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Original Paper

Cold disinfestation of Tankan orange against *Bactrocera dorsalis* (Diptera: Tephritidae)

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Abstract: The Oriental fruit fly *Bactrocera dorsalis* (Hendel) reinvaded Amami Island in Japan in December 2015 and the host fruit of this fly was restricted from moving domestically from the island until the fly was eradicated in July 2016. To allow domestic movement of Tankan oranges even when *B. dorsalis* reinvades a region in Japan in future, a phytosanitary treatment against this fruit fly was developed. First, a suitable infestation method, the development of immature stages in fruits, and the most tolerant immature stage to cold treatment were investigated. Then, cold treatment against the most cold-tolerant stage of *B. dorsalis* at 1.0–1.5℃ for 16–18 days was conducted to determine a treatment schedule to kill all immature stages. Finally, large-scale cold treatment against the same stage at 1.0–1.5℃ for 17 days was conducted to verify the effectiveness of the treatment schedule. As a result, it was concluded that cold treatment at 1.1℃ for 17 days kills all immature stages of *B. dorsalis* infesting Tankan oranges. No remarkable negative effect on the fruit quality was observed after cold treatment under similar schedules to the large-scale treatment mentioned above.

Key words: cold treatment, quarantine treatment, *Citrus*, Oriental fruit fly, Tankan orange

Introduction

The Oriental fruit fly *Bactrocera dorsalis* (Hendel) caused serious damage to agricultural products in the southwestern islands of Japan. The species was eradicated in 1986 by using the male annihilation technique (MAT) over a period of 18 years (Yoshizawa 1997) and the restriction on the movement of the host plant of this fruit fly to Japan's mainland was lifted. MAT for *B. dorsalis* continues to be employed to prevent fruit fly reinvasion because the eradication area is exposed to a constant risk of fruit fly invasion from neighboring countries.

The temporary reinvasion of *B. dorsalis* on Amami-Oshima Island, Kagoshima Prefecture led to the suspension of fruit and vegetable movement from there to Japan's mainland in December 2015. Tankan oranges are a specialty of Amami-Oshima Island, with about 60% of the fruit production and about 20% of the agricultural production on this island (Kagoshima Prefecture, 2014). Fortunately, highly concentrated eradication resulted in the removal of restrictions on host plant movement in July 2016.

Ethylene dibromide (EDB) fumigation had been applied to Tankan oranges and allowed its movement from the domestic area where *B. dorsalis* existed to Japan's mainland before eradication of the species in 1986; however, EDB fumigation has been stopped since the substance is known to be a carcinogenetic chemical to humans.

The establishment of a postharvest phytosanitary treatment for host commodities of *B. dorsalis* in advance would be important as one of the measures to reduce the economic damage even when this fruit fly invades a region in Japan in future as in the case of Amami-Oshima Island. Cold treatment as one of the phytosanitary treatments against fruit flies has been applied to wide range of *Citrus* and research has been conducted; it might be a possible alternative to EDB fumigation for Tankan oranges (Grout et al. 2011ab; Shimada et al. 1974; Sugimoto and Hurusawa 1982).

In developing a quarantine treatment such as heat treatment or

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cold treatment, a large-scale disinfestation test is conducted after determining the most tolerant pest species and stages among the target pests (Corcoran et al. 1993; Heather and Hallman 2008; Hill et al. 1988; Jessup et al. 1993; Santaballa et al. 2009).

In the present study, in order to develop a cold treatment schedule for Tankan oranges against *B. dorsalis*, we studied its infestation methods in Tankan oranges and the development of *B. dorsalis* immature stages in the fruit. We determined the most cold-tolerant stage of *B. dorsalis* and the treatment schedule providing 100% mortality of the most tolerant stage and demonstrated 100% mortality after treatment with more than 30,000 insects.

Materials and Methods

1. Test insects

Laboratory colonies of *B. dorsalis* maintained at the Naha Plant Protection Station (NPPS) and Yokohama Plant Protection Station (YPPS) were used.

