Modeling the Survival of Two Soilborne Pathogens Under Dry Structural Solarization

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ABSTRACT

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Structural (space) solarization of a closed, empty greenhouse for sanitation involves dry heating to 60°C and higher and low relative humidity (RH), under a fluctuating temperature and RH regime. Survival of inocula of *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *Sclerotium rolfsii* during structural solarization was studied for 4 years (total of 12 experiments) in an attempt to develop a dynamic model for expressing the thermal inactivation of the pathogens. After 20 days of exposure, the populations of *F. oxysporum* f. sp. *radicis-lycopersici* and *S. rolfsii* were reduced by 69 to 95% and by 47.5 to 100%, respectively. The Weibull distribution model was applied to describe pathogen survival. The Weibull rate parameter, *b*, was found to follow an exponential (for *F. oxysporum*

f. sp. *radicis-lycopersici*) and the Fermi (for *S. rolfsii*) functions at constant temperatures. To improve the applicability of the model, fluctuating conditions of both temperature and RH were utilized. The Weibull distribution derivative, expressed as a function of temperature and moisture, was numerically integrated to estimate survival of inocula exposed to structural solarization. Deviations between experimental and calculated values derived from the model were quite small and the coefficient of determination (R^2) values ranged from 0.83 to 0.99 in 9 of 12 experiments, indicating that ambient RH data should be considered. Structural solarization for sanitation could be a viable component in integrated pest management programs.

Additional keywords: fluctuating climatic conditions, greenhouse sanitation, inoculum eradication.

Many soilborne plant pathogens survive between seasons, either in the soil or above-ground, by means of resting structures, such as propagules that are either free or embedded in infected plant debris. Sanitation aims to reduce or eliminate the pest population from all sources, thus breaking the continuity of survival in time and space between crops (11,29,30). Soil disinfestation (e.g., by fumigation or solarization) in greenhouses before planting is a routine procedure. However, this practice does not eliminate the inoculum remaining from the previous season, which adheres to the greenhouse structure (e.g., walls and poles), and could become a source for reinfestation of the disinfested soil (14,21). Therefore, structural sanitation of the greenhouse, e.g., by physical or chemical means, has to be carried out as a complementary management tool (11). Greenhouse structural (space) solarization is a thermal, nonchemical approach to structural sanitation and is carried out by closing the greenhouse for a certain period of time during the appropriate season. As a consequence, the temperature is raised to high levels in an attempt to control pathogens persisting on the greenhouse structure or even in the soil (28). This is a modification of an approach previously developed in Japan (22) and northern Italy (15) for solarizing soils in cooler regions, and in Morocco for sanitizing tomato support stakes to prevent *Didymella lycopersici* transmission (2).

The climatic conditions prevailing during structural solarization differ markedly from those of soil solarization. In the former, the temperatures are higher (60°C or above), and the relative humidity (RH) is very low (28,44), whereas in soil solarization, the soil is

kept wet throughout the process (20). Therefore, structural solarization involves a dry heating process which is less effective at thermal inactivation of pathogens than wet heating. Moreover, in contrast to wet heating, studies on microorganism survival under dry heat conditions are scarce.

Studies of thermal inactivation of pathogens utilize data collected under constant elevated temperatures. Based on chemical reaction kinetics at constant temperatures, an exponential inactivation relationship is typically expected (49). Traditionally, thermal inactivation of microorganisms is considered a first-order reaction, characterized by a logarithmic change in the organism population with time (12). A logarithmic relationship was found between time and temperature at constant temperatures for thermal inactivation of four soilborne plant pathogens (35). Studies on thermal inactivation under fluctuating temperatures, such as those naturally prevailing in greenhouses, are much more complicated to perform, because the partial effects of varying temperatures on pathogens are difficult to weigh and mandate numerical integration to account for their complexity.

Pathogen propagules are exposed during solarization to a diurnal, fluctuating temperature regime. Various mathematical models based on ambient climatic data have been developed to simulate heating of solarized soils and closed greenhouses (4,28,53). However, models based on biological-physical principles for simulating pathogen survival during the course of soil solarization are rare, although empirical approaches such as level of mortality versus accumulated hours above a certain temperature (degree-hours [DH]) have been used with varying degrees of success. This approach is often adopted for calculating life cycles of insects (24). It is also used for thermotherapy of propagation material (51) and soil solarization. A set of empirical regression equations of temperature-time showed complete inactivation, during soil solarization,

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of *Botrytis cinerea* sclerotia after the accumulation of 34.5 DH above 38°C (27). However, in studies that were carried out in northern Florida, the DH concept was valid only as a general indication of inactivation (5).

Simulation of survival under fluctuating temperature conditions, using constant temperature survival kinetics, was carried out for *Xanthomonas campestris* pv. *campestris* (41), for food-contaminating microorganisms (12,23), and for thermal inactivation of *Heterodera carotae* (43) and *Phytophthora nicotianae* during soil solarization (7). In cases in which constant temperature inactivation curves did not follow simple exponential behavior, different relationships were suggested for the simulation under constant and fluctuating temperatures. For instance, a model based on a modified Gompertz equation was developed for analyzing nonlinear thermal survival of *Listeria monocytogenes* (25). A different approach, suggested recently, is based on the fundamental consideration that the survival curve should not be treated in kinetics terms at all. Instead, it should be a cumulative form of the temporal distribution of lethal events (32). These, in turn, represent the spectrum of resistances to thermal inactivation within the treated population. One of the most flexible and convenient functions to describe the nonlinear nature of microorganism survival is the Weibull distribution (52), which is based on the assumption that it reflects lethal events. A model based on the Weibull probabilistic distribution was demonstrated to simulate the survival of *L. monocytogenes* and *Clostridium botulinum* exposed to heating at different rates, heating and cooling cycles, oscillating temperature, and exposure to a dissipating chemical agent (31–33). This model fits both constant and fluctuating temperature regimes (50). However, structural solarization is a process in which both temperature and RH fluctuate, and an appropriate approach describing these conditions has yet to be developed.

The soilborne plant pathogen *Fusarium oxysporum* f. sp. *radicis-lycopersici* W.R. Jarvis & Shoemaker, causes Fusarium crown and root rot in tomato. This pathogen produces chlamydospores in the soil and macroconidia on the stems, which are air-borne and can be disseminated, contaminating the greenhouse structure (38,40). Hence, structural solarization is essential when such pathogens are involved.

The pathogen *Sclerotium rolfsii* Sacc. (teleomorph: *Athelia rolfsii* (Curzi) Tu & Kimbrough) is a soilborne fungus that causes the southern blight disease in many crops (36). This pathogen survives in the absence of a host by means of sclerotia which are multicellular structures.

The objectives of this work were to (i) study the survival of *F. oxysporum* f. sp. *radicis-lycopersici* and *S. rolfsii* during structural solarization, (ii) study the pathogens' survival under constant temperatures and moisture, and (iii) develop a model describing thermal inactivation of the pathogens under the dynamic climatic conditions prevailing during structural solarization (namely, fluctuating temperature and RH). Brief reports have been published previously (44,45).

MATERIALS AND METHODS

Media. Potato dextrose agar (PDA) was used to isolate fungi from diseased plants (46). A modified *Fusarium*-selective medium (FSM) (14) was utilized for enumeration of *Fusarium* propagules. Czapek-Dox medium (CDM) (46) was used to grow inoculum for pathogenicity tests. Water agar (0.1% agar) supplemented with 0.1% MgSO₄·7H₂O was used for suspending soil samples for soil dilution. Filter paper moistened with bromocresol green was utilized for viability of *S. rolfsii* sclerotia (13).

