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EFFECT OF LOW TEMPERATURES ON EGGS AND LARVAE OF MEDITERRANEAN FRUIT FLY AND PEACH FRUIT FLY INSIDE FRUITS AS A QUARANTINE PROCEEDURE

BY

Hashem, A.G.; Nehad A. Soliman and Soliman, A.M.
Plant Protection Research Institute, Agricultural research Center, Ministry of Agriculture and Land Reclamation, 7, Nadi El-Said St., Dokki, Giza, Egypt, 12618. The corresponding Author. Tel.: +2-335-6176; FAX: 202-335-6176; e-mail: nehad4@hotmail.com

ABSTRACT

Effect of low temperatures on the eggs and larvae of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) and peach fruit fly, *Bactrocera zonata* (Saunders) inside fruits was studied using guava, orange and mango fruits. It was found that the storage of fruits infested with *B.zonata* at 4°C for 10 days and those infested with *C.capitata* at 1.7°C for 16 days would be acceptable as a quarantine treatment. Eggs, first, second and third larval instars of both pests were found susceptible to low temperatures mentioned above.

Keywords: Low temperature; eggs; larvae; *Bactrocera zonata*; *Ceratitis capitata*.

INTRODUCTION

The export of Egyptian fruits to many countries is restricted unless fruits are guaranteed to be free of pests. Peach fruit fly, *Bactrocera zonata* (Saunders) and Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) are very destructive pests to most fruit crops in Egypt. Chemical treatments such as ethylene dibromide and methyl bromide fumigation can be used for disinfestations of freshly infested fruits with these pests. Unfortunately, such treatments may result in toxic residues. Restrictions on use of chemical treatments increased and it will be completely banned because of these chemicals' potential carcinogenicity (Anonymous 1984).Thus, non-chemical quarantine treatments are needed. Cold temperatures affect the development of different stages of Mediterranean fruit fly, *C. capitata* in citrus (Sproul 1976), *Dacus tryoni* in kiwifruit (Rippon and Smith 1979) and Caribbean fruit fly, *Anastrepha suspensa* (Loew) in oranges (Benschoter 1983 and Hill *et al.*, 1988) and Queensland fruit fly in Australian mandarin (Heather *et al.*, 1996). The present work, was conducted to find out the effect of cold storage at 1.7°C and 4°C on the mortality of eggs and larval stages of *C. capitata* and *B. zonata* in guava, orange and mango fruits, respectively.

MATERIAL AND METHODS

Mature freshly harvested guava, orange (Navel and Valencia) and mango healthy fruits (contains no cuts, scratches or any type of infestation) grown

without use of insecticides were picked from local orchards in Ismailia and Alexandria Governorates, Egypt.

1. Infestation of fruits:

All fruits were artificially infested with eggs of Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) and peach fruit fly, *Bactrocera zonata* (Saunders) separately using a hypodermic syringe (Friend 1957; Hill *et al.*, 1988 and Jessup *et al.*, 1993) to introduce , approximately, equal number of eggs to each fruit. However, the location of eggs and larvae seemed to be easier to detect. In case of orange, small holes were made in fruits at different places by being punctured at depth of 3-4mm using a cork borer (0.2 mm in diameter) to facilitate the injection of eggs inside the fruits. The holes were covered by the core of fruit, then fixed with plaster strips (3cm x 3cm) each to prevent fall of eggs. In case of guava and mango, a "V" letter shape openings were made in fruits core, then eggs were injected beneath them. These artificial openings were covered by plaster strips (3 cm X 3 cm). Eggs aged 12 ± 2h old at the time of injection. The eggs had been collected from water-filled troughs placed under the fabric sides of flies' rearing cages.

