HEAT TOLERANCES OF THE IMMATURE STAGES OF FRUIT FLIES, *BACTROCERA PHILIPPINENSIS and BACTROCERA OCCIPITALIS* (DIPTERA: TEPHRITIDAE)

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ABSTRACT

The heat tolerances of *Bactrocera philippinensis* and *Bactrocera occipitalis* were determined by exposing immature stages, eggs (1-3 hours and 28-30 hours) first instars (48- 50 hours), third instars feeding (120-122 hours) and non-feeding (170-172 hours) to temperatures from 45 to 49°C at 0-128 minutes (general test) and 0-26 minutes (specific test).

The general test showed that at 45, 46 and 47°C , the first instar were the most tolerant stage for *B. philippinensis* and *B. occipitalis*. At higher temperatures (48 and 49°C), the third instar (non - feeding) was the most tolerant stage for both species.

In the specific test, the late egg stage was most tolerant at 45°C for both species at 46°C, B. philippinensis in all stages responded similarly, while B. occipitalis exhibited tolerance in the 3rd instar, non - feeding (LT99 = 11 mins). At temperatures greater than 46°C, first instar was most tolerant with LT 99 = 9 mins and LT99 = 5 mins at 47°C and 48°C, respectively. The late egg was the most tolerant stage at 49°C.

The study revealed that irregardless of the species, late eggs and 1st instars were the most tolerant stages. In both test, B. philippinensis was generally tolerant over *B. occipitalis* except at 45°C.

Imported newspaper, thick and thin waxy magazine were the most promising bagging materials for mango fruits in all seasons while brown paper bags and local newspapers are recommended only during dry season.

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INTRODUCTION

Philippines is among the major mango producing countries in the world with about 508.127 mt in 1994 and 917.471 mt in 1998. The leading mango regions are Ilocos (290.969 mt). Cagayan Valley (177.215 mt). Southern Tagalog (148.535 mt). Western Visayas (83.379 mt) and Central Luzon (65.367 mt). At present, massive expansion of mango plantings are done in Mindanao with estimated production of 123.759 mt. The area provides enough supply of mango fruits during the off-season production in Luzon and Visayas.

About 90 percent of the total production are sold/ consumed locally and only 8 to 10 percent are exported. In 1998, the value of mango export was reported at 51.3 million U. S. dollars, representing 10.8 percent share of the annual fruit export. The important markets for Philippine mango are Hongkong, Japan and Singapore. With the excellent eating quality of 'Carabao' mango, improved production technologies and proximity of Philippines to Asian countries, expansion of export markets for mango is inevitable. This does not include other lucrative markets in the near future.

United States is a potential market for Philippine mango. The demand for the fruit is high especially among Fil-Americans who are familiar with quality and taste of the variety. However, no mango fruit has yet reached US markets because of the strict quarantine regulation imposed on the commodity. Disinfestation treatment that kills 100 percent of the immature stages of fruit flies, (without affecting fruit quality) is an important requirement before any exportation is approved.

The current Vapor heat treatment (VHT) for disinfesting mangoes with fruit flies is not acceptable to APHIS/USDA. This is because earlier efficacy trials involved the Oriental fruit fly, *Bactrocera dorsalis*, a species not present in the country. On the other hand, the test insects used were likely represented by 2 fruit fly species, *B. occipitalis* and *B. philippinensis*. APHIS therefore requires a small-scale test using hot water bath immersion bioassay to determine the most tolerant stage(s) of each species.

OBJECTIVES

A bioassay of temperature-time effect was therefore conducted for *B. occipitalis* and *B. philippinensis* in Guimaras Island, Philippines to: a) determine the mortality response of early egg and late egg, 1st instar and 3rd instar (feeding, non-feeding) to different temperatures and immersion time and b) identify the most heat tolerant stage(s) within the species and between species.

REVIEW OF LITERATURE

Mangoes exported abroad have to undergo disinfestation treatments to meet the quarantine requirement of many importing countries. In the past, mangoes sent to Japan, Australia and New Zealand were subjected to fumigation with ethylene dibromide (EDB) at 16 g/m3 for 2 hours at 26°C (Phil. Recommends for Mango, 1994). However, when EDB was banned in 1987 due to carcinogens, an alternate disinfestation treatment known as Vapor heat treatment (VHT) was developed. Merino **et al.** (1985) reported that fruits subjected to VHT (pulp temperature of 46°C and maintained for 10 mins), resulted in 100 percent mortality of the immature stages of the Oriental fruit fly, *(Dacus dorsalis)* and Melon fly *(Dacus cucurbitae)*. No significant injury was observed on the treated fruits.

Today, export of fresh mangoes to Japan is only permitted if post harvest treatment with VHT is done. The effectiveness of the treatment has shown negative interception of live specimen of the immature stages of fruit fly since the start of the trade. Lately, Korea and Australia also approved similar post harvest treatment for mango and papaya imported from the Philippines.

