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The development of Postharvest Quarantine Treatments for Peppers *Capsicum annuum* L., and Melons *Cucumis melo* L., *reticulatus* against fruit flies (*Ceratitis capitata* and *Dacus ciliatus*)

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Summary (Hebrew) I

LIST OF ABBREVIATIONS

Abbreviation	Meaning
CI	Chilling injury
EFF	Ethiopian fruit fly
L1	First larvae instar
L2	Second larvae instar
L3	Third larvae instar
Medfly	Mediterranean fruit fly
PH	Postharvest
RP	Red pepper
RH	Relative humidity
T.S.S	Total soluble solids
YP	Yellow pepper

Summary

Sweet pepper (*Capsicum annuum* L.) is one of the most important export fruit crops for Israel and other Mediterranean countries. Pepper is a host of the Mediterranean fruit fly (*Ceratitis capitata* Wiedemann) (Medfly), which is a pest of large economic importance also to a wide range of other fruit crops. For this reason, Medfly is considered one of the main quarantine pests in the world and its presence in a geographic region possess serious limitation to the export of crops to Medfly free areas: major pepper-importing countries, such as the USA and Japan, demand strict quarantine security protocols to diminish the risk of accidental introduction of insect pests and diseases through the imported fruits. Thus, the inquiry for post-harvest treatments that will kill the development of the Medfly in the fruit and its introduction to areas free of the insect is a requisite for fruit-exports.

In this study we developed a postharvest cold treatment for yellow bell peppers (YP), which can be implemented as a quarantine protocol. We used a method for artificial infestation of YP with Medfly eggs, allowing Medfly larvae to proceed to the L1, L2 and L3 stages at 25^{o}C inside bagged fruits, and then subjecting the infested fruit to cold-treatment tests using three different temperatures (1.5°C, 4°C and 7°C). The same method was used previously in order to study the effect of this post-harvest protocol on fruit quality and Medfly survival on red bell pepper fruits (RP). We also estimated the mortality temporal-trends of different Medfly larval stages (L1, L2 and L3) incubated at low temperatures on YP and RP. Larval protein and total lipid were estimated during incubation at 25°C as an indication of fruit nutritional suitability to the fly larvae, and as an explanation to different mortality trends seen in the two pepper strains. Finally, we tested the packaging and lining effects on fruit quality for all treatment temperatures.

Our results showed that all immature stages (egg, L1, L2 and L3) were totally killed by cold treatments at 1.5°C or 4°C for 21 days. This time framework simulates the average ship journey from Israel to distant markets such as Japan or the USA. The cold treatment at 7°C did not completely eliminate immature Medflies on YP, although this temperature is commonly suggested for sweet peppers storage.

The kinetics (i.e., mortality trends through time) study showed that L3 is the most cold-tolerant larval stage in YP and in RP. The larval L1 was more cold-tolerant than L2 in RP, while the opposite trend was observed for YP. These differences could be related to the nutritional and phagoestimulative differences between pepper strains, which were expressed in the ability of the developing larvae to accumulate reserves. The larvae that were fed on RP had a higher amount of accumulated lipids, which seems to derive from an increased larval metabolism resulting from the higher phagoestimulative strength of RP. This higher metabolism may have increased the sensitivity of the larvae to cold temperature, thus increasing the speed of mortality in RP. Slower metabolic rate in YP, as expressed by the slower accumulation of lipids, seems to reduce the susceptibility of the larvae to cold environments. Differences seen in the accumulation of lipids were not expressed on the accumulation of protein in developing Medfly larvae in YP and RP.

All bagged fruits presented little chilling injuries during cold quarantine treatments, especially after 21 days at 1.5°C storage and 3 more days at 20°C (shelf life), complying with commercially required levels of quality. In contrast, peppers without any lining suffered significantly higher chilling injuries that led to accelerated decay and water loss.

In conclusion, the quarantine post-harvest treatments at 1.5°C and 4°C, combined with packaging-lining for a period of 21 days, showed excellent results killing Medfly

in YP, while preserving the commercial quality of the fruits. Furthermore, we conclude that larval mortality rates vary not only among different cultivars, but also between varieties of the same cultivar. These results suggest that further investigation on other varieties of peppers is necessary in order to assess whether this cold treatment protocol will be satisfactory as a general quarantine method.

Following the efficient cold treatment of YP and RP against Medfly, we started developing a similar post-harvest protocol for Galia-type melons fruits (*Cucumis melo* L., *reticulatus*) against the Ethiopian Fruit Fly (*Dacus ciliatus* Loew) (EFF). The first step consisted on exploring ways to artificially infest melons with EFF eggs. In order to achieve this, it was necessary to disinfect the eggs before inoculation to prevent the transmission of microbial contamination to the melon, and reduce melon decay and spoilage. This step was not required for bell peppers infested with Medfly eggs. Other investigated aspect included the ability of EFF artificially inoculated eggs to develop on different developmental stages of the melon fruit. For this aim we classified melons into four different developmental stages and investigated whether the sugar content of melon has an effect on EFF egg and larval development. Our results showed that EFF eggs were able to develop in all melons stages, indicating that neither the sugar content of the fruits nor other melon chemical-properties found on the different stages of developing-melon affect the ability of the EFF eggs to complete larval-development and pupate. It was also shown that the egg-disinfection process (up to a certain percentage of chlorine) and the disinfection of the fruit inoculation surface with alcohol did not affect the ability of eggs to hatch and develop. In contrast, fruit decay, and our inability to completely sterilize the inoculation and transmission of spoilage microorganisms, clearly affected our ability to create an artificial system of EFF inoculation into melons, and the exploration of post-harvest

treatments to curtail EFF survival in exportable melons. Additional experiments are required in order to implement a cold quarantine treatment also for melons fruits.

Introduction

Sweet pepper (*Capsicum annuum* L.) is an excellent source of antioxidants, vitamins C, polyphenols and ascorbic acid, components that are important to the nutrition of human (Howard et al., 2000). For this reason it is considered one of the most important export fruit crops worldwide, especially for Israel and other Mediterranean countries. In 2010, approximately 140,000 tons of peppers of various colors were exported from Israel to Europe and the United States, most by sea-freight. Melons fruits (*Cucumis melo* L., *reticulatus*), on the other hand, although have the potential to become an important export crop for Israel, have shown low export rates during the last years: as an example, only 5,000 tons of spring and autumn melons were exported during 2010. The reason for the low export rate of melons is probably related to the high levels of pesticide-residues in the mature fruit resulting from the need of controlling pests and diseases during production, and from market competition. In addition, neither peppers nor melons are exported to the Japanese or other Far Eastern markets, because these fruits are hosts to a number of insects pests considered of quarantine importance in this region of the world.

Various pepper-importing countries, such as the USA and Japan demand quarantine security protocols to diminish the risk of accidental introduction of insect pests and disease on imported fruit. Fruit flies (Diptera: Tephritidae), such as the Mediterranean fruit fly, *Ceratitis capitata* (Medfly) and the Ethiopian fruit fly, *Dacus ciliatus* (EFF), comprise one of the groups with the highest risks of introduction to

countries or regions free of them. The Medfly is considered the most important fruit fly pest of fruit production worldwide (White and Elson-Harris, 1992). Originating from tropical regions of Africa, the Medfly is also found in southern Europe, the Middle East, Central America, the Caribbean, Australia and parts of Oceania, and is distinctly absent in cold regions of the world (Malavasi et al., 2000). The Medfly is highly polyphagous and causes damage to a very wide range of unrelated fruit crops. In Mediterranean countries, it is particularly damaging to citrus, peach, apples, mangos, persimmons, pears, apricot, table's grapes, etc. It may also transmit fruit-rotting fungi (Cayol et al., 1994). Damage to fruit crops by the Medfly is frequently high and, without control, may reach 100% (Fimiani, 1989; Fischer-Colbrie and Busch-Petersen, 1989). In Central America, losses to coffee crops were estimated at 5-15% and the berries matured earlier and fell to the ground with reduced quality (Enkerlin et al., 1989). In areas where the fly is endemic, like in Israel, the economic impacts include reduced production, increased control costs and loss of markets. The Ethiopian fruit fly (*Dacus ciliatus*) (EFF), a pest of many cucurbits, such as cucumbers, watermelons, and melons, was recently discovered in a restricted area in the southern Negev, Israel (Yarom et al., 1997). This pest is also a quarantine fruit fly for Europe, Japan and the USA. The economic importance of *D. ciliatus* in Israel has increased over recent years in the wake of its propagation to the north of Israel and the increased damage that is causing to cucurbitaceous growers. There is little information about the infestation of EFF on melon fruit.

With the increase in global trading of agricultural produce, the hazards and invasion of species of insects into new regions, in which they are considered to be quarantined pests, has increased several fold (Follet and Neven, 2006). Both, the Medfly and the EFF are considered quarantine insects in Europe and the USA

(defined by EPPO as A1; OEPP/EPPO 1990). According to the governments in these importing countries, in the event of infection, the economic damage caused by invasive insect pests is immense, to the extent of endangering local agricultural production in these countries (Enkerlin and Mumford, 1997). In order to enable the export of fresh agricultural produce from Israel to these countries, or to all the countries that demand quarantine treatment, Israel must develop post-harvest protocols that would enable reducing export risks of these insects in fresh produce without affecting the internal and external quality of the fruit.

Currently, there are a number of postharvest quarantine treatments available for fresh produce, which are applied either prior to export or during transit to destination. Some conventional physical treatments against quarantine insects include (1) exposure of fresh produce to extreme cold or heat temperatures treatments, (2) controlled air environments, (3) radiation, and (4) a combination of these. Developing a quarantine treatment against the Medfly and *D. ciliatus*, which would kill larvae of this insects without damaging the fruit's quality, would enable exporting peppers and melons to other markets that demand, or will demand in the future, quarantine treatment.

An effective post-harvest treatment must combine complete insect kill (i.e., limitation of Probit-9 as a general treatment efficacy standard: at least 99.9968329% mortality of a pest after treatment), minimal damage to plant tissue, and reasonable treatment cost (Neven et al., 2003). Heat treatment is, usually, administered by exposing the fruit or insect to hot air, or immersing the fruit in hot water at a temperature that exceeds the insect's survival threshold. Heat treatment is in general applied to tropical fruits. With avocados, the fruit is heated to a temperature of 40°C for a period of 24 hours, which reduces the survival of eggs and larvae of the melon

fruit fly (*Bactrocera curcurbitae*) by almost 100% (Yang et al., 1994). A further heat treatment that has been found to be effective in oranges and grapefruit is hot-air at a temperature of 45-46°C for a number of hours. This treatment is effective in killing eggs and larvae of quarantine insects such as the Mexican fruit fly (*Anastrepha ludens*), and is much shorter than the time required for effective cold-treatment (hours rather than days) (Mangan et al., 1998). Administering cold treatment is performed according to the same principle, but the fruit is exposed to extremely low temperatures (below the optimal storage temperature for fresh fruit).

Cold quarantine treatments, which involve the exposure of fruit to near-freezing temperatures for a period of 10–16 days, is a procedure accepted for Medfly disinfestations of citrus fruit by the regulatory agencies of most importing countries, and is currently applied on a commercial scale (Armstrong et al., 1995). Commercial quarantine treatment of citrus fruit for disinfestations of the Medfly is performed by exposing the fruit to a temperature of 1°C for 16 days (Schirra et al., 2004). In persimmon fruit it is needed exposure to 0.0°C for 12 days (Yoav Gazit, personal communication). Cold treatments by storage at 1.1, 1.6 or 2.2°C for periods of 14, 16 or 18 days, respectively, have been established by APHIS (the USA plant protection agency) to prevent living-infestations of Medfly in imported citrus fruit (Gould and Ware, 2008). Regarding bell peppers and melons, there is little information on post-harvest cold treatments for disinfestations against fruit flies.