The colony at NPPS was originally collected on Okinawa Island before eradication in 1986 (Permit No. 63Y2152) and flies were reared at 27 ± 1℃, 70 ± 10% RH with a photoperiod of 14L:10D and were given an artificial diet and water in adult cages (45×30×30 cm). The studies "Infestation methods and development of *B. dorsalis* immature stages in Tankan", "Determination of the most cold-tolerant stage of *B. dorsalis*", "Small-scale disinfestation tests" and "Fruit quality tests for cold treatment" were conducted with the colony at NPPS. The colony at YPPS was originally collected on Ogasawara Island before eradication in 1985 (Permit No. 59Y1301) and flies were reared at 27 ± 1℃, 65 ± 10% RH with a photoperiod of 13L:11D and were given an artificial diet and water in adult cages (45×30×30 cm). A large-scale disinfestation test was conducted with the colony at YPPS.

2. Infestation methods and development of B. dorsalis immature stages in Tankan

To determine the most appropriate infestation method before the subsequent disinfestation tests, infestation tests were conducted.

For the following three experiments, Tankan fruits (weight: 140.1–169.8 g, average weight: 154.4 g) harvested in Okinawa were used. Fruits infested by 3 different methods were kept at 27 ± 1℃, 70 ± 10% RH, and then 7 fruits per day and per method were dissected and the number of survivors in each immature stage was counted from the next day after infestation to the 8th or 9th day.

(1) Natural infestation

Fifteen to twenty fruits were placed on top of the adult cage containing about 2,000 flies (21–31 days old after adult emergence). Flies laid eggs in these fruits through the mesh roof of the cage for one hour.

(2) Cap method: artificial infestation

Eggs were collected from gravid females (21–31 days old) by placing a polyethylene receptacle (7 cm in diameter, 17 cm in height) with small oviposition holes into the adult cage containing about 2,000 flies for one hour. The inner surface of the receptacle was moistened with lemon juice. Eggs were washed with tap water and transferred onto black gauze using a funnel. The black gauze with the eggs was then placed on moist filter paper in sterile plastic Petri dishes (9 cm in diameter). One hundred eggs were counted onto black filter paper (1×1 cm) using a fine brush under a microscope. Fruit was cut horizontally with a surgical knife and divided into the upper part (1/4 pulp) and the lower part (3/4 pulp). Some slits were made on the surface of the pulp of the lower part by knife and 100 eggs on the filter paper were inoculated on the surface. Some pulp was removed from the upper part and a hole for respiration was made using a cork borer. Finally, the upper part was placed on top of the lower part and affixed with surgical tape, to restore the same appearance as it was before being separated.

(3) Upper triangle method: artificial infestation

Eggs were collected the same way as mentioned above. A triangular pyramid was removed from the upper fruit using a surgical knife and the fruit was divided into a triangular pyramid (upper part) and the remainder (lower part). Some slits were made on the surface of the pulp of the lower part by knife and 100 eggs on the filter paper were inoculated on the surface. Some pulp was removed from the upper part and a respiration hole was made by knife. Finally, the upper part was placed back on top of the lower part and affixed with surgical tape, to restore the same appearance as it was before being separated.

3. Determination of the most tolerant stage of B. dorsalis to cold treatment

Tankan fruits (weight: 128.8–173.7 g (replicate 1: 128.8–173.7 g, replicate 2: 132.7–154.4 g)) harvested in Okinawa were used. Eggs were collected in the same way as described above. Fruits infested with 100 eggs were prepared using the upper-triangle method. The infested fruits were kept at 27 ± 1℃, 70 ± 10% RH for 1 day, 2 days, 3 days and 5 days, and resulted in fruits infested with eggs (29h-old eggs), first instar larvae, second instar larvae and third instar larvae, respectively. The fruits infested with four different stages were introduced into a cold treatment chamber (Hitachi Appliances, Inc., Cosmopia EC-40HHP) simultaneously and exposed to 1.0–1.5℃ for 3, 6, 9, 12 and 15 days. In each replicate, two infested fruits per developmental stage, per exposure day were used, and in addition, three uninfested fruits were used for the measurement of pulp temperature. For measuring the pulp temperature, sensor probes (Chino Co., Ltd., JPT100) were inserted into the core of the uninfested fruits after calibration by the ice-melting method. Treatment was considered to start when 2

of the 3 sensor fruits reached 1.5℃. After a specific exposure period, the infested fruits were removed from the cold chamber and kept at 27℃. Control fruits were prepared and stored in the same way as the treated fruits. For the mortality check, the treated and control fruits infested with eggs and first instar larvae were dissected after 5–6 days of storage at 27℃, fruits with second and third instar larvae were dissected after 3 days of storage at 27℃, and then the number of survivors was counted. Moving larva just after touching with a fine brush or tweezers was assumed as a survivor.