Soils. A natural agricultural sandy soil from Kikar Sedom Experimental Station (Ein Tamar, Israel) was used for *F. oxysporum* f. sp. *radicis-lycopersici* studies. It consisted of 0.1% (wt/wt) organic matter, 93.9% sand, 4% silt, and 2% clay. The moisture content of the air-dried soil was 0.6% (wt/wt). Acid-washed quartz

sand (Sigma Chemical, St. Louis) was used for *S. rolfsii* studies. The moisture content of the air-dried sand was 0.6%.

Inocula. Soil samples from fields with diseased tomato plants naturally infected with *F. oxysporum* f. sp. *radicis-lycopersici* were taken from Kikar Sedom Experimental Station. Soil samples air dried at ambient temperature served as a natural inoculum consisting of chlamydospores, as verified microscopically, and were stored at 5°C until use. *S. rolfsii* was initially isolated from diseased bean seedlings. Sclerotia were obtained from a culture of the pathogen grown on PDA at 27°C for 30 days. Sclerotia were collected after being air dried at room temperature and tested for their viability by plating on PDA prior to each treatment. Only batches of sclerotia that yielded ≥90% viability were used. Fifteen sclerotia were mixed with acid-washed quartz sand and packed into a nylon net bag (3 g) or a microtube (2 g) before being exposed to heating treatments.

Greenhouse experiments. Experiments were performed at an experimental greenhouse (The Hebrew University Experimental Farm, Rehovot, Israel) during the hot season (May to October) from 1995 to 1998. Seven experiments were performed with *F. oxysporum* f. sp. *radicis-lycopersici* and five with *S. rolfsii*. Structural solarization was performed by closing the greenhouse during the indicated period. Its effectiveness in pathogen control was assessed by exposing nylon net bags containing the inoculum (3 g, an amount that enables a rapid adjustment to the greenhouse climatic conditions) in four replications for each treatment. The bags were hung in the evening with strings on horizontal metal supports 1 m from the ground, a height that was found to represent the greenhouse climatic conditions in the middle of the closed greenhouse space. Bags were hung in the evening to enable the inoculum in the bag to equilibrate with the greenhouse atmosphere before being exposed to heating. At the indicated intervals, the inoculum bags were removed in the morning (0700 h) and brought to the laboratory to assess inoculum viability. Therefore, 12 h was considered for adjustment.

Enumeration of pathogen propagules. The density of propagules in *F. oxysporum* f. sp. *radicis-lycopersici*-infested soils was assayed by the soil-dilution method (14). Samples (0.2 ml) of the appropriate dilutions were spread on each of five petri dishes containing FSM. Dishes were incubated in the dark at 28°C for 4 to 6 days. Results were expressed as colony forming units per gram of dry soil. Percent survival was calculated by comparing populations of treated versus untreated inoculum. Inoculum density in the various inoculum batches used ranged between 1,500 and 4,000 CFU/g. Colonies of *F. oxysporum* growing on FSM were randomly transferred to CDM and assessed for pathogenicity as previously described (21) to verify their identity as *F. oxysporum* f. sp. *radicis-lycopersici*. Sclerotia of *S. rolfsii* were separated from the soil by sieving and tested for viability: 15 sclerotia were placed on a filter paper moistened with bromocresol green in each petri dish, and tested for germination over 96 h (13).

Meteorological data. Temperature and RH were recorded continuously by a micrologger (±0.05% of voltage measurements, 21X, Campbell Scientific Inc., Logan, UT). Data were recorded once a minute and were averaged every 10 min. Temperature was measured with T-type thermocouples (±0.5°C, copper-constantan probes), which were either inserted into the center of the inoculum bags in the soil or in an aerated meteorological shelter to prevent heating by radiation. DH values above 50°C were calculated by the trapezoid method. RH data were measured with an electronic hygrometer (±1.5% RH, I-100, Rotronic, Bassersdorf, Switzerland).

Constant temperature heating experiments. Air-dried soil inoculum samples (2 g) of both pathogens were sealed in sterile screw-cap (2 ml) microtubes (Sarstedt, Nümbrecht, Germany) to prevent inoculum wetting, thus ensuring dry heating. Samples were immersed in a water bath in which temperatures were regulated by a thermostat (±0.1°C; Fried Electric, Haifa, Israel) and

measured with copper-constantan probes connected to a micrologger. The temperature of the inoculum in the microtubes reached the desired level within 3 min. Survival studies were conducted at 44.0, 48.0, 52.5, 55.0, 57.5, 60.0, 62.5, and 65.0°C. At each temperature, seven to eight time exposures were tested in four replications. Viability was assessed as described previously, and the survival fraction (percent survival divided by 100) and standard errors were calculated. The number of hours required to achieve 50 and 80% mortality (ED₅₀ and ED₈₀ values, respectively) was calculated from the survival curves of each constant temperature by reading the time corresponding to 50 and 20% survival points, respectively. Every constant temperature experiment was repeated once, and the results of the two experiments were averaged. These studies were conducted to collect kinetic data under constant conditions.

To compare the effect of wet versus dry heat, samples of wetted (to 80% field capacity of the soil) and air-dried inocula of *F. oxysporum* f. sp. *radicis-lycopersici* were exposed for 1 h to 44 and 55°C, respectively. In parallel, samples of wetted and air-dried inocula of *S. rolfii* were exposed for 1 h to 43 and 50°C, respectively.

Momentary moisture. To derive the momentary moisture content from the ambient conditions of RH and temperature, the sorption isotherms (37) of inoculum samples were experimentally measured. Although the effect of temperature on the sorption isotherm is quite small, three representative temperatures (25, 40, and 50°C) were chosen. Air-dried soil samples were placed in small beakers (six replications) and exposed to five levels of water activity ($a_w = \text{RH}/100$) in tightly sealed jars. Known water activities were achieved by using saturated solutions of LiCl, MgCl₂, Mg(NO₃)₂, NaCl, and K₂SO₄ (37). Apparent equilibrium was reached after 3 weeks, and moisture content of the samples was determined by oven-drying (105°C for 24 h).

To express the effect of momentary temperature and RH, the Guggenheim-Anderson-de Boer (GAB) equation (37) was used:

$$m = \frac{m_0 K_c a_w c e^{\frac{E_a}{T}}}{(1 - K_c a_w) \left(1 - K_c a_w + K_c a_w c e^{\frac{E_a}{T}} \right)} \quad (1)$$

where m is the moisture content of the inoculum (g of H₂O per 100 g of dry soil); $a_w = \text{RH}/100$; E_a is the energy of activation (kcal/g mole K); T is the temperature (Kelvin); and m_0 , c , and K_c are constants.

The values of m_0 , c , K_c , and E_a were derived from all the experimental data measured at the three chosen temperatures, using a nonlinear regression. Once the values of m_0 , c , K_c , and E_a were established, equation 1 could be utilized to calculate the momentary moisture content according to the prevailing environmental conditions (temperature and RH) in the greenhouse. This approach assumes that environmental changes in the greenhouse are quite smooth and moderate.

Weibull model. Changes in propagule population survival ($d\phi$) over time (dt) with exposure to high temperature for a duration of time, $T(t)$, was described by the Weibull distribution (32,33) as

$$\frac{d\phi}{dt} = b n t^{n-1} e^{-b t^n} \quad (2)$$

where b and n are the Weibull rate and shape parameters, respectively.

