Round #1
by Cedric Pennetier, 26 Apr 2022 09:34
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Dear Louise van Oudenhove and authors,
Thanks so much for submitted your preprint to PCI Zoology.
Your preprint was reviewed by 3 specialists and all of them agree that your work is highly relevant. Reviewers highlighted few major concerns that should be addressed in ordrer to improve the quality of your manuscript.
So I invite you to go through all comments and submit a revised version of your preprint.
Thanks you again for your support to PCI.
Cédric Pennetier

AUT>> Thank you for your these reviews.
We addressed all reviewers'comments (see detailed answer bellow) and modified our manuscript consequently.
Please apologize the delay of this reply.
Thank you for your understanding.

Louise van Oudenhove in behalf of the authors.

Reviews

Reviewed by anonymous reviewer, 20 Apr 2022 15:49

AUT >> Most suggestions of the annotated document have been accepted. Some were reformulated as detailed bellow :

REV>> L. 41: I am not sure I understand this very well. Did you mean that "BPs should complement natural control agents such as parasitoids and predators"?

AUT>> Not exactly. We meant that "In an Integrated Pest Management (IPM) context, different strategies can be required for crop protection, and BPs can be deployed together with biocontrol agents such as parasitoids and predators." We modified the formulation: [L46-48]

REV>> L. 103: Wonder the basis for choosing these quantities? How they compare with what would happen in the field?

AUT>> Tested doses correspond to 909, 1818 and 3636 ppm. These values were chosen closed to the estimated LC_{01}, LC_{10} and LC_{50} of *Ferula assafoetida* EO on adult female *Trichogramma* wasps (Poorjavad et al., 2014).
This point has been added to the material and methods: [L128-130]. We also added a paragraph about the suitability of this choice and the question of concentration in the discussion: [L449- 470]

REV>> L. 342: what this means?

AUT>> “hardly no impact” was changed to “no observable impact”: [L 391]

REV>> L. 417: Why do you provide a reference here ?

AUT>> We removed the reference.

REV>> L. 417: It would be worth conducting a risk/benefit study. If the benefit of using EOs outweights the risks to parasitoids then there should still be a value in using those EOs

AUT>> We added the following sentence: [L508-509]. Using EOs for plant protection would require to carrefully study the benefits (toxicity on pests) and risks (effects on natural enemies and phytotoxicity) (Dunan et al. 2021).

Reviewed by anonymous reviewer, 14 Apr 2022 08:57

The manuscript by van Oudenhove et al. entitled "Non-target effects of ten essential oils on the egg-parasitoid Trichogramma evanescens" reports laboratory experiments on both parasitoid pre-imaginal development and female movements when exposed to vapor of pure essential oils. Authors selected 5 strains of Trichogramma evanescens which were exposed to different doses of ten essential oils (EOs) either in a tube or in a 4-way olfactometer to investigate effect on development or movements, respectively. The authors used Bayesian statistics to analyze all the data.

REV>> As I am not qualified to review Bayesian analyzes, I did not make any comments on it. Nevertheless, I found it sometimes difficult to comprehend and I had to trust the authors on results interpretations.

AUT>> Since this comment was also risen by the third reviewer, we asked for a review of the Bayesian analysis part to an expert (Julien Papaix, INRAE Avignon, France). Following this, we simplified our analysis, we added an illustration of the model function (Fig 4), and we also present the data together with the Bayesian estimations in the result section (Fig. 6).

REV>> The rationale is well steered and results support conclusions. However, I expected more discussion about the parasitoid movements and the origin of strains. Is it possible to make more link between secondary compounds found in "mother plants", movements of parasitoids while exposed to EOs and the inter-strain variability? I think it could underline the originality of working with several strains of a single parasitoid species.

