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COLD STORAGE AS DISINFESTAION TREATMENT AGAINST THE PEACH FRUIT FLY, *BACTROCERA ZONATA* (SAUNDERS), (DIPTERA: TEPHRITIDAE) ON VALENCIA ORANGE

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ABSTRACT

Peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae), which invaded the Egyptian ecosystem during early nineties of the last century, produced different hazardous to the horticultural products and controlled the export of oranges to other countries. This paper addresses the effectiveness of cold treatment against peach fruit fly infestation in Valencia oranges. The storage temperature and period, which ensured 100% mortality of the most tolerant stage of peach fruit fly on Valencia oranges was determined. Small scale postharvest cold disinfestation test was carried out to study the most tolerant stage against cold, where the infested oranges with different immature stages (egg and 1st , 2nd and 3rd instar larvae) were exposed to 1.7 ±0.5 °C for 3, 5, 7, 10 and 14 days. 3rd instar larvae were the most tolerant stage. When the 3rd instar larvae exposed to 1.7 ±0.5 °C for 10 and 14 days, 100% mortality was demonstrated. Large postharvest cold disinfestaion test was induced to confirm the results of the small scale test. More than 1470 Valencia oranges, which previously infested with 50 eggs each and kept in 25°C and 65% Rh until the 3rd larval instar appears (≈ 33 3rd instar larvae/fruit), were exposed to 1.7 ±0.5°C for 14 days. The fruits were dissected for larval examination. More than 31900 of the 3rd instar larvae were produced and all of them were dead.

INTRODUCTION

Fruit flies (Diptera: Tephritidae) are serious insect pests that their wide distribution and host range affect fruit production worldwide. Different species of Family Tephritidae have been accidentally introduced into Egypt (**Efflatoun, 1924**). During 90's of the last century,

a harmful species of Tephritid, the peach fruit fly, *B. zonata* (Saunders), was reported (**El-Minshawy *et al.*, 1999**) causing considerable damage to fruit production (**Hashem *et al.*, 2001**). It is considered as a polyphagous insect, where it attacks oranges as well as more than 40 species of fruit and vegetables crops (**Oakly, 1948, Narayanan and Batra, 1960, Syed *et al.*, 1970, and Kapoor and Agarwal, 1982**). Orange is considered as one of the most important horticultural sources of income in Egypt, where it is exported to different countries round the world. As a consequence of the presence of this pest, the interstate movement and export of Egyptian oranges are restricted by plant quarantine regulations. These barriers to free trade may be overcome by the application of a suitable postharvest disinfestations treatment. For many years, fumigation of fruit with ethylene dibromide (EDB) was accepted as disinfestation procedure, and fumigation schedules have been developed for the treatment of oranges against fruit pests (**Rigney and Wild 1975 and Bond 1984**). As the EDB has hazard effect on the ecosystem (**Environmental Protection Agency, 1984**), other postharvest treatments was needed to be evaluated. The efficacy of cold storage as a disinfestation treatment has been demonstrated previously for Mediterranean fruit fly, *Ceratitis capitata* (Weidemann) in apple (**Sproul, 1976**), oranges and peaches (**El-Nahal *et al.*, 1984**), Caribbean fruit fly, *Anastrepha suspense* (Loew), in oranges (**Benschoter, 1984, Benschoter and Witherell, 1984 and Hill *et al.*, 1988**) and carambola (**Gould, 1996**) and Queensland fruit fly, *Dacus tryoni* (Froggatt) in Kiwi fruit (**Rippon and Smith, 1979 and Jessup and Baheer 1990**), Valencia oranges (**Hill *et al.*, 1988**), Eureka and Lisbon lemons (**Jessup *et al.*, 1993**).

The present study is undertaken to evaluate the efficiency of cooling treatment against the immature stages of peach fruit fly in oranges to be used as a postharvest method and consequently the Egyptian oranges can be exported to different countries safely. The test limits adopted were those described by **Sproul (1976)** and accepted by the Japanese plant quarantine authorities.

