

Novel Temperature-Dependent Development Rate Models for Postdiapause Egg Eclosion of Three Important Arthropod Pests Found in Commercial Christmas Tree Plantations of Southern Québec, Canada

J.-F. Doherty, J.-F. Guay, and C. Cloutier¹

Département de biologie, Université Laval, 1045 avenue de la Médecine, Ville de Québec, QC, G1V 0A6, Canada, and ¹Corresponding author, E-mail: conrad.cloutier@bio.ulaval.ca

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Abstract

The balsam twig aphid *Mindarus abietinus* Koch (Hemiptera: Aphididae), the spruce spider mite *Oligonychus ununguis* (Jacobi) (Acari: Tetranychidae), and the pine needle scale *Chionaspis pinifoliae* (Fitch) (Hemiptera: Diaspididae) are three important pests for the Christmas tree industry of Québec, Canada. Temperature-dependent development rates for postdiapause overwintering eggs of these species have never been studied for populations of southern Québec, where the vast majority of Christmas trees are grown. The accumulation of physiological time in growing degree days (GDD) is a standard tool to predict egg eclosion dates for pests, when first generation immatures are most vulnerable to insecticidal control. Development rates for postdiapause eggs were tested at four or five constant temperatures in the 11–23°C range under controlled conditions, based on time before hatching for eggs collected in a balsam fir plantation in late winter. The standard linear model and three published nonlinear models were fitted to the data and compared for their ability to estimate key biological temperature parameters and to predict development rates of postdiapause eggs. Validation of model predictions of egg eclosion time on balsam fir in the field was reasonably accurate using the classical linear model where field-accumulated GDD were calculated with a particular interpretation of the average method based on daily maximum and minimum temperatures. The linear model could predict mean egg eclosion within around 5 d of eclosion dates observed in the field.

Résumé

Le puceron des pousses du sapin *Mindarus abietinus* Koch (Hemiptera : Aphididae), le tétranyque de l'épinette *Oligonychus ununguis* (Jacobi) (Acari : Tetranychidae) et la cochenille des aiguilles du pin *Chionaspis pinifoliae* (Fitch) (Hemiptera : Diaspididae) sont d'importants ravageurs des arbres de Noël au Québec. Le développement des œufs hivernants postdiapausants, en fonction de la température, n'a jamais été étudié pour les populations du sud du Québec, où cette culture est concentrée. L'accumulation du temps physiologique en degrés-jours de croissance (DJ) est un outil essentiel pour prédire la date d'éclosion des œufs de ravageurs, alors que les immatures de première génération sont vulnérables aux traitements insecticides. Le taux de développement des œufs hivernants a été mesuré en conditions contrôlées pour quatre ou cinq températures constantes dans la gamme 11–23°C, calculé selon le temps de développement jusqu'à l'éclosion d'œufs collectés sur le sapin baumier. Le modèle linéaire standard et trois modèles non linéaires publiés ont été évalués pour leur capacité à prédire les paramètres biologiques de réponse à la température et le taux de développement. La prédiction de l'éclosion des œufs sur le terrain a été validée avec le calcul du temps physiologique accumulé en DJ avec une interprétation particulière de la méthode des moyennes basée sur les températures quotidiennes maximales et minimales, en association avec le modèle linéaire. Cela a permis de prédire l'éclosion printanière sur le terrain à environ cinq jours près.

Key words: temperature, modeling, postdiapause, development, *Abies balsamea*

Introduction

Temperature-dependent development in insects and mites is an important physiological trait that can strongly influence population dynamics, therefore it is often studied for pest management purposes (Roy et al. 2002, Trudgill et al. 2005, Sandhu et al. 2010, Moore and Remais 2014). Apart from rare exceptions (Heinrich 1974), poikilothermic life processes respond to daily temperature fluctuations and, on a larger time scale, to seasonal trends in temperature (Speight et al. 2008). When testing the effects of temperature on arthropod development, models can be used to describe this relation and predict pest activity. In turn, reasonably accurate models can help reduce the use of pesticides and the costs of treatments. For these reasons, this study focuses on three conifer arthropods notably considered as pests in commercial Christmas tree plantations of southern Québec, Canada.

The rate of development R_{dev} for poikilotherms, simply estimated as the reciprocal of time (e.g., hours or days) taken to reach maturity or to complete specific life stages (Roy et al. 2002), varies systematically according to temperatures above freezing and below the tolerable maximum (Speight et al. 2008, Moore and Remais 2014). This relation has been variously described mathematically first with the classical linear model (Ikemoto and Takai 2000, Trudgill et al. 2005) and also with several nonlinear models (e.g., Table 1 in Zahiri et al. 2010). With nonlinear models, the thermal dependence of development rates is characterized by three theoretical biological temperature parameters: the low and high temperature thresholds (T_{min} and T_{max}), and the optimal temperature (T_{opt}). In this three-parameter mathematical representation, the R_{dev} is nil at T_{min} , increases in a quasilinear fashion between T_{min} and T_{opt} where, rather than increasing further linearly, it is maximal and plummets rapidly to become nil again at T_{max} (Brière et al. 1999, Trudgill et al. 2005, Paaijmans et al. 2013).

For Christmas tree growers targeting timely control of pests during their most vulnerable development stages, thermal requirements are most easily approximated using physiological time or time needed to develop at temperatures above threshold, expressed as growing degree days (GDD), which is derived from the linear model. This time is assumed to be fixed for a given life stage and is thus often reported as a ‘thermal constant’ (K), directly obtained using the reciprocal of the slope from the linear model (Ikemoto and Takai 2000, Trudgill et al. 2005). The linear model can provide an accurate prediction of emergence for a target pest stage if field temperatures generally fall within the intermediate viable range below T_{opt} , where development rate is linearly related to temperature or nearly so (Bergant and Trdan 2006). The most commonly used method to calculate the amount of GDD accumulated daily is based on the difference between the mean temperature, calculated from the maximum and minimum, and the development threshold (McMaster and Wilhelm 1997). In practice, the linear model-based prediction

for the occurrence of a final step (e.g., egg eclosion) is based simply on cumulative daily GDD to reach the specific thermal constant of the development stage of interest. Thus, for linear modeling in pest monitoring, T_{min} and K are needed along with temperatures that generally represent those experienced by arthropods in the field, in order to predict when a pest completes a given life stage.