2. Storage of infested fruits:

Infested fruits were stored at 23 ± 2°C and approximately 65% RH to allow larvae to develop. Trials in which susceptibility of eggs to low temperatures was tested, infested fruits were stored at 1.7°C and 4°C one day after infestation. Young and old larvae were allowed to develop for 5, 7 and 9 days for *Ceratitis capitata* and *for Bactrocera zonata* as well. In both species, young larvae were considered as the first and second instars, but old larvae are mainly third instars. Each infested fruit was packed separately in a fiberboard cage (20x20x20 cm). Temperature of infested fruits was determined using fruit thermometers (measures the temperature inside the fruit) and the period of storage began when the infested fruit reached the desired temperature. Temperatures inside incubators were measured by means of hygrothermographs. Fruits were stored for1,3, 5, 7,10,12,14,16 and 18 days at 1.7°C and 4°C for both species. At completion of storage periods, fruits were stored at 23 ± 2°C on mesh trays over sand (as pupation medium) as described by Seo *et al.*, (1971). A parallel control treatment was also carried out.

3. Examination of infested fruits:

Examination of fruits and sand for survivors was made after 5, 10 and 15 days and formation of normal puparium was considered as the standard survival (Baker 1939). Also, random samples of 20 treated fruits were dissected to determine the effect of cooling treatments on the developmental stages inside fruits. Percentage of mortality was determined by relating number of survivors to the production of pupae expected from the number of treated fruits. This was estimated from the number of pupae produced from check control samples.

4. Determination of the most-tolerant developmental stage:

Fruits contained third larval instar of both species were stored at 1.7°C and 4°C for 10, 16 and 18 days. A sample (20 fruits) was dissected and examined to determine the number of dead and alive larvae present. The fruits were cut and

squeezed gently to extract juice and most of the larvae. The remained fibers and pulp were macerated under the running water using a brush (n° 1 and 2) and a fine sieve to remove any remaining larvae. Microscopic examination determined that this method extracted all the larvae present. Larvae and macerated fruit pulps were examined under magnification and illumination and the number of alive and dead larvae was recorded. The rest of fruits were stored at 23 ± 2°C after each storage period for development of surviving individuals. This experiment was replicated three times for both species for each type of fruits.

After cold storage, fruits were kept at 20°C for 15 days marketing period and were measured in diameter before and after cold storage. Also, examination of the external and internal tissues was done.

5. Statistical analysis

Percentage of mortality was determined for each treatment, then converted to probits and plotted against the logarithm of days of exposure. The data were subjected to computer programmed probit regression analysis for estimation of probit 9 values.

RESULTS AND DISCUSSION

A total of 11,522 guava, orange (Navel and Valencia) and mango fruits containing an estimated population of 430,885 eggs and larvae of *Bactrocera zonata* and *Ceratitis capitata* was tested. Larvae of both species showed the same pattern of development in all types of tested fruits used (Table 1). Data obtained showed that eggs and first instar larvae of both *C. capitata* and *B. zonata* were highly sensitive to cold storage and complete mortality was obtained after 3 days when stored either in 1.7°C or 4°C. Second and third larval instars of both species were most tolerant. Complete mortality of second and third larval instars of *B. zonata* was obtained after 10 days of cold storage in both 1.7°C and 4°C. Also, the same result was obtained for second larval instar of *C.capitata*. Third larval instar of *C. capitata* required 16 days in cold storage at 1.7°C and 18 days at 4°C to obtain complete mortality. There was no pupal-development noticed after cold storage for the previously mentioned periods. The obtained data showed agreement with results obtained by Hill *et al.*, (1988) who found that eggs and first larval instar were the less-tolerant stages of *C. capitata*, when stored at 1.5 °C ± 0.5. Also, these results agree with data published by Jessup *et al.*, (1993) who tested the effect of 1°C± 0.2 on immature stages of *Dacus tryoni* in lemon fruits. Burditt and Balock (1985) stated that cold storage of larvae of *Dacus dorsalis* and *Dacus cucurbitae* for 10 -12 days at 1.7°C were enough to prevent their development. The data also are in agreement with those obtained by Hashem *et al.* (2002) who stated that cold storage at 1°C and 2°C were sufficient to kill any alive stage (eggs and larvae)of *C. capitata*. Slight loss of the volume of fruits was observed after the cold storage at 1.7°C and the fruits kept all their normal, healthy and physical characteristics externally and internally. When the fruits were kept at 20°C after the cold storage, they were found still keeping their healthy appearance and normal marketing characteristics similar to that before cold storage.

