Natural Resources Canada | Ressources naturelles Canada

Validation of a spruce budworm phenology model across environmental and genetic gradients: applications for budworm control and climate change predictions

Interim Report

Jean-Noël Candau, John Dedes, Chris J.K. MacQuarrie, Kerry Perrault, Amanda Roe and Ashlyn Wardlaw

Natural Resources Canada
Canadian Forest Service

Information report GLC-X-20

Canada

Validation of a spruce budworm phenology model across environmental and genetic gradients: applications for budworm control and climate change predictions

Jean-Noël Candau, John Dedes, Chris J.K. MacQuarrie, Kerry Perrault, Amanda Roe and Ashlyn Wardlaw

Published by:
Natural Resources Canada
Canadian Forest Service
Great Lakes Forestry Centre
1219 Queen Street East
Sault Ste. Marie, Ontario P6A 2E5

Information Report Number: GLC-X-20
2018

Cataloguing information for this publication is available from Library and Archives Canada.

Validation of a spruce budworm phenology model across environmental and genetic gradients: applications for budworm control and climate change predictions.
(Information Report, GLC-X-20)

Issued also in French under the title: "Validation d'un modèle de phénologie de la tordeuse des bourgeons de l'épinette pour les gradients environnementaux et génétiques : applications pour la lutte contre la tordeuse et les prévisions relatives aux changements climatiques".
J.-N. Candau, J. Dedes, C.J.K. MacQuarrie, K. Perrault, A. Roe, et A. Wardlaw

Electronic monograph in PDF format.
Includes bibliographical references.
ISBN 978-0-660-25660-3 ISSN 2562-0738 (online)
Cat. no.: Fo123-2/20-2018E-PDF

Table of Contents

List of Tables

List of Figures

List of Appendices and Figures

Appendix A: Simulated vs observed development times of the IPS colony at 7 constant temperatures - Overall development

Appendix B: Simulated vs observed development times of the IPS colony at 7 constant temperatures - Larval stage development

Abstract

This report presents the results of an ongoing investigation into the variability of spruce budworm (*Choristoneura fumiferana* (Clemens)) development rates over its geographic distribution. The first phase of the study involved rearing laboratory colonies from the Insect Production Services (IPS) in Sault Ste. Marie at seven constant temperatures (i.e. 5-35°C), which allowed us to develop and test rearing protocols. It also provided an opportunity to assess the accuracy of BIOSIM's Spruce Budworm Biology Model predictions for the phenology of laboratory colonies that have been kept in isolation from wild populations for many generations. Our results indicate that the model fits the phenology of the laboratory colonies very well over the 15-25°C range. For lower and higher temperatures (5-10°C and 30-35°C, respectively), the model tends to overestimate development rates. In 2017, we successfully completed the second phase of the project, which involved establishing colonies from wild budworm populations sampled in five locations across its geographic distribution, from Inuvik to northern Quebec. A trend analysis of simulated budworm phenology at these five locations for the period 1998-2017 reveals a trend towards a faster overall larval development (L2-L6) that is statistically significant for Inuvik (NT) and almost significant for High Level (AB). The samples collected in these locations were reared through to the next generation to provide enough individuals in each colony to run seven temperature experiments. Preliminary results for the Ontario colony reared at 30°C and 35°C are similar to what was observed with the IPS colonies, i.e. the current phenology model tends to overestimate the development rate at these temperatures. Other temperature treatments for Ontario and Quebec are ongoing while the other colonies have not yet completed their obligatory diapause.

Introduction

The ability to accurately predict spruce budworm phenology is important in many aspects of the management of this insect. In control programs, the efficacy of all current spruce budworm integrated pest management strategies hinges on the ability to target the right development stage. In predicting population dynamics, a reliable model of adult emergence phenology is a key component in modeling landscape-scale dispersal, a factor that is increasingly recognized as being critical in budworm large-scale dynamics. In longer-term predictions, an accurate knowledge of budworm phenology, intrinsically and in relation to its hosts' phenology, is of particular importance in our ability to predict the potential impacts of climate change on the distribution of this insect.

The most accurate model currently available to predict the phenology of spruce budworm over large areas is BIOSIM's Spruce Budworm Biology model (Régnière et al. 2014). This model has been tested against field observations on several occasions. In most cases, it was able to predict budworm phenology with exceptional accuracy. However, it is important to note that the data used so far to develop and validate this model have come from a fairly limited geographic range (i.e., roughly between 45°-50°N and 70°-90°W). A comparison with the current distribution of spruce budworm (between 43°-68°N and 55°-133°W) ultimately questions the limits of applicability of the model. This project investigates the variability of spruce budworm development rates for each larval stage across the geographic distribution of the insect. Particular attention is given to detecting clinal variation in development rates related to

environmental and/or genetic gradients. Comparing the observed rates to Régnière's model for a wide distribution of locations will help better define the geographic limits of applicability of the model.

Part 1: Development rate of laboratory colonies at different temperatures

The first step in this project consisted of rearing laboratory colonies at different constant temperatures. The main objective of this exercise was to test if the relationships between temperature and development rates in these colonies, which had been reared for dozens of generations at constant temperature regimes, had "drifted" from the observations made 30 years prior. It also provided the opportunity to develop and test specific rearing protocols, test the rearing equipment and train staff. To our knowledge, the mass rearing of spruce budworm at different temperatures through its entire life cycle had not been carried since the mid-1980's, so most of the expertise in this type of experiment had to be rebuilt.

Material and Methods

Laboratory colonies were obtained from the IPS at the Great Lakes Forest Centre (Sault Ste. Marie). Each temperature experiment started with a large number of overwintering L2s that were kept at 25±3°C, 55±10%RH and 16L:8D, regardless of the experimental temperature treatment, until emergence from diapause. From the emerged L2s, 250 larvae were sampled, placed in individual cups with artificial diet (provided by IPS), and labeled for the experiment. All 250 cups were then placed in a growth chamber (Conviron) at a set experimental temperature. We tested seven temperature treatments: 5°C, 10°C, 15°C, 20°C, 25°C, 30°C, and 35°C, all ±1°C with 70±20%RH and 14L:10D. The temperature in each chamber was recorded every minute with a data logger (Hobo). Out of the 250 larvae in each chamber, 200 were monitored daily for molting, the remaining 50 were left untouched until pupation and used as backup for mating and genetic analyses. Daily monitoring consisted of checking the status (live/dead) and larval stage of each larva. When the development reached pupal stage, the sex of each individual was assessed by counting the number of abdominal rings. Adults were mated shortly after emergence. After harvesting, the eggs were hatched at 20°C regardless of the temperature treatment. First stage larvae were reared at the same temperature to pre-diapause L2s. The pre-diapause L2s were then placed at 4°C for 24 weeks for diapause. The rearing protocol was repeated for the second generation; the temperature treatment of each F1 was identical to the treatment for its parent population.

Extreme temperatures near development thresholds (high and low) have detrimental effects on larval development and result in high rates of mortality. Development rates at these temperatures have to be estimated using temperature transfers (Régnière et al. 2012). This technique consists of rearing the larval stage at the extreme temperature for a period of time long enough to measure development but not so long as to induce high levels of mortality, then transferring to a more favorable temperature to complete the development stage, then repeating for the next larval stage. Temperature transfers were applied to the 5°C, 10°C, 30°C and 35°C temperature treatments. All transfers were made to 20°C to complete stage developments.

Observed development rates were compared to Régnière's phenology model by applying the set of equations published in Régnière et al. 2012 to the temperatures recorded in each chamber.

Results

We will present the results for 5°C-35°C for the first generation here. The experiments for the second generation are still ongoing at the time of this report. Simulated and observed development rates are represented here as average instar (Appendix A) and larval stage development times (Appendix B).
At 5°C (Fig. A1), the simulated development is consistently faster than observed (R^2=0.69). The differences are particularly pronounced at the L2 and L4 stages (Fig. B1). Observed days to molt at the L4 stage are 15 days longer than simulated. This difference in development time can be partly due to the fact that BIOSIM will simulate an immediate increase in the development rate when the larvae are transferred to 20°C whereas there is likely a delayed response due to physiological constraints. This difference is compounded by the fact that BIOSIM slightly overestimates the development rate at 20°C (see below). Considering the importance of the response of early stages of development to temperatures in the overall timing of the post-diapause phenology and the likelihood that early stages are exposed to low temperatures, it is critical to improve the development model for early stages at low temperatures. The model improves in simulating the overall development at 10°C (R^2=0.82, Fig. A2). The development rates of the L2, L4 and L5 stages at this temperature are overestimated (Fig. B2). At 15°C, the fit of the overall development (Fig. A3) is excellent (R^2=0.98). The development rate of the L2 stage is overestimated by the model. The model underestimates the development rates of later stages (L5 and L6). The model fits very well with the development at 20°C (R^2=0.97) with only a slight overestimation after L3 (Fig. A4). At 25°C (Fig. A5), the model simulates overall development very well (R^2=0.99). L2 and L4 development rates are slightly overestimated while the rate of later instars are underestimated, particularly the female L6 stage. The performance of the model is not as good at higher temperatures. At 30°C and 35°C (Fig. A6 and A7, respectively), the model does not perform as well (R^2=0.68 R^2=0.81, respectively); it overestimates the development rate of the L2 stage at 30°C and 35°C and underestimates the rate at later stages (Fig. B3 and B4).

