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Comparison of three populations of *Bactrocera dorsalis* for efficacy of vapor heat treatment in mangoes

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Abstract

Phytosanitary treatments approved by the International Plant Protection Convention (IPPC) generally are applicable worldwide without regard for the country of origin of the exported commodity. However, circumstantial evidence suggests that quarantine pests may vary in tolerance to phytosanitary treatments by geographic area. If different populations of the same pest species do vary significantly in tolerance to treatments, schedules in the IPPC phytosanitary treatment standard may need to be region-specific instead of globally applicable. This issue has delayed approval of some temperature treatment schedules. The availability of various tephritid colonies at the Insect Pest Control Laboratory of the Food & Agriculture Organization of the United Nations/International Atomic Energy Agency, at Seibersdorf, Austria, is an ideal opportunity to compare species and populations of tephritids for multiple factors including tolerance to phytosanitary treatments. This research compares 3 populations of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) (China, Kenya, and Thailand) for tolerance to vapor heat treatment (47 °C and 95% RH for 150–180 min) in mangoes. Although differences were observed at sub-lethal doses, they became non-significant at levels of control required for phytosanitary treatments, indicating that vapor heat treatments may be broadly applicable geographically.

Key Words: phytosanitation; heated air treatment; quarantine treatment; oriental fruit fly; high temperature-forced air treatment

Resumen

Tratamientos fitosanitarios aprobados por la Convención Internacional de Protección de Plantas (IPPC por sus siglas en inglés) en general son genéricas y pueden ser aplicadas sin tener en cuenta el país de origen del producto exportado. Sin embargo, evidencias sugieren que plagas de importancia cuarentenaria pueden variar su respuesta a los tratamientos fitosanitarios dependiendo de su origen geográfico. Si diferentes poblaciones de la misma especie varían significativamente en la tolerancia a determinados tratamientos fitosanitarios aprobados y listados por la IPPC entonces cada uno de estos tratamientos fitosanitarios deberían ser específicos para determinada región en vez de tener una validez global. Por esta razón la aprobación de algunos tratamientos fitosanitarios ha sufrido retrasos en su aprobación. La colección de colonias de poblaciones de moscas de las frutas mantenidas en el Laboratorio de la Sección de Control de Insectos Plagas de la división conjunta entre la Organización Mundial para la Alimentación (FAO) y el Organismo Internacional de Energía Atómica (OIEA), de las Naciones Unidas en Seibersdorf, Austria, brinda una oportunidad única que permite comparar múltiples factores entre especies y poblaciones de tephritidos, incluido los tratamientos fitosanitarios. En este trabajo de investigación se compara la tolerancia al tratamiento de vapor (47 °C y 95% HR durante 150–180 min) en mangos infestados con 3 poblaciones de *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) (China, Kenia y Tailandia). Aunque algunas diferencias fueron observadas para dosis sub letales, las diferencias no fueron significantes para la dosis requerida para inducir el nivel de control especificado como tratamiento fitosanitario valedero, lo que indica que el tratamiento con alta temperatura por medio del suministro de vapor podría ser considerada como un tratamiento genérico geográficamente.

Palabras Clave: tratamiento fitosanitario, mosca de fruta, vapor caliente

Phytosanitary treatments are used to disinfest agricultural commodities of possible quarantine pests so that the commodities may be shipped from infested areas to areas where the pests do not exist but could become established (Heather & Hallman 2008). The major treatments used worldwide are cold storage, heating in air or water, fumigation, and ionizing radiation. Vapor heat treatments use heated air at about 47 °C with high humidity to kill insects that may be infesting fruit. Vapor heat treatment also is known by other names, such as high temperature-forced air treatment. Vapor heat

treatment schedules dictate at minimum the treatment air temperature and the minimum temperature to be reached in the center of the fruit or at the seed surface for fruits with large seeds, such as mango.