Table (1): Effect of fruit type on egg hatch and larval development of *C. capitata* and *B. zonata* at 23±2°C.

Fruit variety	No. of fruits	Total No. of insects	Mean no. of insects/fruit	% of egg hatch	Mean time needed for larvae to develop from egg in days		
					1st instar	2nd instar	3rd instar
C. capitata							
Guava	1,826	74,866	41.15±0.01	98.45	4.9± 0.01	7.01±0.05	8.11±0.12
Navel orange	1,750	68,250	39.03±0.12	98.12	4.71±0.08	6.8±0.02	8.8±0.2
Valencia Orange	1,670	63,460	38.17±0.03	98.56	4.22±0.01	6.53±0.12	9.01±0.15
Mango	1,290	96,517	75.18±0.04	99.01	4.01±0.03	6.71±0.41	8.63±0.01
B. zonata							
Guava	1,350	58,050	43.72±0.32	98.85	4.5±0.12	7.01±0.45	8.96±0.01
Navel orange	1,270	53,340	42.05±0.24	98.99	4.36±0.23	6.99±0.6	8.11±0.45
Valencia Orange	1,438	43,140	30.01±0.12	99.01	4.96±0.63	7.13±0.25	8.95±0.85
Mango	918	36,720	40.12±0.23	99.12	4.99±0.85	7.08±0.85	8.69±0.18

Table (2): Survival to pupation after cold storage of *C. capitata* and *B. zonata* eggs and larvae in guava fruits at different storage periods at 1.7°C

Fruit variety	No. of fruits	Total No. of insects/fruit	Mean No of insects/fruit	Days in cold storage	Mean No of surviving pupae / fruit when treated as:			
					eggs	1st instar	2nd instar	3rd instar
C. capitata								
Guava	913	30,409	41.15±0.01	0	38.13[a]	38.87[a]	39.15[a]	38.75[a]
				1	10.13	12.03	11.15	18.12
				3	1.01	1.12	8.27	15.13
				5	0	0	5.62	13.95
				7	0	0	2.57	7.95
				10	0	0	0	4.91
				12	0	0	0	2.18
				14	0	0	0	1.02
				16	0	0	0	0
				18	0	0	0	0
B. zonata								
Guava	675	29,513	43.72±0.32	0	43.02[a]	42.13[a]	41.23[a]	41.13[a]
				1	8.97	8.13	9.13	12.15
				3	1.12	1.09	6.78	8.03
				5	0	0	2.01	5.03
				7	0	0	1.03	2.13
				10	0	0	0	0
				12	0	0	0	0
				14	0	0	0	0
				16	0	0	0	0
				18	0	0	0	0

[a] Mean numbers of survivors of fruits

Table 3: Effect of cold storage at 4°C on development of eggs and larvae *C. capitata* and *B. zonata* in guava fruits at different storage periods.

Fruit variety	No. of fruits	Total No. of insects/fruit	Mean No of insects/fruit	Days in cold storage	Mean No of pupae surviving / fruit when treated as:			
					eggs	1st instar	2nd instar	3rd instar
				C. capitata				
Guava	907	35,409	41.15± 0.01	0	40.13[a]	39.87[a]	37.15[a]	35.75[a]
				1	15.13	19.03	19.15	23.12
				3	3.23	1.19	12.08	21.13
				5	0	0	10.27	17.95
				7	0	0	3.49	9.11
				10	0	0	0	6.24
				12	0	0	0	4.03
				14	0	0	0	2.13
				16	0	0	0	1.11
				18	0	0	0	0
				B. zonata				
Guava	775	32,513	43.72± 0.32	0	43.02[a]	42.13[a]	41.23[a]	41.13[a]
				1	8.97	8.13	9.13	12.15
				3	2.56	1.02	5.01	9.03
				5	0	0	3.12	4.12
				7	0	0	1.01	1.09
				10	0	0	0	0
				12	0	0	0	0
				14	0	0	0	0
				16	0	0	0	0
				18	0	0	0	0

[a] Mean numbers of survivors of fruits

Table 4: Effect of cold storage at 1.7°C on development of immature stages of *C. capitata* and *B. zonata* to pupation in Navel and Valencia orange fruits at different storage periods.

