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TECHNICAL NOTE

PATHOLOGY/BIOLOGY

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A Statistical Approach Based on Accumulated Degree-days to Predict Decomposition-related Processes in Forensic Studies*,†

ABSTRACT: Using pig carcasses exposed over 3 years in rural fields during spring, summer, and fall, we studied the relationship between decomposition stages and degree-day accumulation (i) to verify the predictability of the decomposition stages used in forensic entomology to document carcass decomposition and (ii) to build a degree-day accumulation model applicable to various decomposition-related processes. Results indicate that the decomposition stages can be predicted with accuracy from temperature records and that a reliable degree-day index can be developed to study decomposition-related processes. The development of degree-day indices opens new doors for researchers and allows for the application of inferential tools unaffected by climatic variability, as well as for the inclusion of statistics in a science that is primarily descriptive and in need of validation methods in courtroom proceedings.

KEYWORDS: forensic science, accumulated degree-days, decomposition stages, forensic entomology, multiple regression analysis, PMI estimation

In temperate terrestrial ecosystems, the decomposition of carcasses is a complex process driven primarily by biochemical reactions (e.g., [1]), bacterial activity (e.g., [2,3]), and insect activity (2,4). These factors are in turn dependent on environmental conditions, most importantly ambient temperature (4,5). Although decomposition is a continuous process, a classification based on distinct stages representing the physical condition of the carcass is frequently used to document the progression of decomposition. Three viewpoints are commonly expressed in the literature regarding this classification: (i) decomposition stages do not represent breaking points in the faunal succession (6,7), (ii) the nature of the decomposition stages is subjective because it largely depends on the perception of the observer (6), and (iii) decomposition stages are convenient for the documentation of study results only (8–10).

In a previous paper (11), we used a novel approach involving decomposition stages. We developed a linear index via a multiple regression of three levels of accumulated degree-days set arbitrarily (i.e., accumulations over 5, 10, and 15°C) against the decomposition stages. Unexpectedly, the combined degree-day accumulations accounted for nearly all of the variability in the rate of decomposition between pig carcasses. The linear index served as a continuous predictor in a logistic regression analysis to examine the visitation pattern of carrion insects across seasons with different rates of degree-day accumulation. As did other authors (e.g., [12,13]), we argued that accumulated degree-days gave a better representation of the decomposition process than did the standard description based on the number of days or weeks and thus were better suited for the study of insect succession on carcasses. At that time, we did not fully recognize the potential of the linear index alone, but we recently realized that it could be used to verify the relative predictability of the decomposition stages or any other decomposition-related process.

Here, we examine the relationship between accumulated degree-days and the physical condition of carcasses, as described by five decomposition stages. We test the hypothesis that ambient temperature is the main factor influencing the biochemical reactions, bacterial activity, and insect activity that drive decomposition, although these three processes may respond to different temperature thresholds. Because the physical condition of the carcass (i.e., the decomposition stage) is a result of the combination of these processes, we can expect that a combination of degree-day accumulations over nonspecific temperature thresholds (i.e., not related to the specific temperature threshold of a particular process or species) will permit the prediction of the decomposition stages of carcasses throughout the year. Ultimately, the objective is to obtain a temperature-based index applicable not only to decomposition stages, but also to a variety of other decomposition-related phenomena (e.g., microbial succession, vertebrate scavenging) under a given temperature regime (i.e., geographic area).

Material and Methods

The study was performed in a rural area 50 km northeast of Moncton, New Brunswick, Canada, in the Gulf of St. Lawrence lowland forests ecoregion. This ecoregion encompasses two dominant ecosystems: the Acadian Forest and agricultural fields. Two large agricultural fields (sites A and B) were used for the study. Site A was located at the Agriculture Canada experimental farm in Bouctouche, New Brunswick. Site B was on privately owned land located 2.2 km northwest of site A.

