

Dear Professor Gonthier,

We thank the reviewers generously for their time and their feedback on our manuscript, which has been edited to address their concerns. All minor suggestions have been accepted. All other comments have been bulleted below, followed by the way each has been addressed (in red).

Sincerely,

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Reviewer #1:

- Of particular concern is the use of systemic chemical formulations designed for foliar applications only. These formulations contain solvents known to cause phytotoxic effects and may have effected uptake and translocation of active ingredients to targeted areas of the trees.

We would have preferred to use formulations developed for trunk injection, but unfortunately these are not currently available in South Africa. However, care was taken to inject on a day where temperatures weren't too hot, and in a time where soil moisture was not limiting, e.g., under conditions best for rapid uptake to reduce phytotoxicity. The emamectin benzoate product was also highly diluted, as per a previous paper testing the uptake of trunk injected emamectin benzoate at different dilution levels, in order to increase uptake and decrease phytotoxic effects.

The following was added at L392 regarding potential effects of systemic chemical formulations used: "The chemical formulations of these systemic chemicals may have also had an impact, as emulsifiable concentrates of pesticides have demonstrated phytotoxic effects in other studies using trunk injection (Archer et al. 2022; Kiss et al. 2023), which could have inhibited uptake if tree transport tissues were affected. In this study, observation 12 months after injection showed that 3 (of 16 total) trees had

bark discoloration up to *ca.* 10 cm surrounding injection sites, but no visible impacts on overall tree health were observed. Nonetheless, it is recommended that chemical formulations created for trunk injection are used in future research iterations, especially in the case of repeat applications.”

- Need to evaluate the success of additional colonization attempts by beetles for each of the treatments. Contact chemicals will likely effect beetles before they attempt to borer into the host, whereas systemic chemical will effect beetles only after they bore through the bark surface. So, an attempt is made, but the beetle may or may not be successful

In this study, PSHB colonisation was analysed in two ways. In the first, additional PSHB holes at the end of the experimental period (as a result of natural infestation) were counted as new colonisation attempts. These holes were not further excavated/analysed in order to prevent mechanical damage to the trees. Therefore, unfortunately there is not data to conduct further analysis to determine colonisation success on these attempts.

For the second analysis, PSHB beetles were manually introduced into tree branches using 3-D printed traps. Because these branches were removed at the end of the experimental period, the galleries could be excavated and analysed for colonisation success (whether the beetle survived the attempt and/or created a colony). Colonisation success was therefore analysed for the beetles that were manually introduced.

To clarify this, the mention of prophylactic control has been removed from the manuscript. It is now only stated that we tested for therapeutic control, while colonisation attempts are still distinguished from colonisation success. In the case of additional PSHB holes due to natural infestation, only colonisation attempts can be characterised. In the case of manually introduced beetles, colonisation success can be characterised.

Pasted from manuscript:

- L160 “Before applying treatments, the diameter at breast height (DBH) and the number of PSHB holes were recorded for each tree on November 4, 2022.” -From ground to 3 m or whole tree? Clarify

Line changed to: “Before applying treatments, the diameter at breast height (DBH) and the number of PSHB holes (up to a 3 m trunk height) were recorded for each tree on November 4, 2022.”

- L170 “One branch on each selected tree was inoculated with the three *F. euwallaceae* isolates and a control” - Size (diameter) and location on tree?

Line changed to: “The lowest branch (*ca.* 1.5 m height and 10 cm diameter) on each selected tree was inoculated with the three *F. euwallaceae* isolates and a control”

- L232 Response to emamectin benzoate and propiconazole treatments in Table 1: The labels for both Warlock and Bumper indicate that they are formulated for foliar spray applications only; not for trunk injections. Were the phloem and sapwood tissues above the injection sites evaluated for discoloration and necrosis? Based on previous experience with injections of Denim in the USA, it is highly likely that discoloration and necrosis of tissue would also occur with your injected formulations, particularly with Warlock. As a result, upward movement and distribution of the active ingredients within the trees would be reduced/inhibited.

As stated above, although we would have preferred the use of products formulated for injection, they were unfortunately unavailable. However, conditions while injecting and dilution of products were optimised for uptake.

As also stated above, the following excerpt was added at L392 regarding potential effects of systemic chemical formulations used: “The chemical formulations of these systemic chemicals may have also had an impact, as emulsifiable concentrates of pesticides have demonstrated phytotoxic effects in other studies using trunk injection (Archer et al. 2022; Kiss et al. 2023), which could have inhibited uptake if tree transport tissues were affected. In this study, observation 12 months after injection showed that 3 (of 16 total) trees had bark discoloration up to *ca.* 10 cm surrounding injection sites, but no visible impacts on overall tree health were observed. Nonetheless, it is recommended that chemical formulations created for trunk injection are used in future research iterations, especially in the case of repeat applications.”)

- L316 Response to Fig. 3 representing differences among new colonisation attempts across treatment and control trees: It is of interest to know if the number of additional beetle attempted to colonize the host after treatment. However, what is more important is how many of the additional attempts were successful (the beetle survive its attempt). It seems unlikely that many attempts would be detected after application of contact insecticide because of quick knockdown after touching treated surfaces. However, more attempts would be observed with systemics because its necessary for beetles to bore through the bark to come in contact with these chemicals in the phloem and sapwood tissues.