AUT>> We developed the idea on inter-strain diversity and the link between preference and sampled plants’ VOC in the discussion with the following: [L. 481-489]. Strains AM002 and ESP467 were sampled respectively on quince and common bean. None of these plants (or fruits) contains anethole or other phenylpropanoid organic compounds (Tsuneya et al., 1983; Karolkowski et al., 2021). However, these plants might not be fully representative of the local environmental conditions of these strains. On the one hand, parasitoids were reared in the laboratory, without plants, for about 50 generations before experiments. On the other hand, the parasitoid sampling protocol, based on sentinel egg cards exposition, resulted in extremely low capture rate (<5%) and showed no reproducible patterns regarding host-plant associations (Ion Scotta, 2019). It might thus be difficult to connect parasitoid strains’ olfactory preferences with the volatile organic compounds of the sampled plants.

REV>> My main concern is about the doses used to test toxicity on pre-imaginal development. Compared to a

quick search in literature, doses used here seem to be very high (see comment below). The authors should explain this choice and specify why it is acceptable and/or discuss this drawback.

AUT>> We added a justification of our choice in the material and methods [L. 128-130] and we also added a paragraph about the suitability of this choice and the question of concentration in the discussion [L449-470].

Hereafter are some comments intended to help the authors to improve the manuscript.

Introduction

REV>> -lines 65-66: I guess that many EOs have an insecticidal effect. Is there a reason to choose specifically these ten Eos?

AUT>> The EOs were chosen among EOs with insecticidal properties in order to explore Mediterranean plant diversity. Orange and Peppermint EOs belong to the list of natural products acknowledged by the french government for phytopharmaceutical use. Rosemary and Thyme EOs belong to the list of active ingredients eligible for minimum risk pesticide product from the United States Environmental Protection Agency. We chose to explore the Lamiaceae family by including two common aromatic plants: Basil and Oregano (Ikbal and Pavela, 2019). We diversified plant families by including Asteraceae: Mugwort (Wang et al, 2006) and three Apiaceae: Coriander (Khedr and El-Kawas, 2013), Anise and Fennel (Dunan et al., 2021). This explanation was added to the material and method section [L102-112].

REV>> -line 69: "parasitoid" not "paratoid"

AUT>> Ok.

REV>> -line 69: "movement" is not clear enough in the context of the last section of the introduction. Please be more specific.

AUT>> We modified the sentence as follows: [L.83-84]. Behavioral consequences were studied by testing if vapors of EOs influenced parasitoid distribution in a 4-way olfactometer.

Materials & Methods

REV>> -lines 73-78: As for EOs, is there a reason to choose these five strains of T. evanescence? Did the authors expect different results according to the origin of parasitoids? The five strains were collected in 2015 and 2016. When were carried out the experiments? How many generations were obtained between collection and experiments?

AUT>> Strains were chosen among the 20 available living strains of *T. Evanescens* in the BRC Ep-Coll. Strain diversity was maximized by choosing strains from different sampling sites and from different sampled host plants. Experiments were carried out in 2018, so parasitoids were reared in the lab for about 30 to 55 generations before experiments. These information were provided in the main text [L88-95].

REV>> -line 98: Please, change "experiment" for "exposure to Eos"

AUT>> Ok [L.121].

REV>> -line 99: Were all the eggs parasitized? If not known at Day 1, please reword. Is there any chance that around 50 females for around 25 eggs produced superparasitism? If yes, is it possible that it affects results on pre-imaginal development and emergence rate?

AUT>> All eggs were probably not parasitized. In all treatments, host:parasitoid ratios were quite similar to the rearing conditions, in which about 500 eggs are exposed to 300 adults, resulting in about 60% parasitism. At these densities and with this host species, superparasitism is rarely observed. When several eggs are laid in a single host, in most cases, a single adult emerges (Corrigan et al., 1995). So we do not expect superparasitism to be important, or to differ across our treatments. We have clarified this in the text [L.136-138]: “Adults emerged on day (D+14). About one adult emerged per parasitized egg, since superparasitism is rarely observed in these conditions, and in most cases, even when several eggs are laid in a single host, a single adult emerge (Corrigan et al., 1995).”

We’ve also realized we had made a mistake in the description of the experiment, when describing the estimation of the number of eggs per adult. Two cards with 5 patches of eggs each were exposed to 300 adults (that came from the rearing conditions). This represents 2*5*25 = 250 eggs. We have reformulated as follows: [L121-123]. “small patches with about 25 ± 7 *E. kuehniella* eggs were exposed during 24h to emerging parasitoids (two cards with five patches each, i.e. 250 host eggs, were placed with about 300 individuals with a sex-ratio around 0.45 ± 0.11).

