Modelling time-varying low-temperature-induced mortality rates for pupae of *Tuta absoluta* (Gelechiidae, Lepidoptera)

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Abstract
In a series of laboratory experiments, acclimated pupae of *Tuta absoluta* were exposed to various constant low temperatures in order to estimate their maximum survival times (Kaplan–Meier, Lt99.99). A Weibull function was fitted to the data points, describing maximum survival time as a function of temperature. In another experiment at −6°C, the progress of mortality increasing with exposure time was identified. These values were fitted by a sigmoidal function converging asymptotically to 100% mortality for very long exposure times. Analysing mortality data from the maximum survival experiment by a generalized linear model showed a significant common slope parameter (p < .001) that reveals parallelism of the survival curves at each temperature if a log time axis is used. These curves appear stretched (time scaled) if plotted with a nonlogarithmic time axis. By combining these mathematical relations, it was possible to calculate a species-specific ‘mortality surface’ which exhibits mortalities, depending on temperature and duration of exposure. In order to accumulate hourly mortalities for courses of varying temperatures, an algorithm was developed which yields mortality values from that surface taking into account the attained mortality level. In validation experiments, recorded mortalities were compared against modelled mortalities. Prediction of mortality was partially supported by the model, but pupae experiencing intensely fluctuating temperatures showed decreased mortality, probably caused by rapid cold hardening during exposure. Despite this observation, mortality data converged to distinct levels very close to 100% depending on the intensity of temperature fluctuations that were characteristic for different types of experiments. The highest mortality limit occurred at intensely fluctuating temperatures in laboratory experiments. This constituted a benchmark that was not reached under various field conditions. Thus, it was possible to identify temperature limits for the extinction of field populations of *Tuta absoluta* pupae.

Keywords
accumulation algorithm, hourly chilling mortality, mortality surface, overwintering, rapid cold hardening, time scaling
INTRODUCTION

Modelling the overwintering mortality of poikilothermic species (insects, mites, nematodes) offers a useful tool for predicting the successful overwintering of tropical species that invade temperate countries. The goal of this study was to establish a mathematical model for the accumulation of hourly mortalities at variable/ fluctuating temperatures using *Tuta absoluta* (tomato leaf miner; TLM; Lepidoptera: Gelechiidae) as a test organism. This pest of tomatoes originates from tropical South America (with a climate characterized by the absence of seasonal temperature variation) and lives now in Africa and Asia but also in Southern Europe in the field as well as in Northern Europe in greenhouses (Desneux et al., 2010 and Cuthbertson et al., 2013). In South American lowlands, up to 12 generations may develop per year. In highlands, infested tomatoes occur normally up to 1,000 m above sea level (Desneux et al., 2010). Alive adult moths were found in 3,200 m altitude—probably carried by the wind. Larvae may pupate in their feeding galleries but mostly leave their mines in order to spin a cocoon within the surface of the litter near to the host plants. Van Damme et al. (2015) studied aspects of low temperature biology of TLM: pupae were shown to have a small delay of development but no reproductive diapause under short-day conditions (8/16 hr). They were characterized by a \( L_{90} \) of 21.6 days at +5°C and of 25.7 days at 0°C. Adults were even more cold resilient at 5°C, but at 0°C, the difference was not significant. The supercooling point of pupae was situated at −16.7°C and that of adults at −17.8°C, thus suggesting that adults may be better suited for surviving periods of low temperature. Yet the resilience of pupae to negative temperatures was not studied at all. It may be concluded that TLM is most probably freezing intolerant though that was not stated explicitly. Anticipating own results, we state here that principally pupae were used in our study. Generally, the temperature environment in soil or litter a few centimetres below the soil or litter surface differs significantly from the 2 m air temperature or soil surface temperature. In an average situation, the diurnal and day-to-day temperature variations are dampened such that the insects in soil or litter are much less exposed to temperature extremes at the surface (Parton & Logan, 1981).