Data Analysis

All mortality data was corrected using Abbott's formula (Abbott 1925). The mortality data was subjected to Probit analysis (LeOra Software Co., PoloPlus, version 1.0) to estimate LT_{90}, LT_{95} and LT_{99} providing the lethal exposure period for mortality of 90%, 95% and 99%, respectively, and to compare the cold tolerance among the immature stages of *B. dorsalis*.

4. Small-scale disinfestation tests

The objective of this test was to determine a cold treatment schedule providing 100% mortality of the most cold-tolerant stage of *B. dorsalis*.

Experiment 1

Tankan fruits (weight: 125.3–175.8 g (replicate 1: 125.3–175.8 g, replicate 2: 140.4–167.0 g)) harvested in Okinawa were used. Infested fruits were prepared by the upper-triangle method except that the eggs were collected from the adults for 3 hours and 100–120 eggs were inoculated into the fruit in this test. The infested fruits were kept at 27 ± 1℃ and 70 ± 10% RH for 5 days and produced third instar-infested fruits.

The fruits infested with third instar larvae were introduced into a cold treatment chamber (Hitachi Appliances, Inc., Cosmopia EC-40HHP) simultaneously and exposed to 1.0–1.5℃ for 16, 17 and 18 days. An untreated control group was prepared at the same time. In each replicate, 60 infested fruits were used on each exposure day, and 6 uninfested fruits were used for the measurement of pulp temperature during the cold treatment. For measuring the pulp temperature, sensor probes (Chino Co., Ltd., JPT100) were inserted into the uninfested fruits after calibration by the ice-melting method. A commercial carton box (38×30×22 cm) was filled with 60 infested fruits and 6 sensor fruits. Treatment was considered to start when 3 of the 6 sensor fruits reached 1.0℃. After the specific exposure period, the infested fruits were removed from the cold chamber and kept at 27℃. For the mortality check, the treated and control fruit were dissected after 3 days of storage at 27℃ and the number of survivors was counted.

Experiment 2

In Experiment 1, all treated larvae were killed by the cold treatment. In order to investigate the border between life and death, we carried out this additional experiment.

Tankan fruits (weight: 145.1–221.5 g) harvested in Okinawa were used, after being washed with tap water. Fruits infested with 100 eggs were prepared by the upper-triangle method except that the eggs were collected from the adults for 2 hours. The infested fruits were kept at 27 ± 1℃, 60 ± 10% RH and 14L:10D for 5 days, and produced third instar larvae-infested fruits.

Twelve commercial carton boxes (42.5×32×34.5 cm) were loaded into two cold treatment machines (Yamato Scientific Co., Ltd. Program Incubator Model: IQ822) simultaneously and exposed to around 1.0–1.5℃ for 17 days (3 boxes/chamber × 2 chambers/machine). Each box was filled with 20–21 infested fruits, 1 sensor fruit (at the center) and 9–12 uninfested filler fruits (at the outer edge). Wireless sensor probes (T&D Co., Ltd., Model: RTR-502) were used to measure the pulp temperature of the sensor fruits every hour. Treatment was considered to start when 2 of the 3 sensor fruits reached 1.5℃. After the specific exposure period, the infested fruits were removed from the cold chamber and kept at 27℃ for two days. Fifty control fruits were prepared and stored in the same way as the treated fruits. For the mortality check, the treated and control fruits were dissected after two days of storage at 27℃, and the number of survivors was counted.

5. Large-scale disinfestation test

The objective of this test was to verify that were no survivors from more than 30,000 of the most cold-tolerant stage (third instar) of *B. dorsalis* in the total of 3 replicates of cold disinfestation tests (fruit pulp temperature at 1.0℃ for 17 days).