From the many nonlinear models available, some are more commonly used than others to model temperature-dependent development rates in poikilotherms (Roy et al. 2002). Although they do not estimate a thermal constant like the linear model, they could also be used in modeling and provide three critical temperature parameters (i.e., T_{min} , T_{opt} , and T_{max}), which are useful for quantifying the thermal limits of the organism at a particular stage. The Hilbert and Logan, Lactin-2, and Kontodimas-16 models tested here can estimate all three parameters mentioned above (Hilbert and Logan 1983, Lactin et al. 1995, Kontodimas et al. 2004). These nonlinear models account for the curvilinear nature of development rates for the whole range of temperatures, including limiting ones near the thresholds for the studied species.

In commercial Christmas tree plantations, the balsam twig aphid *Mindarus abietinus* Koch (Hemiptera: Aphididae) is an important pest of balsam fir *Abies balsamea* (L.) Miller (Pinaceae) and Fraser fir *Abies fraseri* (Pursh) Poiret (Pinaceae) (Nettleton and Hain 1982, Bradbury and Osgood 1986, Fondren and McCullough 2003). Its life cycle is complex and begins in the spring with the asexual fundatrix, or stem mother, which emerges from an overwintering egg in early May in southern Québec (Deland et al. 1998, Doherty et al. 2017). When fir buds break open, developing aphids of this first generation, which up to bud break had fed on shoots of the previous year, migrate onto the young current-year shoots to feed, where they mature and viviparously produce the second generation of aphids, thus forming a colony (Varty 1968). The growing colony stunts the growth of shoots and deforms their needles, thus creating a pseudogall, which protects the aphid colony, but reduces the aesthetic value of commercial Christmas trees, especially during the last 2 yr before tree harvest (Nettleton and Hain 1982). Therefore, insecticidal treatments against *M. abietinus* should be most effective before the migration of the immature stem mothers onto the newly expanding shoots, when they are most exposed (Kleintjes 1997). Estimates of *M. abietinus* for T_{min} and K for egg eclosion are available for New Brunswick, Canada and southern Québec (Varty 1968, Deland et al. 1998), but are based primarily on field observations (Table 1), instead of determination under controlled conditions at experimentally fixed temperatures, as usually indicated (Brière et al. 1999, Roy et al. 2002, Trudgill et al. 2005).

The spruce spider mite *Oligonychus ununguis* (Jacobi) (Acari: Tetranychidae) is also considered a pest in commercial Christmas tree plantations, especially on Fraser fir (Boyne and Hain 1983,

Table 1. Previous estimates of physiological time required for egg eclosion of three arthropod species overwintering as diapause eggs on fir. GDD estimates assume that development rates increase linearly with temperature

| Species | Estimate of thermal constant over development threshold | Estimation method (source) |
|------------------------------|---|---|
| <i>Mindarus abietinus</i> | 95 GDD over 2°C | Field observations only (Deland et al. 1998) |
| <i>Oligonychus ununguis</i> | 143.3 GDD over 7.1°C | One experimental temperature tested (Richmond and Sherlar 1996) |
| <i>Chionaspis pinifoliae</i> | 136.0 GDD over 10.8°C ^a | Eight experimental temperatures tested (Burden and Hart 1989) |

^aGDD model for *C. pinifoliae* egg development based solely on hatching time at a constant 18.5°C (threshold estimated between 10.8°C and 11.8°C); first generation.

Richmond and Shetlar 1996, Cloutier et al. 2009). In southern Québec, its life cycle typically begins in mid-May when first-generation mites emerge from overwintering eggs (Cloutier et al. 2009). The larvae and nymphs feed by sucking out the cellular contents of fir needles, which causes the yellowing and bronzing of foliage (Kielczewski 1966). During severe infestations of *O. ununguis* on fir, needle abscission, reduction in shoot growth, and even premature death of the host tree can occur (Löytteniemä 1971, Johnson and Lyon 1991). Relatively warm and dry conditions increase the fecundity of females, and four to five generations may be observed from late spring to early fall in southern Québec (Boyne and Hain 1983, Cloutier et al. 2009). As for the balsam twig aphid, the period for optimal control of *O. ununguis* rests just after the larvae of the first generation have emerged from the overwintering eggs. A thermal constant for overwintering egg hatching is available for populations of *O. ununguis* in Ohio (Table 1) (Richmond and Shetlar 1996).

As for the pine needle scale *Chionaspis pinifoliae* (Fitch) (Hemiptera: Diaspididae), its presence is commonly reported not only on both balsam and Fraser firs in commercial Christmas tree plantations but also on other ornamental pine and spruce conifer species (Miller et al. 2005). In Québec, *C. pinifoliae* has the status of an emerging pest of Christmas trees and is not known to cause any significant damage. Although not generally monitored by growers, it can be an obstacle for exportation. In Canada, it is a univoltine species, whereas in the United States, a second generation has been observed (Cumming 1953, Nielsen and Johnson 1973). Both sexual and parthenogenetic forms have been documented (Stimmann 1969, Luck and Dahlsten 1974). Typically, the hatching of overwintering eggs occurs around mid-June, after which the mobile crawlers settle rapidly on the needles to pursue development and secrete their protective scale (Cumming 1953, Martel and Sharma 1968), thus limiting the time window when these insects are exposed and vulnerable to insecticide treatments. GDD models and thermal response data exist for the pine needle scale (Table 1), but are based on populations in Iowa and Michigan, USA (Burden and Hart 1989, Fondren and McCullough 2005).

