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EFFECT OF COLD-STORAGE TEMPERATURES UPON THE MEDITERRANEAN FRUIT FLY

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INTRODUCTION

Since the introduction of the Mediterranean fruit fly (*Ceratitis capitata* Wied.) into the Hawaiian Islands and the subsequent quarantines against Hawaiian fruits, the problem of the fruit grower in these islands has been how to use his fruit to advantage at home. Many host fruits of the fruit fly are ruined long before they are suitable for either the table or storage. There are, however, other fruits, such as the avocado (*Persea gratissima*) and certain varieties of mangos (*Mangifera indica*) and star-apples (*Chrysophyllum cainto*), which, while often becoming too badly infested to be of use if left to ripen normally upon the tree, become infested so late in their development that they may be preserved for commerce if they respond favorably to cold storage, and if such cold storage kills whatever stages of the fruit fly may be present in the fruit when picked.

The experimental work reported in this paper was undertaken primarily with the hope that it would be an aid in solving the discouraging problems of the local horticulturists. But whatever its value in this direction, it now appears that the results may be of much greater commercial importance in defining the conditions under which cold-storage temperatures will kill the fruit fly in stored fruits, thus rendering them free from danger as transporters of this pest from one country to another or even from one infested district to another in host fruits.

HISTORICAL REVIEW

Cold-storage temperatures have been used in economic entomology in the past more to suspend insect activity than to cause death, except in the case of the Mediterranean fruit-fly work in Australia and Africa. The first practical use of cold-storage temperatures known to the writers was made by the manager of a large storage-warehouse company of Washington, D. C., in an attempt to find a safe method of protecting clothing from insect ravages during the warmer period of the year. At the suggestion and with the assistance of Dr. L. O. Howard experiments were carried on to determine the effect of cold-storage temperatures upon still other insects affecting stored goods. Dr. Howard (1),[1] in a paper read before the eighth annual meeting of the Association of Economic Entomologists in 1896, discussed for the first time in professional entomological literature the important use to which cold-storage temperatures may be put in controlling insects. In 1905 Duvel (2), while investigating the storage of cowpeas (*Vigna sinensis*), found that storage at 32° to 34° F. was entirely practicable and economical in combating the common bean weevil (*Bruchus obtectus*), the cowpea weevil (*Bruchus chinensis*), and the four-spotted bean weevil (*Bruchus quadrimaculatus*).

While the work referred to above was carried on primarily to safeguard produce and stored goods from attack during certain periods when pests are active, experiments to determine the effect of cold-storage temperatures upon the Mediterranean fruit fly have been undertaken with the object of killing the various stages within the fruit. The interest in this work in Africa and Australia has grown out of the fact that the growers have sought for their surplus fruit markets in northern Europe, England, and North America, and even in South America, China, and the Hawaiian Islands. To reach these markets their fruits must be in transit a sufficiently long time for infestations overlooked at the packing houses to cause considerable decay unless the cold-storage temperature to which the fruit is subjected en route either suspends or kills chance cases of infestation.

In 1906, Fuller (3) recorded the resistance of fruit-fly larvæ in a certain lot of peaches in Natal to 40° F. for 124 days. The writers question the accuracy of this statement, as they have been unable at this temperature to keep larvæ or eggs alive for more than 22 days, in tests covering several thousand larvæ and eggs (see Table I). Fuller believes from his observation that cold storage as a method of substitution for quarantines involves considerable risk.

Lounsbury (4) states in 1907 that experiments conducted by him in South Africa indicate that a temperature of 38° to 40°, continued for three weeks, is sufficient to insure the death of all fruit-fly larvæ in infested fruit, that two weeks at such a temperature causes considerable mortality, and that one week is thoroughly ineffective. In 1908, in a second paper (6), he records no living larvæ among 511 specimens found in peaches held for 21 and 27 days at 38° to 40°. It is his belief that the storage temperature necessary for the preservation of fruit in transit from Africa to countries of the Northern Hemisphere and to America is amply low to effect the extinction of all life in larvæ and eggs of the fruit fly contained within it.

Hooper (5) recorded in 1907 in West Australia that he had found that larvæ and eggs of the fruit fly could not resist temperatures ranging from

[1] Reference is made by number to "Literature cited, p. 665–666.

33° to 35° for more than 15 days, and advised that fruit kept within this range of temperature for three weeks would be perfectly free from living forms. His report indicates that the work was done carefully.

The work of Wilcox and Hunn (7) in 1914 has shown that such semitropical host fruits as the star-apple, fig (*Ficus* spp.), papaya (*Carica papaya*), mango, and avocado withstand without injury to texture or flavor a temperature slightly above 32° for from 27 days in the case of papaya to two months in the case of the avocado. Such periods at 32° are well above the margin of safety for complete mortality of the larvæ and eggs of the fruit fly.