Tankan fruits (weight: 118.9–298.1 g (rep 1: 118.9–280.0 g, rep 2: 138.0–237.9 g, rep 3: 134.1–298.1 g)) harvested in Okinawa were used after washing with tap water and spraying the fruit surface with 70% ethanol as a fungicide. Infested fruits were prepared by the upper-triangle method. The infested fruits placed in a plastic container (32×40×16 cm) were kept at 27 ± 1℃, 65 ± 10% RH, 13L:11D for 5 days and produced third instar-infested fruits. In each replicate, 330 infested fruits (275 fruits for cold treatment and 55 fruits for untreated control) were prepared. The infested fruits for untreated control were still kept at 27℃ for 1–3 more days before the mortality check. The infested fruits for cold treatment were packed into 10 commercial cartons (31.5×38×18 cm/carton; 27–28 fruits/carton) with uninfested filler fruits. Seven uninfested fruits for the measurement of pulp temperature during cold treatment were placed in 7 of the 10 cartons. For measuring the pulp temperature every hour, sensor probes (T&D Co., Ltd. Model: RTR-52) were inserted into the uninfested fruits after calibration using the ice-melting method. The fruits in cartons were introduced into a cold chamber (Nikkei Panel System Co., Ltd., 21.3 m^3) so that the pulp temperature was exposed to around 1.0℃ for 17 days. Treatment was considered to start when 4 of the

7 sensor fruits dropped to 1.5℃. After 16 days and 15–22 hours, the infested fruits were removed from the cold chamber and kept at 27℃ for 2–3 days. The treated and control fruits were dissected carefully, and the number of survivors was counted for the mortality check.

6. Fruit quality test for cold treatment

The objective of this test was to determine the fruit quality after cold treatment. Tankan fruits (weight: 140.1–159.3 g) harvested in Okinawa were used. Ten fruits were allocated to each experimental lot held at: 20℃ for 15, 20, 25 and 30 days; 1℃ for 15 and 20 days; and 9℃ for 5 and 10 days after 1℃ for 15 and 20 days.

After the completion of each storage schedule, weight loss (%), citric acid (%), Brix (%), and number of decayed fruit were investigated. A saccharic acidity measuring apparatus (Horiba, Ltd., Model: NH-1000) was used for measurement of citric acid and Brix.

Results and Discussion

1. Infestation methods and development of B. dorsalis immature stages in Tankan oranges

The percentages of each immature stage of *B. dorsalis* in Tankan oranges after egg infestation by 3 different methods are shown in Table 1. The hatchability of eggs used was 87.3 ± 2.2%.

In the cap method and upper-triangle method, the composition of each immature stage changed with the number of days after infestation and the dominant immature stage in the fruit was clear, such as egg, first instar, second instar and third instar larvae for 1, 2, 3 and 5 days after inoculation, respectively. On the other hand, in natural infestation, it was not clear.

Decayed fruits were observed 9 days after infestation in natural infestation compared to 3 days and 6 days after inoculation in the cap method and upper-triangle method, respectively.

Furthermore, remarkable fruit softening due to larval feeding was not observed until 9 days after infestation by natural infestation, while it was observed at 7 days with the cap and upper-triangle method.

The upper-triangle method was selected as the infestation technique for subsequent disinfestation tests, because of the clear development of immature stages in the fruits and the good fruit condition after egg inoculation. It was assumed that the fruit at 1 day, 2 days, 3 days and 5 days after egg inoculation was infested with eggs, first instar larvae, second instar larvae, and third instar larvae, respectively.

2. Determination of the most cold-tolerant stage of B. dorsalis

The mean fruit-pulp temperature (mean minimum pulp temp, mean maximum pulp temp) of Tankan during cold treatment for 15 days was 1.34℃ (1.25℃, 1.42℃) and 1.32℃ (1.25℃, 1.43℃) for the first and second replicate, respectively.

The number of survivors and the corrected mortality in the 4 different stages infesting the treated fruits on each exposure day are shown in Table 2. No survivors were observed after 6 days or more of exposure in the egg- and first instar-infested fruits, and after 12 days or more of exposure in the second instar-infested fruits; however, one survivor was observed even after 15 days of exposure in the third instar-infested fruits. Therefore, third instar larvae were considered as the most cold-tolerant stage of *B. dorsalis*.

