RES. BULL. PL. PROT. JAPAN No. 28 : 55~60 (1992)

Short Communication

Acquisition of Cold Tolerance in Immature Stages of Oriental Fruit Fly, *Dacus dorsalis* (Diptera : Tephritidae) in Artificial Diet and Orange Fruits

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Abstract: Immature stages of the oriental fruit fly infesting an artificial larval diet and 'Valencia' orange fruits were cold-treated at 1°C±0.5°C. Difference in speed of the precooling process significantly modified mortality of larvae by the cold treatment. Freshly laid eggs were the most susceptible and their mortality was not influenced by the precooling method. One-day-old eggs which have experienced the quick precooling were relatively tolerant but the slow precooling tended to show cumulative lethal effect on eggs. Young and mature larvae, on the other hand, revealed higher cold tolerance when they were precooled slowly. It is clearly demonstrated that eggs and larvae show different responses to low temperature and that the larvae infesting fruits can acquire cold tolerance during the precooling period.

Key words: *Dacus dorsalis*, cold tolerance, precooling, quarantine treatment

Introduction

Studies on the cold tolerance of immature and adult stages of oriental fruit fly and melon fly were carried out in detail by KOIDSUMI (1933, 1934, 1936, 1937a, 1937b) in order to ecologically evaluate the possible invasion of these flies into the southern regions of Japan and, concurrently, to look for the possible application of cold treatment as a practical quarantine treatment against these fruit flies. In his studies, the cold storage was shown to be effective for infested citrus fruits. Although it was a simple physical treatment that could be applicable to the quarantine field, it was not much ultilized because the fumigation by chemicals such as ethylen dibromide (EDB) became more common and extensive. However, since the use of EDB was banned in the U.S.A. because of its potential carcinogenecity (Environmental Protection Agency, 1984), the cold treatment began to receive attention as a possible alternative quarantine procedure and a number of studies have already been made in this field (BURDITT and BALOCK, 1985 ; GOULD and SHARP, 1990 ; HILL, *et al.*, 1988). In all of these studies, the treatment was carried out at a constant low temperature which did not sufficiently reflect the general commercial cold storage conditions. On the other hand, it has been reported that 'conditioning' of citrus fruits at moderately low temperature prior to the cold storage was positively effective for

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reducing chilling injuries (KITAGAWA, 1989). Still more significant is the report that the immature fruit flies developed cold tolerance to the range of 0~9°C when they were initially exposed, prior to the storage, to the gradually decreasing temperatures at 15°C for 2 days, 10°C for 4 days and then 5°C for 6 days (KOIDSUMI and SHIBATA, 1964).

In the present study, the authers examined two types of precooling which simulated the commercial cold storage conditions in order to evaluate the possible development of cold tolerance in immature stages of oriental fruit fly infesting an artificial larval diet and orange fruits. The term of 'precooling' used in this study dose not apply to any definitive pre-treatment procedures such as the conditioning, the hydro-cooling or the different pressure cooling (KITAGAWA, 1989), but it only implies a transition period before the air temperature or core temperature of fruits in the chamber reaches desired target temperature.

Materials and methods

About 130 of freshly laid eggs (1~3-hour-old eggs) were disinfected with 0.1% sodium hypochlorite solution and put on an artificial larval diet (ICHINOHE and NOHARA, 1976) in petri dish. They were allowed at 27°C to develop into 1-day-old eggs in one day, young larvae in 2~3 days and mature larvae in 5 days, respectively. These test insects were

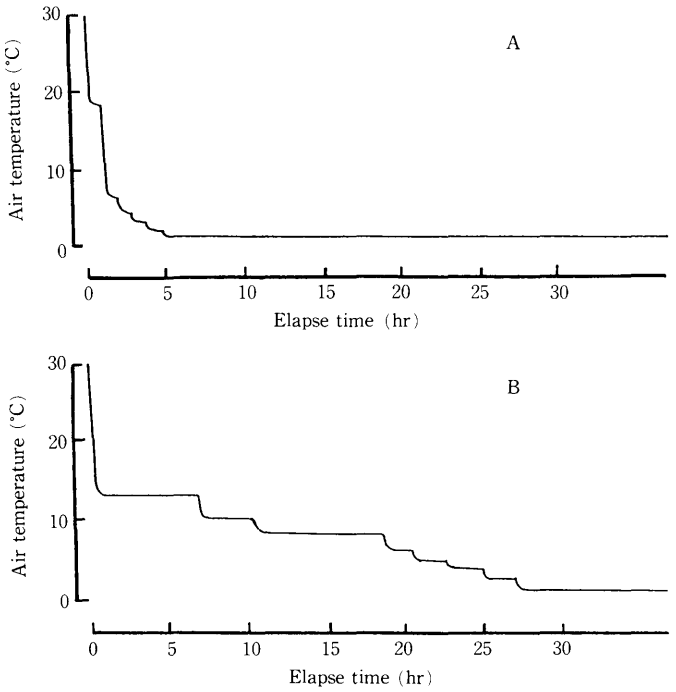

Fig. 1. Decrease of air temperature in treatment chamber
Immature stages of oriental fruit flies were inoculated to artificial larval diet in petri dish.
A, Quick precooling; B, Slow precooling