Discussion

In summary, the current phenology model performs very well in predicting the larval development of IPS laboratory colonies for temperatures ranging from 15-25°C. This suggests that the phenology of the current colonies is not noticeably different from the phenology of the colonies used to parametrize the model over three decades ago. The model does not perform as well at extreme temperatures, particularly 5°C and 30°C. The temperature transfers required at these temperatures (see Methods above) are simulated as instantaneous shifts from one development rate to the other, which is very unlikely to reflect what happens physiologically. Indeed, there is likely a period of time necessary for the physiology of the larva to adjust to the new temperature. This could in part explain why the model almost systematically overestimates the development rate at these temperatures. It also points to the challenges of using estimates of development rates measured at constant temperatures to model development at variable temperatures.

Part 2: Development rates across environmental and genetic gradients

To assess the variability of budworm development rates across its geographical range requires sampling different locations and establishing laboratory colonies from each sample. To our knowledge, no new colonies of spruce budworm have been established from wild populations since the development of the IPS colonies over 30 years ago. The task can be challenging because of the absence of published protocols and potential high levels of mortality and low fecundity due to microsporidia and viruses. In an effort to minimize the risk of disease cross-contamination that would lead to colony collapse, we reared the larvae individually and mated adults in separate pairs (vs a large number of males and females in the same container). Infection loads were checked regularly throughout the process so infected individuals could be discarded. It took three staff from March to Oct. 2017 to complete the rearing of five populations from flushing diapausing L2s to the next generation of L2s in diapause. Below, we will describe the protocol we used and the results of the first temperature treatments for the Ontario population.

Sampling and rearing methods for wild populations

The objective of the sampling was to cover the maximum range of budworm geographical distribution. At the same time, samples had to be collected from areas that were currently or recently at outbreak population levels to maximize the chances of having enough larvae to start colonies. We believe that the five populations we sampled (Fig. 1) were the maximum that could be done considering the current population levels across the range. If outbreaks were to develop in other areas, it would be interesting to add sampling locations between Alberta and Ontario, in the southern edge of the distribution, and in Newfoundland.

Our sampling protocol followed Morris (1955): 3 branches, each between 75-100 cm in length, were sampled from the crown of approximately 30 trees in each location except for the Quebec population, which was sampled in 18 locations along a transect from Manic-5 to Fermont. The branches were separated by source tree and stored in canvas or plastic bags for storage and shipping. The samples were collected from June 2016 to May 2017 (Table 1).

Table 1. Summary of sampling locations.

Prov/T	Date	Location	Lat	Long	Host	Collector credit
NT	02-05-2017	Inuvik	67.5	-133.7	Sw	Martin Callaghan (For. Mgt. Division, Gvt of NT)
AB	05-04-2017	High Level	58.6	-118.2	Sw	R. Hermanutz (Alberta Agriculture and Forestry)
ON	01-03-2017	Timmins	48.6	-82.1	Fb, Sb	G. Brand (CFS-GLFC)
QC	19-09-2016	Manic-5	50.7	-68.7	Fb, Sb	L. deGrandpré and his crew (CFS-LFC)
NB1	28-06-2016	Campbellton	47.8	-66.4	Fb	R. Johns and his crew (CFS-AFC)
NB2	07-03-2017	Balmoral	47.8	-66.7	Fb	R. Johns and his crew (CFS-AFC)

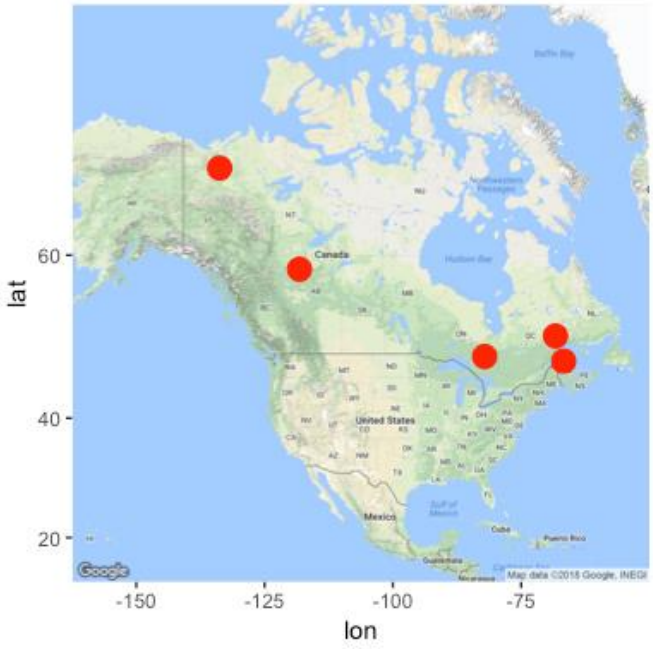


Figure 1. Location of wild populations sampled in 2017.

Flushing Methods

If the samples were collected in the spring, flushing was carried out immediately after receiving the branches. Branches that were collected in fall or winter during diapause were stored at 4°C, 70% RH in the dark until the following spring. Traditionally, there are two methods of flushing overwintering larvae from branches (Sanders, 1980). The "paper cone" method consists of wrapping branch samples cut in small bundles in a cone-shaped paper suspended over a wash basin filled with water. Emerging L2s are collected while they are crawling on the surface of the paper or floating on the surface of the water after they have dropped from the branches. The "enclosed box" method consists of placing the branches in pyramid-shaped opaque boxes fitted with transparent vials at the top that are pointed towards a bank of lights. Phototactic larvae are collected from the vials. The two methods were compared using the Ontario and Quebec samples. The enclosed box method was much less successful than the paper cone method. There were several issues with the enclosed box method that may have limited its success. First, mold developed in the boxes that were set up with water-soaked paper towel around the base of the branches. The high level of mold in the boxes may have caused mortality or limited upward movement of the larvae. Open glass vials containing water-soaked gauze were a second option to allow some moisture in the box without directly touching the branches. This seemed to have little effect on emergence versus completely dry boxes, but did limit mold growth. Third, many of the boxes had noticeable levels of mortality inside the bottom of the box. If this method is attempted, attention needs to be paid to humidity levels. It is also recommended that branch segments placed inside the box stay as intact as possible, and that boxes are not overstuffed, allowing the best conditions for larvae to move upwards. In contrast to the enclosed box method, the branches in the paper cone method are fully visible during emergence, allowing more larvae to be spotted and collected rather than relying on them to maneuver to a collection area on their own. Additionally, moisture levels can more easily be

controlled with the branches exposed. One issue with this method is high levels of needle loss. Water basins need to be cleaned almost daily with a straining device after collection of larvae from the water surface. Sometimes, the amount of needles in the basin hindered collection because they obscured the water's surface. Additionally, water had to be changed weekly in order to prevent mold growth on stray needles floating in the water. A final issue with both methods was the collection of non-target species. When the larvae are in the L2 stage, they are hard to identify by species. Larvae were often not identified as a non-target species until several instars later, when they had already been placed on diet. At that time, they were discarded. The number of larvae flushed from the samples ranged from 4466 in NB to 648 in NT (Table 2).

Rearing Methods

Once larvae were collected, they were individually placed on McMorran artificial spruce budworm diet in ¾ oz cups with a cardboard lid. Since the populations were wild and contained possible pathogens, the diet contained Fumidil-B at 4000 ppm (van Frankenhuyzen et al. 2004). They were labelled by population and collection date, and placed into a 20° C walk-in chamber. Diet was changed every 14 days. For each population, a subsample of 10-12% of L4 or L5 stage larvae was tested for pathogen load. The results of these tests (Table 2) dictated the level of isolation needed for mating the adults in each population. Populations with low or no presence of virus or fungal infection were placed into several group matings as well as several single matings. If pathogen levels were medium to high, only single matings (one to two males and one to two females per mating group) were used. After single matings were completed, every adult female used for mating was sent to the quality control laboratory for pathogen testing. If fungi or more than one type of virus was found, the eggs produced by that mating were discarded. This procedure is necessary because of the potential vertical transfer of microsporidia (van Frankenhuyzen et al. 2007). Microsporidia infection rates were high in AB, while NPV and EPV were high in ON and QC (Table. 2).

Table 2: Infection rates in wild samples collected in 2016-17.

Origin of Population	No. of larvae flushed	% Microsporidia	% NPV	% EPV
AB	2370	36.5	8.7	0.0
NT	648	1.2	9.5	0.0
NB	4466	6.8	15.0	1.6
ON	1084	10.8	41.2	5.9
QC	765	0.0	33.9	32.1

An analysis of 20 years of simulated spruce budworm phenology across its distribution

While our rearing experiments of wild colonies are starting to produce empirical data to measure the variability of spruce budworm phenology across its natural range, we can already establish a baseline by simulating budworm phenology for different locations using historical weather data and the current phenology model. We are presenting here an analysis of the simulation of budworm's phenology for the past 20 years in locations across its distribution range. We downloaded Environment Canada's daily temperature data for the past 20 years for five weather stations close to the wild populations' sampling locations, i.e. Inuvik (NT), High Level (AB), Timmins (ON), Baie Comeau (QC), and Fredericton (NB).