United States is a potential market for 'Carabao' mango. Negotiation for fruit export was done for several years but with no promising results because of the stringent quarantine requirements, particularly on the mango seed weevil, mango pulp weevil and fruit flies. In the case of the mango seed weevil, its absence in the country was proven by the nationwide survey conducted in different mango growing regions (Basio **et al.** 1987). On the other hand, Basio **et. al.** (1994) reported the presence of the pulp weevil in southern Palawan, prompting the Bureau of Plant Industry to issue Special Quarantine Adm. Order No. 20, series of 1987, declaring the pulp weevil as a dangerous pest. It also placed Palawan under quarantine to prevent the spread of the insect. However, the presence of the pulp weevil does not prevent other provinces (free of the pest) from exporting mangoes to the U.S. Hence, after a comprehensive survey in Guimaras which revealed the absence of seed and pulp weevils, the island province was considered as possible source of mangoes for export to the U.S. (Golez **et. al.** 1993).

On the other hand, fruit flies remain hindrance to exportation of Guimaras mango, since USDA does not accept the current disinfestation treatment against the pest. According to APHIS, efficacy data on VHT involved the test insect referred to as the Oriental fruit fly, *B. dorsalis* which today is represented by 2 species, *B. philippinensis* and *B. occipitalis* (Drew and Hancock, 1994). This finding, therefore, renders the result of the previous test indeterminate. Hence, where treatment is needed, APHIS/USDA requires efficacy data for each species before any importation is approved. A small-scale test using hot water bath immersion test is recommended to determine the most tolerant stage(s) of the insect. Once the resistant stage is determined, confirmatory test with VHT has to be conducted.

The effectiveness of any post-harvest quarantine treatment against fruit flies depends on the knowledge of the most resistant stage(s) present in the commodity during a given treatment. In turn, tolerance and susceptibility to the treatment is a function of the physiological condition of the test insect. Jang (1991) studied the kinetics of thermal death for early and late instars of *D. dorsalis* and showed that both stages of development exhibited non-logarithmic death (survivorship curve), characterized by an initial lag in mortality followed by increasing death rate with time at a given temperature.

Vueti **et al.** (1996) reported that the early eggs (<10 hrs) of *B. passiflorae* were most heat tolerant at 45°C. There was no significant difference in mortality response between early and late eggs at 47°C or at higher temperatures. The tolerant stage in Fiji was also compared with the tolerant stage (late eggs) of *B. melanotus* in Cook Is., which showed that the latter species was more tolerant than the former.

Heat tolerances of the immature stages of *B. facialis* and *B. xanthodes* were also determined by Foliaki and Armstrong (1996). The eggs and larval stages of *B. facialis* were significantly more heat tolerant than *B. xanthodes* for all life stages at all temperatures. For temperature less than 46°C, the first instar was most tolerant while at 46°C, the late eggs were more tolerant. At temperatures greater than 46°C, the 3rd instars were more tolerant specially the non-feeding stage.

Sales **et al.** (1996) compared the egg and larval mortalities of 3 fruit fly species after immersion in hot water. Results showed that at all temperatures, the mature eggs and first instar larvae were more tolerant than any other stages at 44°C and 45°C. Any treatment should therefore be directed against the mature eggs of *B. cucurbitae* and 1st instar larvae of the species. This result also provided information necessary for treatment of fruits infested with other species of fruit flies.

In the Philippines, Yaptengco **et al.** (1996) studied the thermal death kinetics of the eggs of Melon fly *(B. cucurbitae)* with the following findings: a) linear regression gave a better fit than the A & S method for temperature range 44 to 46°C and b) kinetic parameters of 26 hrs. old melon fly eggs showed that mortality occurred at a higher rate than fruit quality could deteriorate, allowing optimization of heat treatment.

Manoto **et al.** (1998) showed a positive correlation with temperature of water and percentage mortality at different time of immersion. Older eggs were more tolerant than younger ones. For *B. dorsalis*, the most sensitive stage (2 hrs old eggs) was equated to about 6 percent embryonic development while tolerant eggs have about 60 to 80 percent development. In addition, a linear relationship between immersion time and mortality of *B. dorsalis* was obtained from probit analysis. The estimated lethat time (LT 95) for 2, 20 and 25 hours eggs were 2.79, 5.97 and 16.92 minutes, respectively.

METHODOLOGY

Equipment

The container for the hot water bath bioassay was made of stainless steel with dimensions of 22 in X 28 in X 6 in. This was enclosed in a fitted wooden box to insulate heat.

The water in the bath was heated at a prescribed temperature using an electric heater-circulator (Polyscience Model 7305). The instrument was positioned on one side of the bath with the heating apparatus submerged 3.5 inches below water surface for proper circulation. Three calibrated mercurial thermometers were each inserted in a styrofoam and allowed to float on the water. This monitored temperature on all sides of the bath and also verified temperature reading of the instrument for uniformity. Purified water was used in all the tests.

The holding tubes were made of 30 ml plastic syringes. The base was cut on both sides providing rectangular openings. This area was covered with black muslin cloth, glued onto the walls of the syringe, to allow heated water to pass through during the test. A separate circular black cloth was also provided to contain the test insects. This was attached to the base of syringe by a rubber bond.

