Thermal Death Kinetics of Some Common Postharvest Pathogens of Papaya

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Abstract. Thermal death kinetics, decimal reduction times (D-values), and rate constants, k, at 43 to 49 °C were determined for spore or cell suspensions of *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. in Penz., *Guignardia psidii* Ullasa & Rawl, *Guignardia* sp. Viala & Ravaz., and *Enterobacter cloacae* (Jordan) Hormaeche & Edwards. D-values for *Monilinia fructicola* (Wint.) Honey, *Rhizopus stolonifer* (Ehr. : Fr.) Vuill., and *Stemphylium lycopersici* (Enjoji) Yamamoto were calculated and extrapolated from published reports. We compared the relative heat resistances of the various postharvest pathogens to their expected survival during quarantine heat treatments and found that *Guignardia*, *Rhizopus*, and *E. cloacae* could be expected to survive quarantine heat treatments.

Heat treatments have played an important role in the disinfection of disease and insect pests in fresh fruit for many years (Armstrong, 1994; Couey, 1989). A hot water treatment at 49 °C for 20 min to control postharvest decay in papayas (*Carica papaya* L.) was used commercially in Hawaii for more than 30 years following fumigation with ethylene dibromide (Akamine and Arisumi, 1953) and is currently used in some packinghouses after vapor heat treatment. Hot water treatment of 30 min at 42 °C followed by 20 min at 49 °C (Couey and Hayes, 1986) or hot air treatments with 40% to 60% relative humidity at 48.5 °C for 3.5 h (Armstrong et al., 1995) have been used to disinfest Tephritid fruit flies from papayas. The role of heat in insect and fruit systems have been studied in thermal death kinetics of fruit flies (Jang, 1986; Moss and Chan, 1993) and in thermal inactivation kinetics of fruit ripening enzyme systems, i.e., polygalacturonase (Chan et al., 1981) and 1-aminocyclopropane-1-carboxylate oxidase (ACC oxidase) (Chan, 1986, 1991).

Numerical simulation models integrating thermal diffusion in papayas with temperature stress on a ripening enzyme and on survival of fruit flies have been reported (Laidlaw and Hayes, 1990). However, information on the thermal sensitivity of postharvest fungal spores

and hyphae is lacking (Coates and Johnson, 1993). For example, postharvest quarantine hot air and hot water treatments either reduce or enhance anthracnose (*Colletotrichum gloeosporioides*), *Guignardia* spot (*Guignardia* sp.), and internal yellowing (*Enterobacter cloacae*) diseases of papaya (Alvarez and Nishijima, 1987; Nishijima et al., 1987, 1992), but thermal death kinetics of the pathogens are unreported. This study reports the thermal death kinetics of some postharvest pathogens of papaya to provide insight into the role of heat treatments in control of postharvest diseases.

Materials and Methods

Fungal cultures. *Colletotrichum gloeosporioides* cultures were obtained and identified by K. Nishijima in 1986 and *Guignardia* sp. (*Phyllosticta caricaecola* Saw. anamorph) (papaya strain) (KN 7-14-88, IMI 366584) cultures were obtained in 1988, both from single conidia isolations from diseased papaya fruit collected on the Island of Hawaii. *Guignardia psidii* (guava strain) (KN 12-8-94, IMI 366585) cultures were obtained in 1994 from single conidia isolations from diseased guava (*Psidium guajava* L.) fruit collected in Hawaii (Island of Hawaii). *Guignardia* strains were identified by the International Mycological Institute Biosystematics Services (United Kingdom). All cultures were grown on potato dextrose agar (PDA; Difco Laboratories, Detroit) in continuous fluorescent light (Sylvania Daylight, 15W; Osram-Sylvania Co., Westfield, Ind.) at 24 °C for 12 to 14 days and stored as stock cultures in sterile distilled water (SDW) tubes.