Table 1 Development of *Bactrocera dorsalis* in Tankan oranges stored at 27° C (Percentage of each developmental stage of *B. dorsalis* after oviposition)

Infestation method	Stage	Days after oviposition								
		1d	2d	3d	4d	5d	6d	7d	8d	9d
Natural infestation	Egg	100	23.5	0	0	0	0	0	0	0
	1st instar	0	76.5	99.9	76.1	56.8	65.6	33.9	10.0	8.6
	2nd instar	0	0	0.1	23.9	43.2	34.3	63.8	73.3	63.9
	3rd instar	0	0	0	0	0	0.1	2.4	16.7	27.6
	Pupa	0	0	0	0	0	0	0	0	0
Cap method	Egg	100	11.7	0	0	0	0	0	0	−
	1st instar	0	88.3	27.9	3.9	0	0	0	0	−
	2nd instar	0	0	72.1	20.3	5.5	0	0.4	4.6	−
	3rd instar	0	0	0	75.8	94.5	100	99.3	94.9	−
	Pupa	0	0	0	0	0	0	0	0.5	−
Upper-triangle method	Egg	100	9.4	0	0	0	0	0	0	−
	1st instar	0	90.6	31.1	6.7	0	0	0	0	−
	2nd instar	0	0	68.9	44.7	1.6	0.2	0	6.0	−
	3rd instar	0	0	0	48.6	98.4	99.8	100	94.0	−
	Pupa	0	0	0	0	0	0	0	0	−

Data from this test was subjected to Probit analysis to estimate the number of exposure days at 1.0℃ providing 90% mortality (LT_{90}), 95% mortality (LT_{95}) and 99% mortality (LT_{99}) in each stage of *B. dorsalis* (Table 3). The values of LT_{95} and LT_{99} indicate that a longer treatment period is required for the second instar than the third instar larvae; however, confidence limits of them were overlapped between second instar and third instar larvae, whereas confidence limits of LT_{90} were not overlapped between them.

It is concluded that third instar larva is the most tolerant to cold treatment of 1.0℃ among the 4 different immature stages of *B. dorsalis*.

3. Small-scale disinfestation tests

Experiment 1

The mean fruit-pulp temperature (mean minimum pulp temp, mean maximum pulp temp) of Tankan during cold treatment for 18 days was 1.19℃ (0.96℃, 1.44℃) and 1.14℃ (0.96℃, 1.45℃) for the first and second replicate, respectively.

No survivors were obtained from the fruits infested with third instar larvae (= the most cold-tolerant stage) of *B. dorsalis* treated by cold treatment of pulp temperature at 1.0–1.5℃ for 16 days or more (Table 4).

Experiment 2

The mean temperature of Tankan during cold treatment for 17 days and the number of tested insects and survivors are shown in Table 5. The mean temperatures were 0.92–1.53℃.

One survivor was observed in only one box, in which the mean temperature was 1.53℃, the highest temperature among all the boxes. The larva was infested into a fruit again and reared at 27℃ for two days. After that, it died without pupating. No survivors were observed in the other boxes in which the mean temperatures were 1.44℃ or below.

By assessing the results of these tests, a cold treatment schedule of 17 days at 1℃ was selected for the following large-scale disinfestation test.

Table 2 Number of survivors and corrected mortality of four different stages of *Bactrocera dorsalis* in Tankan oranges treated at 1–1.5° C

Developmental stage	No. of infested fruits / experiment lot / replicate	No. of eggs inoculated / experiment lot / replicate	No. of survivors[1] (Corrected mortality %)[2]					
			Control	3 days	6 days	9 days	12 days	15 days
Egg	2	200	207(−)	76 (63.3)	0 (100)	0 (100)	0 (100)	0 (100)
1st instar	2	200	219(−)	45 (79.5)	0 (100)	0 (100)	0 (100)	0 (100)
2nd instar	2	200	273(−)	134 (50.9)	61 (77.7)	9 (96.7)	0 (100)	0 (100)
3rd instar	2	200	264(−)	46 (82.6)	13 (95.1)	6 (97.7)	1 (99.6)	1 (99.6)

[1] The total of two replicates.
[2] Calculated from the data of two replicates.

