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A Probit Analysis of the Disinfestation of Mangosteens by Cold Treatment Against the Larval Stages of the Oriental Fruit Fly (*Bactrocera dorsalis* (Hendel)) (Diptera : Tephritidae)

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ABSTRACT

The presence of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera : Tephritidae), in Thailand restricts entry of mangosteens to some foreign markets because of plant quarantine regulations. Mangosteens infested with third instar larvae of *B. dorsalis* were held at 5, 6, and 7°C for 1/4, 1/2, 1, 2, 4, 8, 11, 14, 16, and 20 days. Probit regression lines were fitted to the data of the relationship between exposure time and mortality of the insect. The exposure times required to kill *B. dorsalis* in mangosteens at 99.9968% mortality (probit 9), based on the recovery of puparia or adults, were calculated to be 19.4, 24.9, and 24.9 days for fruit treated at 5, 6, and 7°C, respectively. Attempts to find alternative procedures given the criterion of probit 9 were discussed.

Key Words : Insecta, *Bactrocera dorsalis,* cold treatment, probit analysis

INTRODUCTION

Mangosteen, *Garcinia mangostana* L. (Guttiferales : Clusiaceae), has been named "the queen of fruit" by many consumers. The shape of fruit is globular ; when mature, the peel or carpel is pinkish red and turns to deep purple at ripening stage. The meat is pearly white, soft, sweet, and has a refreshing aroma. The fruit has a very high potential for both domestic and overseas markets. Currently, only frozen mangosteens at -17.8°C are allowed to import into country where rigid plant quarantine regulations are imposed such as Japan. The main theme of the frozen fruit is for the elimination of the oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera : Tephritidae). The freezing temperature obviously destroys the natural flavor and the attractive quality of the fruit. Thus, it is necessary to find alternative disinfestation methods against the insect without damaging the quality of the fruit. The overall objective of our research was to establish a cold-temperature treatment as a standard for quarantine regulation of mangosteen against the oriental fruit fly that would also assure superior fruit quality. The response of *B. dorsalis* to cold treatment is death, which is a type of quantal response and suitable for the study by the use of probit regression analysis (Finney, 1971). This paper reports the results of the study on probit analysis of the exposure times in cold temperatures related to mortality of *B. dorsalis.*

The disinfestation treatment using cold temperature has been determined previously for mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), in apples (Sproul, 1976) and in oranges

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(Hill *et al.*, 1988), Queensland fruit fly, *Bactrocera tryoni* (Froggatt), in kiwifruit (Rippon and Smith, 1979), and Caribbean fruit fly, *Anastrepha suspensa* (Loew), in oranges (Benschoter, 1984). The major difficulty in developing quarantine treatments is the condition that no insects survive and capable of becoming established at the destination. The accepted security level is the achievement of probit 9 or no more than 3 survivors per 100,000 treated insects (99.9968% mortality). Unquestionably large numbers of insects are required in order to demonstrate this mortality level. However, Sproul (1976) suggested the limits of three consecutive tests of more than 10,000 insects each with no survivours should be adopted. The treatment was done on *C. capitata* and accepted by the Japanese Plant Quarantine Authorities.

MATERIALS AND METHODS

The research was done during 1989-90 at the KU-APEX Mangosteen Research Laboratory, Bangkhen, Bangkok. Mangosteens were obtained from Rayong Province. Third instar larvae (5-day-old larvae reared at 27 ± 1°C) of *B. dorsalis* were used in this study. The third instar larvae were considered to be most tolerant to cold treatment compared with eggs and young larvae (Hill *et al.*, 1988, Anonymous, 1989). The laboratory insect culture was strengthened by periodically mating with field populations. Mangosteen fruits of color stage 5 or 6 were artificially infested by opening the fruit rind at the equator, 10 third-instar larvae placed on the meat, the larvae allowed to feed or penetrate inside, then the rind put back. Five infested fruits with *B. dorsalis* were placed over sand of 5 cm deep as a pupation medium and confined in a circular plastic container, measuring 24 cm in diameter and 10 cm in height. The lid of the container was cut into a circular opening 12 cm in diameter, and filted with wire screen for ventilation. Ten plastic containers with infested mangosteen fruits inside were stored in a refrigerator, measuring 0.34 m^3 in capacity with accuracy of ± 0.5°C at the temperature setting point. Three refrigerators were employed and set constantly at 5, 6, and 7°C, respectively. The selection of 5, 6 and 7°C as treated temperatures was based on 1989's experiment (Anonymous, 1989). A plastic container with infested mangosteen fruits, randomly selected to be untreated and classified as a control unit, was kept at room temperature (27 ± 1°C). One container was randomly removed from the refrigerator at 6 hr, 12 hr, 1 day, 2 days, 4 days, 8 days, 11 days, 14 days, 16 days, and 20 days. A total of thirty-one containers was used for one experiment. At the end of these exposure periods to cold temperatures, the containers with infested fruits were returned to room temperature of 27 ± 1°C and 50-60% R.H. for the development of surviving insects.

Inspection of the fruit and sand for survivors was carried out over one-week period. Formation of normal puparia (light brown in color) recovered from the treatments, or adults emerged from the recovered pupae, was considered as a standard for survival. The experiment was replicated six times. Percentage mortalities were recorded for both treated and untreated units.

Data Analysis

The analysis of data followed the procedure of probit analysis as described by Finney (1971). The response variable was percent mortality of larvae in terms of probit value, and the explanatory variable was exposure time (hours) of cold treatment. The expected mortality of 99.9968% (probit 9) was calculated at each treated temperature.