At present, the concept of lower lethal limits is used widely for estimating the ability of an insect to overwinter (reviewed by Sinclair, Alvarado, & Ferguson, 2015) but it does not take into account variable temperature courses as encountered in nature. More complicated models were presented by Nedvěd (1998) or Kaliyan, Carillo, Morey, Wilcke, and Kells (2007) which accumulate mean hourly mortality values specific for each temperature—referred to as mortality rates or lethality index, respectively. Hourly mortalities depending only on temperature require a linear temporal increase of mortality—so that the mortality rate (hourly mortality) is constant. This is not at all the case—numerous studies show that mortality is progressing sigmoidal (e.g. Casagrande & Haynes, 1976). Of course, it would be possible to calculate mean hourly mortalities over a long exposure period if the mortality rises nonlinear (e.g. sigmoidal). Yet the resulting mean value would deviate from the instantaneous mortality value in a systematic way depending on the examined mortality level. Hence, the modelled mortality would be calculated from systematically underestimated or overestimated mortality values. Furthermore, accumulated mortalities would exceed 100% after a while—which is impossible in a mortality model. Hence, we decided to use exact hourly mortality values instead of mean values. Nedvěd, Lavy, and Verhoef (1998) described a three-dimensional species-specific ‘mortality surface’ for *Orchesella cincta* (Collembola), which shows accumulated mortality values resulting from various exposure durations at various temperatures. This promised to be a very useful element for our model.

A fundamental study of Stroustrup et al. (2016) enables a simplified mathematical description of such a mortality surface. According to their basic ideas, survival functions (logistic or Weibull) are generally time-scaled with respect to exposure to different intensive stress factors such as temperature, oxidative stress, and diet composition. They demonstrated clearly for *Caenorhabditis elegans* (Nematoda) as an example that for various intensities of the same stressor, survival functions could be made identical by stretching or shrinking of the time scale. As this is also a precondition for the simplified model described here, it was necessary to find out if time-mortality functions at various low temperatures were time-scaled for *Tuta absoluta*. If so, the mortality surface could be described by two trajectories only, that is (a) the function of time needed to reach a certain high mortality level (maximum survival — here \( L_{99.99} \)) depending on temperature and (b) the time-mortality function for a discrete low temperature (e.g. −6°C). For the first trajectory, a curve would be fitted to the prediction values of maximum survival (\( L_{99.99} \)) for several low temperatures between −10°C and +4°C in order to get a functional equation for maximum survival depending on temperature. For the second trajectory, a sigmoidal curve would have to be fitted to progressing mortality data obtained at −6°C. If it could be shown that time-mortality curves yielded from the maximum survival experiment were time-scaled, it would be possible to find an appropriate transformation factor which could stretch or shrink the time-mortality curve for −6°C so that it would fit to any given temperature and would fill in the gap between the starting point at 0/0 and the ‘endpoint’ at \( L_{99.99}/99.99 \). Thereby, it would be possible to create the desired mortality surface in the sense of Nedvěd et al. (1998) in a relatively simple way.

The last cornerstone of our model is the development of an accumulation algorithm which adds the mortality arising during 1 hr to a certain initial mortality. This increase depends on the current temperature and the mortality level which has already been reached. To achieve this, (a) the proper time-mortality curve has to be selected by transforming the empirical −6°C curve into a curve defined by its start at 0/0 and its ‘end’ at \( L_{99.99}/99.99 \)—for the ambient temperature. The transforming factor may be deduced from corresponding values of \( L_{99.99} \) for the requested temperature and for −6°C. As the functional equation of \( L_{99.99} \) depending on temperature will be identified empirically for a range of temperatures, this factor may be calculated for any temperature within this range. (b) Additionally, a virtual time \( t_v \) has to be calculated that would cause just the given
initial mortality by using the selected time-mortality curve appropriate to the current temperature. By using this mortality curve and the virtual time, it would be possible then to continue the mortality curve at the proper mortality level and thus to compute the increase of mortality after 1 hr ($t_v + 1$). The whole process might be repeated as often as required to get an accumulated mortality for arbitrary exposure times at arbitrary temperature courses.

The progressing mortality during exposure at variable temperatures can be compared with the stepwise growth of a polygon governed primarily by the sigmoidal temporal mortality progress function and being modified secondly by particular ambient temperatures. To this end, we establish a method for correctly calculating mortality levels at courses of variable low temperatures. This model may be applied in future studies for analysing cold-temperature-induced mortality in insects of economic or medical relevance.