MATERIALS AND METHODS

Test insect:

Laboratory stock cultures peach fruit fly, *B. zonata* (Saunders) were started from samples of guava and peach infested fruits collected

from Giza, Qalyubia and Fayoum governorates orchards during the period of September 1999 and reinforced periodically with wild strain. Rearing of flies was conducted in the laboratories of the Horticultural Insects Research Department (HIRD), Plant Protection Research Institute (PPRI), Agricultural Research Center (ARC).

Rearing method:

The laboratories were adjusted for constant conditions where the temperature was 25±1°C and the relative humidity was 65±5%. Eggs were collected in an egg receptacle (a yellow plastic transparent ball with fine holes around and internally smeared with guava juice) placed in the cage to receive the oviposited eggs. Eggs were planted on artificial diet used by **Afia *et al.* (2005)**.

Test fruits used in the following experiment were *Citrus sinensis* Var. *Valencia*. The fruits were mature, fresh, well-ripened and grown. Fruits were stored at 4-5 °C until the experiment was started. Fruits were examined to be healthy and free from cuts, scratches or any microbial infection or insect infestation.

Eggs were collected from the egg receptacles by 12 hours interval in sterilized water. Eggs were hanged on card (black filter paper of about 2 cm X 2 cm) using camel hair brush under stereo microscope. Using the Cap method of inoculation, each fruit was inoculated with egg card. Both the treatment and control fruits were inoculated.

There were small and large disinfestation tests. The small scale disinfestation test was conducted to find out the most tolerant stage of peach fruit fly against cooling and the shortest cooling period that induce 100% mortality. The distribution of developmental stages of peach fruit fly in oranges was studied by dissecting 5 fruits every day for seven days to obtain the required developmental stage. There were 4 different treatments, 1 day egg and 1st, 2nd and 3rd instar larvae treatment. Each treatment represented by 6 cooling applications, control and 3, 5, 7, 10 and 14 days exposure. Each cooling application replicated 5 times. Each orange received 150 eggs (3 egg cards) except 3rd instar larvae treatment received 100 eggs (2 egg cards) each.

For large scale disinfestation test, more than 1600 fruits were used in the experiment (more than 1470 fruits for treatment, 100 fruits for control, 21 fruits for larval development and 3 fruits for sensors). Each fruit (except sensor's fruit) received an egg card (50 eggs).

After inoculation, the fruits were distributed in trays (20 fruits / tray) and incubated in 25±2°C for larval development. Incubators and cooling chambers were in Central Laboratory of Date Palm. Pre-cooling treatment were initiated when more than 80% of the larvae reached the desired stage, where fruits transmitted to 4-5°C incubator to prevent the water to evaporate from the fruits hence the cooling were going to be affected. Precooling period extend for 24 hours. On the second day the fruits were moved to the cooling chamber of 1.7 ±0.5 °C and 65 ±5% Rh. The cooler was previously adjusted for the desired temperature and relative humidity for more than 48 hours before the fruit power. After the fruits were powered into the cooler, the used cooler space was increased to be 35% of the total space for load factor.

During the applications of small and large disinfestation tests, 5 sensors were used to estimate the temperature changes inside and outside the fruits (3 sensors were inside the fruits (sensor 1, 3 and 4) and 2 sensors were outside the fruits (sensors 2 and 5). Before the experiment started, calibration of the sensors was performed by immersing the sensors inside a container full of melted ice water. The cooling treatment started when the temperature of all data sensors of inside fruits arrived to be 1.7 ±0.5 °C.

After the fruits were powered into cold chambers at 1.7 ±0.5 °C, the control fruits were moved for larval popping out. Each fruit was incubated in a plastic container of 500 ml volume. The containers were previously lined with a fine layer of sand for larval pupation and tissue paper for water absorption. The containers were covered tightly with perforated piece of texture.

