mortality of *C. capitata* immature stages. The duration is much shorter because we used an artificial diet, which is very different from real fruit in many ways. For example, the cold transfer in fruit can take more time than in artificial diet. Another reason is that we used 0°C, lower than the temperatures used in many previous studies. Since there is a close association

between duration of exposure, temperature and survival in most insects, the lower temperature leads to faster injury and mortality.

We also compared the sex ratios of survived adults after treatments. Our result (Table 2.S2) showed that there is no significant difference in the sex ratios at different stages. While several studies investigate industry-relevant applications of cold treatment of Tephritid fruit flies (Broughton and De Lima, 2002; Badii et al., 2015; Jessup et al., 1998) in various fruits at various stages, a fundamental understanding of the mechanisms underlying why and how cold can kill the flies is currently lacking. Without this knowledge, it is difficult for us to improve and optimize our current cold treatment strategies and develop more efficient treatment methods.

2.6. Conclusions

In this study, cold treatment at 0.0°C with different exposure durations (0–12 days) was applied to the Mediterranean fruit fly *C. capitata* (Wiedemann) fed on a lab diet. The examined developmental stages were early eggs (<6 h), late eggs (>42 h), 1st instar, 2nd instar and 3rd instar larvae. Our results showed that both 3rd instar and 1st instar is among the most cold tolerant stages of *C. capitata*. There were no significant differences between early eggs and late eggs in the tolerance of cold treatment. We studied stage-specific tolerance because, in infested fruit experiments, it is probably unlikely that all fruit flies are in the same developmental stage. Therefore, any treatment should be efficient enough to kill the most tolerant stage. There were no significant differences in *C. capitata* sex ratios among different stages after treatment. In future studies on cold treatments of fruit flies, we recommend further investigation of the interaction between survival times and test temperatures. Different temperatures may induce different responses at cellular and molecular levels. This study will improve our understanding of *C. capitata* responses to cold treatment and assist in the optimization of current treatment strategies to control this horticulturally destructive pest species.

2.7. Supplementary Materials:

The following are available online at www.mdpi.com/xxx/s1, Table 2.S1: Four different models were used in the *C. capitata* mortality data analysis; Table 2.S2: The sex ratios

comparative of *C. capitata* stages which were treated with cold treatment 0.0°C; Figure 2.S1: (A) Mortality (%) based on pupation and adults (B) ratios from the treated *C. capitata*

Table 2.S1. Four different models were used in the *C. capitata* mortality data analysis

	Pupation method							Adult method						
Stage	Name	Log	Type	Residue	BIC	Intercept	Slope	Name	Log	Type	Residue	BIC	Intercept	Slope
Early eggs	Model1	Y	Probit	209.9	345.48	-0.6943	1.52913	Model1	Y	Probit	220	347.11	-0.5642	1.53
	Model2	Y	Logit	271.85	407.43	-1.235	2.729	Model2	Y	Logit	271.14	398.24	-1.011	2.758
	Model3	N	Probit	147.12	282.7	-1.0024	0.5802	Model3	N	Probit	191.82	318.92	-0.915	0.5987
	Model4	N	Logit	152.68	288.25	-1.819	1.049	Model4	N	Logit	188.1	315.21	-1.711	1.104
Late eggs	Model1	Y	Probit	210.73	342.4	-0.2423	1.3071	Model1	Y	Probit	231.9	358.6	0.1045	1.123
	Model2	Y	Logit	288.82	420.49	-0.4303	2.331	Model2	Y	Logit	302.54	429.24	0.1641	2.0417
	Model3	N	Probit	66.96	198.63	-0.5987	0.5247	Model3	N	Probit	82.99	209.69	-0.2035	0.4442
	Model4	N	Logit	90.87	222.54	-1.1329	0.9518	Model4	N	Logit	111.05	237.75	-0.4842	0.8267
1st instar	Model1	Y	Probit	492.6	664.82	-2.225	2.063	Model1	Y	Probit	375.54	530.17	-1.179	1.704
	Model2	Y	Logit	445.94	618.15	-4.48	4.01	Model2	Y	Logit	431.46	586.1	-2.194	3.083
	Model3	N	Probit	351.57	523.79	-2.0967	0.6199	Model3	N	Probit	259.42	414.05	-1.427	0.6155
	Model4	N	Logit	298.65	470.87	-3.87	1.155	Model4	N	Logit	242.01	396.65	-2.6	1.123
2nd instar	Model1	Y	Probit	165.59	282.7	-3.097	2.991	Model1	Y	Probit	214.95	341.94	-1.718	2.178
	Model2	Y	Logit	164.01	281.11	-5.903	5.616	Model2	Y	Logit	241.16	368.14	-3.317	4.068
	Model3	N	Probit	98.43	215.54	-3.089	1.024	Model3	N	Probit	100.8	227.79	-2.1792	0.8784
	Model4	N	Logit	92.74	209.85	-5.601	1.86	Model4	N	Logit	100.45	227.43	-3.873	1.566
3rd instar	Model1	Y	Probit	615.83	761.53	-2.755	2.292	Model1	Y	Probit	363.74	496.24	-1.522	1.94
	Model2	Y	Logit	666.71	812.41	-5.038	4.133	Model2	Y	Logit	422.09	554.6	-2.801	3.503
	Model3	N	Probit	465.81	611.51	-2.354	0.636	Model3	N	Probit	282.62	415.13	-1.8138	0.7244
	Model4	N	Logit	504.37	650.07	-4.069	1.102	Model4	N	Logit	260.63	393.14	-3.125	1.258

The models including probit, logit, non log - probit and non - log logit. Which its blow a line was the selected one to calculate LT_{90} and LT_{99} in table1.

Table 2.S2. The sex ratios comparative of *C. capitata* stages which were treated with cold treatment 0.0°C.

SUMMARY						
Groups	***Count***	***Sum***	***Average***	***Variance***		
Early egg	15	776.6	51.7	98.1		
Late egg	12	585.8	48.8	148.8		
1st instar	18	939.7	52.2	65.6		
2nd instar	14	726.2	51.8	89.8		
3rd instar	18	872.6	48.4	109.5		
ANOVA						
Source of Variation	***SS***	***df***	***MS***	***F***	***P-value***	***F crit.***
Between Groups	208.38	4	52.09	0.52	0.71	2.49
Within Groups	7159.87	72	99.44			
Total	7368.25	76				

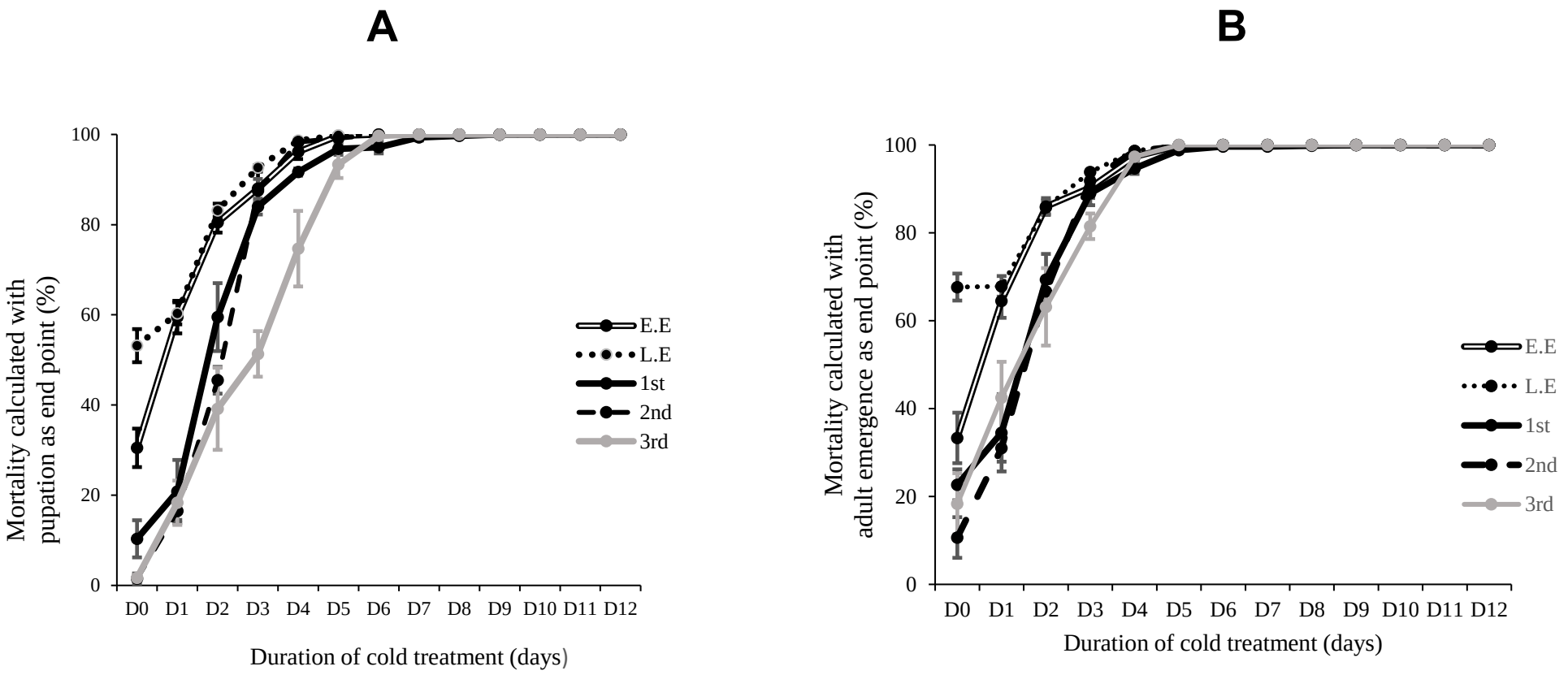


Figure 2.S1. (A) Mortality (%) based on pupation and adults (B) ratios from the treated *C. capitata*.

Statement of Contribution

Title of Paper	Cold Responses of the Mediterranean Fruit Fly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) in Blueberry
Publication Status	published
Publication Details	Insects Journal, 2020, 11(5), 276.
Principal Author	
Name of Principal Author (Candidate)	Farhan J.M. Al-Behadili
Contribution to the Paper	Methodology, Collected Data, Writing- Original Draft Preparation, Conceptualization and Validation
Overall Percentage (100%)	85 %
Signature	Date 28 / 07 /2020

Co-Author Contributions

By signing the statement of contribution, each author certifies that:

- The candidate's stated contribution to the publication is accurate (as detailed above).
- Permission is granted for the candidate to include the publication in the thesis
- The sum of all co-author contributions is equal to 100% less the candidate's stated Contribution

Name of Co-Author	Manjree Agarwal
Contribution to the Paper	Supervision
Overall Percentage (100%)	5 %
Signature	Date 28 / 7 /2020
Name of Co-Author	Yonglin Ren
Contribution to the Paper	Supervision, Funding acquisition
Overall Percentage (100%)	5 %
Signature	Date 28 / 07 /2020
Name of Co-Author	Wei Xu
Contribution to the Paper	Supervision, Funding acquisition, Validation, Conceptualization
Overall Percentage (100%)	5 %
Signature	Date 03 / 08 /2020

Chapter three

Cold Responses of the Mediterranean Fruit Fly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) in Blueberry

3.1. Abstract

In this study, the effects of cold treatment at 1.0±0.2°C were investigated on eggs, 1st, 2nd and 3rd instar larvae of *Ceratitis capitata* in two Australian blueberry cultivars C99-42 and C00-09. Pupariation, emerged adults and sex ratios were examined on *C. capitata* after the treatment. The results showed that exposure time at low temperatures was a key factor to affect pupariation and adult emergence. Eleven days of exposure to cold treatment at 1.0±0.2°C were enough to eradicate all four immature stages in both cultivars. Cold tolerance of the four *C. capitata* stages was not affected when reared on two different blueberry cultivars. The third instar larva is the most tolerant stage for cold treatment in two blueberry cultivars. There were no significant differences in sex ratios from survived *C. capitata* among different stages after treatment. This study improves understanding of *C. capitata* responses to cold treatment in blueberries, which may contribute to phytosanitary required quarantine treatment of this destructive horticulture pest species.

3.2. Introduction

The Mediterranean fruit fly (Medfly), *Ceratitis capitata* (Wiedemann 1824), is one of the major pests of fruits and vegetables. *C. capitata* originated from sub-Saharan Africa and was identified in southern parts of Europe in the early 19th century. Currently, it is present in Mediterranean Europe, the Middle East, in most parts of Africa, including the Indian Ocean islands, South and Central America, Western Australia, and the Pacific region. Their economic losses predestined to be more than 2 billion dollars yearly (Sciarretta et al., 2018). Moreover, it is a major fruit fly species of quarantine importance and capable of causing extensive damage to a broad range of cultivated and wild fruit and vegetable products (Pimentel et al., 2017). Medfly has the excellent dispersive ability and a tolerance of both natural and cultivated habitats over a comparatively wide temperature range, it has successfully established in many parts of the world (Malacrida et al., 2007). Therefore, *C.*

capitata has a significant economic impact, affecting production, control costs and market access.

For fruits, phytosanitary treatments are used to reduce the risk at quarantine areas and to prevent an infestation at non-infested importation areas (FAO and IPPC, 2017). Various areas of the world have a history of repeated detections of fruit fly species, with some resulting in quarantines that prevent the export of host commodities until the pest is declared eradicated (De Meyer et al., 2010), which can result in economic loss and reputation damage.

The cold treatment has been used as a postharvest treatment method since 1916 (Back and Pemberton, 1916), due to its advantages of being environmentally friendly (no pesticide residue) and safe for employers and consumers. It is an effective treatment method to provide phytosanitary control (Heather and Hallman, 2008), increase fresh plant product shelf life (Ghafir, 2009) and maintain fruit quality; also, it is easy to apply compared to other methods, such as sterilizing technology and fumigation (Richardson, 1952).

A plethora of studies have investigated the use of cold treatment against fresh fruit and vegetable pests. These studies continue to show cold impacts on insects. The species of insect pests showed different responses to the cold treatment. The differences may be due to genetics, physiology or effects of host variation (Mangan and Hallman, 11998; Hallman and Sharp, 1994; Gould and Hennessey, 1997). *C. capitata* is possibly the most studied organism in phytosanitation, with numerous studies examining cold treatment (Hallman et al., 2019). However, the cold treatment on *C. capitata* in blueberry has not been examined previously.

Blueberries belong to the genus *Vaccinium* (Vander Kloet 1988) (Ericaceae), which also includes cranberries and huckleberries, one of the few cultivated fruits native to North America (Williamson and Lyrene, 2004). The industry has expanded to Europe, South America and Australasia, but North America remains the major producer (Strik, 2005). Blueberries have high nutritional value (Zheng and Wang, 2003). The Australian blueberry industry is one of the premium blueberry industries in the world, cultivation increased by 70% from 2010 to 2012, with 90% production supplied to the local markets. The Australian blueberry was successfully exported to Japan until 2011; however, the export was suspended because blueberries were infested with Medfly (Brazelton, 2013).

This study aims to evaluate cold treatment against Medfly in blueberry to provide an integrated knowledge for understanding the cold response of Medfly in two blueberry cultivars.

3.3. Materials and Methods

3.3.1. *Ceratitis capitata* colony

C. capitata colony used in this experiment was initially established in 2015 from a stock ancestry kept at the Department of Primary Industries and Regional Development (DPIRD) in Western Australia, which was periodically refreshed with the introduction of more wild flies. *C. capitata* adults were maintained in a Bugdorm-1 cage (30 x 30 x 30 cm ‘‘BioQuip products’’) with about 300 adults per cage, with access to water in a glass vial covered with a plug of cotton and also a dry 3:1 mixture of sucrose and yeast extract in a shallow glass container (Sasso et al., 2020). Mature insects laid eggs through the cloth sidewalls of the cages, which were collected and moved to the artificial breeding medium consisting of 300 g torula yeast, 1 kg ground dehydrated carrot, 4.5 L hot tap water, 36 mL HCl, 30 g nipagin and 500 mL boiling water (Tanaka et al., 1969). After 13–16 days, pupae were collected and moved into the adult breeding cages. The emerged adult insects were reared on crystalline sugar, the yeast hydrolysate and water. Breeding conditions were 26.0±1.0°C, 60-70% RH, and darkness light cycle of 16:8 h (De Lima et al., 2011)). Eggs and the three larval instars (1^{st}, 2^{nd} and 3^{rd}) were used in this study.

3.3.2. Fruits and quality assessment

Two blueberry cultivars used for this research were pesticide-free C00-09 and C99-42 from Western Australia. Both varieties have strong sweetness and weak acidity, which makes them desirable by consumers. Another reason is C00-09 has a later seasonal availability, while C99-42 has an earlier availability (Wright and Lyrene, 2010; Wright and Lyrene, 2012), this provides a permanent host to *C. capitata*. The combination reasons of quality and availability for consumers and *C. capitata* led us to choose these two cultivars.

Whilst at 25.0˚C, thirty fruits from each cultivar were distributed in three replicates, ten fruits per replicate, for physical quality and six replicates, five fruits per replicate for biochemical quality determination. Physical qualities included weight, diameter, colour (red, grey, black),

hardness, and moisture content. Biochemical qualities included the percent of acid, degrees Brix and sugar to acid ratio. The blueberry samples were stored at 3.0±0.5°C at Murdoch University cold storage.

3.3.2.1. Colour test

Colour was measured as RGB colour model (Red, Grey, Black) with a colourimeter (CS-210 Portable Precision Digital Colourimeter, China). The colourimeter was calibrated with white and black colour. Three replications were performed for each blueberry fruit.

3.3.2.2. Size

Fruit diameters were measured using plastic Vernier calipers to the nearest millimeter (mm) (Ningbo, China) and three replications were performed to measure the diameter of each fruit.

3.3.2.3. Weight

Fruit weight was measured by a digital balance (PAL-BX /ACID7, Atago (Tokyo, Japan).

3.3.2.4. Hardness

A small part of fruit peel was removed. The hardness of blueberry fruits was measured by a fruit hardness tester (Model No GY-2, Ningbo/Shanghai, China). The capacity of the hardness tester is 0.2-4 kg/cm² (x105 pa), a pressure diameter is 3.50 mm, the accuracy is ±0.02 mm, a pressure depth is 10 mm and dimensions are 140 × 60 × 30 mm.

3.3.2.5. Moisture content

Relative humidity was measured via a moisture meter (model DH-100 –DELMHORST, USA) two pins that penetrate deeply into the blueberry fruit, measuring from 0-99.9%, with an LCD screen, 80 mm width × 35 mm high × 150 mm diameter and 245 g.

3.3.2.6. Degrees Brix, acid and their ratio (sugar/acid)

Sugar and acidity level in blueberry fruit juices was measured with a Pocket Brix-acidity meter, PAL-BX|ACID7 master kit brand Atago (Tokyo, Japan). The meter was calibrated with water for sugar content and without water for acidity.

Six replicates of five blueberries, from each cultivar, each was juiced through a piece of mesh to measure Brix. An aliquot of undiluted juice (at least 0.3 mL) was applied to the lens with a plastic pipette.

To measure acidity, 1.0 g. of fruit juice was transferred into the beaker with a dilution ratio of 1:50 with distilled water to a total weight of 50 g while stirring gently. Then diluted juice was moved by plastic dropper to the lens of the PAL-BX|ACID meter in percentage (%) and measured (first pressing for acid and the second for sugar). The sugar-acid ratio was displayed and recorded. Six replicates per cultivar were done for acidity, Brix and their ratio.

3.3.3. Natural infestation

The natural infestation method was utilised on blueberries to avoid fruit damage by artificial infestation method, which may lead to microorganism growth affecting *C. capitata* development stages. Additionally, the natural infestation can avoid egg damages through the collection and transferring processes to the fruits when using artificial methods. A representative random assay of blueberry fruits from each cultivar was inspected to ensure the absence of field infestation and determine cultivar health. In preparation for conducting the infestation with Medfly at 26.0±1.0°C, 60-70% RH in the rearing room, approximately 300-400 random healthy blueberry fruit of each cultivar (C00-09 and C99-42) were conditioned at 25.0±1.0°C and 60-70% RH overnight. Fruits were placed in an aluminium tray of the dimensions of 29 cm width, 92 cm length and 2.5 cm depth with metal mesh bottom (0.6 cm). The blueberries were transferred to the aluminium tray and held in the middle of the adult rearing box (200 cm × 150 cm × 40 cm) containing around 250,000-300,000 flies with the sex ratio of 50:50. This set up allowed flies to reach fruits easily. The natural infestation was conducted repeatedly for getting enough amount of infested fruits. Various periods of exposing the fruits to females (30, 45, 90 and 120 minutes) with three replicates for each exposure period were tested to identify an ideal time for females laying an appropriate amount of eggs in each fruit. From each replicate, 16 fruits were randomly selected six times and individually dissected to count the eggs inside the fruit.

3.3.4. Life history study

To study the life history of Medfly in the two blueberry cultivars, two blueberry fruits from the sample of 45-minute exposure time (for each cultivar which was used for life history study) after natural infestation were randomly selected and put in a glass jar (300 mm) with

sand. The jar was closed by a piece of mesh and banded with a rubber band. A total of 300 jars were prepared for both cultivars and kept at 26.1±1.0°C and 60-70% RH. After 24 h incubation, 16 jars were moved to the laboratory to dissect the fruit for recording the stages of the flies under a microscope. This step was repeated every day until the first appearance of the pupae in control jars.

3.3.5. Cold treatment

The temperature (1.0±0.2°C) was selected because it is a relatively safe temperature for fruits. Lower than 1.0°C will be likely to cause serious fruit or vegetable damage. The cold cabinet is a constant temperature and humidity incubator (model HWS, LET code 0574-88000432, Tianjin- China), which was made from stainless steel with a temperature range of 0 to 65°C, a humidity control range of 50 to 90% RH, humidity fluctuation of ±8% RH and an inner stainless-steel mirror. The door consists of toughened metal with an outside observation window. The cold cabinet is equipped with self-diagnosis tools, a sensor failure alarm and over-temperature protection. The temperature inside the cabinet was recorded every 30 minutes by putting two HOBO® data logger units at different heights (Model number H08-004-02, Onset Computer Corporation, MA 02532, USA, www.onsetcomp.com). We calibrated the cabinet temperature sensor and HOBO® data logger units by comparing them with four thermometers red spirit. The thermometers were checked using an ice point check as per the NATA tech note. Then the incubator and HOBO were checked with checked thermometers (NATA), 2019).