The optimum storage temperatures for colored peppers and Galia-type melons are 7°C and approximately 5°C, respectively. Low-temperature storage is considered to be the most effective method for maintaining the quality of most fruits and vegetables (Hardenburg et al., 1986). However, temperatures lower than the optimum cold storage for commodities may cause chilling injury (CI), inducing cold damage

already after one week of storage and reducing fruit quality. Chilling Injury is a serious problem in the post-harvest handling of many tropical and subtropical commodities. Symptoms vary with different crops. In mango most cultivar are susceptible to CI below 10°C; the symptoms include grayish, scald-like discoloration on the skin, followed by pitting, uneven ripening, and poor flavor and color development (Medlicott et al., 1990). In bell pepper, cold storage below 7°C cause fruit decay, weight loss, calyx darkening and an increase to fungal infections such as: *Botrytis cinerea* and *Alternaria alternata* (Barkai-Golan 1981; Lim et al., 2007). In Galia melons, cold storage below 6°C induces to reddish-tan discoloration, pitting, surface decay, and failure to ripen (Wang, 1994). One of the methods to reduce cold damage, enabling cold quarantine treatments, is physical treatment with heat (usually hot water) immediately after harvest, with or without any type of wrapping (Fallik et al., 2009). In addition, packaging of peppers with shrink films has been reported to reduce water loss, delay softening, and extend shelf life (Gonzales-Aguilar et al., 1999; Fallik et al., 2012).

During the last five research years, our laboratory focused on finding post-harvest treatments (hot or cold) that will assure the complete killing of Medfly eggs and larvae in fresh peppers. The objective of this intensive research has been to open the Japanese market, or that of any other country with quarantine regulations for fruit flies, for the Israeli pepper (Fallik et al., 2009). Within this framework, our laboratory found that a warm pre-wash combined with individual cling film or padding, enabled storing the pepper at 1.5°C for 21 days, and an additional 3 days at 20°C, with only minimal cold damage and no effect on other quality measures of the fruit (Bar-Yosef et al., 2009). Our laboratory also demonstrated that this treatment can also assure

complete killing of artificially inoculated Medfly-eggs on red peppers (Fallik et al., 2012).

The aim of the present study was to expand, and investigate, the application of Fallik et al. (2012) protocol to yellow peppers (YP), that is known to be more susceptible to physiological and pathological deterioration than red pepper (RP) fruit (Maalekuu et al., 2003, 2004), and to study the effect of this postharvest protocol on fruit quality and Medfly survival. We also investigated differences in Medfly larvae development trends between the two cultivars, and tolerance to cold environments of the different larval stages. An additional study included the development of *D. ciliatus* eggs inoculation-protocols for melon fruit as a prerequisite to explore post-harvest treatments for this fly and this crop. The exploration was based on our previous experience gained with Medfly and peppers.

The Research Objectives

The main aims of the study were to explore postharvest cold-treatments against the Medfly larvae developing on YP, and establish an egg-inoculation protocol for *D. ciliatus* on melons. Specific objectives include:

1) Investigate a previously developed cold-treatment postharvest protocol for RP on their ability to kill eggs and larvae of the Medfly in YP, and the effect of cold-treatments on the quality of the fruit after prolonged storage and shelf-life simulation.
2) Evaluate and contrast the time-rate of mortality of the Medfly larval stages developing in RP and YP at 1.5°C.

3) Develop a protocol for the inoculation of *D. ciliatus* eggs into melons as a pre-requisite for a later evaluation of post-harvest cold-treatments for this pest in this commodity.

Material and Methods

A) Medfly survival on peppers stored in cold temperature environments

General procedures:

I. Fruit Source and preparation:

Red (cv. Cannon) and yellow (cv. Dinamo) sweet bell peppers (*C. annuum* L.) were picked in the North of the Arava during the winter and spring of 2012. The peppers were collected in January and in February. The fruit were picked at a ripeness stage of approx. 85%. All the fruit were taken from the same growers. Within the first hours after harvest, peppers were rinsed in hot water (at 55°C for approx. 15 sec), as the current established preliminary commercial treatment (Fallik et al., 1999). Following rinsing, peppers were packed in cartoons (5 kg), embedded within one plastic padded wrapping with macro perforations (XF-100 - Xtend, StePac, Tefen, Israel). Rinsed fruit followed two possibilities: (1) direct storage in cold treatments (several tested temperature-environments) and subsequent measurement of fruit quality after 21 days in different temperature-environments, plus 3 days at 20°C (sea transport and marketing simulation to distance markets (Japan), or (2) inoculation with Medfly eggs in order to explore Medfly eggs and larval survival in cold temperature-environments. Temperature environments included: 1.5°C, 4°C, 7°C and 25°C (control).

II. Measurement of fruit quality parameters before and after the cold treatments:

Fruit were stored at 1.5°C, 4°C and 7°C (control) and 95% humidity for 21 days and 3 days more at 20°C. At the end the following indices were examined:

a) Weight loss during storage (%).

b) Differences in fruit firmness resulting from storage. Firmness was measured using a "pressure gauge" with a 2-kg weight placed on top of the fruit for about 10 seconds. Results were expressed in mm deformation (flexibility) (Hamson, 1952).

c) Percentage of fruit and calyx rottenness. This parameter was measured by the presence of mycelium on the fruit peel or calyx.

d) Chilling damage index. This was defined on a scale of four levels, from 0 to 3, according to coverage area and the diameter of the cold damage of the fruit peel: 0 = No damage, 1 = slight damage (up to 10% of the fruit peel), 2 = moderate damage (up to 30% of the fruit peel, 3 = severe damage (over 30% chill damage of fruit peel). Damage index (severity damage) per carton was calculated by the following formula: (number of fruits without damage X 0 + Number of fruit with little damage X 1 + number of fruit with moderate damage X 2 + number of fruit with severe damage X 3 divided by total fruit handling) (Bar-Yosef et al., 2009).

e) Percentage of chilling injury. Fruit with cold damage were classified with the visible dint of at least 2 mm of circle spot or visible brown color in the calyx.

f) Fruit appearance index. Was defined on a scale of 5; with 1 = fruit with rottenness, with cold damage and soft; 2 = fruit decay and softness with low cold injury; 3 = fruit with acceptable quality; 4 = good fruit quality, a bit soft with no cold injury; 5 = excellent fruit quality. Fruit with an appearance index upper to 2.5 is considered the largest sales.

Two experiments were conducted and analyzed using one-way test of JMP software version 6. Each treatment included four export-quality cartons of 5 kg.

III. Artificial inoculation of peppers with medfly eggs

In order to examine the efficacy of cold treatments on the development and survival of the flies, we conducted postharvest artificial infestation of the fruit with Medfly eggs. Medfly eggs were obtained from the medfly colony of the "Israel Cohen" Institute for Biological Control, Plant Production and Marketing Board of Israel, Citrus Division. Infestation of the fruit was conducted by directly injecting a known average amount of eggs into the fruit. Eggs were suspended in an agar media, to homogenize their distribution in the suspension and make injected quantities of eggs more accurate. The inoculated suspension with Medfly eggs was prepared as follows: four drops of recently laid (last 24 hrs.) sedimented-eggs (ca. 1 ml) were dripped into 20 ml of a cold 0.1% agar solution, as suggested for the preservation of medfly eggs during storage and transport between mass rearing facilities (Mamman and Caceres, 2007;Yoav Gazit, personal communication). The solution was then gently shaken to disperse the eggs homogeneously in the agar solution. After shaking, Medfly eggs in this type of suspension spread almost uniformly throughout the solution. Injection into peppers included a volume of 0.2 ml 0.1 % agar-eggs suspension per fruit (Yoav Gazit, personal communication). The injection-hole in the fruit was then sealed with plastic adhesive (Fallik et al., 2012). Average number of eggs suspended in the aliquot of the agar suspension was quantified by counting eggs on a black filter paper (25-30 eggs/0.2ml of injection).

IV. Procurement of different larval stages for temperature environments treatments (Phase I, Fig. 1):

After inoculating the fruit with the Medfly eggs, the fruits were incubated at 25°C and 98 % RH for 5 days to procure first-stage larvae (L1) from hatched eggs, 7 days to

allow L1 larvae to molt into L2, and 9 days to permit most L2 larvae to molt into L3. Fruit incubated at 25°C for different times (5, 7 and 9 days) was inspected to verify larval stage frequency in a sample of fruit by comparing their mouthpart under stereo-microscope, and the group of fruit having the expected larval stage was then assigned to the different temperature-environment treatments. An additional group of fruit was allowed to continue at 25°C until larvae jumped out from the fruit and pupated. This fruit and pupae numbers were used as control for the cold temperature-environment treatments. Pupae were allowed to complete development until adult emergence, in order to verify the identity of the developing pupae. The results of this experiment were analyzed using Chi square-test of independence (STATGRAPHICS software).

V. Incubation of infested peppers in cold temperature-environments (Phase II and III in Fig. 1):

Medfly inoculated pepper fruit with the appropriate egg or larval stage (L1, L2 and L3) was packed in cartoons within one plastic padded wrapping with macro perforations. Cartoons with fruit were then stored at different temperatures for 21 days: 1.5°C, 4°C and 7°C. At the end of the storing period under the different temperature-environments, fruit was transferred to 25°C, and living specimens, if at all, allowed completing development until pupation. Fruit was individually hold for 12 days at 25°C, and produced pupae counted. Differences between temperature-treatments were inferred using a one way ANOVA test (STATGRAPHICS software).

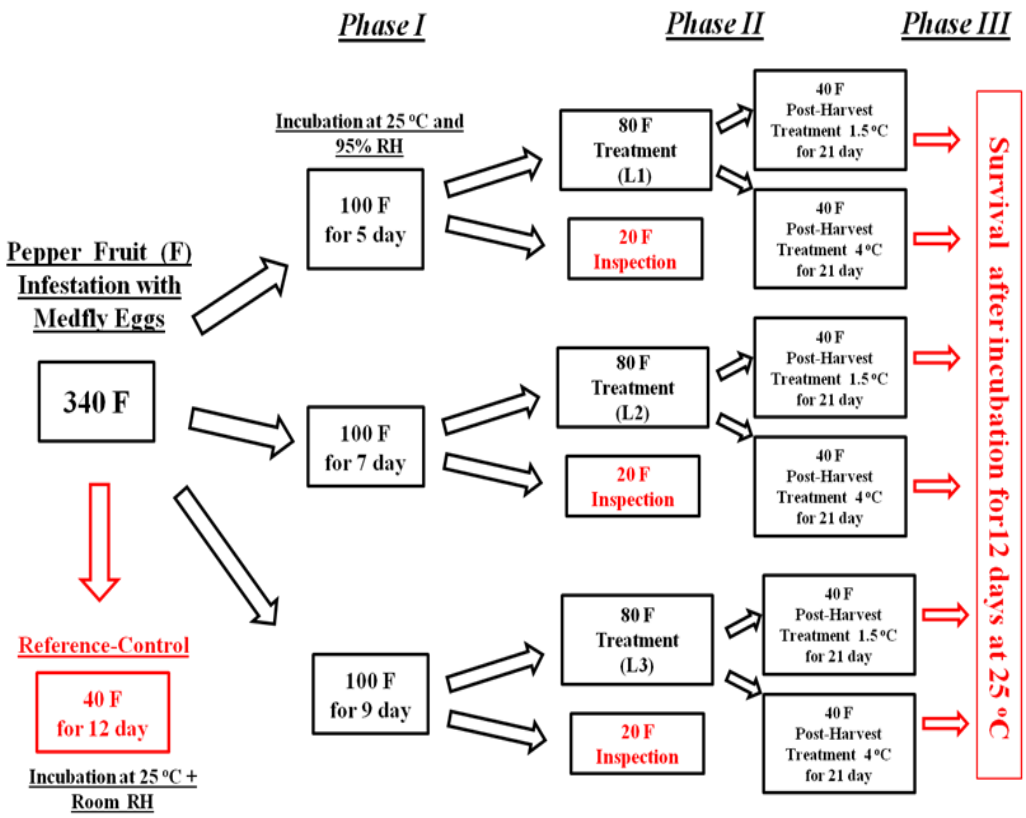

Figure 1: A scheme describing the protocol developed for studying the effect of cold treatments on the Mediterranean fruit fly larvae (Fallik et al., 2012)

B) Kinetics of larval mortality as a function of incubation-time at 1.5°C

I. Daily assessment of larval mortality in peppers hold at 1.5°C

The average time required to kill the different larval stages in a cold temperature environment of 1.5°C was investigated as follows: Procurement of the appropriate larval stage was performed as described in section II.2. Peppers were then stored at 1.5°C. Daily mortality under this temperature storage was assessed by taking daily samples of fruit, opening them and quantifying living and dead larvae under a stereo-microscope (dead larvae can be identified by their lack of motion and by their turning into black). This experiment compared the mortality-kinetics of L1, L2 and L3 both,

in YP and RP. Results were analyzed using Probit-analysis (STATGRAPHICS software).