Procedures. Thermal death studies were performed on *C. gloeosporioides* and two

strains of *Guignardia* spp. using the flask method of Hatcher et al. (1979). Conidia or mycelial plugs were removed from the surface of *C. gloeosporioides* or *Guignardia* cultures, respectively, and suspended in 15 mL SDW, and filtered through sterile stainless steel screens (30-µm pore size) to remove hyphae. Conidia concentrations were determined using a hemacytometer. For *Guignardia* spp., ascospores accounted for <5% of total spores. Spore suspensions were mixed and diluted to 10⁵ to 10⁶ spores/mL.

For the heat treatments, 36 mL of SDW (pH 5.3 to 5.5) contained in a 100-mL beaker was heated with a constant-temperature circulator (PolyScience, Niles, Ill.) in a water bath. A sterile stainless steel propeller was used to circulate the heated water in the beaker. When the desired temperature in the beaker was reached (43, 45, 46, 47, or 49 °C), 4 mL of the spore suspension was pipetted into the beaker and the timer set. Water bath temperatures were typically set at 0.7 to 1.0 °C higher than the desired temperatures in the beaker to attain the desired beaker temperatures. Beaker water temperature was monitored with thermocouples connected to a data logger (Omnicore Polycorder; Omnicore Intl., Logan, Utah). After a designated time interval (0 to 60 min for *C. gloeosporioides* or 0 to 180 min for *Guignardia* strain), 0.5 mL of heated spore suspension was withdrawn from the beaker and mixed into 1.5 mL of cool (8 to 10 °C) SDW in tubes held in a cold water bath. After the tube contents had reached <20 °C (2 to 3 min in cold water bath), the tube was removed and held at room temperature for viability assay.

Viability was assayed by mixing the contents of the test tube on a vortex mixer, withdrawing 0.2 mL from each tube of various time-temperature treatments, and pipetting onto PDA medium. The spores were spread over the agar surface with a sterile, bent glass rod and the agar plates were allowed to air-dry in the laminar airflow hood. Plates were inverted and incubated at 24 °C; germinated and nongerminated conidia were counted under a 20× objective of a light microscope (Bausch & Lomb, Rochester, N.Y.) after 18 to 24 h and again at 48 h to include “delayed” germination. Each temperature represented separate tests and each test was replicated at least twice. Percent germination was based on the mean of two counts of 100 spores each from plates incubated 48 h.

For the control sample, 1.0 mL of filtered spore inoculum was pipetted into 9 mL SDW and mixed with a vortex mixer. From this tube, 0.5 mL was pipetted and mixed into a tube containing 1.5 mL SDW precooled in cold water bath (8 to 10 °C). After ≈2 min, the tube in the cold water bath was removed and mixed, then 0.2 mL of the tube contents was pipetted onto a PDA plate and spread over the agar surface with a sterile bent glass rod. For *C. gloeosporioides*, “0” time (control sample) counts were only counted at 18 h because hyphae were overgrown after that period.

Thermal death kinetics for the fungi *Stemphylium lycopersici* (Glazner et al., 1984),

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Rhizopus stolonifer, and *Monilinia fructicola* (Smith and Blomquist, 1970) were calculated from the literature. Data for *M. fructicola* and *R. stolonifer* were limited to two or three data points; thus, the reliability and precision of these extracted data are uncertain. These studies were included in this report to illustrate the potential use of thermal death kinetics in determining the efficacy of vapor heat treatments (VHT) and hot water treatments for the control of fungal pathogens.

Bacterial cultures. The *Enterobacter cloacae* strains PV-5 and WT-2, obtained in 1986 (Nishijima et al., 1987) from diseased papaya fruits (PV-5) and hot water treatment tanks (WT-2) (collected before and after heat treatments), were used for thermal death studies. The cultures were maintained on yeast-dextrose-calcium carbonate agar (YDC) at 30 °C. A water bath (Haake E-2, Berlin, Germany) containing test tubes (12 × 75 mm) in a test tube rack were equilibrated to 45 or 49 °C. Temperatures were monitored with a YSI telethermometer (model 42 SC; Yellow Springs Instrument Co., Yellow Springs, Ohio) equipped with a temperature probe placed in a test tube containing 1.0 mL of SDW. Aliquots (0.1 mL) of a standard bacterial suspension [$\approx 10^8$ colony-forming units (cfu)/mL] prepared from a 24-h YDC culture were pipetted (in triplicate) into preheated tubes containing 0.9 mL SDW. After measured intervals of 0, 15, 30, 60, and 120 min, the tubes were removed from the bath and cooled in ice water for 30 sec to <20 °C before diluting and plating. Survival of bacterial populations were determined by counting the number of colonies visible on nutrient agar plates after 24 h incubation at 30 °C. Data were reported as percent survival relative to "0 time" (control) colony counts. Each test was done three times and consisted of running one bacterial strain at a single temperature.