2 | MATERIALS AND METHODS

2.1 | Experimental design

In preliminary experiments, young pupae of *Tuta absoluta* were identified to be most frost resistant compared to other developmental stages of the species (after 4 days of exposure at −4°C only pupae survived but no eggs, larvae or adults). The first task in model development was the creation of a three dimensional ‘mortality surface’ which displays accumulated mortality values dependent on the duration of exposure and on temperature. These values were obtained from two basic experiments carried out with young pupae of *Tuta absoluta* reared in the laboratory under standardized conditions (as described in sections 2.2 and 2.4) and subjected to a cold hardening procedure (section 2.3) prior to testing. The first experiment (section 2.5) yielded exposure times that lead to mortalities near 99.99% (Lt99.99) at series of constant low temperatures. The second experiment (section 2.6) yielded mortality data under progressing duration of exposure at −6°C. In order to obtain the desired three-dimensional mortality surface, progressive mortalities at several constant temperatures had to be calculated according to the observed time scalability of mortality curves at various temperatures (Stroustrup et al., 2016). An algorithm was developed that accumulates specific hourly mortality values considering the mortality level attained so far. The model was validated in two kinds of laboratory experiments (section 2.7 and 2.8) and in field experiments (section 2.9).

2.2 | Insect rearing

The stock culture of *Tuta absoluta* was derived from an outbreak (2012 in Vienna, Austria). Tomatoes were cultured in growth chambers (25/15°C; 16-hr light/8-hr dark; 8,000 Lux). The moths were reared in climatic chambers at 22°C (13-hr light/11-hr dark). Adult moths were housed in cages (40 × 40 × 60 cm) and were supplied with diluted invert sugar syrup. Eggs were laid readily on potted tomato plants and plants removed every week. If infested leaves collapsed by an excess of feeding galleries, fresh leaves were added. Three weeks afterwards, strips of corrugated cardboard were added as pupation sites and were changed three times weekly. After 1 day of additional development, the harvested cardboard was pulled apart and released pupae were handled with a fine brush.

2.3 | Cold hardening

Pupae intended for use in mortality experiments were subjected to ~14 days of cooling at +6°C in order to induce cold adaptation. In a series of preliminary experiments, young pupae stored at +6°C for 10–20 days had shown similar levels of cold resistance in mortality experiments at −4°C. Cooling for <5 days or more than 25 days, in contrast, caused increased mortality (data not shown). A single batch of cold-hardened pupae was used for each experimental replicate.

2.4 | Handling of experimental pupae and temperature control during experiments

In all cold exposure experiments, pupae destined for one single experiment were harvested three times over the course of 1 week. Experimental pupae—including those intended for control—were taken randomly from this batch. Pupae that were not exposed to cold temperature after cold hardening served as control; mortalities obtained from the exposure experiments were corrected by the control mortality using Abbott’s formula (Finney, 1952). The cold hardened pupae were wrapped in small envelopes of paper towels that were placed in boxes of foamed polystyrene (22 × 22 × 28 cm) filled partly with fine metal shot (steel balls of 1 mm diameter). Since metal has a high thermal capacity, it is able to smooth abrupt thermal changes. For the transfer from +6°C to various exposure temperatures, certain amounts of metal shot were used for each intended temperature step. Thereby, the following values of a percentage decay in 30 min [%] were achieved: 16°C (4.6 kg) [8.2%], 14°C (3.9 kg) [9.6%], 12°C (3.2 kg) [13.9%], 10°C (2.4 kg) [16.5%], 8°C (1.2 kg) [25.0%], 6°C (0.6 kg) [34.4%] and 4°C (0.4 kg) [39.8%]. After termination of exposure, the Styrofoam boxes were transferred to a +6°C chamber for 24 hr and later on to +23°C for recovery. Pupae were checked for emerging adults 4 weeks later. Moths were regarded as surviving if they were able to hatch, showed no wing deformation and did not adhere to their exuviae. After recording the hatching of adults, these samples were discarded—so all these experiments were ‘destructive’ in a statistical sense. The exact numbers of experimental pupae, temperatures and exposure durations may be seen in the online resources referred for each particular experiment.

2.5 | Identification of maximum temporal survival (Lt99.99)

The Lt99.99 was calculated by exposing pupae to one of the following constant temperatures: −10, −8, −6, −4, −2, 0, +2, +4 and +6°C. Experimental pupae were harvested weekly and divided into 2–5 batches of 25–150 individuals each, and a total of 6,677
experimental pupae were used. Mortality data were analysed by the method of Kaplan–Meier (Table S1).

In a separate experiment, the supercooling point (SCP) of 50 pupae was determined according to a method of Carillo and Cannon (2005). Briefly, the Styrofoam box containing the pupae was transferred to a freezer at −25°C. Hereby, an exponential cooling rate was exerted that averaged −0.13°C/min near the SCP. Thirty pupae at +23°C (data not shown).