REV>> -lines 103-104: Please, provide concentrations to make them comparable to other studies. When I converted doses in concentrations, they seem to be very high. Are they relevant if used in the field? This point could be discussed.

AUT>> Tested doses thus correspond to 909, 1818 and 3636 ppm. These values were chosen closed to the estimated LC_{01}, LC_{10} and LC_{50} of *Ferula assafoetida* EO on adult female *Trichogramma* wasps (Poorjavad et al., 2014). This point was added to the material and methods section [128-130]. A paragraph about the concentration used and its relevance in the field was added in the discussion [449-470].

REV>> -line 106 and throughout the manuscript: Please use "location" instead of "position".

AUT>> Ok.

REV>> -lines 122-130: Please, use the past tense.

AUT>> Ok.

REV>> -line 129: What was the extraction air flow?

AUT>> We don’t know exactly the value of the extraction air flow. But since the flexible ducting hose conducting to the exhaust fan was not hermetically fixed, air was not pulled out. Besides, extraction did not influence the airflow inside the device (checked with flowmeter). We modified the text in order to avoid confusion [L157]. Air from the hole was gently extracted out of the room via an exhaust fan.

REV>> -lines 134-135: Did all the females climb up? What happened if too few females climbed up?

AUT>> No. All the females did not always climb up. Experiments were stopped (and postponed) when too few individuals climbed up (<10) after 15 minutes. This sentence was added to the manuscript [L163-164].

REV>> -line 136: I guess it was with the same EO.

AUT>> Yes. We precised it to avoid confusion [L165].

REV>> -lines 189-191: From which data was the initial parasitism rate estimated? If I understood well, the first data collected are at day 5 to estimate the number of eggs turning black (pupal stage).

AUT>> Initial parasitism rate was not estimated at this step. We only had access to what we called “the effective parasitism rate after five days” meaning the number of pupae divided by all the available eggs in the patch. However, since a need for model simplification was raised by the third reviewer, we modified our model. Effective parasitism rate was no more useful so we removed this definition.

REV>> -line 239 and legend of Table 3: "µL" instead of "mL"

AUT>> Ok.

REV>> -line 240-247: In repeated measurements of individual locations, the probability that an individual is located in a given area at t(n) is dependent from its location at t(n-1). Does the model take account for the previous location of each individuals? Or, are the parasitoid movements fast and long enough (in a 2-3min laps time) to consider as equal the probability for a parasitoid to be located at any point?

AUT>> We do not know individual location, we only have access to the number of individuals in a given zone. We divided parasitoid movement in two steps: (a) to stay or not to stay in their zone (phase ii), and (b) for those who leave their zone, a random allocation in the exposure chamber (phase iii). For this phase iii, we indeed assumed that parasitoids can move freely and that the probability to land in one of the three other zones is similar.

Results

REV>> -lines 272-297: This section is very unclear, I had to read it several times and I am not sure I understood well. Authors provide a C50 (which is in fact a LD50, see below) which seems to correspond to the overall development (<2µl). Then, they provide probabilities for the first phase of development (α) and the second phase (β) for a dose of 5µL. Using the same estimation procedure for survival would help the reader to comprehend. Maybe the authors should consider reworking this section.

AUT>> We’ve totally reworked the model and this section [L318-351]. We now use the same procedure for global survival and the distinction between the two phases.

REV>> -line 272: What the authors named "C50" is in fact a "LD50" (if it can be considered that an absence of development into adults is related to the death of larvae, otherwise it is an ED50).

AUT>> We agree. We've modified the manuscript using LD50. We defined the concept in the materials and methods section [L 251-256].

Discussion

REV>> -lines 349-350: "emergence rate" and "pre-imaginal development" seem to be used indifferently to refer to the same data point. Please pick one and be consistent across the manuscript or clarify.

AUT>> We chose the term "pre-imaginal survival" and made sure to be consistent across the manuscript.

REV>> -line 360: It could also be a consequence of stage duration as pupal stage is almost twice the larval stage duration.