II. Determination of lipid and protein contents in Medfly larvae developing on yellow and red pepper incubated at 25°C.

To determine lipid and protein content of Medfly larvae developing on YP and RP at 25°C and 98% RH, living larvae at the ages of 7, 8, 9 and 10 days after egg-inoculation were sampled from the fruits. Larvae of 7 and 8 days were expected to be at the L2 larval stage, while larvae of 9 and 10 days after inoculation of eggs were expected to be at the L3 stage. For each sampling date and host type, 20 larvae were sampled for protein determination and 20 for lipid determination. Larvae were individually placed in Eppendorf (1.7 ml) vials and kept at -20°C until chemical analysis. For lipid determination we only used the L3 larvae since L2 lipid contents were below detection thresholds.

Lipid and protein extraction were performed according to Nestel et al. (2003, 2004). For lipids determination, individual larvae (L3) instar were homogenized with 0.2 ml of 2% saturated sodium sulfate. Lipid extraction was conducted using Chloroform: methanol (1:2) solution. Samples were centrifuged (10,000 rpm) for 10 min. Lipids were developed with Vanillin Reagent, and colorimetrically determined at 490 nm in an ELISA reader spectrophotometer, using Triolein as a standard.

Protein content was determined by homogenizing individual larvae (L2 & L3 instar) in 0.3 ml of Phosphate Buffer Saline (PBS). After centrifugation (10,000 rpm) for 4 min, an aliquot (25 µl) was taken and diluted in 775 µl of PBS. This solution then was reacted with Bradford Reagent and measured in an ELISA reader at 595 nm, using

Bovine Serum Albumin (BSA) as a standard. Differences were analyzed using a General lineal model (STATGRAPHICS software).

C) Development of *Dacus ciliatus* eggs-inoculation Protocol on melons

I. Artificial procurement of eggs and disinfestation from fungi

Eggs were collected from the colony of *D. ciliatus* held in the dept. of entomology of the ARO. Plastic bottles (560 ml) with small holes created with a pin were used as artificial devices for egg collection. Bottles were loaded with a filter paper previously impregnated with the juice of blended zucchini as an egg-laying stimulating solution. The filter paper touched the walls of the bottle and holes, giving the sensation to the egg-laying female of a zucchini fruit. Bottles were left for an entire night, and eggs collected the following day by washing the bottle and filter paper with water, and concentrating the eggs by decantation.

In order to avoid contamination of the fruit being injected with eggs, the disinfestation of the egg-suspension with a chlorine-solution was first explored. The appropriate concentration of chlorine-solution was determined by investigating the development of fungi on an agar-media plate (1% agar + 10% sugar) and by studying the effect of chlorine concentration on egg-hatching (chlorine-treated eggs were placed on a black filter paper and incubated at 27°C). Chlorine treatments included the following concentrations: 0.25%, 0.5%, 0.75% and 1%. Eggs were suspended in the chlorine solution for 5 minutes. The effect of chlorine-disinfestation on fungal development was studied by spreading the treated eggs (15 drops per dish) on the agar plates, and incubating the plates at room temperature for 10 days. Drops of the egg-suspension with no chlorine washing were used as a control. For each concentration and control

we used 5-replicate agar plates. Development of colonies in the plates was determined qualitatively as positive or negative development.

Effect of chlorine-solution on egg-hatching was only evaluated for 2 concentrations of chlorine: 0.5% and 1% (5 min exposure). No chlorine-treated eggs were used as control. Chlorine-treated and control eggs were spread on black filter paper and incubated at 25°C for 3 days. Number of hatched eggs was determined after the incubation period. Three replicates were used per concentration.

II. Infestation of melons with *D. ciliatus* eggs

A) Fruit source and preparation

Galia melons fruit (cv Glory or 6025) were picked in the north of the Arava (Ein Yahav) twice a year from April to October 2012/2013. The fruit were picked at four different developmental stages, and classified them as: Category A: fruit of average diameter size of 5.6 cm harvested at 20% of coloration; Category B: fruit of average diameter of 8.8 cm, 30% of coloration; Category C: fruit of average size 10 cm, 40% of coloration; Category D: fruit of average size 11 cm, 50% of coloration. Eggs were collected from the colony of *D. ciliatus* held in the dept. of entomology of the ARO following the procedure described above in the artificial procurement of eggs.

B) Measurement of fruit parameters

Fruit-quality parameters were used to further classify the melons. Galia melons were weighted individually (g) and related to diameter. In addition, sugar content (%) was individually estimated by refract-meter. Color classification was performed by comparing with standard tables.

C) Artificial inoculation of melons

In the artificial inoculation of melons with *D. ciliatus* eggs were run four experiments: one of them without egg-disinfection and the other three experiments with pre-disinfected eggs with Chlorine (1% and 0.5% for 5 minutes). After disinfection, eggs were suspended in 20 ml of a cold 0.1% agar, similar to the procedure described for the inoculation of medfly-eggs to peppers (section III).The last two experiments included cleaning of the melon surface with alcohol before the inoculation of disinfected-eggs. The injection-hole in the fruit was then sealed with plastic adhesive (Fallik et al., 2012). Melons were individually incubated into 1 L plastic boxes and placed at 25°C and 42% RH for 8 to 10 days. After this period, melons were inspected and produced pupae of the EFF counted and separated. During incubation, rotten melons were discarded.

Results

A) Medfly survival on peppers stored in cold temperature environments

I. Procurement of different larval stages for temperature-environments treatments

Figure 2 and 3 show the proportion of larvae at different developmental stages after different times of incubation. After 5 days of egg-inoculation, most of the larvae were at the first instar (L1) stage, while after 7 days most were at the second instar (L2) and after 9 days they were at the third instar (L3) (Fig. 2 and 3). Figure 2 shows results for the experiment run in January 2012. Chi square- test of independence (X^2_4 = 37.75, P< 0.01) suggests that the frequency of the larvae stages during the 3 sampling dates is dependent on the time of incubation. Figure 3 shows results for the experiment run

during February 2012. Statistical inference (X^2_4, = 42.43, P< 0.01), also suggests that the frequency of larvae stages is dependent on the time of incubation.

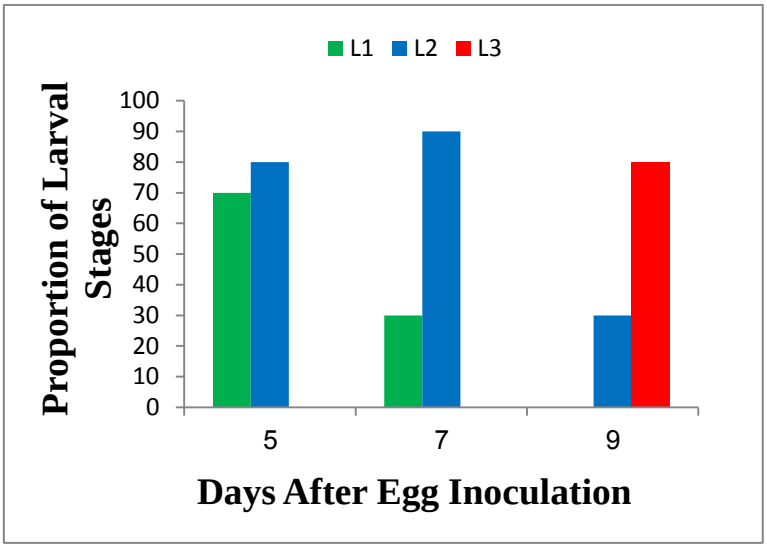

Figure 2: Frequency of larvae at different development stages (L1, L2 and L3) in yellow pepper hold at 25°C and 98% RH after 5, 7 and 9 days. Exp #1 (January 2012).

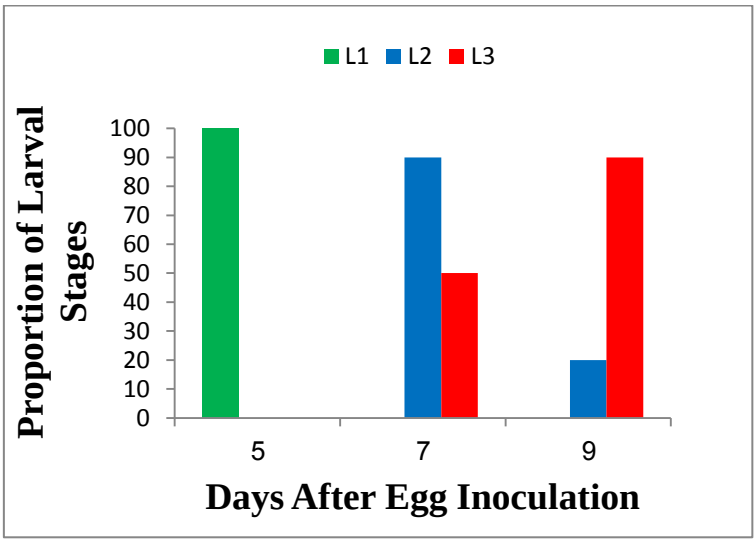

Figure 3: Frequency of larvae at different development stages (L1, L2 and L3) in yellow pepper hold at 25°C and 98% RH after 5, 7 and 9 days. Exp #2 (February 2012).

II. Postharvest temperature-treatment on different larvae stages

Table 1 and 2 provides the results on the effects of post-harvest temperature-treatments on the production of pupae from Medfly eggs on fruits incubated at 25°C and 98% RH (control) and at lower temperatures (1.5°C, 4°C and 7°C). In addition, Table 1 and 2 provide the results for the ability of Medfly larval stages (L1, L2 and L3) incubated on pepper fruits at 1.5°C, 4°C and 7°C for 21 days to reach the pupal stage. During both experiment, Medfly-eggs infested fruits incubated at temperatures of 1.5°C, 4°C, 7°C did not produce pupae at all. They proportion of fruit producing pupae significantly differed from the proportion of fruit in the control, in which 90 % of the peppers produced pupae from eggs (Table 1, for January, 2012, experiment, X^2_3 = 92, $P < 0.01$; Table 2, for February, 2012, experiment, $X^2_3 = 103$, $P < 0.01$). During the 1st experiment, fruit incubated with L1 larvae at the 3 temperatures suggest that pupal development is independent of condition of incubation (Table 1, $X^2_2 = 5.213$, $P = 0.07$), although 14% of peppers incubated at temperature of 7°C did produce some pupae. During the 2nd experiment, statistical inference do shows that production of pupae from peppers with L1 instar stage is dependent on the condition of incubation (Table 2, $X_{22} = 15.96$, $P = 0.0003$), with 30% of peppers producing pupae at temperature of 7° C. In contrast, both experiment results suggest that production of pupae by peppers with L2 larvae incubated at different temperatures is dependent of the condition of incubation (Table1, $X^2_2 = 28.4$, $P < 0.01$; Table 2, $X^2_2 = 23.9$, $P < 0.01$); in experiment 1, 70% of peppers produced pupae from L2 at temperature of 7°C, in the 2nd experiment, 50% of peppers produced pupae from L2 at temperature of 7°C. Similarly, in both experiments, results showed that production of pupae from peppers incubated at different temperatures with L3 larval stage is dependent on the condition of incubation (Table1, $X^2_2 = 26.057$, $P < 0.01$); (Table 2, $X^2_2 = 13.5$, $P = 0.0012$); in

experiment 1, 60% of peppers produced pupae at temperature of 7°C while in the 2nd experiment 30 % produced pupae.