SCP at −17.5°C and checked for survival after recovery for 4 weeks during 4 years: Rax mountain (47.71 N–15.77 E; altitude 1547 m; in winter 2012/2013, 2013/2014, 2014/2015 and 2015/2016). Insect samples were taken to the laboratory according to a time schedule aiming to cover the whole mortality range. They were slowly defrosted at +6°C and observed at 23°C for hatching. Temperatures were recorded hourly by data loggers (Tinytag™, Gemini Data Loggers). Temperatures above +6°C were not used for modelling because pupae were expected to resume their development near this temperature. Corrected mortalities were set in relation to modelled mortalities. In these experiments, a number of 3,029 individuals were used (Table S5).

2.10 | Statistical analyses and modelling

For curve fitting, SigmaPlot™ version 14 was utilized which is based on algorithms using least squares (Levenberg 1944, Marquard 1963). Estimation of maximum survival according to Kaplan–Meier and calculation of a generalized linear model (GLM) was achieved by the software GNU R version 3.5.1 (R Development Core Team, 2016).

2.10.1 | Maximum survival

For estimation of maximum survival, the time needed to kill 99.99% (Lt99.99) of experimental insect populations at several low temperatures was used. These times were calculated by using the Kaplan and Meier (1958) estimates and performing a fourth-degree polynomial interpolation. Resulting prediction values and their confidence intervals (95% probability) were used for curve fitting in order to describe Lt99.99 as a function of temperature. No constraints or weightings were used in this process. Mortality values of control groups (without exposure to frost) that had been recorded were not used for these calculations as they do not influence the prediction values. A three-parameter Weibull curve was fitted to these data. Parameters of the resulting curve including standard errors, $R^2$, F-value, df and p-value are reported.

2.10.2 | Progressing mortality at −6°C

Mortality data were corrected by Abbot’s method to achieve 0% mortality at the start of each experimental group. Data were analysed by curve fitting using a sigmoidal function with five parameters. At the start of curve fitting, a constraint was defined by (a) $a + y_0=100$ which fixes the upper asymptote exactly to 100% and (b) a discrete value for $y_0$ which fixes the lower asymptote by its distance to the x-axis. Curve fitting was repeated with a stepwise refined $y_0$ in every run until at least the fitted curve started exactly from the origin of coordinates and converged to 100% for very long exposure times. Parameters of the resulting Weibull function including standard errors, $R^2$, F-value, df and p-value are reported.

2.10.3 | Time scalability of time-mortality functions at several constant temperatures

Calculations were based on mortalities yielded from the maximum survival experiment. A generalized linear model (McCullagh
& Nelder, 1989) was fitted using a binomial distribution with logit built-in link function in order to prove whether there exists a common slope for all temperatures. A significant common slope parameter would reveal parallelism of the survival curves at each temperature and thus their time scalability if plotted with a non-logarithmic time axis.

2.10.4 | Deduction of a transformation factor— for stretching or shrinking progressing time-mortality curves

The transformation factor \( k_T \) stretches or shrinks the time-mortality trajectory until it fits exactly into the gap between its starting point at 0/0 and its 'endpoint' at \( L_{99.99} \) of time-mortality curves specified by the temperature of the \( L_{99.99} \) calculation. The formula of the stretching/shrinking factor \( k_T \) was deduced by basic geometric and algebraic considerations. Its principally correctness was proved by its application to the empiric time-mortality trajectory with its known function equation so as to transform it to a similar curve stretched/squeezed along the \( x \)-axis. The geometrical similarity (time scalability) of the two curves along the \( x \)-axis was shown by corresponding tangents (having the same \( y \) at their touch points) which need to cross at one point on the \( y \)-axis—if corresponding curves were scaled along the \( x \)-axis.

2.10.5 | Deduction of the accumulation algorithm

For the mathematical deduction of the accumulation algorithm, algebraic operations were necessary being based upon Equations 1-4. It may be helpful to bring the geometric interpretation of any step to the mind.