Table 3 Estimated number of exposure days for LT_{90}, LT_{95} and LT_{99} in cold treatment at 1–1.5° C from Probit analysis for four different stages of *Bactrocera dorsalis* infesting Tankan oranges

Developmental stage	LT_{90} (95%CL)	LT_{95} (95%CL)	LT_{99} (95%CL)
Egg[1]	–	–	–
1st instar[1]	–	–	–
2nd instar	7.35 (6.61 − 8.35)	8.54 (7.66 − 9.85)	10.77 (9.53 − 12.78)
3rd instar	4.67 (2.87 − 6.06)	6.76 (5.39 − 8.79)	10.68 (8.68 − 15.31)

[1] LT value for egg and first instar could not be calculated because the data did not fit the Probit analysis.

Table 4 Results of Experiment 1 in small-scale disinfestation tests on cold treatment against third instars of *Bactrocera dorsalis* infesting Tankan oranges

Group	Replicate 1 (1.19° C)			Replicate 2 (1.14° C)		
	No. of infested fruits[1]	No. of test insects[2]	No. of survivors	No. of infested fruits[1]	No. of test insects[2]	No. of survivors
Control	40	2,371	2,371	40	1,464	1,464
Cold treatment for 16 days	60	3,556	0	60	2,196	0
17 days	60	3,556	0	60	2,196	0
18 days	60	3,556	0	60	2,196	0

[1] One hundred eggs per fruit were inoculated.
[2] Number of test insects in cold treatment group = Number of survivors in control group / Number of infested fruits in control group × Number of infested fruits in treatment group.

Table 5 Results of Experiment 2 in small-scale disinfestation tests on cold treatment against third instars of *Bactrocera dorsalis* infesting Tankan oranges

Chamber	Box	Mean temperature for 17 days (℃)	No. of infested fruits[1)]	No. of test insects[2)]	No. of survivors
1	1	1.02	20	1,341	0
	2	1.22	21	1,408	0
	3	1.44	21	1,408	0
2	1	0.92	20	1,341	0
	2	1.35	21	1,408	0
	3	1.44	21	1,408	0
3	1	0.92	21	1,408	0
	2	1.09	21	1,408	0
	3	1.53	21	1,408	1
4	1	1.01	21	1,408	0
	2	1.18	21	1,408	0
	3	1.29	21	1,408	0

[1)] One hundred eggs per fruit were inoculated.
[2)] Number of test insects in cold treatment group = Number of survivors in control group / Number of infested fruits in control group × Number of infested fruits in treatment group.

4. Large-scale disinfestation test

The mean fruit-pulp temperature of Tankan oranges during cold treatment in large-scale tests was 1.10℃, 1.25℃ and 1.10℃ for the first, second and third replicate, respectively (Fig. 1 and Table 6). Mean fruit-pulp temperature of the mean fruit-pulp temperature of the 3 replicates was 1.15℃ and the temperature difference between this and the mean fruit-pulp temperature in each replicate was ≤ 0.1℃. Therefore, we propose 1.1℃ as the target fruit-pulp temperature of the cold treatment schedule.

No survivors were obtained from the total of 825 fruits infested with 46,563 third instar larvae (= the most cold-tolerant stage), which were treated at 1℃ for 16 days and 15–22 hours in 3 replicates (Table 6 and Table 7).

By assessing these results, it is considered that cold treatment at ≤ 1.1℃ for 17 days kills all immature stages of *B. dorsalis* infesting Tankan oranges.

5. Fruit quality tests for cold treatment

Table 8 shows the results of investigations on weight loss (%), citric acid (%), Brix (%) and number of decayed fruits after each storage schedule.

A strikingly negative effect of cold treatment at 1℃ for 15 and 20 days including subsequent 9℃ storage was not observed in terms of a comparison with the results of fruit quality stored at 20℃ for 15–30 days. Further studies with a greater number of fruits will be needed.

Conclusion

From the results of the tests described above, a cold treatment schedule of pulp temperature at ≤ 1.1℃ for 17 days is an effective phytosanitary treatment for Tankan oranges against *B. dorsalis* to lift the restriction on domestic movement even when this fruit fly invades a region in Japan in future.