Except for the control (peppers incubated at 25°C), no pupae was produced from peppers infested with Medfly-eggs in any of the low temperature incubation treatments in the two experiments (Table 1 and 2) (exp. 1, $F_{3,101}$ = 42.91, P< 0.01; exp. 2, $F_{3,99}$ = 104.5, P< 0.01). Average amount of pupae produced from eggs per pepper at 25°C was 8.1 pupae in experiment 1, and 9 pupae/pepper in experiment 2. In experiments using different larval stages (L1, L2 and L3) as the starting material for temperature treatment, production of pupae by peppers was only observed at 7°C, and significantly differed (except for L1 in the 1st experiment) from the nil production of pupae in peppers incubated at lower temperatures (Table 1, $F_{2,46}$ = 2.42, P = 0.10; Table 2,$F_{2,52}$ = 7.83, P< 0.01). The lack of statistical significant differences in the production of pupae from peppers bearing L1 stages in experiment 1 is due to the relatively low amount of pupae produced by peppers at 7°C (average of 0.21 pupae/pepper); in the 2nd experiment pupal production from L1 at 7°C was in average 0.6. Similar to peppers with L1, peppers incubated at 7°C with L2 and L3 larval stages produced pupae (see Table 1 and 2), which significantly differed from the production of pupae in lower temperatures (exp. 1, L2, $F_{2,43}$= 25.15, < 0.01; exp 2, L2,$F_{2,55}$= 10.53, P< 0.01; exp. 1,L3, $F_{2,45}$ = 16.63, P < 0.01; and exp. 2, L3 $F_{2,77}$= 7.11, P =0,0015).

The egg-pupal efficacy rate in peppers incubated at 25°C was 0.25 in exp. 1 (Table 1) and 0.27 in exp. 2 (Table 2). L1 larvae-pupae efficacy rate in pepper incubated at 7°C was 0.007 in exp.1 (Table1) and 0.02 in exp. 2 (Table 2). L2 larvae-pupae rate in peppers incubated at 7°C was 0.03 in exp.1 (Table 1) and 0.06 in exp.2. L3 larvae-pupae rate in pepper incubated at 7°C was 0.04 in exp.1 and 0.009 in exp.2.

Table 1: Effect of storage conditions (cold treatment) on the development of fruit fly eggs artificially injected in yellow peppers. January 2012.

Incubation Temperature Treatment	Frequency of Fruit Producing Pupae (amount of peppers in treatment)	Avg. no. of Pupae/pepper (±SD)	Avg. Efficacy (avg. no. of pupae/avg. no. of inoculated eggs)
Control (25°C)	0.9 (42)	8.1 ± 5.0	0.25
Peppers with egg stage (immediate incubation at temp. after inoculation) **PH treatment 1.5°C**	0 (22)	0	0
Peppers with egg stage (immediate incubation at temp. after inoculation) **PH treatment 4°C**	0 (20)	0	0
Peppers with egg stage (immediate incubation at temp. after inoculation) **PH treatment 7°C**	0 (21)	0	0
Peppers with L1 stage (5 days in 25°C and 98%RH, see Fig. 2) **PH treatment 1.5°C**	0 (17)	0a	0
Peppers with L1 stage (5 days in 25°C and 98%RH, see Fig. 2) **PH treatment 4°C**	0 (18)	0^{a}	0
Peppers with L1 stage (5 days in 25°C and 98%RH, see Fig. 2) **PH treatment 7°C**	0.14 (14)	0.21^{b} ± 0.58	0.007
Peppers with L2 stage (7 days in 25°C and 98%RH, see Fig. 2) **PH treatment 1.5°C**	0 (19)	0^{a}	0
Peppers with L2 stage (7 days in 25°C and 98%RH, see Fig. 2) **PH treatment 4°C**	0 (18)	0^{a}	0
Peppers with L2 stage (7 days in 25°C and 98%RH, see Fig. 2) **PH treatment 7°C**	0.7 (9)	0.89^{b} ± 0.78	0.03
Peppers with L3 stage (9 days in 25°C and 98%RH, see Fig. 2) **PH treatment 1.5°C**	0 (14)	0^{a}	0
Peppers with L3 stage (9 days in 25°C and 98%RH, see Fig. 2) **PH treatment 4°C**	0 (24)	0^{a}	0
Peppers with L3 stage (9 days in 25°C and 98%RH, see Fig. 2) **PH treatment 7°C**	0.6 (10)	1.3^{b} ± 1.4	0.04

***In column 2 (avg no. of pupae/pepper), different letters stand for significantly different amount of pupae produced for each one of the larval stage (no statistics was applied to eggs, since only the control produced pupae).**
***PH= Post-harvest**

Table 2: Effect of storage conditions (cold treatment) on the development of fruit fly eggs artificially injected in yellow peppers. February 2012

Incubation Temperature Treatment	Frequency of Fruit Producing Pupae (amount of peppers in treatment)	Avg. no. of Pupae/pepper (±SD)*	Avg. Efficacy (avg. no. of pupae/avg. no. of inoculated eggs)
Control (25°C)	1 (29)	9.0 ± 4.4	0.27
Peppers with egg stage (immediate incubation at temp. after inoculation) **PH treatment 1.5°C**	0 (26)	0	0
Peppers with egg stage (immediate incubation at temp. after inoculation) **PH treatment 4°C**	0 (24)	0	0
Peppers with egg stage (immediate incubation at temp. after inoculation) **PH treatment 7°C**	0 (24)	0	0
Peppers with L1 stage (5 days in 25°C and 98%RH, see Fig. 2) **PH treatment 1.5°C**	0 (18)	0^{a}	0
Peppers with L1 stage (5 days in 25°C and 98%RH, see Fig. 2) **PH treatment 4°C**	0 (20)	0^{a}	0
Peppers with L1 stage (5 days in 25°C and 98%RH, see Fig. 2) **PH treatment 7°C**	0.3 (19)	0.6^{b} ± 0.9	0.02
Peppers with L2 stage (7 days in 25°C and 98%RH, see Fig. 2) **PH treatment 1.5°C**	0 (19)	0^{a}	0
Peppers with L2 stage (7 days in 25°C and 98%RH, see Fig. 2) **PH treatment 4°C**	0 (18)	0^{a}	0
Peppers with L2 stage (7 days in 25°C and 98%RH, see Fig. 2) **PH treatment 7°C**	0.5 (21)	2^{b} ± 2.6	0.06
Peppers with L3 stage (9 days in 25°C and 98%RH, see Fig. 2) **PH treatment 1.5°C**	0 (36)	0^{a}	0
Peppers with L3 stage (9 days in 25°C and 98%RH, see Fig. 2) **PH treatment 4°C**	0 (29)	0^{a}	0
Peppers with L3 stage (9 days in 25°C and 98%RH, see Fig. 2) **PH treatment 7°C**	0.2 (15)	0.3^{b} ± 0.72	0.009

***In column 2 (avg no. of pupae/pepper), different letters stand for significantly different amount of pupae produced for each one of the larval stage (no statistics was applied to eggs, since only the control produced pupae).**
***PH= Post-harvest**

III. Effect of cold treatment on fruit quality of yellow pepper

Table 3 and 4 show the effect of cold treatment on fruit quality of YP picked in January and February (2012). We found that, compared to fruit stored in without lining, fruit packing XF lining and stored at 4°C or 1.5°C' for three weeks plus 3 days at 20°C, significantly reduced the severity of cold damage index and the percentage of chilling injury.

No significant difference was found on fruit stored without lining compare to lining fruit at 7°C (Table 3 and 4). On the other hand, we found (in both experiments) that fruit packed with XF lining, and stored in the three difference temperature (1.5°C, 4°C, 7°C), loosed less weight as compared to the weight lost by fruits stored without lining (Table 3 and 4). In both experiments, fruit packed with XF lining were maintained at acceptable levels in firmness, except fruits stored at 4°C, in which we found loss of turgor (Table 3 and 4). Fruit decay was found less on fruits packed with XF lining in both experiment except on fruits stored at 4°C (Table 3 and 4). For the three different temperatures, results from the two experiments suggest that fruits packed with XF lining had "better appearance" compare to fruits stored without lining.

Table 3: Effect of cold treatment on fruit quality of yellow pepper picked in January (2012). Parameters describe situation after 21 days of low temperatures + 3 days at 20°C (average of 4 cartons) (CIN-chilling index; CI-chilling injury).

Handling / storage temperature	Weight loss (%)	Flexibility (mm)	T.S.S (%)[2]	Decay (%)	CIN (3-0)	CI (%)	Appearance (1-5)
7°C without lining	4.2b	3.0a	7.4a	6.1b	0b	0a	**2.3 b**
7°C + lining XF100[1]	1.9cd	2.3b	7.7a	5.8b	0b	0a	**2.5 ab**
4°C without lining	2.5c	2.8ab	7.7a	3.8c	0.3b	5.2b	**2.5 ab**
4°C XF100[1]	1.5d	1.7c	7.4a	5.1b	0.03b	3.0c	**2.7a**
1.5°C without lining	5.1a	3.3a	7.6a	7.8a	1.9a	33a	**2.0 c**
1.5°C + lining XF100[1]	1.9cd	1.6c	7.3a	1.6d	0.05b	4.8b	**2.6[a]**

***Means with the same letter in the same column are not significantly different**
[1] fruit packed lining XF-100
[2]T.S.S= total soluble solids, sugar content

Table 4: Effect of cold treatment on fruit quality of yellow pepper picked in February (2012). Parameters describe situation after 21 days of low temperatures + 3 days at 20°C (average of 4 cartons) (CIN-chilling index; CI-chilling injury).

Handling / storage temperature	Weight loss (%)	Flexibility (mm)	T.S.S[2] (%)	Decay (%)	CIN (3-0)	CI (%)	Appearance (1-5)
7°C without lining	5.6a	3.9a	7.1a	9.3a	0b	0d	**2.4b**
7°C + lining XF100[1]	2.1cd	2.9b	6.9a	5.6b	0b	0d	**2.2c**
4°C without lining	2.6c	2.3c	7.1a	2.4c	0.5b	2.6b	**2.6b**
4°C + lining XF100[1]	1.9d	2.6b	7.0a	5.6b	0.02b	1.5c	**2.9a**
1.5°C without lining	4.2b	3.2b	6.7a	1.9c	1.3a	16.1a	**2.2c**
1.5°C + lining XF100[1]	1.7d	2.4c	7.2a	1.4d	0.1b	2.9b	**2.6b**

***Means with the same letter in the same column are not significantly different**
[1] fruit packed lining XF-100
[2]T.S.S= total soluble solids, sugar content

B) Kinetics of larval mortality as a function of incubation-time at 1.5°C

Figure 4 shows the average of two experiments (run in March 2012 and February 2013) on the kinetics of larval-mortality (L1, L2 and L3 instars stages) developing on YP and RP that were exposed to 1.5°C for different times (inset showed the modeled probit). On YP (Fig. 4A), L1 larvae dies faster than L2 and L3 instar larvae exposed to 1.5°C. Similarly, L2 dies faster than L3. LT_{50} (probit analysis) for L1 in YP was 1.8 days (for Confidence Intervals refer to Table 5) and LT_{99} 6.23 days, while LT_{50} for L2 and L3 were 3.27 and 3.97 days (Table 5). LT_{99} for L2 and L3 in YP were 8.16 and 8.96 (Table 5). Mortality of larval stages exposed to 1.5°C in RP can be observed in Fig. 4B. LT_{50} for L1, L2 and L3 on RP was 2.47, 1.95 and 2.88, respectively. LT_{99} was 5.94, 5.16 and 7.02, respectively. In contrast to the observed in YP, L2 larva in RP was relatively more susceptible than L1. In general, larval stages exposed to 1.5°C were more susceptible to the low temperature when they were developing in RP and YP (Fig. 4 and Table 5).