3 | RESULTS

3.1 | Creation of the mortality surface

3.1.1 | Maximum survival (\( L_{99.99} \))

TLM pupae were incubated at constant temperatures between \(-10^\circ\mathrm{C}\) and \(+4^\circ\mathrm{C}\) to calculate survival as a function of temperature. Pupae held at \(-10^\circ\mathrm{C}\) displayed the shortest \( L_{99.99} \), and pupae held at \(+4^\circ\mathrm{C}\) with the longest \( L_{99.99} \) (Figure 1, Table S1). Control groups (not exposed to low temperatures) showed an overall mortality of \( 27.15\% \). (\( \mathrm{SE} = 1.67 \)). A three-parameter Weibull function described 99.8% of the variation in \( L_{99.99} \) values (with \( \mathrm{SE} y = 31.1 \) hr) by temperature (\( F = 1774.28 \), \( df \) (regression) = 2, \( df \) (residuals) = 6, \( p < .0001 \)) (Equation 1, Figure 1). Numerical values of parameters \( a, b, c \) including their standard errors may be seen from the Appendix S1. \( T \) = temperature, and \( f_1 \) yields the dimension time [hours].

\[
 f_1(T) = a \times \left[ 1 - e^{-\left(\frac{T - b}{c}\right)} \right] \quad (1)
\]

FIGURE 1 Maximum temporal survival (\( L_{99.99} \)) of cold adapted young pupae of \( Tuta absoluta \) at various low temperatures. The data were fitted by a Weibull function presented as Equation 1. The dashed line shows the 95% confidence band.

3.1.2 | Temporal progress of mortality at \(-6^\circ\mathrm{C}\)

The relationship between time and mortality was tested by holding pupae at a fixed temperature of \(-6^\circ\mathrm{C}\). Mean mortality over time is shown in Figure 2; data may be taken from Table S2. Standard error is biggest at 144 hr and decreases for shorter or longer exposure periods. A five-parameter sigmoidal function described 93.6% of the variation in mortality values (with \( \mathrm{SE} y = 11.1 \)) by time (\( F = 799.49 \), \( df \) (regression) = 2, \( df \) (residuals) = 109, \( p < .0001 \)) (Equation 2, Figure 2). Numerical values of parameters \( a', b', c', x_0', y_0' \) including their standard errors may be seen from the Appendix S1.
\[ f_3(t) = y' + \frac{a}{1 + e^{-\left(\frac{t - x'}{b}\right)}} \]  

\[ f_3(t) \] yields mortality (\(m_3, \%\)) of TLM pupae at −6°C depending on \(t\) (exposure time \(t\), [hr]). The parameters in Equation 2 are marked with an apostrophe to improve clarity. Control groups (not exposed to low temperatures) showed a mean mortality of 22.50\% (\(SE = 3.21\)). The curve crosses the origin and converges asymptotically to 100\% mortality for very long exposure times.

### 3.1.3 Time scalability of the time-mortality function at several low temperatures

Analysing mortality data from the maximum survival experiment with a generalized linear model (logit built-in link function) showed a significant \((p < .001)\) common slope parameter of temperature of −0.317 with \(SE\) being 0.019. That reveals parallelism of the survival curves at each temperature if a log time axis is used. In other words, these curves appear stretched (time scaled) if plotted with a non-logarithmic time axis. The temperature as well as the time had a statistically significant influence on the mortality.

### 3.1.4 Calculation of the mortality surface

The time-mortality curve of Equation 2 was obtained at −6°C and is most basic for the entire mortality surface. Appropriate time-scaled curves for other low temperatures may be obtained from it by transforming (stretching/shrinking) this curve with a specific transformation factor. This factor refers to any data point of couples of curves at a certain temperature. It is unknown but it may be derived for any couple of mortality values known from the maximum survival function (Equation 1). For the development of a formula for the transforming stretching/shrinking factor, the parameter \(x'_0\) has to be corrected. It was originally responsible for moving the temporal progressing mortality curve \(f_3(t)\) along the \(x\)-axis but now is governed also by time- and by the temperature-specific factor \(k_T = (t^\prime \text{Lt}_{99.99} - x'^\prime \text{Lt}_{99.99})/\text{Lt}_{99.99}\) with \(T_i\) being the current ambient temperature at the time \(t\).

\[ f_3(t) = k_T \times t + x'_0 = \left(\frac{t^\prime \text{Lt}_{99.99} - x'^\prime \text{Lt}_{99.99}}{\text{Lt}_{99.99}}\right) \times t + x'_0 \]  

\[ (3) \]