We used Sen's (1968) non-parametric method to test for linear trends in the time-series, and Pettit's (1979) test to identify change points (i.e. abrupt changes in the time-series). For the period 1998-2017, annual mean daily temperatures for each location (Fig. 2 show a significant warming in Inuvik (+0.11°C/year, $p<0.001$). The graphs seemed to indicate a slight cooling in eastern Canada but no significant trend was detected. The change point analysis detected a probable change in Inuvik in 2007 with an increased warming rate (+0.17°C/year, $p<0.001$ for 2007-2015). Change points were detected in the other locations but they did not reveal significant trends.

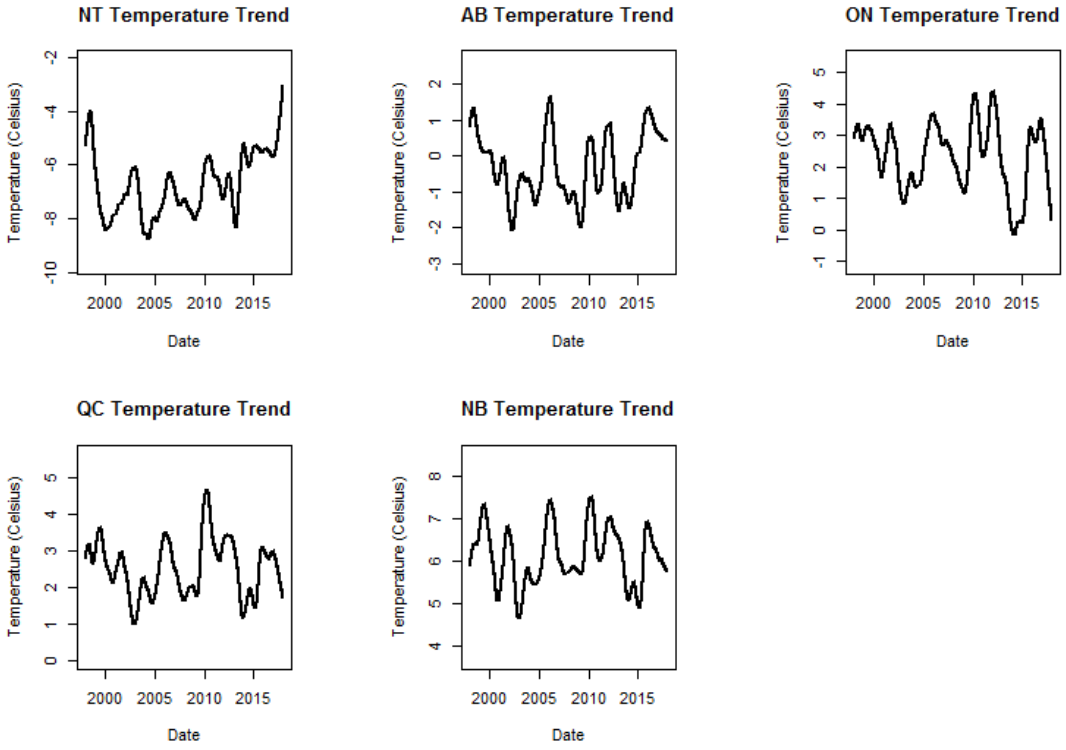


Figure 2. Trends in mean daily temperature between 1998-2017 for 5 weather stations close to the sampling locations: Inuvik (NT), High Level (AB), Timmins (ON), Baie Comeau (QC), and Fredericton (NB).

Annual spruce budworm phenology was simulated for each location, using daily temperature records and BIOSIM. Development times at each location (Fig. 3) show a clear segregation of the number of days at the overwintering L2 (L2o) stage with a longer duration in NT, followed by QC, AB, ON, and NB. On average, the duration at the L2o stage is 38 days longer in Inuvik than in Fredericton. It is noticeable that the ranking among locations is nearly reversed at the L3 stage: the duration in that stage is shorter in the NT followed by AB, ON, QC and NB.

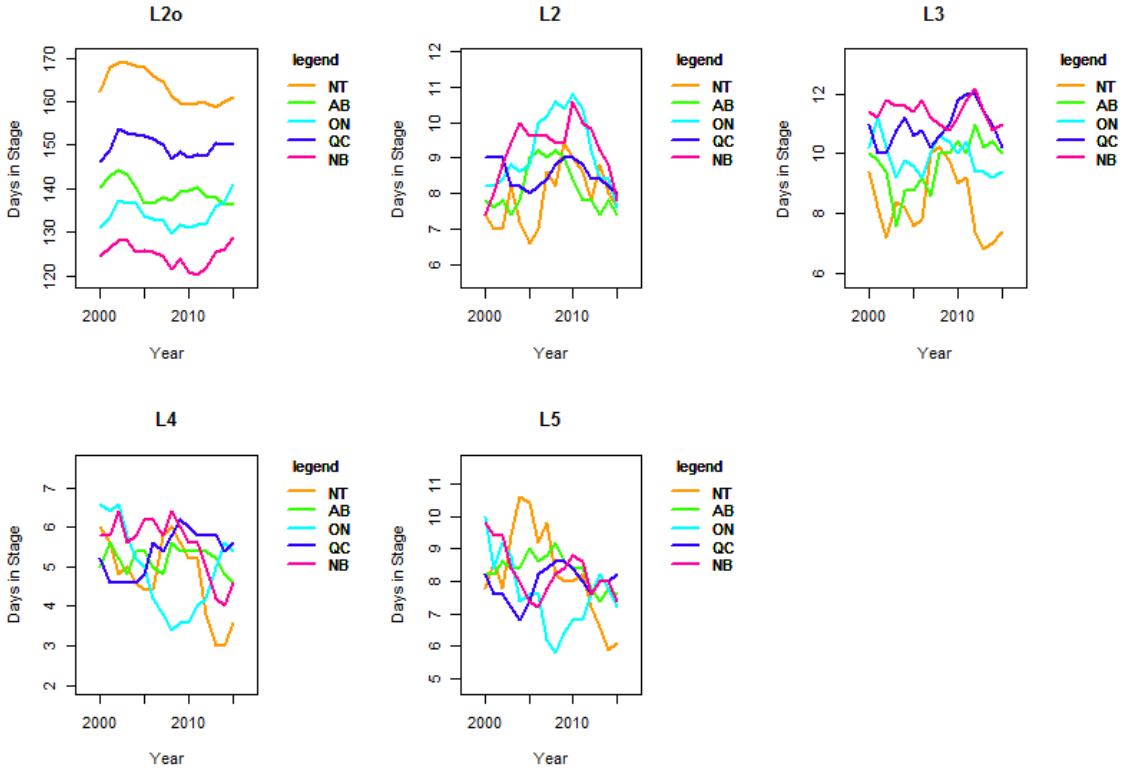


Figure 3. Simulated development times at each larval stage for five weather stations close to the sampling locations.

Trend analyses reveal significant trends for different larval stages in various locations. In the NT, the time in stage decreases significantly for L2o (-0.7d/yr), L4 (-0.2d/yr), L5 (-0.2d/yr) and L6 (-0.4d/yr) since 1999. In AB, stage duration decreases at the L2o and L6 stages (-0.4d/yr and -0.1d/yr respectively), but it increases at the L3 stage (+0.1d/yr). In ON, the only significant trend is a small increase in the duration of the L6 stage (+0.1d/yr). In QC, the L4 stage shows a small increase (+0.1d/yr). In NB, the duration of the L6 stage shows a significant positive trend (+0.2d/yr).

When the duration of development is summed across stages from L2o to pupa (Fig. 4), the total duration decreases significantly in the NT (-0.7d/year, $p<0.05$) and almost significantly in AB (-0.6d/yr, $p=0.06$).