The objective of this study was to test the effect of temperature on postdiapause egg development rates for populations of *M. abietinus*, *O. ununguis*, and *C. pinifoliae* in southern Québec. Also, we aimed to develop practical models to estimate thermal requirements (i.e., physiological time) for egg eclosion and associated temperature parameters (T_{\min} , T_{opt} , T_{\max} , and K) for all three species. We hypothesize that the available thermal constants previously estimated for these arthropod pests are not representative of the populations found in southern Québec. Optimizing development models for local populations of these species could provide a better understanding of the effects of climate on spring hatching and facilitate their control, thus maximizing the efficacy of pesticide treatments and reducing their frequency in commercial Christmas tree plantations.

Materials and Methods

Sample Collection and Experimental Arenas

Eggs from *M. abietinus* and *O. ununguis* were collected on balsam fir shoots in a commercial Christmas tree plantation (45.905°N, 71.036°W) near the municipality of Courcelles, in the Estrie administrative region of southern Québec, Canada. Samples were collected on the 6-III-2017, 13-III-2017, and 20-III-2017, when fir trees were still partially covered with snow. Since development rates calculated from all three collection dates did not vary significantly and only a small number of eggs were collected at each date, all data were

pooled for analysis. Shoots ($n = 200$) were randomly cut in a plot of ~675 trees (i.e., 15 rows of 45 trees), from their southern side. To avoid premature development within the egg after collection, all samples were kept at ~2°C, assumed to be near T_{\min} , until examined in the laboratory. When an egg was found, the shoot was cut down to about 1 cm in length and the needles were carefully removed with a razor blade. Shoot sections with eggs were placed in 2-ml microtubes (Starstedt AG & Co., Nümbrecht, North Rhine-Westphalia, Germany) with small holes pierced through the lid for air circulation. For manipulation purposes, two *M. abietinus* eggs were put into each microtube. *Oligonychus ununguis* eggs were usually clustered onto single shoots, so one randomly selected shoot section, containing several eggs, was allotted per microtube. All *C. pinifoliae* eggs were collected on the 13-III-2017 from another plantation (45.950°N, 70.923°W) near the municipality of Saint-Évariste-de-Forsyth, ~10 km from the first site, from a single-generation parthenogenetic form of the scale. Egg clusters, hidden beneath the waxy scale, were carefully removed with a fine brush, mixed together, and randomly distributed in microtubes (~20 eggs per microtube, see above). Throughout the experiment, we ensured that around 10 µl of water was kept inside the lid of all microtubes with eggs, in order to maintain constant high humidity.

Preliminary data, obtained in 2015 for a 25°C constant temperature regime for *M. abietinus* and *O. ununguis* and a fluctuating temperature regime for *C. pinifoliae* (i.e., 16 h at 25°C and 8 h at 30°C), were used as starting points to estimate the high temperature threshold T_{\max} for nonlinear development rate models. In 2016, eggs of all three species were collected similarly in the plantations described above on the 4-IV-2016, for a preliminary experiment on their developmental response to temperature. Unfortunately, eggs were collected too late in 2016 to provide meaningful estimates, but these preliminary data provided key information on appropriate collection dates and experimental temperatures for the critical experiment in 2017.

Experimental Temperatures

Experiments on postdiapause development of overwintering eggs as a function of temperature were started in March 2017. We used Conviron E15 (Controlled Environments Ltd., Winnipeg, Manitoba, Canada) growth chambers set to provide a photoperiod of 16:8 (L:D) h and 65% RH. On the basis of preliminary data (see above), five constant temperature regimes (11, 14, 17, 20, and 23°C) were used. Only the four higher temperatures were tested for *O. ununguis* due to a limiting number of egg clusters. Experimental temperature and relative humidity were recorded hourly inside each growth chamber with an HOBO Pro v2 data logger (Onset, Cape Cod, MA, USA), which confirmed that experimental temperatures remained within $\pm 0.5^\circ\text{C}$.

Analytical Methods

Eggs were inspected daily for hatching and mortality (i.e., failure to hatch). The estimated time to egg eclosion was assumed to have occurred at midpoint between two consecutive observations (± 12 -h precision). In addition, all unhatched eggs were kept for at least a week after the last observed hatch in the growth chamber, in order to assure that an accurate rate of survival was recorded.

Development Rate Models

Apart from the GDD linear model, three nonlinear development rate models, commonly used for illustrating temperature-dependent development in poikilotherms (Kontodimas et al. 2004), were tested

(Table 2). For model selection, two criteria were used (Roy et al. 2002): first, the model should describe the experimental data accurately, therefore the coefficient of determination (R^2) and the mean squared error (MSE) were compared between nonlinear models; second, the model should be able to predict field egg eclosion within an acceptable timeframe in order to apply the appropriate control measures in a timely fashion in a plantation (Trudgill et al. 2005).