RESULTS AND DISCUSSION

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 meat of mangosteen. There was approximately 18% mortality in the control unit. Abbott's formula (Abbott, 1925) was used as a correction factor of larval mortality. The mortality of larvae and the exposure period at each temperature are presented in Table 1, and the results of probit analysis are summarized in Table 2. Figure 1 shows the exposure period of larvae in refrigerator as a function of mortality in probit at 5, 6, and 7°C. The data are well represented by a line as indicated by the closeness of the points to that line, and non-significant chi-square value (Table 2 ; P-values > 0.05).

The regression lines of 5, 6, and 7°C in Figure 1 are very close together and difficult to differentiate from each other. This is an indication of an insignificant difference whether 5, 6, or 7°C were used for the cold treatment. However, we cannot lower the temperature below 5°C because of the chilling effect on mangosteens, or raise it to higher than 7°C which would be difficult to kill the larvae. Exposure time longer than 16 days is required in order to kill *B. dorsalis* larvae in mangosteens treated at 8°C. At that interval in the refrigerator the cold temperature could give some adverse effects to mangosteens (Anonymous, 1989). The higher the slope of the regression line the lower the temperature with the highest 3.752 at 5°C (Table 2). It shows that lower temperatures are better to eliminate the larvae. The projection at probit 9 was 465.6, 599.8, and 599.8 hr, or 19.4, 24.9, and 24.9 days, for infested mangosteens with *B. dorsalis* larvae at 5, 6, and 7°C, respectively (Table 2). These figures are very much higher than the results of

Table 1 **Time-mortality relationship of third instar larvae of *Bactrocera dorsalis* in mangosteens exposed to cold treatments .**

Temperature (°c)	Exposure time (hours)	No.of insects	No.of experiments	Mortality[1] (%)
5	6	276	6	19.9
	12	281	6	21.4
	24	290	6	39.3
	48	293	6	64.8
	96	281	6	95.0
	192	296	6	99.7
	264	286	6	100.0
6	6	273	6	18.7
	12	289	6	20.8
	24	286	6	42.3
	48	279		66.3
	96	287	6	93.0
	192	289	6	99.3
	264	288	6	100.0
7	6	292	6	18.8
	12	295	6	20.7
	24	275	6	43.6
	48	282	6	72.0
	96	284	6	91.9
	192	280	6	99.6
	264	288	6	100.0
27	0	286	6	18.2

[1] Based on the number of normally formed puparia, or the number of adult fllies emerged from the pupae.

Table 2 Fitness test of linearity of the relationship between logarithm of exposure time of *Bactrocera dorsalis* larvae in cold treatment versus mortality in probit, with the projection for exposure times at probit 9 (data from Table 1).

Temperature (°C)	Regression equation[1]	Chi-square df. = 5	P-value	Exposure time at probit 9	
				Logarithms	Hours
5	Y = -1.009 + 3.752X	9.290	0.902	2.668	465.6
6	Y = -0.287 + 3.344X	5.241	0.613	2.778	599.8
7	Y = -0.084 + 3.271X	6.282	0.720	2.778	599.8

[1] Maximum likelihood method as described by Finney (1971); Y = mortality in probit; X = exposure time (hours) in logarithm.

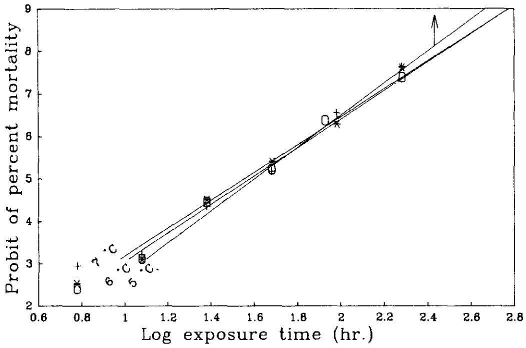

Figure 1 Relationship between probit of kill of *Bactrocera dorsalis* larvae and time of exposure to cold treatment at 5, 6, and 7°C. The up arrow indicates the point at 100% of observed mortality.

previous preliminary study in which no larvae survived (2,300 larvae tested) after 11-12 days at 6°C. Nevertheless, no survivors were obtained after 11 days (264 hr) in cold treatment at all treated temperatures (Table 1). Another aspect may be due to the property of the regression equation itself, in which less accuracy will be obtained if the projection is far beyond the average, in this case more than probit 5.

According to the discrepancy of the results of probit 9 between probit analysis and the observed 100% mortality, longer exposure times would be required to reach 99.9968% mortality by the estimation of the regression equations compared to the observed data. The criterion of Sproul (1976) should be used instead for disinfestation treatment of *B. dorsalis*. That is no survivors from the test limits of over 30,000 individuals, involving three consecutive tests of more than 10,000 insects each. Any combination of cold treatments and exposure times that passes the test mentioned above shall be accepted as candidates for quarantine treatment of mangosteen fruits.

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LITERATURE CITED

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

Anonumous. 1989. Fruit flies of mangosteen and disinfestation treatments. Final Report for the APEX Corporation. Department of Entomology, Kasetsart University, Bangkok. 43 p.

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

Finney, D.J. 1971. Probit Analysis. 3rd ed., Cambridge University Press, London. 333 p.

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* (Froggatt) and *Ceratits capitata* (Wiedemann) (Diptera : Tephritidae). J. Econ. Entomol 81:257-260.

Rippon, L. E. and R. J. Smith. 1979. Postharvest treatment of Chinese gooseberries (kiwifruit) for the control of the 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.