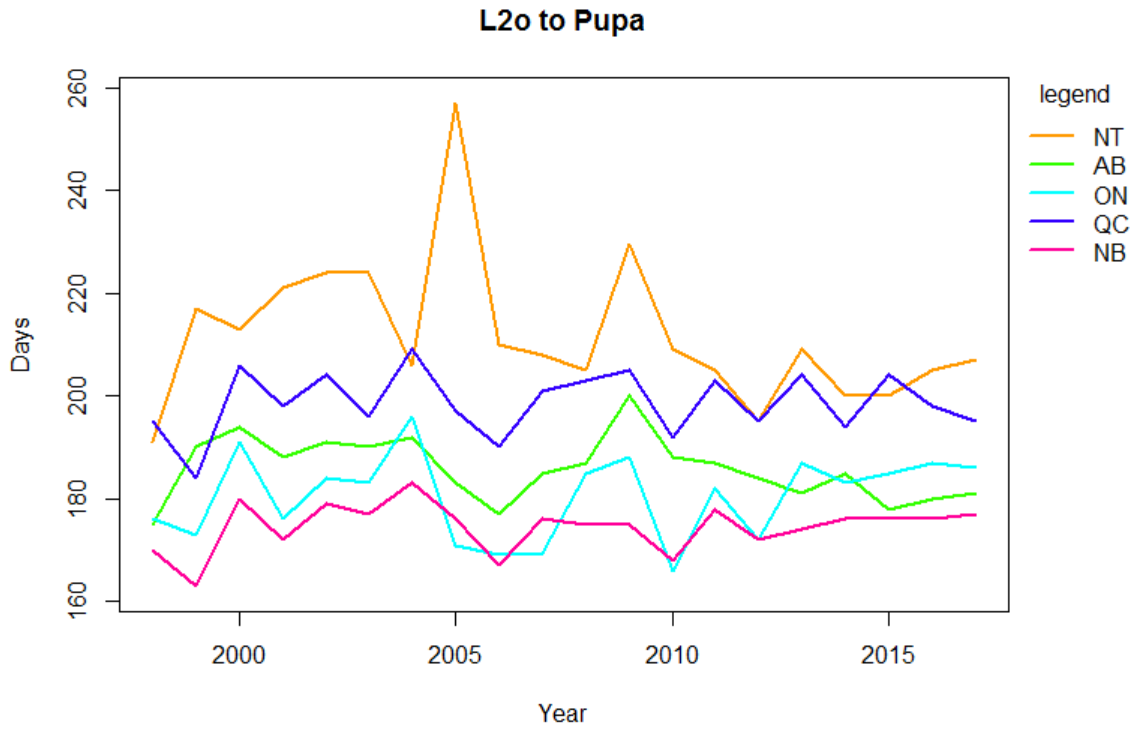


Figure 4. Simulated development time to reach pupal stage close to the sampling locations of the wild populations.

Development rates of the Ontario colony at 30°C and 35°C

At the time of this report, we have completed the rearing for the Ontario colony at 30°C and 35°C. The rest of the temperature treatments (i.e. 5, 10, 15, 20, 25°C) are ongoing. We also started to rear the Quebec colony. The other colonies are still in diapause. The expected initiation of temperature treatments for these is between the end of January (NB) and the beginning of April (NT). The experimental protocol used for rearing the wild colonies is identical to the protocol used for the IPS laboratory colonies.

At 30°C, the development of the Ontario colony appears slower than BIOSIM predictions (Fig. 5). This is similar to what was observed with the IPS colony. The observed development at the L2 stage (Fig. 6) is slower than simulated and slightly slower than the development of the IPS colony. This is partly compensated by faster development of the Ontario and IPS colonies at the L5 stage compared to the model predictions. The model predicts well the development of L3 (better than it predicts IPS colonies) and L6 stages.

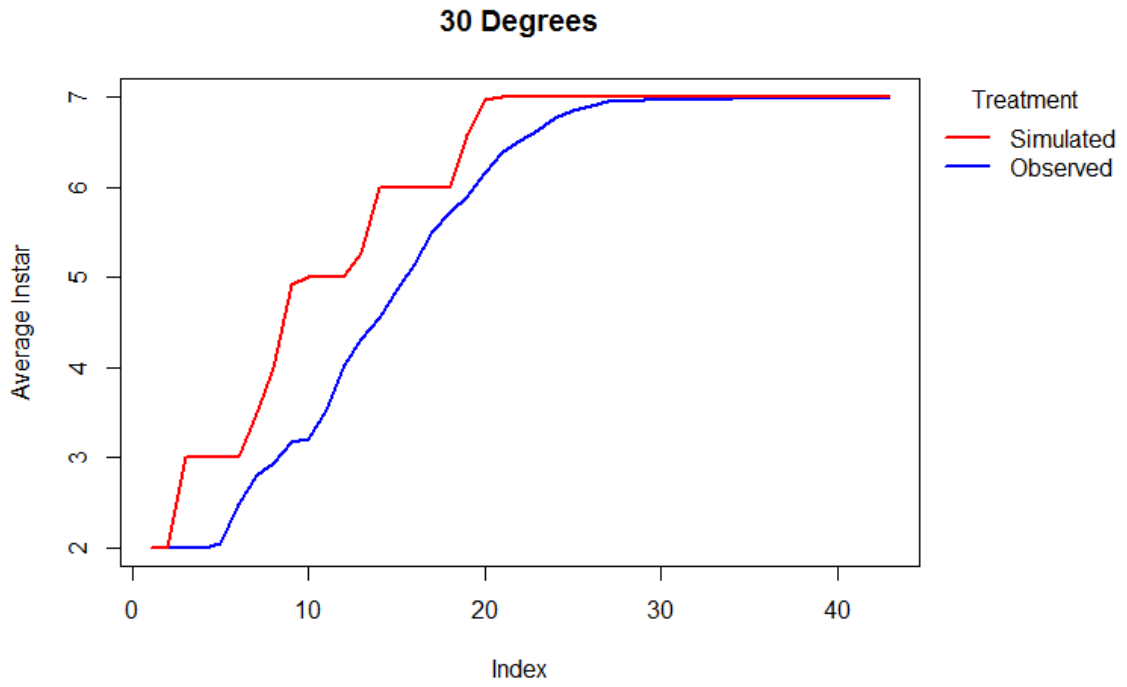


Figure 5. Average instar development of the Ontario colony compared to BIOSIM simulation at 30°C.

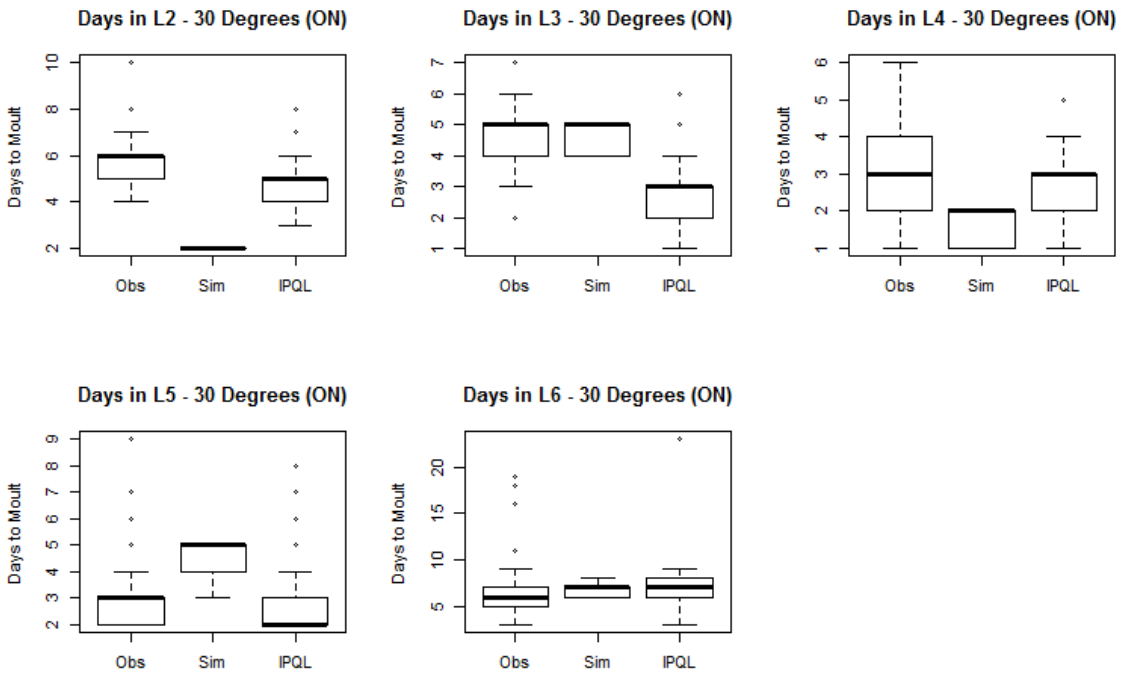


Figure 6. Instar development of the Ontario colony compared to BIOSIM simulation and IPS colony at 30°C.

At 35°C, the observed development is again slower than simulated (Fig. 7). The development of the Ontario colony is slower than predicted by the model at the L2 and L4 stages, and to a lesser degree at the L6 stage (Fig. 8). The model agrees with the observations at the L3 and L5 stages.