As stated above, in this study, PSHB colonisation was analysed in two ways. In the first, additional PSHB holes at the end of the experimental period (as a result of natural infestation) were counted as new colonisation attempts. These holes were not further excavated/analysed in order to prevent mechanical damage to the trees, especially on the main trunk. Therefore, unfortunately there is not data to conduct further analysis on new colonisation attempts (additional PSHB holes).

For the second analysis, PSHB beetles were manually introduced into tree branches using 3-D printed traps. Because these branches were removed at the end of the experimental period, the galleries could be excavated and analysed for colonisation

success (whether the beetle survived the attempt and/or created a colony). Colonisation success was therefore analysed for the beetles that were manually introduced.

- L376 “The systemic treatments, emamectin benzoate and propiconazole, did not reduce colonisation attempts and thus did not offer prophylactic control.” This statement is no necessarily correct. Yes, EB and propi will not reduce attempts to colonize the host. However, these treatments will sig reduce the success rate of colonization (either survival of adult beetles or symbiotic fungi) Need to know how many of the attempts were successful? Successful attempts/attack sites almost always bleed (the bark is wet around the attack site). Whereas unsuccessful attempts are indicated by dry bark surface around the attack site.”

As stated above, there is not data to analyse the colonisation success rate of new PSHB holes (as a result of natural infestation), only of galleries created by manually introduced PSHB. To clarify this, the mention of prophylactic control has been removed from the manuscript. It is now only stated that we tested for therapeutic control, while colonisation attempts are still distinguished from colonisation success. In the case of additional PSHB holes, only colonisation attempts can be characterised. In the case of manually introduced beetles, colonisation attempts and colonisation success can be characterised.

- L447 “While the fungal growth rate was decreased, propiconazole did not kill the fungus and it would therefore still likely be available to the beetles in the galleries.” Propiconazole is not a true fungicide, rather it is referred to as a fungistat; it doesn't kill the fungi, but instead holds it at bay. As long as the concentration of the propi in the sapwood is above a threshold the fungi will not grow and expand, but once the concentration drops below the threshold (usually after 18 month) the fungi is able to resume growth.

Line changed to: “It seems plausible that during the current study, propiconazole concentration in sapwood tissues were sufficient to reduce *F. euwallaceae* growth, but insufficient to entirely halt growth (Peyton et al. 2015). Therefore, the fungus was likely still available as a food source to the beetles in the galleries.”

- L470 “Importantly, the environmental impact of these treatments, especially the contact chemicals, as well as the possible phytotoxic effects, especially for the systemic chemicals, should be evaluated.” Yes, see comment above related to use of systemic chemical formulations designed specifically for foliar applications. Consider using formulation designed for tree injection and known not to cause phytotoxic injury.

Comment addressed in L392 with same addition as a previous: “The chemical formulations of these systemic chemicals may have also had an impact, as emulsifiable concentrates of pesticides have demonstrated phytotoxic effects in other

studies using trunk injection (Archer et al. 2022; Kiss et al. 2023), which could have inhibited uptake if tree transport tissues were affected. In this study, observation 12 months after injection showed that 3 (of 16 total) trees had bark discoloration up to ca. 10 cm surrounding injection sites, but no visible impacts on overall tree health were observed. Nonetheless, it is recommended that chemical formulations created for trunk injection are used in future research iterations, especially in the case of repeat applications.”

Reviewer #2:

- L70: “The PSHB vectors multiple species of fungi, which grow within galleries that the beetle constructs in the xylem tissues of woody hosts.” Is it really an advantage to apply a systemic compound when dead tissue needs to be protected? Authors, please comment on how a systemic compound can end up in dead tissue like wood / xylem in the discussion, potentially where you write about the limitations in efficacy.

Though xylem cells die once mature (apart from parenchyma cells), they are still functional as vessels that transport water and minerals within the tree, which is how injected systemic chemicals are then distributed. As many systemic pesticides are not phloem mobile, many injectable products instruct injection into the xylem. Further, since the PSHB beetle and its pathogenic fungus colonise in the xylem, injection into the xylem provides the best target. The ability of a xylem-injected pesticide to establish and distribute within the areas of a tree most vulnerable to attack by PSHB was demonstrated in a paper referenced in the manuscript (Byrne et al. 2020).

- L84: “The fungus may persist and cause disease symptoms, but the infection is usually not fatal.” For being fatal, the pathogen must reach the living tissue of the plant. I am not sure if fungal growth on dead tissue is considered as an infection. The authors may wish to check their choice of words, here

Line changed to: “The fungus may persist and cause disease symptoms, but fungal establishment alone is usually not fatal.”

- L212: The meaning of PanAf n.d. is not clear to me. Please explain at a more prominent position.

The full name of PanAf is expanded in L124 as PanAfrican Farms. Line L124 has been expanded to include “(PanAfrican Farms [PanAf], Parys, South Africa, <https://www.panafricanfarms.co.za>)”

- L339 and L355: “These treatments should therefore be considered viable options as part of an integrated pest management program against PSHB on American sweetgum.” Prophylactic treatments do not seem to be in line with the rationales of integrated pest management, where low levels of infestation are usually left uncontrolled and control thresholds are advertised. Please avoid suggestions that a

prophylactic treatment could be in agreement with the principles of integrated pest management.

As per a previous comment, mentions of prophylactic control have been removed. However, L355 has also been changed to “management program” rather than “integrated pest management program”