Field Validation

To validate development rate models parametrized in the laboratory, the appearance of newly hatched immatures for the three species was recorded in the field, if present, during three consecutive springs (i.e., from 2015 to 2017) and compared with model predictions. For *M. abietinus* and *O. ununguis*, ~50 eggs or larvae were examined on shoots in the plantations described above on a weekly basis in 2015 and 2016, around the time when eggs supposedly hatched in the region, for at least 3 wk. For *C. pinifoliae*, shoots bearing scales were brought to the laboratory for closer inspection. In 2017, model predictions for egg eclosion of *M. abietinus* and *C. pinifoliae* were validated by observing shoots collected on the mean hatching day as predicted by the linear model. To calculate the accumulation of GDD in the plantation for a given day, we used an alternative interpretation of the average method (Equation 1) employed primarily in crop research (McMaster and Wilhelm 1997, Moore and Remais 2014), which accounts for frequent temperature fluctuation around the developmental threshold (T_{\min}):

$$\text{GDD} = \left(\frac{\delta_{\max} + \delta_{\min}}{2} \right) - T_{\min} \quad (1)$$

In this interpretation, the daily temperature minimum δ_{\min} equals T_{\min} if it falls below the developmental threshold (the same would go if the daily temperature maximum δ_{\max} falls below T_{\min}). This modification allows for the accumulation of physiological time even when daily minimum temperatures do not exceed T_{\min} . In 2015 and 2016, field temperatures were recorded hourly in both plantations throughout the spring with a HOBO Pro v2 data logger installed in the middle height section of a tree in the southeastern corner of the plantation. Since temperatures from both plantations actually varied within 1°C from each other (not shown here), we assumed that these recordings were representative of all eggs located in the area. Therefore, only one temperature logger was used in the Courcelles plantation in 2017.

Table 2. Linear and three nonlinear models of development rate as a function of ambient temperature, which are among the most commonly used to estimate insect biological temperature parameters (T_{\min} , T_{opt} , T_{\max} , and K)

| Model | Equation | Estimated parameters |
|--|---|--|
| Linear (GDD) | $R_{\text{dev}} = a + bT$ | T_{\min} and K_{GDD} |
| Hilbert and Logan (1983) | $R_{\text{dev}} = \Psi \left(\frac{(T - T_{\min})^2}{(T - T_{\min})^2 + d^2} \right) - e^{\left(\frac{-(T_{\max} - (T - T_{\min}))}{\Delta T} \right)}$ | T_{\min} , T_{opt} , and T_{\max} |
| Lactin-2 (Lactin et al. 1995) | $R_{\text{dev}} = e^{\rho T} - e^{\left(\rho T_{\max} - \frac{T_{\max} - T}{\Delta T} \right)} + \lambda$ | T_{\min} , T_{opt} , and T_{\max} |
| Kontodimas-16 (Kontodimas et al. 2004) | $R_{\text{dev}} = a(T - T_{\min})^2(T_{\max} - T)$ | T_{\min} , T_{opt} , and T_{\max} |

Empirical constants to estimate are a , b , d , and λ ; ψ represents the maximal development rate and ρ represents the development rate at T_{opt} . In the Lactin-2 and Hilbert and Logan models, ΔT is the range of temperatures above T_{opt} over which physiological breakdown becomes the overriding factor causing rapid development rate decline.

Statistical Analyses: Fitting Development

Rate Models

Data were analyzed with SAS 9.4 (SAS Institute, Cary, NC), using the reciprocal of eclosion time as the development rate for each experimental temperature T , in order to fit the four development rate models (Table 2). For nonlinear models, development rate data were used to parametrize each model by iterative nonlinear regression based on the Marquardt algorithm (Marquardt 1963). PROC NLIN was used to fit all nonlinear development rate models with initial values of graphically-estimated parameters (Brière et al. 1999). Later, biological temperature parameters were estimated either directly from the model or indirectly using other model parameters or the first derivative, where $d(R_{\text{dev}})/d(T) = 0$ for T in the Kontodimas-16 model. PROC REG was used to fit the linear model, with T_{\min} calculated indirectly as the unique extrapolated value of T when $R_{\text{dev}} = 0$, and the thermal constant K calculated as the reciprocal of the regression slope b (Table 2) (Ikemoto and Takai 2000, Trudgill et al. 2005).

Results

Development Time Between Species

All results are provided in Table 3 for this section. Of all three species, *M. abietinus* always had the shortest egg eclosion time for a given temperature regime. In comparison with the aphid, the development of *O. ununguis* eggs required almost twice the amount of time before hatching for a given temperature. Eggs of *C. pinifoliae* took the longest to hatch for a given temperature regime, more than doubling the development times of the mite eggs.

Accuracy and Precision of Development Rate Models

For *M. abietinus*, explained variation of development rate by the tested models (R^2) was relatively high, varying from 0.8784 to 0.9995, with MSE varying accordingly (Table 4A). The Kontodimas-16 model failed to match the developmental rates of *M. abietinus* eggs observed for temperatures below 14°C and above 20°C (Fig. 1A). Of the three nonlinear models tested, the Lactin-2 model scored highest overall and best predicted the development rates measured for *M. abietinus* as a function of temperature.

Table 3. Mean development time and percentage of hatching for postdiapause eggs of three species of Christmas tree herbivores reared in the laboratory under five experimental constant temperature regimes.

| Species | Temp (°C) | Initial no. of eggs | Mean development time ± 95% CI (d) | Hatching (%) [95% CI ^a] |
|--|-----------|---------------------|------------------------------------|-------------------------------------|
| <i>Mindarus abietinus</i> | 11 | 52 | 17.9 ± 0.5 | 78.8 [65.8 to 87.9] |
| | 14 | 46 | 11.3 ± 0.4 | 71.7 [57.4 to 82.8] |
| | 17 | 45 | 9.3 ± 0.4 | 84.4 [70.9 to 92.6] |
| | 20 | 46 | 7.7 ± 0.3 | 76.1 [61.9 to 86.2] |
| | 23 | 46 | 6.2 ± 0.3 | 65.2 [50.7 to 77.4] |
| <i>Oligonychus ununguis</i> ^b | 14 | 31 | 20.6 ± 0.8 | 87.1 [70.5 to 95.5] |
| | 17 | 44 | 17.1 ± 0.8 | 77.3 [62.8 to 87.3] |
| | 20 | 39 | 13.4 ± 0.5 | 89.7 [75.9 to 96.5] |
| | 23 | 29 | 10.4 ± 0.8 | 82.8 [65.0 to 92.9] |
| <i>Chionaspis pinifoliae</i> | 11 | 97 | 144.9 ± 12.9 | 33.0 [24.4 to 42.9] |
| | 14 | 87 | 52.4 ± 2.6 | 19.5 [12.5 to 29.2] |
| | 17 | 122 | 35.6 ± 1.2 | 29.5 [22.1 to 38.2] |
| | 20 | 124 | 27.3 ± 0.7 | 49.2 [40.6 to 57.9] |
| | 23 | 125 | 21.1 ± 0.7 | 64.0 [55.3 to 71.9] |

^a95% CI of hatching rates were estimated with the modified Wald method for binomial proportions.