The tubes were placed in wooden rack (ten tubes/rack) and immersed in hot water. The temperature inside the tube was allowed to equilibrate with the surrounding temperature before any test was done. Water temperature was monitored using a digital thermometer provided with a thermo couple wire.

Test insects

Colonies of adult *Bactrocera philippinensis and B. occipitalis* were separately reared and maintained at the fruit fly laboratory of the National Mango Research and Development Center, Guimaras. Adults were feed with protein hydrolysate, sugar and water under natural light, supplemented by artificial light. Room temperature was maintained from 26 to 28°C and relative humidity of about 80 percent.

Eggs. The eggs of the two fruit flies species were collected from matured females (3 to 5 weeks old) by introducing pin-pricked, ripe papaya domes, inside cages for two hours (oviposition time). Eggs were collected from the dome by spraying them with fine mist of water. Collected eggs were divided into 3 parts (aliquot). One aliquot was used for the early eggs and the remaining 2 parts were used for the late eggs and larvae (first and third instars, feeding and non-feeding).

The age of the early egg was from 1 to 3 hours after oviposition while the late egg stage was from 28 to 30 hours. The eggs were viable with good hatchability (85 to 90 percent).

First larval instar. After removing the eggs from the papaya dome, these were placed on black, moist cloth with fine brush and transferred in petri dishes for emergence at room temperature. The age of the first instars were 48 to 50 hours from oviposition. These were minute, whitish larvae ready for the immersion test.

Third larval instar. Similarly, eggs collected from the same cohort were placed on moist cloth and transferred in plastic trays containing larval food. The natural diet consisted of blended ripe papaya, torula yeast and nipagen (methyl paraben). Water was added to adjust diet consistency. The eggs were held at room temperature (26 to 28°C) and allowed to hatch to first instar and finally to the third instars.

The feeding third instars have well developed mandibular hooks and were about 120 to 122 hours from oviposition. These were collected by mixing the diet with concentrated sucrose/water solution. The feeding larvae float on top of the solution and were carefully removed using a nylon sieve. Larvae were thoroughly rinsed with distilled water (remove the sugar solution) and precounted for the hot water immersion test.

On the other hand, the non-feeding ("popping") third instar, jumped out of the media upon reaching this stage (170 to 172 hours from oviposition). These were collected by placing the tray with diet over a basin containing water. The third larval instars were collected from the water and pre-counted for the test using a soft forcep.

Hot water bath immersion test

General test. This was done to determine the response of the immature stages of fruit flies to a wide range of temperatures and immersion time. The general test included the following treatments: 45°C (1, 2, 4, 8, 16, 32, 64 and 128 mins.); 46°C (1, 2, 4, 8, 16, 32 and 64 mins.); 47°C (1, 2, 4, 8, 16 and 32 mins.); 48°C (0.5, 1, 2, 4 and 8 mins.); and 49°C (0.25, 0.5, 1, 2 and 4 mins.).

At a desired water temperature, the tubes were placed in the rack with the bottoms immersed in heated water. The temperature inside the tube was allowed to equilibrate with the surrounding water and monitored using a digital thermometer provided with a thermo-couple wire.

A drop of aliquot (100 to 200 early egg stage) was placed in each tube using a small plastic syringe (3 ml capacity). To ensure that eggs were submerged in hot water, the tubes were slightly shaken and any floating eggs were collected and discarded. After the required dipping time, the tubes were removed from the hot water bath and immediately immersed in water at ambient temperature (26 to 28°C) for 1 minute. One hundred healthy and viable looking eggs were counted on a moistened black cloth inside a petri dish. These were allowed to hatch to first larval instars and percent hatchability was checked under a stereoscope. Similar procedure was also done for the late egg stage using different temperature-time combinations.

From the same cohort, the first instars which emerged were collected using a soft, fine brush. Twenty-five larvae in 2 drops of water were placed in 3 ml plastic pipette with the handle cut longitudinally to provide wide opening. This facilitated easy transfer of larvae in the tubes. The larvae were emptied in the tubes, similar to the early and late eggs. After dipping, the tubes were placed in water at ambient temperature for 1 minute. Treated larvae were carefully transferred on moist, black cloth and placed over diet. The larvae were allowed to develop in the papaya diet for 7 days and were later washed and transferred to sterilized sawdust for pupal development. Survivors were determined by counting the number of pupae developed in the sawdust.

The feeding larvae (third instars) were sieved from the diet and rinsed with distilled water. These were placed in small plastic cups (30 ml) with small amount of water using a soft forcep. These were later emptied in tubes at various temperatures and immersion time. Similarly, the non-feeding larvae were sieved from the basin and placed in small cups with water. The latter were poured inside plastic tubes immersed in heated water.

Twenty-five or more larvae of each stage was used per treatment. After dipping, the larvae (feeding) were placed in cups with larval diet. The larvae were allowed to develop for 2 days and were latter washed and transferred in sterilized sawdust for pupation. On the other hand, the non-feeding larvae were placed directly in sterilized sawdust for pupation. Survivors were determined by counting the number of pupae developed from the 25 treated larvae.

