Review of "Biennial aerial application of Bacillus thuringiensis var. kurstaki provides good protection against spruce budworm (Choristoneura fumiferana [Clemens])"

Andrea Battisti, David Coyle, Aurélien Sallé, Erwin Dreyer, Eric Bauce, Alain Dupont, Christian Hébert, Richard Berthiaume, Roberto Quezada-García, Alvaro Fuentealba

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Review of "Biennial aerial application of *Bacillus thuringiensis* var. kurstaki provides good protection against spruce budworm (*Choristoneura fumiferana* [Clemens]) "

Andrea Battisti [R 1], David R. Coyle [R 2], Aurélien Sallé [HE 3], Erwin Dreyer [EIC 4], Éric Bauce[A 5], Alain Dupont [A 6], Christian Hébert [A 7], Richard Berthiaume [A 6], Roberto Quezada-García [A 5], Alvaro Fuentealba [A 5*]

[R] Reviewer
[HE] Handling Editor
[EIC] Editor in chief
[A] Author
[*] Corresponding author

[1] Universita di Padova, Italy.
[2] Clemson University, College of Agriculture, Forestry and Life Sciences, USA.
[3] Université d'Orléans, France.
[4] Université de Lorraine, AgroParisTech, INRAE, France.
[5] Université Laval, Québec, Canada.
[6] Société de Protection des Forêts contre les Insectes et Maladies (SOPFIM), Québec, Canada.
[7] Natural Resources Canada, Canadian Forest Service, Québec, QC G1V 4C7, Canada.

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Original submission – R0

R0 - Reviewer 1 (Andrea Battisti)

Reviewer Recommendation Term: Accept with minor modifications.

General comment

This manuscript synthesizes 11 years of *Bacillus thuringiensis* var. kurstaki (Btk) applications against the spruce budworm *Choristoneura fumiferana* in the coniferous forests of Quebec, Canada. The data were carefully collected and analysed, and the results offer interesting outcomes for the future management of the pest. Having a biennial spraying will result not only in saving costs but also in reducing the impacts of the treatment on non-target Lepidoptera and it will also allow better recovery of the natural enemy's complex. Unfortunately, weather data across the 11 years of the study are not reported so the reader would believe weather is not important in tree recovery and ring growth, something that seems rather unlikely. Perhaps a *caveat* sentence is required in this context. What is missing from the analysis is the consideration of non-target effects of the Btk applications and the inclusion of a landscape approach in the management. These two aspects are of increasing importance in the management of forest insects and should be briefly considered, at least in the discussion and future issues. In the introduction, the authors state that Btk has negligible or no effects on the ecosystem; this is hardly acceptable as there is evidence of such effects in various parts of the world, even if the effects are often transient for the short persistence of Btk, at least for defoliating Lepidoptera.

I would also suggest including landscape traits in the future design of Btk applications, as for example mature and overmature stands seem to be more susceptible than younger stands to the spruce budworm. This is reported in a few points of the manuscript although it does not come up

in the discussion and conclusions. I do believe that future management of the spruce budworm should include such a landscape approach, especially if benefits will be estimated beyond the commercial value of the timber. Under the current climate change scenario, it would be good to project the management of the spruce budworm into a perspective where multiple factors are considered.

R0 - Reviewer 2 (David R. Coyle)

Reviewer Recommendation Term: Accept with minor modifications.

Level of interest: An exceptional article.

Quality of written English: Needs some language corrections before being published.

Declaration of competing interests: I declare that I have no competing interests.

This paper summarizes and reports on a long-term, large-scale study to evaluate the efficacy and cost-effectiveness of a treatment program for spruce budworm on three species (balsam fir, white spruce, black spruce) in Canada. While anyone can find something to criticize while performing a manuscript review (i.e., no manuscript is perfect), I found very little to criticize or question at all, and frankly found this to be one of the better papers I have read in some time. The authors carried out a relevant evaluation of standard and experimental treatments for spruce budworm, and their results have real on-the-ground implications for how authorities treat this forest pest as well as the financial implications of different treatment methods. They found that treating every two years instead of the standard treatment was just effective (statistically) and saved money – thus potentially allowing a greater area to be treated in future years. I believe these are the types of studies that can help improve management and impact policy on a large (e.g., Provincial) scale.