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We used 12 domestic pig (*Sus scrofa* L.) carcasses, all weighing between 20 and 27 kg, as surrogates for human cadavers. Albeit this was only empirically confirmed once (14), pig carcasses of this size are considered the most appropriate surrogates because they (i) are approximately the same size as an average human torso where most of the decomposition occurs, (ii) have a relatively hairless skin and a gut fauna similar to that of humans, (iii) are accessible and affordable, and (iv) do not seem to attract negative public and media attention when used in field experiments (15,16). Eight of the pigs were killed via a pin gun shot to the head in an establishment certified for slaughtering pigs and brought to the sites within 2 h postmortem. At the sites, a composite board consisting of a styrofoam sheet (1.22 × 0.61 m, 5 cm in thickness) glued under a pressed-wood sheet (1.22 × 0.61 m, 6.3 mm in thickness) and wrapped in a layer of polyurethane was placed directly on the ground. It was then covered with *c.* 5 cm of soilless potting mix (Canadian Growing Mix #2; Fafard, Shippagan, NB., Canada), on top of which was placed a carcass. This was repeated for all the carcasses to isolate them from the ground and its organisms, and allowed for homogeneous soil conditions between exposition sites. Because it prevented insect burrowing, the board also delineated a sampling area, which was essential for two concurrent studies on insect taxa. The carcasses were then enclosed individually within wooden framed chicken wire cages. Throughout the study, the carcasses were always separated by at least 50 m (see [10]). One carcass was exposed on May 11, 2007, at site A; one on June 7, 2007, at site B; two on June 27, 2007, at site A; two on August 2, 2007, at site B; one on September 5, 2007, at site A; and one on May 6, 2008, at site A. We also included data from a previous study (11) documenting the decomposition of four carcasses placed directly on top of the vegetation in the summer and fall of 2006.

Minimum and maximum air temperatures were obtained from a federal weather station located at site A. A portable data logger installed at site B indicated that the weather station at site A provided an accurate representation of the temperature in both sites. Pictures were taken daily to facilitate the subsequent determination of the decomposition stages. We slightly modified the definitions of the decomposition stages detailed in Anderson and VanLaerhoven (10) and kept only the information needed for consistent determination in the field:

Fresh stage (0): begins at the moment of death; ends with the onset of the bloated stage;
Bloated stage (1): abdomen distention is obvious; the limbs are usually raised and not in the relaxed position characteristic of the fresh stage; the neck area is distended; the anus is often protruded;
Active decay stage (2): marked by a complete deflation of the abdomen under the action of maggots; presence of large maggot masses on the carcass;
Advanced decay stage (3): maggots leave to pupate away from the carcass, causing a relatively sudden decline in the number of maggots on the carcass;
Dry decay stage (4): only the dried skin, bones, and hairs remain; for most of the year, this stage is characterized by a total absence of maggots; however, in cold weather, a few maggots (usually 10 or less) sometimes remain on the carcass as they strive to complete their life cycle.

Statistical Analysis

Briefly, the analysis was carried out in five phases. First, degree-day accumulations since death were calculated. Second, a scale was defined to represent decomposition with realistic intervals. Third, a multiple regression analysis including degree-day accumulation and the decomposition scale was carried out to obtain a degree-day index. Fourth, the probability that a carcass belongs to a given stage along the degree-day index was calculated. Last, the index was validated using original data from another study. All the analyses were performed using SAS version 9.0 (17). Residuals were examined to ensure that postulates of parametric analyses were respected.

For each carcass on each day, the accumulation of degree-days (ADD) since death, over a given temperature threshold, was calculated with one unit increments for threshold values of 0–20°C using the following formula:

$$ADD_{thd} = [\{T_{\min} + T_{\max}\}/2] - \text{thd}$$

where $T_{\min}$ and $T_{\max}$ represent the minimum and maximum air temperatures reached that day, respectively, and thd represents the minimum threshold over which the accumulation is considered. Thus, to approximate the daily temperature cycle, the temperature profile of each day was treated as a sine wave with amplitude equal to the minimum and maximum air temperatures. Negative ADD values were set to zero.

Because stages are not of equal duration (e.g., [10,11,18]), we created a new scale that would allow stages to be represented with realistic intervals instead of rigid values as described earlier (i.e., stages 0–4). To determine the average length of each decomposition stage at relatively steady day-to-day temperatures, we combined the data from our summer sequences only and calculated the average ADD_5 value (i.e., value of degree-day accumulation over 5°C) needed to reach the onset of each decomposition stage. We then divided these values by the ADD_5 value needed to reach the dry decay stage to obtain a new scale of decomposition going from 0 (fresh stage) to 1 (dry decay stage). On this new scale, the bloated stage, active decay stage, and advanced decay stage have values of 0.214, 0.465, and 0.596, respectively. For this calculation, the use of other thresholds than five degrees did not improve the explanatory power (i.e., R^2) of the degree-day model developed later. Conversely, the transformation of decomposition stage values into a continuous scale increased the explanatory power of the degree-day model.