Control was provided for all treatments. The eggs, first instar or third instar were immersed in water at ambient temperature for duration of the longest heat treatment. One control for every test temperature was done using the same number of test insects at each stage of development.

In addition, a check was also provided to determine normal egg hatching. The check consisted of similar number egg samples placed on moist cloth and allowed to hatch at room temperature. Checks were also done for the first and third instars (feeding and non-feeding) to determine the normal larval development using the same number of insects from a cohort. The first larval instars were placed on moist black cloth, inside a cup containing larval diet and allowed to pupate. On the other hand, the feeding larvae were placed in small cups with natural diet. The non-feeding larvae were placed directly in sawdust and allowed to pupate.

Specific test. This was conducted to provide information on the precise time necessary to obtain 100 percent mortality of the test insects at a given temperature. The specific test included the following temperature-time combinations: 45°C (2, 6, 10, 14, 18, 22, 26 mins.); 46°C (2, 4, 8, 10, 12, 14, 16 mins.); 47°C (0.5, 1, 2, 4, 6, 8 mins.); 48°C (0.5, 1, 2, 3, 4 mins.) and 49°C (0.25, 0.5, 1, 1.5 and 2 mins.).

As has been done with the general test, the immature stages of B. occipitalis and B. philippinensis were subjected to different temperatures and immersion time. These included the early egg, late egg, first instar and third instar (feeding and non-feeding). Similar procedures were followed in terms of operating the hot water bath, handling of test insects before and after treatment, treatment proper and evaluation of hatchability and number of survival/mortality. Checks and controls were also provided as descried earlier.

The general and the specific tests were replicated 3 times, using 1 cohort per replication. For each temperature and time combination, 10 tubes were used with 100 eggs each for early and late eggs, 25 larvae each for first and third instars (feeding and non-feeding).

Probit analysis was used to derived the relationship between mortality at different temperatures and immersion time. Similar procedure was used to estimate lethal time (LT99). A graph was also constructed to show the mortality trends and identify the tolerant stage(s) between the two species.

RESULTS AND DISCUSSIONS

The response of the immature stages (eggs and larvae) of *B. philippinensis* and *B. occipitalis* to heat as measured by percent mortality increases with increasing temperature and immersion time. The estimated lethal time to kill 99 percent of the samples is presented in Table 1 (General test) and Table 2 (Specific test).

Table 1. Estimated LT99 (min) and 95% Confidence Interval (fiducial limits) for each life stage - temperature combination for B. philippinensis and B. occipitalis (General test).

Temperature	Lifestage	Time for 99% mortality	
		B. philippinensis	*B. occipitalis*
45C	Early egg	12.498 (11.176 - 14.164)	17.795 (16.025 - 19.961)
	Late egg	42.687 (38.649 - 47.488)	44.075 (39.318 - 49.876)
	1st instar	41.005 (33.370 - 52.387)	61.407 (48.588 - 80.748)
	3rd instar (F)	20.655 (18.806 - 22.943)	21.529 (19.199 - 24.469)
	3rd instar (NF)	36.664 (32.937 - 41.325)	23.273 (21.065 - 26.020)
46C	Early egg	9.895 (8.776 - 11.347)	5.925 (5.362 - 6.644)
	Late egg	18.825 (17.157 - 20.800)	12.378 (11.119 - 13.948)
	1st instar	22.449 (18.588 - 28.035)	17.176 (14.010 - 21.983)
	3rd instar (F)	16.915 (14.919 - 19.505)	8.503 (7.647 - 9.603)
	3rd instar (NF)	15.892 (14.805 - 17.270)	16.094 (14.332 - 18.344)
47C	Early egg	5.561 (4.963 - 6.358)	3.427 (3.125 - 3.824)
	Late egg	6.541 (6.045 - 7.139)	7.363 (6.510 - 8.503)
	1st instar	11.940 (9.701 - 15.488)	10.620 (8.596 - 13.898)
	3rd instar (F)	7.831 (6.947 - 9.004)	6.159 (5.437 - 7.145)
	3rd instar (NF)	8.975 (8.489 - 9.544)	6.099 (5.473 - 6.923)
48C	Early egg	2.173 (1.917 - 2.534)	1.927 (1.712 - 2.227)
	Late egg	5.249 (4.742 - 5.907)	2.687 (2.453 - 2.980)
	1st instar	5.769 (4.640 - 7.611)	4.061 (3.240 - 5.492)
	3rd instar (F)	4.118 (3.635 - 4.767)	2.702 (2.427 - 3.065)
	3rd instar (NF)	7.752 (6.645- 9.279)	3.846 (3.443 - 4.369)
49C	Early egg	0.538 (0.491 - 0.605)	0.558 (0.501 - 0.630)
	Late egg	2.295 (2.111 - 2.515)	1.457 (1.355 - 1.578)
	1st instar	1.260 (1.073 - 1.548)	1.901 (1.568 - 2.435)
	3rd instar (F)	1.323 (1.237 - 1.432)	1.608 (1.446 - 1.818)
	3rd instar (NF)	2.514 (2.251 - 2.852)	2.725 (2.427 - 3.111)

Table 2. Estimated LT99 (min) and 95% Confidence Interval (fiducial limits) for each life stage - temperature combination for B. philippinensis and B. occipitalis (Specific test).