After 45 minutes of exposure to female flies, at laboratory condition, the fruits were used for cold tolerance study. Two berries from cultivar C00-09 were randomly selected and put in a glass jar (30 mm) with sand. Three hundred and twelve jars containing C00-09 were prepared with two berries in each jar, which were divided into four groups (78 jars in each group). Each group was put on a plastic plate labelled with the insect stage (eggs, 1st instar, 2nd instar and 3rd instar), date and cultivar. For eggs used in the experiment, the plastic plate (group 1) with 72 jars was directly transferred to cold treatment cabinet (1.0±0.2°C chamber) and six jars to rearing cabinet as control. Life-cycle results were used to determine how long each stage remained in the rearing cabinet before transferring it to the cold cabinet (Table 3.S1). After three days and 12 hours, 72 jars were moved from the rearing cabinet (1st instar, group 2) to the cold cabinet, which was set up at 1.0±0.2°C, six jars were left in the rearing cabinet

as a control. Five days and 12 hours later, the second plate (2^{nd} instar, group 3) was moved from the rearing cabinet, with 72 jars for cold cabinet and six for a control cabinet. Eight days later, the third plate (3^{rd} instar, group 4) was moved from the rear cabinet, with 72 jars for cold treatment and 6 for a control cabinet.

After 24 hours of cold treatment, six jars from each group were moved to the rearing cabinet and labeled. Every day, six jars were collected from the 1.0±0.2°C cold cabinet and transferred to the rearing cabinet. Every six jars that were moved from cold cabinet to rearing cabinet from day one to day twelve (72 jars) were left 28 days in a rearing cabinet and checked every day. After pupae were first seen, the sand inside each jar was sieved three times weekly; and the dry blueberries were checked well for pupae. After that, the pupae were counted and placed in a sterile Petri dish, which was left in the rearing cabinet. Additional observations on the number of emerged flies were considered. This provided information on the viability of pupae and whether they were able to develop into adults, and the sex ratios observations were recorded and analyzed. Firstly, all the surviving adults from treated eggs and the three instars were collected and the percentage of females was calculated. The same protocol was applied with cultivar C99-42. Secondly, to examine if a certain period of days treatment influences the sex development, female ratios from treated flies on different days were analysed If the numbers of adults emerged were too low (<10), results were not included in the analysis.

3.3.6. Statistical analysis

The control pupariation and adult emergence rates were used to normalize treatment pupariation and adult emergence by Schneider-Orelli's formula (Püntener, 1981)). Firstly, the test of normal distribution was conducted via the Kolmogorov-Smirnov test and Shapiro-Wilk (Table 3.S2). Once the data met the normal distribution test, the pupariation and emerged adults' rates of the insects under cold treatment were statistically estimated following the general linear model-univariate. The mortality rate of the insect under cold treatment was statistically estimated following the Median Lethal Time method (LT). The 90, 95 and 99% mortality (LT_{90}, LT_{95} and LT_{99}) were estimated by using the selected models. According to the results of the normality test, the regression models (probit analysis) were selected. Pupariation and emerged adults were counted as the survival (and mortality) to calculate the lethal time (LT). If a treated fruit fly egg or larva can successfully develop to a

pupa or an adult, it is a survived fly; otherwise, it is a dead fly. The LT value estimated under a generalized linear model with probit link function on cold treatment days. The model can be written as:

$$\eta = \beta 0 + \beta 1\, x \qquad (1)$$

Where η is the response or proportion mortality, x is the dose, $\beta 0$ is the intercept and $\beta 1$ is the coefficient of the dose. An ANOVA single factor test was used to compare the sex ratio of adult insects emerging from different stages after cold treatment in two blueberry cultivars. The fruit quality was statistically estimated following the comparing means /independent samples t-test. In all the statistical methods, the probability level was ≤ 0.05 and SPSS software (SPSS, IBM version 24 Armonk, New York, America) was used.

3.4. Results

3.4.1. Fruit quality assessment

To investigate the effect of different blueberry varieties impact on the fruit fly response to cold treatment, the fruit qualities of two blueberry varieties, C00-09 and C99-42, were compared. The results of biochemical tests of two blueberry cultivars (Table 3.1) indicated no significant differences in percentages of acid, Brix and the ratio of sugar to acid between the two cultivars ($p > 0.05$), while there was a significant difference in water content of the cultivars ($F = 13.600$, $P < 0.0005$) (Table 3.1). The water content in cultivar C00-09 was higher than cultivar C99-42, where the water content was 92.4 at C00-09 while 79.7 in C99-42. The physical measurements of cultivars C00-09 and C99-42 (Table 3.1) showed that there were significant differences in weight ($F = 13.600$, $P < 0.0005$), diameter ($F = 133.000$, $P < 0.0005$) and density blue colour ($F = 9.180$, $P = 0.002$) while there were no significant differences in density red colour, density green colour and hardness between both cultivars.

Table 3.1. Fruit quality assessment includes biochemical and physical quality of blueberry cultivars C00-09 and C99-42 before cold treatment.

Fruit quality	Test	Blueberry cultivars	Mean ± SE	unit	Significance
Biochemical	Sugar	C00-09	13.5 ± 0.14	%	0.3
		C99-42	11.3 ± 1.9	%	
	Acid	C00-09	0.5 ± 0.03	%	0.1
		C99-42	0.4 ± 0.04	%	
	Sugar to acid	C00-09	26.1 ± 1.6	%	0.05
		C99-42	34.1 ± 3.3	%	
	Water content	C00-09	92.7 ± 0.7	%	0.0
		C99-42	79.4 ± 1.4	%	
Physical	Weight	C00-09	3.2 ± 0.1	gm	0.0
		C99-42	2.0 ± 0.1	gm	
	Diameter	C00-09	20.6 ± 0.3	mm	0.0
		C99-42	17.9 ± 0.3	mm	
	Colour R	C00-09	91.1 ± 21.5	nm	0.5
		C99-42	104.5 ± 1.9	nm	
	Colour G	C00-09	90.8 ± 4.5	nm	0.7
		C99-42	92.7 ± 2.3	nm	
	Colour B	C00-09	108.3 ± 4.2	nm	0.0
		C99-42	92.2 ± 2.5	nm	
	hardness	C00-09	3.1 ± 0.1	Kg/cm	0.3
		C99-42	6.2 ± 3.5	Kg/cm	

3.4.2. Natural infestation.

Four time-points (30, 45, 90 and 120 min) for the natural infestations were tested on two blueberry cultivars. The results of this experiment (average ± Standard error) indicated that after 30 minutes only 0.6±0.3 and 0.8±0.3 egg/fruit were produced, in cultivars C00-09 and C99-42 respectively. Ninety- and 120-minutes natural infestation yielded 78.1±0.7 and 76.3±0.6 eggs/fruit in cultivar C00-09 while 93.3±0.6 and 91.6±0.4 eggs/fruit in C99-42 respectively. This high population of larvae will result in larval competition on a limited food

amount (one fruit). Therefore, 45 minutes was selected as the average egg number per fruit as optimal, 6.8±0.4 and 6.5±0.4 eggs/fruit in cultivars C00-09 and C99-42, respectively. There were no significant differences in the rate of eggs between the two cultivars ($p > 0.16$), while there was a significant difference in the egg rates between the exposure times ($F = 50866.1$, $P < 0.00$) (Table 3.S3)

3.4.3. Life history study.

Before proceeding with cold treatment, the life history of *C. capitata* immature stages in blueberry at 26.1±1.0 °C and 60-70% RH were examined (Table 3.S1). Two days after infestation, eggs started to hatch. Four and a half days after the infestation, the maximal 1st instar was 231 and 201 for cultivar C00-09 and C99-42 respectively. Five days after the infestation, 2nd instar larvae were observed. Six days after the infestation, suitable numbers 208 and 152 for cultivar C00-09 and C99-42 respectively of 2nd instar was obtained. Nine days later, suitable numbers 146 and 125 for cultivar C00-09 and C99-42 respectively of 3rd instar larvae were ready to use, and pupae started to be observed on day 10.

3.4.4. Responses (Mortality) to cold treatment.

The temperature and humidity inside the cabinet during cold treatment were 1.0±0.2°C and 60-70% RH. Significant differences in pupariation ratios ($F = 323.567$, $P < 0.0005$) and adult emergence ($F = 387.389$, $P < 0.0005$) were found among the four stages (eggs and three larvae instars) that were treated with cold treatment (1.0±0.2°C) for 12 days (Table 3.2).

3.4.5. Cold treatment Bioassay

In the control group of pupariation, the natural mortality was considered zero, following common practice in calculating mortality where large populations of insects are tested inside the host fruits (Sun and Shepaud, 1947). Based on pupariation ratios as survival indicator, the stage with the highest survival rate was 3rd in star followed by2nd and 1st instar, while the egg was the most susceptible in both blueberry cultivars.

There was a significant difference between the pupariation rates of eggs and the three larval instars in both blueberry cultivars, which were decreasing with increased exposure time to cold (Table 3.2). The exposure times to the low temperature, which completely eradicated eggs and larvae instars, were different for different fly stages. In both cultivars, nine-day

treatment was enough to reach 0 survival in egg samples. Ten-day treatment to 1st and 2nd larvae instars can reach 0 survival flies, while 11-day treatment is needed for 3rd instar larvae to achieve 100% mortality. The results of the statistical analysis showed significant differences during cold days in pupariation rates ($F = 380.335$, $P < 0.0005$) and adult emergence ($F = 320.710$, $P < 0.0005$). It was concluded that a longer duration of exposure to cold resulted in fewer opportunities for insect survival; in both blueberry varieties.

Based on emerged adults as survival indicators, fly eggs were the most susceptible stage to the cold treatment because nine-day treatment leads to 0 adults in both blueberry cultivars. The 3rd instar is the most tolerant stage in both cultivars, which required 11-day treatment to achieve 0 adults. There was a significantly negative relationship between exposure time to cold and survival emerged rates in eggs and larvae instars in both cultivars. The control mortality of emerged adults was below 5%; therefore, it was ignored (WHO, 2016).

There was no significant effect between blueberry cultivars on pupariation means among the four stages during all exposure times (Table 3.2), while there were significant effects on emerged adults ($F = 23.962$, $P < 0.0005$) (survival insects) (Table 3.2). Blueberry cultivar C00-09 had a significant impact on an increase of emerged adults in eggs and all larvae instar compared to cultivar C99-42. Therefore, the results showed that the survival rates in cultivar C00-09 were higher than those in the C99-42.

Table 3.2. pupariation rates and emerged adults of four immature stages of *C. capitata* after exposure to cold treatment (1.0±0.2°C) for 12 days in 2 western Australian blueberry cultivars (C00-09 and C99-42). SE means standard error.

Blueberry	Treatment (Days)	Egg		1st instar		2nd instar		3rd instar	
		pupariation (%) Mean (SE)	Emerged adult (%) Mean (SE)	pupariation (%) Mean (±SE)	Emerged adult (%) Mean (±SE)	pupariation (%) Mean (±SE)	Emerged adult (%) Mean (±SE)	pupariation (%) Mean (±SE)	Emerged adult (%) Mean (±SE)
C00-09	0	100(0)	98(0.2)	100(0)	99(0.1)	100(0)	99(0.1)	100(0)	100(0)
	1	52.6(0.4)	48.0(0.4)	59.2(0.3)	54.8(0.3)	74.8(0.3)	76.3(0.2)	82.7(0.4)	83.9(0.4)
	2	37.5(0.5)	32.8(0.6)	37.7(0.3)	30.8(0.4)	56.2(0.6)	58.0(0.6)	79.6(0.3)	763(0.3)
	3	37.5(0.5)	31.2(0.4)	29.6(0.6)	28.5(0.6)	56.2(07)	53.4(0.4)	75.9(0.4)	74.8(0.3)
	4	33.8(0.8)	26.7(0.5)	29.6(0.5)	24.8(0.6)	54.0(0.5)	48.4(0.5)	69.9(0.3)	68.7(0.3)
	5	27.0(0.5)	25.1(0.3)	26.6(0.8)	22.5(0.3)	51.8(0.7)	45.8(0.5)	62.4(0.9)	61.0(0.8)
	6	19.5(0.4)	17.5(0.4)	207.(0.6)	19.5(0.6)	48.1(0.6)	42.7(0.4)	58.6(0.4)	50.3(0.6)
	7	12.0(0.2)	9.9(0.2)	19.2(0.6)	17.2(0.5)	34.5(0.4)	29.0(0.4)	45.1(0.2)	42.7(0.3)
	8	4.5(0.2)	3.8(0.2)	17.0(0.5)	13.5(0.3)	19.7(0.4)	17.5(0.5)	37.5(0.3)	32.8(0.4)
	9	0	0	11.8(0.4)	4.5(0.2)	11.1(0.2)	6.1(0.1)	15.7(0.3)	15.2(0.2)
	10	0	0	0	0	0	0	9.7(0.4)	7.6(0.2)
	11	0	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0	0
C99-42	0	100(0)	99(0.1)	100(0)	100(0)	100(0)	98(0.2)	100(0)	99(0.1)
	1	49.6(0.3)	46.1(0.3)	58.6(0.6)	53.8(0.7)	70.4(0.3)	72.0(0.2)	81.4(0.3)	81.4(0.3)
	2	37.5(0.2)	33.0(0.3)	34.5(0.7)	30.7(0.9)	58.4(0.3)	56.(0.5)	78.5(0.6)	77.7(0.5)
	3	33.8(0.3)	27.6(0.2)	27.0(0.7)	25.3(0.7)	53.8(0.5)	48.8(0.6)	74.8(0.4)	76.3(0.3)
	4	30.0(0.5)	30.0(0.5)	26.3(0.7)	23.0(0.7)	52.3(0.4)	48.0(0.4)	66.6(0.3)	66.6(0.3)
	5	22.5(0.3)	19.2(0.2)	23.3(0.6)	21.5(0.6)	50.0(0.3)	48.0(0.2)	62.9(0.2)	56.2(0.2)
	6	17.2(0.5)	12.3(0.4)	19.5(0.8)	15.3(0.6)	46.1(0.2)	38.4(0.4)	52.5(0.4)	45.1(0.6)
	7	9.7(0.3)	6.1(0.1)	18.7(0.8)	13.8(0.6)	31.5(0.4)	33.3(0.2)	40.7(0.6)	37.0(0.5)
	8	4.5(0.2)	2.3(0.2)	15.0(0.3)	12.3(0.2)	20.0(0.3)	14.4(0.4)	33.3(0.2)	26.6(0.3)
	9	0	0	9.7(0.3)	4.6(0.5)	10(0.2)	4.8(0.2)	13.3(0.4)	11.8(0.4)
	10	0	0	0	0	0	0	8.1(0.3)	4.4(0.3)
	11	0	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0	0

3.4.6. Modeling analysis

When using the recovered pupariation as end-point for mortality modeling, the results appeared that the 3rd instar is the most tolerant stage with LT_{90}, LT_{95} and LT_{99} values being 10.3, 11.6 and 14.1 days respectively in cultivar C00-09, while 10.0, 11.3 and 13.8 days in cultivar C99-42 (Table 3.3). The 2nd instar larvae were the second most tolerant stage. Eggs were more susceptible (LT_{99} = 10.8 and 10.5 in cultivar C00-09 and cultivar C99-42, respectively) than larvae (Table 3.3). Interestingly, based on the emerged adult ratios in cultivar C00-09, LT_{99} was 10.7, 12.4, 12.7 and 13.7 days for eggs, 1st instar, 2nd instar and 3rd instar respectively, further confirming that 3rd instar was the most cold-tolerant stage (LT_{99} = 13.7). While in cultivar C99-42 LT_{99} values were 9.7, 12.0, 12.8 and 13.1 days for eggs, 1st instar, 2nd instar and 3rd instar respectively. Also, the results confirmed that the 3rd instar was the most cold-tolerant stage and the egg was the most susceptible (Table 3.3).

Table 3.3. Cold treatment duration to induce 90, 95 and 99% mortality of *C. capitata*.

Development stage	Blueberry cultivars	Mortality (LT)%	Pupae recovery as end point			Adults recovery as end point		
			Treatment (days)	95% Confidence Limits		Treatment (days)	95% Confidence Limits	
				Lower	Upper		Lower	Upper
Eggs	C00-09	90	6.7	6.0	7.7	6.3	5.8	6.9
		95	8.1	7.2	9.4	7.8	7.2	8.6
		99	10.8	9.5	12.9	10.7	9.7	11.9
	C99-42	90	6.4	5.9	7.0	5.7	5.3	6.2
		95	7.8	7.2	8.6	7.1	6.5	7.8
		99	10.5	9.6	11.7	9.7	8.8	10.9
1st	C00-09	90	8.0	6.9	9.6	7.1	6.2	8.5
		95	9.8	8.5	12.1	9.0	7.8	10.9
		99	13.3	11.3	17.0	12.4	10.6	15.6
	C99-42	90	7.6	6.6	9.2	6.9	6.0	8.8
		95	9.5	8.2	11.7	8.6	7.6	10.3
		99	13.0	11.0	16.6	12.0	10.3	14.7
2nd	C00-09	90	9.1	8.0	11.1	8.7	7.7	10.1
		95	10.7	9.2	13.2	10.1	8.9	11.9
		99	13.6	11.5	17.2	12.7	11.1	15.3
	C99-42	90	9.2	8.0	10.9	8.7	7.6	10.3
		95	10.7	9.3	12.9	10.1	8.8	12.2
		99	13.4	11.5	16.7	12.8	10.9	15.9
3rd	C00-09	90	10.3	9.3	11.8	10.0	9.1	11.2
		95	11.6	10.4	13.5	11.3	10.3	12.8
		99	14.1	12.5	16.9	13.7	12.3	15.8
	C99-42	90	10.0	9.1	11.4	9.5	8.8	10.5
		95	11.3	10.2	13.0	10.7	9.8	11.9
		99	13.8	12.3	16.1	13.1	11.9	14.7

3.4.7. Sex ratios

To determine, if the cold treatment affected the sex development of the emerged adults, the sex ratios of the treated eggs and larval instars were compared. There were no significant differences at each stage in both blueberry cultivars, which were all close to 50% (Figure 3.1). From Day 0 to Day three, there were no significant differences between eggs, 1st, 2nd and 3rd larval instars on each daily treatment (Figure 3.S1).

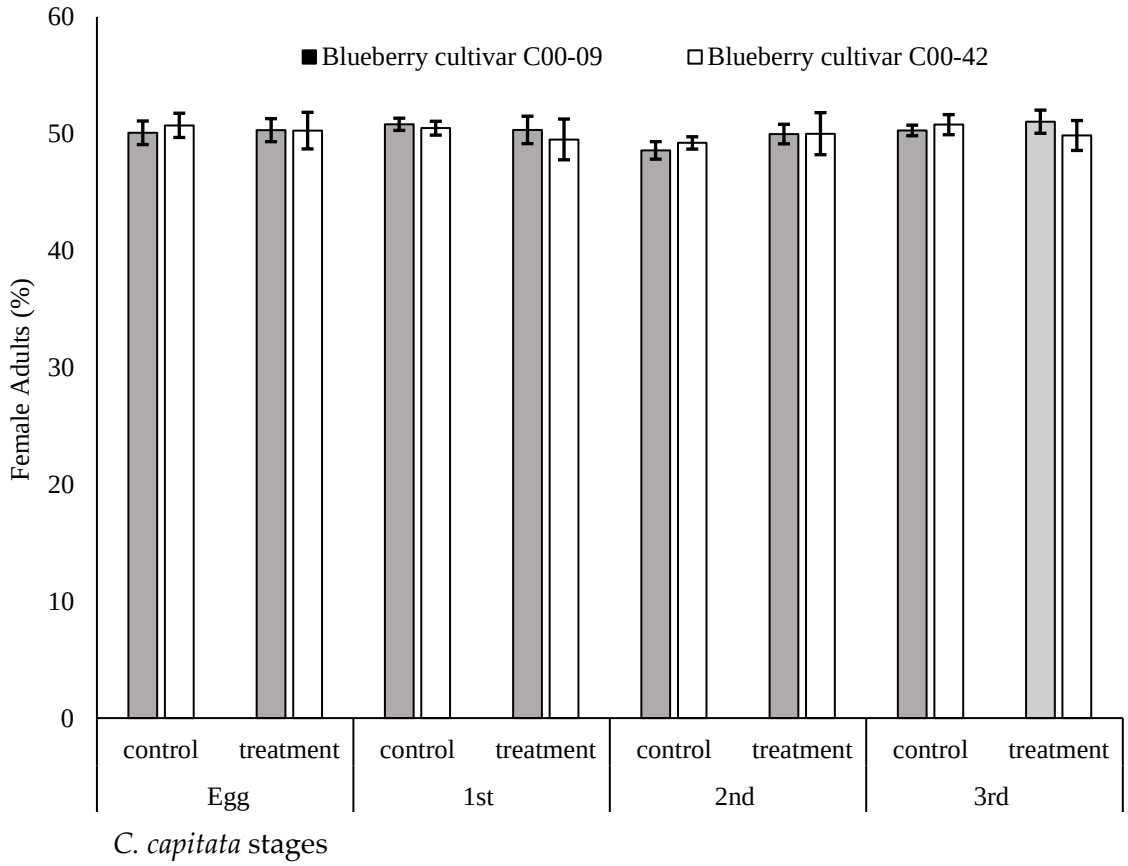


Figure 3.1. The average female adult ratios of survived adults for each stage in blueberry cultivar C00-09 and C99-42 after cold treatment. Error bar means standard error. An ANOVA single factor test was used to compare sex ratios, $p > 0.05$.

3.5. Discussion

The life history study results were consistent with a previous study in which, 30 hosts (including the blueberry) were used to analyze the life history of *C. capitata* at 30.0°C, RH 65±10% and 12:12 L:D (Krainacker et al., 1987).