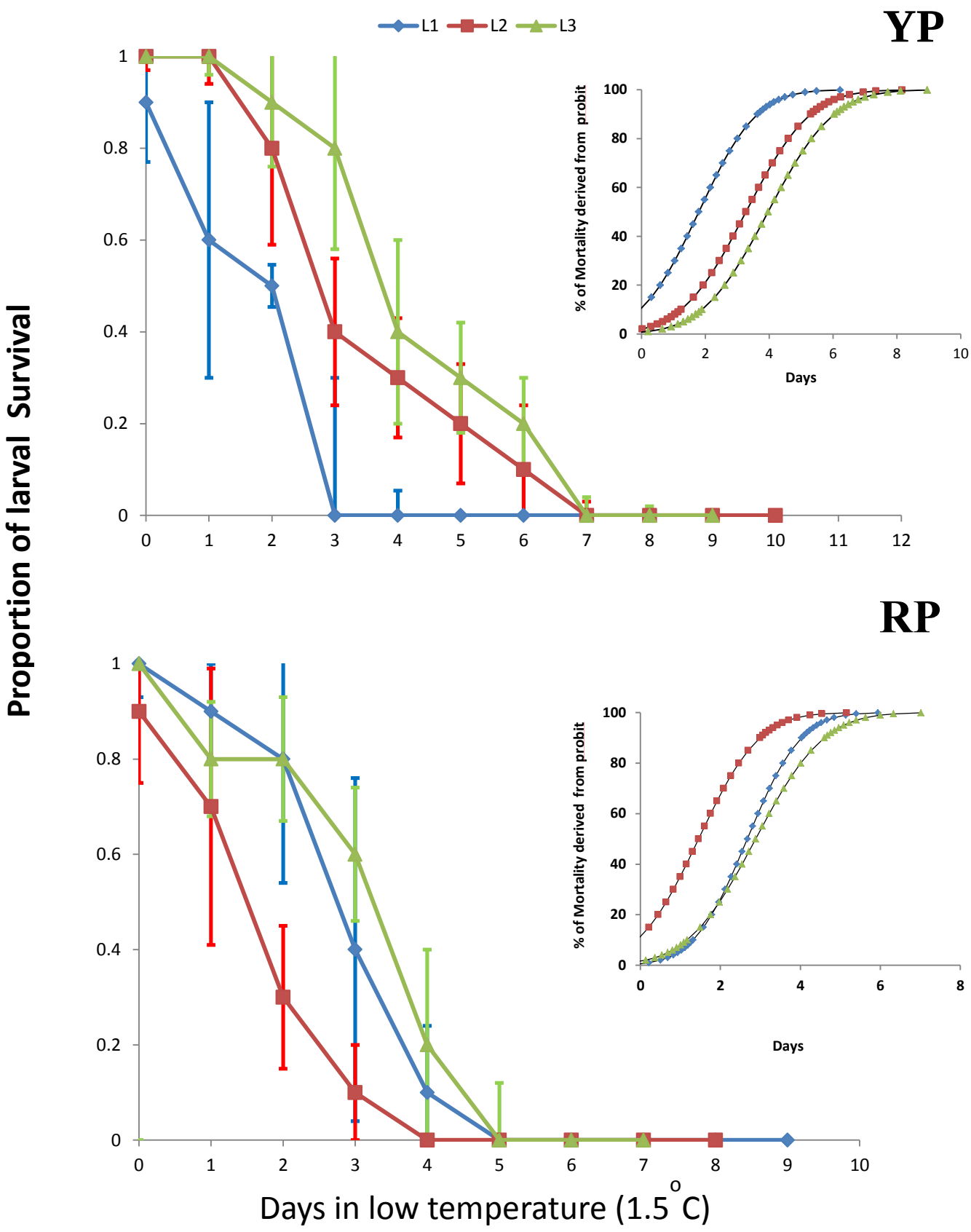

Figure 4: Kinetics of larval survival/mortality as a function of exposure-time at 1.5°C. yellow pepper (YP) and red pepper (RP).

Table 5: Calculated 50% and 99.9% lethal time (LT_{50}, $LT_{99.9}$), and 95% upper and lower limits, using Probit-Analysis for different Medfly larval stages developing

Host/stage	LT_{50} (days)	lower 95%	upper 95%	$LT_{99.9}$ (days)	lower 95%	upper 95%
Yellow pepper L1	1.80	1.65	1.94	6.23	5.87	6.65
Yellow pepper L2	3.27	3.16	3.39	8.16	7.84	8.54
Yellow pepper L3	3.97	3.86	4.08	8.96	8.64	9.32
Red pepper L1	2.67	2.58	2.77	5.94	5.67	6.27
Red pepper L2	1.45	1.36	1.55	5.16	4.87	5.51
Red pepper L3	2.88	2.79	2.98	7.02	6.74	7.34

C) Determination of lipid and protein contents in Medfly larvae developing on yellow and red pepper incubated at 25°C.

Figure 5 shows the average lipid content after 9 and 10 days of larvae (L3 instar) developing on YP and RP incubated at 25°C. Lipid content on L3 larva instar feeding on YP and RP significantly differ between them ($F_{1,36}$ = 5.5, P< 0,05). No differences were found in the lipid contents of larvae sampled during the two age-periods ($F_{1,36}$ = 0.27, P = 0.6). Figure 6 describes protein content of developing larvae in RP and YP incubated at 25°C. No significant differences were found on the content of protein between larvae feeding on YP and RP ($F_{1,76}$ = 0.01, P = 0.9). On the other hand, larval stage (L2 and L3) significantly differed in their amount of protein ($F_{1,76}$ = 76.7, P< 0.01). No significant interactions were found between type of pepper and larval stage ($F_{1,76}$ = 1.08, P = 0.3).

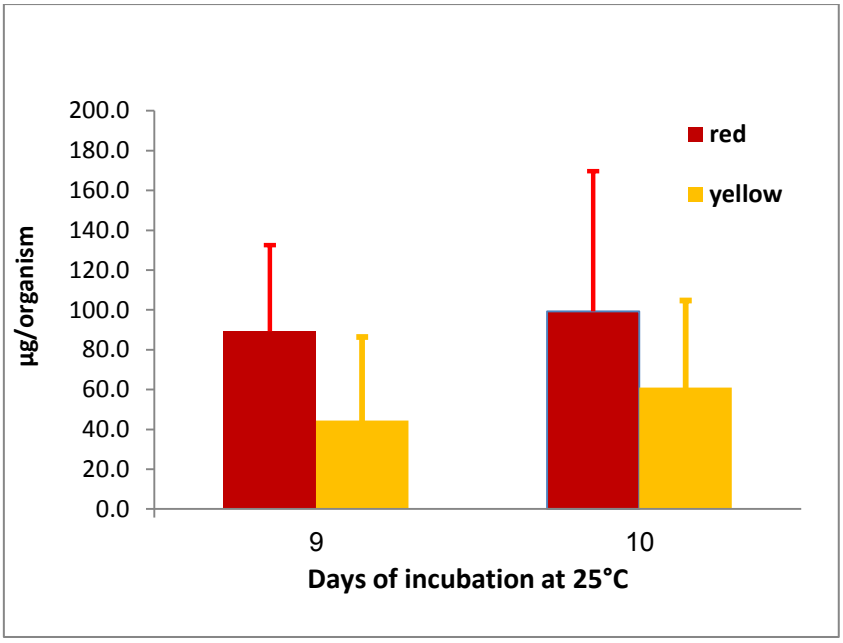

Figure 5: Lipid content of larvae (stage- L3) developed in yellow and red peppers.

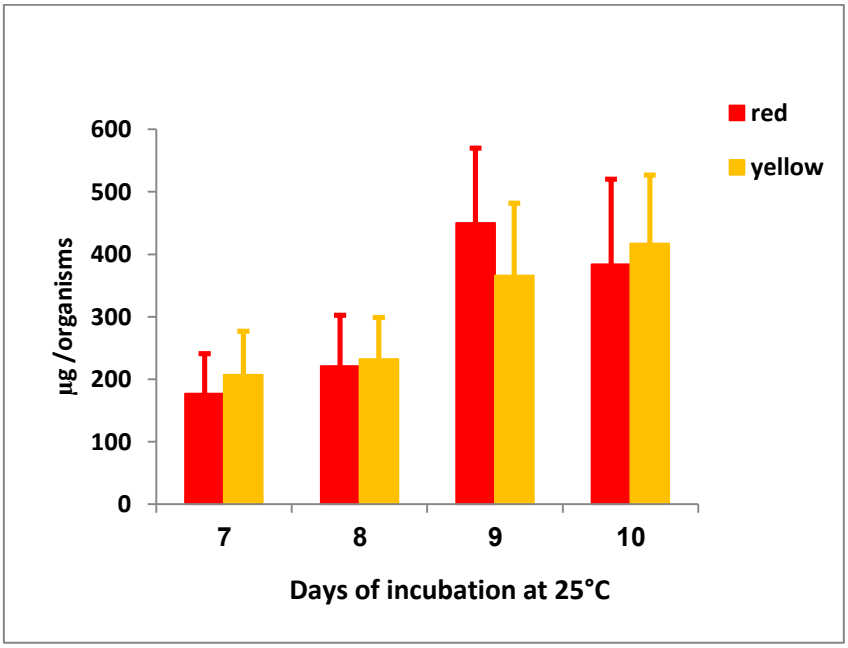

Figure 6: Protein content of larvae (stage-L2, L3) developed in yellow and red pepper.

D) Development of a protocol for the inoculation of *Dacus ciliatus* eggs to melons

I. Fungi-development on AGAR- media plates at different chlorine-concentration, and the effect of chlorine-solution on egg-hatching.

Table 6 shows the proportion of fungi-development on AGAR–media plates (1% agar+10% sugar), seeded with *D. ciliatus* eggs after chlorine disinfection (at 1%, 0.75%, 0.5%, 0.25%). As a control, eggs were washed only with water. Results show that the lower the chlorine concentration, the higher the probability of fungi to develop (X^2_4= 18.34, P = 0.001) (Table 6). Level of eggs-hatching is also dependent to the concentration of chlorine solution (X^2_4= 77.78, P <0.01): the higher the chlorine concentration the lower the hatching level (Table 7).

Table 6: Proportion of agar petri-dishes seeded with eggs after chlorine disinfestation showing fungi development

	Chlorine- Concentration				
	0% (control)	0.25%	0.5%	0.75%	1%
% Fungi Development	100%	80%	20 %	0 %	0 %

Table 7: Percentage of *D. ciliatus* egg-hatching after treatment with chlorine

	Chlorine- Concentration				
	0% (control)	0.25%	0.5%	0.75%	1%
% egg-hatching	91%	86%	78%	49%	47%

II. Production of larvae from melon-fruit artificially-inoculated with *D. ciliatus* eggs: effect of chlorine disinfection

Tables 8, 9, 10, 11 describe the production of larvae of *D. ciliatus* on melon fruits developing from artificially inoculated eggs. Towards the development of the protocol, four different experiments were performed, each adding a different step (disinfection type and form) that will improve the outcome and the integrity of the melons. Melons used for the experiments were initially classified by color and size into four categories during field sampling (A, B, C, and D). In addition, within each category melons were described by their average diameter, weight and sugar content.

In all the experiments, melon diameter significantly differed between melon categories (Table 8, 9, 10, 11) ($F_{3,12}$ = 5.01, P = 0,0176 for 1st exp.), ($F_{3,18}$= 61.4, P < 0.01 for 2nd exp.), ($F_{3,35}$= 3.15, P < 0.01 for 3rd exp.), ($F_{3,16}$= 19.1, P < 0.01 for 4th exp.). Similarly, fruit weight significantly differed between all categories. Regarding weight, fruit in different categories significantly differed between them (Table 8, 9, 10, 11) ($F_{3,12}$ = 8.4, P = 0,003 for 1st exp.), ($F_{3,18}$= 11.46, P < 0.01 for 2nd exp.),

($F_{3,35}$= 8.78, P < 0.01 for 3rd exp.), ($F_{3,16}$= 19.05, P < 0.01 for 4th exp.). Regarding melon sugar-contents, sugar significantly increased t with more developed phenological stage (i.e., melon category), which peaks in mature melons (Table 8, 9, 10, 11) ($F_{3,12}$ = 389.3, P< 0.01 for 1st exp.), ($F_{3,18}$= 105.17, P < 0.01 for 2nd exp.), ($F_{3,35}$= 32.9, P < 0.01 for 3rd exp.), ($F_{3,16}$= 81.9, P < 0.01 for 4th exp.). Mature melons (category D) in general had 2-3 times more sugar than early development stages (e.g., category A and B).