The replacement of \(x'_0\) in Equation 2 by \(f_3(t)\) allows the calculation of temporal progressing mortality at any low constant temperature between −10°C and +4°C. Equation 4 \((f_4(t,T))\) describes temporal accumulated mortality at arbitrary temperatures in this range. Dependence on temperature is not explicit but introduced by the temperature depending factor \(k_T\):

\[ f_4(t,T) = m_4 = y' + \frac{a}{1 + e^{-\left(\frac{t - x'}{b}\right)}} \]  

\[ (4) \]

\[ f_4(t,T) \] may be calculated, thereby making it possible to solve Equation 4. For a given mortality, it is possible now to calculate a virtual exposure time \(t_0\) that would give rise just to the desired mortality value if it were subjected solely to the current constant temperature. Thus, it would be possible to continue the mortality curve from the point representing the actual mortality. Solving Equation 4 for the time \(t_0 + 1\) will yield the new mortality according to the current temperature and the already attained mortality level. This new mortality can be used as the initial mortality for the next calculation cycle.
3.3 Validation of the model

For validation of the model, the mortalities from exposure experiments under various temperature regimes were compared with referring modelled mortalities (Figure 5). In the first validation experiment, temperatures changed slowly in a monthly cycle ('half cycle': +6°C/−8.5°C/+2°C) — individual data are listed in Table S3; the mean mortality of control groups was 28.40%, SE = 3.64. In the second validation experiment, temperatures fluctuated daily between 0°C and −8°C (individual data may be taken from Table S4; the mean mortality of control groups was 36.57%, SE = 4.59). In the third series, experimental pupae were exposed to completely irregular field temperatures (individual data in Table S5; the mean mortality of control groups was 26.74%, SE = 2.20). Visual data inspection showed the following patterns of distribution of data points: one data point (Figure 5, left side) combines high observed mortality with low modelled mortality values and is classified as statistical outlier. A crowd of data points is concentrated near the 100/100 endpoint on the right side of the graph and needs further evaluation (see below). The rest of the data points seem to represent two ‘mortality responses’: one broadband representing the normal mortality response following more or less the modelled reference line — such experimental pupae react as predicted by the model. The second pattern represents a low mortality response and displays nearly constant mortality at ~40% for a wide range of modelled mortalities with a drastically increasing slope at ~95% modelled mortalities. This is confined mainly to individuals
having experienced intensive daily temperature fluctuations but comprise also few individuals from other groups. In the above-mentioned high mortality zone (right margin in Figure 5), mortality values are squeezed towards the 100% limit. For further analysis of this zone, corresponding negative log-transformed survival rates were used instead of formerly used mortalities. Only data sets with observed mortalities higher than 90% with the exclusion of 100% were selected for that purpose. Levene’s test was used to check for equal variance ($p = .49116$). Modelled mortalities in all variants differed significantly from each other (Figure 6) as shown by ANOVA (F ratio: 25.23, $df$ (total) = 47, $df$ (between groups) = 2, $p = .0000$) and multiple range test (95% LSD). As modelled mortalities converge steadily and slowly towards 100%, the significantly differing modelled mortalities from Figure 6 will differ accordingly also in the exposure time being necessary to induce mortality. This means that mortality in the variant ‘monthly fluctuation’ appears earlier than in the variant ‘daily fluctuation’—the latter appearing to be delayed. The variant ‘irregular’ is situated in between.

4 | DISCUSSION

The objective of this study was to find a method for predicting mortality of *Tuta absoluta* after exposure to variable low temperatures. Numerous experiments at constant temperatures were carried out with young pupae, the most frost tolerant stage of this species—according to initial experiments at negative temperatures. In experimental series at diverse constant temperatures, the maximum survival ($LT_{99.99}$) could be described as a function of temperature (Figure 1, Equation 1). In time series at $-6^\circ$C, the sigmoidal course of progressing mortality was determined and described as a function of time (Figure 2, Equation 2). These two lines together with time scalability as a feature of the latter function made it possible to calculate a ‘mortality surface’ (Figure 3) introduced by Nedvěd et al. (1998). An accumulation algorithm was developed to calculate proper hourly mortalities by taking into account the already obtained level of mortality and was used to yield mortalities for arbitrary temperature courses. In many validation experiments, mortality of pupae followed this model. In contrast, in experiments with intensive daily fluctuating temperatures, recorded mortalities were reduced compared to the model. Recorded mortalities converged to specific limits very close to 100%, dependent on the intensity of experienced temperature fluctuations (Figure 6). The statistical parameters of their mortality/survival distribution may serve as benchmarks for mortality/survival limits in field experiments.