It is, however, fairly annoying to have to find another paper (in this case, Fuentealba et al. 2019) to get the necessary details on the treatments and site selection, and I strongly recommend including some sort of summary of this information in this manuscript. As a reviewer and reader, I feel that papers should be stand-alone units, and not dependent on another manuscript to make or support methodological details.

A question that did arise in my mind is how did the authors know that trees were killed by spruce budworm and spruce budworm only? What rationale was applied to ensure that mortality seen was a direct result of defoliation, and not other factors such as drought, root-rot, or some other combination? Typically, defoliation is but one stressor that can contribute to tree mortality, but it is generally not the only factor. It would be good if the authors could provide some details on this aspect of the study.

There are also several instances where some editing is needed (e.g., words should be pluralized and are not and vice versa) but these will likely be caught during the editorial process. Further, some of the figures are very low resolution and will need to be much higher quality for publication.

Overall, I commend the authors for this work and I look forward to seeing it published.

R0 - Handling Editor (Aurélien Sallé)

Recommendation R0: Accept with minor modifications.

Dear authors,

Two reviewers have evaluated your manuscript. They both appreciated the relevance of your approach and the quality of the presented data, as well as the way the data were presented. Overall, they only have minor comments to make on your manuscript. I suggest you take them into account as they will contribute to further improving the quality of your article. It would be particularly relevant to expand the discussion on non-target effects, as there is indeed a body of literature on the subject, and to discuss the consideration of landscape characteristics. I also recommend better linking to the previous article (Fuentealba et al., 2019) to facilitate the reading of your new manuscript.

R0 - Editor in chief (Erwin Dreyer)

Decision letter R0: Accept with minor modifications.

We have received the reports from our advisors on your manuscript (ANFS-D-24-00018), 'Comparative analysis of different forest protection scenarios using aerial applications of Bacillus

thuringiensis var. kurstaki to reduce spruce budworm-related tree mortality and growth losses ', submitted to Annals of Forest Science.

Based on the advice received (see comments below), we reached the conclusion that your manuscript will be suitable for publication after you have carried out the revision as suggested by the referees. Below, please find the reviewers' comments for your perusal. You are kindly requested to also check the website for possible reviewer attachment(s).

The reviewers and the handling editor provided a very careful and insightful review of the paper, and their comments should be of help in revising the manuscript. My personal quick reading of the manuscript lead to a full agreement with the produced comments and suggestions, and to a few additional point (see also the comments in the attached version of the manuscript):

1. The title needs be simplified and should mention the most striking result;

2. I insist that Annals of Forest Science strongly supports the open-data movement and in particular the sharing of datasets generated and/or analyzed during the study (see our blog page: https://ist.blogs.inrae.fr/afs/2019/07/24/guidelines-for-authors-how-to-share-your-datasets/). We do invite you to deposit the data set (raw/primary data) into a public repository, where it would become a citable item with a DOI. It could then be cited in the reference list of your paper and enhance the impact of your paper and the visibility of your work. This would be a very nice added value to the paper;

3. I agree with the reviewers and handling editor that there should be a mention to the negative impact of Bt sprays on Lepidoptera populations. The cost-benefit analysis cannot solely rely on the cost of the treatments vs. the gains in production;

4. I agree also that the manuscript should better build upon the results of the previous paper on the topic and be presented as a follow-up to the earlier publication. This does not preclude that all details required to understand the approach need be provided here (as required by one of the reviewers).