The basic assumptions of the model are that the temperature effect is additive and that there is no growth or recuperation of injured cells under dry conditions. The cumulative form of the Weibull distribution is described by the equation

$$S(t) = e^{-b t^n} \quad (3)$$

where $S(t)$ is survival as a function of time.

Alternatively, it could be presented as a semilogarithmic relationship:

$$\text{LS} = \log_e[S(t)] = -b t^n \quad (4)$$

Discussions and a justification of the Weibull distribution model's applicability, as well as its usefulness in numerous cases, have been recently published (31,32,50). The shape of the distribution curve is governed by the values of n and b (equation 4). For example, when n is <1 (the distribution is skewed to the right), the logarithmic survival curve has a noticeable upward concavity. When n is >1 (the distribution is skewed to the left), the logarithmic survival curve is concave downward. Only when $n = 1$ is the logarithmic survival curve linear (i.e., apparent first-order). The exact nature of the underlying distribution is usually unknown and cannot be assumed a priori (19,32).

Because the conditions in the greenhouse experiments are not constant, both the temperature, $T(t)$, and the sample's moisture content, $m(t)$, change continuously as a function of time. Hence, to develop a mathematical model describing microorganism survival, experiments with constant temperature and moisture content were initially carried out. The rationale for using constant conditions was based mainly on the need to establish a relationship between the Weibull rate parameter, b , and $T(t)$ when m was kept fixed. Once the temperature relationship was established, the model could be expanded to include the effect of the variable moisture content, as has been demonstrated in similar studies (17,18,23,41,43).

Fig. 1. Survival of two soilborne pathogens over time at a constant temperature of 57.5°C: **A**, *Fusarium oxysporum* f. sp. *radicis-lycopersici* and **B**, *Sclerotium rolfii*. Bars represent $\pm$ SE.

Model and parameter estimations. Figure 1A and B depict typical survival curves for *F. oxysporum* f. sp. *radicis-lycopersici* and *S. rolfisii*, respectively, exposed to a constant heating temperature of 57.5°C. Experiments were also conducted at seven other constant temperatures (44 to 65°C, as indicated earlier). The survival data, $S(t)$, at each specific time, t , and temperature, T , were then utilized to establish the effect of temperature on the value of the Weibull rate constant, b , while keeping moisture content, $m(t)$, constant:

$$b = b[T(t)] \quad (5)$$

Rewriting equation 4 yields

$$LS = -b[T(t)] t^{F[T(t)]} \quad (6)$$

where both $b[T(t)]$ and $F[T(t)]$ are functions describing the effects of temperature that need to be derived. This model takes into consideration that n is affected only by the external temperature.

For *F. oxysporum* f. sp. *radicis-lycopersici*, the Fermi equation (32) was initially applied for estimating $b[T(t)]$. However, due to the large error of the residuals, it was found unsatisfactory. Lower mean error squares with normal distribution of the residuals were achieved when an exponential model was applied:

$$b = k_{\text{ref}} e^{-E \left[\frac{1}{T(t)} - \frac{1}{T_{\text{ref}}} \right]} \quad (7)$$

and

$$F[T(t)] = C_1 + \frac{T(t)}{C_2} \quad (8)$$

where k_{ref} , E , T_{ref} , C_1 , and C_2 are constants.

Equation 7 can be considered identical to the Arrhenius expression. In this case, T_{ref} is a reference temperature, E is the equivalent of the energy of activation divided by the universal gas constant, and k_{ref} is the rate constant at T_{ref} , an arbitrary temperature

typically chosen with the range of the temperature at which the experiment is carried out. This approach does not suggest that b follows the Arrhenius equation. It is based on data fitting an exponential relationship that could describe the survival curve as a function of time and temperature. Many other functions could probably be used.

For *S. rolfisii*, the Fermi equation (32) was applied for estimating the dependence of b on the temperature ($b[T(t)]$), expressed as

$$b = \frac{1}{1 + \exp \left(P - \frac{T(t)}{X} \right)} \quad (9)$$

$F[T(t)]$ was expressed as in equation 8, where P , X , C_1 , and C_2 are constants and $T(t)$ is given in degrees Celsius.

Once the dependence of b on temperature was established, equation 6 was rewritten to express the effect of moisture as

$$LS = -b[T(t)] t^{\{f[m(t)]\} \{F[T(t)]\}} \quad (10)$$

where $f[m(t)]$ expresses the effect of moisture, which like the temperature may change over time. The actual function $f[m(t)]$ needs to be derived from another set of experiments. We used the data collected during the greenhouse solarization to establish this function. This approach makes the fundamental assumption that moisture affects only the value of the Weibull shape parameter, n . Thus, n depends on external moisture conditions and augments the history of the process. Following numerous trials, the best results were obtained when a quadratic model was applied:

$$f[m(t)] = P_1 m(t) + P_2 m^2(t) + P_3 \quad (11)$$

where P_1 , P_2 , and P_3 are parameters.

Once the values of the parameters are derived, the Weibull distribution model is explicit and can be utilized for the survival experiments.

Fig. 2. A flow-chart describing the numerical algorithm used to estimate the Weibull parameters.

To quantify the values of the parameters P_1 through P_3 , the model was rewritten:

$$LS = -b[T(t)]t \left[C_1 + \frac{T(t)}{C_2} \right] [P_1 m(t) + P_2 m^2(t) + P_3] \quad (12)$$

The analytical derivative of equation 12 with respect to time, taking into consideration that both temperature and moisture are a function of time, is

$$\frac{d}{dt} \left(-b[T(t)]t \left[C_1 + \frac{T(t)}{C_2} \right] [P_1 m(t) + P_2 m^2(t) + P_3] \right) \quad (13)$$

The derivation of equation 13 was carried out with MathCAD 7 Student version (MathSoft Inc., Cambridge, MA) and was used as the model for the numerical integration. Utilizing the derivative (equation 13) rather than the function itself (equation 12) requires smooth conditions of temperature and moisture and elimination of sudden but erratic fluctuations of temperature and RH curves during the day. This was achieved by smoothing the data before the derivative was calculated. Smoothing was achieved by presenting data in an Excel worksheet and calculating every data point as an average of 10 time points—five points forward and five points backward. Derivation was carried out from $t = 0$ to $t = t_F$ (end of the process), while in each step the experimental data values of $T(t)$ and $RH(t)$ (converted to $m(t)$ via the GAB equation) were utilized. The parameters of the kinetic model were estimated by the Complex method (42) and utilized a fourth-order Runge-Kutta numerical integration with a variable time step (initially set to 0.5 min). The tolerance for the numerical integration was set to 10^{-5} .

Fig. 3. Typical temperature and relative humidity (RH) data during structural solarization.

The objective function for the minimization was defined by the minimum sum of squares:

$$SSQ = \text{Minimum} \sum_{i=1}^m (LS_c - LS_e)^2 \quad (14)$$

where SSQ is the sum of squares, m is the number of data points in the greenhouse experiment, LS_c is the calculated survival at each interval, and LS_e is the actual measured survival.

A schematic presentation of the numerical algorithm used to estimate the parameters P_1 , P_2 , and P_3 is presented in Figure 2. To begin the numerical process, initial values of the parameters and their limits were guessed. The values were improved via the Complex method until no further reduction in the objective function was feasible. For the three parameters estimated, the objective function was evaluated at seven mutually equidistant points, which are the vertices of the simplex, in the space created by the three independent variables (namely P_1 , P_2 , and P_3). A projection was made from the point that yields the highest objective function value through the center of the remaining points. Iterations continued until convergence was reached. The decision to terminate the iterations was based on two criteria: (i) the relative changes in the objective function value, and (ii) the independent variable values during two consecutive iterations (42). Both convergence criteria values were set to 10^{-4} .