EXPERIMENTAL WORK

In determining the effect of cold-storage temperatures upon the eggs and larvæ of the Mediterranean fruit fly, the writers have been fortunate in securing the cooperation of an ice company during 1913 and of an electric company during 1914 and 1915. At the cold-storage plants of these companies there were to be had all the facilities found in modern, well-regulated cold-storage plants. While an abundance of fruit-fly material is to be had in and about Honolulu, the writers have preferred in their work to infest in the insectary host fruits known to be previously free from attack. As no such fruits can be found in Hawaii under natural conditions, apples (*Malus* spp.) from California were used. These fruits were suspended for several hours in jars containing several hundred ovipositing fruit flies and then removed and held in the insectary for the number of days which experience had shown was necessary for the flies within to reach the stages desired for experiment. In this way larger amounts of material in definite stages could be used at one time than otherwise. While much of the data recorded in Table I was secured from fruit flies in apples, a sufficient amount, including observations on many thousands of eggs and larvæ, has been secured from fruit flies in peaches and kamani nuts (*Terminalia catappa*), as checks, to prove that there is no probability that the nature of the host fruit affects the action of temperatures.

No examination of material to determine the effect of various temperatures was made until the host fruits had been removed from storage from 24 to 48 hours. By placing the host fruits within storage the eggs and larvæ were under normal conditions. On examination the eggs were dissected out of the punctures and placed in moist chambers where all that hatched might be recorded. Larvæ found torpid though normal in color on examination within 24 to 48 hours after removal from storage invariably failed to resume activity.

THE EGG

No eggs hatch in cold storage if held at temperatures below 50° F.

A temperature of 32° proved quickly fatal to eggs. A total of 6,747 eggs were under observation. No eggs hatched upon removal from storage after the ninth day of refrigeration. Only one egg hatched on the ninth day, and but 2 out of 2,327 removed on the seventh, eighth and ninth days. After the tenth to fifteenth days of refrigeration, 2,221 eggs were removed to warmer temperature, but none hatched. Mortality increased rapidly after the fourth day of refrigeration; thus, on the fifth day only 15 out of 735 eggs hatched. (See Table I.)

TABLE I.—*Effect of cold-storage temperatures upon eggs and larvæ of the Mediterranean fruit fly*

Number of days in cold storage.	Temperature of storage room.	Eggs.		Larvæ.					
		Number under observation.	Number hatching after removal from storage.	First instar.		Second instar.		Third instar.	
				Number alive.	Number dead.	Number alive.	Number dead.	Number alive.	Number dead.
	°F.								
1	32	81	81	.....	.....	252	40	33	7
2	32	528	520	94	0	463	9	53	2
3	32	150	135	37	1	226	15	16	75
4	32	336	216	285	26	152	0	101	3
5	32	735	15	196	202	71	175	.....	.....
6	32	469	12	26	165	18	50	105	10
7	32	659	1	11	454	14	64	135	132
8	32	834	0	2	845	20	423	38	200
9	32	734	1	0	339	11	473	20	429
10	32	.....	.....	0	701	0	257	.....	.....
11	32	635	0	0	450	0	332	6	374
12	32	887	0	0	440	0	493	0	157
13	32	.....	.....	0	355	0	276	0	173
14	32	699	0	0	273	0	248	0	152
15	32	.....	.....	.....	.....	0	262	0	144
2	32–33	.....	.....	86	0	78	0	3	0
3	32–33	.....	.....	154	1	146	2	89	0
4	32–33	.....	.....	46	0	73	0	32	0
5	32–33	.....	.....	96	0	39	0	30	0
6	32–33	152	23	279	7	8	1	24	0
7	32–33	.....	.....	31	1	16	11	9	0
8	32–33	401	5	35	163	3	27	10	16
9	32–33	.....	.....	0	169	0	167	2	14
10	32–33	357	0	2	179	0	110	0	31
12	32–33	784	0	0	880	0	86	0	35
13	32–33	900	0	0	637	0	35	0	2
14	32–33	1,001	0	0	425	0	42	0	28
15	32–33	1,121	0	0	255	.....	.....	.....	.....
16	32–33	312	0	0	519	0	43	.....	.....
17	32–33	.....	.....	0	143	0	29	0	3
3	33–34	.....	.....	60	0	94	0	55	0
4	33–34	.....	.....	108	2	107	2	68	0
5	33–34	.....	.....	42	26	79	28	.....	.....
6	33–34	.....	.....	68	32	286	169	8	5
7	33–34	.....	.....	75	20	81	100	55	1
8	33–34	300	45	46	20	35	175	51	48
9	34–34	500	0	38	207	48	456	31	189
10	33–34	541	0	4	1,446	32	296	0	48
11	33–34	.....	.....	0	72	0	314	4	126
12	33–34	358	0	1	215	0	509	0	48
13	33–34	.....	.....	2	632	0	385	0	4