Fruit variety	No. of fruits	Total No. of insects/fruit	Mean No of insects/ fruit	Days in cold storage	Mean No of pupae surviving / fruit when treated as:			
					eggs	1st instar	2nd instar	3rd instar
			C. capitata					
Navel	875	24.154	27.03±0.12	0	26.13[a]	26.87[a]	26.15[a]	26.75[a]
				1	9.13	8.03	10.15	14.12
				3	2.12	1.92	5.19	11.13
				5	0	0	2.99	11.95
				7	0	0	1.02	5.11
				10	0	0	0	4.87
				12	0	0	0	2.13
				14	0	0	0	1.18
				16	0	0	0	0
				18	0	0	0	0
Valencia	813	27.179	33.17±0.03	0	32.98[a]	31.12[a]	32.03[a]	32.02[a]
				1	8.15	10.01	10.12	20.14
				3	1.33	1.04	6.02	18.01
				5	0	0	3.14	15.62
				7	0	0	0.93	8.02
				10	0	0	0	6.03
				12	0	0	0	5.12
				14	0	0	0	1.99
				16	0	0	0	0
				18	0	0	0	0
			B. zonata					
Navel	675	29,513	43.72± 0.32	0	41.02[a]	42.13[a]	40.23[a]	41.13[a]
				1	8.97	8.13	9.13	12.15
				3	1.95	3.12	6.13	8.03
				5	0	0	3.96	2.11
				7	0	0	1.01	1.02
				10	0	0	0	0
				12	0	0	0	0
				14	0	0	0	0
				16	0	0	0	0
				18	0	0	0	0
Valencia	635	26,701	41.62± 1.02	0	40.79[a]	38.12[a]	39.54[a]	38.94[a]
				1	7.02	10.12	13.02	10.65
				3	3.12	2.65	8.13	9.69
				5	0	0	6.39	7.12
				7	0	0	1.13	3.18
				10	0	0	0	0
				12	0	0	0	0
				14	0	0	0	0
				16	0	0	0	0
				18	0	0	0	0

[a] Mean numbers of survivors of fruits.

Table 5: Survival to pupation after cold storage at 4°C of eggs and larvae of *C. capitata* and *B. zonata* in Navel and Valencia orange fruits at different storage periods.

Fruit variety	No. of fruits	Total No. of insects/fruit	Mean No of insects/ fruit	Days in cold storage	Mean No of pupae surviving / fruit when treated as:			
					eggs	1st instar	2nd instar	3rd instar
			C. capitata					
Navel	865	27.154	30.93±0.12	0	26.13[a]	25.87[a]	26.15[a]	25.75[a]
				1	5.13	8.03	10.15	19.12
				3	1.12	3.32	5.65	14.13
				5	0	0	2.11	9.12
				7	0	0	1.17	9.09
				10	0	0	0	7.36
				12	0	0	0	4.19
				14	0	0	0	3.01
				16	0	0	0	1.15
				18	0	0	0	0
Valencia	813	28.179	31.17±0.03	0	28.98[a]	28.92[a]	28.03[a]	27.02[a]
				1	8.15	10.01	10.12	19.14
				3	1.32	3.38	1.02	13.01
				5	0	0	1.02	10.23
				7	0	0	1.01	7.35
				10	0	0	0	4.12
				12	0	0	0	3.66
				14	0	0	0	1.96
				16	0	0	0	1.01
				18	0	0	0	0
			B. zonata					
Navel	678	27,513	30.72± 0.32	0	28.02[a]	27.13[a]	27.23[a]	29.13[a]
				1	8.97	8.13	9.13	12.15
				3	2.19	3.99	4.17	6.19
				5	0	0	2.77	3.09
				7	0	0	1.93	1.99
				10	0	0	0	0
				12	0	0	0	0
				14	0	0	0	0
				16	0	0	0	0
				18	0	0	0	0
Valencia	635	28,701	30.01± 1.02	0	27.79[a]	28.12[a]	29.54[a]	29.94[a]
				1	7.02	10.12	13.02	12.23
				3	3.91	5.16	9.13	8.03
				5	0	0	3.98	3.18
				7	0	0	1.11	1.99
				10	0	0	0	0
				12	0	0	0	0
				14	0	0	0	0
				16	0	0	0	0
				18	0	0	0	0