Statistical analyses. Greenhouse and laboratory experiments were repeated at least once. Variances between experimental trials were small, and thus, average results were calculated. Analyses included analysis of variance, linear and nonlinear regression, Student's t test, and calculations of standard error. Significance of main effects and interactions were determined by the F test, and the differences between means were detected using Duncan's new multiple range test. SAS program (Release 6.04 for PC and 6.12 for Windows; SAS Institute, Cary, NC) was used for all statistical analyses, except the nonlinear regressions (Systat version 7.1 for Windows; SPSS Inc., Chicago).

RESULTS

Survival of pathogens under structural solarization: greenhouse studies. Seven greenhouse experiments were performed with *F. oxysporum* f. sp. *radicis-lycopersici* and five with *S. rolfssii* over 4 years, from May to October. In all experiments, the maximal temperatures were reached between 1300 and 1400 h and frequently exceeded 60°C, while RH declined to $\approx 15\%$ (Fig. 3). This system therefore involved dry heating. At night, the temperature decreased to 22 or 23°C and the corresponding RH reached approximately 80%. The heating intensity, expressed as the index of DH above 50°C (DH > 50°C, Table 1), varied among experiments and depended on the time period. The DH values of experiments 1, 3, 4, 5, 6, 8, 10, and 11, which were performed during the sum-

TABLE 1. Characterization of structural solarization experiments for the control of *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *Sclerotium rolfssii*

Experiment	Year (month)	Pathogen	DH > 50°C ^a	% Survival (after 372 h) ^b	% Survival (after 492 h) ^c
1	1995 (6,7)	<i>F. oxysporum</i> f. sp. <i>radicis-lycopersici</i>	859	28.0 ± 3.2	20.0 ± 4.9
2	1995 (9,10)	<i>F. oxysporum</i> f. sp. <i>radicis-lycopersici</i>	450	42.0 ± 2.5	31.0 ± 4.1
3	1996 (7,8)	<i>F. oxysporum</i> f. sp. <i>radicis-lycopersici</i>	779	30.0 ± 1.2	14.2 ± 2.7
4	1996 (8,9)	<i>F. oxysporum</i> f. sp. <i>radicis-lycopersici</i>	1,213	17.5 ± 0.7	9.8 ± 2.1
5	1997 (7)	<i>F. oxysporum</i> f. sp. <i>radicis-lycopersici</i>	623	8.6 ± 0.5	5.5 ± 0.9
6	1997 (9)	<i>F. oxysporum</i> f. sp. <i>radicis-lycopersici</i>	766	23.0 ± 1.9	17.3 ± 1.0
7	1998 (9,10)	<i>F. oxysporum</i> f. sp. <i>radicis-lycopersici</i>	345	25.1 ± 1.1	21.1 ± 0.7
8	1995 (6,7)	<i>S. rolfssii</i>	800	41.2 ± 5.3	0
9	1995 (9,10)	<i>S. rolfssii</i>	423	87.1 ± 3.1	62.5 ± 3.2
10	1996 (6,7)	<i>S. rolfssii</i>	600	82.7 ± 4.6	3.2 ± 0.3
11	1996 (7)	<i>S. rolfssii</i>	750	63.5 ± 1.7	0
12	1998 (9,10)	<i>S. rolfssii</i>	345	71.3 ± 2.1	42.0 ± 3.7

^a Degree-hours above 50°C after 492 h.

^b Refers to 15 days + 12 h of adjustment (±SE).

^c Refers to 20 days + 12 h of adjustment (±SE).

mer (July to September), were above 600. In comparison, the DH values of experiments 2, 7, 9, and 12, carried out toward the end of the summer (September and October), ranged from 345 to 450.

The population of both pathogens exposed to structural solarization declined to various levels according the relevant temperature regimes (Table 1; Figs. 4 and 5). The population in experiments 2 and 7 (*F. oxysporum* f. sp. *radicis-lycopersici*), as well as in experiments 9 and 12 (*S. rolfsii*), in which the heating intensity was lower (Table 1), exhibited a relatively smaller decrease. In all experiments, the survival curves followed a typical pattern: a rapid

decline in survival during the first 3 to 8 days (72 to 192 h), to about 50%, followed by a slower rate of decline (Figs. 4 and 5).

Effect of constant heating regimes on survival. Air-dried inoculum samples of each pathogen were exposed to dry heating at controlled constant temperatures, and the survival curves were plotted for each temperature. The patterns of the curves under constant temperatures (Fig. 1A and B) were quite similar to those described previously for the greenhouse experiments (Figs. 4 and 5), namely, an initial rapid thermal inactivation followed by a slower rate. In the highest temperature range, 62.5 to 65°C, only

Fig. 4. Effects of structural solarization on survival of *Fusarium oxysporum* f. sp. *radicis-lycopersici* in seven greenhouse experiments (experiments 1 to 7). The Weibull model was utilized to describe the decline in survival over time ($\diamond$ represents experimental data; line represents model). R^2 (coefficient of determination), RMS (residual mean square between experimental and calculated values), and calculated/experimental (CE) values after 228 (CE 228) and 492 h (CE 492) are given for each experiment.

the rapid decline was observed (data not shown). Survival data were related to temperature and exposure duration, as reflected by ED₅₀ and ED₈₀ values (Table 2). Calculating the *b* values (the Weibull rate parameter) of *F. oxysporum* f. sp. *radicis-lycopersici* for the eight temperatures tested revealed increasing values with

temperature: 8.61×10^{-3} and $2.62 \times 10^0 \text{ h}^{-n}$ (*n* is the Weibull shape parameter) at 44 and 65°C, respectively (Table 2; Fig. 6). These results showed that survival data under constant temperatures can be expressed by a function that resembles the Arrhenius relationship. The exponential equation parameters *E* and *k*_{ref} (equation 7)

Fig. 5. Effects of structural solarization on survival of *Sclerotium rolfsii* in five greenhouse experiments (experiments 8 to 12). The Weibull model was utilized to describe the decline in survival over time (◇ represents experimental data; line represents model). *R*² (coefficient of determination), RMS (residual mean square between experimental and calculated values), and calculated/experimental (CE) values after 228 (CE 228) and 492 h (CE 492) are given for each experiment.

TABLE 2. Evaluation of survival data (ED₅₀ and ED₈₀)^a and the Weibull rate parameter, *b*, after dry heating of *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *Sclerotium rolfsii* at constant temperatures

Temperature (°C)	<i>F. oxysporum</i> f. sp. <i>radicis-lycopersici</i>				<i>S. rolfsii</i>			
	ED ₅₀ (h)	ED ₈₀ (h)	<i>b</i> value ^b (h ⁻ⁿ) ^c	<i>R</i> ^{2d}	ED ₅₀ (h)	ED ₈₀ (h)	<i>b</i> value ^c (h ⁻ⁿ) ^c	<i>R</i> ^{2d}
44.0	73.0	202.0	8.61×10^{-3}	0.97	100.3	115.5	1.68×10^{-7}	0.98
48.0	31.2	104.0	1.45×10^{-2}	0.95	48.7	68.1	2.22×10^{-6}	0.97
52.5	14.2	37.0	4.58×10^{-2}	0.96	33.3	39.2	4.05×10^{-5}	0.95
55.0	1.9	8.2	1.82×10^{-1}	0.94	21.3	26.5	2.03×10^{-4}	0.93
57.5	1.7	6.2	2.15×10^{-1}	0.95	12.0	16.4	1.02×10^{-3}	0.96
60.0	0.9	2.4	6.33×10^{-1}	0.97	8.7	10.5	5.09×10^{-3}	0.95
62.5	0.5	1.7	1.11×10^0	0.95	4.1	4.9	2.50×10^{-2}	0.97
65.0	0.3	0.7	2.62×10^0	0.98	1.7	3.2	1.14×10^{-1}	0.94

^a Number of hours required to achieve 50 and 80% mortality, respectively.