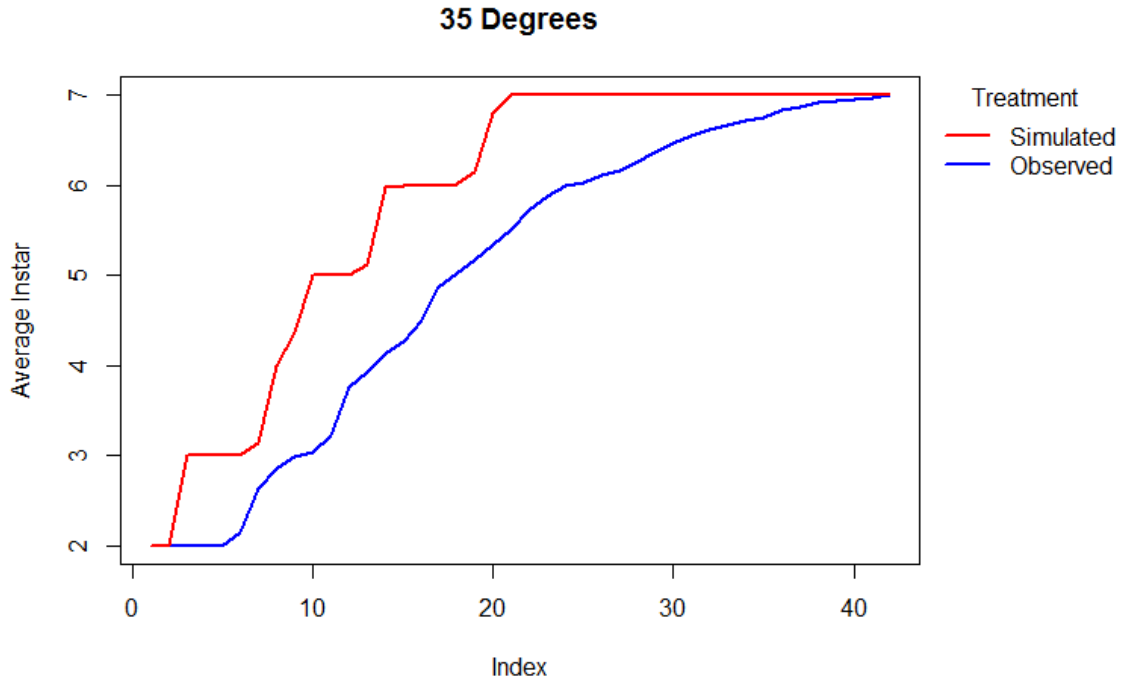


Figure 7. Average instar development of the Ontario colony compared to BIOSIM simulation at 35°C.

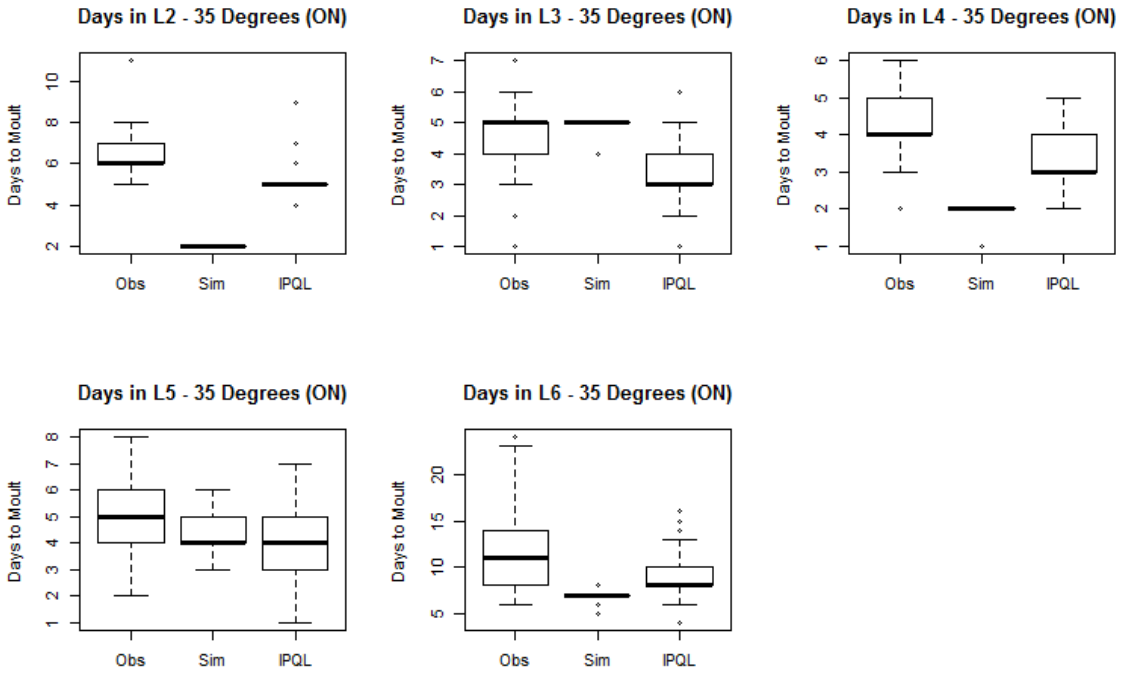


Figure 8. Instar development of the Ontario colony compared to BIOSIM simulation and IPS colony at 35°C.

Summary

In summary, the preliminary results for the Ontario colony suggest that the model overestimates the development rate at 30°C and 35°C, a result that is consistent with what was observed on the IPS colony. However, it is important to point out that: (1) the temperature transfers that are required to rear budworm at these temperatures are probably not well addressed in the model, as pointed out earlier; and (2) differences between observed and simulated development rates of early instars as observed here likely have little impact on the usability of the model because early instars are very unlikely to encounter these temperatures.

Acknowledgements

This work was carried-out with financial support from SERG-international members (NSDNR, SOPFIM, AB-AAF, SK MOE, OMNRF) and in-kind support from AB-AAF and NT government. The Great Lakes Forestry Centre of the Canadian Forest Service contributed significant financial and in-kind support. We thank Courtney Irwin, Alice Liu, and Emilie Vaillancourt for helping with lab rearings. We thank Stephane Bourassa (CFS-LFC), Gord Brand (CFS-GLFC), Roger Brett (CFS-NoFC), Martin Callaghan (NT Environment and Natural Resource), Louis De Grandpré (CFS-LFC), Ryan Hermanutz (AB Agriculture and Forestry), Rob Johns (CFS-AFC), Greg Pohl (CFS-NoFC), and Joris Wiersinga (FPL) for their help in sampling branches for this experiment. Without them, this large-scale sampling would not have been possible.

References

Cooke, B.J.; Régnière, J. 1999. Predictability and measurability of *Bacillus thuringiensis* efficacy against spruce budworm (Lepidoptera: Tortricidae). Environmental Entomology 28(4): 711-721.

Morris, R.F. 1955. The development of sampling techniques for forest insect defoliators, with particular reference to the spruce budworm. Canadian Journal of Zoology, 1955, 33:225-294

Régnière, J.; Saint-Amant, R.; Béchard, A. 2014. BIOSIM 10 – User's manual. Nat. Resour. Can., Can. For. Serv., Laurentian For. Cent., Québec (Quebec). Inf. Rep. LAU-X-137E

Régnière, J.; St-Amant, R.; Duval, P. 2012. Predicting insect distributions under climate change from physiological responses: spruce budworm as an example. Biol. Invasions 14:1571-1586.

Régnière, J. 1987. Temperature-dependent development of eggs and larvae of *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricide) and simulation of its seasonal history. The Canadian Entomologist 119: 717-728.

van Frankenhuyzen, K.; Ebling, P.M.; McCron, B.; Ladd, T.R.; Gauthier, D.; Vossbrinck, C. 2004. Occurrence of Cytosporogenes sp. (Protozoa, Microsporidia) in a multi-species insect production facility and its elimination from a colony of the eastern spruce budworm, *Choristoneura fumiferana* (Clem.) (Lepidoptera: Tortricidae). Journal of Invertebrate Pathology 87: 16-28.

Volney, W.J.A.; Cerezke, H.F. 1992. The phenology of white spruce and the spruce budworm in northern Alberta. Canadian Journal of Forest Research 22(2): 198-205.

Weber, J.D.; Volney, W.J.A.; Spence, J.R. 1999. Intrinsic development rate of spruce budworm (Lepidoptera: Tortricidae) across a gradient of latitude. Environmental Entomology 28 (2): 224-232.Alexander, M.E.; Stocks, B.J.; Lawson, B.D. 1991. Fire behavior in the black spruce lichen woodland: the Porter Lake Project. For. Can., North. For. Cent., Edmonton, AB. Inf. Rep. NOR-X-310. 44 p.

Appendix A: Simulated vs observed development times of the IPS colony at 7 constant temperatures - Overall development

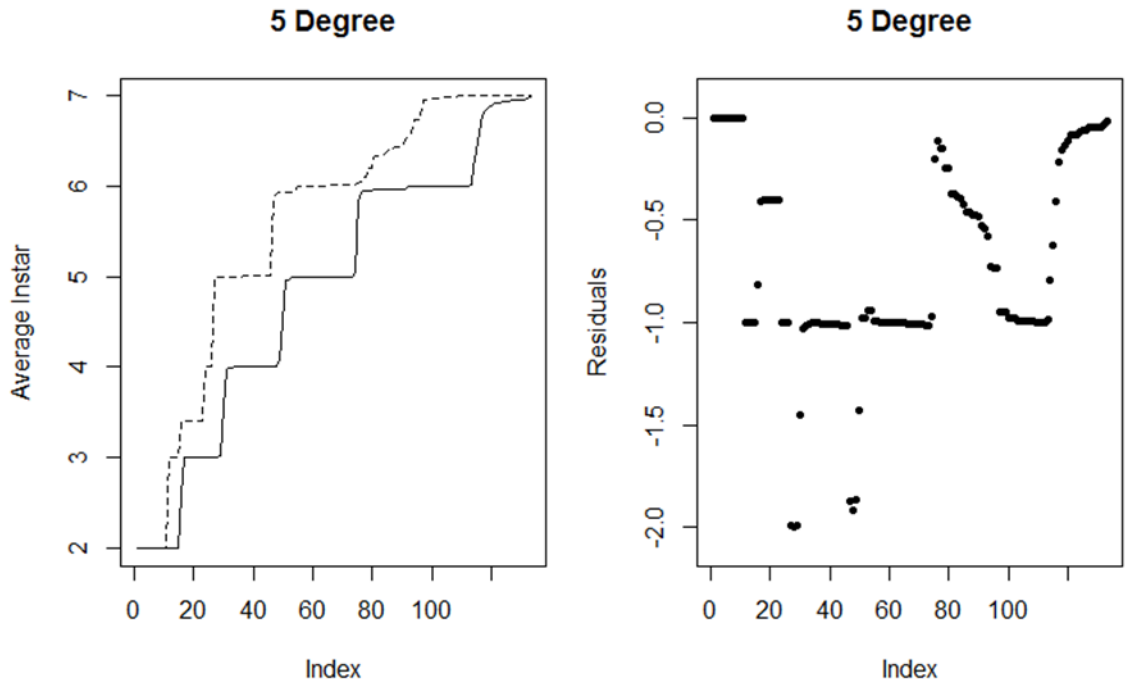


Figure A1. Comparison between simulated (dotted line) and observed (solid line) development times at 5°C for IPS colony generation F0.