When the cooling period conducted, the fruits were powered into the 500 ml containers and kept at 25 ±2 °C and 65% Rh as in the control treatment to allow larvae which might still alive to pop out. After 5 days, the sand of the control and treatment containers was sieved to collect and count the pupae for each fruit. The fruits were dissected for larval examination.

A One-way analysis of variance (ANOVA) with a complete block design was used to compare among treatments using SPSS computer program version 16. Larval mortality data were presented as percentage mortality after correction in comparison to the control of the experiment using Abbott's formula (**Abbott, 1925**). Least Significant Difference

(LSD) was used for multiple mean comparisons between each treatment and other (**Green and Salkind, 2003**).

RESULTS AND DISCUSSION

Different national plant protection organizations have a number of polices to determine phytosanitary measures for one same pest. The Animal Plant and Inspection Service (APHIS), from the United States Department of Agriculture (USDA) has standardized cold quarantine treatments for various fruit fly species, regardless of the fruit fly type. Recently modified cold treatments established by **APHIS (2009)** include T107-a for *C. capitata* at 1.1°C, 1.67°C and 2.2°C for the period of 14, 16 and 18 days, respectively. On the other hand, the Ministry of Agriculture, Forestry and Fisheries (MAFF) from Japan requires each country to develop its own treatments for all the varieties proposed for export (**MAFF, 1996**). Referring to the biological information of peach fruit fly, *B. zonata* (Saunders), it was found that egg duration at 25°C was 2.3 days with rate of development of 43.1% and the larval duration was 8.5±0.2 days with a rate of development of 11.8%.

Small scale cold disinfestation test:

The dead larvae induced by cold treatment were rigorous, stick like and with some spots of their bodies turned blackish. They usually came up and died on the upper surface of the orange's tissues. Abbott's formula (**Abbott, 1925**) was used as a correction factor of immature stage mortality for each treatment. Lethal effects of 5 durations of cold temperature at 1.7 ±0.5°C on peach fruit fly egg and larvae are presented in Table (1). The test concluded that increasing of the cooling duration reflected by increase of mortality of the different immature stages. When the correlation coefficient between cooling duration and corrected mortality of the different applications was calculated the results indicated that there was a complete direct correlation. 1st instar larvae was the highest susceptible stage where 100% of the individuals died on the 7th day of cooling, followed by the 1 day old egg and 2nd instar larvae, while the highest tolerant stage was the 3rd instar larvae. Unlike **Jessup *et al.*, (1993)** who concluded that 2nd instar larvae of *C. capitata* were the most tolerant stage and in 1993 and 1998 concluded that 1st instar larvae of *B. tryoni* were more tolerant than other stages (**Jessup et al., 1993 & 1998**), the results of this study concluded that the 3rd instar larval stage of peach fruit fly was the most tolerant stage against cooling and this coincides

with **Hill *et al.*, (1988)** and **Willink *et al.*, (2006)** studies, which consider 3rd instar larvae was the most tolerant stage of *C. capitata*. By 10 days of exposure to the 1.7 ±0.5°C temperature all the immature stages of peach fruit fly in Valencia oranges were destroyed. **Benschoter (1983)** concluded that exposure of the immature stage of Caribbean fruit fly *Anastrepha suspense* (Loew) to 1.7°C and 4.4°C for 14 days and 28 days respectively, produced 100% mortality. Also **Burditt and McAlister (1982)** reported that refrigeration of 1.7°C or lower was effective in destroying *A. suspense* and *A. oblique* infestations in several subtropical fruits including grapefruit.