^b Calculated by the exponential model (equation 7).

^c *n* is the Weibull shape parameter.

^d Coefficient of determination. All coefficients are significant (*P* < 0.01).

^e Calculated by the Fermi equation (equation 9).

were estimated by plotting $\ln(b)$ versus $1/T - 1/T_{\text{ref}}$ (Fig. 6). These values were estimated for $T_{\text{ref}} = 70^\circ\text{C}$ (342 K): $E = 30,387$ K, $k_{\text{ref}} = 68.12 \text{ h}^{-n}$, and $R^2 = 0.98$. Analyzing b values for *S. rolfisii* by the Fermi equation (equation 9) revealed increasing values with temperature: 1.68×10^{-7} and $1.14 \times 10^{-1} (\text{h}^{-n})$ at 44 and 65°C , respectively (Fig. 7; Table 2). The derived Fermi equation parameters were $P = 43.99$ and $X = 1.55^\circ\text{C}$. Utilizing these values for both pathogens resulted in a coefficient of determination (R^2) higher than 0.93 for all temperatures tested.

The survival of inocula of *F. oxysporum* f. sp. *radicis-lycopersici* exposed for 1 h to dry heat at 55°C and wet heat at 44°C was 100 and 76.2%, respectively. The survival of *S. rolfisii* inocula exposed for 1 h to dry heat at 50°C and wet heat at 43°C was 77.6 and 29.5%, respectively. These results demonstrate the lower effectiveness of dry heat in pathogen control.

Fluctuating-temperature-based studies. The Weibull model was utilized to describe *F. oxysporum* f. sp. *radicis-lycopersici* and *S. rolfisii* survival under fluctuating temperatures, because plotting semilogarithmic survival versus exposure time showed a nonlinear pattern. For *F. oxysporum* f. sp. *radicis-lycopersici*, initially only the temperature effect was considered (equations 7 and 8). However, using the model with the data from experiment 3 as an example, complete inactivation of the inoculum within 3 days was calculated, while in practice, this took place only after 28 days (Fig. 8A). Similarly, for *S. rolfisii*, only the temperature effect was considered initially (equations 8 and 9). Using the model with

Fig. 6. Dependence of the Weibull rate parameter (b) on temperature (T) of *Fusarium oxysporum* f. sp. *radicis-lycopersici*, derived from experiments conducted at constant temperatures (measured in Kelvins; $T_{\text{ref}} = 342.2$ K).

Fig. 7. Dependence of the Weibull rate parameter (b) on temperature of *Sclerotium rolfisii*, derived from experiments conducted at constant temperatures.

typical data (experiment 10, as an example), complete inactivation of the inoculum within 3 days was calculated, while in practice, this took place only after 13 days (Fig. 8B). A similar discrepancy was observed with the other experiments with the two pathogens. Therefore, the model that considers only the temperature effect seems inadequate, and additional variables should be included in order to improve its adequacy under fluctuating climatic conditions. Because thermal inactivation is affected by both temperature and moisture, further studies were carried out in an attempt to develop a model describing thermal inactivation of the pathogen under fluctuating temperature and moisture conditions. We therefore measured the temperatures and RH and calculated moisture content values of exposed inoculum in a closed greenhouse at three times during the day at 0600, 1010, and 1225 h, in an attempt to determine moisture regime in the inoculum during the day. The respective temperatures were 25, 40, and 50°C , the respective RH values were 80, 55, and 25%, and the respective moisture content values were 5.6, 3.6, and 0.85 g of H_2O per 100 g of dry soil.

Fluctuating conditions of temperature and RH-based model. Initially, the relationship between ambient RH and water content of the inoculum sample at any temperature was described using the GAB relationship (equation 1), which enables a calculation of the momentary moisture content at any temperature. The parameters were derived by using all the sorption data and applying a nonlinear regression. For *F. oxysporum* f. sp. *radicis-lycopersici* inoculum, the constants of the GAB equation were estimated as $m_0 = 2.81$ (g of H_2O per 100 g of dry soil), $c = 1.56 \times 10^{-18}$, $E_a = 13,389$ kcal/g mole K, $K_c = 0.611$, and $R^2 = 0.95$. For *S. rolfisii* inoculum, the GAB equation parameters were derived,

Fig. 8. Experimental versus calculated (based on temperature model only) survival curves of **A**, *Fusarium oxysporum* f. sp. *radicis-lycopersici* and **B**, *Sclerotium rolfisii*, during structural solarization. Bars represent $\pm$ SE.

yielding $m_0 = 2.15$ (g of H_2O per 100 g of dry soil), $c = 2.1 \times 10^{-15}$, $E_a = 13,200$ kcal/g mole K, $K_c = 0.521$, and $R^2 = 0.93$. The momentary moisture content value at any temperature was then utilized in the Weibull model, describing the survival curves under fluctuating temperatures during structural solarization. The parameters C_1 and C_2 were calculated (equation 8) as described for *F. oxysporum* f. sp. *radicis-lycopersici* and for *S. rolfii*, and their values were $C_1 = 1.018$ and 5.76 , and $C_2 = 5,979$ K and -18.05°C , respectively.

Values of P_1 , P_2 , and P_3 (equation 11) for all greenhouse experiments were derived from the numerical integration of equation 12 and minimizing the SSQ (equation 14) between the experimental and calculated values as presented in Table 3. The values of the parameters ranged widely. For instance, P_1 for *F. oxysporum* f. sp. *radicis-lycopersici* ranged from -0.205 to 9.343 and for *S. rolfii* ranged from -3.41 to 3.41 ; P_2 for the former ranged from -1.158 to 6.336 , and for the latter ranged from -0.99 to 2.79 ; and P_3 for the former ranged from -0.090 to 0.330 and for the latter ranged from 0.50 to 1.42 . SSQ values were in the range of 10^{-2} (indicating a deviation of 1 to 6%), except for experiments 5, 6, 11, and 12 where they were on the scale of 10^{-1} .

Modeling pathogen survival under fluctuating temperatures and RH in solarization experiments. Once the values of the parameters P_1 , P_2 , and P_3 had been estimated for each experiment, the derived estimates were used to assess the agreement between the experimental and calculated values (Figs. 4 and 5). This was performed by calculating the ratio between the derived thermal inactivation and the experimental data at two time points, namely, after 228 h, at about the middle of the exposure period, and at 492 h, at the final stage. The data show that, except for experiment 12, the ratios for the indicated time points ranged from 0.90 to 1.24 and from 0.82 to 1.03, respectively, while the ratio for experiment 12 at 492 h was 1.32. The R^2 values of the fitness of the model were significant ($P < 0.05$) and high: 0.83 to 0.99 in 9 of 12 experiments (Figs. 4 and 5).