Revised Version R1

R1 – Authors

Author's response to editors' and reviewers' comments

Dear Sir,

Please find a revised version of our manuscript entitled "Comparative analysis of different forest protection scenarios using aerial applications of *Bacillus thuringiensis* var. kurstaki to reduce spruce budworm-related tree mortality and growth losses". We have changed the title to: "Biennial aerial application of *Bacillus thuringie*nsis var. kurstaki provides good protection against spruce budworm (*Choristoneura fumiferana* [Clemens]) and clarified pertinent sections in the text in relation to the issues that were raised by the reviewers. We hope that we have satisfactorily addressed all of the reviewers' concerns. Please note that a native English speaker edited the final text. Our responses (in bold) to issues raised by each reviewer (in italics) are the following:

Answers to Editor in chief

1. The title needs be simplified and should mention the most striking result.

The title was simplified as requested. The new title is "Biennial aerial application of *Bacillus thuringiensis* var. kurstaki provides good protection against spruce budworm (*Choristoneura fumifera*na [Clemens])".

2. I insist that Annals of Forest Science strongly supports the open-data movement and in particular the sharing of datasets generated and/or analyzed during the study (see our blog page: https://ist.blogs.inrae.fr/afs/2019/07/24/guidelines-for-authors-how-to-share-your-datasets/).
We do invite you to deposit the data set (raw/primary data) into a public repository, where it would become a citable item with a DOI. It could then be cited in the reference list of your paper and enhance the impact of your paper and the visibility of your work. This would be a very nice added value to the paper.

We have deposited the raw data in the repository BOREALIS. The DOI of the dataset is: https://doi.org/10.5683/SP3/TTWHHF

3. I agree with the reviewers and handling editor that there should be a mention to the negative impact of Bt sprays on Lepidoptera populations. The cost-benefit analysis cannot solely rely on the cost of the treatments vs. the gains in production.

We now mention the ecological impact that Btk sprays may have on non-target Lepidoptera in the introduction (lines 65-71) and also have added a paragraph in the discussion (lines 439-452) to discuss the benefits that spraying every two years may have in terms of allowing better recovery of non-target lepidopterans. Answers to Reviewer 1

4. I agree also that the manuscript should better build upon the results of the previous paper on the topic and be presented as a follow-up to the earlier publication. This does not preclude that all details required to understand the approach need be provided here (as required by one of the reviewers).

We now give more details about the previous paper in the introduction (lines 99-110), and we have added the rationale behind every protection scenario in Table 1 in order to better connect the current manuscript with our previous study.

Answers to Handling Editor

1. It would be particularly relevant to expand the discussion on non-target effects, as there is indeed a body of literature on the subject, and to discuss the consideration of landscape characteristics.

We now mention the ecological impact that Btk sprays may have on non-target Lepidoptera in the introduction (lines 65-71) and have also added a paragraph in the Discussion to discuss the benefits that spraying every two years may have in terms of allowing a better recovery of non-target Lepidoptera (lines 439-452). Furthermore, we mention in the discussion (lines 453-466) and the conclusion (lines 497-499) the effects that landscape characteristics may exert on the efficacity of Btk spraying operations and how important it would be to consider these characteristics in future studies.

2. I also recommend better linking to the previous article (Fuentealba et al., 2019) to facilitate the reading of your new manuscript.

We now provide more details about the previous paper in the Introduction (lines 99-110), and we have added the rationale behind every protection scenario in Table 1 in order to better connect the current manuscript with our previous study.

Answers to Reviewer 1

1. Unfortunately, weather data across the 11 years of the study are not reported so the reader would believe weather is not important in tree recovery and ring growth, something that seems rather unlikely. Perhaps a caveat sentence is required in this context.

We agree that climate may have an important role in tree growth, vigour and vulnerability to insect attack. We did not consider it pertinent to include weather data in this manuscript because the main goal of this study was to evaluate the impact of spruce budworm defoliation and protection scenarios on tree growth and mortality, rather than determining the causes of growth reduction. However, given the importance of this abiotic factor, we included in the discussion a sentence to suggest that the effects of climate should also be incorporated into the decision process given that adverse climate may affect tree growth, resulting in loss of vigour and greater vulnerability to spruce budworm attack (lines 461-463).