TABLE I.—*Effect of cold-storage temperatures upon eggs and larvæ of the Mediterranean fruit fly*—Continued

Number of days in cold storage.	Temperature of storage room.	Eggs.		Larvæ.					
				First instar.		Second instar.		Third instar.	
		Number under observation.	Number hatching after removal from storage.	Number alive.	Number dead.	Number alive.	Number dead.	Number alive.	Number dead.
	° *F.*								
14	33–34	1,035	0	0	76	0	245	0	49
15	33–34	746	0	0	710	0	301	3	154
16	33–34	1,058	0	1	763	0	65	0	53
17	33–34	513	0	0	521	0	45	0	134
18	33–34	1,000	0	0	514	0	46	.....	.....
19	33–34	.....	.....	0	221	0	67	0	18
8	34–36	.....	.....	.....	.....	0	11	7	170
9	34–36	.....	.....	.....	.....	0	21	1	176
10	34–36	.....	.....	0	44	0	8	5	321
11	34–36	236	0	0	192	0	60	0	225
12	34–36	.....	.....	0	74	0	138	4	399
13	34–36	241	0	.....	.....	0	84	0	436
14	34–36	.....	.....	0	111	0	19	0	354
15	34–36	.....	.....	0	42	0	6	0	158
2	36	167	131	.....	.....	120	5	242	2
3	36	281	261	166	3	261	1	260	6
4	36	419	419	127	2	245	4	180	22
5	36	433	405	288	2	473	25	256	24
6	36	365	254	75	57	334	12	158	77
7	36	184	150	28	142	147	43	62	157
8	36	454	264	1	382	0	323	33	363
9	36	858	335	1	475	0	300	2	402
10	36	301	27	0	494	0	385	0	160
11	36	652	2	0	588	0	437	0	186
12	36	728	0	0	670	0	858	0	213
13	36	534	0	0	504	0	91	0	364
14	36	463	0	0	443	0	54	1	261
15	36	568	0	0	573	0	22	1	198
16	36	480	0	.....	.....	0	38	0	251
17	36	532	0	.....	.....	.....	.....	.....	.....
3	36–40	.....	.....	.....	.....	42	2	.....	.....
4	36–40	.....	.....	.....	.....	127	46	.....	.....
5	36–40	.....	.....	.....	.....	123	3	.....	.....
6	36–40	.....	.....	.....	.....	127	25	.....	.....
7	36–40	.....	.....	.....	.....	18	94	.....	.....
8	36–40	.....	.....	.....	.....	0	13	60	258
9	36–40	136	0	.....	.....	0	25	3	112
10	36–40	128	0	.....	.....	.....	.....	.....	.....
11	36–40	125	0	0	102	0	18	0	275
12	36–40	122	0	0	23	0	12	0	256
13	36–40	.....	.....	.....	.....	0	25	0	352
14	36–40	185	0	0	32	0	275	0	522
15	36–40	.....	.....	.....	.....	0	218	0	163
16	36–40	.....	.....	0	48	0	69	0	324
17	36–40	106	0	.....	.....	0	131	.....	.....
18	36–40	.....	.....	0	118	0	18	0	97
19	36–40	210	0	.....	.....	.....	.....	.....	.....
20	36–40	.....	.....	0	16	0	64	.....	.....

TABLE I.—*Effect of cold-storage temperatures upon eggs and larvæ of the Mediterranean fruit fly*—Continued