DISCUSSION

Sanitation of the greenhouse structure and thermal inactivation of *F. oxysporum* f. sp. *radicis-lycopersici*, *S. rolfii* (Figs. 4 and 5), and other pathogens (44) can be achieved by structural solarization. However, due to the lower effectiveness of dry heating, complete elimination of the pathogens involves a relatively long process, despite the high temperatures reached (Table 1; Figs. 4 and 5). In Italy, significant reduction of an *F. oxysporum* f. sp. *radicis-lycopersici* population was achieved by this method (9). Thermal inactivation of this pathogen during structural solarization was governed by diurnal fluctuations in both temperature and RH.

Structural solarization involves dry heating, because low RH prevails during the hottest hours of the day (Fig. 3). Dry heating is

less effective than wet heating at thermal inactivation of pathogens, as was demonstrated in this study, with several other soil fungi and with *Bacillus cereus* spores (10,34). Survival curves of *Alternaria porri* f. sp. *solani* spores during exposure to 53°C and 14, 51, and 75% RH showed maximal longevities of 40, 4, and 3 days, respectively (39).

Estimating the effect of fluctuating temperatures on the thermal inactivation process is a much more complicated task than its counterpart, carried out under constant heating conditions. The DH concept has been attempted in various studies as a tool for evaluating the effectiveness of thermal inactivation of pathogens (5,26,27,51). However, when using this approach with *F. oxysporum* f. sp. *radicis-lycopersici* (Table 1), some noticeable discrepancies were found. For example, the most extensive heating took place in experiment 4 ($DH > 50^\circ\text{C} = 1,213$), but the lowest survival was observed in experiment 5 ($DH > 50^\circ\text{C} = 623$), which was ranked only fifth with regard to heating intensity. However, better results were obtained with *S. rolfii*. It should be emphasized that the DH concept is an empirical and not a physical-biological-based model, and hence it can only provide a general indication of the thermal inactivation, especially since it gives equal weight to all temperatures above a certain point. Also, because this concept does not take into consideration the effect of moisture fluctuations, it does not appear to be a suitable tool for describing thermal inactivation during structural solarization. This approach was successfully used as a guiding tool in thermotherapy of *Cucumber mosaic virus* in *Nicotiana rustica* tissue cultures (51). The DH concept was used with regular soil solarization (namely wet heating) studies. In one study (27), a significant regression coefficient was achieved when a set of empirical equations was developed. In another study, in which soil solarization for controlling *Phytophthora* root rot in avocado was investigated, regression equations could be established (26). An alternative approach is based on a dynamic model, i.e., incorporating thermal inactivation rates derived at constant temperatures into a fluctuating-temperature-regime model, as demonstrated previously with a variety of organisms (12,23,41). Parameters derived under constant temperature were utilized to simulate soil solarization for thermal inactivation of *P. nicotianae* in the laboratory (7) and of the nematode *H. carotae* under soil solarization conditions (16, 43). Utilizing the LD_{90} (exposure time necessary to kill 90% of the propagules) curvilinear regression of *Verticillium dahliae* that was calculated under constant temperatures allowed the estimation of the efficacy of soil solarization in the greenhouse (47). Hence, the dynamic approach was used as the starting point of our study (equations 6 and 8).

Traditionally, the efficacy of thermal processes has been calculated on the basis of the assumption that microbial inactivation follows first-order kinetics (1,12,49). This dependence was also found for four soilborne pathogens (35). The approach is based on

TABLE 3. The derived moisture quadratic equation parameters values and sum of squares (SSQ) of the residuals values for *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *Sclerotium rolfii* under structural solarization experiments

Experiment ^a	Pathogen	P_1^b	P_2^b	P_3^b	SSQ
1	<i>F. oxysporum</i> f. sp. <i>radicis-lycopersici</i>	8.405	-0.702	0.330	5.94×10^{-2}
2	<i>F. oxysporum</i> f. sp. <i>radicis-lycopersici</i>	9.343	1.281	0.075	1.26×10^{-2}
3	<i>F. oxysporum</i> f. sp. <i>radicis-lycopersici</i>	2.849	-1.158	-0.090	3.11×10^{-2}
4	<i>F. oxysporum</i> f. sp. <i>radicis-lycopersici</i>	-0.205	4.344	0.003	4.93×10^{-2}
5	<i>F. oxysporum</i> f. sp. <i>radicis-lycopersici</i>	2.786	4.547	0.284	3.20×10^{-1}
6	<i>F. oxysporum</i> f. sp. <i>radicis-lycopersici</i>	0.121	6.336	-0.006	1.79×10^{-1}
7	<i>F. oxysporum</i> f. sp. <i>radicis-lycopersici</i>	0.846	2.189	-0.026	4.91×10^{-2}
8	<i>S. rolfii</i>	0.001	0.934	0.781	3.37×10^{-2}
9	<i>S. rolfii</i>	3.410	-0.998	1.180	8.59×10^{-2}
10	<i>S. rolfii</i>	1.550	-0.119	1.420	8.29×10^{-2}
11	<i>S. rolfii</i>	0.548	0.614	0.877	1.59×10^{-1}
12	<i>S. rolfii</i>	-3.410	2.790	0.504	4.44×10^{-1}

^a Refers to experiments described in Table 1.

^b P_1 , P_2 , and P_3 are the parameters of the quadratic moisture equation (equation 11).

the assumption that all cells or spores in a population have similar sensitivity to heat, and that it is the chance of a quantum of heat striking the cell, spore, or target within the cell that will determine the death rate (32). Utilizing an exponential relationship was adequate for describing survival curves for *F. oxysporum* f. sp. *radicis-lycopersici* at constant temperatures (Table 2). However, plotting semilogarithmic survival curves for the structural solarization experiments, which involve fluctuating temperatures, showed nonlinear behavior. The model that we used to describe this behavior is based on the assumption that lethal events reflect the population's spectrum of thermal sensitivities or resistances rather than assuming that the propagules do not differ in their sensitivity to heat (32). Thus, nonlinear semilogarithmic survival curves of all structural solarization experiments were followed by the Weibull probabilistic distribution (equation 4). This model is sufficiently flexible to account for different survival patterns, whereas previously, the assumption of different kinetic-order reactions had to be made. Hence, the Weibull distribution was selected because of its relevance in describing similar situations. The Weibull rate parameter, b , is characteristic to each reaction and reflects the thermal reaction rate. This model has also been used extensively in life-testing and survival studies, and for describing models of growth, dose-response, and plant disease progress (3). The applicability of this model to describe thermal inactivation of microbial vegetative cells has been shown recently in dozens of cases (50). We analyzed our data utilizing this model and established significant relationships between the Weibull rate parameter, b , and temperature, T , for the two pathogens (equations 7 and 9). This analysis was carried out utilizing the survival data from the constant temperature experiments under fixed moisture conditions. Because the temperature-only-based model showed a large discrepancy (Fig. 8A and B), it was further expanded to account for diurnal fluctuating temperatures and moisture (equation 10). The relationship between the Weibull rate parameter, b , and temperature, T , for *F. oxysporum* f. sp. *radicis-lycopersici* at constant temperature was exponential, because a significant linear relationship was shown between the natural logarithm of b , the Weibull rate parameter, and $1/T - 1/T_{\text{ref}}$ (Table 2; Fig. 6). According to the Weibull model, the exponential dependence (apparent first-order reaction) is a special case of equation 4, with $n = 1$. Several studies (1,12,49) have indicated a similar exponential relationship between the thermal inactivation rate (which can also be regarded as analogous to the Weibull parameter, b) and temperature. The dependence for *S. rolfii* was found to follow the Fermi function (equation 9; Fig. 7), which was previously also shown for *L. monocytogenes* (33). The different functions describing the dependence of b on T for *F. oxysporum* f. sp. *radicis-lycopersici* versus *S. rolfii* might be connected with their different types of resting structures. While those of *Fusarium* are typically unicellular chlamydospores, those of *S. rolfii* are sclerotia, which are macroscopic multicellular structures protected by a melanized layer. Our data show that if an energy of activation, E_a ($= 1.987 \times E$), is considered, as in many microbiological kinetics, a value of $\approx 60,000$ kcal/mol is derived. This is a typical value for microorganisms (48).