The 3rd instar larvae showed a higher survival rate than the other stages. A previous study of cold susceptibility and disinfestation of *Bactrocera invadens* (syn. *B. dorsadis*) (Dew 2003) in oranges showed that the 1st instar is more susceptible than 2nd and 3rd (Grout et al., 2011). However, this result disagreed with another study on *Bactrocera tryoni* (Froggatt 1897) in cold storage (1.0°C for 12 days) in three cultivars of Australian blueberry, in which the 1st instar was the most cold-tolerant stage (1.0°C for 12 days) (Jessup et al., 1998). This current study results also disagree with a study on *C. capitata* and *B. tryoni* in five Australian citruses (*Citrus* spp.) in which the 2nd instar was reported the most cold-tolerant stage at 2.0 and 3.0°C (De Lima et al., 2007). Hallman et al. (2011) reported that the 3rd instar *C. capitata* is the most cold-tolerant stage (1.5±0.5°C) when eggs and 1st, 2nd and 3rd larvae were tested in oranges (Hallman et al., 2011). In comparison with a previous study, when the *C. capitata* treated at 0.0°C with different exposure durations (0–12 days) and fed on a lab diet; the eggs were the more susceptible immature stage, while the 1st instar and 3rd instar had the highest survival rates (Al-Behadili et al., 2019). All these studies demonstrated that different fruits fly species or temperatures may show different tolerance responses during cold treatment, which should be studied case by case.

Eleven-day cold treatment is enough to get no survival from any of the four Medfly stages (eggs and larvae instars) in both blueberry cultivars at 1.0±0.2°C. Results were similar to those of Mason et al. (1934), who confirmed that 11 days were enough to kill the four stages of Medfly at 1.0±0.5°C (Mason and McBride, 1934). These results disagreed with those of Hill (1990), who found that after 16 days of exposure to 0.0-1.5°C there was no insects survival in disinfection of *C. capitata* in oranges (Hill et al., 1988). The difference in fruit may affect the results of the same phytosanitary treatment (Hallman et al., 2019).

It was shown there was no significant effect of cold treatment on sex ratios among the four stages of *C. capitata* throughout all exposure time. Similar results have been reported by

Maurizio et al. (2019) on Queensland fruit fly *B. tryoni* (eggs and larvae) (Maurizio et al., 2019).

To understand the influences of blueberry cultivars on survival of Medfly during cold treatment, pupariation rates, emerged adults and sex ratio were used as indicators. There were no significant effects of varieties on pupariation means (Table 3.2). Where in both cultivars the order of survival stages was similar, the survival was progressive from the lowest (eggs) to the highest (3rd instar). The time required to eradicate each stage also was the same in both cultivars, where nine days was enough to eradicate eggs, ten days for 1st instar and 2nd instar, and 11 days for 3rd instar, respectively. These results agreed with the results from previous studies, where the blueberry cultivars had no effect of 1st instar survival of *B. tryoni* at 1.0 °C. In their experiment, three cultivars, Premier blueberries, Climax blueberries and Sharpe Blue blueberries were used. In all these cultivars, the time required to ensure no survival of 1st instar was ten days (Jessup et al., 1998). Further, they found that the eradication time of 2nd instar and 3rd instar of *B. tryoni* at 1.0°C was different in some blueberry cultivars. Jessup et al. (1998), reported that the Premier blueberries and Sharpe Blue blueberries have the same effect on eradicating time of 2nd and 3rd instars where they found that eight days of exposure to cold were enough to ensure no survival insect while Climax blueberries increased the eradication time of 2nd and 3rd instars to ten days.

Interestingly, results of this research indicated that there was a significant impact of blueberry cultivars on emerged adult rates although there were no significant differences in biochemical quality such as sugar, acid and their ratio between the two cultivars. It is possibly attributed to the significant difference in the water content, which was higher in the cultivar C00-09 (92.7) than in C99-42 (79.4), as shown in table 3.1. All living organisms require available water to grow and function (Jordan and Tomberlin, 2017). For insects, the water content of the blueberry affected the consumption of diet (Vanderzant, 1969).

There were significant differences in certain physical qualities, including weight, diameter and blue light density (Table 3.1). In cultivar C00-09, the larger weight and diameter provided a larger amount of food and area for insects comparing to cultivar C99-42. The reflection intensity of blue light in cultivar C00-09 was higher than cultivar C99-42. These results were consistent with those by Shibuya et al. (2018). They found blue light (Short-

wavelength visible light 400–500 nm) has affected the survival of fruit fly *Drosophila melanogaster* and increased mortality of eggs and larvae stage (Shibuya et al., 2018).

3.6. Conclusions

In this study, we provided the first evidence of the Medfly responses to the cold treatment at 1°C in two blueberry cultivars. Eleven days of exposing *C. capitata* to 1°C in both blueberry cultivars was enough to achieve 0 alive insects. The 3rd instar was the most cold-tolerant stage of *C. capitata* in blueberries. This small-scale treatment study is a preliminary investigation for Medfly cold treatment in blueberries, which will help develop or refine the large-scale cold treatment of Medfly for the blueberry trade-in industry. This study also provided information for the cold treatment of *C. capitata* in other fruits, especially small fruits such as strawberries, raspberries, blackberries and grapes.

3.7. Supplementary Materials

Table 3.S1. Life history of immature stages of *C. capitata* in blueberry cultivar C00-42 and cultivar C00-09. Table 3.S2. Test of normality according to Kolmogorov-Smirnov value and Shapiro-Wilk, the significant was > 0.05 in all *C. capitata* tests, which mean all data follow the normal distribution. Figure 3.S1. The average female adult ratios of survived adults on each day treatment of immature stages in blueberry cultivar C00-09 and C99-42. Error bar means standard error. An ANOVA single factor test was used to compare sex ratios, $p > 0.05$. Table 3.S3. ANOVA table of natural infestation of *C. capitata* to blueberry cultivar C00-42 and cultivar C00-09 with four exposure times.

Table 3.S1. Life history of immature stages of *C. capita* in blueberry cultivar C00-42 and cultivar C00-09.

Time	Cultivar C00-42								Cultivar C00-09						
	Eggs	1st		2nd		3rd		pupae	Eggs	1st		2nd		3rd		pupae
		alive	dead	alive	dead	alive	dead			alive	dead	alive	dead	alive	dead	
D1	95	0	0	0	0	0	0	0	126	0	0	0	0	0	0	0
D2	121	0	0	0	0	0	0	0	103	0	0	0	0	0	0	0
D3	33	124	1	0	0	0	0	0	52	124	0	0	0	0	0	0
D4	14	64	0	0	0	0	0	0	22	83	0	0	0	0	0	0
D5	13	13	3	118	0	0	0	0	33	24	4	139	0	0	0	0
D6	18	0	3	90	0	0	0	0	30	3	5	13	9	0	0	0
D7	14	1	3	55	12	51	0	0	21	1	2	36	0	24	0	0
D8	10	0	6	11	13	38	0	0	10	1	6	51	4	38	2	0
D9	12	0	1	7	1	57	0	0	1	1	0	18	31	63	0	0
D10	7	0	0	9	0	42	0	1	19	0	0	1	1	38	0	5
D11	7	0	0	6	0	42	0	7	4	0	1	2	47	16	14	5
D12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
∑	344	202	17	296	26	230	0	8	421	237	18	260	92	179	16	10
	E.H.P		1st.D. P		2nd.D. P		3rd. D. P			E.H. P	1st. D. P		2nd.D. P		3rd. D. P	
Percent	63.66279	0	8.41584	0	8.78378	0	0	0		60.57007	7.59494		35.38462		8.93855	

E.H.P is eggs hatching percent.

1st D.P, 2nd D.P and 3rd D.P are 1st ,2nd and 3rd larvae instar death percent (%).

Table 3.S2. Test of normality according to Kolmogorov-Smirnov value and Shapiro-Wilk, the significant was >0.05 in all *C. capitata* tests, which mean all data follow the normal distribution.

Blueberry cultivar	***C. capitata* stages**	**Kolmogorov-Smirnov (sig)**	**Shapiro-Wilk (sig)**	**Development stage**
C00-09	Eggs	0.20	0.08	Pupation
	1st instar	0.20	0.34	Pupation
	2nd instar	0.18	0.11	Pupation
	3rd instar	0.20	0.12	Pupation
C00-42	Eggs	0.20	0.09	Pupation
	1st instar	0.20	0.20	Pupation
	2nd instar	0.20	0.12	Pupation
	3rd instar	0.20	0.12	Pupation
C00-09	Eggs	0.20	0.09	Emerged adult
	1st instar	0.20	0.19	Emerged adult
	2nd instar	0.22	0.21	Emerged adult
	3rd instar	0.20	0.21	Emerged adult
C00-42	Eggs	0.20	0.06	Emerged adult
	1st instar	0.20	0.14	Emerged adult
	2nd instar	0.20	0.12	Emerged adult
	3rd instar	0.20	0.16	Emerged adult

Table 3.S3. ANOVA table of natural infestation of *Ceratitis. capitata* to blueberry cultivar C00-42 and cultivar C00-09 with four exposition times.

R Squared = .998 (Adjusted R Squared = .997)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	653138.539	7	93305.506	21802.169	.000
Intercept	761395.315	1	761395.315	177910.934	.000
cultivars	7.878	1	7.878	1.841	.176
time	653066.904	3	217688.968	50866.149	.000
cultivar * time	63.758	3	21.253	4.966	.002
Error	1609.146	376	4.280		
Total	1416143.000	384			
Corrected Total	654747.685	383			

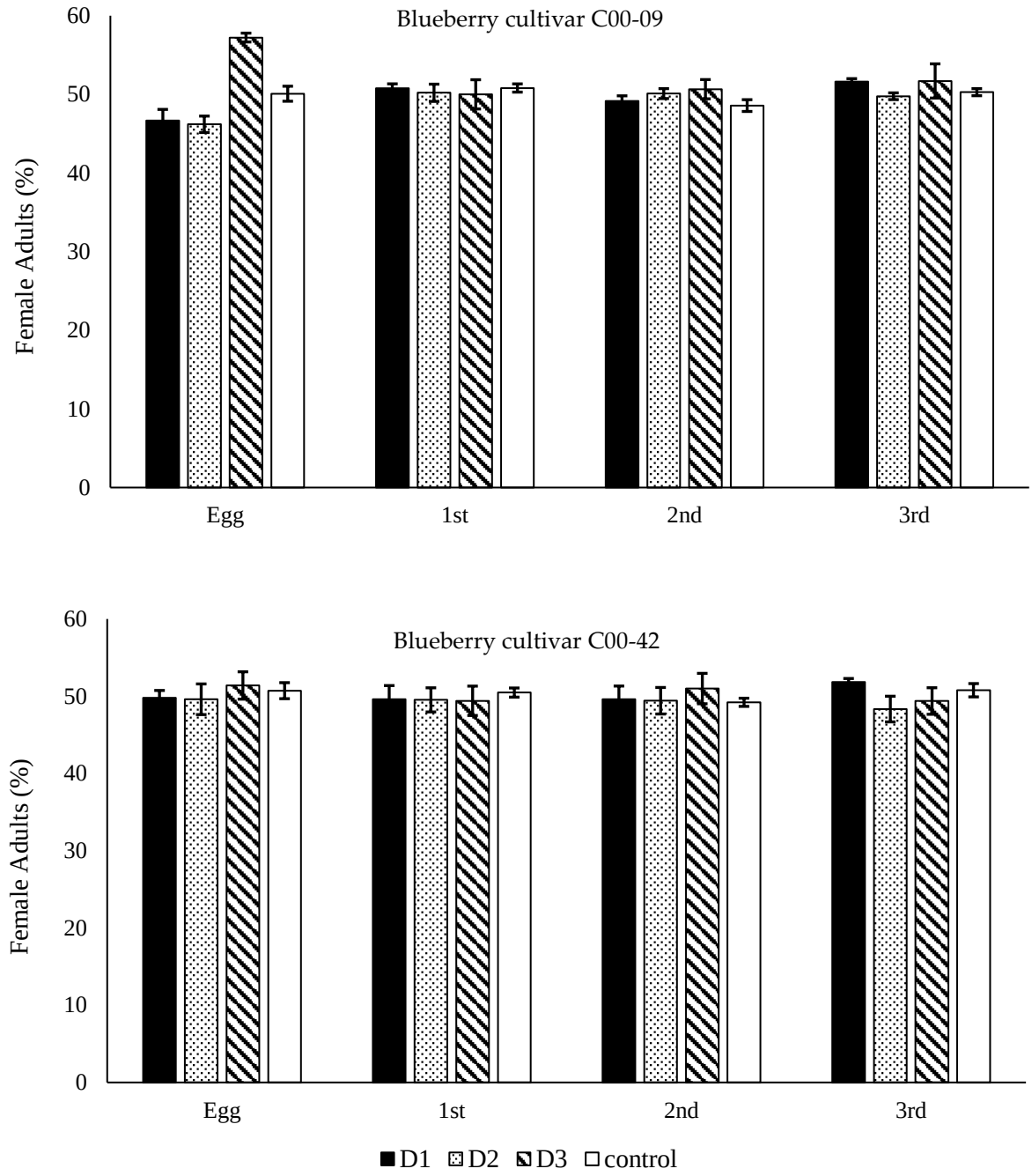


Figure 3.S1. The average female adult ratios of survived adults on each day treatment of immature stages in blueberry cultivar C00-09 and C99-42. Error bar means standard error. An ANOVA single factor test was used to compare sex ratios, $p > 0.05$.

Statement of Contribution

Title of Paper	Mediterranean fruit fly *Ceratitis capitata* (Diptera: Tephritidae) eggs and larvae responses to a low-oxygen/high-nitrogen atmosphere
Publication Status	In press
Publication Details	Insects Journal
Principal Author	
Name of Principal Author (Candidate)	Farhan J.M. Al-Behadili
Contribution to the Paper	Methodology, Collected Data, Writing- Original Draft Preparation, Conceptualization and Validation
Overall Percentage (100%)	85 %

Signature

Date 17/10 /2020

Co-Author Contributions

By signing the statement of contribution, each author certifies that:

- The candidate's stated contribution to the publication is accurate (as detailed above).
- Permission is granted for the candidate to include the publication in the thesis
- The sum of all co-author contributions is equal to 100% less the candidate's stated Contribution

Name of Co-Author	Manjree Agarwal
Contribution to the Paper	Supervision
Overall Percentage (100%)	5 %

Signature

Date 17 /10 /2020

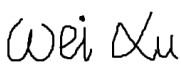
Name of Co-Author	Yonglin Ren
Contribution to the Paper	Supervision, Funding acquisition
Overall Percentage (100%)	5 %

Signature

Date 17/10/2020

Name of Co-Author	Wei Xu
Contribution to the Paper	Supervision, Funding acquisition, Validation, Conceptualization
Overall Percentage (100%)	5 %

Signature



Date 17 /10/2020

Chapter four

Mediterranean fruit fly *Ceratitis capitata* (Diptera: Tephritidae) eggs and larvae responses to a low-oxygen/high-nitrogen atmosphere

4.1. Abstract

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), is one of the most damaging horticultural insect pests. This study used a low-oxygen/high-nitrogen bioassay to control *C. capitata*. Two low-oxygen treatments were applied (0.5% O_2 + 99.5 N_2 and 5% O_2 + 95% N_2) to *C. capitata* eggs, 1st, 2nd and 3rd instar larvae from 0 to nine days on a carrot diet at 25°C; 70-75% RH. The pupariation, adult emergence, and sex ratios of survived flies were examined. The results demonstrated increased mortality of all tested life stages correlated with increased exposure times at both levels of low-oxygen treatments. Complete control of eggs was achieved after eight days and nine days for larvae using 0.5% O_2 at 25°C; 70-75% RH. The 3rd instar was the most tolerant stage, while the egg was the most susceptible stage to the low-oxygen environment. There were no significant differences in sex ratios between emerged adults after low-oxygen and control treatments. This study demonstrates and confirms the mortalities of *C. capitata* caused by low-oxygen treatment, which may help develop new postharvest strategies to control this destructive fruit fly pest.

4.2. Introduction

Globally, one of the most damaging horticultural insect pests is the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) (Thomas et al., 2001). *C. capitata* has been recorded feeding on over 300 host plants. After mating, one female adult can lay as many as 800 eggs during its lifetime (Woods et al., 2005). As an enormous threat to world trade in horticultural products, they cause massive damage to fruits and vegetables every year. Consequently, it is a world-wide biosecurity threat (White and Elson-Harris 1992, Malacrida, et al., 2007).

In recent years, strict government biosecurity policies have been implemented to minimize the risks and damage *C. capitata* causes. Pre-harvest actions include sterile insect technique (SIT), insecticides, monitoring and inspections, and postharvest treatments such as

fumigation, low-oxygen, irradiation, heat or cold treatments have been widely applied to control fruit fly pest species. Due to public concerns on chemical residues, insect resistance and environmental pollution, non-chemical techniques have become more and more preferred in postharvest control (Broughton and De Lima, 2002; Al-Behadili, et al. 2019). Controlled atmosphere using carbon dioxide, oxygen, and/or nitrogen, together with controlled temperature and humidity, have been used for postharvest treatments against various fruit flies (Villers et al., 2008; Mditshwa et al., 2018).

Oxygen plays a vital role in growth and development of organisms or animals (Frazier et al., 2001). A previous study showed that low-oxygen affects the developmental rate of *Drosophila melanogaster* (Kloek, 1979). Reducing oxygen concentration is one of the methods that has been increasingly used to control postharvest pests at room temperature (De Lima, 1990; Chiappini et al., 2009). Low-oxygen treatment to control fruit fly is one of the most promising options for the horticulture industry because low oxygen can kill insects while improving the quality of some fruits (Agnello et al., 2002). For example, prevention of surface browning caused by the enzyme polyphenol oxidase (Gorny, 1997), increasing the shelf life of plant products by reducing their respiration rates and slowing the use of the finite energy supplies that are available in any living tissue (Herner, 1987), and maintaining edible quality, firmness, soluble solids and acidity (Chen et al., 1985). Furthermore, low-oxygen treatment can slow down metabolic processes, senescence, and conversion of starch, hinder fruit ripening, suppress ethanol production, inhibit certain storage disorders and decrease the growth of decay organisms (Mathooko, 1996; Ke and Kader, 1992). In general, lower survival rates of insect pests can be achieved at a lower oxygen level, whenever the temperature rises above 25ºC (Cao et al., 2019).

Previous studies on insect control in low-oxygen treatment have focused on CO_2 instead of N_2 (Lehmann et al., 2000). However, there have been concerns about using CO_2 because it is a greenhouse gas and causes global warming (Jacobson, 2009). Moreover, CO_2 can react with water to produce carbonic acid depending on temperature (generally at 25ºC), while nitrogen does not (Steiner, 1993). In addition, high levels of CO_2 can also damage fruit quality, including appearance (browning, impair the skin) and the nutritional value (enhance fruit ripening and off-flavor) (Smilanick and Fouse, 1989; Yahia et al.; 1992). In postharvest treatment, when using a low-oxygen method with elevated N_2 to control insects, the fruit

quality is acceptable (Nishizawa et al., 2002). Therefore, more and more attention has been paid to using N_2 to replace CO_2 in the low-oxygen treatment (Kehat and Dunkelblum, 1990; Yahia, 1998). The high-nitrogen and low-oxygen atmosphere have proven to be environmentally friendly, safe for fruit quality and workers and an effective method for treating insect pests (Hanlon et al., 1992).

Many studies confirmed that there is no anaerobic respiration within a controlled atmosphere. It has less decay in stored fruit quality than at room temperature with low O_2 (< 1%) levels compared to those at average O_2 (20.9) because 1% O_2 level inhibits the growth of most bacteria and molds which cause anaerobic respiration (Burg, 2004).

The atmosphere associated with anaerobic paths due to low oxygen has a significant role in the occurrence of anaerobic respiration. Nishizawa et al. (2002) confirmed that fruit decay did not occur under anaerobic N_2 atmospheres and low oxygen concentration. Furthermore, anaerobic nitrogen atmospheres resulted in inhibition of depolymerization of polyuronides and non-cellulosic neutral sugars in the cell walls, high flesh firmness and low ethylene production when they exposed "Andesu" netted melon fruit to low oxygen concentration with pure nitrogen for 12 days.

In this study, we examined low-oxygen/high-nitrogen treatments on *C. capitata* eggs and larvae on a carrot diet, to understand its responses to low- oxygen. An artificial diet was chosen, as different fruits vary in size, composition, nutrients and chemicals, which can influence the outcomes of the treatment, fly development and infestation methods (Hallman 2014). Therefore, the use of an artificial diet minimizes these risks and allows for the focus on fly mortalities in the low-oxygen environment.

4.3. Materials and Methods

4.3.1. Insect culture

Ceratitis capitata used in this study was established in 2015 from a laboratory colony preserved at the Department of Primary Industries and Regional Development (DPIRD) in Western Australia. It was reared in the National Centre for Postharvest Disinfestation Research on Mediterranean Fruit Fly at Murdoch University (Perth, Western Australia). The

flies were reared in a temperature-controlled cabinet at 26.1.0±1.0°C, 60-65% RH and darkness light cycle of 16:8 hrs (D:N).

For the artificial diet, 1 kg dehydrated dried carrot (Spices Australia - Herbs & Spices, Dehydrated Food-Ballina, NSW-Australia) was soaked in 5.0 litres of hot water for 15 minutes and homogenised with 330 g Torula Yeast (Lotus - organic foods store- Perth, Western Australia), 30 g methyl propyl hydroxybenzoate (Nipagin) and 33 mL hydrochloric acid (32%, w/w). Approximately 20 kg of the artificial diet was stored in 4 × 5 L containers (10 cm × 20 cm × 25 cm) (Tanaka et al., 1969).

The eggs were then placed on the diet in a plastic tray (20 × 15 × 3 cm^3), which was moved to vermiculite filled plastic tray with 1 cm depth of medium vermiculite size at the bottom), covered with mesh cloth and transferred to an incubator at 25.0±1.0°C; 70-75% RH until the larvae start to develop into pupae. By sieving the vermiculite, pupae were collected and transferred to screen cages (30 cm^3 in size) until they developed to adults. Adults were fed with crystalline sugar (Bidvest, Australia), yeast hydrolysate (Australian Biosearch) and water. Each substance was placed in a container separately. The adult females laid their eggs on the sides of the screen cages and those that fell into the water tray adjacent to the cage were collected each day for preparing *C. capitata* required for treatments (AL-Khshemawee et al., 2017). To prepare 1^{st}, 2^{nd} and 3^{rd} larval instars, different groups of eggs were placed on a carrot diet and incubated at 25.0±1.0°C; 70-75% RH for different periods (three days for 1^{st}, five days for 2^{nd} and eight days for 3^{rd} instar). For greater accuracy in the preparation of the larvae, after incubation, a magnifying glass (5 x) was used to check the morphological characteristics of each larva to confirm its instar (Frías et al., 2006). After verification of each instar, the required number was picked by 5 mL plastic dropper and counted using a manual cell counter.