Number of days in cold storage.	Temperature of storage room.	Eggs.		Larvæ.					
				First instar.		Second instar.		Third instar.	
		Number under observation.	Number hatching after removal from storage.	Number alive.	Number dead.	Number alive.	Number dead.	Number alive.	Number dead.
	°*F.*								
10	38–40	......	......	38	8	19	8	10	1
12	38–40	......	......	4	25	26	19	36	6
13	38–40	......	......	3	60	15	0	......	......
14	38–40	......	......	0	36	17	40	......	......
15	38–40	......	......	15	46	5	24	......	......
16	38–40	......	......	0	99	14	148	1	25
20	38–40	......	......	0	42	0	39	4	3
23	38–40	......	......	0	43	0	84	......	......
25	38–40	......	......	0	18	0	133	0	1
28	38–40	......	......	0	33	0	27	0	9
30	38–40	......	......	0	44	......	......	......	......
2	40–45	12	12	......	......	......	......	......	......
3	40–45	55	19	......	......	......	......	......	......
4	40–45	26	0	......	......	......	......	......	......
5	40–45	8	3	......	......	......	......	......	......
6	40–45	16	12	......	......	......	......	......	......
8	40–45	14	7	......	......	......	......	......	......
9	40–45	31	17	......	......	......	......	......	......
11	40–45	14	1	......	......	......	......	......	......
14	40–45	31	1	......	......	......	......	......	......
15	40–45	30	0	......	......	......	......	......	......
17	40–45	26	6	......	......	......	......	......	......
19	40–45	21	0	......	......	37	34	80	56
20	40–45	67	2	......	......	79	79	138	135
21	40–45	127	0	......	......	107	130	187	103
22	40–45	50	0	......	......	......	......	......	......
23	40–45	15	0	......	......	92	97	160	226
24	40–45	21	0	......	......	68	182	125	220
25	40–45	38	0	......	......	14	281	89	88
26	40–45	......	......	......	......	30	95	106	320
28	40–45	......	......	......	......	0	9	27	208
29	40–45	......	......	......	......	1	131	57	112
31	40–45	......	......	......	......	0	161	8	201
32	40–45	......	......	......	......	0	8	4	139
33	40–45	......	......	......	......	0	290	5	318
36	40–45	......	......	......	......	0	218	7	397
37	40–45	......	......	......	......	0	345	3	393
38	40–45	......	......	......	......	0	204	7	377
39	40–45	......	......	......	......	0	42	1	385
40	40–45	......	......	......	......	0	84	0	401
41	40–45	......	......	......	......	0	112	2	330
42	40–45	......	......	......	......	0	92	0	292
44	40–45	......	......	......	......	0	39	0	200
45	40–45	......	......	......	......	0	36	1	689
46	40–45	......	......	......	......	0	23	0	476

Temperatures ranging from 32° to 33° proved equally fatal, the effect on 5,055 eggs being practically identical with that recorded for an even 32° F. Thus, no eggs hatched from batches removed between the ninth and sixteenth days of refrigeration, although 4,475 were under observation. Only 5 eggs hatched out of 401 removed on the eighth day, and 23 out of 152 removed on the sixth day.

Temperatures ranging from 33° to 34° proved fatal after the eighth day; 45 eggs out of 300 removed on the eighth day hatched. No eggs hatched out of 6,051 removed between the ninth and eighteenth days of refrigeration.

At 34° to 36° eggs were examined only on the eleventh and thirteenth days of refrigeration. No eggs hatched out of 236 and 241 removed after these periods of refrigeration.

All the eggs subjected to a temperature of 36° were not killed until after the eleventh day of refrigeration. Out of 652 eggs removed from storage on the eleventh day, 2 hatched; and out of 301 eggs removed after 10 days, 27 hatched. No eggs hatched out of 3,305 removed after from 12 to 17 days of refrigeration. No appreciable mortality occurred at this temperature until after one week.

No eggs held at 36° to 40° were examined until the ninth day of refrigeration. Out of 1,012 eggs removed in small batches daily between the ninth and nineteenth days of refrigeration, none hatched.

Only 602 eggs were used for refrigeration at 40° to 45°. No eggs hatched after a refrigeration of 21 days. Two eggs out of 67 refrigerated for 20 days hatched on removal to the laboratory. No eggs hatched of those removed after 21 to 25 days of refrigeration.

THE LARVA

Larvæ in the third instar proved more resistant to cold than larvæ in the first and second; and all instars are generally more resistant to low temperatures than are the eggs. (See Table I.)

A temperature of 32° F. was found fatal to larvæ of the first instar after the eighth day of refrigeration; 2,558 larvæ removed after refrigeration from 9 to 14 days were found to be dead. The data in Table I show that 2 out of 845 were alive on the eighth day of refrigeration and only 11 out of 454 on the seventh day. This temperature did not appear to affect the first-stage larvæ appreciably until after the fifth day of refrigeration. Larvæ of the second instar failed to live after the ninth day, and very few lived that long; but 11 out of 473 and 20 out of 423, respectively, were alive after the eighth and ninth days of refrigeration. All of 1,868 second-instar larvæ were found dead on removal from storage after the tenth to fifteenth days of refrigeration. Only 6 out of 332 larvæ of the third instar were alive on the eleventh day of refrigeration; 626 larvæ removed after 12 to 15 days of refrigeration were found dead.

A temperature of 32° to 33° had practically the same effect upon 5,352 larvæ as did 32°.