Survival under fluctuating temperatures and RH during structural solarization was calculated by utilizing the derivative of equation 12 as the general Weibull model, where both moisture and temperature are a function of time. Using the derivative of the survival function rather than the survival function itself requires smooth conditions of moisture and elimination of sudden tiny but erratic fluctuations in the RH curves during the day. As described, this was achieved by smoothing the moisture data before calculating the derivative. The model parameters were estimated by numeric integration of the derivative ds/dt , the momentary survival (equation 13). The parameters were used to calculate the theoretical survival (Fig. 2). The model is also based on the assumption that under the conditions examined, the momentary rate of thermal

inactivation is only a factor of the momentary heating intensity and survival fraction, but not of the rate at which the survival fraction has been reached (33). The low SSQ obtained and the high R^2 values indicate that the suggested model was satisfactory for describing the survival of both pathogens under natural diurnal fluctuating temperatures and RH conditions. In the survival studies, we found that in 11 of 12 greenhouse experiments, the ratio between experimental and calculated mortality values of the pathogen for two periods (228 and 492 h) ranged between 0.90 and 1.24 (Figs. 4 and 5). This indicates that moisture fluctuations play a role in thermal inactivation, even under structural solarization conditions. Since these results were also obtained with natural inoculum, and under various climatic regimes and collected over a 4-year period, their applicability could be extended to other cases. However, the wide range of parameters used in the model highlights the fact that prediction models need to be further developed. A natural inoculum is less uniform than a laboratory-produced one, but represents the realistic situation. Previous attempts to use the Weibull model for simulating survival at low temperatures have not been very accurate (33). Several studies (33,50) applied the Weibull model to short processes of a few minutes in which there were 8 to 12 decimal reductions of bacteria. However, with soilborne fungal pathogens, 2 to 3 decimal reductions are sufficient to reduce inoculum density to an acceptable sanitary level. It should be emphasized that our study differs from most of those dealing with thermal inactivation of microorganisms, including standard soil solarization, because the latter involve wet heating, whereas structural solarization involves dry heating with fluctuations in both temperature and RH. The survival curve patterns of both pathogens in greenhouse studies (Figs. 4 and 5) can be explained by the assumption that the most heat-susceptible propagules are affected rather quickly, while it takes longer to inactivate the more resistant ones, which is also in line with the Weibull model approach.

The dynamic processes taking place during thermal inactivation were described using a novel apparatus not hitherto used for solar heating. This approach might be adopted for other cases of dry heating, e.g., thermotherapy of propagation material (6). Our approach for describing thermal inactivation of pathogens can also be partially adopted for simulating regular (wet) soil solarization, which is a hydrothermal process (8). Our studies demonstrate the potential of structural solarization as a nonchemical and inexpensive sanitation tool that could become an important component of disease management in greenhouses. In addition to the elimination of soilborne pathogens, solar sanitation has the potential to eliminate additional pests, including foliar pathogens, e.g., *Botrytis cinerea* (E. Shlevin, I. S. Saguy, Y. Mahrer, and J. Katan, unpublished data) and *D. lycopersici* (2), as well as arthropods and weeds (47). Sanitation is especially important with soilborne pathogens, which produce airborne conidia on the stems that disseminate and can contaminate the greenhouse structure, such as some formae speciales of *F. oxysporum* including *radicis-lycopersici* (38, 40), *basilici* (14), *lycopersici*, and others (21). Hence, the multiple mechanisms of survival and dissemination of soilborne pathogens require the adoption of a holistic and integrated management approach that deals with all of the inoculum sources. Simulation models are useful when developing such management strategies.