Table (1): Corrected mortality of peach fruit fly, *B. zonata* (Saunders) eggs and larvae infesting Valencia oranges treated at 1.7 ±0.5°C for different periods

Exposed stage	Corrected mortality percentages after certain period of cooling				
	3 days	5 days	7 days	10 days	14 days
1 day egg	68.9±4.9	85±4.3	99.8±0.2 a¥	100±0.0 a	100±0.0 a
1st instar larvae	71.2±4.4	93.8±2.9 a	100±0.0 a	100±0.0 a	100±0.0 a
2nd instar larvae	38.5±4.3	89.7±1.8 a	93.7±1.6 a	100±0.0 a	100±0.0 a
3rd instar larvae	13.8±9.5	61.1±6.9 a	81.3±2.1 ab	100±0.0 b	100±0.0 b
Average	54.3±8.7	82.5±4.1a	95.3+2.8ab	100+0.0b	100+0.0b

¥ Means followed by the same letter within the same row are not significantly different (P≥0.05)

Large scale cold disinfestation test:

Eggs started to be inoculated, where every fruit had 50 eggs. 1570 fruits were inoculated and kept in 25±2°C and 65% Rh incubator. After 13 days, sand under the control treatment was sieved and number of pupae for each fruit was counted. From 100 fruits, 3296 pupae were collected with an average of about 33 pupae/fruit.

Table (2) appropriates the distribution of the different larval stages of peach fruit fly collected from the control fruits sample after dissection. Fruits dissection started on the 6th days after inoculation where results indicated that 68.8% of the larvae were in their second stage. On the next day, the maximum number of larvae (66.3%) was third stage. By the 8th day, the percentage of the third stage increased to be 94.5% of the larvae. So the fruits were moved to the precooling room (4-5 °C) on 9th day. On the 10th day, the treatment fruits (1470 fruits) were powered to the 1.7 ±0.5°C incubator.

Table (2): Distribution of larval stages of peach fruit fly, *B. zonata* (Saunders) in Valencia orange sample examined for initiating cooling application in the large scale cold disinfestation test.

Date	Number examined Fruits		larvae			Total
			First	Second	third	
6th day	9	Total number	30	216	68	314
		Percentage	9.6	68.8	21.7	
7th day	15	Total number	0	136	267	403
		Percentage	0	33.7	66.3	
8th day	15	Total number	0	25	430	455
		Percentage	0	5.5	94.5	

Referring to the sensors results, it could be concluded that the temperature inside the fruits arrived to 1.7 ±0.5°C (Zero time for cooling) on the 13th day after inoculation at 2.59 PM. After 14 days of cooling, the cold-treated fruits were moved from 1.7 ±0.5°C incubator to the 25 ±2°C incubator for 5 days (Fig. 1). After that, 1470 fruits were dissected for larval counting and examination for their life as well as sifting the sand under the fruits. The results indicated that, there was not any pupa in the sand. Dissection of the fruits produced 31900 dead larvae.

So it could be concluded that 3rd instar larva e of peach fruit fly *B. zonata* (Saunders) was the most tolerant immature stage against the cold and the cooling of the Valencia orange fruits which previously infested with peach fruit fly, *B. zonata* (Saunders) at 1.7±0.5°C for 14 days is considered as efficient method to terminate the immature stages of this pest.