For phytosanitary treatment schedules to be broadly applicable geographically there should not be significant differences in efficacy among populations of the same pest species from different areas. The phytosanitary treatment literature and treatment schedules indicate possible differences in efficacy among different populations of the

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same species of tephritids. For example, in a comprehensive review of the literature, Dohino et al. (2017) note that vapor heat treatment schedules for mangoes for *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) differ in severity from a low of seed surface temperature ≥ 46 °C for 10 min in the Philippines to a high of seed surface temperature ≥ 48 °C for 20 min in India. Reasons why these schedules are different might include differences in susceptibility to heat among pest populations, the large-scale confirmatory testing was done at different time or temperature combinations, different manners of measuring efficacy were used, results were interpreted differently, and importing plant protection organizations required different levels of efficacy or simply added time or temperature to the treatment for additional security. The possibility that differences might exist may obstruct the evaluation and approval of phytosanitary treatments by international treaties dealing with trade, such as the International Plant Protection Convention (IPPC).

The objective of this study was to determine if populations of *B. dorsalis* from different geographic areas vary significantly in tolerance to the vapor heat treatment. A secondary objective was to estimate final seed surface temperatures required for efficacy for *B. dorsalis* in mango. *Bactrocera dorsalis* was chosen for this research because vapor heat treatments for this insect are being evaluated by the IPPC and the question of possible variation in tolerance to heat among different populations was raised by countries reviewing the recommendations.

A review of the literature indicates that the mid-egg stage is generally the most thermo-tolerant when studies are done with fruit flies infesting fruit and a common measure of efficacy of survival to the 3rd instar or pupariation is used. Only studies conducted with insects feeding their entire larval lives in fruit were considered, and not studies using insects reared in artificial diet and then inserted into fruit or done completely in vitro, to avoid making untested assumptions about the effect of artificial infesting techniques on relative efficacy. Hallman (2014) observed that 3rd instars of Mexican fruit fly, *Anastrepha ludens* (Loew) (Diptera: Tephritidae), reared on diet and inserted into grapefruit seemed more susceptible to hot water immersion treatment than those reared naturally in grapefruit via oviposition, although the results were not statistically significant in that case.

Data supporting the conclusion that the mid-egg stage is the most heat tolerant are from 8 species of tephritids including *B. dorsalis* (Table 1). In 10 of 13 studies (77%) the egg was the most thermo-tolerant stage or of equal tolerance as other stages that were among the most tolerant in that study. In the 3 studies where the egg was not the most tolerant stage it was the next most tolerant after 1st or 3rd instars.

Materials and Methods

SOURCES OF *BACTROCERA DORSALIS* AND INFESTATION OF MANGOES

Bactrocera dorsalis populations from China (Fujian Province), central Kenya, and Thailand (Saraburi Province), 45, 48, and 60 generations in culture, respectively, were reared at 25 ± 0.5 °C and $65 \pm 5\%$ RH under a photoperiod of 14:10 h (L:D) with semi-artificial diet using standard procedures at the Insect Pest Control Laboratory (IPCL) of the Food & Agriculture Organization of the United Nations/International Atomic Energy Agency, Seibersdorf, Austria, as detailed by Hallman et al. (2013).

Twelve physiologically mature but non-ripe mangoes (cultivars 'Kent,' 'Keitt,' 'Palmer,' or 'Tommy Atkins,' obtained from Brazil during late Oct through Nov 2016; mean \pm SE mass = 640 ± 12.6 g) were infested by placing them in screen cages containing approximately 2,000 2- to 5-wk-old ovipositing flies of each population for 1 to 2 h to obtain a reasonable infestation rate. The infested mangoes were held at about 25 °C for 24 h before being vapor heat treated. The infestation and the following vapor heat treatment were replicated 11 times. Each replicate consisted of 3 batches of 12 mangoes of the same cultivar infested by each of the 3 fly populations at the same time. Cultivars were selected depending on which was available at the time. The experimental design is randomized complete block.