2. What is missing from the analysis is the consideration of non-target effects of the Btk applications and the inclusion of a landscape approach in the management. These two aspects are of increasing importance in the management of forest insects and should be briefly considered, at least in the discussion and future issues. In the introduction the authors state that Btk has negligible or no effects on the ecosystems, this is hardly acceptable as there is evidence of such effects in various parts of the world, even if the effects are often transient for the short persistence of Btk, at least for defoliating Lepidoptera.

We now mention the ecological impact that Btk sprays may have on non-target Lepidoptera in the Introduction (lines 65-71) and have also added a paragraph in the Discussion to discuss the benefits that spraying every two years may have in terms of allowing a better recovery of non-target

Lepidoptera (lines 439-452). Furthermore, we mention in the Discussion (lines 453-466) and the Conclusion (lines 497-499) the effect that landscape characteristics may exert on the efficacity of Btk spraying operations and how important it would be to consider these characteristics in future studies.

3. I would also suggest including landscape traits in the future design of Btk applications, as for example mature and overmature stands seem to be more susceptible than younger stands to the spruce budworm. This is reported in a few points of the ms although it does not come up in the discussion and conclusions. I do believe that future management of the spruce budworm should include such a landscape approach, especially if benefits will be estimated beyond the commercial value of the timber. Under the current climate change scenario, it would be good to project the management of the spruce budworm into a perspective where multiple factors are considered.

We now discuss the importance of landscape traits in Btk spraying operations (lines 453-466) and we mention in the Conclusion that future studies should consider the impact of these traits in the efficacy of spraying operations (lines 497-499).

4. Please remove the full stop at the end of the title.

Full stop was removed as requested.

5. L 7 replace the first protection with application.

The word "protection" was replaced by "application" as requested.

6. L 16 add from defoliating Lepidoptera.

The term was added as requested.

7. L 23 benefit/cost ratio is fine although in the literature it is better known as cost/benefit analysis. Please try to use the most accepted terms as at page 6.

A cost/benefit analysis is the process of comparing the projected or estimated costs and benefits associated with a given project. It involves tallying up all costs and deducting that amount from projected benefits. The benefit/cost ratio in turn is an indicator used in cost-benefit analysis to determine how much financial value a project may generate in relation to the investment that is required to see through to the end of the project (Keefe et al. 2012). In that sense, we consider that it is appropriate to use the term benefit/cost ratio here because we are making reference to the indicator, rather than to the analysis. In contrast, we use cost-benefit analysis as a heading in section 2.5 because we are making reference to the process.

8. L 32 spraying scenarios are mentioned here and then reported in the result section, it would be good to briefly list them.

We agree that it would be good to provide more information about the spraying scenarios here, but word restrictions prevent us from doing so.

9. L 40 it is odd starting with spruce when fir was mentioned above as most susceptible and main target of the treatment.

We now start by describing the impact of protection scenarios on balsam fir.

10. L 28 I would be cautious saying there are few or not-existent non-target effects as many examples are known from the literature. Perhaps saying that those effects are transient as Btk is not persistent and recolonization by non-target may easily occur.

We now mention that non-target Lepidoptera may be affected by spraying operations, but that this impact is transient (lines 65-71).

11. L 8-14 it is not clear if the previous paper (Fuentealba et al. 2019) included the scenarios considered in this study, please specify.

We now clarify that the previous study tested the same scenarios that are considered in this study (lines 99-105, 116-119).

12. L 28-29 specify the origin of the defoliation area data and how they were retrieved.

We briefly explained how defoliation data were measured and that they were used to estimate the residual photosynthetic capacity (lines 99-110).

13. L 34-55 the experimental design is difficult to follow and the reader should not be reported to the previous paper. A schematic presentation or a table would be useful. Also specify the distinction from what was done in the previous paper should be presented (longer time series?).

We have added to Table 1 table the rationale behind the protection scenarios that were tested in this study.

14. See comment above for cost/benefit analysis and benefit/cost ratio.

See response above.

15. L 39-43 benefits are calculated as timber growth although there could be other benefits that were not considered, such as ecological benefits that are difficult to estimate. Did the authors think about them?