Data calculations and equations. Procedures for calculations and equations that were applied in our kinetic studies are as follows:

For those organisms that followed logarithmic death, a first-order rate equation of

$$\frac{dN}{dt} = -kN \quad [1]$$

was applied. Rearranging and integrating Eq. [1] results in:

$$\int \frac{dN}{N} = -k \int dt \quad [2]$$

subsequently, integrating this equation results in:

$$\log N = -kt/2.303 + \log N_0 + \log C \quad [3]$$

When Eq. [2] is integrated between the limits, N_1 at t_1 and N_2 at t_2 , it results in:

$$\int_{N_1}^{N_2} \frac{dN}{N} = -k \int_{t_1}^{t_2} dt \quad [4]$$

in which N_1 = number of organisms at time t_1 , N_2 = number of organisms at t_2 . If $N_1 = N_0$ (initial number of organisms) at $t_1 = 0$, and N

= number of surviving organisms at t , the resulting integrated Eq. [4] results in the first-order rate equation (Kittsley, 1955; Stumbo, 1973):

$$\log N = -kt/2.303 + \log N_0 \quad \text{Eq. [5]}$$

The above equations were used in the following manner: Eq. [3] was applied to the thermal death data of all organisms to screen for fitness to a first-order logarithmic decay model using least squares fitting of the equation (Table Curve 2D V2.0; AISN Software; Jandel Scientific, San Rafael, Calif.), which also reported the R^2 values for each curve. The criterion employed was that N_0 must approximate the value 100 because survival data were expressed in percentages that would require $N_0 \approx 100$. When this criterion was met, then Eq. [5] was applied to calculate rate constants, k , and D -values. When N_0 values were several magnitudes >100, we concluded that the thermal death curves were nonlogarithmic, with potential for an extensive lag phase. However, in certain cases, we found that at >46 °C, the lag phase becomes minimal and thermal death kinetics approached the classical first-order models, in which case the first-order models were applied. However, at <46 °C, where extensive lag phases were evident, other equations, such as that of King et al. (1979) or Pruitt and Kamau (1993), which describe nonlogarithmic thermal death, were more appropriate and therefore applied.

The equation derived by King et al. (1979):

$$(\log N_0 - \log N)^a = kt + c \quad [6]$$

was used when there was sufficient data to calculate the nonlogarithmic thermal death rate constant, k , where N_0 and N are the original and surviving number of spores after heat treatment and t the time in minutes. The exponential constant a was determined from the reciprocal of the slope of a least squares best fit of the log survival [$\log (\log N_0 - \log N)$] against log time. The a value of the least severe treatment showing mortality of at least 10% was used to determine the subsequent c and k values at the more severe temperatures. The rate constant k was determined from the slope of the best fit line of a plot of $(\log N_0 - \log N)^a$ vs. time. Use of an iterative method further improved the value of the constants, resulting in a closer approximation to the data and a smaller variance (Jang, 1986). The a value determined by Jang's (1986) method was then used to calculate new c and k values for each temperature. Iterative fitting was done using Table Curve 2D V2.0 using a Levenburg-Marquardt algorithm for the least square fitting and reported R^2 values for fitted curves.

Decimal reduction time (D -values) represent the time required to attain 90% death at a certain temperature and is a convenient way of comparing heat resistance. D -values are calculated from the rate constant, k , where:

$$D = 2.303/k \quad [7]$$

and D is the time required for the survival curve to traverse one log cycle. For example, if 1- D represents 90% kill, then 2- D would be

99% kill, 3- D would be 99.9% kill (Stumbo, 1973).

