Re: AFE(2022)4394 entitled "Risk assessment for non-crop hosts of Pea Enation Mosaic Virus and the aphid vector Acyrthosiphon pisum" by Clark, Robert Emerson; Oeller, Elisabeth Constance ; Eigenbrode, Sanford; Crowder, David; Basu, Saumik  
  
Dear Dr. Clark,  
  
Manuscript ID AFE(2022)4394 entitled "Risk assessment for non-crop hosts of Pea Enation Mosaic Virus and the aphid vector Acyrthosiphon pisum" which you submitted to Agricultural and Forest Entomology, has been reviewed.  
  
The reviewers have recommended revisions to your manuscript.  Therefore, I invite you to respond to the reviewers' comments and revise your manuscript.  
  
Sincerely,  
  
Hefin Jones  
Editor, Agricultural and Forest Entomology  
Organisms and Environment Division  
Cardiff  
United Kingdom of Great Britain and Northern Ireland

**Thank you for facilitating the review of our manuscript in Agricultural and Forest Entomology. We have addressed the suggested changes from two reviewers below. The comments have helped to improve the manuscript and we appreciate their critical feedback.**

**All responses are bolded below, with the original text from the reviewers unchanged.**

Reviewer(s)' Comments to Author:  
Reviewer: 1  
  
Referees Comments  
In this article, the authors studied which non-crop (weedy) hosts serve as i) aphid (pea aphid) and pathogen (PEMV) reservoir, and whether the presence and density of one host-plant reservoir correlated with pea aphid presence.  
This is an original and interesting piece of work highlighting the importance of Vicia villosa as a host plant for both a virus of agronomic importance and its aphid vector.  
The manuscript is well written; however, I have a few questions and some suggestions that might improve the MS.  
  
L. 12. I disagree (or I don’t understand properly) with the term “exclusively” here, as in Fig. 2 pea aphid densities are quite important on 2-3 other host plants (even if smaller than on V. villosa) and in Fig. 4 high probability of pea aphids occurs even with ~0m of hairy vetch.

**Edited to highlight that aphids were relatively more abundant in sites dominated by hairy vetch, not exclusive to these sites. Line 12 now reads:**

**“Relatively high densities of *A. pisum* were found in habitats dominated by hairy vetch (*Vicia villosa*), which was the only legume other than cultivated pulses where PEMV was detected.”**

L. 92. The authors mention 60 locations but all do not appear on the map Fig. 1. Please reorganize the Figure.

**We changed the text to better reflect the sampling strategy. Also, see the next comment on removing some points that are overlapping.**

**“This so-called “outbreak year” thus provided an opportunity to discover the non-crop hosts for A. pisum and PEMV in a season when aphids are widespread, thus we targeted sampling at areas with patches of weedy legumes in 60 sites (30 locations >1km apart, each with two repeated visits but samples taken 150m apart).”**

Please complete/correct Figure 1, triangles and circles overlap on the map, hiding some red PEMV location sites.

**This is a good suggestion since the goal is to show the spread of locations and presence of PEMV rather than the survey design. Therefore, the shapes modified to show overlapping points better, especially places with PEMV.**

**To prevent overlapping points, we slightly reduced the size of points drawn on the map. For locations were overlapping still occurred, we removed some points that occurred within 150m to 250m. Due to the scale of this map, this overlap and do not provide any new information. The text in the figure caption is modified accordingly with the following sentence at the end:**

**“Repeated sampling locations 150m to 250m in proximity not shown to prevent overlapping points on the map.”**

L. 103. The authors mention “10m line transect” but 20 m transect in Fig. S2. Which size is correct?

**10m line transect was a typo. 20m is correct and this was verified with the original 2018 field data during this revision. The text has been updated.**

L. 115. 60 locations (L. 103) turned into 65 locations. Please correct.

**Five additional opportunistic plants were collected, but this is mentioned later in the methods and these five are not the result of transect sampling. Thank you to the reviewer for catching this mistake. The text is modified.**