Control groups were subjected to cold hardening at $+6^\circ$C but were not exposed to lower temperatures. They showed a mean mortality of 27.8% ($SE = 1.23$). This seems to be a high value yet it is still lower than 90% mortality observed after 21.6 days of exposure at $+5^\circ$C as reported by Van Damme et al. (2015). Therefore, mortality values were usually corrected by Abbott’s method except in experiments for maximum survival. In this case, the Kaplan–Meier estimation does not require such corrections.

The herein presented mortality model processes variable temperatures. Previously developed models (Kaliyan et al., 2007; Nedvěd, 1998) required clearly defined hourly mortalities for each low temperature—which do not exist if temporal progressive mortalities are sigmoidal, whereas this model accumulates hourly mortalities in a mathematically correct way; however, uncertainty remains if cold adaptation is not constant, for example if it changes during the experiment. Cold adaptation may be induced by several environmental factors, and fluctuating temperatures seem to play an important role (Colinet, Sinclair, Vernon, & Renault, 2015; Kelty & Lee, 2001; Meats, 1976). In our experiments, we subjected pupae to a pretreatment cooling of 14 days at $+6^\circ$C, that is near to the basic temperature for development (Barrientos, Apablaza, Norero, & Estay, 1998; Cuthbertson et al., 2013). This procedure led to moderately cold adapted experimental pupae, whereas more intense adapted pupae would have exhibited lower mortalities under the same conditions. The basic problem of changing cold adaptation during exposure will
be discussed in detail further below. For an accurate estimation of mortality, this lack of knowledge about underlying cold adaptation is a clear limitation. However, under fluctuating exposure temperatures, it was possible to identify limits of thermal resilience above which no survival was possible. The model will be best suited for predicting mortality thresholds in episodes of low temperatures. Another limitation of the model may be seen if pupae have to pass longer periods of temperatures above their threshold of development. Such conditions are considered in contrast detrimental because *Tuta absoluta* has no diapause and resumes its development if warmed, thereby leaving its best suited physiological stage. The collapse of its cold adaptation may also be possible (Sobek-Swant, Crosthwaite, Lyons, & Sinclair, 2012). On the other hand, sublethal effects may be restored (Nedvěd et al., 1998) in such periods. In our studies, experimental pupae experiencing long-lasting warm periods were excluded from the field validation experiments as we felt it would be impossible to model mortality under such ambiguous conditions. However, sometimes pupae were subjected to short temperature peaks above +10°C. It was impossible to avoid such conditions entirely. This might have lowered mortality to a certain extent. Contrastingly, in laboratory daily fluctuation experiments, such high-temperature peaks were prevented, which led to very distinct results (Figure 5).

Mortality experiments revealed that at −17.5°C (SCP), no survival was possible. The SCP in our experiments was situated slightly lower than reported by Van Damme et al. (2015) at −16.7°C. Different cooling rates may be responsible for this. However, in our experiments, the same cooling rate was used for determination of the SCP and the identification of corresponding mortality. This data point (−17.5/0) was used in Figure 1—yet the highest temperature with no survival for very short exposure times will be situated presumably somewhat above −17.5°C. The exact position of the latter is of low relevance for the curve as it proceeds almost parallel to the axis in this temperature range (Figure 1).

Analysis of mortality data from the maximum survival experiment by a generalized linear model demonstrated that time-mortality curves were time-scaled for various low temperatures. This feature is very important for the development of our model as it allows the calculation of the mortality surface by the utilization of two trajectory functions only. Additionally, it is an essential part of the accumulation algorithm as it enables the calculation of any time-mortality curve for arbitrary low temperatures. The general feature of time scaling was firstly described by Stroustrup et al. (2016) in Nematodes but seems to be prevalent also in insects in respect of their mortality at low temperatures (Bale, 1991; Beerwinkle, Berry, & Kunz, 1978; Brokerhof, Banks, & Morton, 1992; Casagrande & Haynes, 1976; Nedvěd et al., 1998; Turnock & Bilodeau, 1992; Turnock, Lamb, & Bodnaryk, 1983).