In cases where the specific data points were not available, rate constants (k) were calculated using the Arrhenius equation (Kittsley, 1955):

$$E_a = \frac{2.303RT_1T_2}{(T_1 - T_2)} \log \frac{k_2}{k_1} \quad [8]$$

where E_a = energy of activation, $R = 8.314$ J-degree⁻¹·mol⁻¹, k_1 and k_2 are the rate constants at the absolute temperatures T_1 and T_2 , respectively.

Results and Discussion

Effects of time and temperature on the survival of *C. gloeosporioides* (Fig. 1A), *Guignardia* sp. (papaya) (Fig. 1B), and *G. psidii* (guava) (Fig. 1C) on semilogarithmic plots showed nonlinear kinetics for all three organisms. Therefore, a nonexponential model of death kinetics (Eq. [6]) was applied. Using the equation of King et al. (1979) the data produced high correlation coefficients (Table 1). From the thermal death rate constants, the energy of activation E_a was calculated (Table 1). E_a values for *C. gloeosporioides* and the two species of *Guignardia* were very close, indicating that similar death rates would be expected from increases in temperature. Based on the D -values (Table 1), the relative heat resistance was *G. psidii* > *Guignardia* sp. > *C. gloeosporioides*. Interestingly, the fungal pathogens with the highest heat resistance, the two strains of *Guignardia*, also required heat to activate germination, since for *Guignardia* sp. (papaya) (Fig. 1B) and *G. psidii* (guava) (Fig. 1C) a thermal activation phase was evident. Spore germination increased in the early stages of heating at 43 to 49 °C before thermal death set in. Thermal kinetics were calculated only for the death phases and not for the activation phases of *Guignardia*. The heat of activation for spore germination and heat resistance phenomena correlated with the observation that *Guignardia* rarely occur on papaya except when papaya are treated too long during quarantine heat treatments at 48 to 49 °C (Alvarez and Nishijima, 1987). Since the activation phenomenon complicates the interpretation of thermal death kinetics for *Guignardia*, other thermodynamic models need to be considered or developed.

For the survival of *R. stolonifer* and *M. fructicola*, Eqs. [3] and [5] were applied and the resulting survival curve for each respective organism was then analyzed for linearity ($N_0 \approx 100\%$) (Table 2). High N_0 values were obtained for: *Monilinia*-dormant and -germinated at 43 °C, and *Rhizopus*-dormant at 46 °C. However, as the temperature increased, linearity of survival increased for *R. stolonifer* and *M. fructicola*, in which case Eq. [3] was applied. Equation [3] also was more appropriate than Eq. [6] in the case of *Monilinia* and *Rhizopus* because of the meager data given. High D -values at 43 °C for *Monilinia* and at 46 °C for *Rhizopus*-dormant indicated that attempts to control postharvest decay at these

Fig. 1. Thermal death plots using King's equation (Eq. [6]) for (A) *Colletotrichum gloeosporioides*, (B) *Guignardia sp.* (papaya), and (C) *G. psidii* (guava) in distilled water at pH 5.3–5.5. Curve fitting for (B–C) *Guignardia sp.* and *G. psidii* was done only for the death phase and not for the activation phase. Results are the means of at least three tests in which percent germination in each test was based on the mean of two counts of 100 spores each from PDA plates incubated at 24 °C for 48 h.

temperatures could be problematic. Another organism that appears to be highly heat resistant is the bacterium *Enterobacter cloacae* (Zhu et al., 1986). *Enterobacter cloacae* causes internal yellowing and the infection site is in the internal seed cavity and surrounding tissue of papayas (Nishijima et al., 1987; Tang et al., 1972). Under the present quarantine (VHT) regime, the internal temperatures of papayas are required to reach 47.2 °C after which the fruit are hydrocooled (Armstrong et al., 1989). D-values for *E. cloacae* at 45 and 49 °C (Table 3) indicated potential survival of *E. cloacae* during the VHT, since the internal cavity of papayas was <60 min at the critical thermal death temperatures (i.e., >45 °C) (Armstrong et al., 1995).