[a] Mean numbers of survivors of fruits.

Table 6: Survival to pupation, after cold storage at 1.7°C, of eggs and larvae *C. capitata* and *B. zonata* in mango fruits.

Fruit variety	No. of fruits	Total No. of insects/fruit	Mean No of insects/ fruit	Days in cold storage	Mean No of pupae surviving / fruit when treated as:			
					eggs	1st instar	2nd instar	3rd instar
			C. capitata					
Mango fruits	645	27.179	38.93±0.12	0	38.13[a]	38.87[a]	38.15[a]	38.75[a]
				1	9.13	12.03	12.15	20.12
				3	4.19	6.09	7.14	15.02
				5	0	0	3.27	11.13
				7	0	0	1.67	5.95
				10	0	0	0	3.11
				12	0	0	0	3.09
				14	0	0	0	1.28
				16	0	0	0	0
				18	0	0	0	0
			B. zonata					
Mango fruits	459	18,365	40.01±0.33	0	39.02[a]	39.13[a]	39.23[a]	39.13[a]
				1	8.97	8.13	9.13	12.19
				3	3.12	5.15	8.65	8.79
				5	0	0	3.62	5.89
				7	0	0	1.01	2.98
				10	0	0	0	0
				12	0	0	0	0
				14	0	0	0	0
				16	0	0	0	0
				18	0	0	0	0

[a] Mean numbers of survivors of fruits.

Table 7: Survival to pupation after cold storage at 4 °C of eggs and larvae of *C. capitata* and *B. zonata* in mango fruits.

Fruit variety	No. of fruits	Total No. of insects/fruit	Mean No of insects/ fruit	Days in cold storage	Mean No of pupae surviving / fruit when treated as:			
					eggs	1st instars	2nd instars	3rd instars
			C. capitata					
Mango fruits	641	27.062	37.93±0.12	0	38.13[a]	38.87[a]	38.15[a]	38.75[a]
				1	10.13	13.03	14.15	29.12
				3	1.14	3.99	7.27	22.13
				5	0	0	3.89	15.79
				7	0	0	1.19	7.16
				10	0	0	0	7.02
				12	0	0	0	5.78
				14	0	0	0	4.66
				16	0	0	0	2.33
				18	0	0	0	0
			B. zonata					
Mango fruits	450	18,315	39.01±0.33	0	38.92[a]	38.83[a]	38.23[a]	38.13[a]
				1	3.97	9.13	17.13	20.15
				3	0.93	3.12	9.16	15.03
				5	0	0	4.16	6.36
				7	0	0	2.01	4.33
				10	0	0	0	0
				12	0	0	0	0
				14	0	0	0	0
				16	0	0	0	0
				18	0	0	0	0

[a] Mean numbers of survivors of fruits.

Table (8): Responses to, cold storage at 1.7°C, of immature stages of *C.capitata* and *B. Zonata* in different fruit types.