VAPOR HEAT TREATMENT

In each replicate, 6 of the 12 mangoes of the same cultivar infested with 24-h-old eggs of each population (18 mangoes total) were placed in an environmental chamber (Pol-Eko Aparatura, Model KK 700 TOP+, Warsaw, Poland, about 1 m³ volume). This machine provides the basic features for a vapor heat treatment, namely a sustained source of heat with high humidity. The 18 mangoes infested with the 3 populations were randomly mixed together in the treatment chamber and heated at 47 °C and 95% RH for 150 to 180 min. The other 6 mangoes from each population were used as a control treatment. These were not treated and were held at 25 °C to allow larvae to develop to the size where they could be counted (3rd instar) and used to estimate egg population sizes in the treated mangoes.

Temperatures at the seed surface in 3 mangoes (1 from each population) were recorded with thermocouples (HOBO data logger, Onset Computer Corporation, Bourne, Massachusetts, USA) calibrated at

Table 1. Summary of thermo-tolerance studies among stages of Tephritidae using common measures of efficacy among the stages (egg and all 3 instars) and reared and treated within fruit.

Species	Fruit	Relative tolerance	Reference
<i>Bactrocera aquilonis</i>	mango	1st > egg > 3rd > 2nd	Corcoran (2001)
<i>Bactrocera cucumis</i>	zucchini	Egg > 1st > 2nd > 3rd	Corcoran et al. (1993)
<i>Bactrocera cucurbitae</i>	eggplant	Egg > 1st > 3rd	Furusawa et al. (1984)
<i>Bactrocera cucurbitae</i>	pepper	Egg > 1st > 3rd	Sugimoto & Sunagawa (1987)
<i>Bactrocera cucurbitae</i>	momordica	Egg > 3rd > 2nd > 1st	Sunagawa et al. (1988)
<i>Bactrocera dorsalis</i>	mango	All same	Corcoran (2001)
<i>Bactrocera fraunfeldti</i>	mango	1st = egg > 3rd > 2nd	Corcoran (2001)
<i>Bactrocera jarvisi</i>	mango	1st = 3rd > egg > 2nd	Corcoran (2001)
<i>Bactrocera tryoni</i>	mango	Egg > 3rd > 2nd > 1st	Heard et al. (1992)
<i>Bactrocera tryoni</i>	mango	3rd > egg > 2nd > 1st	Heard et al. (1992)
<i>Bactrocera tryoni</i>	mango	Egg = 3rd	Heather et al. (1997)
<i>Bactrocera tryoni</i>	tomato	Egg > 1st > 2nd > 3rd	Heather et al. (2002)
<i>Ceratitidis capitata</i>	mango	Egg = 3rd	Heather et al. (1997)

46.0 °C with a certified thermometer (H-B Instrument-SP Scienceware, Trappe, Pennsylvania, USA) traceable to the US National Institute of Standards and Technology.

The goal was to vapor heat treat the infested mangoes long enough to kill almost all of the *Bactrocera dorsalis* eggs, but leaving a few survivors so that possible differences in survival would be measurable and allow for comparisons among populations. The decision on when to terminate a treatment was made taking into account results from previous replicates; i.e., if no insects survived a treatment, the subsequent treatment target temperature time was reduced, and if many survived it was increased. All of the mangoes within a replicate were removed at the same time.

STATISTICAL ANALYSES

Analysis of variance (ANOVA) was used to compare survival among the 3 populations of *B. dorsalis* after vapor heat treatment. Proportional data should be transformed before analysis if the data are close to one extreme, as in the objective of this research (McDonald 2014). There are 2 transformations recommended for proportional data: logit and arcsine. Logit is preferred for regression analysis, while arcsine is preferred for ecological count data (<http://strata.uga.edu/8370/rtips/proportions.html>). Proportional survival data for the 3 populations were arcsine transformed prior to ANOVA (SAS 9.4, Cary, North Carolina, USA). The fixed factor was the 3 fly populations and the random factor replicates.