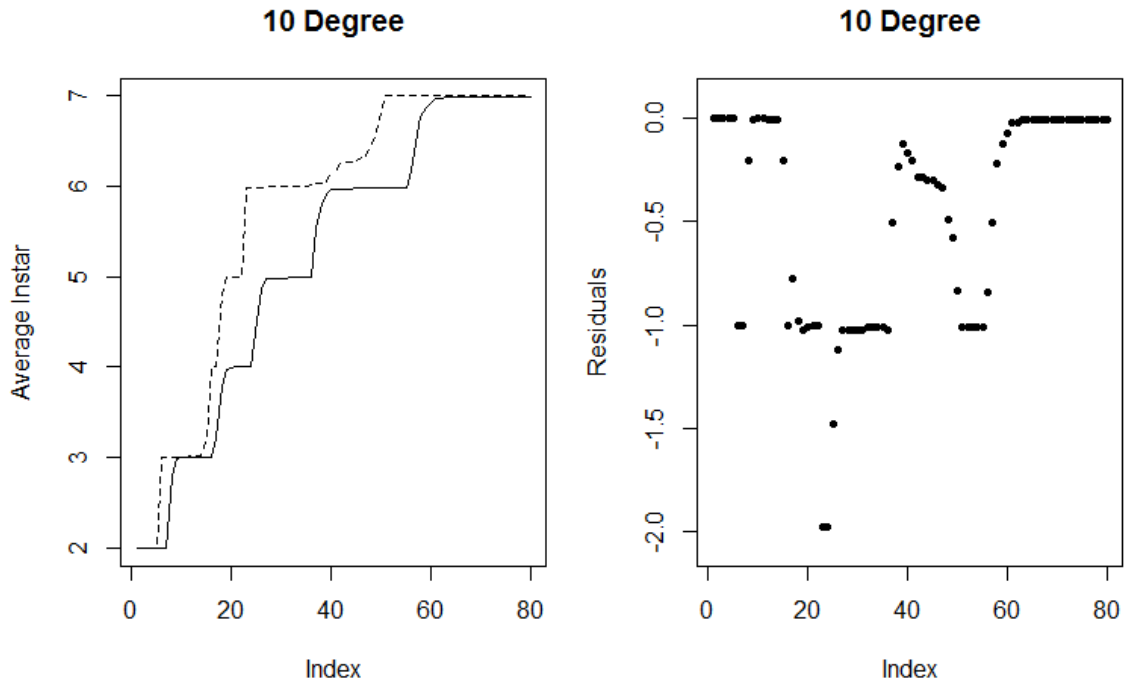


Figure A2. Comparison between simulated (dotted line) and observed (solid line) development times at 10°C for IPS colony generation F0.

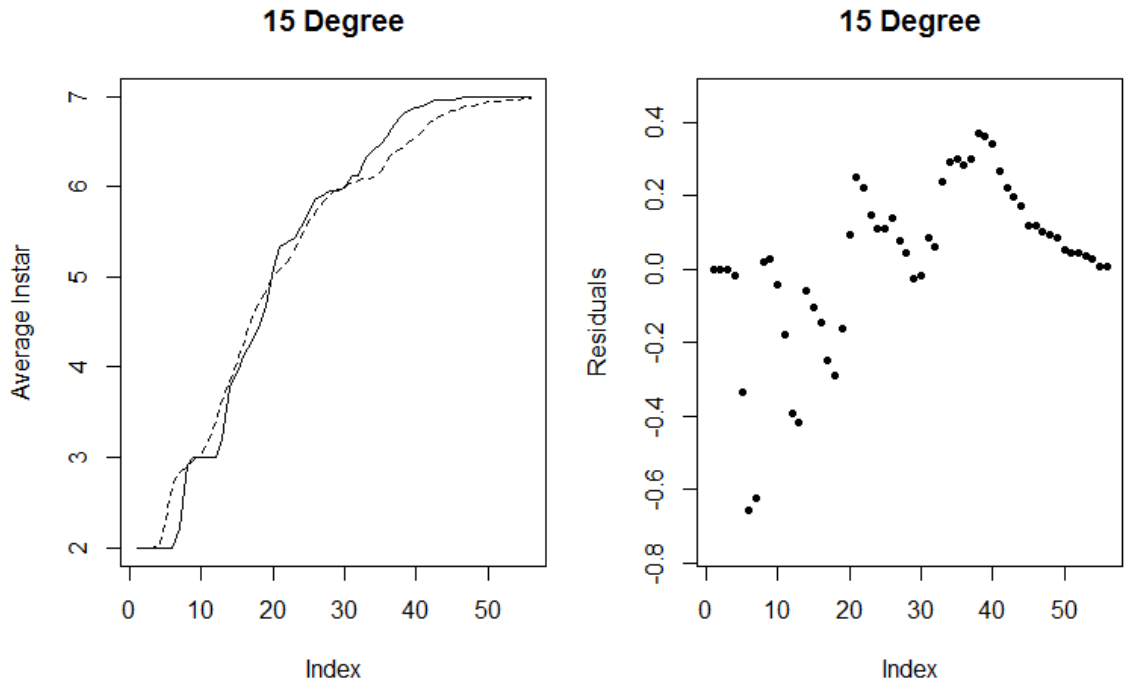


Figure A3: Comparison between simulated (dotted line) and observed (solid line) development times at 15°C for IPS colony generation F0.

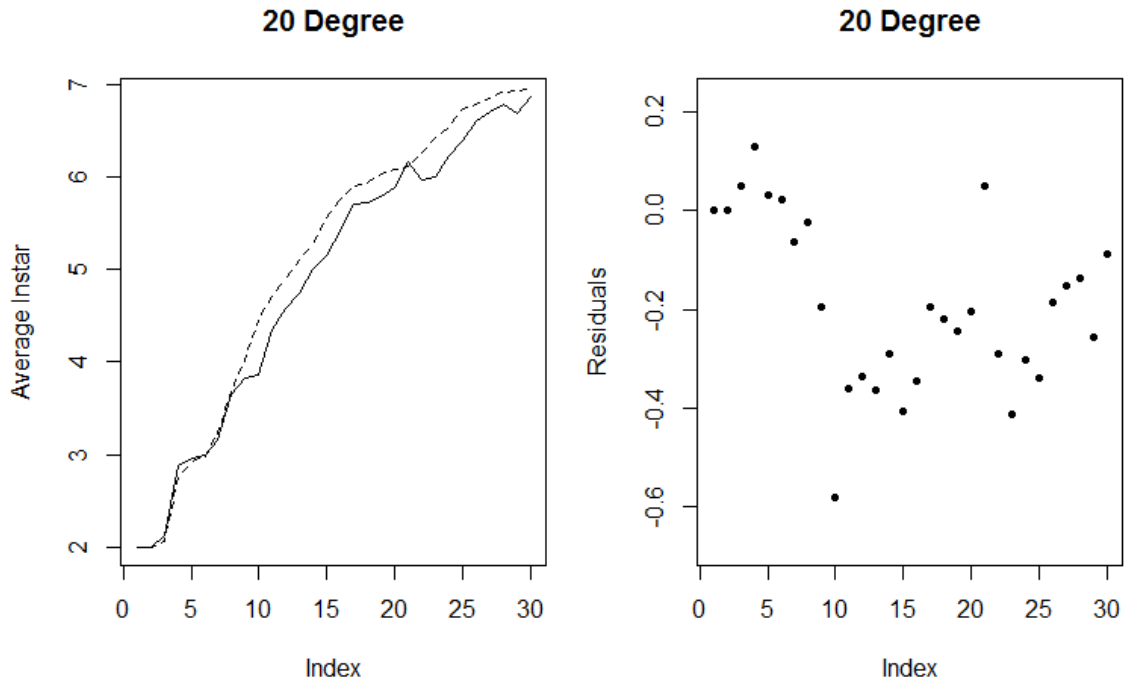
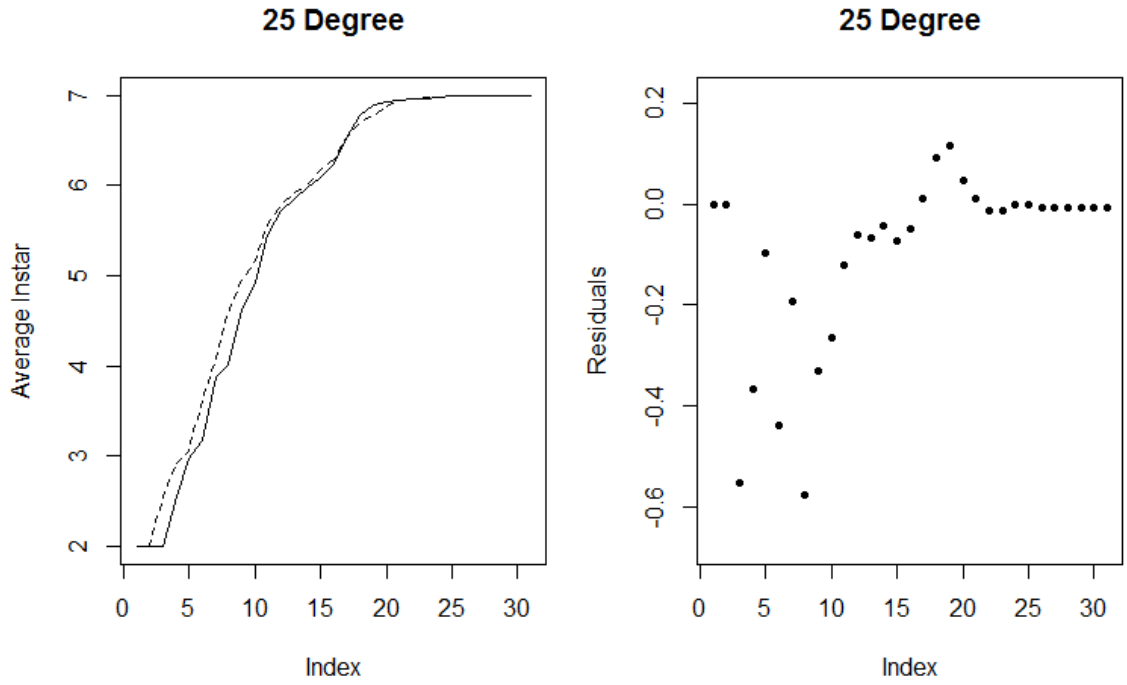


