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Effects of cold post-harvest treatments of sweet bell peppers on the development of the Mediterranean fruit fly (*Ceratitis capitata*)

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ABSTRACT

We investigated the survival of Mediterranean fruit fly (Medfly) larval stages on yellow peppers stored for 21 d in cold environments (1.5, 4 and 7 °C), and the differences in larval mortality rates of Medflies exposed to 1.5 °C storage environments between yellow and red peppers. In addition, we investigated the effect of packing yellow peppers and exposing them to cold environments for 21 d on the quality of the fruits. The aim of the study was to investigate packing and shipping post-harvest protocols as a preliminary investigation to explore cold environments as a system to assure the export of quality peppers with low risks of surviving Medfly immature stages. This exploratory study showed that storing yellow peppers for 21 d in lined boxes (20-μm-thick macroperforated Xtend film) at 1.5 and 4 °C protected the quality of the fruit, and precluded the survival of Medfly eggs and larval stages (L1, L2 and L3). In contrast, peppers stored at 7 °C for 21 d did not completely kill immature stages of the Medfly. In addition, the study showed that Medfly larval stages exposed to 1.5 °C environments on yellow and red peppers have different tolerances to cold. Sensitivity to cold in red pepper was higher than in yellow for all larval stages. In addition, sensitivity to cold between larval stages varied between yellow and red peppers: for yellow, sensitivity preceded as follows L1 > L2 > L3, while in red peppers it was L2 > L1 > L3. Abilities to accumulate lipids and protein by feeding Medfly larvae suggest that palatability and nutrition may be partially responsible for the differences in cold tolerance.

1. Introduction

With the increase in global trading of fresh agricultural produce during the last decades, the invasion of species of insects into new regions, in which they are considered to be quarantined pests, has increased by several fold (Follett and Neven, 2006; Lockwood et al., 2007). Importing countries, such as the USA and Japan, demand quarantine security protocols to diminish the risk of accidental introduction of insect pests and diseases through imported fruits (Follet and Neven, 2006). Fruit flies (Diptera: Tephritidae), such as the Mediterranean fruit fly (Medfly), *Ceratitis capitata*, comprise one of the insect groups with the highest risks of introduction to regions free of them (Papadopoulos et al., 2013).

Cold quarantine storage of fresh produce is an acceptable approach adopted by the U.S. Department of Agriculture (APHIS) to reduce the risk embedded in the importation of fresh produce potentially infested with Tephritidae larvae (Hallman et al., 2011). Exposure to cold environments for several d has become a common practice for shipped commodities reaching commercial ports with quarantine regulations and inspections services. Cold treatment protocols involving storage at 1.1, 1.7 or 2.2 °C for periods of 14, 16 or 18 d, respectively, have been established by APHIS (e.g., T 107 a) to prevent living-infestations of Medfly in imported citrus fruit (https://www.regulations.gov/#!documentDetail;D=APHIS-2006-0050-0008; https://www.aphis.usda.gov/import_export/plants/manuals/ports/downloads/fv.pdf Gould and Ware 2008; Hallman et al., 2011; Gazit et al., 2014). Cold treatment protocols to reduce the introduction of living Medfly larvae into quarantine regions have also been proposed and developed for fruits such as date (Gazit et al., 2014) and red peppers (Fallik et al., 2012).

Cold tolerance of immature fruit flies varies with Tephritidae species and type of fruit where the larvae are developing (Aluja et al., 2010). Cold tolerance is also larval-stage dependent (Gazit et al., 2014). Thus, although certain generalizations can be made to

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determine and suggest possible post-harvest cold-treatment protocols, the adoption of a treatment by the importing country requires the investigation of the cold tolerance of the fruit fly species in the fruit of interest. We recently investigated several cold-treatment protocols for sweet red bell peppers, an important export commodity for Israel. These studies were aimed at reducing damage of fruit by cold treatment (e.g., chilling injury), and to ensure the killing of Medfly larvae inside the fruit (Bar-Yosef et al., 2009; Fallik et al., 2012). Successful protocols include a short rinse of red bell peppers over brushes at 55 °C for 15 s followed by storage and packing at 1.5 °C for 21 d in macroperforated Xtend® plastic bags (Fallik et al., 2012). The present study reports the results of a preliminary study aimed at expanding this post-harvest cold-treatment protocol to sweet yellow bell pepper, which is more susceptible to physiological and pathological deterioration after prolonged storage than red peppers (Maalekuu et al., 2003). The study also compared the effect of cold environments on the larval stages of the Medfly developing in the two different varieties of bell peppers (red and yellow fruit), and investigated possible causes explaining the different cold tolerances found for the same fruit fly species in the two varieties. The purpose of the manuscript and this exploratory study is to provide a basis for future full scale trials to validate the observed patterns and develop export protocols for both yellow and red pepper.