Temperature	Lifestage	Time for 99% mortality	
		B. philippinensis	*B. occipitalis*
45C	Early egg	18.696 (16.805 - 21.090)	24.962 (22.622 - 27.836)
	Late egg	38.508 (36.073 - 41.286)	42.550 (39.469 - 46.123)
	1st instar	29.406 (26.615 - 32.873)	24.652 (22.307 - 27.582)
	3rd instar (F)	21.394 (19.304 - 24.020)	20.252 (18.275 - 22.741)
	3rd instar (NF)	20.051 (18.852 - 21.522)	19.647 (18.458 - 21.110)
46C	Early egg	9.869 (8.849 - 11.231)	8.300 (7.552 - 9.273)
	Late egg	10.164 (9.498 - 10.955)	9.128 (8.460 - 9.944)
	1st instar	9.302 (8.437 - 10.428)	7.797 (7.097 - 8.716)
	3rd instar (F)	10.581 (9.627 - 11.802)	8.239 (7.484 - 9.230)
	3rd instar (NF)	10.555 (10.099 - 11.092)	11.871 (10.521 - 13.670)
47C	Early egg	3.288 (3.059 - 3.560)	3.033 (2.833 - 3.268)
	Late egg	7.959 (7.469 - 8.515)	6.458 (6.011 - 6.976)
	1st instar	9.146 (7.822 - 11.044)	6.296 (5.516 - 7.330)
	3rd instar (F)	5.637 (5.195 - 6.181)	6.145 (5.488 - 6.985)
	3rd instar (NF)	6.756 (6.328 - 7.270)	7.257 (6.695 - 7.937)
48C	Early egg	2.170 (1.990 - 2.397)	1.909 (1.779 - 2.068)
	Late egg	5.006 (4.563 - 5.549)	3.397 (3.119 - 3.735)
	1st instar	5.361 (4.509 - 6.620)	3.218 (2.783 -3.844)
	3rd instar (F)	3.577 (3.193 - 4.082)	2.168 (1.981 - 2.409)
	3rd instar (NF)	4.429 (3.996 - 4.978)	3.642 (3.334 - 4.025)
49C	Early egg	1.549 (1.426 - 1.700)	1.111 (1.038 - 1.198)
	Late egg	2.222 (2.070 - 2.399)	2.204 (2.043 - 2.393)
	1st instar	1.343 (1.185 - 1.561)	1.546 (1.370 - 1.782)
	3rd instar (F)	1.228 (1.555 - 1.320)	1.452 (1.336 -1.596)
	3rd instar (NF)	2.135 (1.942 - 2.376)	1.640 (1.550 - 1.748)

General Test

At 45°C, the late egg stage of *B. philippinensis* was tolerant compared to other stages of development except for the 1st larval instar where the LT99 did not differ significantly with each other. In addition, the response of the 3rd instar (non-feeding) to heat was also comparable to the 1st instar. For *B. occipitalis*, the 1st larval instar was the most tolerant stage. This species showed some degree of tolerance over to *B. philippinensis* at early egg and 1st instar except in 3rd instar (non-feeding) where *B. philippinensis* was observed tolerant.

At 46°C, the 1st instar of *B. philippinensis* was the most tolerant stage. Similarly, this stage was also tolerant in B. occipitalis. However, its LT99 was lower and comparable to the 3rd instar (non-feeding). At this temperature, *B. philippinensis* was tolerant over *B. occipitalis* in most stages except in the 3rd instar (non-feeding) where both species gave similar response.

At 47°C, the first instar of *B. philippinensis* still remained tolerant compared to other stages. The same result holds true for *B. occipitalis* where the LT99 did not vary significantly with the 2 species. This indicates that the tolerant stage of *B. philippinensis* and *B. occipitalis* (1st instar) had similar response to the given temperature. However, *B. philippinensis* was tolerant compared to *B. occipitalis* at early egg and 3rd instar (feeding and non-feeding) except in the late egg stage.

At 48°C, the 3rd instar (non-feeding) of *B. philippinensis* exhibited heat tolerance compared to all stages. In *B. occipitalis*, it was the 1st instar however, its LT99 was comparable to the 3rd instar (non-feeding). Between the 2 species, *B. philippinensis* showed tolerance over *B. occipitalis* in most stages except in early egg where the LT99 was also comparable.

At 49°C, shorter time was required to attain the 99 percent mortality for the 2 species. All stages were destroyed between 0.5 to 2.5 minutes for *B. philippinensis* and 0.5 to 2.7 minutes for *B. occipitalis*. The tolerant stage for *B. philippinensis* and *B. occipitalis* was 3rd instar (non-feeding). *B. philippinensis* showed tolerance over *B. occipitalis* only at the late egg stage.