4.3.2. Low-oxygen treatment facility

The experimental equipment was installed in a laboratory (25.0±1.0°C**;** 35- 45% RH) at Murdoch University, Murdoch, WA 6150, Australia. A 2-L glass desiccator (Sigma-Aldrich, America) was used for the low-oxygen treatment of *C. capitata* eggs and different instars. The total volume of the desiccator with lid was 2.610 L. Through the rubber stopper, a plastic tube (5 mm ID clear vinyl non-toxic PVC tubing – 5 m. I/N: 3130558, conforming to

AS/NZS 2070:1999 for food contact applications) was inserted into the bottom of the desiccator (Figure 4.1). The air and nitrogen (cylinders G Size, Brand BOC, Industrial Grade, purity >99.5%) flowed through regulators, and tubes to a gas system device (Shimadzu GC-9A, American subsidiary). The device has gauges, nipples and airflow meters to mix, adjust and monitor the gas concentrations. The desired concentration (0.5% or 5.0%, v/v) of oxygen and nitrogen (99.5% v/v) (mixture) were adjusted by the nipples. The gas mixture (O_2+N_2) exited from the gas system through one tube to the desiccator, where it was bubbled through 500 mL distilled water (Figure 4.1).

Once the desiccator was saturated with the desired O_2 concentration (0.5% or 5.0%), the gas flow was maintained for the duration of the experiment and flowed through an outlet port. A Witt OXYBABY® 6.0 (WIT-Gasetechnik GmbH and Co KG T, Germany) was used to monitor levels of oxygen and nitrogen (gas mixture) in the desiccators which were placed in an incubator. The O_2 and N_2 levels were monitored daily three times with the OXYBABY to avoid any changes in the Shimadzu GC-9A device. The distilled water was added to the desiccator to maintain the humidity inside the desiccator. The volume (500 mL) of distilled water was already calibrated with the gas mixture flow rates, desiccator volume and exposure time. When a monitoring value was observed it changed from the desired system setup, thenwas immediately rectified accordingly. The temperatures and relative humidities inside of desiccator were recorded every 30 minutes by HOBO® data logger units (Model number H08-004-02, Onset Computer Corporation, MA 02532, USA, www.onsetcomp.com). The HOBO® units had previously been calibrated against a standardised mercury glass thermometer for temperature, and with a range of glycerol/water solutions for relative humidity.

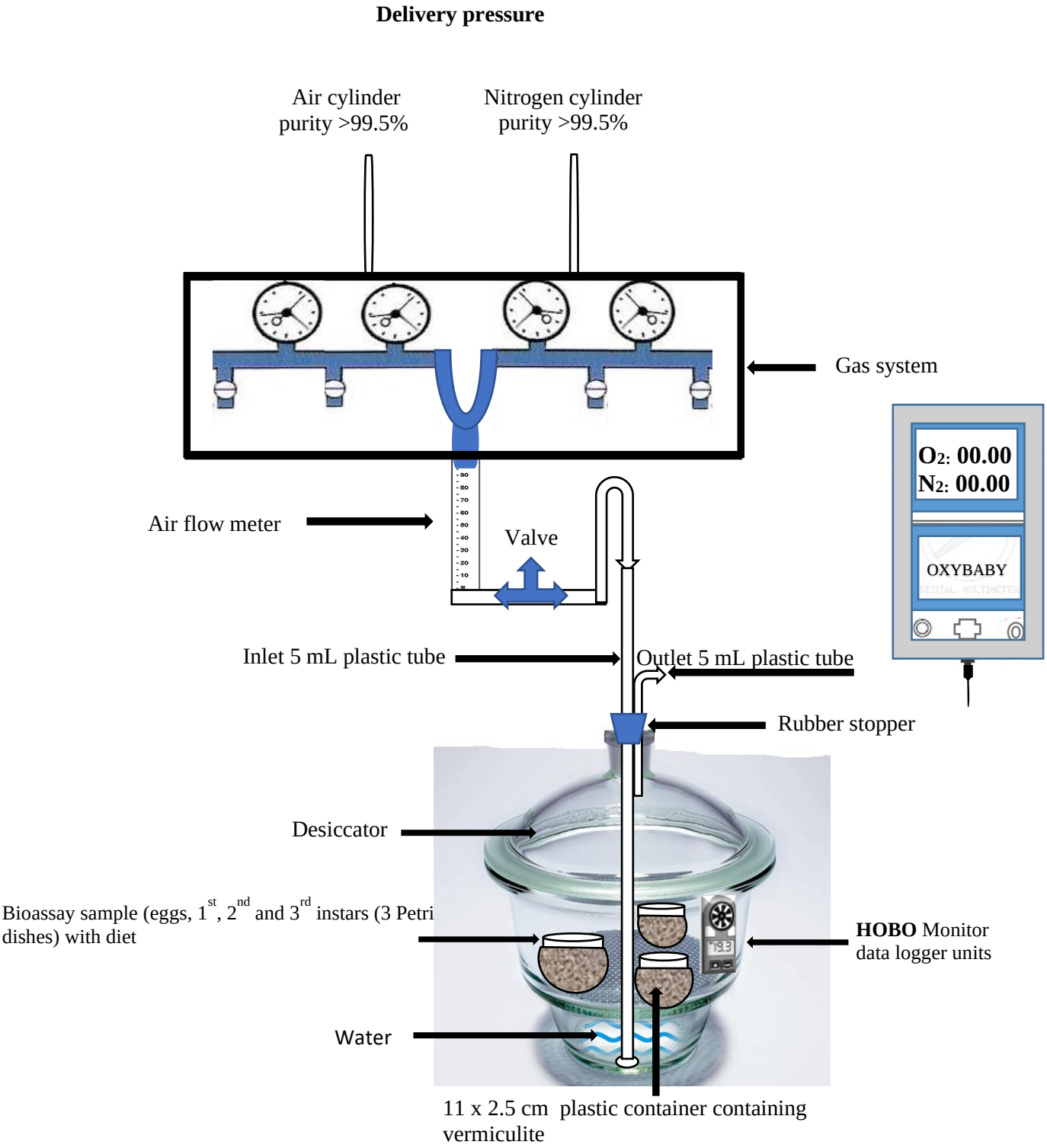


Figure 4.1. Schematics of low-oxygen treatment experimental setup.

4.3.3. Low-oxygen treatment

The prepared diet (55 g) was placed in sterile 9 cm × 10 mm plastic Petri dishes. Eggs were collected from the Medfly colony using a plastic pipette. One hundred 24-hr-old eggs were placed on the diet surface in each Petri dish. For each low-oxygen treatment (0.5% and 5.0%), a total of 27 Petri dishes were prepared, triplicates (including control) for the nine days of exposure time, the total number of Petri dishes are shown in Table 4.S1. Each prepared petri dish was transferred to a plastic container filled with vermiculite (11 cm diameter and 2.5 cm depth) the container, in turn, was covered with a piece of cloth (mesh) and fixed with a rubber band to prevent the escape of larvae. The three replicate plastic containers containing eggs, 1^{st}, 2^{nd} and 3^{rd} instar larvae were placed into each 2-liter treatment desiccator (9 desiccators for each treatment and 1 for control), and transferred into the incubator (model HWS, LET code 0574-88000432, Tianjin- China) set at 25.0±1.0°C and 70-75% RH. The low-oxygen treatments (0.5% and 5.0%) were applied to each stage of *C. capitata* and each replicate included 100 individuals of each insect stage. Gas cylinders placed in the laboratory controlled by an air conditioner, which has an auto sensor set up at 25.0ºC (Acson, AWM-GWRCM-DW/EW ACK-AW/CW ACC-CW ADB-BW, ISO 5151, the temperature setting range is from 16°C to 30°C, Malaysia). The time required to obtain the desired oxygen concentration from the gas mixture (O_2+N_2) into the desiccators was four hours at a flow rate of 0.6 cubic foot/hour. The gases from the N_2 and O_2 cylinders were released into the gas system (Shimadzu GC-9A) then to the desiccators, as shown in Figure 4.1. The O_2 and N_2 concentrations in control were 20.9% and 78.09%, respectively and monitored through out, which were regulated at constant rates using the same methods and instruments. One difference between the treatment desiccators and the control desiccator was that an air cylinder was joined to the gas system to maintain the control samples with air only. After the required O_2 concentrations (0.5% or 5.0%) and planned exposure times were achieved, the Petri dishes were removed from desiccators and placed in the incubator at ambient environmental conditions until they became pupae. The pupae were counted and transferred to sterile 9 mm × 50 mm plastic Petri dishes until they emerged as adults, and counted. The emerged adults were investigated to determine if the low-oxygen treatment leads to unbalanced sex ratios of the surviving flies. A total of 24,000 eggs and larvae for 0.5% O_2 and 5.0% O_2 (3,000 for each stage of egg, 1^{st}, 2^{nd} and 3^{rd} instar) were used through the 0-9 days of exposure to low-oxygen treatment Table 4.S1.

4.3.4. Statistical analysis

Pupariation and adult emergence rates from control fly groups were used to normalize the pupariation and adult emergence rate from treated fly groups according to Schneider-Orelli's formula (Püntener, 1981).

The mortality rate of the insect under low-oxygen treatment was statistically estimated following the lethal time method (LT). Pupariation and emerged adult ratios (%) were utilised to calculate the LT. If a treated fly, egg or larva successfully developed into a pupa or an adult, it was considered as a survived fly. If not, it was counted as a dead fly. Some data of samples were not subject to the normal distribution; therefore, two different models were used on the low-oxygen treatment of eggs, 1st instar, 2nd instar and 3rd instar larvae. They are probit model on log-transformed treatment days and (Eq. 1) logit model on log-transformed treatment days. The LT value was (50, 90 and 99%) estimated under a generalized linear model with both probit and a logit link function on low-oxygen treatment days. The model is written as:

$$\eta = \beta 0 + \beta 1 * \chi \qquad \text{Eq. 1}$$

Where η is the response or proportion mortality, $\beta 1$ is the coefficient of the dose, $\beta 0$ is the intercept and χ is the dose. The best model was selected based on the Chi-square value. The smaller values of Chi-square, the less values of residual will be (residual is the difference between observed values and expected values). The 50, 90 and 99% mortality ($LT_{50,\ 90\ and\ 99}$) were estimated by using the selected models. A 95% confidence interval was reported. Statistical Package for the Social Sciences (SPSS, IBM version 24 Armonk, New York, America) was used to analyse the dose-response data. Experiments were analysed using one-way ANOVA, followed by Tukey's honestly significant difference (HSD).

4.4. Results

Pupariation and emerged adult rates after treatment under both low-oxygen concentrations (0.5% and 5.0%) were significantly lower than the control for all tested stages (Figures 4.2 and 4.3). At 0.5% O_2 treatment, eight-day treatment on eggs lead to 0.0 pupae (Figure 4.2). Nine-day treatment on larvae (including 1st, 2nd and 3rd instars) resulted in no pupae. However, nine-day treatment under 5.0% O_2 resulted in 9.3, 15.3, 42.6 and 59.0%

pupariation ratios for eggs, 1st, 2nd and 3rd instar larvae, respectively (Figure 4.2). This result showed that 0.5% O_2 treatment at 25.0ºC for nine days offers complete control of all immature Medfly stages than at 5.0% O_2 (Figure 4.2). The pupariation rates decreased with increasing exposure time at low O_2 treatment. After the treatment, the highest pupariation rate was always recorded from the 3rd instar larvae while the lowest was from the eggs (Figure 4.2), indicating that the 3rd instar was the most tolerant stage while the egg was the most susceptible stage at the low-oxygen level tested in this study.

When using pupariation as the endpoint for mortality analysis, the probit model was a better model for eggs, 1st, 2nd and 3rd instar under 0.5% O_2 treatments and eggs, 2nd and 3rd instar under 5.0% O_2 treatments where the values of the chi-square with probit model were less than in the logit model. For 1st instar larvae under 5.0% O_2 treatments, the logit model was the better model. The modeled results demonstrated that the 3rd instar was the most tolerant stage with $LT_{50,\ 90\ and\ 99}$ values being 3.8, 7.0 and 10.4 days, respectively under 0.5% O_2 treatment, while 9.5, 16.8 and 22.8 days under 5.0% O_2 treatment (Table 4.1). Similar results were observed in the bioassay that the 3rd instar mortality was always the lowest in both O_2 concentrations and during all exposure times (Table 4.1). The 2nd instar was the second most tolerant stage. Eggs were more susceptible (LT_{99} = 8.6) than larvae and their mortality was the highest (Table 4.1). The results of the emerged adults followed the same pattern as the pupariation , but at a lower rate, as is evident in figure 4.3.

When using adult emergence as the endpoint for mortality analysis, the logit model was a better model for eggs, 1st and 3rd instar under 0.5% O_2 treatments, while the probit model was a better model than logit for the 2nd instar stage. The probit model was a better model for all four stages under 5.0% O_2 treatment. We modeled the duration of low-oxygen treatment to induce 50, 90 and 99% mortality at four immature stages of *C. capitata* fed on a lab diet by using selected models. The modelled results revealed that under 0.5% O_2 treatments, LT_{99} was 8.5, 8.8, 9.5 and 9.6 days for eggs, 1st instar, 2nd instar and 3rd instar, respectively (Table 4.1), further confirming ($F = 349.334$, $P < 0.0005$, CL= 95%) that 3rd instar was the most low-oxygen tolerant stage (LT_{99}=9.6) (Table 4.1). The second is the 2nd instar (LT_{99}=9.5) (Table 4.1), while 1st instar was the third (LT_{99}=8.8) (Table 4.1). The egg was the most susceptible stage to low-oxygen treatment (LT_{99}=8.5 and 14.3, respectively) (Table 4.1)

Table 4.1. Low-oxygen treatment duration to induce 50, 90 and 99% mortality based on pupariation and emerged adults from four developmental stages of *C. capitata*.

Treatment	stage	Mortality based on Pupariation			Mortality based on emerged adults		
		LT_{50} (95% *CL)	LT_{90} (95% CL)	LT_{99} (95% CL)	LT_{50} (95% CL)	LT_{90} (95% CL)	LT_{99} (95% CL)
0.5% O_2	Egg	2.8 (2.6-3.1)	6.0 (5.6-6.4)	8.6 (8.0-9.3)	2.1 (1.3-2.8)	5.2 (4.3-6.6)	8.5 (6.9-11.5)
	1^{st}	2.8 (2.3-3.3)	6.5 (5.9-7.4)	9.5 (8.5-11.1)	2.6 (2.1-3.0)	6.0 (5.4-6.8)	8.8 (7.8-10.2)
	2^{nd}	3.2 (2.9-3.4)	6.7 (6.3-7.1)	9.5 (8.9-10.3)	3.3 (3.0-3.5)	6.7 (6.3-7.1)	9.5 (8.9-10.2)
	3^{rd}	3.8 (3.5-4.0)	7.0 (6.6-7.4)	10.4 (9.7-11.3)	3.7 (3.5-4.0)	6.9 (6.6-7.4)	9.6 (9.0-10.2)
5.0% O_2	Egg	5.2 (4.6-5.7)	9.5 (8.5-10.9)	14.1 (12.4-16.8)	4.9 (4.5-5.2)	9.4 (8.8-10.1)	14.3 (13.2-15.7)
	1^{st}	5.6 (5.3-6.0)	10.4 (9.7-11.3)	15.6 (14.4-17.3)	5.3 (5.0-5.6)	10.2 (9.5-11.0)	15.2 (14.2-17.1)
	2^{nd}	6.8 (6.3-7.4)	13.1 (11.9-14.7)	18.2 (16.4-20.7)	6.0 (5.3-6.9)	11.8 (10.3-14.3)	16.6 (14.1-20.6)
	3^{rd}	9.5 (8.6-10.7)	16.8 (14.8-19.7)	22.8 (19.8-27.1)	7.7 (7.2-8.4)	13.9 (12.6-15.7)	19.0 (17.0-21.7)

*CL= confidence limits; LT = lethal time.

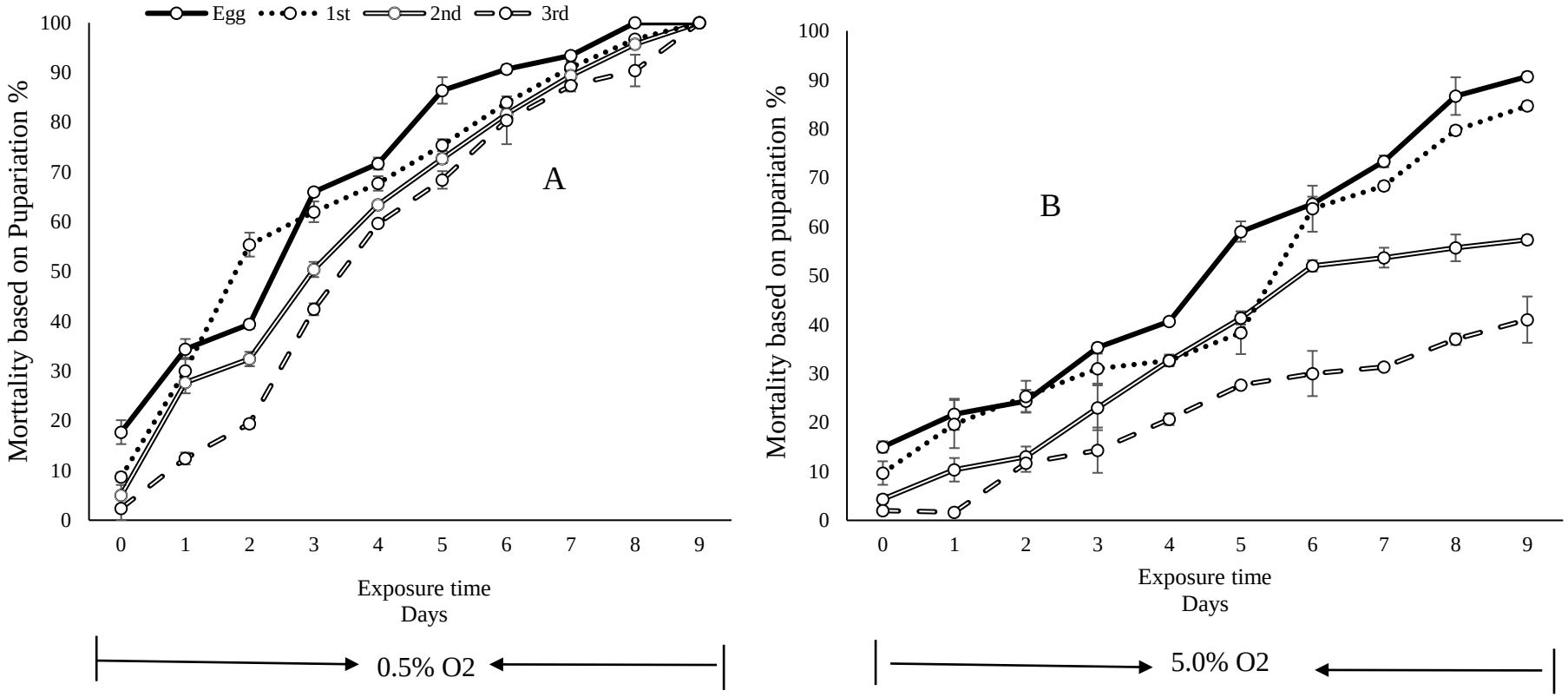


Figure 4.2. Mortality (SE) of eggs and 1st, 2nd and 3rd stage larvae based on pupariation rate. A- 0.5% O_2 and B - 5.0% O_2

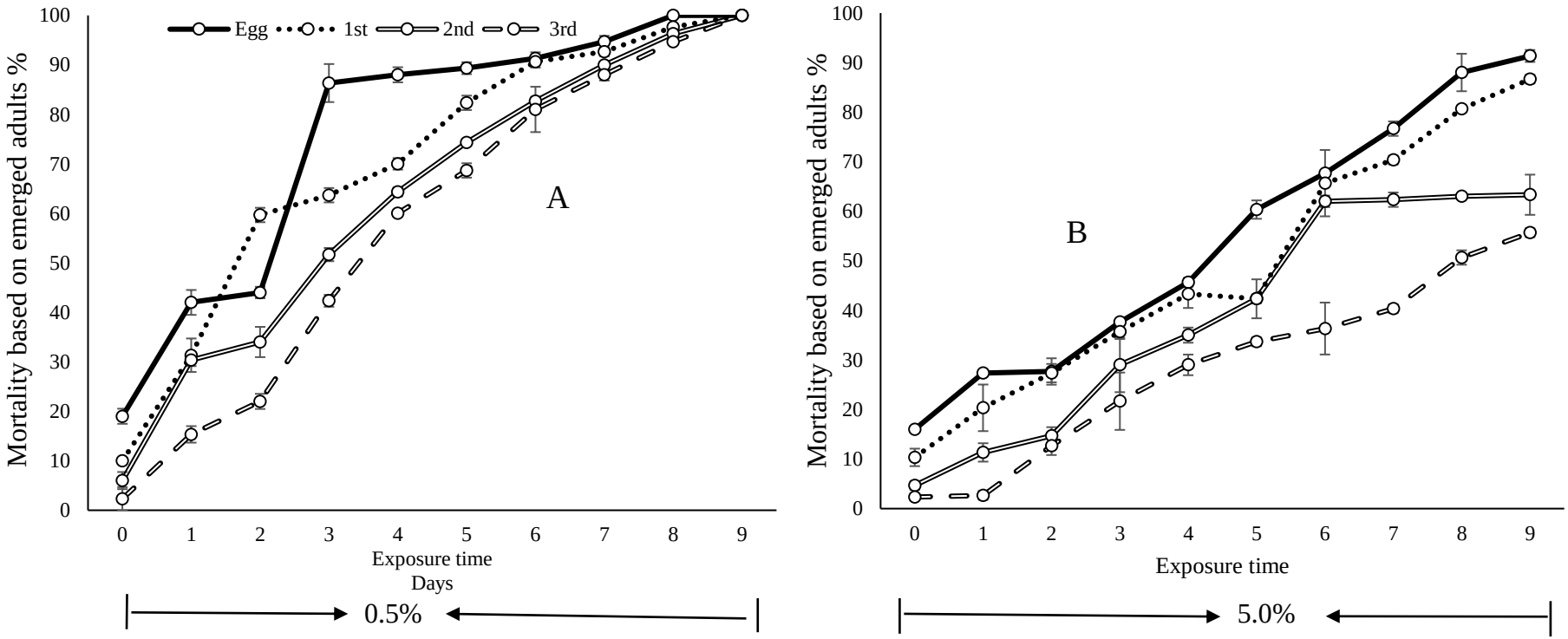


Figure 4.3. Mortality (SE) of eggs and 1st, 2nd and 3rd stage larvae based on emerged flies rate. A- 0.5% O_2 and B - 5.0% O_2.