Figure A4. Comparison between simulated (dotted line) and observed (solid line) development times at 20°C for IPS colony generation F0.



FigA5. Comparison between simulated and observed development rates at 25°C for IPS stock generation F0.

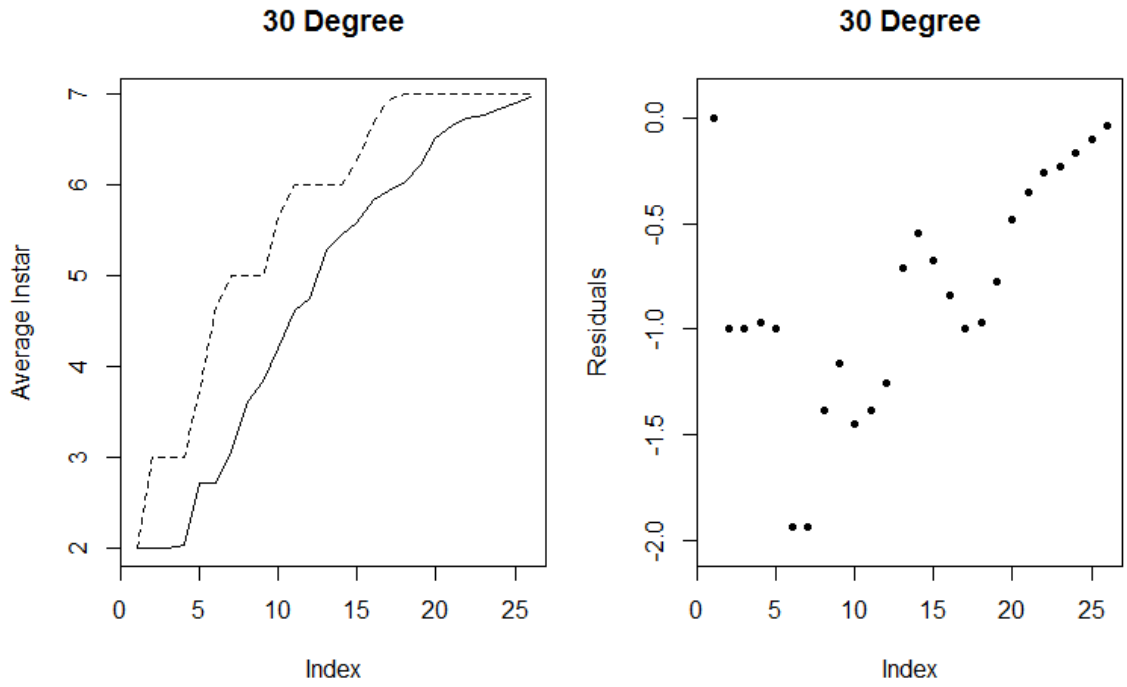


Figure A6. Comparison between simulated (dotted line) and observed (solid line) development times at 30°C for IPS colony generation F0.

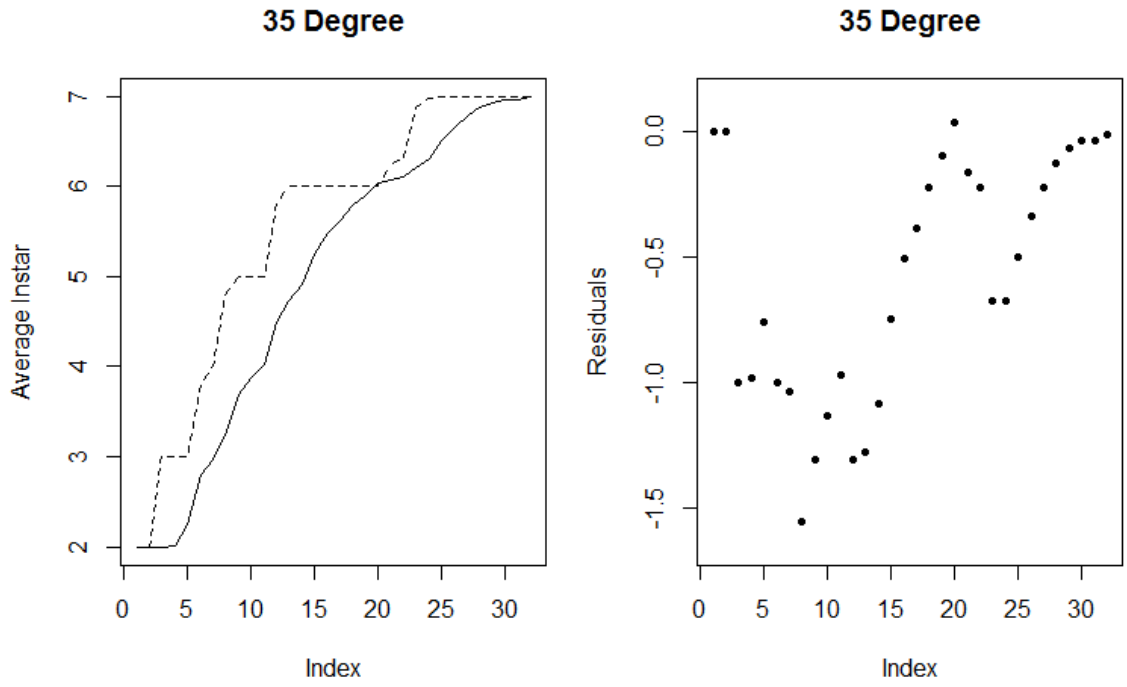


Figure A7. Comparison between simulated (dotted line) and observed (solid line) development times at 35°C for IPS colony generation F0.