Type of fruits	Estimated population	Life stage	$LT_{99.998}$ (95% FL)			
			C. capitata	Estimated population		*B. zonata*
Guava	30,409	Eggs	3.87 (3.01 , 5.79)	29,513	Eggs	3.71 (2.92 , 5.38)
		1st instars	3.82 (3.04, 5.45)		1st instars	3.71 (2.91, 5.47)
		2nd instars	9.28 (8.33 , 11.16)		2nd instars	8.18 (6.89 , 10.73)
		3rd instars	14.74 (13.81 , 17.22)		3rd instars	8.82 (7.79 , 11.15)
Navel Orange	24,154	Eggs	4.79 (2.82 , 6.38)	27,179	Eggs	4.77 (3.59 , 7.65)
		1st instars	4.62 (3.04 , 5.95)		1st instars	4.89 (3.67 , 7.81)
		2nd instars	8.85 (6.46, 14.72)		2nd instars	8.01 (7.02 , 10.93)
		3rd instars	15.83 (14.59 , 19.67)		3rd instars	8.13 (6.84 , 14.24)
Valencia Orange	27,179	Eggs	4.48 (3.43 , 7.24)	26,701	Eggs	4.45 (3.96 , 5.97)
		1st instars	4.11 (3.27, 6.14)		1st instars	4.83 (3.97 , 6.03)
		2nd instars	9.8 (7.86 , 14.44)		2nd instars	7.95 (7.11 , 9.92)
		3rd instars	15.77 (14.79 , 17.99)		3rd instars	9.78 (8.16 , 14.65)
Mango	27,179	Eggs	4.12 (3.05 , 6.19)	18,365	Eggs	4.66 (3.16 , 7.32)
		1st instars	4.65 (3.31 , 6.64)		1st instars	4.65 (3.85 , 7.99)
		2nd instars	9.14 (7.70 , 14.27)		2nd instars	8.14 (7.13 , 11.36)
		3rd instars	15.24 (14.22 , 18.25)		3rd instars	10.11 (8.71 , 13.61)

CONCLUSION

It is clearly obvious from the results obtained from this work that fruits which may be infested with either *C. capitata* or *B. zonata* could be stored at 1.7°C for 16 days as a quarantine security procedure for these pests.

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تأثير درجات الحرارة المنخفضة على الأطوار المختلفة لذبابة فاكهة البحر المتوسط وذبابة ثمار الخوخ داخل الثمار لإعتمادها كإجراء حجرى .

عبد الفتاح جاد هاشم، نهاد عبد الحميد سليمان و على محمود سليمان .

معهـد بحوث وقاية النباتات، مركز البحوث الزراعية، وزارة الزراعة. ٧، ش نادى الصيد الدقى – الجيزة – جمهورية مصر العربية.

يمثل وجود حشرتى ذبابة فاكهة البحر المتوسط و ذبابة ثمار الخوخ (الدراق) عائقـا أمام تصدير معظم أنواع الفاكهة المنتجة فى جمهورية مصر العربية طبقا لنظم الحجـر الـزراعى المتعارف عليها. ولذلك تم دراسة تأثير درجات الحرارة المنخفضة على الأطوار الحية التى يمكن أن تتواجد داخل الثمار، و قد أجريت الدراسة على ثمار الجوافة و البرتقال بنوعى أبو سرة و الصيفى و المانجو و أثبتت النتائج ان حفظ الثمار علـى درجة ٤م لمدة ١٠ أيام كان كافيا لقتل أى طور حى داخل الثمار بالنسبة لحشرة ذبابـة ثمار الخوخ (الدراق) ؛ أما بالنسبة لحشرة ذبابة فاكهة البحر المتوسط فإن حفظ الـثمار على درجة ١,٧م لمدة ١٦ يوما كان كافيا لقتل أى طور حى داخل الثمار. وقد تبيـن أن طور البيضة و الأطوار اليرقية الثلاث كانت ذات حساسية لدرجات الحرارة المنخفضـة بالنسبة للحشرتين. كما تم دراسة تأثير ذات درجات الحرارة السابق ذكرها علـى الثمار و صفاتها و قد وجد أن الثمار احتفظت بجودتها و صفاتها الطبيعية و لم تتأثر بدرجات الحرارة المنخفضة .