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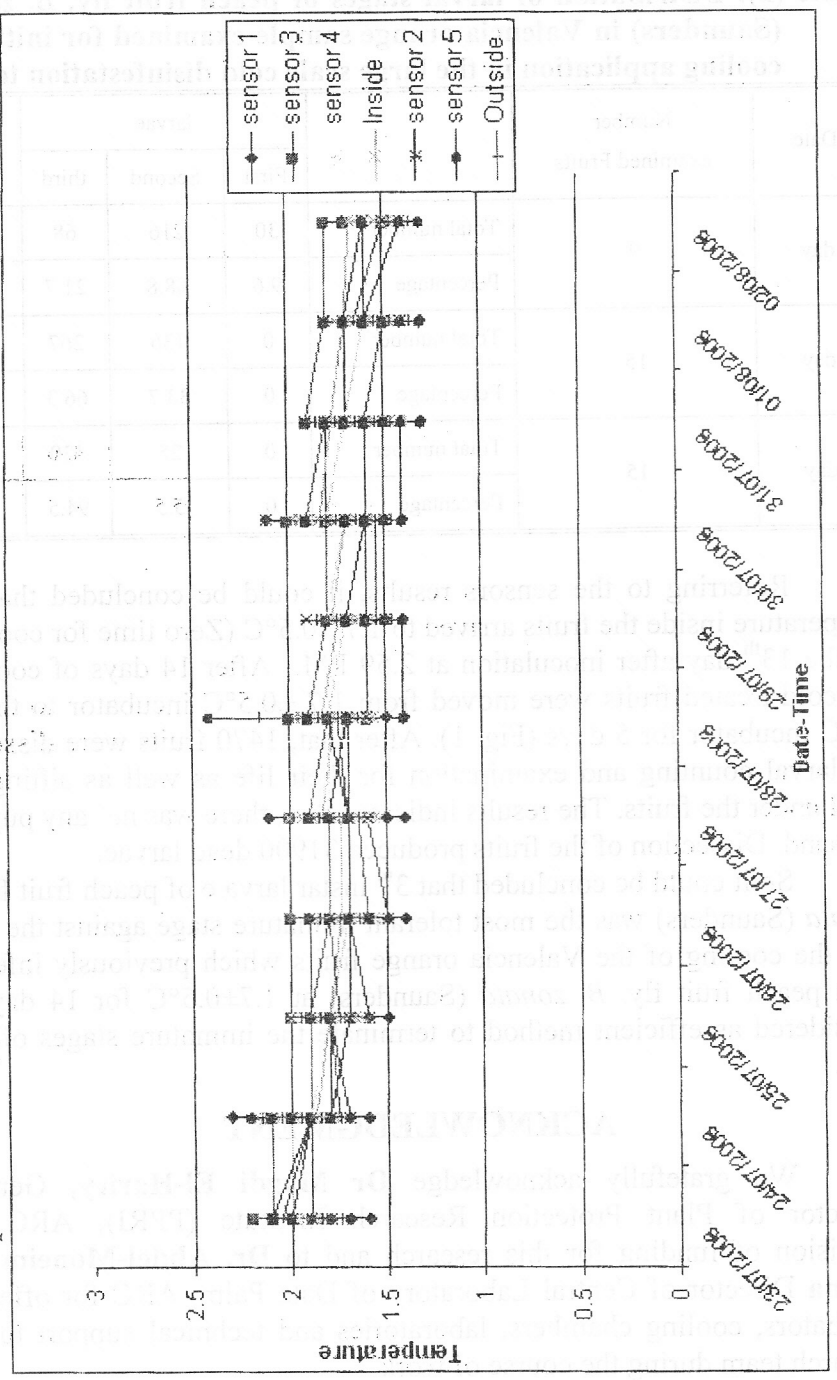

Fig. (1): Distribution of temperatures inside and outside Valencia oranges infested with 3rd instar's larvae of the peach fruit fly, *B. zonata* (Saunders) during large scale cooling disinfestations test

REFERENCES

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

Afia, Y.I. ; M.A. El-Sheikh ; A.G. Hashem and S. El-Nagar (2005). Influence of different constant temperatures on the biological aspects of the two fruit flies (Diptera: Tephritidae) in Egypt. Egypt. J. of Appl. Sci. 20(7): 257-276.

APHIS-PRQ. (2009). Animal and plant health inspection service – plant protection and quarantine PPQ treatment manual. http://WWW.aphis.usda.gov/ppq/manuals/online_manuals.html

Benschoter, C.A. (1983). Lethal effect of cold storage temperatures on Caribbean fruit fly in grapefruit. Proc. Fla. State Hortic. Soc. 96: 318-319.

Benschoter, C.A. (1984). Low-temperature storage as a quarantine treatment for the Caribbean fruit fly (Diptera: Tephritdae) in Florida citrus. J. Econ. Entomol. 77: 1233-1235.