We performed a multiple regression analysis using the values calculated earlier for decomposition and degree-day accumulation since death. Because we hypothesized that decomposition does not progress in response to a unique threshold, we used the degree-day accumulations of 0–20°C (i.e., ADD_0 to ADD_{20}) calculated earlier to determine the best model. The regressions included the onset of each decomposition stage (i.e., five values—stages—for each of the 12 pigs) as the dependent variable and the ADD values as independent variables. Criteria for choosing the best model were parsimony and accuracy (i.e., the model with the least number of independent variables and $R^2 \geq 95\%$). Multicollinearity was not assessed because it would not affect statistical significance, fit or overall prediction capacity of the model (19,20). This model was used to create a temperature-based, continuous index of decomposition.

To ensure that the pooling of data from the different experiments, fields, and years was appropriate, as well as to examine whether or not a repeated-measures analysis was required, (i) a mixed-model multiple regression including the study protocol (either 2007–2008 or 2006) as a random effect was performed and (ii) a repeated-measures mixed-model regression including individual pigs as subjects was performed. These analyses were carried out using the procedure MIXED, a procedure appropriate to model mixed effect data, as well as repeated measures. The fit statistics specified by the procedure MIXED (i.e., −2 Log Likelihood, Akaike's information criterion, Schwarz's Bayesian information criterion) were used to compare the performance of models and indicated that pooling was appropriate and that random effects were negligible. The absence of a difference in model performance between the two experiments also confirmed that the composite boards used in this study did not affect decomposition.

In an analysis that included all the field data, we estimated the probability of a carcass belonging to a given stage along the degree-day index using partial proportional-odds logistic regression because the proportional-odds assumption of the ordinal logistic regression was not met. The partial proportional-odds model was developed with the use of the procedure GENMOD and included adjustments for repeated measurements on carcasses. The procedure GENMOD is appropriate to fit logistic models that account for correlated data arising from repeated measurements. The index was then validated using the original data from Michaud and Moreau (11), with insect occurrence being used as dependent variables instead of decomposition stages. For the validation, the explanatory power of the logistic models was compared using the R^2 values.

Results

The multiple regression model selected on the basis of parsimony and accuracy was

$$Y = -0.0124\,\mathrm{ADD}_6 + 0.0112\,\mathrm{ADD}_{10} - 0.239\,\mathrm{ADD}_{12} + 0.151\,\mathrm{ADD}_{13}$$

where ADD_6, ADD_{10}, ADD_{12}, and ADD_{13} represent the degree-day accumulation over 6, 10, 12, and 13°C, respectively, and Y represents the onset of each decomposition stage. The model accounted for 97% of the variability in decomposition with respect to temperature ($R^2 = 0.97$; $F_{4,56} = 398.9$; $p < 0.01$). All individual parameter estimates were significant ($p < 0.01$).

Using the equation above-mentioned, a daily value of heat accumulation, hereafter called degree-day index, was calculated for all the field data. For this calculation, the Y in the equation above-mentioned becomes the index value. This resulted in index values ranging from 0 (fresh stage) to 1.75 (carcasses beyond the onset of the dry decay stage). Although the onset of decomposition stages has been used to obtain parameter estimates in the multiple regression, the calculation of degree-day index values is solely dependent on ambient temperature.

The probability of a carcass belonging to a given stage along the degree-day index was obtained from an ordinal logistic regression with partial proportional odds that accounted for 89% of the variability ($R^2 = 0.89$; df = 4; $\chi^2 = 246.12$; $p < 0.01$) (Fig. 1). All individual parameter estimates were significant ($p < 0.01$). The regression indicates that every stage, except the active decay stage, has a high probability to occur at a precise moment somewhere along the degree-day index.