Specific test

At 45°C, the late egg was the most tolerant stage observed between the 2 species of fruit flies. However, *B. occipitalis* exhibited greater tolerance over *B. philippinensis* as shown by higher LT99. In addition, this species also showed some degree of tolerance in the early and first larval instars. The LT99 of *B. occipitalis* was however, comparable to *B. philippinensis* in the 3rd instar (feeding and non-feeding).

At 46°C, all the immature stages of *B. philippinensis* gave similar response to the prescribed temperature as shown by the overlapping confidence intervals (C.I.). This means that at 10 minutes, immersion time, 99 percent mortality is expected for the eggs and larval stages of the species. In *B. occipitalis*, the most tolerant stage was the 3rd instar (non-feeding). This was followed by the late eggs where LT99 value was comparable to the early egg and 3rd instar (feeding). Between the two species, *B. philippinensis* exhibited more tolerance in most stages except for the 3rd instar (non-feeding).

At 47°C, the 1st instar larvae of *B. philippinensis* was most tolerant while in *B. occipitalis*, it was the 3rd instar (non-feeding). *B. philippinensis* was tolerant over *B. occipitalis* at late egg and 1st instar. However, the response to heat was comparable for both species at early egg stage and 3rd instar (feeding and non-feeding).

At 48°C, the 1st instar was the most tolerant stage of *B. philippinensis*. However, its LT99 was comparable to the late eggs, indicating similar response to temperature. In contrast, the tolerant stage of *B. occipitalis* was 3rd instar (feeding). However, its LT99 was also comparable to the late egg stage. Between the 2 species, *B. philippinensis* was tolerant over *B. occipitalis* at all stages of development.

At 49°C, the late egg was the most tolerant stage in *B. philippinensis*. However, its LT99 was also comparable to 3rd instar (non-feeding). Similarly, the most tolerant stage for *B. occipitalis* was the late egg. Again, *B. philippinensis* was tolerant over *B. occipitalis* at early egg and 3rd larval instar (non-feeding). Opposite result was observed in the 1st instar and 3rd instar (feeding), where these stages became tolerant.

Figure 1 Mortality response of Bactrocera philippinensis to heat (General test).

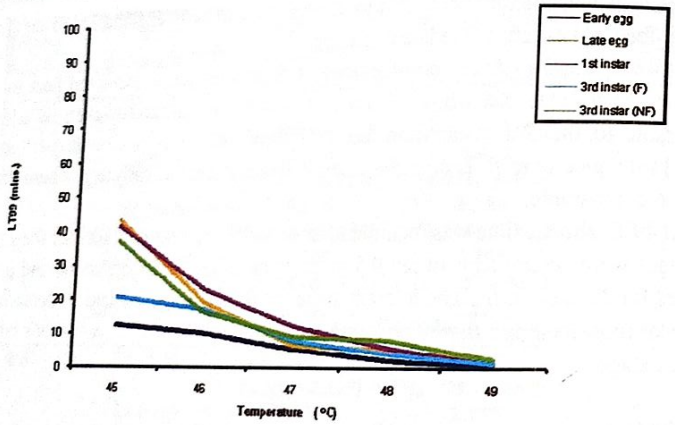


Figure 2 Mortality response of Bactrocera occipitalis to heat (General test).

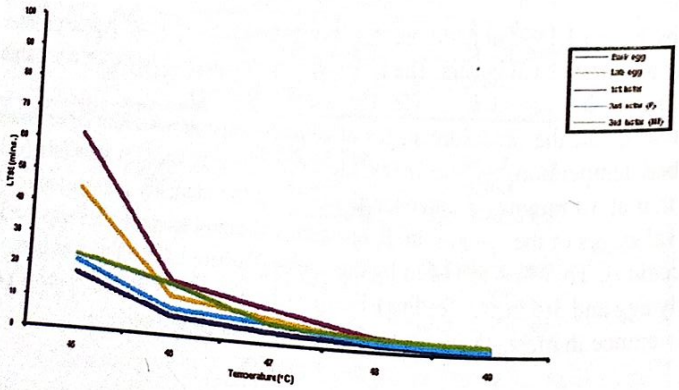


Figure 3 Mortality response of Bactrocera philippinensis to heat (Specific test.)

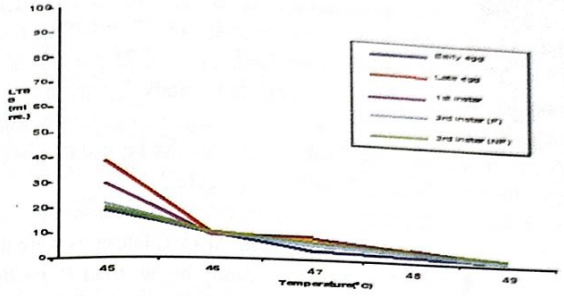
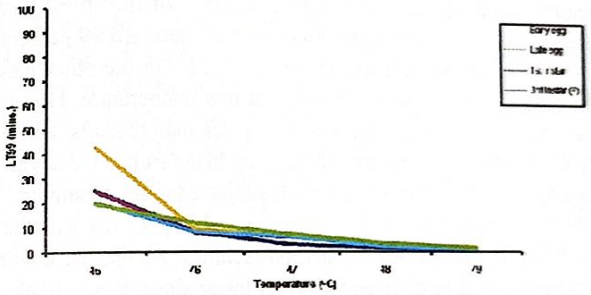


Figure 4 Mortality response of Bactrocera occipitalis to heat (Specific test).