Joint results of slowly or rapidly fluctuating temperatures in validation experiments (Figure 5) are analysed by the method of visual data exploration: it becomes evident that experimental pupae may exhibit two responses to exposure at low temperatures: (a) recorded mortalities are similar to predicted values—visualized by the band of data points grouped along the reference line, and (b) recorded mortality values remain constant at −40% for a long period. At approximately 95% modelled mortality, such reduced recorded mortalities increase drastically (Figure 5). There are several ways to explain this observation. Several authors attributed reduced mortality at significant positive temperature pulses of 1- to 2-hr duration to repair mechanisms: Nedvěd et al. (1998) for *Orchesella cincta*; Yocum, Rinehart, and Kemp (2012) for *Megachile rotundata*; Renault, Nedvěd, Hervant, and Vernon (2004) and Colinet, Lalouette, and Renault (2011) for *Alphitobius diaperinus*. Yet in these experiments, markedly recovery occurred at least at +10°C, mostly at +15°C or +20°C. Contrastingly, in our laboratory validation experiment under intensely fluctuating temperatures, the highest experienced temperature was 0°C. Accordingly, we consider reduced mortalities at fluctuating temperatures as a result of increased cold adaptation known as rapid cold hardening (Lee, Chen, & Denlinger, 1987, review by Teets & Denlinger, 2013). That suits very well to reported induction of rapid cold hardening by temperatures near 0°C. Rapid cold hardening may increase with the number of experienced thermocycles as demonstrated by Kelty and Lee (2001) for *Drosophila melanogaster*. It seems possible hence that cold adaptation will increase during each fluctuation cycle and will keep mortality constant in a wide range in Figure 5. This mortality reducing effect was substantially stronger during rapid laboratory fluctuations compared to field experiments (Figure 6). This decrease of recorded versus modelled mortality comes to an end at approximately 95% modelled mortality. Obviously, the effect of retarding mortality is limited—maybe this is based on an energy consuming active process which stops as energy reserves are exhausted. However, even after its breakdown, some mortality retarding mechanisms seem to remain active in the high mortality zone. Consequently, maximum survival is significantly delayed in those variants that had been subjected to intensive temperature fluctuations (cf. Figure 6). Attempting not to overinterpret our results, it seems possible that more than one process is involved in this mortality delay. In nature, cold adaptation and mortality expression will occur at the same time and are not separated like in many laboratory experiments. The mortality accumulation model presented here enables the visualization of such opposing processes. In particular, the incomplete cold adaptation at the start of exposure offered an excellent chance to study increasing cold adaptation during exposure. The results of this study make clear that for a refinement of the here described model, the numerical influence of rapid cold hardening to survival functions would be essential.

As the mortality for long exposures at constant temperatures converges to 100%, mortality at variable temperature courses will also converge to 100%. This is fundamentally different to growth models working with an accumulation of degree days, which will exceed certain thresholds that coincide with specific phenological events (e.g. hatching of larvae or adults). So, we come to the delicate question that the model accumulates hourly mortalities in a mathematically correct way but those calculations might be useless because any high mortality value will yield values very near to 100%. This shortage can be overcome if mortalities are displayed as corresponding negative log-transformed survival rates (e.g. Candy, Sfiligoj, King, & Mondon, 2015). Thereby a situation known from growth models appears (Figure 6). It
seems remarkably that modelled high mortalities in different validation experiments (monthly or daily fluctuation cycles or irregular ‘field fluctuation cycles’) are not merged during the process of accumulation and differ significantly. They remain discernible and may be displayed by appropriate methods (see above). The highest observed limit occurred at daily fluctuating temperatures (−8 to 0°C) in laboratory experiments (Figure 6). This benchmark was not even reached under various field conditions. Thus, it was concluded that mortality limits (upper confidence interval or upper whiskers) are defined and laboratory data under fluctuating temperatures might be used in identifying mortality limits for the field populations studied.

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CONFLICT OF INTEREST

No conflict of interest appeared.

AUTHORS’ CONTRIBUTIONS

Andreas Kahrer, Anna Moyses and Helfried Scheifinger conceived research. Andreas Kahrer, Anna Moyses, Alois Egartner, Teresa Miglbauer, Katharina Müllner, Lara Reinbacher, Christina Pilz and Julia Votzi conducted experiments. Lisa Hochfellner analysed data and conducted statistical analyses. Wolfgang Tiefenbrunner provided an important contribution in the successful implementation of time scaling into the model. Andreas Kahrer wrote the manuscript. All authors read and approved the manuscript.

PERMITS

No permits were necessary in the course of this study.

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