# Assessing Trogoderma variabile as a behavioral surrogate for the quarantine pest, Trogoderma granarium (Coleoptera: Dermestidae), through dual choice assays

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## Introduction

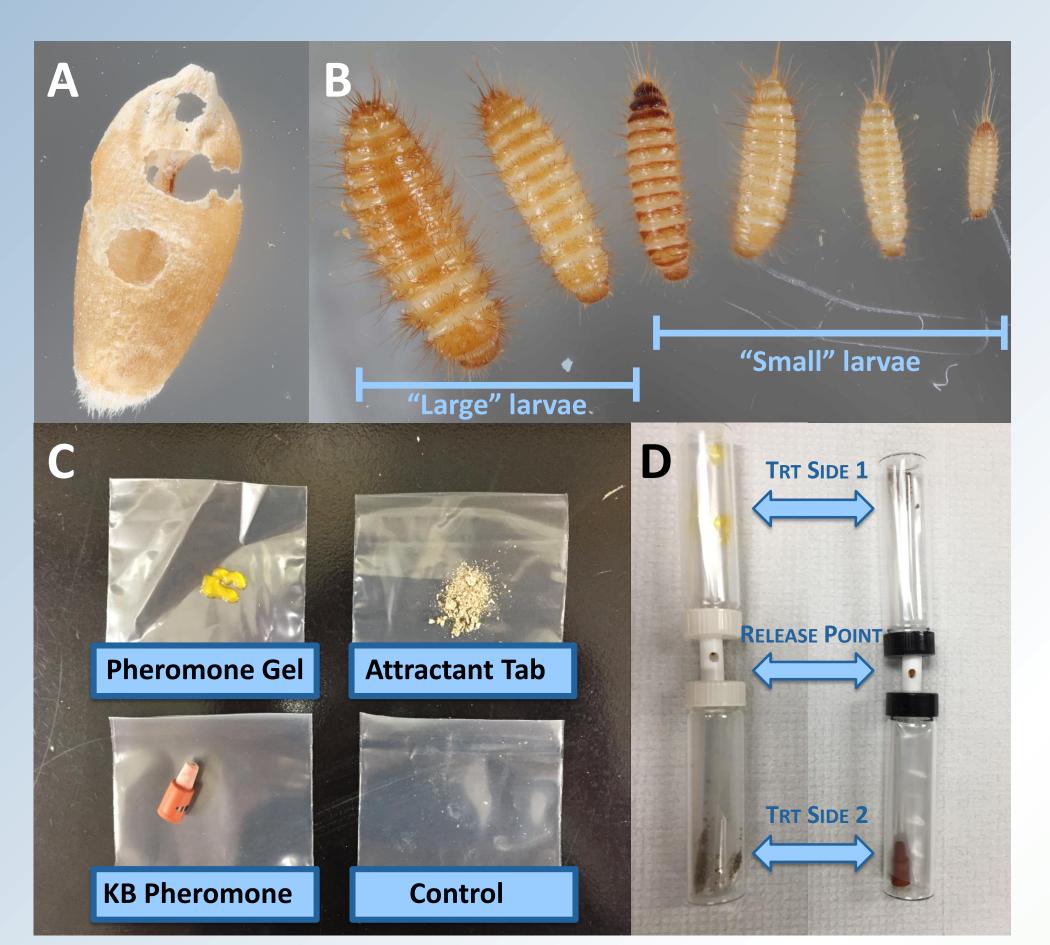
Stored product pests are a leading cause of post harvest losses in nations around the world, accounting for up to 20% of losses in some developing nations.<sup>1,2</sup> The Khapra beetle (or KB), *Trogoderma granarium* (Fig. 1A), is a threat to the biosecurity of the U.S., and represents the only stored product species under quarantine in the country.<sup>3</sup> In the past several years, there has been an increasing frequency of *T. granarium* at ports of entry and borders in the U.S. Because researchers can only work with the pest in quarantine, research on *T. granarium* is necessarily limited because only a handful of facilities have permission to keep colonies of the species. The closely related beetle, Trogoderma variabile (the warehouse beetle or WHB; Fig. 1B) is commonly present throughout stored product facilities in the U.S., causes economic losses (Fig. 2A),<sup>4</sup> and is not under quarantine. Due to physical similarities (Fig. 1), and their close relatedness, it is possible that their behavioral responses are also similar. This raises the question of whether one can act as a behavioral surrogate for the other, which would speed research into how *T. granarium* would respond to stimuli if it gained entry in the U.S.

#### **Objective**

The goal of this study was to investigate the response of *T. variabile* to commonly available beetle attractants, and compare its responses to those of the quarantine pest, *T. granarium*. This was done by assessing the attractants in a dual choice assay.



Figure 1. The khapra beetle, *Trogoderma granarium* (A), and the warehouse beetle, *Trogoderma variabile* (B) are physically and phylogenetically very similar.