^bNote that only four constant temperature regimes were tested for *O. ununguis*.

Table 4. Parametrization of the linear and three nonlinear models for (A) *Mindarus abietinus*, (B) *Oligonychus ununguis*, and (C) *Chionaspis pinifoliae* postdiapause egg development rate, with goodness-of-fit statistics (MSE and R^2) and biological temperature parameters estimated directly ($\pm 95\%$ CI) or indirectly from the model.

| Species | Model | Mean squared error | R^2 | Biological temperature parameters | | | |
|---------|-----------------|--------------------|--------|-----------------------------------|-----------------------|-----------------|--------------|
| | | | | T_{\min} (°C) | T_{opt} (°C) | T_{\max} (°C) | K^a (GDD) |
| A | Linear | 0.0002 | 0.9992 | 5.4 ± 0.1 | n/a | n/a | 105.9 ± 6.3 |
| | Hilbert & Logan | 0.0002 | 0.8401 | -0.3 ± 3.0 | 25.7 | 25.7 ± 3.1 | n/a |
| | Lactin-2 | 0.0002 | 0.9995 | 4.9 | 25.5 | 25.7 ± 0.0 | n/a |
| | Kontodimas-16 | 0.0008 | 0.8784 | 7.7 ± 0.6 | 19.6 | 25.6 ± 0.2 | n/a |
| B | Linear | 0.0001 | 0.9922 | 7.1 ± 0.1 | n/a | n/a | 161.2 ± 16.6 |
| | Lactin-2 | 0.0001 | 0.9982 | 6.8 | 25.6 | 25.8 ± 0.0 | n/a |
| | Kontodimas-16 | 0.0002 | 0.8963 | 10.5 ± 0.6 | 20.5 | 25.5 ± 0.1 | n/a |
| C | Linear | 0.0000188 | 0.9999 | 9.3 ± 0.1 | n/a | n/a | 277.8 ± 11.2 |
| | Hilbert & Logan | 0.0000189 | 0.6294 | 6.7 ± 1.1 | 22.3 | 23.1 ± 1.1 | n/a |
| | Lactin-2 | 0.0000188 | 0.9999 | 9.4 | 24.1 | 26.9 ± 1.3 | n/a |
| | Kontodimas-16 | 0.0000284 | 0.5818 | 8.3 ± 0.7 | 23.3 | 30.9 ± 0.0 | n/a |

^a K is the reciprocal of the slope parameter b in the linear model.

As for *O. ununguis* eggs, R^2 values varied between 0.8963 and 0.9982, with the MSE varying accordingly (Table 4B). Similar to *M. abietinus*, the Lactin-2 model scored highest and best predicted the development rates of the mite for all experimental temperatures. The Kontodimas-16 failed to predict development rates for at least half of the experimental temperatures (Fig. 1B) and the Hilbert and Logan model failed to converge and was thus excluded for *O. ununguis*.

Finally, *C. pinifoliae* development rate models showed similar trends, with the Lactin-2 model scoring highest among the nonlinear models and predicting similar development rates for experimental temperatures as the linear model (Table 4C, Fig. 1C). For this species, the Hilbert and Logan model failed to predict the development rates for all five experimental temperatures. For all three species, the linear model had a relatively high R^2 and accurately predicted development rates (Table 4).

Biological Temperature Parameters

Estimated parameters provided by all four tested models are given in Table 4, according to species. Although the nonlinear models estimated all three critical temperature parameters (i.e., T_{\min} , T_{opt} , and

T_{\max}), the Lactin-2 model provided the T_{\min} closest to those estimated by the linear model. Since T_{\max} was estimated based on preliminary data only, nonlinear model predictions most likely provide a minimum estimate for the actual thermal maximum. Fitted model parameter estimates (\pm SE) can be found in the Supp Table 1 (online only).

Field Validation of the Linear and Lactin-2 Development Rate Models

For 2015, 2016, and 2017, predicted dates for egg eclosion in the field were estimated using temperatures recorded in situ from the plantation where eggs came from (Table 5). For validation of the Lactin-2 model, hourly development rates (and corresponding physiological time) were calculated for continuously recorded field temperatures (i.e., one measure per hour) and added sequentially until a total of 1.00 was reached, indicating that egg development should then be complete on average. For all three species, the accumulation of physiological time in the field was assumed to have begun on March 1, based on preliminary data (not shown here). Parameters of the linear model, in conjunction with daily maximum and minimum temperatures (see Equation 1), were used to follow