Benschoter, C.A. and P.C. Witherell (1984). Lethal effects of suboptimal temperatures on immature stages of *Anastrepha suspensa.* Fla. Entomol. 67: 189- 193.

Bond, E.J. (1984). Manual of fumigation for insect control. Food and Agricultural Organization, Rome, Italy, pp 432.

Burditt, A.K. and L.C. McAlister (1982). Refrigeration as a quarantine treatment for fruit infested with eggs and larvae of *Anastrepha* species. Proc. Fla. State Hort. Soc. 95: 224-226.

Efflatoun, H.C. (1924). A monograph of Egyptian Diptera, part II., Fam. Trypaneidae. Memoires de la societe entomologique de egypte. 2(2): 1-132.

El-Minshawy, A.M. ; M.A. El-Eryan and A.I. Awad (1999). Biological and morphological studies on the Guava fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) found recently in Egypt. 8th Nat. Conf. Of pests & Dis. Of Veg. and Fruits in Ismailia, Egypt, pp. 71-82.

El-Nahal, A.K. ; A.M. Ali ; M.K. Ahmed and A.M. El-Hakim (1984). Effect of low temperature on egg and larval instars of the Mediterranean fruit fly, *Ceratitis capitata* (Weid.). Egypt Agric. Res. Rev. 62(1): 261-266.

Environmental Protection Agency, (1984). Revocation of tolerances for ethylene dibromide. Environmental Protection Agency, 40CFR Part 180, Federal Rigester 49(103): 22082-22085. U.S. Government Printing Office, Washington, DC.

Gould, W.P. (1996). Cold treatment the Caribbean fruit fly and carambolas, pp.489-493. In McPheron, B. A. and G. J. Steck (eds.), Fruit fly pests. A world assessment of their biology and management, St. Lucie Press, Florida, USA.

Green. S.B. and N.J. Salkind (2003) Using SPSS for the Macintosh and Windows: Analyzing and understanding data, 3rd edition. Prentice Hall PTR Upper Saddle River, NJ, USA pp 496.

Hashem, A.G. ; S.M.A. Mohamed and M.F. El-Wakkad (2001). Diversity and abundance of Mediterranean and peach fruit flies (Diptera: Tephritidae) in different horticultural orchards. Egypt. J. Appl. Sci. 16(1): 303-314.

Hill, A.R. ; C.J. Rigney and A.N. Sproul (1988). Cold storage of oranges as a disinfestation treatment against the fruit flies *Dacus tryoni* (Frogatt) and *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae). J. Econ. Entomol. 81(1): 257-260.

Jessup, A.J. and A. Baheer (1990). Low-temperature storage as a quarantine treatment for kiwi fruit infested with *Dacus tryoni* (Diptera: Tephritidae). J. Econ. Entomol. 83: 2317-2319.

Jessup, A.J., C.P.F. DE Lima, C.W. Hood, R.F. Sloggett, A.M. Harris and M BecKlngham (1993). Quarantine disinfestations of ' lemons against *Bactrocera tryoni* and *Ceratitis capitata* (Diptera: Tephritidae) using cold storage. J. Econ. Entomol. 86 (3) 798-802.

Jessup, A.J. ; R.F. Sloggett and N.M. Quinn (1998). Quarantine disinfestation of Blueberries against *Bactrocera tryoni* (Froggatt) (Diptera: Tephritidae) by cold storage. J. Econ. Entomol. 91(4): 964-967.

Kapoor, V.C. and M.L. Agarwal (1982). Fruit flies and their increasing host plants in India. Proc. CEC/IOBC Intern. Symp. Athens/ Greece, 16-19, November, 1982, p. 252-257.

MAFF. (1996). Ministry of Agriculture, Forestry and Fisheries. Cold treatment. In MAFF (eds.), Textbook of plant quarantine treatments, Tokio, Japan pp. 136 -144.