**“…pooled samples of all tissue collected from each transect (n = 60).”**

**The end of this section now reads:**

**“Five additional *V. villosa* samples were to rule out contamination as the cause of PEMV detection at this site.”**  
  
L. 133. Which one (not shown in Fig. 1)? And why this precision?

**The site is not included in the text to avoid cluttering Fig 1. During the field season all technicians are handling plant tissue potential infected with PEMV, and even though clean protocols were used, we felt it necessary to validate through additional sampling that PEMV was present and persistent in the site with the strongest PCR signal as proof of our methodology.**

**The text now reads:**

**“For one large population of hairy vetch that contained PEMV (Wawawei Park Road, 46.630, -117.378), we revisited the site later in the season and sampled a living, adjacent hairy vetch population, validating that PEMV was indeed persistent in this location.”**  
  
Fig. 2. The figure is difficult to understand, i) the axes are badly positioned and the text overlaps with the bar plots. ii) the axis title is incomplete and shifted up. iii) the authors mention a density but the x-axis is "log aphid per meter" (so not per surface). I'm not sure I fully understand the calculation used here and why this unit.

Also, are the two 180° “sweeps” fully representative of the total length of the transect (10 or 20 meters)?

I would like more details on this protocol and the calculations made after it.

The authors mention 23 host species but only 17 names are presented in Fig. 2. I guess the missing plants are included under the categories Trifolium sp. Vigna sp. and Astralagus sp., right? Please give some more details.

**We thank the author for providing many suggestions on how to improve this figure, so we have made changes accordingly. However, some of the issues with text overlapping were not found in the PDF we submitted, so we suspect there might have been a file conversion issue in the original submission.**

**The following changes were made in the figure to address these comments:**

**We changed the axis labels in Fig. 2, with the y-axis being “Legume species from transects” and the x-axis being “Aphids per meter sampled” log scale. For additional information we rewrote the Figure caption to include the description of how these values are calculated.**

**“Fig 2. Cumulative aphid counts per meter of sampled plants (log transformed). Bar length equals the total abundance of aphids divided by the total meters covered by each individual host plant. Bar colors indicate whether a host plant was discovered with PEMV through rtPCR.** **Six host plant species are not shown as they occurred only incidentially in a single transect and did not have aphids or PEMV.”**

**To clarify, there is no statistical test for this figure, it is merely a data visualization showing the range of aphid abundances found on different hosts and a quick summary of which plants had aphids & PEMV at all.**

L. 153. How are the transects distributed in the 65 sites? And why is it not homogeneous?  
The authors mention 5 "opportunistic" sites, are they included in the 65? Is it why they first mentioned n=60 L. 103 and n=65 now?

**This issue is addressed in the updated methods section (60 sites, 5 follow up opportunistic collections).**  
  
L. 157. I think the authors reversed the figure numbers.

**This paragraph has been rewritten following the above suggestion as well:**

**“Among all transects, we collected 15,289 A. pisum aphids in total and assayed 1,076 candidate plant tissue samples for PEMV. In our transects we recorded 145 species of annual plants, of which 23 were in the family Fabaceae. We observed a range of abundances of aphids on non-crop hosts (Fig. 2) and abundance of non-crop legumes (Fig. 3).”**  
  
Fig. 3. Figure 3 has the same axis and label problems as Fig. 2. What is the total length of the surveys? Again, I'm confused by the y-axis unit. Isn't it simpler to present a coverage percentage?

**The axis label has been modified following Fig 2. And the figure caption is also updated to provide more information. We chose to stay with cumulative plant cover since it is the same value being used as the denominator for the calculation in Fig. 2.**  
  
Fig. 4. The x-label is incomplete and shifted up. (The printed version is even more incomplete).

**Formatting error in the pdf production process. We did not see this in our submitted manuscript but will check upon resubmission.**

Isn't it interesting to add an R² to this kind of correlation?