As a validation, we compared the performance of the degree-day index using forensic entomology successional data from a previous study (11). The latter used an index that (i) did not correct for stage duration, (ii) used equidistant thresholds of degree-day accumulation set arbitrarily, and (iii) was built from data collected over two seasons in a single year with limited replication. Overall, the index developed here allowed for a more accurate modeling of the occurrence of five carrion-related insects along the degree-day index than the index used in the original study. The explanatory power (i.e., R^2) of the logistic models used to predict insect occurrence increased for three species (*Lucilia illustris* Meigen [from 0.67 to 0.69], *Necrophila americana* L. [from 0.77 to 0.79], and *Necrodes surinamensis* Fabricius [from 0.37 to 0.39]), remained unchanged for one species (*Ontholestes cingulatus* Gravenhorst [0.14]), and declined for one species (*Phormia regina* Meigen [from 0.55 to 0.52]).

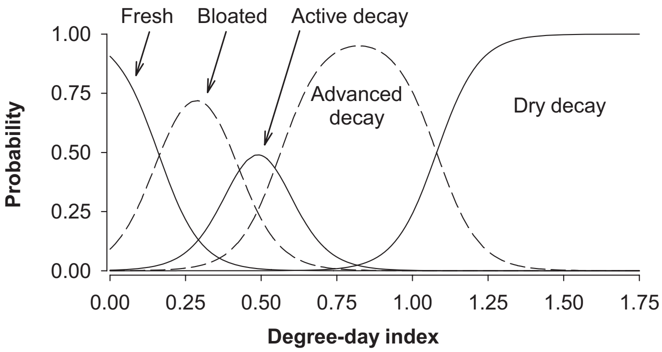

Fig. 1—Probability associated with the fresh, bloated, active decay, advanced decay, and dry decay stage of decomposition with respect to the degree-day index.

Discussion

Results from this study support the prediction that the physical condition of a carcass, as described by decomposition stages, is a reliable representation of the decomposition process. To the best of our knowledge, this is only the second study (see [12]) that statistically assessed the potential of decomposition as a prediction tool. However, our study differs from Megyesi et al. (12) in many aspects, including that here, (i) the exact moment of death was known for all carcasses, (ii) a greater percentage of the variability was explained, (iii) five distinct stages were used instead of a broad scale of decomposition, and (iv) with respect to postmortem heat accumulation, probabilities can be associated with each decomposition stage. This latter aspect is of particular value because it opens new doors for researchers and allows for the inclusion of statistics in a science that is primarily descriptive (6,21) and in urgent need of validation methods in courtroom proceedings (see [22,23]).

Once a degree-day index has been developed for a given geographic area, the methodology requires a reconstruction, from temperature records, of the ADD since 24, 48, 72 h, and so forth. Subsequently, these accumulations are used to calculate index values for the same intervals. Inferences are then possible. For example, at a constant temperature of 21°C, the decomposition model presented here predicts that carcasses have a 5% probability of reaching the dry stage in 9 days (index = 0.88), 50% in 11 days (index = 1.08), and 95% in 13 days (index = 1.28). Different temperature regimes would yield different probabilities.

At first glance, one could argue that some level of subjectivity exists in the discrimination between decomposition stages, a point of view previously expressed in the literature (see [6]). However, there is very little error associated with the determination of the fresh stage and the dry decay stage, and because the model accounts for 97% of the variability, there remains very little space for subjectivity in determining between the other three stages. Nevertheless, the need for standard stage definitions that would reduce the potential for personal interpretation cannot be overemphasized. As an example, the current definition of the advanced decay stage may pose operational problems as an investigator arriving at a crime scene has no basis to compare between the number of maggots at the scene and the number that was there previously.

In forensic studies, the use of a model based on a degree-day index has many advantages compared to the common approach based on the number of days. Because this approach incorporates adjustments for inter-year, between-season, and within-season variability, it allows for the development of prediction models valid throughout the year for a given geographic area. Because the index is linear, it also allows for the study of phenomena that occur after the onset of the dry decay stage, as well as for comparisons between causes of death, seasons, locations, and other variables of interest. In addition, a degree-day index can be used as baseline in prediction models studying different decomposition-related processes, such as carrion-insect fauna and microorganism fauna. As an example, the validation indicated that the model performed well with insect occurrence data, but the explanatory power declined slightly for the calliphorid *P. regina*, a result that is consistent with a previous observation (11) that the species is not a reliable pioneer of exposed carcasses in our geographic area. Ultimately, the degree-day index allows for a mathematical combination of the probabilities associated with a number of decomposition-related processes along the index to obtain a robust estimation of the postmortem interval.

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