Temperatures ranging from 33° to 34° did not prove entirely fatal to the first-instar larvæ until the seventeenth day of refrigeration; one larva out of 763 was alive on the sixteenth day. This was very exceptional and demonstrates the value of using an abundance of material and of continuing examinations after all larvæ seem to have been killed. Only 4 out of 1,446 were alive after 10 days of refrigeration; 1 out of 215 after 12 days, and 2 out of 632 after the thirteenth day of refrigeration. First-instar larvæ to the number of 1,256, removed after the seventeenth, eighteenth, and nineteenth days of refrigeration, were all dead. No second-instar larvæ subjected to 33° to 34° were found alive after the tenth day of refrigeration; 1,997 removed after 11 to 19 days of refrigeration were all dead. A few third-instar larvæ subjected to 33° to 34° lived until the fifteenth day of refrigeration, but none for a longer time. After the ninth day no larvæ were found alive, except during the examinations made after the eleventh and the fifteenth days of refrigeration, when 4 out of 126 and 3 out of 154, respectively, were found alive. A study of the data in Table I shows that a temperature of 34° to 36° had practically the same effect upon 1,615 larvæ as did that of 33° to 34°.

A temperature of 36° proved fatal to first-instar larvæ after the tenth day. After the ninth day of refrigeration 1 out of 476 was found alive. No living first-instar larvæ out of 3,272 were found alive after refrigeration from 10 to 15 days. The mortality at this temperature among first-instar larvæ became very noticeable after the sixth day of refrigeration, when 57 out of 132 larvæ were found dead. No second-instar larvæ were found alive after the eighth day of refrigeration; thus, all of 2,508 removed after refrigeration from 8 to 16 days were found dead. No third-instar larva was found alive after the ninth day of refrigeration, except on the fourteenth and fifteenth days, when 1 living larva was found out of 262 and 199 larvæ examined. After the ninth day but 2 out of 404 larvæ were found alive.

Temperature, 36° to 40° F.: No examinations were made to determine the effect of this temperature on the first-instar larvæ until after the tenth day of refrigeration. Of 339 larvæ removed after refrigeration from 11 to 20 days, none was alive. No living second-instar larva was found alive after the eighth day of refrigeration; after the seventh day 18 out of 112 were found alive. All of 868 second-instar larvæ removed after refrigeration from 8 to 20 days were dead. No living third-instar larva was found after refrigeration for 10 days, 3 out of 115 being alive after refrigeration for 9 days. All of 1,989 larvæ removed after refrigeration from 11 to 18 days were dead.

Temperature, 38° to 40° F.: All of 279 first-instar larvæ removed from storage after refrigeration from 16 to 30 days were dead, 15 out of 61 being alive after refrigeration for 15 days. No living second-stage larva was found after refrigeration from 20 to 28 days. No examinations were made on the seventeenth, eighteenth, and nineteenth days; on the sixteenth day of refrigeration 14 out of 162 second-instar larvæ were alive. Third-instar larvæ were found alive after refrigeration for 20 days. No examinations were made between the twenty-first and twenty-fourth days, but no living third-instar larvæ were found during examinations of larvæ after the twenty-fifth and twenty-eighth days of refrigeration.

The warmest temperatures to which fruit flies were subjected ranged from 40° to 45°. Only larvæ of the second and third instars were used. One second-instar larva was alive on the twenty-ninth day, but no living second-instar larvæ were found thereafter, although a total of 1,658 larvæ were examined after refrigeration from 31 to 46 days. One third-instar larva was alive on the forty-fifth day. All of 476 third-instar larvæ examined on the forty-sixth day of refrigeration were dead. More data at this temperature are desirable to fix the limit safely in so far as the mature larvæ are concerned. Fruit is not, however, held at such high temperature as 40° to 45° for periods sufficiently long to kill the fruit-fly larvæ; hence, the effect of these temperatures is of far less importance than that of temperatures ranging from 32° to 40°.

CONCLUSION

The data contained in this paper show that no eggs or larvæ of the Mediterranean fruit fly survived refrigeration at 40° to 45° F. for seven weeks, at 33° to 40° for three weeks, or at 32° to 33° for two weeks. They may lead to the modification of existing quarantines and encourage the refrigeration of fruit subject to fruit-fly attack. It seems reasonable to conclude that sooner or later the certification of properly refrigerated fruit will be practicable. When an association of fruit growers or a people find it financially worth while there is no reason why they can not operate a central refrigeration plant under the supervision of an official whose reputation shall be sufficient to guarantee all fruits sent out from the plant to be absolutely free from danger as carriers of the Mediterranean fruit fly.

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