We agree that there are other potential benefits that could be included in the cost-benefit analysis, and we intend to do so in future evaluations. One of the goals of this project is to evaluate the effects of the protection scenarios on forest carbon budgets, which can be included in future cost-benefit analyses.

16. Discussion. The benefits of spraying every two years may also consist in reduced impact on non-target species of Lepidoptera.

We added a paragraph in the discussion to discuss the benefits that spraying every two years may have in terms of allowing better recovery of non-target Lepidoptera (lines 433-452).

Answers to Reviewer 2

1. It is, however, fairly annoying to have to find another paper (in this case, Fuentealba et al. 2019) to get the necessary details on the treatments and site selection, and I strongly recommend including some sort of summary of this information in this manuscript. As a reviewer and reader I feel that papers should be stand-alone units, and not dependent on another manuscript to make or support methodological details.

We added to Table 1 the rationale behind the protection scenarios that were tested in this study.

2. A question that did arise in my mind is how did the authors know that trees were killed by spruce budworm and spruce budworm only? What rationale was applied to ensure that mortality seen was a direct result of defoliation, and not other factors such as drought, root rot, or some other combination? Typically defoliation is but one stressor that can contribute to tree mortality, but it is generally not the only factor. It would be good if the authors could provide some details on this aspect of the study.

Host trees that were killed during the outbreak were first identified by the complete absence of needles. Mortality was then confirmed by examining the cambium near breast height for discoloration and dryness (MacLean 1979). Only trees that were still standing and those present on the forest floor with bark and branches were included in mortality estimates. Given the low annual natural mortality of hosts (MacLean & Ostaff 1989) and that host wood decomposes rapidly (e.g., Campbell and Laroque 2007), the criteria that were used to select trees greatly reduce the likelihood of including natural mortality occurring prior to the outbreak (Bergeron et al. 1995). Furthermore, to remove suppression-related mortality, only trees with DBH ≥ 10 cm were included in mortality estimates. This information is now included in the methodology (lines 175-182).

We hope that you will be pleased with this revision and accept the manuscript for publication in your journal. In addition, we want to thank the reviewers for their useful comments on the manuscript, which allowed us to improve this work.

Sincerely,

Éric Bauce, (on behalf of the co-authors: Alain Dupont, Christian Hébert, Richard Berthiaume, Roberto Quezada-Garcia and Alvaro Fuentealba).

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R1 - Handling Editor (Aurélien Sallé)

Recommendation R1: Accept

Dear authors,

You have adequately taken into consideration the various minor comments that were made to your manuscript. The manuscript is now acceptable, and will constitute a quality contribution to the journal. I am consequently pleased to recommend it for publication.

Thanks for submitting your work to Annals of Forest Science

Sincerely yours

R1 – Editor in chief (Erwin Dreyer)

Decision R1: Accept with minor modifications

Thank you for the careful revision of your manuscript (ANFS-D-24-00018R1), 'Biennial aerial application of Bacillus thuringiensis var. kurstaki provides good protection against spruce budworm (Choristoneura fumiferana [Clemens]).', submitted to Annals of Forest Science.

The revised version of the manuscript was reviewed by the handling editor and by myself. We both found it was improved with respect to the earlier version. We both believe this is a very interesting and important experiment with valuable and useful results. However, when I went through the manuscript myself, I detected many areas for further improvements. Based on our reading, we reached the conclusion that your manuscript will be suitable for publication after a minor but substantial revision. Below, please find the very positive comments of the handling editor.

My own comments and suggestions are mostly reported in the attached version of the manuscript. My main concerns relate to the writing of the text, which should be improved. It is in places overlong and contains a few awkward sentences. I suggested many minor changes to help you produce a more fluid version.

I suggest also that the introduction ends up with a few explicit hypotheses and/or questions, to guide the reader along the rationale of this very interesting and detailed experiment. As it stands the whole paragraph at the end of the introduction sounds a little confusing.

The presentation of the experimental design should be improved. It is clear in general, but we need to understand which is the reference treatment used as control (is the the one without any Btk spray, or on the contrary the one with the current practice used in Québec? Similarly, we would like to see the actual growth data (like was done in the figures for defoliation and other features) before presenting relative changes. This requires changes in the tables and figures.