Probit analysis (Polo Plus Version 2.0, LeOra Software, Petaluma, California, USA) was used to estimate final seed surface temperatures that would provide 95, 99, and 99.9968% (probit 9) mortality of eggs of *B. dorsalis* as measured by failure to develop to the 3rd instar inside the fruits. The number of eggs treated was estimated using the number of larvae counted in the non-treated controls. Although this undoubtedly underestimates the number of eggs laid, it automatically separates those that die due to factors not related to the treatment, negating the need for correction for mortality caused by other factors not related to the treatment. It is acknowledged that the estimate of number of eggs laid is variable and can affect estimates of efficacy; however, this effect is reduced as efficacy increases to the very high levels required of phytosanitary treatments.

Results

VAPOR HEAT TREATMENT

In 1 replicate with the Kenyan population, no larvae were found in the controls, indicating lack of infestation by that population for that

replicate (Table 2). In all other combinations the infestation rate ranged from 3.8 to 194.5 larvae per fruit in the non-treated control group. Infestation values do not necessarily reflect the population of eggs laid, only the population of larvae surviving to a size where they could be found. It is assumed that differences in the number of eggs deposited in mangoes and the number of larvae found later are relatively analogous among populations, enabling comparisons among populations.

No survivors were found when the mean mango seed surface temperature reached $\geq 45.01 \pm 0.56$ °C in the Chinese population and $\geq 45.38 \pm 0.29$ °C in the other 2 populations (Table 2). Mean infestation levels at these points when no survivors were found were 28.5, 22.7, and 26.3 larvae per fruit for China, Kenya, and Thailand, respectively.

In 4 of 11 replicates the vapor heat treatment resulted in no survivors for any of the populations, leaving 7 replicates for analysis of differences in efficacy among the 3 populations (Table 2). ANOVA of the 7 replicates and 3 populations found that they were not significantly different at the 95% level of confidence ($F = 3.22$; $df = 2,12$; $P = 0.076$). The arcsine transformed means \pm SEM for the Thai, Chinese, and Kenyan populations were 7.45 ± 4.00 , 1.70 ± 1.28 , and $0.79 \pm 0.65\%$, respectively.

Estimates of final seed surface temperatures for 95, 99, and 99.9968% mortality for the *B. dorsalis* populations from Kenya and Thailand at 47 °C are presented in Table 3. The software did not provide estimates for the population from China due to lack of convergence in the model.

Estimates were obtained for all 3 populations combined and the data for the populations from Kenya and Thailand combined. The mean seed surface temperature to achieve 99.9968% control was estimated to be 47.1 °C when all data were pooled.

Discussion

Decisions on phytosanitary measures by plant protection organizations are sometimes made in situations that will not be entirely defined and would require an inordinate amount of time and resources to clarify beyond a reasonable doubt. Trade and international relations may influence the need to arrive at a decision. The consequences for concluding that there are significant differences among populations of the same pest species for tolerance to a phytosanitary treatment, when actually there are not, may result in treatments being region-specific and create unnecessary obstacles to trade. The consequences of concluding that there is no significant difference, when actually

Table 2. Results of vapor heat treatment (air temperature 47 °C, RH 95%) of mangoes infested with 24-h-old eggs of *Bactrocera dorsalis* from 3 locations.

Cultivar	Mean mango mass \pm SE (g)	Treatment time (min)	Final mean seed surface temperature \pm SE (°C)	Total number of larvae from 3 populations of <i>B. dorsalis</i> in 6 mangoes each in control and heated fruit					
				China		Kenya		Thailand	
				control	heated	control	heated	control	heated
Palmer	643 \pm 5.5	180	43.33 \pm 0.47	997	161	265	3	374	154
Palmer	651 \pm 5.8	180	43.09 \pm 0.96	1167	35	977	79	339	120
Palmer	624 \pm 7.0	180	45.55 \pm 0.16	268	0	244	0	101	0
Palmer	628 \pm 7.0	180	45.40 \pm 0.30	584	0	32	0	251	2
Kent	678 \pm 8.9	165	45.01 \pm 0.56	171	0	245	0	294	0
Kent	679 \pm 8.7	165	43.68 \pm 0.69	768	1	25	0	373	0
Keitt	661 \pm 9.2	160	44.45 \pm 0.88	403	0	0	0	291	33
Keitt	652 \pm 7.9	150	45.45 \pm 0.26	392	0	23	0	351	0
Kent	646 \pm 7.5	150	45.19 \pm 0.31	924	0	481	2	808	3
Kent	675 \pm 6.9	165	44.94 \pm 0.55	596	16	310	0	184	0
Tommy Atkins	620 \pm 5.3	160	45.38 \pm 0.29	43	0	136	0	158	0