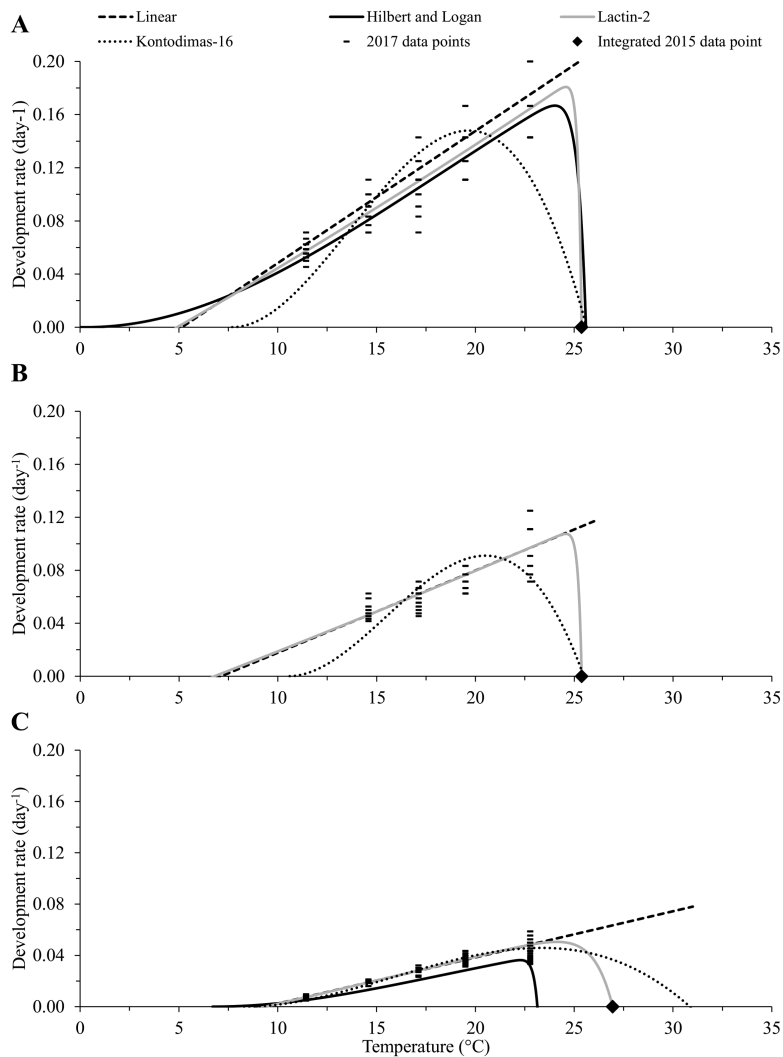


Fig. 1. Observed eclosion of postdiapause eggs of (A) *Mindarus abietinus*, (B) *Oligonychus ununguis*, and (C) *Chionaspis pinifoliae* (data points), as a function of temperature, with the linear and three nonlinear development rate models fitted to the 2017 data.

the accumulation of physiological time (i.e., K in GDD) in the field for all 3 yr (Fig. 2). Here, daily GDD accumulation predicts eclosion, based on the linear model, to occur where it crosses the gray band. On the basis of field observations, the egg hatching period lasted less than a week in 2015 and 2016 (not shown here). Therefore, we can conclude that the linear model predicted egg eclosion within more or less 5 d of the observed date in the field for all three species, most likely within 1 or 2 d of the actual median date of eclosion (Table 5). In 2017, we observed near complete hatching of *M. abietinus* eggs on 28 April, according to the linear model prediction. For *C. pinifoliae*, we observed ~50% hatching on the very date predicted by the linear model. The Lactin-2 model predicted field egg eclosion to occur between 4 and 11 d later than field observations, relatively late in the actual eclosion period, depending on species (Table 5).

Discussion

No published study has experimentally examined the effect of such a range of ambient temperatures on the development rates of *M. abietinus* and *O. ununguis*, the two most important arthropod pests in commercial Christmas tree plantations of southern Québec. As for

C. pinifoliae, which is known mainly to attack *Pinus* trees (Nielsen and Johnson 1973), we hereby provide new insight on its temperature requirements for populations of southern Québec. Interestingly, the Lactin-2 model did not provide sufficiently accurate predictions for egg eclosion in the field when compared with the linear model. Predicted eclosion dates with this model using hourly-cumulated field temperatures apparently overestimated development time in the field because predictions for all three species fell behind those provided by the traditional linear model (Table 5). In the case of *C. pinifoliae*, the Lactin-2 model predicted mean egg eclosion by up to 11 d later than field-observed hatching. Since the scale eggs hatched around a month later than both other species, the predictive error of the Lactin-2 model on hatching could have increased with time since March 1, which may explain such a difference between predicted and observed eclosion dates for this species. Temperatures around 25°C were recorded in May of 2015 and 2017 (J.-F. Doherty, Université Laval, unpublished data), which would have decreased development rates according to the Lactin-2 model for *C. pinifoliae* and delayed hatching predictions for both years (Fig. 1).

Fitting the Lactin-2 model including the high temperature threshold, based on preliminary observations, may have decreased

Table 5. Predicted eclosion time (date, dd-mm) for eggs of *Mindarus abietinus*, *Oligonychus ununguis*, and *Chionaspis pinifoliae* in Christmas tree plantations, with observed proportions of egg eclosion for 2015, 2016, and 2017

| Species | 2015 | | | 2016 | | | 2017 | | |
|---|-----------------------|--------------|----------------|-----------------------|--------------|----------------|-----------------------|--------------|----------------|
| | Date (% egg hatch) | Linear model | Lactin-2 model | Date (% egg hatch) | Linear model | Lactin-2 model | Date (% egg hatch) | Linear model | Lactin-2 model |
| <i>Mindarus abietinus</i> | 5-V (~90%) | 6-V | 9-V | 5-V (~75%) | 5-V | 12-V | 28-IV (~75%) | 28-IV | 6-V |
| <i>Oligonychus ununguis</i> ^a | 15-V (~40%) | 17-V | 25-V | n/a | n/a | n/a | n/a | n/a | n/a |
| <i>Chionaspis pinifoliae</i> ^b | 11-VI (~45%) | 9-VI | 22-VI | n/a | n/a | n/a | 14-VI (~50%) | 15-VI | 24-VI |

Predictions based on the linear model (summed with Equation 1) and the Lactin-2 model. See Table 2 for model equations.

^aDue to very low population densities of *O. ununguis* in the field, accurate validation of the models based on field observations of egg eclosion in 2016 and 2017 was not possible.