In contrast to *E. cloacae*, most of the postharvest fungal pathogens, including *Rhizopus sp.*, were initially surface infectants exposed to the maximum severity (48.5 °C) of the quarantine temperature regime. *Rhizopus stolonifer* is one of the most destructive postharvest pathogens of papaya treated in hot water (Alvarez and Nishijima, 1987). The very long D-values of dormant spores of *R. stolonifer*, as calculated from data of Smith and Blomquist (1970) (>1400 min at 46 °C), were indicative of the heat resistance of these spores (Table 2) and may provide a rationale for the occasional ineffectiveness of heat treatments in controlling postharvest decay due to *R. stolonifer*. This heat resistance of dormant spores emphasized the importance of sanitation in the control of *Rhizopus* rot. Poor sanitation before quarantine treatment that allows the proliferation of inoculum, including dormant spores, could reduce the effectiveness of VHT and hot water quarantine treatments as a postharvest decay control method.

A comparison of the relative heat sensitivity for various life stages of the Oriental fruit fly expressed as 5-D (Jang, 1986), the dormant spores of *R. stolonifer*, the bacterial pathogen (*E. cloacae*) and the fruit ripening enzyme, ACC oxidase (Chan, 1986, 1991), expressed as 1-D-values (90% kill) is appropriate (Fig. 2). The 5-D values indicate that 99.999% is the expected kill range for Oriental fruit flies under probit 9 quarantine protocol. At 43 °C, the egg stage is more heat resistant than the first- and third-instar larval stages, while at 46 °C the larval stages are more heat resistant than the egg stages (Jang, 1986). The relationship between the heat sensitivity of the bacterial pathogen, *E. cloacae*, the fungal pathogen, *R. stolonifer*, and the disinfestation of larval stages of the Oriental fruit fly are important at 46 and 47 °C, which are typical temperatures encountered during quarantine VHT. At 46 °C and 40 min, death at 5-decimal reduction (5-D) is attained for third-instar larvae of Oriental fruit fly, while death at 1-D is attained for *E. cloacae*, PV-5, indicating a possible 10% survival of *E. cloacae*. The survival of *E. cloacae* (papaya strain PV-5) was confirmed by a packinghouse survey that reported up to 9.7% internal yellowing disease in hot-water-treated papayas and recovery of *E. cloacae* from hot water treatment tanks (water tank strain WT-2) (Nishijima et al., 1987). A 1-D value of

Table 1. Decimal reduction times (D-values), rate constants (k), and coefficients of determination (R^2), for *Colletotrichum gloeosporioides*, *Guignardia* sp. from papaya, and for *G. psidii* from guava, derived from King et al. (1979) where $(\log N_0 - \log N)^a = kt + C$, in which N_0 and N are the original and surviving number of spores and t = time in minutes and a = exponential constant.

Fungus	t (C)	k (min ⁻¹)	R^2	1-D (min)	3-D (min)	a^b	E_a^c (kJ·mol ⁻¹)
<i>C. gloeosporioides</i>	43	0.0155	0.98	63.8	77.8	0.1781	447
	45	0.0355	0.91	21.4	27.5		
	46	0.0721	0.93	11.2	14.2		
	47	0.1154	0.89	7.5	9.4		
	49	0.3620	0.91	2.9	3.5		
<i>Guignardia</i> sp.	43	0.0091	0.97	110	143	0.2385	394
	46	0.0491	0.98	20.4	26.5		
	47	0.0537	0.93	18.6	24.2		
	49	0.1504	0.98	6.6	8.6		
<i>G. psidii</i>	43	0.0047	0.94	213	244	0.1250	418
	46	0.0258	0.97	38.8	44.5		
	47	0.0248	0.98	40.3	46.3		
	49	0.1026	0.92	9.8	11.2		

^c E_a = energy of activation.

^b a = exponential constant.

Table 2. Thermal death constants (k), 1-D-values (min), and estimated original or percentage of population, N_0 at heating time, $t = 0$, calculated from published data for spores of various postharvest pathogens.