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LITERATURE CITED

1. Ball, C. O., and Olson, F. C. W. 1957. Sterilization in Food Technology. McGraw-Hill, New York.

2. Besri, M. 1983. Lutte contre le chancre a *Didymella lycopersici* de la tomate par chauffage solaire (solarisation) de tuteurs. *Phytopathol. Z.* 108:333-340.
3. Campbell, C. L., and Madden, L. V. 1990. Introduction to Plant Disease Epidemiology. John Wiley & Sons, New York.
4. Cenis, J. L. 1989. Temperature evaluation in solarized soils by Fourier analysis. *Phytopathology* 79:506-510.
5. Chellemi, D. O., Olson, S. M., and Mitchell, D. J. 1994. Effects of soil solarization and fumigation on survival of soilborne pathogens of tomato in northern Florida. *Plant Dis.* 78:1167-1172.
6. Clear, R. M., Patrick, S. K., Turkington, T. K., and Wallis, R. 2001. Effect of dry heat treatment on *Fusarium gramineum* in seed. Pages 84-89 in: Proc. Canadian Workshop on Fusarium Head Blight, 2nd. Canadian Grain Commission, Winnipeg, MB.
7. Coelho, L., Mitchell, D. J., and Chellemi, D. O. 2000. Thermal inactivation of *Phytophthora nicotianae*. *Phytopathology* 90:1089-1097.
8. DeVay, J. E., and Katan, J. 1991. Mechanisms of pathogen control in solarized soils. Pages 87-101 in: Soil Solarization. J. Katan and J. E. DeVay, eds. CRC Press, Boca Raton, FL.
9. Di Primo, P., and Cartia, G. 1998. Control by space solarization and genetic variability of *Fusarium oxysporum* f. sp. *radicis-lycopersici*. (Abstr.) *J. Plant Pathol.* 80:257.
10. Dunn, P. H., Barro, S. C., and Poth, M. 1985. Soil moisture affects survival of microorganisms in heated chaparral soil. *Soil Biol. Biochem.* 17:143-148.
11. Fletcher, J. T. 1984. Diseases of Greenhouse Plants. Longman Scientific & Technical, London.
12. Fujikawa, H., and Itoh, T. 1998. Thermal inactivation analysis of mesophiles using the Arrhenius and z-value models. *J. Food Prot.* 61:910-912.
13. Gamliel, A., Grinstein, A., Klein, L., Cohen, Y., and Katan, J. 1998. Permeability of plastic films to methyl bromide: Field study. *Crop Prot.* 17:241-248.
14. Gamliel, A., Katan, T., Yunis, H., and Katan, J. 1996. Fusarium wilt and crown rot of sweet basil: Involvement of soilborne and airborne inoculum. *Phytopathology* 86:56-62.
15. Garibaldi, A., and Tamietti, G. 1983. Attempts to use soil solarization in closed greenhouses in northern Italy for controlling corky root of tomato. *Acta Hort.* 152:237-243.
16. Greco, N., D'Addabbo, T., Sasanelli, N., Seinhorst, J. W., Stea, V., and Brandonisio, A. 1998. Effects of temperature and length of exposure on the mortality of the carrot cyst nematode, *Heterodera carotae*. *Int. J. Pest Manag.* 44:99-107.
17. Haralampu, S. G., Saguy, I., and Karel, M. 1986. The development of dynamic stability tests for moisture sensitive products. *Math. Model.* 7:1-13.
18. Haralampu, S. G., Saguy, I., and Karel, M. 1986. The performance of a dynamic stability test for moisture sensitivity. *Math. Model.* 7:15-25.
19. Hutchinson, T. P. 2000. Graphing the death of *Escherichia coli*. *Int. J. Food Microbiol.* 62:77-81.
20. Katan, J., and DeVay, J. E. 1991. Soil Solarization. CRC Press, Boca Raton, FL.
21. Katan, T., Shlevin, E., and Katan, J. 1997. Sporulation of *Fusarium oxysporum* f. sp. *lycopersici* on stem surfaces of tomato plants and aerial dissemination of inoculum. *Phytopathology* 87:712-719.
22. Kodama, T., and Fukui, Y. 1979. Solar heating solarization in the closed vinyl house against soil-borne diseases. I. The movements of soil temperature and determination of thermal lethal conditions for some soilborne pathogens. (In Japanese, with English summary.) *Bull. Nara Prefect. Agric. Exp. Stn.* 10:71.
23. Labuza, T. P. 1979. A theoretical comparison of losses in foods under fluctuating temperatures sequences. *J. Food Sci.* 44:1162-1168.
24. Lindblad, M., and Sigvald, R. 1996. A degree-day model for regional prediction of first occurrence of frit flies in oats in Sweden. *Crop Prot.* 15:559-565.
25. Linton, R. H., Carter, W. H., Pierson, M. D., and Hackney, C. R. 1994. Use of a modified Gompertz equation to model nonlinear survival curves for *Listeria monocytogenes* Scott A. *J. Food Prot.* 58:946-954.
26. Lopez-Herrera, C. J., Perez-Jimenez, R. M., Basallote-Ureba, M. J., Bonilla, T. Z., and Melero-Vara, J. M. 1997. Effect of soil solarization on the control of *Phytophthora* root rot in avocado. *Plant Pathol.* 46:329-340.
27. Lopez-Herrera, C. J., Verdu-Valiente, B., and Melero-Vara, J. M. 1994. Eradication of primary inoculum of *Botrytis cinerea* by soil solarization. *Plant Dis.* 78:594-597.
28. Mahrer, Y., Avissar, R., Naot, O., and Katan, J. 1987. Intensified soil solarization with closed greenhouse: Numerical and experimental studies. *Agric. For. Meteorol.* 41:325-334.
29. Palti, J. 1980. Cultural Practices and Infectious Crop Diseases. Springer-Verlag, Berlin.
30. Palti, J., and Katan, J. 1997. Effect of cultivation practices and cropping systems on soilborne diseases. Pages 377-396 in: Soilborne Diseases of Tropical Crops. R. J. Hillocks and J. M. Waller, eds. CAB International, Wallingford, UK.
31. Peleg, M. 2000. Modeling and simulating microbial survival in foods subjected to a combination of preservation methods. Pages 163-181 in: Innovations in Food Processing. G. V. Barbosa-Canovas and G. W. Gould, eds. Technomic Publishing, Lancaster, PA.
32. Peleg, M., and Cole, M. B. 1998. Reinterpretation of microbial survival curves. *Crit. Rev. Food Sci. Nutr.* 38:353-380.
33. Peleg, M., and Penchina, C. M. 2000. Modeling microbial survival during exposure to a lethal agent with varying intensity. *Crit. Rev. Food Sci. Nutr.* 40:159-172.
34. Pfeifer, J., and Kessler, H. G. 1994. Effect of relative humidity of hot air on the heat resistance of *Bacillus cereus* spores. *J. Appl. Bacteriol.* 77:121-128.
35. Pullman, G. S., DeVay, J. E., and Garber, R. H. 1981. Soil solarization and thermal death: A logarithmic relationship between time and temperature for four soilborne plant pathogens. *Phytopathology* 71:959-964.
36. Punja, Z. K. 1985. The biology, ecology and control of *Sclerotium rolfsii*. *Annu. Rev. Phytopathol.* 23:97-123.
37. Rahman, S. 1995. Food Properties Handbook. CRC Press, Boca Raton, FL.
38. Rekah, Y., Shtienberg, D., and Katan, J. 2000. Disease development following infection of tomato and basil foliage by airborne conidia of the soilborne pathogens *Fusarium oxysporum* f. sp. *radicis-lycopersici* and *F. oxysporum* f. sp. *basilici*. *Phytopathology* 90:1322-1329.
39. Rotem, J. 1968. Thermoxerophyte properties of *Alternaria porri* f. sp. *solani*. *Phytopathology* 58:1284-1287.
40. Rowe, R. C., Farley, J. D., and Coplin, D. L. 1977. Airborne spore dispersal and recolonization of steamed soil by *Fusarium oxysporum* in tomato greenhouses. *Phytopathology* 67:1513-1517.
41. Ruissen, M. A., Van der Vossen, R. T. M., and Kocks, C. G. 1993. Growth of *Xanthomonas campestris* pv. *campestris* populations at constant and variable temperatures. *Neth. J. Plant Pathol.* 99 Suppl. 3:173-179.
42. Saguy, I. 1983. Optimization of dynamic systems using the maximum principle. Pages 343-356 in: Computer-Aided Techniques in Food Technology. I. Saguy, ed. Marcel Dekker, New York.
43. Sasanelli, N., and Greco, N. 2000. Formulation of a model to relate nematode populations with exposure times to a range of temperatures. *Acta Hort.* 532:131-135.
44. Shlevin, E., Katan, J., Mahrer, Y., and Kritzman, G. 1995. Space solarization for sanitation of inocula of plant pathogens in the greenhouse structure. (Abstr.) *Phytopathology* 85:1209.
45. Shlevin, E., Saguy, S., Mahrer, Y., and Katan, J. 2001. Utilization of the Weibull model to quantify survival of soilborne pathogens under dynamic conditions during structural solarization. *Phytoparasitica* 29:247-248.
46. Singleton, L. L., Mihail, J. D., and Rush, C. M. 1992. Methods for Research on Soilborne Phytopathogenic Fungi. The American Phytopathological Society, St. Paul, MN.
47. Tamietti, G., and Valentino, D. 2001. Soil solarization: A useful tool for control of Verticillium wilt and weeds in eggplant crops under plastic in the Po valley. *J. Plant Pathol.* 83:173-180.
48. Thompson, D. R., and Norwig, J. F. 1986. Microbial population, enzyme and protein changes during processing. Pages 202-265 in: Physical and Chemical Properties of Food. M. R. Okos, ed. American Society of Agricultural Engineers, St. Joseph, MI.
49. Toledo, R. T. 1999. Fundamentals of Food Process Engineering. 2nd ed. Aspen Publishers, Gaithersburg, MD.
50. van Boekel, M. A. J. S. 2002. On the use of the Weibull model to describe thermal inactivation of microbial vegetative cells. *Int. J. Food Microbiol.* 74:139-159.
51. Walkey, D. G. A., and Freeman, G. H. 1977. Inactivation of cucumber mosaic virus in cultured tissues of *Nicotiana rustica* by diurnal alternating periods of high and low temperatures. *Ann. Appl. Biol.* 87:375-382.
52. Weibull, W. 1951. A statistical distribution of wide applicability. *J. Appl. Mechanics* 18:293-297.
53. Wu, Y., Perry, K. B., and Ristaino, J. B. 1996. Estimating temperature of mulched and bare soil from meteorological data. *Agric. For. Meteorol.* 81:299-323.