^bDue to insufficient sampling, model validation based on field observations of eclosion for *C. pinifoliae* in 2016 was not possible.

overall development rate predictions for lower temperatures. Since nonlinear models usually include critical temperature thresholds, the assumption that development rates are nil at precisely-determined temperatures may limit their capacity to predict actual development rates around T_{\min} and T_{\max} . Experimental fluctuating temperature regimes around these critical thresholds could be tested to better estimate corresponding development rates, which might improve our capacity to predict field egg eclosion using nonlinear models.

We conclude that the linear model predicted within 5 d before or after the mean date of eclosion, despite the assumption that physiological time for egg hatching in the spring is a constant (K) in this modeling approach. This evidently cannot be true if the development rate–temperature relation is curvilinear, except for temperatures between T_{\min} and T_{opt} . However, our field data from 2017 indicate that a linear model was useful to predict postdiapause egg eclosion for the last 3 yr, especially for *M. abietinus*. The hatching periods in the field for all three species, depending on temperature conditions, were short and rarely took more than 5 d. On relatively hot days, hatching could be near complete in a single day, which was observed in 2017 for *M. abietinus*. Therefore, the 95% CI of K could indicate the emergence period of first generation immatures of the aphid with sufficient accuracy. Since field consultants and growers usually monitor pest emergence on a weekly basis, these models can help focus screening efforts in the field to a certain period of the season for plantations located in southern Québec.

The average method (i.e., Equation 1) for calculating daily accumulation of GDD is generally accurate when daily temperatures oscillate between $\delta_{\min} < T_{\min}$ and $\delta_{\max} < T_{\max}$ (Moore and Remais 2014), which usually occurs during springtime. Since all five experimental temperatures tested are most likely within the intermediate linear response range, sufficient data to efficiently parametrize the models were provided only by the linear part of the realistic asymmetrical curve. However, more experimental temperatures, especially below those of this study, may still improve field predictions of egg eclosion with nonlinear development rate models, five temperatures being a recommended minimum (Bergant and Trdan 2006). The estimation of T_{\min} by extrapolation beyond the linear fit from five temperatures tested exposes a weakness in the linear model's ability to properly estimate this lower threshold, as it is quite possible that the actual T_{\min} lies somewhere below the one provided by the linear model and that some development does occur below T_{\min} , despite being very slow (Lamb 1992).

There is a trend between all three species with regard to their development times and hatching periods in the field. Predictions based on the linear model with daily GDD updating indicate a similar

pattern of eclosion (Table 5), verified by field monitoring, except for *O. ununguis* in 2016 and 2017, due to very low mite population densities. This pattern of field eclosion is inversely correlated with postdiapause development rates. *Chionaspis pinifoliae* had the lowest egg development rates at all experimental temperatures and its eggs also hatched later in the field. The development times of these species are also correlated with estimated low temperature thresholds. *Chionaspis pinifoliae*, which has the highest T_{\min} estimated at $9.3 \pm 0.1^\circ\text{C}$, is also the last species to emerge in late spring.

The first species to emerge is *M. abietinus*. Its Holarctic distribution matches that of its principal host tree, the balsam fir (Martineau 1984). An estimated T_{\min} of $5.4 \pm 0.1^\circ\text{C}$ and a relatively fast postdiapause development rate in the 11–23°C range can help explain its early eclosion in the field, relative to both other species. Since stem mothers must hatch and complete their early nymphal stages before bud break around mid-May (Doherty et al. 2017), faster postdiapause development rates than the mite and the scale are expected. Early egg eclosion may assure the timely migration of stem mothers onto newly exposed shoots to start producing colonial offspring on the youngest stages of fir shoot growth. For this timely development to occur in southern Québec, postdiapause egg development should be possible at temperatures as low as 5°C, which currently are most likely experienced in late April. Previous estimates for T_{\min} and K dating back from the 1990s in southern Québec for *M. abietinus* (i.e., 95 GDD above 2°C), based solely on field observations (Deland et al. 1998, Berthiaume et al. 2001), underestimate development times when compared with those estimated here (i.e., 105.9 GDD above 5.4°C). For a slightly more southern climate, thermal requirements for *M. abietinus* egg eclosion were estimated at around 53 GDD above 10°C in Lower Michigan, based on field observations (in this case, T_{\min} was arbitrarily set to an agricultural standard for crop plants) (Fondren and McCullough 2003).

The emergence of *O. ununguis* came second during springtime, about 2 wk later than the aphid in the plantation. We speculate that unrequired synchronicity between the hatching of *O. ununguis* immatures and fir bud break, assuming that the earliest active stages of the mite can feed on both the new and past-year foliage, may partly explain why the mite eggs hatch later in the season than *M. abietinus*, whose colonies in pseudogalls can only form on early-stage shoots before elongation. In Ohio, spruce spider mite eggs hatched during the final 2 wk of April 1993 and 1994 at 143.3 ± 30.1 GDD above a T_{\min} of 7.1°C (Richmond and Shetlar 1996). In this Québec study, the mite population of a higher latitude requires approximately the same amount of physiological time above a similar threshold for egg eclosion (i.e., 161.2 GDD above 7.1°C). Since only 1 yr of field