**Figure 2.** Feeding damage by *T. variabile* on oat (**A**), size comparison of *T. variabile* larvae used in this study (**B**), attractants used in dual choice assay (**C**), and dual choice chambers used in assay (**D**).

## Materials and Methods

**Source Insects.** For all assays, small (1-3<sup>rd</sup> instars) and large *T. variabile* larvae (4<sup>th</sup>-6<sup>th</sup> instars; Fig. 2A) from a field-derived strain were used that had been continuously reared on pulverized dog food (300 g SmartBlend, Purina One), with rolled oats, and a moistened paper towel on top in a 800 ml mason jar, and held at 27.5°C, 60% RH, and 14:10 L:D. All individuals were starved 24 h prior to use in experiments. *Trogoderma granarium* were kept under similar conditions in the quarantine facility in Buzzards Bay, Ma.

**Attractants.** The following attractants were used: an unbaited control (ctrl), 0.13 g dermestid pheromone gel (gel, hereafter; IL-2700 from Insects Limited), 0.13 g food attractant tab (tab, hereafter; from Insects Limited), or 1 Khapra beetle pheromone Storgard Cap (Trécé, Inc.; KB hereafter) (Fig. 2C). Each attractant was placed on 7.6 × 6.4 cm L:W pieces of plastic, and used in the assay below. Every pairwise combination of treatments was tested for both *T. variabile* and *T. granarium*.

**Dual Choice Assay.** The assay consisted of two glass vials (8.3 × 2.5 cm H:D) connected by a 4 cm long piece of PVC pipe (6 mm ID) with a 4-mm hole drilled in the center to release larvae (Fig. 2D). Each larva had 5 min to respond, otherwise they were marked as non-responsive. Large and small larvae were tested. At least 25 replicates were performed for every pairwise combination of attractants. This was done for small and large larvae of both species.

**Data Analysis.** The data were analyzed with  $\chi^2$ -square tests with a Bonferroni-corrected p-value to account for multiple comparisons on the same dataset. The data was compared to the null hypothesis of equal response between attractants. All data was analyzed using R Software, with  $\alpha = 0.05$ .