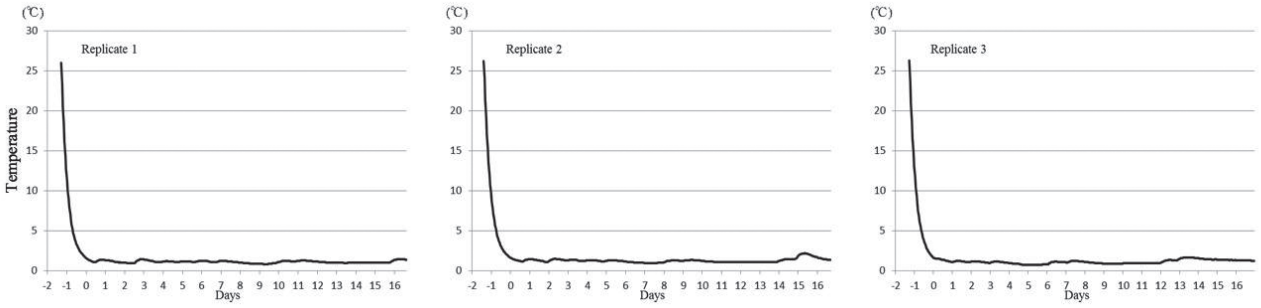

Fig. 1 Change in mean temperature of Tankan oranges (fruit pulp) in three replicates of large-scale test.
Fruit pulp temperatures were monitored and measured by seven sensors every hour in each replicate.
Treatment was considered to start when four of the seven sensors dropped to 1.5° C, and was continued for 17 days (16 days and 15–22 hours).

Table 6 Summary of temperatures of Tankan fruit pulp in large-scale disinfestation test

Replicate	Period of cold treatment[1)]	Mean temperature of fruit pulp[2)]	Mean minimum temperature of fruit pulp[2)]	Mean maximum temperature of fruit pulp[2)]
1	16 days + 15 hours	1.10° C	1.00° C	1.19° C
2	16 days + 16 hours	1.25° C	1.14° C	1.39° C
3	16 days + 22 hours	1.10° C	1.02° C	1.22° C

[1)] Cold treatment period from when four of the seven sensors measuring the fruit pulp temperature dropped to 1.5° C to when infested fruits were removed from the cold treatment chamber.
[2)] Mean minimum and mean maximum temperature of fruit for the cold treatment period were calculated from the mean minimum and maximum temperature of the fruit pulp temperature measured by seven sensors every hour.

Table 7 Results of large-scale test on cold treatment (1.0° C for 17 days) against third instars of *Bactrocera dorsalis* infesting Tankan oranges

Replicate	Control			Cold treatment at 1° C for 17 days			
	No. of infested fruits	No. of eggs inoculated	No. of survivors	No. of infested fruits	No. of eggs inoculated	No. of survivors	No. of 3rd instars tested[1)]
1	55	5,500	3,736	275	27,500	0	14,981
2	55	5,500	3,851	275	27,500	0	15,442
3	55	5,500	4,025	275	27,500	0	16,140
Total	165	16,500	11,612	825	82,500	0	46,563

[1)] Number of third instars tested in cold treatment = [Number of survivors in control group × Percentage of third instars in fruit 5 days after egg inoculation (80.2%: percentage of third instars in fruit when treatment lots were introduced into the cold chamber) / Number of infested fruit in control group] × Number of infested fruit in cold treatment.

Table 8 Results of fruit quality test for cold treatment at 1.0° C for 15–20 days