AUT>> In our analysis, we modeled a daily survival probability elevated to the power "number of days spent in that stage". The duration of the stage was thus explicitly taken into account. We tried to made this clearer in the "materials and methods" section (Fig. 4).

REV>> -lines 386-390: Unclear

AUT>> We modified this part [442-448].

REV>> -lines 373-390: The conclusion of this section could be that exposure mode could be highly important to evaluate parasitoid susceptibility to Eos.

AUT>> We integrated this sentence: [L448]. Exposure mode is thus crucial to evaluate parasitoid susceptibility to EOs.

End of comments.

Reviewed by anonymous reviewer, 14 Apr 2022 11:35

The work presented in the manuscript by VanOudenhove and collaborators entitled "Non-target effects of ten essential oils on the egg-parasitoid Trichogramma evanescens" investigates the fumigant effect of 10 essentials oils on the parasitoid's development and behavior. To that purpose, authors conducted 2 experiments. First, they exposed eggs of 5 strains of T. evanescens to vapor of 3 doses of each EO and characterized the probability of further development into pupae and into adult. In a second experiment, they exposed females parasitoids to 2 doses of each EO in a 4-ways olfactometer to investigate attractant/neutral/repellent properties of the EO. Authors found that some EO have toxic effect on the development of the parasitoids while other EO have significant effect on their behavior (repellency/attraction).
I found this work highly relevant as it could highlight adverse effect of a promising new method for pest control. Nevertheless, I have some concerns and remarks about the methodology, presentation and interpretations of the results that I have detailed in the part below.

REV>> I suggest the authors to re-organize and clarify the results presentation, particularly the effect of EO on development.

AUT>> We simplified this analysis, clarified the methods and homogenized the presentation of the results.

Introduction

REV>> - Are EO tested already available as fumigant bio pesticides ?

AUT>> The EOs were chosen among EOs with insecticidal properties in order to explore Mediterranean plant diversity. Orange and Peppermint EOs belong to the list of natural products acknowledged by the french government for phytopharmaceutical use. Rosemary and Thyme EOs belong to the list of active ingredients eligible for minimum risk pesticide product from the United States Environmental Protection Agency. We chose to explore the Lamiaceae family by including two common aromatic plants: Basil and Oregano (Ikbal and Pavela, 2019). We diversified plant families by including Asteraceae: Mugwort (Wang et al, 2006) and three Apiaceae: Coriander (Khedr and El-Kawas, 2013), Anise and Fennel (Dunan et al., 2021). This explanation was added to the material and method section [L102-112].

REV>> - Authors should justify the choice of the fumigant form for assessing the insecticidal and repellent potential of chosen Eos.

AUT>> There are two reasons for focusing on fumigant form. First, contact toxicity on *Trichogramma* species is much more studied than fumigant toxicity. We made it clear in the introduction by giving more details on the existing litterature [L. 55-61]. Second, contact assay might reveal higher risk of phytotoxicity on crops, then fumigant formulations of EO-based BP might be favoured (Cloyd et al. 2009, Ikbal and Pavela, 2019, Werrie et al. 2020). We added this idea in the introduction [L78-80].

REV>> § 2 : I recommend to the authors to define clearly « non-target » term as it looks confusing during reading. I first understood this term as an effect on non target species (l.36) and later as a synonym of sub lethal effect (l.37:39).

AUT>> Ok. We define as “non-target effects” any consequences, different from the desired effect on the target pest species (e.g. death, repulsion, attraction for trapping, etc), caused by the use of a pest-control strategy. Non-target effects might be helpful (e.g. effects on other pest species) or problematic (e.g. effects on pollinators or natural enemies of the pests), and might affect very different ecological levels from individual organisms to ecosystems (Kohler and Triebskorn, 2013). We included this definition in the manuscript [L38-52].

REV>>- L65:70 : Use of terms « direct » and « indirect » effect seems confusing because I first though that « direct » mean after a contact with the EO and « indirect » without contact. I suggest to authors to change these words and directly talk about toxic effect and behavioral (or other word) effect.