**Since this is a logistic GLM, it is possible to calculate the Pseudo- R², which in this case is 0.049, but these coefficients cannot be interpreted the same way as OLS regression, so we decided it does not add anything to the figure or figure caption.**

On the figure, we can read that on one transect, there is more than a 15m cover of hairy vetch, so the transects are indeed 20m and not 10m long as stated in L. 103. Please correct.

**All transects were 20m and the text has been updated in the manuscript and figures.**  
  
Fig. S4. If I read Fig. S4, no positive samples are shown on the gels. It's a little confusing to propose a gel presenting only "negative" samples as an example of detection... Moreover, the lanes do not follow the same order in the title of the figure "DNA ladder, positive control and negative control" and in the gel (negative, positive, Ladder), please harmonize.

**Figure caption updated. “In this case, samples from sites in lanes 1-6 and 10-15 were negative for PEMV. On the far right both gels are the negative controls (7 and 16), positive controls (8 and 17), and DNA ladder (9 and 18).”**

**We also changed the figure in order to show a gel with positive results.**  
  
L. 217. please correct “corps”.  
  
**Changed to crops, thank you for finding that typo. We did not mean to imply that aphids could use a military formation as a host.**

Reviewer: 2  
  
Referees Comments  
The paper has merit to be published. However, some points must be revised.

I suggest that the authors describe the current status of the virus in the first paragraph of the introduction. Suggestion: According to Hema et al. (2014), pea ination mosaic virus is an important virus disease of pea caused by two viruses in an obligate symbiosis: Pea enation mosaic virus-1 (Enamovirus, Luteoviridae), transmited in a circulative manner by aphids and Pea enation mosaic virus-2 (Umbravirus, Tombusviridae).  PEMV-1 occurs as part of a complex with PEMV-2 and induces mosaic symptoms and enations. Unlike other members of the family Luteoviridae, PEMV-1 is readily transmitted mechanically, a property dependent on its multiplication in cells co-infected with PEMV-2. Aphid transmissibility is conferred by PEMV-1. Virions are found in mesophyll tissue as well as in vascular tissue. The genome of PEMV-1 is capable of autonomous replication in protoplasts, but is dependent on PEMV-2 to support systemic invasion (Hema, M. et al. Adv. Virus Res. 90: 431, 2014).  
  
Line 11: pea enation mosaic virus (PEMV) “According to ICTV this is the correct form to describe virus isolates (lowercase letters, not italics).

**Change made.**  
  
Lines 22-23: The plant viruses transmitted in a circulative manner a need insect for spread, especially phloem-feeding hemipterans like aphids (Power 2000; Hogenhout et al. 2008).

**Change made with slight modifications.**

**“Circulative-transmitted viruses require an insect vector, often phloem-feeding hemipterans like aphids (Power 2000; Hogenhout et al. 2008).”**  
  
Line 69: ... including PEMV (Rashed et al. 2018; Chatzivassiliou 2021)

**Change made to just use the abbreviation.**  
  
Line 88: Alate aphids were counted...

**This change is made along with some slight modifications to improve the clarity in this section.**

Line 112: In the item “PEMV detection in plant”, emphasize that RT-PCR was specific for detection of PEMV-1 which is responsible for transmission by aphids.

**Subsection header is now “*PEMV-1 detection in plants by RT-PCR*”.**  
  
Line 192: (Paudel et al. 2018)

**Change made.**  
  
Papers present in the item "Reference" but not mentioned in the text:  
Al-Karaki, G. N. (1999) **removed**  
Ali, M. P. et al (2014) **removed**  
Chisholm, P. J. et al (2018) **added to methods section**  
Northfield, T. D. et al (2008) **added to discussion section**  
Pernek, M. et al (2008) **added to discussion section**  
Takahashi, H. et al (2019) **added to introduction**  
Teasdale, J. R. et al (2004) **removed**  
Wenninger, E. J. et al (2019) **added to introduction**  
Zalucki, M. P. & Furlong, M. J. (2005) **removed**

Paper cited in the text and not present in the item "Reference"  
Damgaard, C. et al (2019)

**Changed to 2020.**

Figures 2 and 3: sp. it's not in italic.  
  
  
**Figure updated.**