2. Material and methods

2.1. Fruit source and preparation

Yellow (cv. Dinamo) and red (cv. Cannon) sweet bell peppers (*C. annuum* L.) were collected in the North of the Arava, Israel. During 2012 we concentrated on yellow peppers (collection during March 2012), while in 2013 we collected and contrasted Medfly differential tolerance to cold in yellow and red peppers (red pepper collected during January and February 2013, while yellow pepper was collected during February 2013). Fruit was picked at a ripeness stage of approximately 90%, with almost fully developed color. Within the first hours after harvest, peppers were rinsed in hot water (at 55 °C for approx. 15 s) and spore-kill with 0.05% quaternary ammonium compound, as suggested by the current established preliminary commercial treatment (Fallik et al., 1999). Following rinsing, peppers were packed in boxes (5 kg) inside 20-μm-thick macroperforated Xtend film (XF-100) (Set-Pac, Ltd., Tefen, Israel). Rinsed fruits were then treated with either of the following: (1) direct storage in cold (1.5, 4 and 7 °C) and subsequent measurement of fruit quality after 21 d in the cold environment and 3 more d at 20 °C (simulating commercial transportation and marketing) or (2) inoculation with Medfly eggs in order to explore Medfly eggs and larval survival in cold temperature-environments. All the experiments were incubated in cooling rooms build by Herut Cooling Systems, Ltd., Tel Aviv, Israel. Each cooling room was 62.5 m^3 equipped with humidifier (Optiguide Controlled Humidity Solutions Ltd., Yokneam Iiilit, Israel). Temperature was maintained, and automatically regulated by in-built environmental data-loggers, at 1.5 ± 0.5 °C, 7 ± 1 °C, 25 °C ± 1 °C and 98% ± 1% RH. No data-logger probes were installed inside the pulp of fruit.

2.2. Assessment of fruit quality before and after cold treatments

The quality of fruit after incubation in cold environments (1.5, 4 and 7 °C) for 21 d plus 3 d at 20 °C, and with or without XF-100 packing, was assessed following Fallik et al. (1999, 2012). Measured indexes included: (a) Percentage of weight loss from the initial weight during storage, (b) Fruit elasticity (firmness) was measured by a pressure-gauge instrument placing a 2 kg weight for 10 sect and measuring deformity in mm elasticity; the higher the measure the softer the fruit, (c) Total soluble solids (TSS) were estimated by squeezing juice from fruits into an Atago digital refractometer (Atago, Tokyo, Japan), (d) Decay incidence was considered from the moment fungal mycelia appeared on fruit pericarp and/or calyx; decay was expressed as a percentage of the total initial fruit number, (e) Chilling injury (CI) was considered when a fruit presented a sunken pitting of more than 2 mm on the skin or calyx; CI was expressed as a percentage of the total number of fruits in the sample showing signs of injury, (f) The severity of the chilling injury was expressed as chilling index (CIN). CIN fluctuated between 0 and 3: 0 = no chilling injury; 1 = minor damage, covering less than 10% of the fruit peel; 2 = moderate damage, were injury covered between 10 and 30% of the peel; and 3 = severe damage were injury covered more than 30% of the peel. The CIN index was calculated as follows: number of fruits without damage multiplied by 0 + number of fruits with minor damage multiplied by 1 + Number of fruits with moderate damage multiplied by 2 + Number of fruits with severe damage multiplied 3, all divided by the total number of fruit (see Fallik et al., 2012). Differences between treatments were inferred using one-way ANOVA (Statgraphics 5 Plus). Each treatment included four replicate cartons boxes of 5 kg each. In each carton box we had approximately 25 peppers.

2.3. Artificial inoculation of peppers with Medfly eggs and procurement of different larval stages for cold-treatments

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. In 2012 the study concentrated on yellow peppers and explored the effect of cold treatments (1.5, 4 and 7 °C) on eggs and larval survival, while in 2013 we explored the daily mortality of different larval stages on both pepper varieties maintained at 1.5 °C. 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 predetermined amount of eggs into the fruit (Fallik et al., 2012). Eggs were suspended in an agar medium to homogenize their distribution in the suspension and make injected quantities of eggs more accurate and quantifiable (Gazit et al., 2014). Inoculations were done with hardened eggs (after 24 h of incubation).

To investigate the effect of cold treatments on Medfly egg survival in yellow peppers, a sample of newly infested peppers was directly transferred to the different cold treatment rooms (between 45 and 50 peppers per temperature—see Table 2). To study the effect of cold treatment on the different larval stages, Medfly-eggs infested fruits were incubated at 25 °C and 98% R.H. for 5 d to obtain first-stage larvae (L1), 7 d to allow most L1 larvae to molt into L2, and 9 d to permit most L2 larvae to molt into L3 (see Fallik et al., 2012). Medfly larval stage was identified mainly by body length and by mouth hooks appearance (Hardy 1949). In addition, 2nd instar was distinguished from 3rd instar by the ability of 3rd instar larvae to float from its anal spiracles as a snorkel when immersed in water. Samples of these fruits (at least 10 peppers per sampling date) were inspected at 5, 7 and 9 d to verify larval stage presence and frequency in the sampled peppers. After verification, remaining fruits were transferred to 1.5, 4 and 7 °C to continue with the cold-treatment experiment. Number of infested fruits with the appropriate larval stage depended on availability of the proper stage and the amount of available fruit (between 30 and 50 infested fruit per larval stage and temperature—see Table 2). An additional group of fruits (71) was kept at 25 °C and 95% R.H until larvae jumped out from the fruit and pupated. These fruits and pupae were used as controls. In order to