In the general test, the tolerant stages of *B. philippinensis* were the late eggs (45°C), 1st instar (46°C and 47°C), 3rd instar, non-feeding at 48°C and 49°C. This indicates that early eggs were most susceptible to heat compared to other stages of development. This result also confirmed the work of Manoto **et. al.** (1998) where early egg was sensitive to heat compared to the late egg. The LT95 for 2-hour eggs was 2.08 minutes while 5.0 minutes for 20-hour eggs.

Below 47°C the 1st larval instar of *B. philippinensis* was most tolerant, except at 45°C where the late eggs became the tolerant stage. However, its LT99 was still comparable to the 1st larval instar. The estimated lethal time to kill 99 percent of the samples were 42.60, 22.45 and 11.94 minutes for late egg and 1st instars at 46°C 47°C, respectively.

At temperatures above 47°C, the 3rd instar larvae was the most tolerant stage with an estimated lethal time of 7.75 and 2.51 minutes for 3rd instar non-feeding (48°C) and 3rd instar non-feeding (49°C), respectively. The order of heat tolerance for *B. philippinensis* was 1st instar > late egg > 3rd instar (non-feeding) > 3rd instar (feeding) > early egg (Figure 1).

In the case of *B. occipitalis*, the most tolerant stage was the 1st instar for most temperatures except at 49°C where the 3rd larval instar became tolerant. The LT99 of the 1st instars were 61.41, 17.17, 10.62 and 4.06 minutes at 45, 46, 47 and 48°C, respectively. On the other hand, the LT99 of the 3rd instar (non-feeding) was 2.75 minutes at 49°C.

B. occipitalis was tolerant over *B. philippinensis* in the early egg stage and 1st instar. However, at temperatures greater than 45°C, *B. philippinensis* was generally tolerant over *B. occipitalis*. The order of heat tolerance for B. *occipitalis* was 1st instar > late egg >3rd instar (non-feeding) > 3rd instar (feeding) > early egg (Figure 2).

On the other hand, the specific test gave a more precise time necessary to attain the 99 percent mortality for the different stages of development. At 45°C late eggs were the tolerant stage although the LT99 of *B. occipitalis* was significantly higher than *B. philippinensis*, indicating some degree of tolerance. However, at 46°C all the stages of *B. philippinensis* was killed by heat and only the 3rd instar (non-feeding) of *B. occipitalis* exhibited tolerance. At this temperature, *B. philippinensis* was tolerant over *B. occipitalis*.

VHT which disinfests mango fruits for export, makes use of the pulp temperature (46oC for 10 minutes) necessary to kill the eggs and larvae of fruit flies. Result of this bioassay also revealed that immature stages of both species attained 99 percent mortality when subjected to similar temperature and immersion time. On the other hand, the 3rd instar (non-feeding) of *B. occipitalis* was tolerant at this temperature. However, in the field, only the eggs and 1st larval instars are present in green mature fruits. These stages are easily killed by VHT. The 3rd larval instars only develop in the fruit at the advance stage of maturity hence, are not found in green mature fruits subjected to the treatment.

At 47 and 48°C, the most tolerant stage of the *B. philippinensis* was the 1st instar while for *B. occipitalis*, it was the 3rd instar (non-feeding). Again, the concern of any disinfestation treatment should be directed to the 1st instar since, this is likely present in green fruits intended for export. At this temperature, B. philippinensis was tolerant over *B. occipitalis* in many of the stages.

At 49°C, it took shorter time to attain LT99 for the 2 species. All the stages succumbed to heat and mortalities were achieved between 1.22 to 2.22 minutes for *B. philippinensis* and 1.11 to 2.20 for *B. occipitalis*.

Figure 3 represents the response of *B. philippinensis* to heat. The order of tolerance was late egg > 1st instar > 3rd instar (non-feeding) > 3rd instar (feeding) > early egg. The mortality lines were steeper and wider for late egg and 1st instar at 45°C, indicating high tolerance as compared to the rest of the stages. However, at 46°C the lines became closer and tapered down towards 49°C. At this temperature, all stages of *B. philippinensis* responded similarly to heat as shown by the overlapping confidence intervals. Similar trend was observed for *B. occipitalis* (Figure 4) but the lines were wider as the species became tolerant and closer as they became less tolerant.

CONCLUSIONS AND RECOMMENDATIONS

The general test showed that at 45, 46 and 47°C, the most tolerant stage of *B. philippinensis* and *B. occipitalis* was the 1st larval instars. However, at lower temperatures (45°C), the late eggs showed some degree of tolerance but the LT99 did not differ significantly with the 1st instar. At temperatures 48 and 49°C, the 3rd instar (non-feeding) was more tolerant compared to the other stages. *Bactrocera occipitalis* was tolerant over *B. philippinensis* only at 45°C (early egg and 1st instar). However, at temperatures greater than 45°C, *B. philippinensis* was generally tolerant over *B. occipitalis* for most of the stages.