There are no significant differences in the female adult ratios among the four stages (egg, 1st, 2nd and 3rd larvae instar), which were all close to 50% (Figure 4.4). To examine if a certain period of days of treatment influences females survival more than males or opposite, we analysed the female adult ratios from treated flies at Day 0, Day one, Day two, Day three and Day four 4 (Figure 4.S1). The data after Day four was not included in the analysis because the numbers of surviving adults are very low (<10). From Day 0 to Day four, based on the ANOVA test, there are no significant differences between days for the four stages, and the female adult ratios were still close to 50% (Figure 4.S1). Overall, there were no significant effects of low-oxygen treatment on the sex ratio of surviving *C. capitata* in all treatments (insect stages, exposure times and O_2 concentrations) (Figure 4.4 and Table 4.S1).

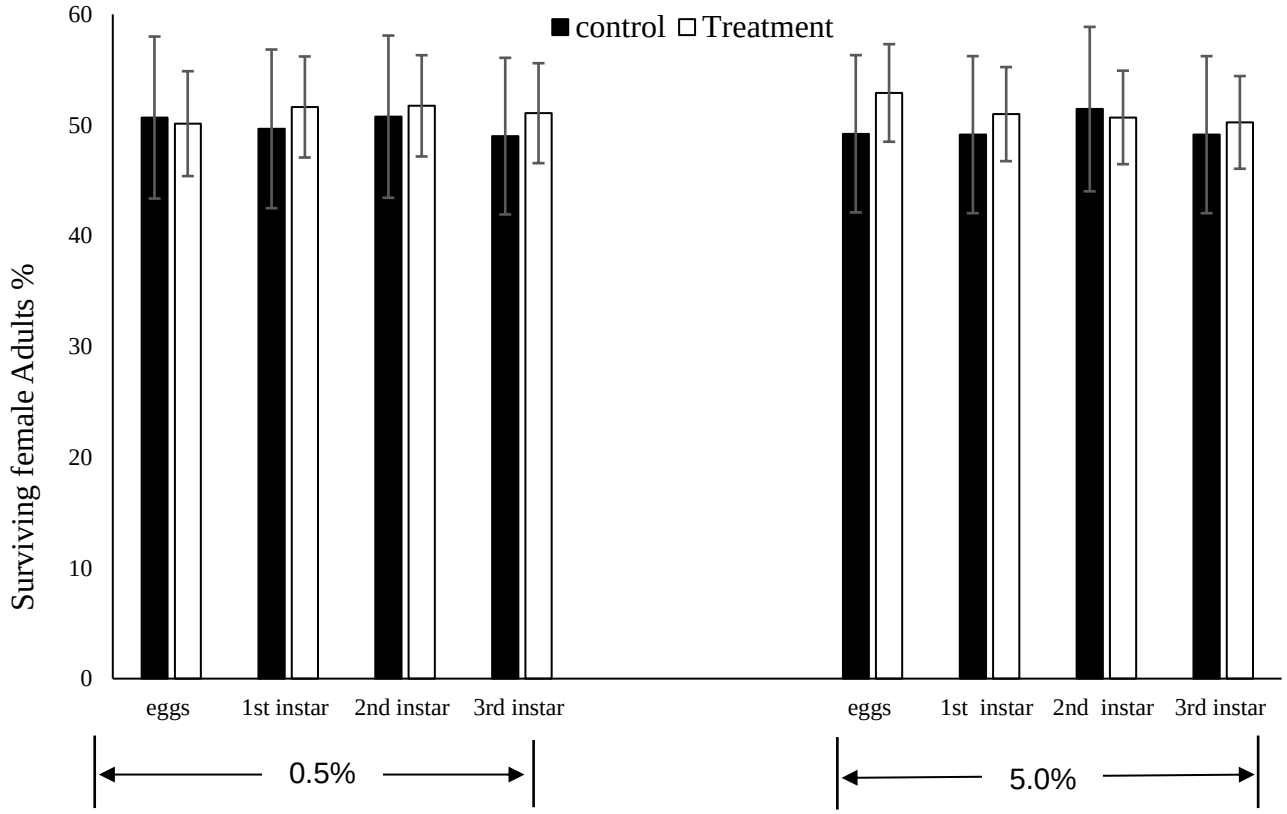


Figure 4.4. Percentage of surviving adult females under (A) 0.5% O_2, and (B) 5.0% O_2. Error bars represent the standard error of the mean. An ANOVA single factor test was used to compare sex ratios, $p > 0.05$.

4.5. Discussion

In this study, we chose to test a low-oxygen treatment to *C. capitata* in the lab diet rather than fruits, to avoid host influence on fly physiology and development, which would result in significant differences in fly response. Of the two oxygen treatments, 0.5% O_2 was more effective than 5.0% O_2 at killing *C. capitata* eggs and larvae. This result is consistent with Maekawa and Elert (2003), who found that there was no survival of *Anthrenus flavipes* (LeConte) under 0.3% oxygen over seven days at 25.6 ºC and 33-75% RH while control insects developed naturally.

Exposure time is one of the significant factors in a controlled atmosphere. We designed the exposure time for nine days according to our preliminary data, where nine days under 0.5% O_2 were enough to kill all stages (eggs and larvae stages) of *C. capitata* on an artificial diet at 25.0±1ºC. Our experimental results showed that the number of surviving flies decreased when continually exposed to the 0.5% O_2 treatment. This finding was consistent with earlier studies that refer to increased mortality of flies after they were exposed to low-oxygen concentrations (Van Voorhies, 2009). Yan et al. (2016) reported that the number of *Callosobruchus maculatus* larvae that developed to pupae and the number of pupae that emerged to adults was significantly reduced when exposured to low concentrations of oxygen (5.0%) compared to control samples.

In the present study, the 3rd instar was found to be the most tolerant of low-oxygen. However, Yahia and Zaleta (2000), found that eggs of *Anastrepha obliqua* were more tolerant than 3rd instar under low-oxygen treatment at 49.4ºC and 54.8ºC with 55%RH for 220-240 minutes. Contrarily, in the present study, eggs were more susceptible to low- oxygen treatments (0.5% and 5.0%) than larvae. These results agree with those of Shellie (2006) who found that the third instar of the Mexican fruit fly (*Anastrepha ludens*), which was artificially infested into grapefruit, was more tolerant than other stages when exposed to 1% O_2 and 50% CO_2 at room temperature. All these results suggest more attention should be placed on the third instar stage of fruit fly during low-oxygen treatment as it is the most tolerant stage. It is likely that there are many inter-related reasons for the increased survival of the larvae compared to the eggs such as ecological, morphological, physiological and behavioral (Kingsolver et al., 2011; Callier, 2015; Harrison and Haddad; 2011).

Low-oxygen atmosphere leads to anaerobic metabolism, which results in the production of lactic acid and CO_2. To release CO_2 from insect tissues, the insect tracheae spiracles would open fully and rapidly. Thus, faulty spiracular control leads to rapid death due to dehydration. (Hetz and Bradley, 2005; Lighton and Schilman, 2007).

The evolution of sex ratio is a complex field of evolutionary ecology, in some cases, environmental factors directly regulate the sex ratio. The evolution of sex ratio may be constrained, e.g., by genetic determination of sex (Fišer, 2019). Sex ratios indicate both the relative survival of females and males and the future breeding potential of a population (Skalski et al., 2010). Walder et al. (1993) observed a difference in sex ratio survival, with mortality rates of females markedly higher than males when they studied the effects of gamma radiation on the sterility and behavioral quality of the Caribbean fruit fly. Some new control strategies of insect pests focus on using methods that affect the sex ratio; for example, environmental effects can create a prejudiced sex ratio by excess production of the sex that is easy to produce under poor environmental conditions (Sapir, 2008).

In the present study, the sex ratios of the emerged adult flies were examined to investigate differences of the male and female larvae mortalities under low-oxygen treatment. There was no significant difference in the sex ratios of flies under low-oxygen treatments at different stages, oxygen concentrations and exposure times. When López-Martínez and Hahn (2012) studied the effect of short-term low-oxygen treatment conditioning and irradiation on the life history of Caribbean fruit fly (*Anastrepha suspense*), they found a 10% increase in the ratio of females to males. In their research, they included a second factor (irradiation) besides low oxygen. Furthermore, Moffitt and Albano (1972) found that exposure to low oxygen changed the sex ratios of codling moth (*Laspeyresia pomonella*) with more females than males. However, in the present study, we observed that there were slightly more female flies in all oxygen treatments than males, but no significant differences were detected.

This study was a preliminary study to examine Medfly responses to low oxygen plus high nitrogen; more work is required before it can be used as a practical application to control flies in fresh produce.

This study will help us to develop protocols that use combinations of lower-dose stresses (low oxygen, irradiation, or cold), which could achieve both higher pest mortality and lower any qualitative impact on the fruits in the future. Nine days are long to control fruit flies in fresh produce, and it is difficult to maintain fruits in good quality. However, this low-oxygen control method can be considered in combination with other treatment stressors (e.g., low temperature, irradiation etcetera) to make the whole treatment shorter and more efficient. This preliminary study provides knowledge to proceed to develop new treatment methods combined with different stresses.

Furthermore, this study established a lab-based low oxygen treatment bioassay for the Medfly. Based on this method, it is possible to study the molecular basis of Medfly responses to low oxygen treatment at specific treatment times. Comparing to flies treated inside fruits, this bioassay using naked fly in diet removed impacts of the fruits and help collect flies quickly and easily. If the treated Medfly samples are collected from fruits, the time taken to process the flies is much longer, and the risk of contamination is increased together with the degradation of the samples, particularly RNA samples.

4.6. In summary

This is the first study to use low-oxygen combined with high-nitrogen to control the Medfly, *C. capitata*. The third instar larvae were the most tolerant stage of low-oxygen treatment. There are no significant differences in the sex ratios of the treated medflies and control flies. Lastly, a laboratory low-oxygen system was established to study fruit fly under low-oxygen treatments, which can be utilized in further studies, for example, fruit flies in various fruits.

4.7. Supplementary Materials

Table 4.S1. Experimental design for control of Medfly on carrot diet at different O_2 concentrations and different exposure time at 25±1ºC with 70-75% RH.

Figure 4.S1. The female percentage (%) of survived adults during the first four days of 0.5% and 5.0% low-oxygen treatments. Error bar means standard error. An ANOVA single factor test was used to compare sex ratios, $p > 0.05$.

Table 4.S1. Experimental design for control of Medfly on carrot diet at different O_2 concentrations and different exposure time at 25±1ºC with 70-75% RH.

Oxygen con.	Insect developmental stage	Insect numbers	Number of replicates	Exposure time	Total number of stages in each test
0.5%	Eggs	100	3	0-9 days	3000
	1st instar	100	3	0-9 days	3000
	2nd instar	100	3	0-9 days	3000
	3rd instar	100	3	0-9 days	3000
5.0%	Eggs	100	3	0-9 days	3000
	1st instar	100	3	0-9 days	3000
	2nd instar	100	3	0-9 days	3000
	3rd instar	100	3	0-9 days	3000
Total			24		24000

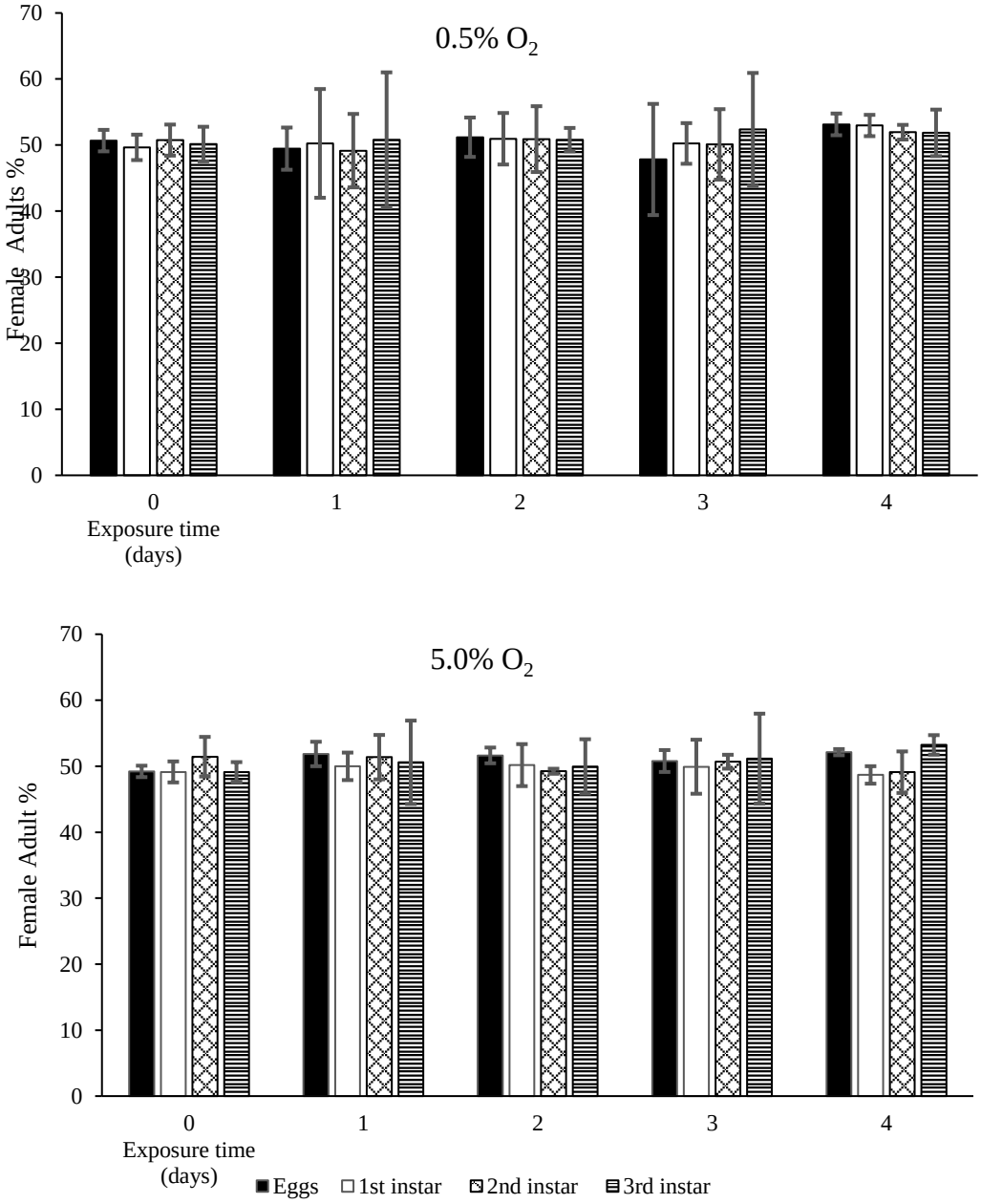


Figure 4.S1. The female adults (%) survived during four exposure periods (days) 0.5% and 5.0% low-oxygen treatments. Error bar means standard error. An ANOVA single factor test was used to compare sex ratios, $p > 0.05$.

Chapter five

5.1. General discussion

The Mediterranean Fruit Fly (*Ceratitis capitata*, or Medfly) attacks over 400 fruit and vegetables. Therefore, it is considered a global food biosecurity pest (Capinera, 2020). There are continued attempts to find effective integrated pest management methods or to modify and enhance the previous methods to ensure protection against Medfly while providing fresh agricultural products.

Postharvest technologies are critical in achieving this goal. They have allowed horticultural industries to meet the global demands of local and large-scale production and intercontinental distribution of fresh produce that has high nutritional and sensory quality (Mahajan et al., 2014). The fresh produce industry should begin to place increased emphasis on integrated methods of pest management (IPM) that are environmentally friendly, healthy and safe, reduce pest resistance, and do not threaten biological diversity and maintained fruit quality (Damos et al., 2015). IPM programs are integrated approaches of science (knowledge) with technologies - used concomitantly or sequentially - to manage pests below economic thresholds. Integrated post-harvest (IPH) is a system approach towards pest management where good management practices are combined with one or more contributory treatments to result in an acceptable level of pest control (Mitcham, 1999). By manipulation of one of the three factors (pest, host and conditions), we can reduce the level of pest problems. The environment can be manipulated easily after harvest to provide a temperature or an atmosphere that is unfavourable to pests and favourable to the host (Mitcham, 1999). Despite these convincing arguments for IPM and IPH, in practice few technologies are blended into most pest management programs (Radcliffe et al., 2009).
In this context, the principal aims of this thesis were to:

- Understand the mechanism of cold treatment to control the Medfly in a homogeneous medium to get rid of the effects of heterogeneity of fruits. This will contribute to developing environmentally safe and healthy alternatives for the end users.

- Test the medfly response to low- oxygen at high nitrogen atmosphere. That will open a new, affordable, available trends without involvement of environmental and health risks such as fumigation with pesticides.
- Evaluate cold treatment to control *C. capitata* in the blueberry as threatening pest to the growing global blueberry trade industry.
- Contribute to developing integrated pest management and built a bridge between industry and knowledge by providing urgent basic data for modern methods of pests control such as molecular biology and others.

Cold treatment is a common post-harvest method of pest control. Naturally, species of pests have varied response to low cold treatment because of different physiological and genetic factors between the species (Marshall et al., 2012). This can be exploited to achieve control. It is striking that Medfly showed different responses to cold treatment on the same fruit cultivar, and the reason still unknown (Sala et al., 2004). Despite the similarity of the biochemical components of these varieties (water, carbohydrates, fats and proteins), their ratios are inevitably different. The different ratios of those components effect the response of the pest to cold treatment (Storey and Storey, 1991; Bale, 2002; Koštál, 2010; Duman, 1991). Also, there are individual differences between the fruit even at the same maturity stage such as size, colour, hardness and water content (Barrett et a., 2010). Therefore, those differences impact the accuracy and reliability of the previous studies' results, and it became necessary to study the response of pests on a homogeneous artificial diet in properties and characteristics.

Consequently, an artificial diet (carrot diet) was used to determine with accurate Medfly's response to low temperature (0°C) with different exposure times. Inevitably, the results of this study will be a fundamental base for understanding the response of medfly to cold treatment and thus understanding and developing cold treatment for postharvest control of medfly. This study was the first to evaluate the response of five bioassays of Medfly at the same time to cold treatment on the carrot diet. Also, it's the first study to evaluate cold treatment effect on the Medfly sex ratio. Cold treatment at 0.0°C with various, exposure durations (0–12 days) was applied to the *C. capitata* fed on a carrot diet. The tested developmental stages were early eggs (<6 h), late eggs (>42 h), 1st instar, 2nd instar and 3rd instar larvae. Our findings showed that both 3rd instar and 1st instar larvae are amongst the most cold tolerant stages of *C. capitata* (Hallman et at., 2011). Moreover, there were no

statistically significant differences between early eggs and late eggs in the tolerance of cold treatment. A further matter, there were no statistically significant on *C. capitata* sex ratios among different stages after treated with cold treatment 0 °C for 12 days. Maurizio et al. (2019 have been reported similar results on Queensland fruit fly B. *tryoni* (eggs and larvae) (Jessup et al., 1998). In future studies on cold treatments of fruit flies, it is recommended to further investigate the interaction between survival times and test temperatures. Different temperatures may induce different responses at cellular and molecular levels.

Some fruit and vegetables are chill sensitive; therefore, the controlled atmosphere may be mutually supportive to cold treatment as well as supporting other treatments such as radiation. Before combining low-oxygen treatment with other methods, it is necessary to know the insect's response to low oxygen at room conditions. The effective low-oxygen concentration, the required exposure time and to determine susceptible and tolerant stage of pests are critical issues which must be accurately studied. Therefore, this first preliminary study was setup using two low-oxygen concentration with high nitrogen atmosphere 99.5% for 12 days. Two levels of low-oxygen treatments were applied (0.5% O_2+99.5 N_2 and 5% O_2+95% N_2) to the *C. capitata* eggs, 1st, 2nd and 3rd instar larvae on a carrot diet at 25°C; 70-75% RH. The pupariation, adult emergence, and sex ratios of survived flies were examined.

This study utilised a laboratory system to investigate low-oxygen, high-nitrogen treatment on the Medfly, *C. capitata*. For the first time, sex ratio of mature treated Medfly was investigated; with no statistically significant difference found between the control and treated groups. Third instar larvae were found to be the most tolerant stage to the treatment; in disagreement to the findings of Yahia and Ortega-Zaleta (2000), where they found that the egg of *Anastrepha obliqua* were more tolerant to low- oxygen treatment at 49.4°C. The difference in the results have practical implications for pest treatments. As eggs are considered the starting point for pests, therefore when the eggs were more tolerant than larvae, that will increase their chances to development into the next harmful stages. Also, the probability of a damage time will be longer because the period of pest development from egg to adult is longer than the larvae to the adults. In addition, stings can occur in apples and peaches when they are unripe, but the eggs do not hatch at this stage. As the fruit ripens the eggs can develop.

The low-oxygen, high-nitrogen treatment was found to be effective to control Medfly in fresh produce after nine days. This effective treatment time would need to decrease to maintain fresh produce nutritional and sensory qualities. Whilst this laboratory-based study indicated the low-Oxygen, high-Nitrogen treatment to be effective in the control of Medfly, further studies into the practical application to control Medfly in fresh produce needs further investigation; including the examination of a combined treatment method that could be utilised to achieve efficient and cost-effective methodology. The laboratory system established in this preliminary study could be utilised in further studies to explore variables such as various fruits or vegetable and temperature.

This study established a laboratory-based low oxygen treatment bioassay for the Medfly. Based on this method, it is possible to study the molecular basis of Medfly responses to low oxygen treatment at specific treatment doses (e.g., 70% mortality). Using naked Medfly in this study, removed impacts of the fruit and enabled quick and easy collection of flies at all stages. If the treated Medfly samples are collected from fruits, it would take a much longer time and increase the risk of contamination and cause the degradation of samples, especially RNA samples.