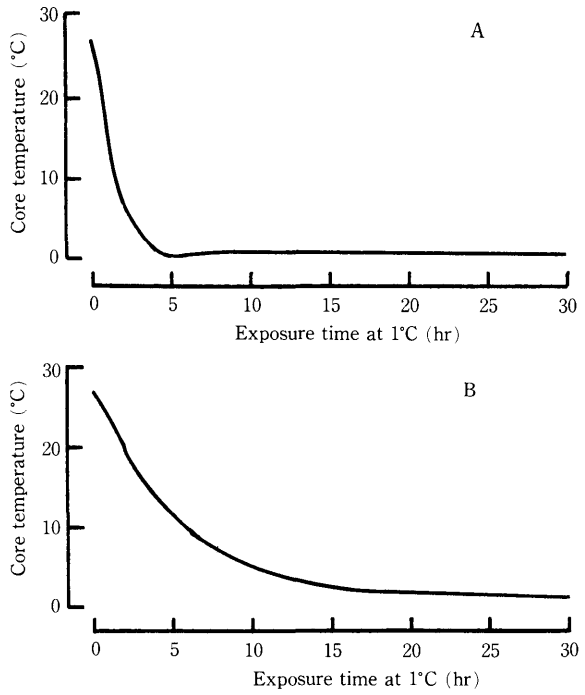

Fig. 2. Decrease of core temperature of orange fruit infested with immature oriental fruit flies
A, Quick precooling; Fruits were stored at 1°C±0.5°C.
B, Slow precooling; Fruits in polystyrene foam container were stored at 1°C±0.5°C.

cold-treated in thermo- and humid-static chamber (Model IR-41, YAMATO SCIENTIFIC Co. Ltd.) equipped with resistace bulbs (Pt 100 Ω) connected to recorder (Model AH-220, CHINO Co. Ltd.). Air temperature in the chamber was lowered quickly in 5 hours and slowly in 27 hours, respectively, to 1°C±0.5°C and then maintained at this treating temperature (Fig. 1). Test was replicated 4 times. Treated insects were reared at 27°C. Mortality of treated eggs, young and mature larvae was examined, respectively, after 5 days, 3～4 days and 2 days.

To demonstrate the possible effect of precooling on the cold tolerance of immature stages of oriental fruit fly in fruits, 1-day-old eggs and mature larvae were employed for the test. One hundred each of freshly laid eggs and 4-day-old larvae reared on artificial diet were inoculated into 'Valencia' orange fruit and then incubated for one day at 27°C. In order to provide a quick and a slow precooling processes, the half of the inoculated fruits were put into polystyrene foam container and, together with the other half, were exposed to constant low temperature. The core temperature of the fruits decreased as shown in Figure 2. Immediately after the cold treatment, the eggs were removed onto moistened blotter papers and the larvae were transferred to the artificial diet. Mortality was determined 48 hours after the treatment. Tests were replicated twice.

Results and discussion

In the case of quick precooling, the minimum time of exposure to 1°C required for killing the test insects in the artificial diet was in the order; 1-day-old eggs≧mature larvae>young larvae≧freshly laid eggs (1~3-hour-old eggs), while, in the case of slower precooling, it was in the order; mature larvae>1-day-old eggs>young larvae>freshly laid eggs (Table 1). It is clear that the extended precooling period decreased the mortality of larvae by the cold treatment. This result concurs with that of KOIDSUMI and SHIBATA (1964) who reported that the gradual temperature decrease acclimated immature stages of *D. dorsalis* and *D. cucurbitae* and that the cold tolerance was influenced, not by the length of the pre-treatment period, but rather by the degree of temperature of the pre-treatment period.

Eggs revealed defferent responses to low temperature according to their age. No influence of precooling method was observed on the mortality of freshly laid eggs but 1-day-old eggs was shown distinctively higher cold tolerance than freshly laid eggs. Although the reason for the irregular and sometimes reversed mortalities of 1-day-old eggs between the two precooling methods after the exposure to 1°C cannot be defined, it is reported that the cold tolerance of eggs of *Anastrepha suspensa* changes by the stage of

Table 1. Effect of precooling method on survival of immature stages of D. dorsalis infesting artificial diet during cold treatment at 1°C.