In the specific test, the late eggs stage for both species was tolerant to heat at 45°C. At 46°C, most of the immature stages were killed at 10 minutes immersion time, except for the 3rd instar (non-feeding).

At higher temperatures, the first larval instars showed some degree of tolerance as well as the 3rd instars. However, the time (minutes) required to achieve LT99 was much shorter compared to lower temperatures.

In both test, *B. philippinensis* was generally tolerant over *B. occipitalis*.

Irregardless of the species, the late eggs and 1st instars were observed as the tolerant stage. As such, any disinfestation treatment required should be directed to these stages.

The tolerance to heat of 3rd instars should not be overlooked. However, the feeding stage is present only when the fruits have reached their advanced stage of maturity. The non-feeding stage on the other hand, leaves the fruit and pupate in the ground. Hence, mango fruits intended for export (green mature) are not infested with this stage.

IMPLICATIONS

1. The study identified the heat tolerant stages of the two species of fruit flies, *Bactrocera occipitalis* and *B. philippinensis* attacking mango.
2. The result serves as basis for confirmatory test using vapor heat treatment to control fruit fly.
3. The methods used could be adapted in carrying out similar tests to determine response of other fruit fly species to heat.
4. Expansion of more markets for mango particularly in countries where postharvest treatment for fruit fly is required.

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UTILIZATION OF ENTOMOPATHOGENIC FUNGI AS BIOLOGICALCONTROL AGENT AGAINST MAJOR INSECT PESTS OF MANGO

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ABSTRACT

Survey and collection of entomopathogenic fungi infecting important pests of mango were done and incidence of infection was recorded. Isolates were also collected from different sources such as *Sporothrix insectorum* and *Aphanocladium album* from Insect Pathology Resource Center, Boyce Thompson Institute, New York (ARSEF). *Metarhizium anisopliae*(ARSEF, Leyte State University and RCPC, Cebu). *Beauveria bassiana* and *Verticillium lecanii* (ARSEF and Leyte).

The potential of the fungal isolates for maximum growth and conidial production was studied using locally available substrates in combination with water and coconut milk. Conidia production of *Metarhizium anisopliae* (Cebu and Leyte) did not vary significantly for most types of substrates used except on palay plus coconut milk. However, *M. anisopliae* (ARSEF) produced the significantly highest spores on Rice broken + water. For *B. bassiana* (Leyte), Rice broken + water supported the highest conidial production of 7.78/gram while, for *Bb*(ARSEF) comparable results were obtained on Corn grits + water, Rice broken + water, Rice whole + water and Palay + water. Spore production for *Verticillium lecanii* grown on both types of rice and corn grits with addition of water did not vary significantly. On the other hand, no sporulation was observed on either types of corn substrate combined with coconut milk. For *Aphanocladium album* (ARSEF), Rice whole + water supported the highest conidial production. Satisfactory yield of conidia/ gram was achieved after 14 days of incubation. Rice with water gave the best results for conidial production and corn can be a good substitute for some isolates.

The efficacy of different entomopathogenic fungi like *Metarhizium anisopliae, Beauveria bassiana, Verticillium lecanii* and *Aphanocladium album* as biological control agent against mirid bugs *(Helopeltis collaris.)*; mango leafhoppers *(Idioscopus clypealis)* and fruit flies *(Bactrocera philippinensis* and *Bactrocera occipitalis*) were evaluated under laboratory and field condition.

B. bassiana at 1x108 and 1x109 conidia/ml caused 100% mortality on mirid bug at 2-4 days after treatment.

Mortality and mycosis of fruit flies differed significantly based on conidial concentrations of the four fungi. *B. bassiana* and *M. anisopliae* at 1x108 and 1x109 conidia/ml caused the highest mycosis of 82 – 93% on *B. philippinensis* and 88 – 92% on *B. occipitalis*. *V. lecanii* at 1x109 conidia/ml caused 57 and 68% mycosis on *B. philippinensis* and *B. occipitalis*, respectively. The lowest mycosis of 35% on *B. philippinensis* and 40% on *B. occipitalis* was caused by *A. album* at 1x109 conidia/ml.

The LT50 occurred at 3.29 days at 1x109 conidia/ml on *Beauveria bassiana* for *Bactrocera philippinensis* and 3.48 for *Bactrocera occipitalis.*

Field trial on mango leafhoppers treated with *Beauveria bassiana* and *Metarhizium anisopliae* 16 days after treatment showed a cumulative mortality of 56.50 and 42.83 percent, respectively. Mycosis on insects treated with *Beauveria bassiana* ranged from 0.83 to 7.67% while no mycosis was observed on *Metarhizium anisopliae* treatments. It was observed that at high insect population, the application of entomopathogenic fungi alone did not give good control, such that combination of other control strategies should be applied for effective control.

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