The global blueberry trade rocketed 24% in value to US$3.45 billion in 2018. The total volumes of the precious fruit shipped across the world have reached 560,101 tons (Eurofresh, 219). Blueberries suffer attacks from several arthropod pests and diseases (Liburd and Arevalo, 2006). Phytosanitary treatments are used to reduce the risk in quarantined areas and to prevent an infestation in non-infested importation areas (FAO, 2017). *C. capitata* is one pest threatening the blueberry industry in the world. *C. capitata* has a significant economic impact, affecting production, control costs and market access of blueberry (Brazelton et al. 2013). Therefore, the effects of cold treatment at 1.0±0.2°C were investigated on the eggs of the 1st, 2nd, and 3rd instar larvae of *C. capitata* in two blueberry cultivars C99-42 and C00-09. The study provided the first evidence of the medfly responses to the cold treatment at 1°C in two blueberry cultivars. Eleven days of exposing *C. capitata* to 1°C in both blueberry cultivars was enough to achieve a100% mortality rate. The 3rd instar was the most cold-tolerant stage of *C. capitate* in blueberries. Cold tolerance of the four *C. capitata* stages was not affected when reared on two different blueberry cultivars. There were no statistically significant differences in sex ratios from surviving *C. capitata* among different stages after treatment. Our findings will help develop or refine the large-scale cold treatment of medfly

for the blueberry trade industry. This study also provided information for the cold treatment of *C. capitate* in other fruits, especially small fruits such as strawberries, raspberries, blackberries, and grapes.

5.2. Conclusion

Postharvest IPM is a system approach towards pest management where good management practices are combined with one or more contributory treatments to result in an acceptable level of disease and insect control. The future success of integrated pest management of postharvest will be driven by the need to deliver high-quality fresh products to the consumer consistently. Indeed, the strategies of this study focused on the use of postharvest treatments as it would boost the global trade of fresh products and would also make it possible to provide fruits throughout the year and thus keep the fruit prices from rising. The current method of the postharvest chemical strategy is widely used but it needs to work towards lowering its negative effects or development of alternative treatments to manage postharvest decay. This will provide a marketing advantage for the export markets as fresh products have increased longevity. The most important method of postharvest management is the cold treatment, but the understanding of cold was not clear because the pests respond differently and that rely on fruits cultivars and the fruit itself. Therefore, the current study contributed to a better knowledge of cold treatment. Some promising fruit industries are suffering from insect pest effects, but they are still not tested with cold treatment. Blueberry is one of these industries which treated by the cold to eradicate the Medfly the first time. The initial results are promising, and the possibility of applying to the global fruit trade should be considered. The study identified the key low-oxygen treatment at high nitrogen atmosphere to control the pests. The low O_2 and high N_2 atmosphere was found to be effective in controlling Medfly after nine days and can be applied to other pests. Managing insects using low O_2 at high N_2 is safer to the environment as compared to chemical treatments.

Recommendations for future research and development of postharvest management methods include innovations and development of postharvest best practice strategies. We recommend further investigation of the interaction between survival times and test temperatures of Medfly treatments. Improving the low oxygen treatment with a nitrogen atmosphere and apply it to fresh fruit and vegetable. Conduct further studies related to interference between cold treatment and a low oxygen and high nitrogen atmosphere. We recommend further

development to the laboratory low O_2 system, which was established to study Medfly response in our research.

References

Abdalla, A., Millist, N., Buetre, B. and Bowen, B., 2012. Benefit–cost analysis of the National Fruit Fly Strategy Action Plan. Australian Bureau of Agricultural and Resource Economics and Sciences, Canberra.

Agnello, A.M., Spangler, S.M., Minson, E.S., Harris, T. and Kain, D.P., 2002. Effect of high-carbon dioxide atmospheres on infestations of apple maggot (Diptera: Tephritidae) in apples. *Journal of economic entomology*, 95(2), pp.520-526.

Al-Behadili, F.J., Bilgi, V., Li, J., Wang, P., Taniguchi, M., Agarwal, M., Ren, Y. and Xu, W., 2019. Cold response of the Mediterranean fruit fly (*Ceratitis capitata*) on a lab diet. *Insects*, 10(2), p.48.

Al-Khshemawee, H., Agarwal, M. and Ren, Y., 2017. Evaluation of stable isotope 13C6-glucose on volatile organic compounds in different stages of Mediterranean fruit fly (Medfly) *Ceratitis Capitata* (Diptera: Tephritidae). *Entomology, Ornithology & Herpetology: Current Research*, 6(3), p.1000195.

Alonso, M., Del Río, M.Á. and Jacas, J.A., 2005. Carbon dioxide diminishes cold tolerance of third instar larvae of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) in '*Fortune'mandarins*: implications for citrus quarantine treatments. *Postharvest biology and technology*, 36(1), pp.103-111.

Annis, P. C., 1987. Towards rational controlled atmosphere dosage schedules: a review of current knowledge. *Proceedings 4th International Workshop Conference on Stored-Product Protection*. Tel Aviv, Israel, Reference 63, pp. 128-148.

Arevalo-Vigne, M.L.I., 2017. Community engagement in biosecurity: Evaluating the role of knowledge and incentives in the area-wide management of Mediterranean fruit fly in Western Australia.

Armstrong, J. W., 1989. High-temperature forced-air quarantine treatments for fresh fruits infested by tephritid fruit flies. *In Symposium on Tropical Fruits in International Trade 269*, pp. 449-452.

Armstrong, J.W., Laidlaw, W.G., Chan Jr, H.T. and Jang, E.B., 1996. The effect of temperature profiles in heat-treatment disinfestation on mortality of pests and on fruit quality. Malaysia Agricultural Research and Development Institute (MARDI).

Armstrong, J. W., and Mangan, R. L., 2007. Commercial quarantine heat treatments. Heat Treatments for Postharvest Pest Control: Theory and Practice. Tang J, Mitcham E, Wang S, and Lurie S (eds.). Wallingford, UK: CAB *International*, pp.311-340.

Armstrong, J.W., Brash, D.W. and Waddell, B.C., 2014. Comprehensive literature review of fumigants and disinfestation strategies, methods and techniques pertinent to potential use as quarantine treatments for New Zealand export logs. *Plant & Food Research* SPTS, (10678).

Australian Government- Agriculture Department - Plant Pests and Diseases- Fruit Flies (AG- AD- PPA- FF), 2020. Exotic fruit fly, how to identify fruit flies. https://www.agriculture.gov.au/pests-diseases-weeds/plant/fruit-flies#how-to-identify-fruit-flies.

Australian Government- Plant Biosecurity- Biosecurity Australia (AG-PB-BA), 2008. Draft Import Risk Analysis Report for Fresh Stone Fruit from California, Idaho, Oregon and Washington. © *Commonwealth of Australia* 2008. https://www.agriculture.gov.au/sites/default/files/sitecollectiondocuments/ba/plant/ungroupeddocs/Stone_Fruit_USA_Draft_IRA_Report.pdf

Back EA, Pemberton C. E., 1915. Life history of the Mediterranean fruit fly from the standpoint of parasite introduction. United States Department of Agriculture, Washington. *Journal of Agricultural Research,* 3, pp. 363-374.

Back, E.A., Pemberton, C.E., 1916. Effect of cold-storage temperatures upon the Mediterranean fruit fly. *Journal of Agricultural Research*., 5, pp. 657–666.

Badii, K. B., Billah, M. K., Afreh-Nuamah, K., Obeng-Ofori, D., and Nyarko, G., 2015. Review of the pest status, economic impact and management of fruit- infesting flies (Diptera: Tephritidae) in Africa. *African Journal and Agricultural research,* Vol 10 (12), pp. 1488-1498.

Bale, J. S., Van Lenteren, J. C., and Bigler, F., 2008. Biological control and sustainable food production. Philosophical Transactions of the Royal Society B. *Biological Sciences*, 363 (1492), 761-776.

Bell, C.H., Price, N. and Chakrabarti, B., 1996. The methyl bromide issue. *John Wiley and Sons*, New York.

Benelli, M., Ponton, F. and Taylor, P.W., 2019. Cool storage of Queensland fruit fly eggs for increased flexibility in rearing programs. Pest management science, 75(4), pp.1056-1064.

Benschoter, C A and Witherell, P. C., 1984. lethal effects of suboptimal temperatures on immature stages of *Anastrepha suspensa*. *Florida Entomologist,* 67, pp. 189–193.

Benschoter, C.A., 1987. Effects of modified atmospheres and refrigeration temperatures on survival of eggs and larvae of the Caribbean fruit fly (Diptera: Tephritidae) in laboratory diet. *Journal of economic entomology*, 80 (6), pp. 1223-1225.

Benschoter, C.A., Spalding, D.H. and Reeder, W.F., 1981. Toxicity of atmospheric gases to immature stages of *Anastrepha suspensa*. *Florida Entomologist*, pp.543-544.

Berg GH., 1979. Pictorial key to fruit fly larvae of the family Tephritidae. San Salvador,*Organismo Internacional Regional de Sanidad Agropecuaria*, pp 36.

Biosecurity New Zealand, 2009, b. Draft Import Risk Analysis, Fresh stone fruit from Idaho, Oregon and Washington. Ministry of Agriculture and Forestry, Wellington, New Zealand. http://www.biosecurity.govt.nz/regs/imports/ihs/risk.

Bond, E.J. and Buckland, C.T., 1979. Development of resistance of carbon dioxide in the granary weevil. *Journal of economic entomology*, 72(5), pp.770-771.

Brazelton, C., 2013. World blueberry acreage and production. 26 Aug. 2013.

Brenner, R.J., Focks, D.A., Arbogast, R.T., Weaver, D.K. and Shuman, D., 1998. Practical use of spatial analysis in precision targeting for integrated pest management. *American Entomologist*, 44(2), pp.79-102.

Broughton, S. and De Lima, F., 2002. Control of Mediterranean fruit fly (Medfly) in backyards.

Burditt Jr, A.K. and Balock, J.W., 1985. Refrigeration as a quarantine treatment for fruits and vegetables infested with eggs and larvae of *Dacus dorsalis* and *Dacus cucurbitae* (Diptera: Tephritidae). *Journal of economic entomology*, 78(4), pp.885-887.

Burditt Jr, A.K., Balock, J.W., Hinman, F.G. and Seo, S.T., 1963. Ethylene dibromide water dips for destroying fruit fly infestations of quarantine significance in papayas. *Journal of economic entomology*, 56(3), pp.289-292.

Burg, S.P., 2004. Postharvest physiology and hypobaric storage of fresh produce. *CABI Pub*, Wallingford, Oxon, UK; Cambridge, MA, USA. P. 654. ISBN: 0-85199-267-6.

Bustos R, M.E., Enkerlin H, W., Toledo A, J., Reyes F, J. and Casimiro G, A., 1991. Irradiation of mangoes as a quarantine treatment (No. IA--91-19). Instituto Nacional de Investigaciones Nucleares.

Calderon, M. and Barkai-Golan, R., 1990. Food Preservation by Modified Atmospheres CRC *Press Boca Raton*. Florida., USA.

Callier, V., Hand, S.C., Campbell, J.B., Biddulph, T. and Harrison, J.F., 2015. Developmental changes in hypoxic exposure and responses to anoxia in *Drosophila melanogaster*. *Journal of Experimental Biology*, 218(18), pp.2927-2934.

Cao, Y., Xu, K., Zhu, X., Bai, Y., Yang, W. and Li, C., 2019. Role of modified atmosphere in pest control and mechanism of its effect on insects. Frontiers in physiology, 10, p.206.

Capinera, J., 2020. Handbook of vegetable pests. *Academic press*.

Castillo, M.A., Moya, P., Hernández, E. and Primo-Yufera, E., 2000. Susceptibility of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) to entomopathogenic fungi and their extracts. *Biological control*, 19(3), pp.274-282.

Cattlin, N., 2010. Mediterranean fruit fly *(Ceratitis capitata)* damage gallery with larvae in a satsuma fruit section. http://www.dijitalimaj.com/alamyDetail.aspx?img=%7B12473A93-58FD-4366-87BC-193924B8C0A4%7D.

Cayol, J. P., and R. Causse., 1993. Mediterranean fruit fly *C. capitata* back in Southern France. *Journal of Applied Entomology* 116:94-100.

Canadian Food Inspection Agency (CFIA), 2008. Import and domestic phytosanitary requirements to prevent the introduction and spread of apple maggot (*Rhagoletis pomonella* spp. (Walsh). *The Canadian Food Inspection Agency*. Webpage, http://www.inspection.gc.ca/english/plaveg/protect/dir/d-00-07e.shtml#c3.

Chen, P.M., Olsen, K.L. and Meheriuk, M., 1985. Effect of low-oxygen atmosphere on storage scald and quality preservation of «Delicious» apples. *Journal of the American Society for Horticultural Science*, 110(1), pp.16-20.

Christenson, L.D. and Foote, R.H., 1960. Biology of fruit flies. *Annual review of entomology*, 5(1), pp.171-192.

Cladera, J.L., Vilardi, J.C., Juri, M., Paulin, L.E., Giardini, M.C., Cendra, P.V.G., Segura, D.F. and Lanzavecchia, S.B., 2014. Genetics and biology of *Anastrepha fraterculus*: research supporting the use of the sterile insect technique (SIT) to control this pest in Argentina. *BMC genetics*, 15(2), pp.1-14.

Cladera, J.L., Viscarret, M.M., Carabajal Paladino, L.Z., Pietrek, A., Soria, M.A. and Ovruski, S.M., 2006. "More than two": integrating biological control and sterile insects, from factory to field, and the possibility of its implementation in Argentina. From Basic to Applied Knowledge *Proceedings of the 7th*. International Symposium on Fruit Flies of Economic Importance 10-15 September 2006, Salvador, Brazil, pp. 143-155.

Copeland, R.S., Wharton, R.A., Luke, Q. and De Meyer, M., 2002. Indigenous hosts of *Ceratitis capitata* (Diptera: Tephritidae) in Kenya. *Annals of the Entomological Society of America*, 95(6), pp.672-694.

Corcoran, R. J., 1993. Heat-mortality relationship for eggs of *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) at varying ages. *Australian Journal of Entomology*, 32(4), pp.307-310.

Corcoran, R.J. and Waddell, B.C., 2003. Ionizing energy treatments for quarantine disinfestation. *Horticulture Australia Ltd.*, Sydney.

Cornelius, M.L., Duan, J.J. and Messing, R.H., 1999. Visual stimuli and the response of female oriental fruit flies (Diptera: Tephritidae) to fruit-mimicking traps. *Journal of economic entomology*, 92(1), pp.121-129.

Curtis, C., Clark, H.D., Tebbets, J.S. and Mackey, B.E., 1992. Incidence of arthropods found in packed nectarine fruit in central California. *The Southwestern entomologist* (USA) V. 17, pp. 29–39.

Daane, K.M. and Johnson, M.W., 2010. Olive fruit fly: managing an ancient pest in modern times. Annual review of entomology, 55, pp.151-169.

Dawson, A., Hassenpflug, S., Sloan, J. and Yoshioka, I., 1998. California Agricultural Trade: Combating the Medfly Menace. Center for Trade and Commercial Diplomacy, Monterey Institute of International Studies, Monterey, California.

De Lima, C.P.F., Jessup, A.J., Cruickshank, L., Walsh, C.J. and Mansfield, E.R., 2007. Cold disinfestation of citrus (Citrus spp.) for Mediterranean fruit fly (*Ceratitis capitata*) and Queensland fruit fly (*Bactrocera tryoni*) (Diptera: Tephritidae). *New Zealand Journal of Crop and Horticultural Science*, 35(1), pp.39-50.

De Lima, C.P.F., 1990. Airtight storage: principle and practice. Food Preservation by Modified Atmospheres. *CRC Press Inc*., Boca Raton, Florida, pp.9-19.

De Lima, C.P.F., 1998. Disinfestation of mandarins for export to Japan. In Proceedings of the Fifth International Symposium on Fruit Flies of Economic Importance, Penang, Malaysia, 1–5 June, *Poster* No. 205.

De Lima, C.P., Jessup, A.J., Mansfield, E.R. and Daniels, D., 2011. Cold treatment of table grapes infested with Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) and Queensland fruit fly *Bactrocera tryoni* (Froggatt) Diptera: Tephritidae. *New Zealand Journal of Crop and Horticultural Science*, 39(2), pp.95-105.

De Meyer, M. and Freidberg, A., 2005. Revision of the fruit fly genus Capparimyia (Diptera, Tephritidae). *Zoologica Scripta*, 34(3), pp.279-303.

De Meyer, M., Robertson, M.P.; Mansell, M.W., Ekesi, S.; Tsuruta, K., Mwaiko, W., Peterson, A.T., 2010. Ecological niche and potential geographic distribution of the

invasive fruit fly *Bactrocera invadens* (Diptera, Tephritidae). *Bulletin of Entomological Research*, 100, pp. 35–48.

De Meyer, M., Delatte, H., Mwatawala, M., Quilici, S., Vayssieres, J.F. and Virgilio, M., 2015. A review of the current knowledge on *Zeugodacus cucurbitae* (Coquillett)(Diptera, Tephritidae) in Africa, with a list of species included in Zeugodacus. ZooKeys, (540), p.539.

Department of Agriculture and Food, 2012. Controlling Mediterranean fruit fly. The Chief Executive Officer of the Department of Agriculture and Food and the State of Western Australia. Western Australian Agriculture Authority, 2012. www.agric.wa.gov.au.

Department of Agriculture and Food, 2014. Controlling Mediterranean fruit fly, bait spraying, Government of Western Australia.

Dewey, D.H., 1984. Guidelines for Constructing and Operating Controlled Atmosphere Storages for Apples. Cooperative Extension Service, Michigan State University.

Diamantidis, A.D., Carey, J.R., Nakas, C.T. and Papadopoulos, N.T., 2011. Population-specific demography and invasion potential in medfly. *Ecology and evolution*, 1(4), pp.479-488.

Dohino, T., Hallman, G.J., Grout, T.G., Clarke, A.R., Follett, P.A., Cugala, D.R., Minh Tu, D., Murdita, W., Hernandez, E., Pereira, R. and Myers, S.W., 2017. Phytosanitary treatments against *Bactrocera dorsalis* (Diptera: Tephritidae): current situation and future prospects. *Journal of economic entomology*, 110(1), pp.67-79.

Dominiak, B.C. and Mapson, R., 2017. Revised distribution of *Bactrocera tryoni* in eastern Australia and effect on possible incursions of Mediterranean fruit fly: development of Australia's eastern trading block. *Journal of Economic Entomology*, 110(6), pp.2459-2465.

Dominiak, B.C. and Daniels, D., 2012. Review of the past and present distribution of Mediterranean fruit fly (*Ceratitis capitata Wiedemann*) and Queensland fruit fly (*Bactrocera tryoni Froggatt*) in Australia. *Australian Journal of Entomology*, 51(2), pp.104-115.

Donahaye, E., 1990. Laboratory selection of resistance by the red flour beetle, *Tribolium castaneum* (Herbst), to a carbon dioxide-enriched atmosphere. *Phytoparasitica*, 18(4), pp.299-308.

Donahaye, E.J., 2000. Current status of non-residual control methods against stored product pests. *Crop Protection*, 19(8-10), pp.571-576.

Drake, S.R. and Neven, L.G., 1998. Irradiation as an alternative to methyl bromide for quarantine treatment of stone fruits. *Journal of Food Quality* 21(6), pp. 529–538.

Drew, R.A.I., Tsuruta, K. and White, I.M., 2005. A new species of pest fruit fly (Diptera: Tephritidae: Dacinae) from Sri Lanka and Africa. *African Entomology*, 13(1), pp.149-154.

Dyck, V.A., Hendrichs, J. and Robinson, A.S. eds., 2006. Sterile insect technique: principles and practice in area-wide integrated pest management. *Springer Science & Business Media.*

Economopoulos, A.P., 1989. Use of traps based on colour and /or shape in fruit flies; their biology, natural enemies and control. *Elsevier*, Amsterdam, V. 3B, PP. 315-327.

Eilenberg, J., 2006. Concepts and visions of biological control. In An ecological and societal approach to biological control. *Springer*, Dordrecht, pp. 1-11

Elsevier Inc. Sasso, R., Gualtieri, L., Russo, E., Nugnes, F., Gebiola, M. and Bernardo, U., 2020. The establishment of a rearing technique for the fruit fly parasitoid *Baryscapus silvestrii* increases knowledge of biological, ecological and behavioural traits. BioControl, 65(1), pp.47-57.

EPPO/CABI., 1997. *Ceratitis capitata*, Quarantine Pests for Europe, 2nd edition, pp. 146–152. CAB International, *Wallingford* (GB). http://www.eppo.int/.

Eurofresh Distribution, 2019. World blueberry market expands 24% in 2018. https://www.eurofresh-distribution.com/news/world-blueberry-market-expands-24-2018.

Exotic fruit flies FS - Plant Health Australia (EFF- FS- PHA), 2015. Fact Sheet, Exotic Fruit Flies. https://www.planthealthaustralia.com.au/wp-content/uploads/2015/01/Exotic-fruit-flies-FS.pdf.

Fields, P., 1994. Physical methods for insect control in stored-grain ecosystems. Stored-grain ecosystems, 39, p.353.

Fišer, C., 2019. Life histories. In Encyclopedia of Caves (pp. 652-657). *Academic Press*.

Fisher, K.T., Hill, A.R. and Sproul, A.N., 1985. Eradication of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) in Carnarvon, Western Australia. *Australian Journal of Entomology*, 24(3), pp.207-208.

Follett, P.A., 2006. Irradiation as a phytosanitary treatment for white peach scale (Homoptera: Diaspididae). *Journal of economic entomology*, 99(6), pp.1974-1978.

Follett, P.A. and Lower, R.A., 2000. Irradiation to ensure quarantine security for *Cryptophlebia spp.* (Lepidoptera: Tortricidae) in sapindaceous fruits from Hawaii. *Journal of economic entomology*, 93(6), pp.1848-1854.

Follett, P.A. and Snook, K., 2013. Cold storage enhances the efficacy and margin of security in postharvest irradiation treatments against fruit flies (Diptera: Tephritidae). *Journal of economic entomology*, 106(5), pp.2035-2042.

Food and Agricultural Organization of the United Nations and International Plant Protection Convention, 2016. International Standards for Phytosanitary Measures (ISPM). ISPM No. 18 Guidelines for the use of irradiation as A Phytosanitary measure. FAO website (www.fao.org/publications) and publicationssales@fao.org.

Food and Agriculture Organization (FAO) and International Plant Protection Convention, 2017. Establishment of areas of low pest prevalence for fruit flies (Tephritidae). *In International Standards for Phytosanitary Measures* (ISPM. 30). Rome, Italy.