Regarding the effect of disinfection protocols, proportion of fruit decay (i.e., fruit removed from the experiment without allowing eggs to reach the pupal stage), is independent of the fruit development stage in all experiments (Table 8, 9, 10, 11),

(X^2= 2.5, P = 0.48 for 1st exp.), (X^2= 0, P = 1 for 2nd exp.), (X^2= 2.1, P = 0.56 for 3rd exp.), (X^2= 4.3, P = 0.23 for 4th exp.). On the other hand, in the 1st experiment, all the melons inoculated with no chlorine pre-washed-eggs produce pupae (Table 8). Pupal production was independent from melon categories (X^2_3= 1.8, P = 0.6). Similarly, all the melons inoculated with chlorine (1%) pre-washed-eggs produced pupae (Table 9). Again, in this experiment, pupal production was independent from melon category (X^2_3= 3.2, P = 0.3). In contrast to the previous two experiments, in experiment 3, where eggs were pre-washed with chlorine (1%) and the whole melon wiped with alcohol, melons in category D (completely mature) did not produce pupae (Table 10), while the other 3 categories produced pupae (X^2= 7.8, P = 0.05). Similarly, in the last experiment, when we only used 0.5% chlorine pre-washing and the whole melon was submerged and cleaned with water, pupae were only produced in categories A and B (X^2= 13, P = 0.018) (Table 11).

Table 8: Characterization of melon stages and their ability to withstand decay and produce pupae with no disinfection treatment of eggs and melon before inoculation

Experiment 1 Without Chlorine disinfection					
Melon developmental stages	**Circumference (cm) Avg ±SD**	**Weigth (gr) Avg ±SD**	**Sugar content (%) Avg±SD**	**Proportion of fruit removed from batch for decaying**	**Proportion of fruit producing pupae (amount of melons in treatment)**
A	**$5.24^{a} \pm 1.04$**	**$118.15^{a} \pm 32.6$**	**$4.63^{a} \pm 0.37$**	**20%**	**1(4)**
B	**$11.37^{ab} \pm 0.38$**	**$809.10^{b} \pm 533$**	**$5.3^{a} \pm 0.42$**	**20%**	**0.7(4)**
C	**$16.4^{b} \pm 7.32$**	**$773.94^{bc} \pm 212.8$**	**$12.24^{b} \pm 0.67$**	**0%**	**0.8(5)**
D	**$10.36^{b} \pm 0.31$**	**$885.9^{c} \pm 89.9$**	**$14.03^{c} \pm 0.15$**	**40%**	**1(3)**

***Means with the same letter in the same column are not significantly different**
****Colors on lines describe the relative color of the melon stage**

Table 9: Characterization of melon categories, and their ability to withstand decay and produce pupae after chlorine (1%) pre-washing of eggs as a disinfection treatment before egg-inoculation (Experiment 2)

Experiment 2 With Chlorine (1%) disinfection					
Melon developmental stages	**Circumference (cm) Avg ±SD**	**Weigth (gr) Avg ±SD**	**Sugar content (%) Avg±SD**	**Proportion of fruit removed from batch for decaying**	**Frequency of fruit producing pupae (amount of melons in treatment)**
A	**$4.63^{a} \pm 0.7$**	**$63.5^{a} \pm 15.7$**	**$5.4^{a} \pm 0.79$**	**20%**	**1(4)**
B	**$9.65^{b} \pm 1.2$**	**$587.1^{a} \pm 180.3$**	**$4.8^{a} \pm 0.55$**	**20%**	**1(4)**
C	**$11.27^{bc} \pm 0.46$**	**$1001.8^{b} \pm 274.9$**	**$5.74^{a} \pm 0.27$**	**20%**	**1(4)**
D	**$10.32^{c} \pm 1.06$**	**$830.33^{b} \pm 460.2$**	**$12.3^{b} \pm 1.22$**	**20%**	**0.75(4)**

***Means with the same letter in the same column are not significantly different**
****Colors on lines describe the relative color of the melon stage**

Table 10: Characterization of melon categories, and their ability to withstand decay and produce pupae after chlorine (1%) pre-washing of eggs and alcohol wiping of melon surface as a disinfection treatment before egg-inoculation (Experiment 3).

Experiment 3 With Chlorine disinfection 1% + alcohol disinfection all the melon					
Melon developmental stage	**Circumference (cm) Avg ±SD**	**Weigth (gr) Avg ±SD**	**Sugar content (%) Avg±SD**	**Proportion of fruit removed from batch for decaying**	**Frequency of fruit producing pupae (amount of melons in treatment)**
A	**7.07^{a}±0.46**	**188.59^{a}±37.5**	**4.5^{a}±0.91**	**20%**	**0.13(8)**
B	**7.7^{a}±0.79**	**244.45^{a}±79.8**	**6.9^{b}±1.22**	**0%**	**0.5(10)**
C	**8.67^{b}±0.57**	**341.36^{b}±60.74**	**9.14^{c}±1.45**	**30%**	**0.29(7)**
D	**8.55^{b}±0.69**	**342.31^{b}±111.33**	**11.12^{d}±2.14**	**33%**	**0(10)**

***Means with the same letter in the same column are not significantly different**
****Colors on lines describe the relative color of the melon stage**

Table 11: Characterization of melon categories, and their ability to withstand decay and produce pupae after chlorine (0.5%) pre-washing of eggs and alcohol wiping of melon surface as a disinfection treatment before egg-inoculation (Experiment 4).

Experiment 4 With Chlorine disinfection (0.5%) + pre-washing +alcohol disinfection all the melon					
Melon developmental stage	**Circumference (cm) Avg ±SD**	**Weigth (gr) Avg ±SD**	**Sugar content (%) Avg±SD**	**Proportion of fruit removed from batch for decaying**	**Frequency of fruit producing pupae (amount of melons in treatment)**
A	**5.5^{a}±0.87**	**99.22^{a}±38.12**	**5.8^{a}±0.56**	**10%**	**0.2(9)**
B	**6.65^{b}±0.28**	**175.73^{b}±26.3**	**7.08^{b}±0.23**	**10%**	**0.6(9)**
C	**7.72^{c}±0.38**	**224.63^{c}±24.37**	**9.08^{c}±0.79**	**0%**	**0(10)**
D	**7.69^{c}±0.4**	**255.2^{c}±46.07**	**11.24^{d}±0.6**	**0%**	**0(10)**

***Means with the same letter in the same column are not significantly different**
****Colors on lines describe the relative color of the melon stage**

Discussion

I. Effect of cold environment on the development of medfly eggs and larvae and quality of fruit

In order to procure the proper stage of Medfly (*C. capitata*) larvae to be tested on the investigation of post-harvest cold treatments, we followed the same protocol that has been developed previously for RP by Fallik et al. (2012). This protocol allows the acquirement of the required instars stages (L1, L2 and L3) using different times of incubation (5 days, 7 days and 9 days, respectively) at 25°C with 98% RH. Our results confirm the usefulness of this system to study effects of cold treatments on different stages of the Medfly larvae developing on YP. Similar protocols were developed by De Lima et al. (2010) for the Medfly and Queensland (*Bactrocera tryoni*) fruit flies developing on citrus cultivars (orange, mandarin and lemon). However, in contrast to our study, they classified the larval instars using a period of time (L1: 3-5 days, L2: 6-8, L3 to 9-10 days) instead of a single day, which allowed them to procure a more normal distribution of the larval-stage population.

Our results showed the effectiveness of cold treatment in arresting the development of Medfly-eggs, and the 3 larval stages on YP, for the first time. The fact that no pupae was found after 21 days of storage at 1.5°C followed by 12 days of incubation at 25°C, suggest that eggs, L1, L2 and L3 larvae were unable to withstand the cold-temperature for such a long period of time and proceed with normal development (Table 1 & 2). Similar results were reported by Fallik et al. (2012) for all Medfly immature stages developing on RP. In the same way, cold treatment of infested table-grapes at 1°C for 16 days successfully arrested Medfly development of

all stages (eggs, L1, L2 and L3) as well as the development of the Queensland (*Bactrocera tryoni*) fruit fly immature stages after 14 days (De Lima et al., 2012). Santaballa et al. (2009) also reported the effectiveness of cold treatment to kill Medfly on clementin-mandarin fruit when stored at 2°C for 14 days. In addition, cold treatment protocols (1.5^{o}C for 16 days) against the Medfly have been established by the Israel Cohen Institute for Biological Control to export citrus to Japan (oranges, pomelo, grapefruit, lemon and oro blanco; for 'Or' mandarin at 2^{o}C for 18 days) (Yoav Gazit, personal communication).

Our study confirmed the fact that development of Medfly eggs, L1, L2 and L3 also stopped even at 4°C for 21 days on YP (Tables 3 & 4), similarly to what was obtained by Fallik et al. (2012) and for Medfly eggs developing on table-grapes stored at 3°C for 20 days (De Lima et al., 2011). The effectiveness in stopping egg-development of the Mexican fruit fly (*Anastrepha ludens*) in avocado fruit maintained at 4°C for 9 days was reported by Aluja et al. (2010), suggesting that temperatures below 4°C seem to effectively stop development of eggs, and immature stages, in several species of fruit fly. In contrast, and as suggested by the results of this study, storage of peppers at 7°C, even for 21 days, does not succeed in arresting Medfly egg, L1, L2 and L3 stages on YP. This same tendency was previously observed on RP (Nestel and Fallik, unpublished results).

Quarantine treatment for pest disinfestations of fruit commodities is essential for international trade (Follett and Neven, 2006). As known, the implementation of different post-harvest treatment (heat or cold) may lead to lower quality and deterioration of the fruit-commodities (Hansen, 1992; Wang et al., 1994). The results of this study, and the previous one on RP (Fallik et al., 2012), suggest that the utilization of Xtend packaging-lining, maintained the quality and turgidity of YP

stored at 1.5 and 4°C for 21 days. This result is encouraging and opens the possibility of shipping peppers with different colors from Israel to far-away markets keeping the quality of the produce in good conditions, and securing the disinfestation of peppers from Medfly.

Cold treatment is gaining ground as a postharvest treatment of agricultural commodities for phytosanitary purposes (Heather and Hallman, 2008). This work contributes to the general trend suggesting that cold treatments can be effectively applied as a post-harvest treatment, reducing the risk of exporting pests to other regions and minimizing damage to the fruit.

II. Temporal-trends (kinetics) of larval mortality on fruit maintained under cold-temperatures

Inquiring on the larval mortality patterns is an important aspect in the study of the effectiveness of post-harvest treatment. It is also suggested that patterns of larval mortality in cold environments will be investigated separately for different fruit types, and even varieties, since fruit fly larvae, even from the same species, are known to have different developmental rates on different hosts. Our study confirmed that even within the same host, but different variety, larval development, and mortality varies. Medfly larval stages developing on YP at storage temperatures of 1.5°C die within the first 8 days of cold-storage. In contrast, Medfly larvae developing on RP at storage temperatures of 1.5°C die earlier: all the larval-population (of all stages) was dead within the first 6 days of cold-storage. Similar differences in time required to kill all larvae with cold-environments treatments on other fruits have been reported by

Santaballa et al. (2009) on Medfly developing on clementine mandarin maintained at 2°C (required 12 days of cold-storage are required to kill all the larval population), and on Medfly larvae developing on table grapes maintained at 1°C: complete Medfly larval population mortality required 12 days (De Lima et al., 2011).

It is commonly agreed that the most cold-tolerant Medfly immature stage is the L3 instar. Powel (2003) analyzed the data of Back and Pemberton (1916) who identified and determined that L3 instar is the most cold tolerant in apples and peaches. In fact, our results showed that it took longer for cold-temperatures (1.5°C) to completely kill the population of L3 instar developing on YP, suggesting, as expected, that the L3 instar is the most resistant of the larval instars to cold temperature. Additionally, L3 larvae developing on RP maintained at 1.5°C was more cold tolerant than L1 and L2 instars larvae. However, L2 instar was relatively more susceptible than L1, suggesting that in certain fruits the more tolerant stage may differ. Some studies with other fruits and fruit fly species have shown similar patterns. The Queensland fruit fly (*Bactrocera tryoni*) L1 larvae developing on table grapes maintained at 1°C, as an example, showed stronger resistance to cold-temperatures than L2 and L3. In contrast, Medfly L2 developing in grapes was found more tolerant to cold-temperatures than Medfly L1 and L3 (De Lima et al., 2011). The same authors (Jessup and De Lima, 1993) found that Medfly L2 instar and Queensland fruit fly L1 instar are more cold-tolerant than the other larval instars when developing on lemon fruit maintained at 1°C.