Table 3. Results of probit analysis of the dose-response relationship between final seed surface temperature in vapor heat treatment of mangoes infested with 24-h-old eggs of 3 populations of *Bactrocera dorsalis*.

Population(s)	χ^2	Degrees of freedom	Heterogeneity	Lethal temperature (LT) (95% fiducial limits)		
				LT ₉₀	LT ₉₉	LT _{99.9968}
Kenya	18.3	8	2.3	42.9 (42.3–43.1)	44.1 (43.8–45.3)	46.2 (45.1–49.9)
Thailand	168	9	18.7	44.0 (43.6–44.5)	44.9 (44.4–46.4)	46.5 (45.5–49.8)
Kenya + Thailand	547	19	28.8	44.0 (43.6–45.0)	44.9 (44.3–47.0)	47.0 (45.7–52.1)
China + Kenya + Thailand	1022	30	34.1	43.8 (43.5–44.5)	44.8 (44.5–46.4)	47.1 (45.8–51.5)

there are, may result in failed treatments and new pest infestations if the treatment was developed with a particularly susceptible population and subsequently applied commercially to commodities infested with more tolerant populations. These potential consequences should be considered when treatments are approved by plant protection organizations.

Although differences in survival after vapor heat treatment among the 3 *B. dorsalis* populations studied were not significant at the 95% confidence level, they would be significantly different at the 92% level of confidence. The population from Thailand appeared to be more tolerant than the populations from Kenya and China. However, the apparently most susceptible population (China) still required a final seed surface temperature of 45.0 ± 0.56 °C to kill 100% of an estimated minimum of 171 eggs, which was only a mean of about 0.4 °C lower and within the standard error limits of the other 2 populations (Table 2). Vapor heat treatment of *B. dorsalis* infested mangoes in the Philippines found a similar result; at a temperature between 45 to 46 °C all eggs were killed (Merino et al. 1985).

Relatively large differences in survival among the 3 populations at < 45 °C (Table 2) may be largely due to the increased error in estimating populations from non-treated fruit as the proportion surviving increases. As the proportion surviving decreases these differences are minimized.

Considerable overlap in confidence intervals among the probit mortality estimates (Table 3) suggests there are no significant differences in susceptibility to vapor heat treatment among these 4 groups. Final seed surface temperature estimates to achieve the very high level of control demanded of phytosanitary treatments may give impractical results. For example, the mean final seed surface temperature for 99.9968% control when data from all 3 populations are combined (47.1 °C) exceeds the treatment air temperature (47.0 °C). Therefore, to confirm a phytosanitary treatment, normally large-scale testing is done where many thousands of insects are treated at a specific set of treatment parameters to demonstrate that a treatment protocol results in an acceptable level of efficacy (Heather & Hallman 2008).

Because the number of generations of each population in culture at the IPCL was 45 to 60, it could be argued that they may not reflect the heat tolerance of the original field populations from which they were collected. However, Corcoran (2001) found that *Bactrocera* spp. reared for 120 to 220 generations in the laboratory were not reduced in heat tolerance compared with wild flies.

This research has aided the approval process of vapor heat treatments being considered by the IPPC resulting in treatment approval without the need for considering country of origin. We feel this decision is justified by the results of this research.

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