Item	Storage schedule of fruit		Total storage duration			
			15 days	20 days	25 days	30 days
Weight loss (%)	Storage at 20℃		10.1	11.5	15.1	15.4
	Cold treatment at 1℃		4.8	7.1	−	−
	Cold treatment at 1℃ for 15 days	+ 9℃ for 5 days	−	6.2	−	−
		+ 9℃ for 10 days	−	−	8.4	−
	Cold treatment at 1℃ for 20 days	+ 9℃ for 5 days	−	−	7.9	−
		+ 9℃ for 10 days	−	−	−	13.1
Citric acid (%)	Storage at 20℃		0.68 ± 0.11	0.71 ± 0.06	0.62 ± 0.07	0.68 ± 0.10
	Cold treatment at 1℃		0.71 ± 0.08	0.68 ± 0.07	−	−
	Cold treatment at 1℃ for 15 days	+ 9℃ for 5 days	−	0.72 ± 0.09	−	−
		+ 9℃ for 10 days	−	−	0.69 ± 0.18	−
	Cold treatment at 1℃ for 20 days	+ 9℃ for 5 days	−	−	0.64 ± 0.07	−
		+ 9℃ for 10 days	−	−	−	0.70 ± 0.08
Brix (%)	Storage at 20℃		10.4 ± 0.7	10.6 ± 0.7	10.4 ± 0.7	10.8 ± 0.9
	Cold treatment at 1℃		10.1 ± 0.8	10.0 ± 0.6	−	−
	Cold treatment at 1℃ for 15 days	+ 9℃ for 5 days	−	10.1 ± 0.9	−	−
		+ 9℃ for 10 days	−	−	9.9 ± 0.7	−
	Cold treatment at 1℃ for 20 days	+ 9℃ for 5 days	−	−	10.7 ± 1.1	−
		+ 9℃ for 10 days	−	−	−	10.0 ± 1.1
Fruit decay (Number of decayed fruits / fruits used)	Storage at 20℃		2 / 10	3 / 10	3 / 10	6 / 10
	Cold treatment at 1℃		0 / 10	1 / 10	−	−
	Cold treatment at 1℃ for 15 days	+ 9℃ for 5 days	−	0 / 10	−	−
		+ 9℃ for 10 days	−	−	0 / 10	−
	Cold treatment at 1℃ for 20 days	+ 9℃ for 5 days	−	−	0 / 10	−
		+ 9℃ for 10 days	−	−	−	4 / 10

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和 文 摘 要

タンカンにおけるミカンコミバエ *Bactrocera dorsalis* の低温殺虫（英文）

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1．奄美大島におけるミカンコミバエの発生により、未発生地域へのミカンコミバエ寄主植物の移動が制限された。このため、タンカンの移動を可能とするため、過去に国内で発生したミカンコミバエの系統を用いて殺虫試験を実施し、低温処理による同虫の殺虫処理技術を開発した。

2．まず、ミカンコミバエのタンカンにおける発育を調査した。タンカン生果実にミカンコミバエの卵を Upper-Triangle 法で接種し、27℃で保管後、毎日果実を切開して発育ステージを確認した結果、採卵及び接種後 1 日目は卵、2 日目に 1 齢幼虫、3 日目に 2 齢幼虫、5 日目に 3 齢幼虫の割合が多いことが明らかとなった。

3．次に、ミカンコミバエの各発育ステージを寄生させたタンカン生果実（各処理区 2 果 / 反復、2 反復実施。）を 1.0～1.5℃で 3,6,9,12,15 日低温処理し、ステージ毎の低温耐性を比較したところ、卵及び 1 齢幼虫は 6 日目以降で補正死亡率 100%、2 齢幼虫は 12 日目以降で補正死亡率 100%となったが、3 齢幼虫は 15 日処理区でも生存虫が確認された。このことから、3 齢幼虫を低温に対して最も耐性のある発育ステージとした。

4．次に、試験規模を大きくして（2 反復実施。1 反復につき各処理区 60 果、対照区 1 果あたりの生存虫数は反復 1,2 でそれぞれ、59.3 頭 / 頭、36.6 頭 / 果であった。）、3 齢幼虫が寄生したタンカン生果実を 1.0～1.5℃で 16,17,18 日間低温処理し、生存虫の有無を確認した結果、全ての処理区で 100%の殺虫効果が得られた。各反復の処理中の平均果実温度は、1.19℃、1.14℃であった。

5．最後に、更に試験規模を大きくして（3 反復実施。1 反復につき処理区 275 果、対照区 1 果あたりの生存虫数は反復 1～3 でそれぞれ 67.9 頭 / 果、70.0 頭 / 果、73.2 頭 / 果であった。）、3 齢幼虫が寄生したタンカン生果実を 1.0～1.5℃で 17 日間低温処理し、生存虫の有無を確認した結果、100% の殺虫効果が得られた。各反復の処理中の平均果実温度は 1.10℃、1.25℃、1.10℃であった（3 反復の平均値 1.15℃）。

6．以上のことから、1.1℃以下で 17 日間の低温処理を行うことで、タンカン生果実に寄生したミカンコミバエは十分殺虫できるものと判断した。

7．25℃で 15-30 日間保管した果実の品質と比較した限り、低温処理 1℃で 15 日間及び 20 日間処理による品質への顕著な悪影響は、処理後 9℃で 5 日間及び 10 日間保管した場合も含めて、観察されなかった。

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