A possible explanation to the cold-tolerance differences between larval stages may be related to the degree of phagostimulation of the fruit and to the rate of food consumption found in the different larval stages. A study in *Drosophila melanogaster* indicated that larval nutrition has a strong impact on larvae stress tolerance (Andersen

et al., 2010). That is, it is possible that a fruit that is more palatable and nutritious to the fruit fly larvae will increase the rate and speed of consumption in larval stages that are naturally more actively feeding, like L3, increasing the metabolic rate of the organism. A higher metabolically rate, on the other hand, may increase the vulnerability of the organism to stressful environments and killing agents, like insecticides. This may be the case with RP, in which L2 was more vulnerable than L1 and L3 stages to the cold environment. In contrast, were the fruit is less palatable to the larvae, the vulnerability to cold-environments may not be completely determined by the feeding and metabolic rate of the organism. The fact that L1 is more vulnerable than L2 and L3 to cold-environments when developing on YP suggests that feeding rates may be slower than in RP, and that the organismal-size may be responsible for the vulnerability of the organism to the stressful environment. That is, the lower metabolic rate, which derives from the slower feeding-rates and lower-palatability, protected L2 and L3 from dying faster in the cold environment. Although we do not have direct behavioral evidence of the feeding rates of Medfly larvae in RP and YP, indirect measurements point in this direction. Lipid accumulation in larval Medfly has been shown to depend on the quality of the food (Nestel et al., 2004). The faster accumulation of lipids in Medfly larvae developing on RP (Fig. 5) suggest that RP, in contrast to YP, allows Medfly to develop faster and accumulate more reserves earlier, pointing at a higher phagoestimulative and nutritious quality of the red variety. This effect was not evident with protein, which seems to be less responsive to diet effects (Nestel et al., 2004). The nutritional quality of the fruit for the Medfly larvae development and its effects upon the nutritional physiology of fly, however, requires a deeper study before any final conclusion can be drawn in this respect.

III. Development of *Dacus ciliatus* eggs: inoculation protocol on melons

The aim of this section of the thesis was to develop a protocol to artificially infest *Dacus ciliatus* eggs on melons fruit. Four experiments were conducted using melon categories that were established based on their maturation stage, which was determined by some physical measurements, including sugar content. We found that the sugar content is directly related to the developmental stages of the fruit, as characterized by size and color. This relationship has been previously reported for melons by Beaulieu et al. (2004) and provided us with a good indicator of melon developmental stage for our study.

There is very little information on the development of Ethiopian fruit fly (EFF) on melons. Field observations in Israel point at the fact that only the immature stages of the 'Galia' melon can be colonized by the EFF (Nestel and Gazit, unpublished data). In order to check the possibility of mature 'Galia' melons being attacked by the EFF, Nestel and Gazit conducted a small experiment in field-cages with unattached melon-fruit (including similar categories of melon to the ones studied in this thesis) and found that the EFF develops in all categories of the fruit, including mature and rough-skin melons. Their hypothesis was that EFF can lay eggs only in soft places of the mature melon-skin and, thus, the exposure of the soft calyx after its removal from the plant allowed the fly to lay eggs in this area of the melon. Their study, however, provided ambiguous results. One of the intentions of our study was to re-evaluate this hypothesis by artificially inoculating EFF eggs into 'Galia' melons. Our study also evaluated the hypothesis that sugar content levels in mature melons can affect the development of the EFF larvae. The results of this study provided initial evidence to the fact that all categories of melon are able to produce EFF pupae, and that sugar

contents, as affected by melon-developmental stage, does not seems to inhibit EFF larvae from developing.

The hardness or roughness of fruits has been previously linked to their resistance to insects, and fruit fly attack. Oi and Mau (1989) reported that the thickness and hardness of fruit skin seems to be a barrier for fruit flies infestations in immature avocados. This mechanical barrier may also be acting in the mature 'Galia' melons, reducing the ability of EFF to lay eggs. The lack of damage of mature melons in the commercial fields may be related to the inability of EFF to lay eggs on the rough-skink of the mature melon, and the results obtained by Nestel and Gazit (unpublished) may have in fact resulted from the exposure of soft tissue in the melon by detaching the calyx. This hypothesis, which is of importance to the need of postharvest treatments, requires further investigation.

On the other hand, the process of artificial inoculation of EFF eggs on melons fruit, may lead to the induction of pathogenic spores, which cause the decay of fruit, preventing EFF larvae to develop. This study showed that the inoculation of EFF eggs previously washed with different chlorine concentration (1% and 0.5%), can reduce the contamination of pathogen agents, and the spoilage of the melon fruit. Similar process was used in the artificial infestation of Medfly on orange, using as disinfectant methyl-benzyl-ammonium- chloride to prevent the induction of pathogen agent while injecting the fruit (Santaballa et al., 2009). More research is still required in order to develop and effective artificial inoculation protocol to investigate post-harvest treatments against the EFF in melon fruit.

Conclusions

Quarantine post-harvest treatment at 1.5°C and even 4°C for 21 days plus 3 days at 20°C (time that represents an average ship journey from Israel to distant markets such as Japan or the USA) has shown total effectiveness against the Medfly (*Ceratitis capitata*) on YP. Cold treatment eliminated all the immature stages, while keeping the fruits in acceptable commercial quality with the combination of Xtend bag (XF-100), that helped to reduce chilling injuries, fruit decay and water losses. Treatment at 7^{o}C did not succeed as a cold-temperature method for killing Medfly on YP. We conclude that lower temperatures (1.5-4^{o}C) are necessary in order to arrest Medfly development, suggesting that this protocol could be used as a post-harvest quarantine treatment for this pest.

The kinetics-mortality study at the temperature of 1.5°C showed that L3 is the most cold-tolerant larval stage in YP and in RP, but L1 was more cold-tolerant than L2 in RP. Total larval mortality was achieved after 8 days in yellow peppers and after 6 days in red peppers. These differences can probably be related to the phagostimulant power of the fruit on the feeding larvae and metabolic rate, as indicated by the accumulation of lipids in Medfly larvae

Overall, Medfly larval mortality following exposure to stressful conditions varied not only among different host cultivars, but also between varieties of the same cultivar. Further investigation including other varieties of peppers is necessary in order to assess whether this cold treatment protocol will satisfy as a general quarantine method.

The results from the artificial infestation of Ethiopian Fruit fly (*Dacus ciliatus*) (EFF) eggs on melon fruits indicated that this method requires disinfection of eggs in order to prevent microbial contamination following inoculation. Moreover, the sugar

content of melon fruits presenting different stages of maturation did not affect the EFF egg development. The results suggest that EFF eggs can hatch within melons that are mature and ready for exporting, therefore, requiring post-harvest treatments to reduce EFF survival and introductions to other regions.

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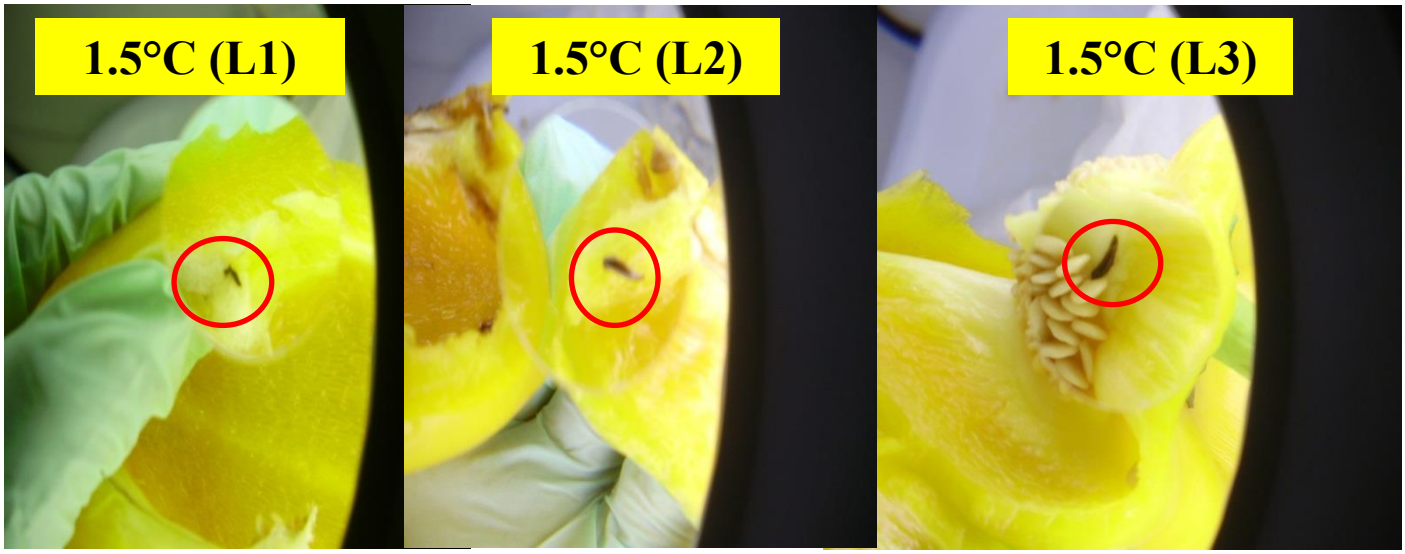
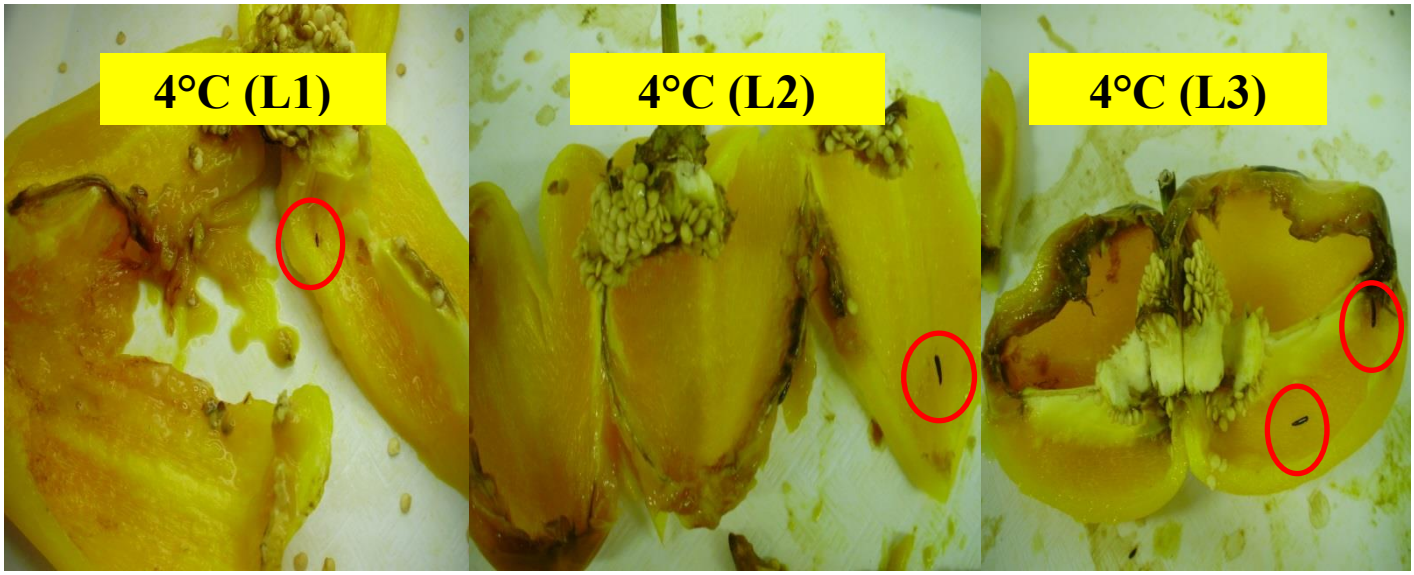
Appendix