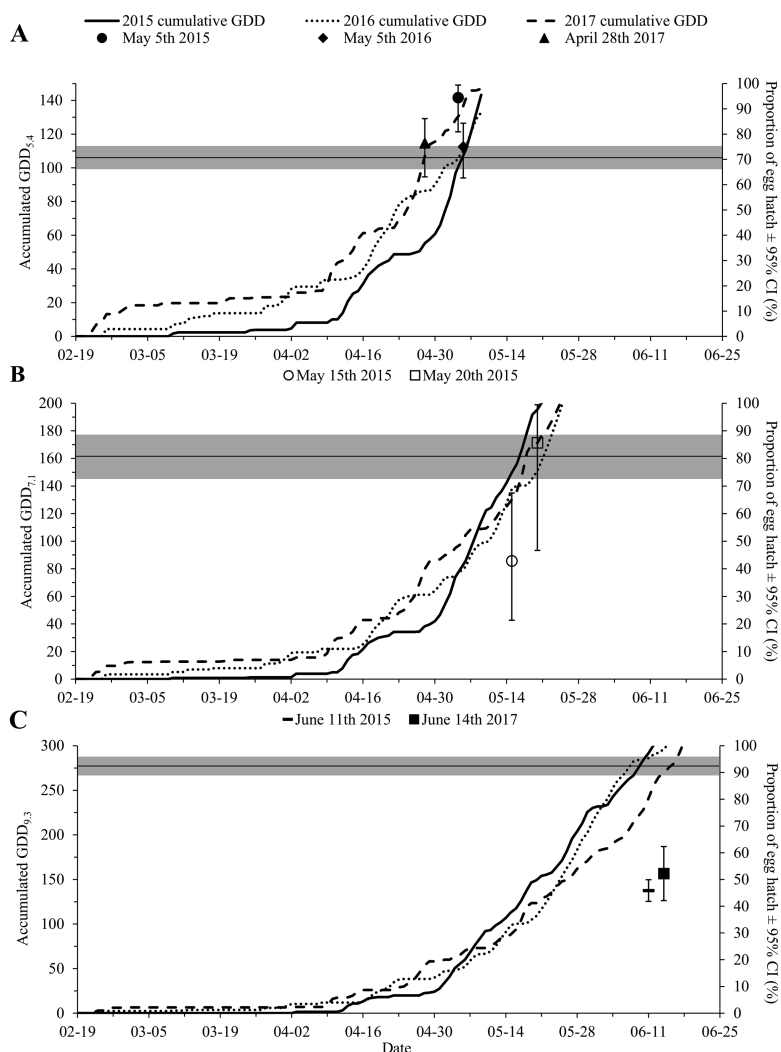


Fig. 2. Cumulative field GDD above threshold T_{\min} ($^{\circ}\text{C}$) (summed with Equation 1) based on the linear model, with the observed proportion of eggs hatched in the field \pm 95% CI (calculated with the modified Wald method for binomial proportions) for (A) *Mindarus abietinus*, (B) *Oligonychus ununguis*, and (C) *Chionaspis pinifoliae*. Gray horizontal bands delimit the 95% CI of the thermal constant K , which is represented by a thin horizontal line.

sampling in 2015 was performed due to very low mite population densities in the area, it is difficult to say to which extent this new model is generally valid for populations in southern Québec.

The last species to emerge in late spring, *C. pinifoliae*, has the highest estimated T_{\min} and clearly requires more physiological time than the aphid and the mite for spring egg hatching, with field eclosion occurring considerably later during the second week of June (Table 5). Relatively lower hatch rates were observed for this species in the growth chambers (Table 3), possibly due to the removal of the protective waxy scale covering egg clusters, which was done to allow random assignment to experimental temperatures. Since eggs in the microtubes were not protected by their scale as in the field, these unnatural conditions could partly account for the lower survivorship observed (Table 3). The presence of the waxy scale on unaltered fir needles and the fact that *C. pinifoliae* scales were concentrated on the lower branches of fir trees (personal observation), microclimatic conditions could differ when temperatures plummet at sunset. This could partly account for the slight differences in the observed proportions of egg eclosion in the field when compared with what was predicted by the linear model and the average method of GDD accumulation (Table 5). Because proper identification of the pine needle scale is complex, due to its close proximity

with *C. heterophyllae* (Philpott et al. 2009), the possibility of cryptic species (Gwiazdowski et al. 2011, Veà et al. 2012), and the existence of both sexual and parthenogenetic forms (Stimmann 1969, Luck and Dahlsten 1974), our development rate models for this scale may not apply generally to different populations. More work would be needed to determine whether heat requirements vary among these forms/species. As for the existing model for populations of lower latitudes (Table 1), it underestimates field egg eclosion for temperatures recorded in southern Québec.

The fact that all three species overwinter as diapausing eggs implies that the collection date is a crucial point in calculating the accumulation of physiological time in the field, assuming that the linear model is correct and that GDD accumulation for egg development is a constant. In this study, we assumed that eggs collected around mid-March had not yet accumulated GDD. On the basis of preliminary observations (2015 and 2016, unpublished data), we assumed that overwintering eggs had completed their diapause by this time and were thus ready to accumulate physiological time in our experiment. However, if eggs were collected either prior to diapause termination or after egg development had started, the models could either overestimate or underestimate eclosion time in the field. For this experiment, we can assume that eggs had not started

developing by mid-March when they were collected, or that prior development was negligible, based on temperature recordings in the plantation (data not shown here).

In conclusion, this study has provided new linear models for postdiapause egg development of three foliage-feeding arthropod pests in commercial Christmas tree plantations of southern Québec. The data support our hypothesis that the existing prediction tools, based on thermal requirements for other parts of North America or only from field observations, are not applicable to current populations from southern Québec, except perhaps to *O. ununguis*, whose low population densities prevented model validation. The sequential pattern of field eclosion for each species can be predicted from egg development rates measured in the laboratory for temperatures within their tolerable range. The linear models predicted mean hatching in the field with sufficient accuracy, as confirmed using field data from three consecutive years. Modeling based on experimental temperatures near the low and high thresholds (i.e., T_{min} and T_{max}) should increase the value of nonlinear models in predicting development rates and field egg eclosion, considering that they do not depend on the unrealistic assumption that the effect of temperature on development rates remains linear over the whole range of viable and developmentally useful thermal conditions.

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Supplementary Data

Supplementary data is available at *Environmental Entomology* online.

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