Treatment time	Freshly laid eggs	1-day-old eggs	Young larvae	Old larvae
		Quick precooling*		
0 hour	527 (89.7±3.3)**	433 (82.0±1.6)	394 (87.1± 1.8)	432 (76.4± 2.0)
19	99 (18.8±2.2)	258 (59.6±7.4)	224 (57.5±16.6)	367 (85.1±13.9)
67	6 (1.1±2.2)	242 (55.9±3.7)	24 (6.1± 0.9)	194 (44.9± 6.7)
115	0	102 (23.5±2.7)	0	51 (11.8± 3.5)
163	0	0	0	0
211	0	0	0	0
259	0	0	0	0
Test insects***	588	528	452	566
		Slow precooling*		
0	497 (89.2±7.4)	403 (80.7±11.2)	471 (87.9± 7.8)	454 (80.3± 1.3)
19	76 (15.6±4.3)	444 (111.1±11.1)	395 (85.0±20.4)	363 (80.8±24.1)
67	6 (1.4±2.3)	238 (60.2±11.7)	184 (38.9± 9.9)	294 (65.2±14.9)
115	0	15 (3.7± 1.4)	4 (0.8± 1.1)	151 (33.1± 5.9)
163	0	0	1 (0.2± 0.4)	26 (5.7± 0.8)
211	0	0	0	6 (1.3± 0.8)
259	0	0	0	0
Test insects***	556	403	471	454

* Air temperature in the treatment chamber reached 1°C, respectively, in 5 hours in quick procooling and 27 hours in slow precooling (See Fig. 1).

** Value is a sum of survivors in four trials. Values in parenthesis are mean survival rate corrected with Abbott's formula and the standard deviation.

*** Number of test insect is of eggs inoculated into the artificial larval diet.

Table 2. Effect of precooling method on survival of 1-day-old eggs and mature larvae infesting 'Valencia' oranges during cold treatment at 1°C for 144 hours.

Stage	Fruits	Effective number of test insects	Precooling*	Replication	Number of survivors
1-day-old eggs	5	447 (89.4±2.6)**	Quick	1	14
	5	455 (91.0±1.4)	Quick	2	11
	5	447 (89.4±2.6)	Slow	1	3
	5	455 (91.0±1.4)	Slow	2	1
Mature larvae	5	455 (91.0±1.6)	Quick	1	5
	5	455 (91.0±2.0)	Quick	2	2
	5	455 (91.0±1.6)	Slow	1	54
	5	455 (91.0±2.0)	Slow	2	12

* Fruit core temperature reached 1°C, respectively, in 5 hours in quick precooling and in 22 hours in slow precooling (See Fig. 2).

** One hundred insects were inoculated into each fruit. Values in parenthesis are mean survival rate and standard deviation.

their development (BENSHOTER and WITHERELL, 1984).

Response of 1-day-old eggs to the precooling methods was opposite to that of young and mature larvae. Such tendency was more pronounced in the case of infested orange (Table 2). It is likely that the larvae can acquire the cold tolerance by metabolic changes during the precooling at intermediate temperature while eggs lack such ability. The metabolic changes to induce cold tolerance may well require a certain length of time at a certain range of low temperature. This is because the mortality of the immature stages of oriental fruit fly and melon fly that are exposed to low temperature intermittently every 12 hours is invariably higher than those exposed to constant low temperature (KOIDSUMI, 1934 ; 1937b). Further studies are needed to clarify the cause of different responses of larvae under varying low temperature conditions.

For the development of quarantine treatment for fruit flies, the most tolerant life stage is selected by preliminary studies and this stage is employed for commercial scale experimentation (SUGIMOTO and FURUSAWA, 1982 ; BURDITT and BALOCK, 1985 ; HILL, *et al.*, 1988 ; GOULD and SHARP, 1990 ; JESSUP and BAHHEER, 1990). Due to the different test conditions, it is difficult to make a critical evaluation of the authers' results in comparison with those reported in the foregoing studies. In addition, the methods of experimentation in the earlier studies did not fully take into consideration such practical conditions as the fluctuation of temperature in commercial storage facilities. For instance, the conditioning of citrus fruits at an intermediate temperature proir to the cold storage may reduce chilling injulies, but it may also induce cold tolerance of fruit flies and increse chances of survival of fruit flies. Therefore, a careful analysis of the possible acquisition of cold tolerance during the precooling process should be made for the establishment of reliable cold treatment for quarantine purposes.

Acknowledgments

The authers thank Kunio SUNAGAWA, Research Division, Yokohama Plant Protection Station, for his advice in the present study and Mr. Takushi OBATA, Japan Plant Quarantine Association, for critical reading of the manuscript.

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