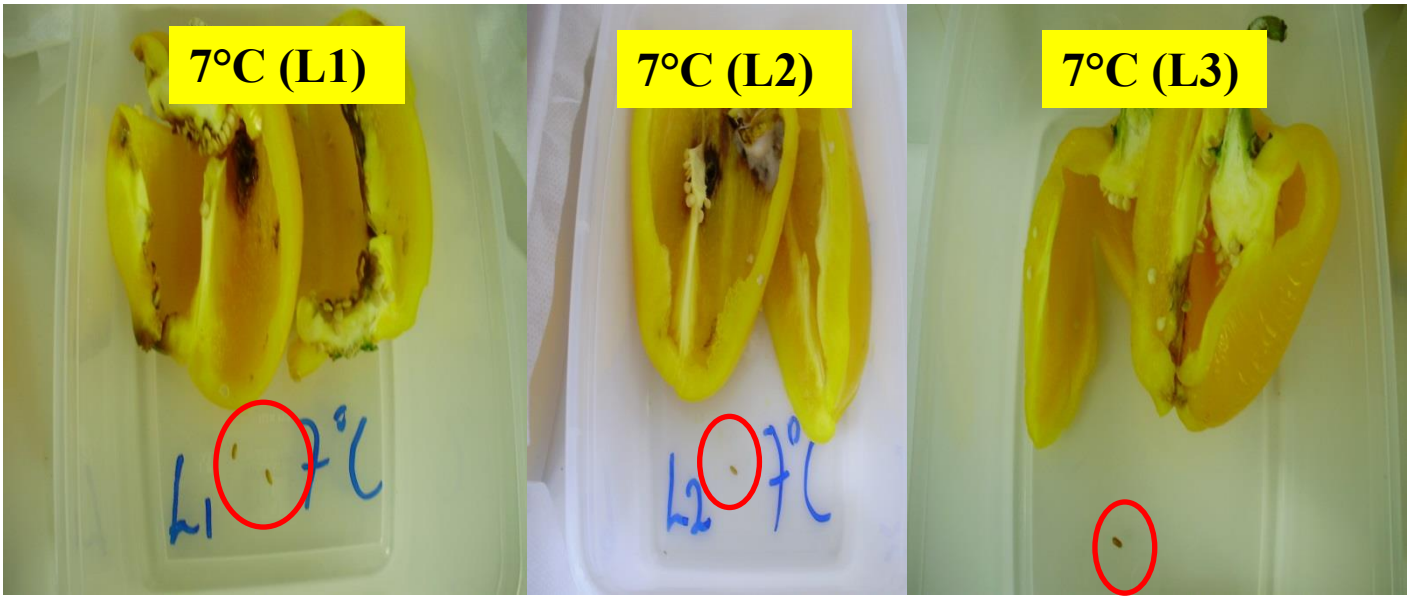
I. Post-Harvest temperature-treatment on different larvae stages



Picture I. Effectiveness of cold-treatment at **1.5°C**, all the stages were killed (L1, L2, and L3) of Medfly infested in yellow pepper.



Picture II. Effectiveness of cold-treatment at **4°C**, all the stages were killed (L1, L2, and L3) of Medfly infested in yellow pepper.



Picture III. Cold treatment at 7°C allowed Medfly eggs to complete its development to pupa.

II. Effect of cold treatment on fruit quality of yellow pepper

Picture I. Chilling injury caused by low temperatures

Picture II. Effect of cold treatment on yellow pepper with (Xtend-film) packaging-lining, which maintained the quality and turgidity of yellow peppers stored at 1.5 and 4°C for 21 day

תקציר

פרי הפלפל (*Capsicum annuum* L) הנו אחד מגידולי הייצוא החשובים של ישראל ובמקומות אחרים בים התיכון. פלפל מהווה פונדקאי לזבוב הים התיכון (זה"י) (*Ceratitis capitata* Wiedemann) הגורם לנזקים כבדים בפירות וירקות אחרים. מסיבה זו, זה"י נחשב כאחד מחרקי ההסגר בכל העולם ולכן הימצאותו בישראל מהווה מגבלה לייצא את הפלפל, בעיקר למדינות הסגר כמו ארה"ב, ובעיקר יפן. לכן מדינות אלה דורשות פרוטוקולים ברורים לקטילת מזיק זה, על מנת למנוע לחלוטין כניסתו לאותן מדינות שם הוא לא קיים ועלול להגיע אליהן על ידי הפירות המיובאים. לכן, קיימת חשיבות רבה ודחופה בפיתוח טיפול לאחר הקטיף שיכחיד מזיק הסגר זה במהלך היצוא ולפני כניסתו למדינות הסגר.

במחקר זה פיתחנו טיפול קור עבור פלפל צהוב הידוע ברגישותו הרבה והמהירה להתכלות פיסיולוגית ופתולוגית לאחר הקטיף, בהשוואה לפרי אדום, אשר יכול להיות מיושם כפרוטוקול הסגר. שיטת ההדבקה המלאכותית של פרי הפלפל בביצי הזבוב שימשה לפיתוח הפרוטוקול, אשר אפשרה לרימות הזבוב להתפתח לדרגות L1, L2, ו-L3 ב-25 מעלות צלסיוס (מ"צ) ולהתגלם, כאשר הפירות נארזו בביטנת פלסטיק מיוחדת. מיד לאחר קבלת שלושת דרגות הרימות, הפירות נחשפו לטיפולי קור בטמפרטורות של 7 מ"צ (טמפרטורת אחסנה מיטבית של פלפל), 4 מ"צ ו-1.5 מ"צ. שיטה זו שימשה בעבר לפתח פרוטוקול הסגר בקור, של פלפל אדום. הערכנו, לראשונה, את קצב תמותת דרגות שלושת הרימות, הן בפרי צהוב והן בפרי אדום. חלבוני הרימות ותכולת השומנים ברימות נמדדו במשך הדגרה ב-25 מ"צ כמדד להתאמת המרכיבים התזונתיים של הפרי בתזונת הרימה, ועל מנת לנסות ולהסביר את ההבדלים ברגישות ותמותת הרימות בפרי הצהוב והאדום. בנוסף, בחנו את איכות הפרי הצהוב שנשטף במים חמים ונארז בביטנת פלסטיק מיוחדת לאחר 21 ימי אחסנה בשלושת הטמפרטורות השונות ועוד 3 ימים נוספים ב-20 מ"צ (הדמיה להובלה ימית ליפן וחיי מדף).

תוצאות המחקר הראו כי ארבעת השלבים הראשוניים (צעירים) של הזבוב; ביצה, L1, L2 ו-L3 נקטלו לחלוטין על ידי טיפול הסגר בטמפרטורה של 1.5 מ"צ ואף ב-4 מ"צ, לאחר 21 ימים בקור וחיי מדף. אחסנת הפרי הצהוב ב-7 מ"צ לא קטלה את שלבי הזבוב הצעירים למרות שזוהי טמפרטורת האחסנה המיטבית של פרי הפלפל לאחר הקטיף.

לימוד קנטיקת תמותת הרימות הראה כי רימת L3 הייתה הסבילה ביותר לטיפול קור (1.5 מ״צ) בפרי הצהוב וקטילתה הושגה רק לאחר 7 ימים, כאשר L1 נקטל על ידי טיפול הקור לאחר 4 ימים. לעומת זאת, קטילת הרימות בשלבים שונים הייתה מהירה יותר בפרי האדום, בהשוואה לפרי הצהוב. אולם, L1 ו-L2 היו העמידים ביותר בפרי אדום וקטילתן הושגה לאחר 5 ו-4 ימים, בקור, בהתאמה. הבדלים אלה נובעים, ככל הנראה, מהשוני ברמת והרכב חומרי המזון בפרי האדום, בהשוואה לפרי הצהוב, והיכולת של הרימות לצבור חומרי מזון. רימות שגודלו בפרי אדום הראו צבירה גבוהה יותר של שומנים, הנובעת מעלייה במטבוליזם של הרימה כתוצאה מרכב המרכיבים התזונתיים בפרי האדום שהשרה הצטברות זאת. יתכן כי העלייה במטבוליזם הגבירה את רגישות הרימות לטיפול בקור בפרי האדום. מטבוליזם איטי של רימה שגודלה בפרי הצהוב, כפי שנמדד על ידי הצטברות איטית יותר של שומנים, הקטין, ככל הנראה, את רגישות הרימה לטיפול קור.

אריזת הפרי בביטנה, הקטינה באופן מובהק את רגישות הפרי לנזקי צינה ואיכותו של הפרי שנארז וטופל, אף ב-1.5 מ״צ, הייתה טובה ומכירה גם כעבור 21 ימים בקור + 3 ימים נוספים בחיי מדף (20 מ״צ). אריזת הפרי השטוף במים חמים הקטינה באופן מובהק את איבוד המים והתפתחות הריקבון.

המסקנה, טיפול הסגר של פלפל לאחר הקטיף, שנארז בביטנה פלסטית, ב-1.5 מ״צ, או 4 מ״צ קטל את זבוב הים התיכון בפרי הצהוב, תוך שמירת איכות הפרי מההיבט המסחרי. בנוסף, שיעור קצב קטילת הרימות נמצא שונה בשני זני הפלפל השונים. ולכן, יש צורך ולהמשיך ולבחון את תגובתם של זני פלפל נוספים, בצבעים שונים, לטיפולי קור על מנת למצוא את טיפול ההסגר הטוב ביותר שמחד, ישמור על איכות הפרי, ומאידך, יקטול את הזבוב בכול שלבי התפתחותו.

על פי תוצאות המחקר שנערך עם זה״י, התחלנו לפתח פרוטוקול קטילת זבוב הדלועיים (ז״ד) (*Dacus ciliates* Loew) הפוגע בפירות מלון (*Cucumis melo*). הצעד הראשון בפיתוח פרוטוקול הסגר היה לפתח שיטה להדביק את הפרי באופן מלאכותי בביצי הזבוב. על מנת להשיג זאת, היה צורך לחטא את הביצים כדי למנוע התפתחות ריקבון בפרי במקום הפציעה וההדבקה. צעד זה לא היה נחוץ בהדבקת פרי פלפל בזה״י. בנוסף, היה צורך לבחון כיצד שלב ההבשלה של פרי המלון ישפיע על הצלחת ההדבקה המלאכותית בביצי הזבוב. לשם כך הודבקו 4 שלבי הבשלה שונים של המלון (ירוק לא בשל ועד פרי צהוב בשל) ובחנו האם קיים קשר בין רמת הסוכר (מרכיבים תזונתיים) ליכולת הביצים להתפתח לרימות ולהתגלם. תוצאות המחקר הראשוני הראה כי ביצי

הזבוב התפתחו בכול ארבעת שלבי ההבשלה של הפרי, דבר המראה כי לא נמצא קשר בין יכולת התפתחות הביצים לרימות ואחר כך לגולם, לבין תכולת , או מרכיבים תזונתיים אחרים הנמצאים בפרי המלון, בדרגות הבשלה שונות. חיטוי הביצים בכלור וחיטוי מקום הפציעה של הפרי באתנול לא השפיע על הביצים לבקוע ולהתפתח. אולם התפתחות ריקבון בפרי ואי יכולתנו לחטא לחלוטין את ביצי הזבוב, השפיע על יכולתנו, בשלב זה של המחקר, לפתח פרוטוקול להדבקת מלון בז״ד. לכן, יש צורך ולהמשיך המחקר זה שיוביל, בסופו של דבר לפתח פרוטוקול הדבקה וקטילת ז״ד על ידי טיפול הסגר כלשהו של מלון לאחר הקטיף.

עבודה זו נעשתה בהדרכתו של:

פרופ' אלי פליק

ודר' דוד נסטל

מחלקה לחקר תוצרת חקלאית לאחר הקטיף והמחלקה ולאנטומולוגיה, מינהל המחקר החקלאי, מרכז וולקני

פיתוח טיפול הסגר לאחר הקטיף לפלפל (*Capsicum annuum* L) ומלון (*Cucumis melo* L. *reticulatus*) נגד זבובי פירות (*Certitis capitata* and *Dacus ciliates*)

עבודת גמר

מוגשת לפקולטה לחקלאות, מזון וסביבה ע"ש רוברט ה. סמיט, האוניברסיטה העברית בירושלים, לשם קבלת תואר "מוסמך במדעי החקלאות"

על ידי

רוסנה קסטרו

ניסן תשע"ד רחובות מאי 2014