Narayanan, E.S. and H.N. Batra (1960). Fruit flies and their control. I.C.A.R. Publ. New Delhi. pp. 1 – 68.

Oakly, W. (1948). Manual of foreign plant pests. pp. 216-217.

Rigney, C.J. and B.L. Wild (1975). *Dacus tryoni*, ethylene dibromide treatment of oranges. J. Econ. Entomol. 68: 653-654.

Rippon, L.E. and R.J. Smith (1979). Postharvest treatment of Chinese gooseberries (kiwifruit) for the control of Queensland fruit fly. Rural Newsl., 73: 34-35.

Sproul, A.N. (1976). Disinfestation of Western Australian granny smith apples by cold treatment against the eggs and larval stages of the Mediterranean fruit fly, *Ceratitis capitata* (Wied.). Aust J. Exp. Agric. Anim. Husb., 16: 280-285.

Syed, R.A. ; M.A. Ghani and M. Murtaza (1970). Studies on trypetids and their natural enemies in West Pakistan.III *Dacus (Strumeta) zonata* (Saunders). Tech. Bull. Common. Inst. Biol. Cont. 13: 1 - 16.

Willink, E. ; G. Gastaminza ; A. Salvatore ; M.C. Gramajo ; M. Acenolaza ; R. Avila and P. Favre (2006). Quarantine Cold Treatments for *Ceratitis capitata* and *Anastrepha fraterculus* (Diptera: Tephritidae) for citrus in Argentina: Conclusions after 10 years of research. Fruit Flies of Economic Importance: From Basic to Applied Knowledge Proceedings of the 7th International Symposium on Fruit Flies of Economic Importance 10-15 September 2006, Salvador, Brazil, pp. 285-293

التبريد كوسيلة للقضاء على ذبابة ثمار الخوخ باكتروسيرا زوناتا (ثنائيات الأجنحة: تيفرتيدى)

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منذ هاجمت ذبابة ثمار الخوخ البساتين المصرية خلال تسعينات القرن الماضى، أحدثت مخاطر لمنتجات الحاصلات البستانية وأثرت على تصدير الموالح إلى البلدان الأخرى. هذه الدراسة تهدف إلى بحث تأثير التبريد كوسيلة للقضاء على الأطوار الغير كاملة لذبابة ثمار الخوخ داخل ثمار الموالح (البرتقال الصيفى). تمت دراسة تأثير درجة حرارة تخزين الثمار والفترة التى تحدث نسبة ١٠٠% موت لأكثر الأطوار مقاومة للتبريد. أجريت تجربة للتبريد على نطاق ضيق حيث تعرضت الأطوار الغير كاملة داخل الثمار (بيضة، عمر

يرقى أول، عمر يرقى ثان، عمر يرقى ثالث) لدرجة حرارة ١,٧ ± ٠,٥°م لمدة ٣، ٥، ٧، ١٠، ١٤ يوم. أثبتت الدراسة أن العمر اليرقى الثالث هو أكثر الأطوار مقاومة للتبريد. عند تعرض العمر اليرقى الثالث لدرجة ١,٧± ٠,٥°م لمدة ١٠، ١٤ يوم تم القضاء على جميع اليرقات. لتأكيد تجربة النطاق الضيق، أجريت تجربة موسعة للتبريد حيث استخدمت أكثر من ١٤٧٠ ثمرة برتقال تحتوى كلا منها على كرت يحمل ٥٠ بيضة ثم حفظت فى درجة حرارة ٢٥°م ، ٦٥% رطوبة نسبية لحين ظهور العمر اليرقى الثالث(≈ ٣٣ يرقة عمر ثالث/ ثمرة) وتم تعريضها إلى درجة ١,٧± ٠,٥°م لمدة ١٤ يوم. عند فحص الثمار بحثا عن اليرقات وجد أن أكثر من ٣١٩٠٠ يرقة عمر ثالث قد تم القضاء عليها نهائيا ولم تخرج يرقة واحدة حية.