AUT>> We meant direct/indirect effect on fitness. To avoid confusion, we adopted the proposition and chose the terms “toxicity” and “behavioral consequences” [L76-84].

Method

REV>> - Insects : the descriptions of strains used and rearing conditions are well done. I suggest to the authors to justify the choice of these 5 strains.

AUT>> Strains were chosen among the 20 available living strains of *T. Evanescens* in the BRC Ep-Coll. Strain diversity was maximized by choosing strains from different sampling sites and from different sampled host plants. These information were provided in the main text [L88-90].

REV>> - Essential oils : the description and origins of the EOs are well written. Full description of EO composition in supplementary Information is clearly presented.

REV>> - L89 : Typo « ; » instead of « . »

AUT>> Ok.

REV>> - L103 : How did the authors chose the volume of essential oil ? Are there previous experiments done that studied the volume to use? Please add a reference if any.

AUT>> Tested doses thus correspond to 909, 1818 and 3636 ppm. These values were chosen closed to the estimated LC_{01}, LC_{10} and LC_{50} of *Ferula assafoetida* EO on adult female *Trichogramma* wasps (Poorjavad et al., 2014). This has been specified in the text [L128-130].

REV>> - L104: The volume of air in the tube seems to be very small in comparison to the volume of essential oil deposited in the cotton. Do the authors have an idea about the concentration of EOs in the tube? Is it the concentration used in the field for biocontrol method?

AUT>> Tested doses thus correspond to 909, 1818 and 3636 ppm (See comment above). A paragraph was added in the discussion to question the relevance of the concentration used [L449-470].

REV>> - As tubes were closed during all the experiment, maybe it could be a risk of saturation/increase of concentration of EO along days. I have a concern about the influence that could have on development.

AUT>> Tubes were not hermetically closed (cotton plugs). So, we could face three mechanisms: saturation ; evaporation, and degradation. We don’t know how the concentration evolves inside the tube. We discussed this point in the discussion [L.449-470].

REV>> - The tubes are in dark all the time, is this natural condition? If not, can't the lack of light/dark alternance and therefore, daily rhythm influence pupation or the emergence of adults?

AUT>> We don’t think that the lack of light alternance significantly affects parasitoid development. It might thought be important for future behavior. But in this experiment, we only check if parasitoids emerge. To be more rigorous about experimental condition, we did not add artificial light, but since the hood did not close hermetically and there are windows in the lab, light naturally varied between days and nights. We added the following sentence to the manuscript [L133]: There was no artificial light.

- Olfactometry bioassays: The device is clearly and precisely described, allowing to replicate experiment if needed. I particularly appreciate the verification of air flow before experiment.

REV>> - As EOs could be toxic as vapor on adult, there was a risk of toxic effect in the olfactometer. Could authors explain their choice of the EOs volume to test?

AUT>> EO volumes were chosen closed to concentration potentially used for pest control (e.g. around 20 uL.L^{-1} air estimated by Dunan et al., 2021) in order to minimize the risk of toxic effect in the olfactometer (lower than the estimated C_{01} for adult parasitoid wasps in Poorjavad et al, 2014). This was added to the text [L.166-169].

REV>> - L131: what is meant for « lm » ?

AUT>> “lm” stands for Lumens. We modified the text [L159].

REV>> - L133: Could authors give the age of the parasitoids used for olfactometer experiment?

AUT>> Wasps were 1 or 2 days old. This was added to the text [L162].

REV>> - L167 : « NbB the number of parasitoid pupae NbB = EggsB) » this sentence implies the assumption of a one to one relation between the number of brown egg and pupae. As the number of females parasitoids was twice higher than the number of eggs of E. kuehniella , could it be possible that an egg could be parasitized with more than one T. evanescens egg?
Note : typo «) » at the end of the sentence.

AUT>> At these densities and with this host species, superparasitism is rarely observed. When several eggs are laid in a single host, in most cases, a single adult emerges (Corrigan et al., 1995). So we do not expect superparasitism to be important, or to differ across our treatments. We have clarified this in the text [L.136-138]: “Adults emerged on day (D+14). About one adult emerged per parasitized egg, since superparasitism is rarely observed in these conditions, and in most cases, even when several eggs are laid in a single host, a single adult emerge (Corrigan et al., 1995).”