Franz, G., 2005. Genetic sexing strains in Mediterranean fruit fly, an example for other species amenable to large-scale rearing for the sterile insect technique. In Sterile Insect Technique (pp. 427-451). *Springer*, Dordrecht.

Frazier, M. J., Kleinkopf, G. E., Brey, R. R., Olsen, N. L., 2006. Potato sprout inhibition and tuber quality after treatment with high energy ionizing radiation. *American Journal of Potato Research* 83(1), pp. 31–39.

Frazier, M.R., Woods, H.A. and Harrison, J.F., 2001. Interactive effects of rearing temperature and oxygen on the development of *Drosophila melanogaster*. *Physiological and Biochemical Zoology*, 74(5), pp.641-650.

Frías, D., Hernández-Ortiz, V., Vaccaro, N.C., Bartolucci, A.F. and Salles, L.A., 2006. Comparative morphology of immature stages of some frugivorous species of fruit flies (Diptera: Tephritidae). *Israel Journal of Entomology*, 35(36), pp.423-457.

Fujii, J.K. and Tamashiro, M., 1972, January. Nosema tephrititae sp. n., a microsporidian pathogen of the Oriental Fruit Fly, *Dacus dorsalis* Hendel. *In Proc. Hawaii. Entomological Society* (Vol. 21, pp. 191-203).

Gazit, Y., Akiva, R. and Gavriel, S., 2014. Cold tolerance of the Mediterranean fruit fly in date and mandarin. *Journal of economic entomology*, 107(5), pp.1745-1750.

Ghafir, S.A., Gadalla, S.O., Murajei, B.N. and El-Nady, M.F., 2009. Physiological and anatomical comparison between four different apple cultivars under cold-storage conditions. *African Journal of Plant Science*, 3(6), pp.133-138.

Gillott, C., 2005. Entomology, third Edition University of Saskatchewan Saskatoon, Saskatchewan, Canada. Springer Science & Business Media.

Gordon, K.H. and Waterhouse, P.M., 2007. RNAi for insect-proof plants. *Nature biotechnology*, 25(11), pp.1231-1232.

Gormley, T.R., 1985. Chilled Foods, the State of the Art, *Elsevier App. Sc i*., London, New York.

Gorny, J.R., 1997. Modified atmosphere packaging in fresh-cut revolution. *Perishables Handling Newsletter*, 90, pp.4-5.

Gould, W.P. and Hallman, G.J., 2004. Irradiation disinfestation of Diaprepes root weevil (Coleoptera: Curculionidae) and papaya fruit fly (Diptera: Tephritidae). *Florida Entomologist*, 87(3), pp.391-392.

Gould, W.P. and Hennessey, M.K., 1997. Mortality of *Anastrepha suspensa* (Diptera: Tephritidae) in carambolas treated with cold water precooling and cold storage. *Florida Entomologist*, pp.79-84.

Grout, T.G., Stephen, P.R., Henry Daneel, J. and Hattingh, V., 2011. Cold treatment of *Ceratitis capitata* (Diptera: Tephritidae) in oranges using a larval endpoint. *Journal of economic entomology*, 104(4), pp.1174-1179.

Gupta, R. L., 1960. Preliminary trial of bait-spray for the control of fruit-flies in India. *Indian Journal of Entomology* 1960 Vol. 20 (4) pp. 304-306.

Hallman, G.J., 2004. Ionizing irradiation quarantine treatment against oriental fruit moth (Lepidoptera: Tortricidae) in ambient and hypoxic atmospheres. *Journal of economic entomology*, 97(3), pp.824-827.

Hallman, G.J., 2014. Insect thermotolerance comparing host infestation methods: *Anastrepha ludens* (Diptera: Tephritidae) reared in grapefruit or diet. *Journal of economic entomology*, 107(4), pp.1377-1384.

Hallman, G.J. and Armstrong, J.W., 1994. Heated air treatments. In: Sharp, J.L. and Hallman, G.J. (eds.). Quarantine Treatments for Pests of Food Plants. *Westview Press*, Boulder, CO, pp. 149-163.

Hallman, G.J., Myers, S.W., Jessup, A.J. and Islam, A., 2011. Comparison of in vitro heat and cold tolerances of the new invasive species *Bactrocera invadens* (Diptera: Tephritidae) with three known tephritids. *Journal of economic entomology*, 104(1), pp.21-25.

Hallman, G.J., Wang, L., Demirbas Uzel, G., Cancio-Martinez, E., Cáceres-Barrios, C.E., Myers, S.W. and Vreysen, M.J., 2019. Comparison of Populations of *Ceratitis capitata* (Diptera: Tephritidae) from Three Continents for Susceptibility to Cold Phytosanitary Treatment and Implications for Generic Cold Treatments. *Journal of economic entomology*, 112(1), pp.127-133.

Hanlon, G., Daniel, V., Ravenel, N. and Maekawa, S., 1992, October. Dynamic system for nitrogen anoxia of large museum objects: A pest eradication case study. In Papers presented at the Wooden Artifacts Group specialty session, June 4, 1993, *AIC annual meeting*, Denver, Colorado (pp. 52-70).

Hansen, J.D., Drake, S.R., Moffitt, H.R., Albano, D.J. and Heidt, M.L., 2000. Methyl bromide fumigation of five cultivars of sweet cherries as a quarantine treatment against codling moth. *HortTechnology*, 10(1), pp.194-198.

Hansen, J.D., Hara, A.H. and Tenbrink, V.L., 1992. Vapor heat: a potential treatment to disinfest tropical cut flowers and foliage. *HortScience*, 27(2), pp.139-143.

Harman, J.E., Lay-Yee, M., Billing, D.P., Yearsley, C.W. and Jackson, P.J., 1990. Effects of methyl bromide fumigation, delayed cooling, and controlled atmosphere storage on the quality of 'Redgold'and 'Fantasia'nectarine fruit. *New Zealand journal of crop and horticultural science*, 18(4), pp.197-203.

Harrison, J.F. and Haddad, G.G., 2011. Effects of oxygen on growth and size: synthesis of molecular, organismal, and evolutionary studies with *Drosophila melanogaster*. *Annual review of physiology*, 73, pp.95-113.

Heather, N.W., Whitfort, L., McLauchlan, R.L. and Kopittke, R., 1996. Cold disinfestation of Australian mandarins against Queensland fruit fly (Diptera: Tephritidae). *Postharvest Biology and Technology*, 8(4), pp.307-315.

Heather, N.W. and Hallman, G.J., 2008. Pest management and phytosanitary trade barriers. CABI, Wallingford, UK.

Heaven, D., 2006. Important information on fruit fly, Government of South Australia, Primary Industries and Resources SA. *F act sheet*, ISSN 1323-0409. FS 21/77/07. https://www.eliminatorspc.net/pdf/fruitfly.pdf

Hendrichs, J., Katsoyannos, B.I., Papaj, D.R. and Prokopy, R.J., 1991. Sex differences in movement between natural feeding and mating sites and trade-offs between food

consumption, mating success and predator evasion in Mediterranean fruit flies (Diptera: Tephritidae). *Oecologia*, 86(2), pp.223-231.

Herner, R.C., 1987. High CO_2 effects on plant organs. *Postharvest physiology of vegetables*, pp.239-253.

Hetz, S.K. and Bradley, T.J., 2005. Insects breathe discontinuously to avoid oxygen toxicity. *Nature*, 433(7025), pp.516-519.

Hill, S.B., 1990. Cultural methods of pest, primarily insect, control. *In Proceedings of the Annual Meeting of the Canadian Pest Management Society* (Vol. 36, pp. 35-49).

Hill, A.R., Rigney, C.J. and Sproul, A.N., 1988. Cold Storage of oranges as a disinfestation treatment against the fruit flies *Dacus tryoni* (Froggau) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). *Journal of economic entomology*, 81(1), pp.257-260.

Hood-Nowotny, R., Watzka, M., Mayr, L., Mekonnen, S., Kapitano, B. and Parker, A., 2011. Intrinsic and synthetic stable isotope marking of tsetse flies. *Journal of Insect Science*, 11(1), p.79.

Hoy, L.E. and Whiting, D.C., 1997. Low-temperature storage as a postharvest treatment to control *Pseudococcus affinis* (Homoptera: Pseudococcidae) on Royal Gala apples. *Journal of economic entomology*, 90(5), pp.1377-1381.

Hulasare, R., Payton, M.E., Hallman, G.J. and Phillips, T.W., 2013. Potential for hypobaric storage as a phytosanitary treatment: mortality of *Rhagoletis pomonella* (Diptera: Tephritidae) in apples and effects on fruit quality. *Journal of economic entomology*, 106(3), pp.1173-1178.

Integrated Pest Management, 2009. IPM reduces reliance on pesticides, especially broad-spectrum pesticides, limiting the opportunity for resistance and promoting populations of beneficial species. *Fact Sheet (National)*, Order number, GRDC454. 529.58 kb Integrated Pest Management Integrated Pest Management**.**

International Atomic Energy Agency, 2001. Economic evaluation of three alternative methods for control of the Meditereanean fruit fly in Isreal, Jorden, Lebanon, Syria Arab Republic. ISSN 1011-4289. December 2001, IAEA, VIENNA. https://inis.iaea.org/collection/NCLCollectionStore/_Public/33/004/33004596.pdf

Israely, N., Ziv, Y. and Galun, R., 2005. Metapopulation spatial–temporal distribution patterns of Mediterranean fruit fly (Diptera: Tephritidae) in a patchy environment. *Annals of the Entomological Society of America*, 98(3), pp.302-308.

Iwahashi, O., 1972. Movement of the oriental fruit fly adults among islets of the Ogasawara Islands. *Environmental Entomology*, 1(2), pp.176-179.

Jacobi, K.K., Wong, L.S. and Giles, J.E., 1993. Lychee (Litchi chinensis Sonn.) fruit quality following vapour heat treatment and cool storage. *Postharvest biology and technology*, 3(2), pp.111-119.

Jacobson, M.Z., 2009. Review of solutions to global warming, air pollution, and energy security. *Energy & Environmental Science*, 2(2), pp.148-173.

Jacxsens, L., Luning, P.A., Van der Vorst, J.G.A.J., Devlieghere, F., Leemans, R. and Uyttendaele, M., 2010. Simulation modelling and risk assessment as tools to identify the impact of climate change on microbiological food safety–The case study of fresh produce supply chain. *Food Research International*, 43(7), pp.1925-1935.

Jessup, A.J., Dominiak, B., Woods, B., De Lima, C.P.F., Tomkins, A. and Smallridge, C.J., 2007. Area-wide management of fruit flies in Australia. *In Area-wide control of insect pests* (pp. 685-697). S*pringer*, Dordrecht.

Jessup, A.J., De Lima, C.P.F., Hood, C.W., Sloggett, R.F., Harris, A.M. and Beckingham, M., 1993. Quarantine disinfestation of lemons against *Bactrocera tryoni* and *Ceratitis capitata* (Diptera: Tephritidae) using cold storage. *Journal of economic entomology*, 86(3), pp.798-802.

Jessup, A.J., Sloggett, R.F. and Quinn, N.M., 1998. Quarantine disinfestation of blueberries against *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) by cold storage. *Journal of economic entomology*, 91(4), pp.964-967.

Jordan, H.R. and Tomberlin, J.K., 2017. Abiotic and biotic factors regulating inter-kingdom engagement between insects and microbe activity on vertebrate remains. *Insects*, 8(2), p.54.

Katsoyannos, B.I., Kouloussis, N.A. and Papadopoulos, N.T., 1997. Response of *Ceratitis capitata* to citrus chemicals under semi-natural conditions. *Entomologia experimentalis et applicata*, 82(2), pp.181-188.

Katsoyannos, B.I. and Papadopoulos, N.T., 2004. Evaluation of synthetic female attractants against *Ceratitis capitata* (Diptera: Tephritidae) in sticky coated spheres and McPhail type traps. *Journal of economic entomology*, 97(1), pp.21-26.

Katsoyannos, B.I., 1994. Evaluation of Mediterranean fruit-fly traps for use in sterile-insect-technique programmes. *Journal of Applied Entomology*, 118(1-5), pp.442-452.

Ke, D. and Kader, A.A., 1992. Potential of controlled atmospheres for postharvest insect disinfestation of fruits and vegetables. *Postharvest News and Information*, 3(2), pp.31N-37N.

Ke, D. and Kader, A.A., 1989, June. Tolerance and responses of fresh fruits to oxygen levels at or below 1%. *In Proceedings of the 5th International Controlled Atmosphere Research Conference*, Wenatchee, Washington, 14 (Vol. 1, pp. 209-216).

Kehat, M. and Dunkelblum, E., 1990. Behavioral responses of *maleHeliothis armigera* (Lepidoptera: Noctuidae) moths in a flight tunnel to combinations of components identified from female sex pheromone glands. *Journal of insect behavior*, 3(1), pp.75-83.

Keiser, I., Kobayashi, R.M., Chambers, D. and Schneider, E.L., 1973. Relation of sexual dimorphism in the wings, potential stridulation, and illumination to mating of oriental fruit flies, melon flies, and Mediterranean fruit flies in Hawaii. *Annals of the Entomological Society of America*, 66(5), pp.937-941.

Kiaya, V., 2014. Postharvest losses and strategies to reduce them. Technical Paper on Postharvest Losses, *Action Contre la Faim* (ACF), 25.

Kingsolver, J.G., Arthur Woods, H., Buckley, L.B., Potter, K.A., MacLean, H.J. and Higgins, J.K., 2011. Complex life cycles and the responses of insects to climate change.

Kiwifruit Vine Health (KVH), 2014. Financial impact of a fruit fly incursion to New Zealand's kiwifruit industry. New Zealand's kiwifruit industry. https://www.kvh.org.nz/vdb/document/98983.

Klassen, W. and Curtis, C.F., 2005. History of the sterile insect technique. In Sterile insect technique. *Springer*, pp 3-36. Dordrecht- Netherlands.

Kloek, G.P., 1979. Oxygen levels safe for continued reproduction of Drosophila in normal and hypobaric atmospheres. Aviation, *Space, and Environmental Medicine*, 50(11), pp.1126-1128.

Krainacker, D.A., Carey, J.R. and Vargas, R.I., 1987. Effect of larval host on life history traits of the Mediterranean fruit fly, *Ceratitis capitata. Oecologia*, 73(4), pp.583-590.

Kroder, S. and Messing, R.H., 2010. A new parasitoid from Kenya, *Fopius ceratitivorus*, complements the extant parasitoid guild attacking Mediterranean fruit fly in Hawaii. *Biological Control*, 53(2), pp.223-229.

Lacroix, M. and Vigneault, C., 2007. Irradiation treatment for increasing fruit and vegetable quality. *Stewart Postharvest Review* 3(3) p. 7.

Lamichhane, J.R., Aubertot, J.N., Begg, G., Birch, A.N.E., Boonekamp, P., Dachbrodt-Saaydeh, S., Hansen, J.G., Hovmøller, M.S., Jensen, J.E., Jørgensen, L.N. and Kiss, J., 2016. Networking of integrated pest management: A powerful approach to address common challenges in agriculture. Crop protection, 89, pp.139-151.

Leftwich, P.T., Koukidou, M., Rempoulakis, P., Gong, H.F., Zacharopoulou, A., Fu, G., Chapman, T., Economopoulos, A., Vontas, J. and Alphey, L., 2014. Genetic elimination of field-cage populations of Mediterranean fruit flies. *Proceedings of the Royal Society B: Biological Sciences*, 281(1792), p.20141372.

Lehane, M.J. and Lehane, M., 2005. The biology of blood-sucking in insects. *Cambridge University Press.*

Lehmann, F.O., Dickinson, M.H. and Staunton, J., 2000. The scaling of carbon dioxide release and respiratory water loss in flying fruit flies (*Drosophila spp*.). *Journal of Experimental Biology*, 203(10), pp.1613-1624.

Lester, P.J. and Petry, R.J., 1995. Gamma Irradiation for Mter Harvest Disinfestation of Diapausing Two spotted Spider Mite (Acari: Tetranychidae). *Journal of economic entomology,* 88(5), pp.1361-1364.

Levinson, H., Levinson, A. and Osterried, E., 2003. Orange‐derived stimuli regulating oviposition in the Mediterranean fruit fly. *Journal of applied entomology*, 127(5), pp.269-275.

Liu, Y.B., 2010. Recent advances in development of ultralow oxygen treatment for postharvest pest control on perishable commodities. *Stewart Postharvest Review,* 3, p.9.

Liu, Y.B., 2008. Ultralow oxygen treatment for postharvest control of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), on iceberg lettuce: I. Effects of temperature, time, and oxygen level on insect mortality and lettuce quality. *Postharvest biology and technology*, 49(1), pp.129-134.

Liu, Y.B., Daane, K.M., Steve Tebbets, J. and Bettiga, L.J., 2008. Ultralow oxygen treatment for control of *Latrodectus hesperus* (Araneae: Theridiidae) on harvested table grapes. *Journal of economic entomology*, 101(5), pp.1515-1518.

Liebhold, A.M., Zhang, X., Hohn, M.E., Elkinton, J.S., Ticehurst, M., Benzon, G.L. and Campbell, R.W., 1991. Geostatistical analysis of gypsy moth (Lepidoptera: Lymantriidae) egg mass populations. *Environmental Entomology*, 20(5), pp.1407-1417.

Lighton, J.R. and Schilman, P.E., 2007. Oxygen reperfusion damage in an insect. *PloS one*, 2(12), p.e1267.

James Niland, 2011. Queensland Fruit Fly - *Bactrocera tryoni*. *Flickr*. Brisbane. https://commons.wikimedia.org/wiki/File:Queensland_Fruit_Fly_-_Bactrocera_tryoni.jpg

López-Martínez, G. and Hahn, D.A., 2012. Short-term anoxic conditioning hormesis boosts antioxidant defenses, lowers oxidative damage following irradiation and enhances male sexual performance in the Caribbean fruit fly, *Anastrepha suspensa*. *Journal of Experimental Biology*, 215(12), pp.2150-2161.

Lux, S.A., Vilardi, J.C., Liedo, P., Gaggl, K., Calcagno, G.E., Munyiri, F.N., Vera, M.T. and Manso, F., 2002. Effects of irradiation on the courtship behavior of medfly (Diptera, Tephritidae) mass reared for the sterile insect technique. *Florida Entomologist*, 85(1), pp.102-112.

Maekawa, S. and Elert, K., 2003. The use of oxygen-free environments in the control of museum insect pests. *Getty Publications*.

Malacrida, A.R., Gomulski, L.M., Bonizzoni, M., Bertin, S., Gasperi, G. and Guglielmino, C.R., 2007. Globalization and fruitfly invasion and expansion: the medfly paradigm. *Genetica*, 131(1), p.1.

Mangan, R.L. and Hallman, G.J., 1998. Temperature treatments for quarantine security: new approaches for fresh commodities. *Temperature sensitivity in insects and application in integrated pest management*, pp.201-34.

Mason, A.C. and McBride, O.C., 1934. Effect of low temperatures on the Mediterranean fruit fly in infested fruit. *Journal of economic entomology*, 27(5), pp.897-902.

Mathooko, F.M., 1996. Regulation of respiratory metabolism in fruits and vegetables by carbon dioxide. *Postharvest Biology and Technology*, 9(3), pp.247-264.

Malan, A.P. and Manrakhan, A., 2009. Susceptibility of the Mediterranean fruit fly (*Ceratitis capitata*) and the Natal fruit fly (*Ceratitis rosa*) to entomopathogenic nematodes. Journal of Invertebrate Pathology, 100(1), pp.47-49.

May, A.W.S., 1963. An investigation of fruit flies (Trypetidae: Diptera) in Queensland. 1. Introduction, species, pest status and distribution. *Queensland Journal of Agricultural Science*, 20(1).

Mazor, M., Peysakhis, A. and Reuven, G., 2002. Release rate of ammonia-a key component in the attraction of female Mediterranean fruit fly to protein-based food lures. *IOBC wprs Bulletin*, 25(9), pp.323-328.

Mazzi, D. and Dorn, S., 2012. Movement of insect pests in agricultural landscapes. *Annals of Applied Biology*, 160(2), pp.97-113.

McPheron, B.A. and Steck, G.J. eds., 2020. Fruit fly pests: a world assessment of their biology and management. *CRC Press*.

Mditshwa, A., Fawole, O.A. and Opara, U.L., 2018. Recent developments on dynamic controlled atmosphere storage of apples-A review. *Food packaging and shelf life*, 16, pp.59-68.

Meats, A. and Edgerton, J.E., 2008. Short-and long-range dispersal of the Queensland fruit fly, *Bactrocera tryoni* and its relevance to invasive potential, sterile insect technique and surveillance trapping. *Australian Journal of Experimental Agriculture*, 48(9), pp.1237-1245.

Mitcham, E. and Yahia, E., 2009. Alternative treatments to hot water immersion for mango fruit. *Report to the National Mango Board*.

Mitcham, E.J., Lee, T., Martin, A., Zhou, S. and Kader, A.A., 2001, July. Summary of CA for arthropod control on fresh horticultural perishables. *In VIII International Controlled Atmosphere Research Conference* 600 (pp. 741-745).

Moffitt, H.R. and Albano, D.J., 1972. Effects of commercial fruit storage on stages of the codling moth. *Journal of economic entomology*, 65(3), pp.770-773.

Mohamed, S.M.A. and El-Wakkad, M.F., 2003. The role of killing bags on the reduction of Peach fruit fly in horticultural orchards. *Journal- Egyptian German Society of Zoology*, 42(E), pp.11-20.

Moss, J.I. and Jang, E.B., 1991. Effects of age and metabolic stress on heat tolerance of Mediterranean fruit fly (Diptera: Tephritidae) eggs. *Journal of economic entomology*, 84(2), pp.537-541.

Muthuthantri, W.S.N., 2013. Citrus host utilisation by the Queensland fruit fly, *Bactrocera tryoni* (Frogatt)(Diptera: Tephritidae): from individuals to populations (Doctoral dissertation, Queensland University of Technology).

Nascimento, A.S., Malavasi, A., Morgante, J.S. and Duarte, A.L.A., 1992. Hot-water immersion treatment for mangoes infested with *Anastrepha fraterculus*, *A. obliqua*, and *Ceratitis capitata* (Diptera: Tephritidae) in *Brazil. Journal of economic entomology*, 85(2), pp.456-460.