Appendix B: Simulated vs observed development times of the IPS colony at 7 constant temperatures - Larval stage development

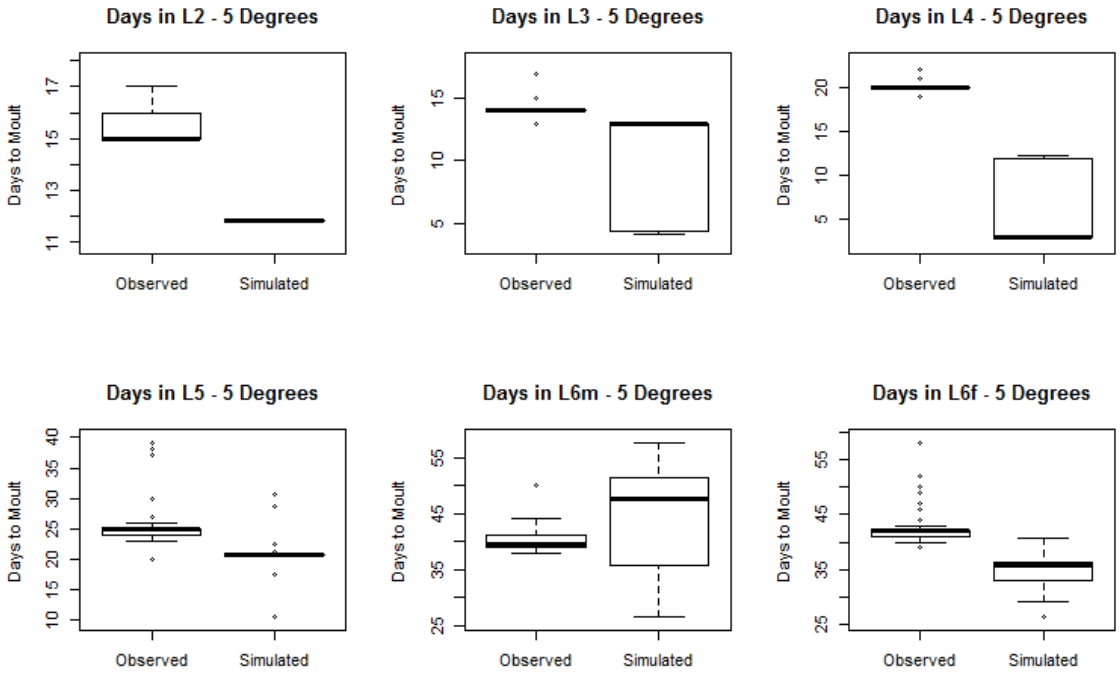


Figure B1. Comparison between simulated and observed development times at 5°C for IPS colony generation F0.

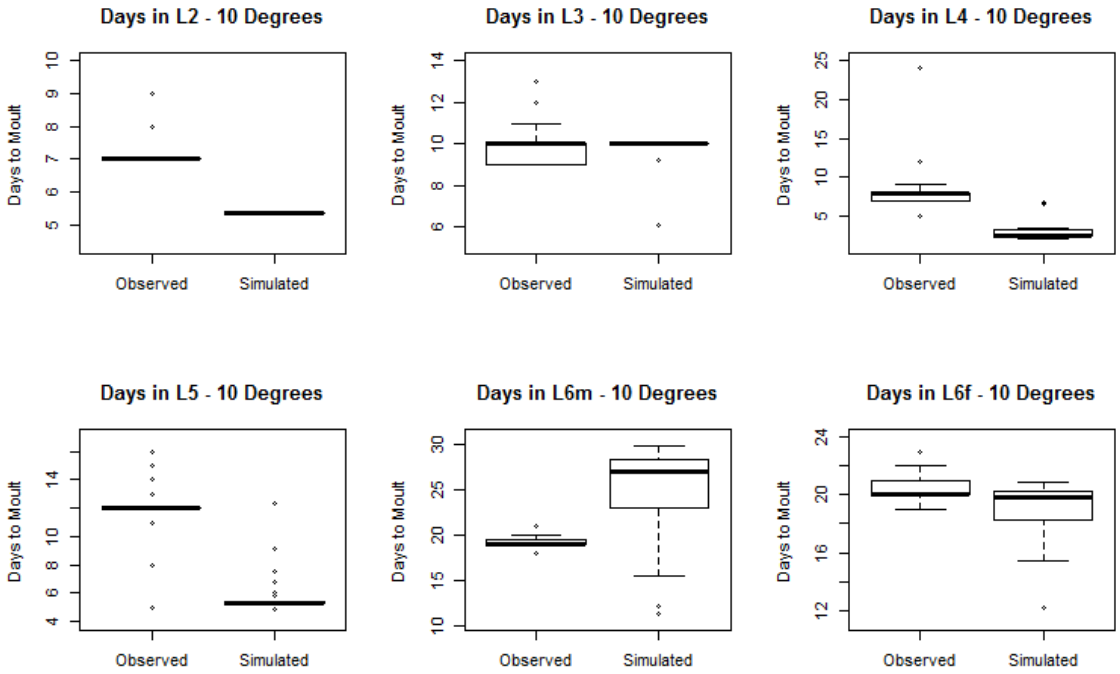


Figure B2. Comparison between simulated and observed development times at 10°C for IPS colony generation F0.

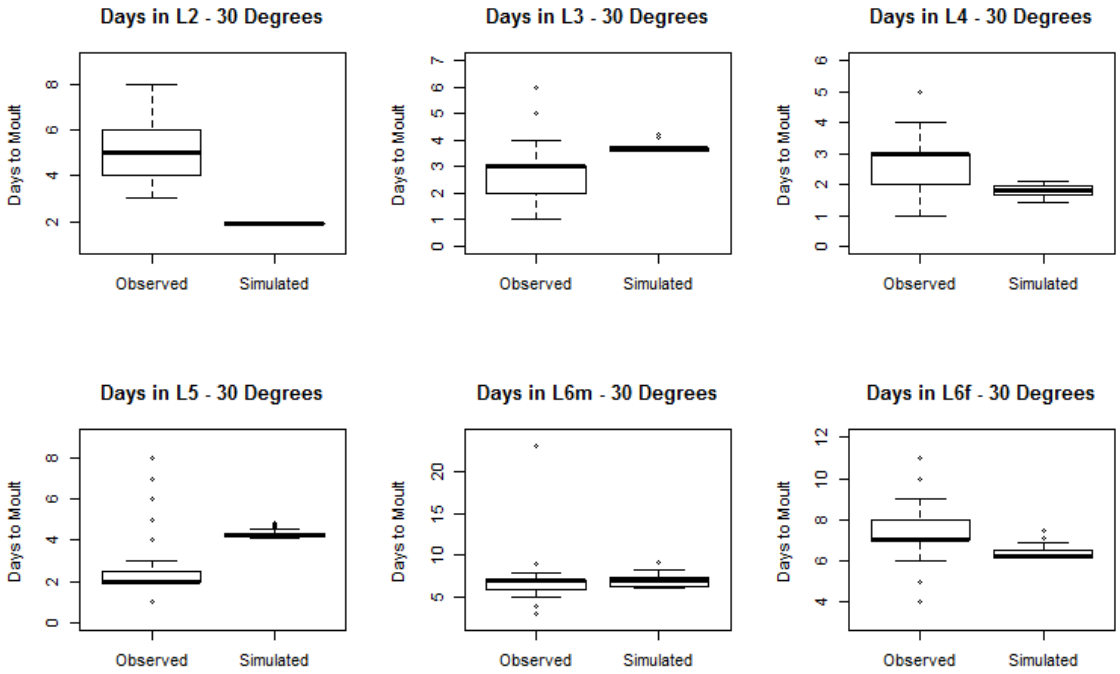


Figure B3: Comparison between simulated and observed development times at 30°C for IPS colony generation F0.

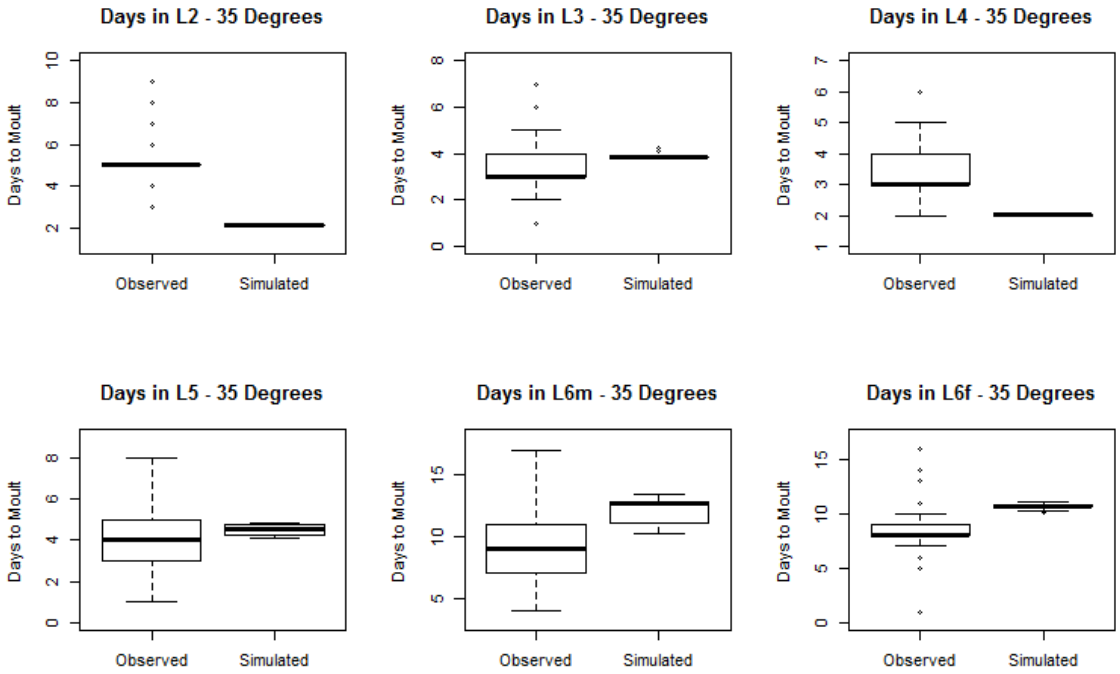


Figure B4. Comparison between simulated and observed development times at 35°C for IPS colony generation F0.