We’ve also realized we had made a mistake in the description of the experiment, when describing the estimation of the number of eggs per adult. Two cards with 5 patches of eggs each were exposed to 300 adults (that came from the rearing conditions). This represents 2*5*25 = 250 eggs. We have reformulated as follows: [L121-123]. “small patches with about 25 ± 7 *E. kuehniella* eggs were exposed during 24h to emerging parasitoids (two cards with five patches each, i.e. 250 host eggs, were placed with about 300 individuals with a sex-ratio around 0.45 ± 0.11).

Statistical analysis
REV>> R Scripts are not joined to the manuscript.

AUT>> R scripts were deposited in an open access depository. This was detailed in the section “Data, script and code availability”.

Pre imaginal survival :

REV>> L190 : Could authors precise how was estimated the number of parasitized eggs? In supplementary informations this parameter is in circle, meaning that it has to be estimated, but I do not understand how.

AUT>> The number of parasitized eggs needed to be estimated because parasitized eggs might die before turning black. It was estimated from the number of available eggs in the patch (NbEggs) and the parasitism rate (ρ_l). The parasitism rate was modeled to integrate variability between strains and between patches. However, since this seems to be confusing, we've modified our model so that we now directly model the number of black eggs, as a function of both parasitism rate and treatment. We've modified the text accordingly [L220-225], as well as the supplementary information S.3.2, the analysis and the results, in order to reflect this simplification. Conclusions are slightly affected: (ii) parasitism rate estimation is higher, (ii) parameter alpha estimation is higher (higher sensitivity in this first phase) and (iii) parameter beta is now strains-dependent. However, the global conclusion of EO effect and the hierarchy between EO toxicity remains unchanged.

REV>> L192, 195,198 and supplementary informations : Why a binomial distribution is used for the estimation of the number of parasitized/turning brown eggs? As the binomial distribution is used for modeling the frequency of successes, I am confused. Do the authors want to say "proportion of eggs" instead of "number" ?

AUT>> The binomial distribution is used to model a number of successes indeed, as is common practice. This distribution has two parameters : the probability of success (p) and the number of trials (n). The number of successes (number of parasitized/turning black eggs) can thus be considered as the realization of a random variable following a binomial distribution with parameters n and p. Perhaps what you found confusing is that in GLM analyses, the "binomial" family can be used indifferently to model the frequency of successes or bernoulli GLM (e.g. glm(y~x, family=binomial) with y being a sequence of 0 and 1) or the number of success or binomial GLM (e.g. glm(cbind(successes, failures)~x, family=binomial)).

REV>> L193 : EO concentration is noted Cl in the text, but Ci in the equation. Is it because one model is run for each dose (5-10-20µL) ? Could the authors specify in which sense the term C should be understood?

AUT>> We did mix C_l (l being the patch) and C_i (i being the EO). We corrected the text by defining C_l as the volume of EO to which patch l was exposed.

REV>> L199 : the description of the beta parameter is a little bit confusing. Authors said it represents the difference of the sensibility between eggs/larva and pupae but that it could also be viewed as the representation of a cumulative effect. As accumulation of EO in a tube is very likely, is it possible to add a parameter to separate this cumulative effect from the real sensitivity to EO of the different stages?

AUT>> We took into account your suggestion by explicitily modelling an accumulation effect and comparing different alternative models. This was added in the material and methods [L232-242], the result [L. 318-323] and in the discussion [413-415]. The new Figure 4 illustrates the different mortality models. The bottom line is that our statistical analyses are more in support of the "two phases" model, so we have no good support for an accumulation effect.

Olfactometry :
REV>> L224-225: Same comment as previously about the use of a binomial distribution for number estimation.

AUT>> See previous explanation.

Results
General comment:
REV>> - I am not familiar with Bayesian analysis, but I am wondering if estimated parameters could be given with an equivalent of confidence interval as in frequentist way? It would allow the reader to quickly assess the precision of the estimation.

AUT>> Yes, the posterior credible intervals. We've added these CI to the estimates.