Nakagawa, S., Prokopy, R.J., Wong, T.T., Ziegler, J.R., Mitchell, S.M., Urago, T. and Harris, E.J., 1978. Visual orientation of *Ceratitis capitata* flies to fruit models. *Entomologia Experimentalis et Applicata*, 24(2), pp.193-198.

National Association of Testing Authorities (NATA), 2019. General Accreditation Guidance: Liquid-in-Glass Thermometers-Selection and Use. Copyright National Association of Testing Authorities, Australia.

Navarro-Llopis, V., Alfaro, C., Primo, J. and Vacas, S., 2011. Response of two tephritid species, *Bactrocera oleae* and *Ceratitis capitata*, to different emission levels of pheromone and parapheromone. *Crop Protection*, 30(7), pp.913-918.

Navarro, S. and Calderon, M., 1979. Mode of action of low atmospheric pressures on *Ephestia cautella* (Wlk.) pupae. *Experientia*, 35(5), pp.620-621.

Navarro, S., Dias, R. and Donahaye, E., 1985. Induced tolerance of *Sitophilus oryzae* adults to carbon dioxide. *Journal of Stored Products Research*, 21(4), pp.207-213.

Neifert, I.E., Cook, F.C. and Roark, R.C., 1925. Fumigation against grain weevils with various volatile organic compounds (No. 1313). US Department of Agriculture.

Nestel, D. and Klein, M., 1995. Geostatistical analysis of leafhopper (Homoptera: Cicadellidae) colonization and spread in deciduous orchards. *Environmental Entomology*, 24(5), pp.1032-1039.

Neven, L.G. and Mitcham, E.J., 1996. CATTS (Controlled Atmosphere Temperature Treatment System): A Novel Tool for the Development of Quarantine Treatments. *American Entomologist*, 42(1), pp.56-59.

Neven, L.G. and Rehfield-ray, L., 2006. Combined heat and controlled atmosphere quarantine treatments for control of western cherry fruit fly in sweet cherries. *Journal of economic entomology*, 99(3), pp.658-663.

New South Wales Government (NSW), 2012. Queensland Fruit Fly (QFF). Published by the *Department of Primary Industries*, Department of Trade and Investment, Regional Infrastructure and Services 2012, ISSN 1832-6668. https://www.dpi.nsw.gov.au/__data/assets/pdf_file/0014/230612/queensland-fruit-fly.pdf.

Nicolas, G. and Sillans, D., 1989. Immediate and latent effects of carbon dioxide on insects. *Annual review of entomology*, 34(1), pp.97-116.

Nishizawa, T., Tamura, A., Mitsuzuka, S., Aikawa, T., Togashi, M. and Motomura, Y., 2002. Water-soaked symptom of*'Andesunetted* melon fruit does not develop under anaerobic nitrogen atmospheres during ripening. *Plant growth regulation*, 38(1), pp.7-14.

Norrbom, A.L., Carroll, L.E., Thompson, F.C., White, I. and Freidberg, A., 1999. Systematic database of names. Myia.

Ollett, P.A., Wall, M. and Bailey, W., 2013. Influence of modified atmosphere packaging on radiation tolerance in the phytosanitary pest melon fly (Diptera: Tephritidae). Journal of economic entomology, 106(5), pp.2020-2026.

Ortega Zaleta, D.A. and Yahia K, E.M., 2000. Mortality of eggs and larvae of *Anastrepha obliqua* (Macquart) and *A. ludens* (Loew) (Diptera: Tephritidae) in controlled atmospheres and high temperature on mango (Mangifera indica) cv. Manila. *Folia Entomológica Mexicana*, (109), pp.43-53.

Ouna, A.E., 2010. Entomopathogenicity of hyphomycete fungi to fruit *fly Bactrocera invadens* (Diptera: Tephritidae) and their potential for biological control on mango (*Doctoral dissertation, The School of Pure and Applied Sciences of Kenyatta*).

Panhwar, F., 2005. Mediterranean fruit fly (*Ceratitis capitata*) attack on fruits and its control in Sindh, *Pakistan. Publisher*.

Papachristos, D.P., Papadopoulos, N.T. and Nanos, G.D., 2008. Survival and development of immature stages of the Mediterranean fruit fly (Diptera: Tephritidae) in citrus fruit. *Journal of economic entomology*, 101(3), pp.866-872.

Papadopoulos, N.T., Katsoyannos, B.I., Kouloussis, N.A., Hendrichs, J., Carey, J.R. and Heath, R.R., 2001. Early detection and population monitoring of *Ceratitis capitata* (Diptera: Tephritidae) in a mixed-fruit orchard in northern Greece. *Journal of economic entomology*, 94(4), pp.971-978.

Paull, R.E., 1994. Response of tropical horticultural commodities to insect disinfestation treatments. *HortScience*, 29(9), pp.988-996.

Peck, L.S. and Maddrell, S.H., 2005. Limitation of size by hypoxia in the fruit fly *Drosophila melanogaster*. *Journal of Experimental Zoology* Part A: Comparative Experimental Biology, 303(11), pp.968-975.

Peñarrubia María, E., 2010. Biology studies and improvement of *Ceratitis capitata* (Wiedemann) mass trapping control technique. Universitat de Lleida.

Pest Tracker exotic pest reporting, 2016. Malaysian fruit fly *Bactrocera latifrons.* http://pest.ceris.purdue.edu/pest.php?code=IOBMCQA.

Pimentel, R., Lopes, D.J.H., Mexia, A.M.M. and Mumford, J.D., 2017. Seasonality of the Mediterranean Fruit Fly (Diptera: Tephritidae) on Terceira and Sao Jorge Islands, Azores, *Portugal. Journal of Insect Science*, 17(1), p.iew097.

Prange, R.K. and Lidster, P.D., 1992. Controlled-atmosphere effects on blueberry maggot and lowbush blueberry fruit. *HortScience*, 27(10), pp.1094-1096.

Pretty, J. and Bharucha, Z.P., 2015. Integrated pest management for sustainable intensification of agriculture in Asia and Africa. *Insects*, 6(1), pp.152-182.

Project Noah, 2015. Melon fruit fly. https://www.google.com/search?q=melon+fruit+fly+project+noah&rlz=1C1GCEA_enAU903AU903&oq=melon+fruit+fly+project+no&aqs=chrome.1.69i57j33i160l2.28587j0j7&sourceid=chrome&ie=UTF-8.

Prokopy, R.J., Cooley, S.S., Prokopy, J.J., Quan, Q. and Buonaccorsi, J.P., 1994. Interactive effects of resource abundance and state of adults on residence of apple maggot (Diptera: Tephritidae) flies in host tree patches. *Environmental Entomology*, 23(2), pp.304-315.

Prokopy, R.J., Miller, N.W., Duan, J.J. and Vargas, R.I., 2000. Local enhancement of arrivals of *Ceratitis capitata* females on fruit mimics. *Entomologia experimentalis et applicata*, 97(2), pp.211-217.

Püntener, W., 1981. Manual for Field Trials in Plant Protection; *Ciba-Geigy; Limited*: Basle, Switzerland.

Radonjić, S., Čizmović, M. and Pereira, R., 2013. Population dynamics of the Mediterranean fruit fly in Montenegro. *International Journal of Insect Science*, 5, pp. IJIS-S12964.

Raga, A. and Sato, M.E., 2006. Time-mortality for fruit flies (Diptera: Tephritidae) exposed to insecticides in laboratory. *Argentina Instituto Biológico*, 73(1), pp.73-77.

Reppchen, A., Schöller, M., Prozell, S., Adler, C., Reichmuth, C. and Steidle, J.L.M., 2010. 10-Physical control of stored product insects. *Journal of Stored Products Research*, 38, pp.43-53.

Retamales, J., Manríquez, D., Castillo, P. and Defilippi, B., 2001. Controlled atmosphere in bing cherries from Chile and problems caused by quarantine treatments for export to Japan. *In VIII International Controlled Atmosphere Research Conference* 600 (pp. 149-153).

Richardson, H.H., 1952. Cold treatment of fruits. United States Department of Agriculture (USDA)(eds.), *Insects: the yearbook of agriculture*. USDA, Washington, DC, pp.404-406.

Rigamonti, I.E., 2004. Contributions to the knowledge of *Ceratitis capitata* Wied. (Diptera, Tephritidae) in Northern Italy. I. Observations on the biology.

Rini, L.A., 2003. Modifying an artificial diet for mass rearing Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae), using locally available maize meal (Doctoral dissertation, Stellenbosch: Stellenbosch University).

Rwomushana, I., 2008. Bioecology of the invasive fruit fly *Bactrocera invadens* (Diptera: Tephritidae) and its interaction with indigenous mango infesting fruit fly species. Unpublished PhD thesis, Kenyatta University, Kenya.

Saleh, A. and El-Hamalawii, M., 2004. The Population Dynamics of the Mediterranean Fruit Fly, *Ceratitis capitata* Wied. Diptera: Tephritidae in Some Fruit Orchards in Gaza Strip.

Sapir, Y., Mazer, S.J. and Holzapfel, C., 2008. Sex ratio. In Encyclopedia of Ecology, Five-Volume Set (pp. 3243-3248).

Schetelig, M.F. and Wimmer, E.A., 2011. Insect transgenesis and the sterile insect technique. In *Insect biotechnology* (pp. 169-194). Springer, Dordrecht.

Schutze, M.K., Aketarawong, N., Amornsak, W., Armstrong, K.F., Augustinos, A.A., Barr, N., Bo, W., Bourtzis, K., Boykin, L.M., Caceres, C. and Cameron, S.L., 2015. Synonymization of key pest species within the *Bactrocera dorsalis* species complex (D iptera: T ephritidae): taxonomic changes based on a review of 20 years of integrative morphological, molecular, cytogenetic, behavioural and chemoecological data. *Systematic Entomology*, 40(2), pp.456-471.

Sciarretta, A., Tabilio, M.R., Lampazzi, E., Ceccaroli, C., Colacci, M. and Trematerra, P., 2018. Analysis of the Mediterranean fruit fly [*Ceratitis capitata* (Wiedemann)] spatio-temporal distribution in relation to sex and female mating status for precision IPM. *PloS one*, 13(4), p.e0195097.

Sharma, A., Kumar, V., Shahzad, B., Tanveer, M., Sidhu, G.P.S., Handa, N., Kohli, S.K., Yadav, P., Bali, A.S., Parihar, R.D. and Dar, O.I., 2019. Worldwide pesticide usage and its impacts on ecosystem. *SN Applied Sciences*, 1(11), p.1446.

Shellie, K.C., 2006. Vine and berry response of Merlot (Vitis vinifera L.) to differential water stress. *American Journal of Entomology and Viticulture*, 57(4), pp.514-518.

Shellie, K.C. and Mangan, R.L., 2000. Postharvest disinfestation heat treatments: response of fruit and fruit fly larvae to different heating media. *Postharvest Biology and Technology*, 21(1), pp.51-60.

Shellie, K.C., Mangan, R.L. and Ingle, S.J., 1997. Tolerance of grapefruit and Mexican fruit fly larvae to heated controlled atmospheres. *Postharvest Biology and Technology*, 10(2), pp.179-186.

Shibuya, K., Onodera, S. and Hori, M., 2018. Toxic wavelength of blue light changes as insects grow. *PloS one*, 13(6), p.e0199266.

Smilanick, J.L. and Fouse, D.C., 1989. Quality of nectarines stored in insecticidal low-O_2 atmospheres at 5 and 15C. *Journal of the American Society for Horticultural Science*, (USA).

Smith, K.G., 1989. An introduction to the immature stages of British flies: Diptera larvae, with notes on eggs, puparia and pupae (Vol. 10, No. 14). *Royal Entomological Society*.

Sproul, A.N., 1976. Disinfestation of Western Australian Granny Smith apples by cold treatment against the egg and larval stages of the Mediterranean fruit fly (*Ceratitis capitata* (Wied.)). *Australian Journal of Experimental Agriculture*, 16(79), pp.280-285.

Srimartpirom, M., Burikam, I., Limohpasmanee, W., Kongratarporn, T., Thannarin, T., Bunsiri, A. and Follett, P.A., 2018. Low-dose irradiation with modified atmosphere packaging for mango against the oriental fruit fly (Diptera: Tephritidae). *Journal of economic entomology*, 111(1), pp.135-140.

Steiner, R.W., 1993. Carbon dioxide's expanding role. *Chemical Engineering*, 100(3), p.114.

Stibick, J.N., 2004. Natural enemies of true fruit flies (Tephritidae) (p. 86). United States Department of Agriculture, *Animal and Plant Health Inspection Serv*.

Strik, B.C. and Yarborough, D., 2005. Blueberry production trends in North America, 1992 to 2003, and predictions for growth. *HortTechnology*, 15(2), pp.391-398.

Sun, Y.P. and Shepaud, H.H., 1947. Methods of calculating and correcting the mortality of insects. *Journal of economic entomology*, 40(5).

Szyniszewska, A.M. and Tatem, A.J., 2014. Global assessment of seasonal potential distribution of Mediterranean fruit fly, *Ceratitis capitata* (Diptera: Tephritidae). *PLoS One*, 9(11), p.e111582.

Tanaka, N., Steiner, L.F., Ohinata, K. and Okamoto, R., 1969. Low-cost larval rearing medium for mass production of oriental and Mediterranean fruit flies. *Journal of economic entomology*, 62(4), pp.967-968.

Tang, J. ed., 2007. Heat treatments for postharvest pest control: theory and practice, CAB International, Wallingford, Cambridge.

Thomas, D.B. and Loera-Gallardo, J., 1998. Dispersal and longevity of mass-released, sterilized Mexican fruit flies (Diptera: Tephritidae). *Environmental Entomology*, 27(4), pp.1045-1052.

Thomas, M.C., Heppner, J.B., Woodruff, R.E., Weems, H.V., Steck, G.J. and Fasulo, T.R., 2001. Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann) (Insecta: Diptera: Tephritidae). Florida Department of Agriculture and Consumer Services, DPI. *Entomology Circulars*.

Tilton, E., Burkholder, W.E. and Cogburn, R.R., 1966. Effects of gamma radiation on *Rhyzopertha dominica, Sitophilus oryzae, Tribolium confusum*, and *Lasioderma serricorne*. *Journal of economic entomology*, 59(6), pp.1363-1368.

Todoriki, S., Mahbub Hasan, M., Miyanoshita, A. and Lmamura, T., 2006. Comet assay: A novel technique to detect DNA damage in larvae of *Curculio sikkimensis* (Heller) exposed to gamma irradiation. *International pest control*, 48(1).

United States Department of Agriculture (USDA), 2003. Mediterranean fruit fly Action, 2003.https://www.aphis.usda.gov/import_export/plants/manuals/emergency/downloads/medfly_action_plan.pdf.

Valentin, N. and Preusser, F., 1990. Insect control by inert gases in museums, archives and libraries. *Restaurator*, 11(1), pp.22-33.

Van Voorhies, W.A., 2009. Metabolic function in *Drosophila melanogaster* in response to hypoxia and pure oxygen. *Journal of Experimental Biology*, 212(19), pp.3132-3141.

Vanderzant, E.S., 1969. Physical aspects of artificial diets. *Entomologia Experimentalis et Applicata*, 12(5), pp.642-650.

Vargas, R.I., Piñero, J.C. and Leblanc, L., 2015. An overview of pest species of *Bactrocera* fruit flies (Diptera: Tephritidae) and the integration of biopesticides with other biological approaches for their management with a focus on the Pacific region. *Insects*, 6(2), pp.297-318.

Vargas, R.I., Miller, N.W. and Prokopy, R.J., 2002. Attraction and feeding responses of Mediterranean fruit fly and a natural enemy to protein baits laced with two novel toxins,

phloxine B and spinosad a. *Entomologia Experimentalis et Applicata*, 102(3), pp.273-282.

Vargas, R., Stark, J.D., Prokopy, R.J. and Green, T.A., 1991. Response of oriental fruit fly (Diptera: Tephritidae) and associated parasitoids (Hymenoptera: Braconidae) to different-color spheres. *Journal of economic entomology*, 84(5), pp.1503-1507.

Vayssieres, J.F., Sinzogan, A., Korie, S., Ouagoussounon, I. and Thomas-Odjo, A., 2009. Effectiveness of spinosad bait sprays (GF-120) in controlling mango-infesting fruit flies (Diptera: Tephritidae) in Benin. *Journal of economic entomology*, 102(2), pp.515-521.

Vaysslères, J.F. and Kalabane, S., 2000. Inventory and fluctuations of the catches of Diptera Tephritidae associated with mangoes in Coastal Guinea. *Fruits* (Paris), 55(4), pp.259-270.

Viel, G. and Catelot-Goldman, C., Efficacite'sur *Ceratitis capitata* Wied de 1957 fumigation des p^ fleches au dibromoethane. *Phytiatr. Phytopharm*, 6, pp.15-17.

Villers, P., Navarro, S. and DeBruin, T., 2008, September. Development of hermetic storage technology in sealed flexible storage structures. *In Proceedings of the 8th International Conference on Controlled Atmosphere and Fumigation in Stored Products*, CAF (pp. 21-26).

Vincent, C., Panneton, B. and Fleurat-Lessard, F., 2001. Physical control methods in plant protection. *Springer Science & Business Media.*

Vincent, C., Weintraub, P. and Hallman, G., 2009. Physical control of insect pests. In *Encyclopedia of Insects* (pp. 794-798). Academic Press.

Vontas, J., Hernández-Crespo, P., Margaritopoulos, J.T., Ortego, F., Feng, H.T., Mathiopoulos, K.D. and Hsu, J.C., 2011. Insecticide resistance in Tephritid flies. *Pesticide biochemistry and physiology*, 100(3), pp.199-205.

Walder, J.M.M. and Calkins, C.O., 1993. Effects of gamma radiation on the sterility and behavioral quality of the Caribbean fruit fly, *Anastrepha suspensa* (Loew) (Diptera: Tephritidae). *Scientia Agrícola*, 50(2), pp.157-165.

Ware, A.B., Du Toit, C.L.N., Mohamed, S.A., Nderitu, P.W. and Ekasi, S., 2012. Cold tolerance and disinfestation of *Bactrocera invadens* (Diptera: Tephritidae) in 'Hass' avocado. *Journal of economic entomology*, 105(6), pp.1963-1970.

Warthon, R., 1989. Classical Biological Control of fruit infesting Tephritidae, Fruit flies their Biology and Natural Enemies and Control, 3. p. 303–313.

Weems, H.V. and Fasulo, L.M., 2002. Apple Maggot, Apple Maggot Fly, *Rhagoletis pomonella* (Walsh) (Insecta: Diptera: Tephritidae). University of Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, EDIS.

White, I.M. and Elson-Harris, M.M., 1992. Fruit flies of economic significance: their identification and bionomics. *CAB international*.

White, N.D., Jayas, D.S. and Muir, W.E., 1995. Toxicity of carbon dioxide at biologically producible levels to stored-product beetles. *Environmental Entomology*, 24(3), pp.640-647.

Whittier, T.S. and Shelly, T.E., 1993. Productivity of singly vs. multiply mated female Mediterranean fruit flies, *Ceratitis capitata* (Diptera: Tephritidae). *Journal of the Kansas Entomological Society*, pp.200-209.

Whittier, T.S. and Kaneshiro, K.Y., 1991. Male mating success and female fitness in the Mediterranean fruit fly (Diptera: Tephritidae). *Annals of the Entomological Society of America*, 84(6), pp.608-611.

Wiedemann, C. R. W., 1824. Munus rectoris in Academia Christiana Albertina aditurus Analecta entomologica ex Museo Regio Havniensi maxime congesta profert iconibusque illustrat. Kiliae, pp. 1-60.

Williamson, J.G. and Lyrene, P.M., 2004. Blueberry varieties for Florida. EDIS, 2004(7).

Wolfenbarger, D.O., 1962. Tolerance of avocados to ethylene chlorobromide and ethylene dibromide dipping and fumigation. *Journal of economic entomology*, 55(4), pp.556-557.

Woods, B., Lacey, I.B., Brockway, C.A. and Johnstone, C.P., 2005. Hosts of Mediterranean fruit fly *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) from Broome and the Broome Peninsula, Western Australia. *Australian Journal of Entomology*, 44(4), pp.437-441.

World Health Organization, 2016. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. 2nd ed.; WHO Press, *BioMed Central journal*, UK,

Wright, G. and Lyrene, P., CostaExchange Ltd and Florida Foundation Seed Producers Inc, 2012. Blueberry plant named ‘C00-09’. U.S. *Patent Application* 12/802,255.

Wright, G. and Lyrene, P., CostaExchange Ltd, 2010. Blueberry plant named ‘C99-42’. U.S. *Patent Application* 12/228,525.

Yahia, E.M. ed., 2009. Modified and controlled atmospheres for the storage, transportation, and packaging of horticultural commodities. *CRC press*.

Yahia, E.M., 2006. Modified and controlled atmospheres for tropical fruits. *Stewart Postharvest Review*, 5(6), pp.1-10.

Yahia, E.M., 1998. Modified and controlled atmospheres for tropical fruits. *Horticultural Reviews-Westport, New York-*, 22, pp.123-183.

Yahia, E.M. and Ortega-Zaleta, D., 2000. Mortality of eggs and third instar larvae of *Anastrepha ludens* and A. *obliqua* with insecticidal controlled atmospheres at high temperatures. *Postharvest biology and technology*, 20(3), pp.295-302.

Yahia, E.M., Rivera, M. and Hernandez, O., 1992. Responses of papaya to short-term insecticidal oxygen atmosphere. *Journal of the American Society for Horticultural Science*, 117(1), pp.96-99.

Yan, Y., Williams, S.B., Baributsa, D. and Murdock, L.L., 2016. Hypoxia treatment of *Callosobruchus maculatus* females and its effects on reproductive output and development of progeny following exposure. *Insects*, 7(2), p.26.

Yokoyama, V.Y., Miller, G.T. and Hartsell, P.L., 1992. Pest-free period and methyl bromide fumigation for control of walnut husk fly (Diptera: Tephritidae) in stone fruits exported to New Zealand. *Journal of economic entomology*, 85(1), pp.150-156.

Zeki, C., Er, H., Özdem, A. and Bozkurt, V., 2008. Distribution and infestation of Mediterranean fruit fly (*Ceratitis capitata*) (Diptera: Tephritidae) on pome and stone fruits in Isparta and Burdur provinces (Turkey). *Munis Entomology and Zoology*, 3, pp.231-238.

Zheng, W. and Wang, S.Y., 2003. Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. *Journal of Agricultural and food Chemistry*, 51(2), pp.502-509.