There is a mistake in the figures: the figure with the cumulated mortality for balsam fir is missing in this copy of the manuscript. Captions of figures and table should be completed as to provide all information needed to understand the content of the tables and the figures.

The mention about the data in the general statements needs be changed as you deposited the data into a public repository. Please provide a link to the repository and add the reference of the data set to the reference list.

The logic of the result presentation needs be improved. I suggest to always start with the actual growth data (absolute values) and only present the relative changes in a second step.

I did not (yet) read through the discussion. It needs anyway to be restructured along the different hypotheses/questions listed at the end of the introduction.

With kind regards, many thanks for this very interesting contribution, and for your willingness to consider our suggestions and comments that all aim at helping to improve the presentation of this very interesting experiment and set of results.

Revised Version R2

R2 – Authors

Author's response to editors' and reviewers' comments

Dear Sir,

We think it better expresses our most striking result as it integrates the economic analysis carried out in the paper, which makes our study innovative compared with others in the literature. We have clarified pertinent sections in the text with the cost-effectiveness concept in mind, which we think will respond to most of the issues raised by the editor. We hope that we have satisfactorily addressed all of the editor' concerns. Please note that a native English speaker edited the final text.

Answers to Editor in chief

I was not convinced by the structure of result presentation. The current presentation mixes actual data and relative values. I would recommend that actual growth data are presented somewhere before analyzing them as relative changes. The loss/gain logic is difficult to understand, and should be

As requested, we modified the structure of the result presentation by beginning the result section with data of cumulative volume losses through mortality and growth reduction expressed in m3. However, we are not sure to understand why the editor considers it difficult to understand the loss/gain logic. The indices used in this study (AVI and SVI) are widely used in North America to analyze growth losses provoked not only by spruce budworm (e.g. MacLean et al. 2024) but also by other defoliators (e.g. balsam fir sawfly (Parsons et al. 2003) and jack pine budworm (Gross 1992, 1996). The logic behind using a pre-treatment growth period to evaluate growth losses is to be able to compare growth rate with and without defoliation. This in turn makes possible to determine the growth rate reduction produced by defoliation and how protection scenarios mitigate this negative impact. In contrast, the use of actual growth data may cause confusion or make it difficult to understand the impact of protection scenarios because it does not consider the growth rate prior to the start of the outbreak. For example, trees from the "no protection" scenario

selected for the stem analysis were those that were still alive after at least 10 years of moderate to severe defoliation. This means that these trees were more vigorous than the typical tree. This is corroborated by the higher SVI exhibited by these trees (see figure 4). Consequently, if we compare actual growth data, we may erroneously conclude that the protection scenarios were not effective in protecting volume growth simply because trees in the "no protection" scenario exhibited higher cumulative volume growth. For this reason, we do not think it is appropriate to include actual growth data as suggested by the editor. However, we believe it could be easier to understand the impact of protection scenarios if we begin the result section presenting data of cumulative volume losses through mortality and growth reduction expressed in m3 (see table 4).

I would suggest to present the actual mortality values rather than the relative increments in mortality.

We decided to begin this section presenting mortality values expressed in m3. However, we think that mortality values expressed in percentage and the relative reductions produced by the protection scenarios are also important and more meaningful because the quantification of the relationship between volume increment and defoliation level is key to using growth models to determine effects of outbreaks on stand and forest productivity, and this is the first study, to our knowledge, to quantify percentage reductions as a function of host species, protection intensity and the number of years after the beginning of treatment application.

Thediscussion needs anyway to be restructured along the different hypotheses/questions listed at the end of the introduction.

We have clarified the hypothesis of this study in lines 116-119 and its goals (lines 119- 124). We consider that the discussion as it is fits well with the hypothesis and the goals of this study, so we think it is not necessary to restructure this section. However, we made some modifications to the discussion to reinforce the key message of this study, namely that applying Btk every 2 years is the most effective and cost-efficient scenario.

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