REV>> - Raw data are not available to the reader.

AUT>> Raw data were deposited in an open access depository. This is explained in the section "Data, script and code availability".

REV>> L272: 276 : concentration word is used but authors talk about volume (µL), not concentration (quantity/ volume). It would be more precise to talk about doses of EO.

AUT>> Indeed. We've changed "concentration" to "dose" all along the manuscript.

REV>> There is no description in the method part of the C50 estimation, could the authors add a description of this point in the method part ?

AUT>> We added a description of the C50 (now called LD50) in the method part [L251-256].

REV>> Fig 4 legend :
Authors should precise the « development » term as it could be used for egg to pupae or pupae to adults etc.
Authors should precise what represents the ribbon around lines.
Authors should clarify the signification of ±0.01 » in « 0.95±0.01 ».

AUT>> Figure 4 was totally modified. With the corrections, it became Fig 6

REV>> L286 : Authors should clarify the signification of « ±0.0005 » in « 0.997±0.0005 ».

AUT>> The result section was totally rewritten.

REV>> Figure 5: I do not understand if estimation parameters are given for one given dose or for all ? Could authors clarify this point?

AUT>> The alpha parameter acts as a multiplicating factor of the dose, and does not depend on the dose. Anyway, we've modified the results presentation, and we now preferentially present the LD50.

REV>> L298: Olfactometry bioassay.
It could be interesting to provide the total number of females that enter in the olfactometer ats the same time. Is 60 the maximum number of individuals that avoid group effect response in the olfactometer? Is group effect

behaviour known for T. evanescens? Please provide a reference if any.

AUT>> About half of the female parasitoids in the inoculation tube climbed up into the olfactometer chamber (mean = 30 individuals and standard deviation = 10). We've added this sentence to the results [L.353-354]. To our knowlegde, there are no reports of group effects in this species, apart from avoidance of hosts already parasitized, especially at such low densities (relative to the size of the experimental arena). This is consistent with our personal observations..

REV>> L302 : Authors should precise the signification of the interval.

AUT>> It corresponds to the Credible Interval at 95%. We've specified this in the text [L.358].

REV>> L304 : Authors need to homogenize the terms they want to use. I suggest that dose is better than concentration in this context.

AUT>> We agree. We modified the text.

REV>> § L329 & Fig 9: I do not really understand why authors make such hypothesis about the relation between toxicity and behavior.
First, the authors do not know the concentrations to which the development stages and adults are exposed, then the comparison doesn't seem relevant. Indeed, it is very likely that the concentration in the tube of the developmental experiment is more important than in the olfactometry bioassays because of the low volume of the tubes.
As the relation between toxicity and behavior is highly complex and dose dependent, I suggest the authors to modify this part of their results and to explicit more clearly their hypothesis in the discussion section.

AUT>> Ok. We have put this part in the discussion and we added a reference to explain our hypothesis [L396-402, Fig11].

Discussion
REV>> Global comment : Authors should add a paragraph to discuss the fact that they do not know the actual air concentration in both experiment.

AUT>> We added a paragraph about EOs concentration in the discussion [L.449-470].

REV>> L337 : same comment as in introduction about direct and indirect terms use.

AUT>> We modified with the terms "toxicity" and "behavioral consequences" [L.386].

REV>> L360-364 : Do the authors have an hypothesis about the higher sensibility to EO of pupal stage compared to larva stage ? It could be interesting to develop this point.

AUT>> This is very difficult to tell. We would think that early instar parasitoids, that have very minute size, might be protected inside the host egg, while after growth and host feeding (Volkoff et al., 1995), parasitoid pupae might be more directly exposed to air pollution. We added this hypothesis in the text [L.420-422].

REV>> § L402 : experimental design means : olfactometry design ?

AUT>> Yes. We modified the text [L. 490].

REV>> I suggest to remove « but had no oviposition experience » (L403) because it suggests that females in the olfactometer are searching for egg to parasitized during the experiment and we do not know that. Authors could make this assumption if there was a signal of egg presence in the olfactometer, but it was not the case if I well understood.

AUT>> It was indeed not the case. We removed this part to avoid confusion.

/