Temp (°C)	Organism	D-value (min)	k (min ⁻¹)	N_0 (%)
43	<i>Monilinia</i> ^a - dormant	1865 ^a	0.001	1250
	<i>Monilinia</i> - germinated	1436 ^a	0.002	8870
	<i>Rhizopus</i> ^a - germinated	0.58 ^w	3.97	82.1
46	<i>Stemphylium</i> ^y	17.3 ^w	0.133	74.4
	<i>Monilinia</i> - germinated	44.7 ^w	0.520	161.3
	<i>Rhizopus</i> - dormant	1422 ^a	0.002	5589
	<i>Rhizopus</i> - germinated	0.26 ^w	8.96	86.5
	<i>Stemphylium</i>	8.65 ^w	0.266	99.2
48	<i>Stemphylium</i>	8.65 ^w	0.266	99.2
49	<i>Monilinia</i> - dormant	1.73 ^w	1.33	109
	<i>Rhizopus</i> - dormant	2.39 ^w	0.96	101
	<i>Rhizopus</i> - germinated	0.33 ^w	7.03	90.9
50	<i>Stemphylium</i>	8.46 ^w	0.272	99.9
52	<i>Monilinia</i> - dormant	0.84 ^w	2.74	97.3
	<i>Rhizopus</i> - dormant	19.05 ^w	0.120	365
	<i>Rhizopus</i> - germinated	0.40 ^w	5.73	94.4
54	<i>Stemphylium</i>	0.58 ^w	3.98	99.4

^aData for *Monilinia fructicola* and *Rhizopus stolonifer* from Smith and Blomquist (1970).

^yData for *Stemphylium lycopersici* from Glazener et al. (1984).

^wD-values were derived from the formula, $\log N = -kt/2.303 + \log N_0 + \log C$, based on the large N_0 value following nonlinear death.

^vD-values were derived from k, $\log N = -kt/2.303 + \log N_0$.

1422 min at 46 °C and 600 min at 47 °C for dormant spores of the fungal pathogen, *R. stolonifer*, indicates potential survival of this pathogen during and after postharvest vapor-heat treatments since most treatments range from 270 to 360 min (Armstrong, 1994).

The long D-values for dormant spores of *R. stolonifer* indicate that any efforts to completely eradicate this pathogen with more severe heat treatments would disrupt normal fruit ripening by reducing ACC oxidase activity, a fruit-ripening enzyme (Chan, 1986). Thus, alternative postharvest decay control strategies (such as fungicides or biological control) for *Rhizopus* rot may be necessary.

In the case of the nonlogarithmic thermal death curves due to the existence of an extensive lag phase, especially at <46 °C, effective kill did not begin until the lag phase was complete. The death rate increased dramatically at higher temperatures where the lag phase of death was shorter (Fig. 1). Thus, heat treatment regimes programmed by temperature ramping or profiling should be designed to incorporate control of postharvest patho-

gens and disinfestation of insects while maintaining fruit quality.

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Table 3. 1-D-values (min) and thermal death rate constant (k) derived from the first-order kinetic equation (Eq. [5]), $\log N = -kt/2.303 + \log N_0$, where N_0 and N are the original and surviving numbers, respectively, of two strains of the internal yellowing bacterium, *Enterobacter cloacae*, strain PV-5 from papaya fruit and strain WT-2 from hot water treatment tanks.

Temp (°C)	Strain	D-value (min)	k (min ⁻¹)
45	PV-5	63.56	0.036
	WT-2	35.30	0.065
46 ^a	PV-5	40.93	0.056
	WT-2	26.92	0.086
47.2 ^a	PV-5	24.14	0.095
	WT-2	19.45	0.118
49	PV-5	10.93	0.211
	WT-2	11.94	0.193

^aD and k values for these temperatures were calculated from an Arrhenius plot using the energy of activation, E_a , derived from thermal death data at 45 and 49 °C.

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Fig. 2. Effects of temperature on 5-D values of Oriental fruit fly eggs; first and third instar larvae (Jang, 1986); and 1-D values for papaya ACC oxidase (EFE) (Chan, 1991), *Rhizopus stolonifer* dormant spores (Smith and Blomquist, 1970), and *Enterobacter cloacae*, PV-5.

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