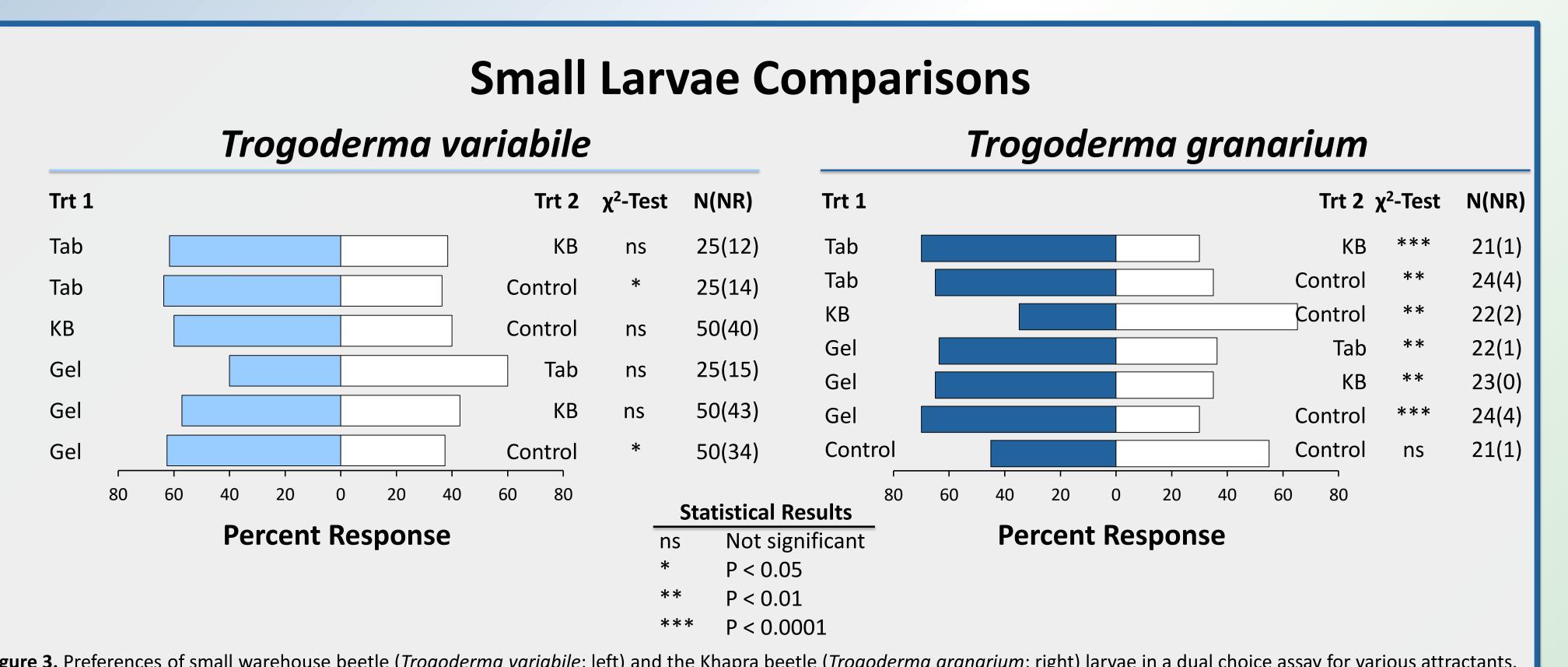
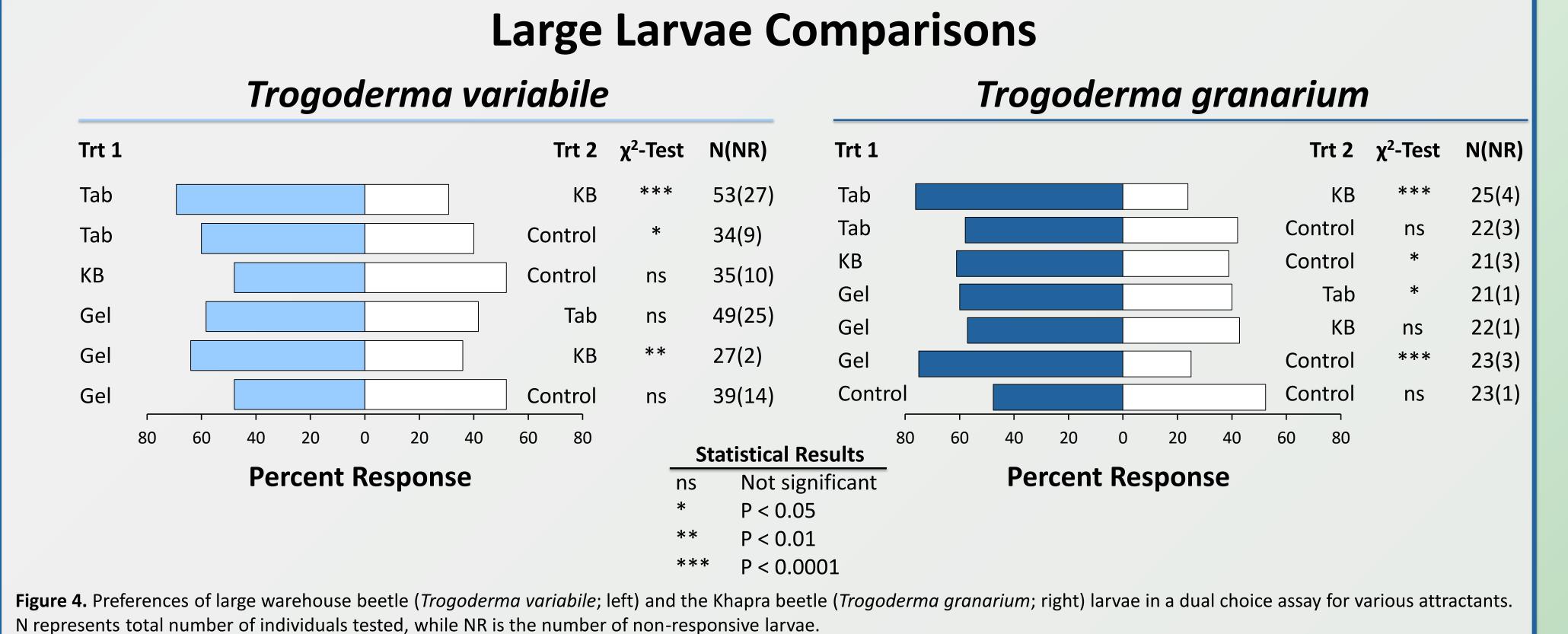


Figure 3. Preferences of small warehouse beetle (Trogoderma variabile; left) and the Khapra beetle (Trogoderma granarium; right) larvae in a dual choice assay for various attractants. N represents total number of individuals tested, while NR is the number of non-responsive larvae.



# Results & Discussion

#### **Small larvae comparisons:**

- Overall, there are few similarities in the behavioral response between small larvae of *T. variabile* and *T. granarium* (Fig. 3).
- However, both species preferred the food attractant tab to control by nearly two-fold.
- Additionally, both species preferred the pheromone gel 1.7-2.3 times compared to the control.

#### Large larvae comparisons:

- There are also very few similarities between the responses of *T. variabile* with *T. granarium* (Fig. 4).
- But, both species preferred the tab by 2 to 3-fold over the KB lure.

#### Conclusions

Both species were most attracted to the tab and gel attractants. However, overall in the dual choice assay, it is apparent that the choices of larval *T. variabile* and *T. granarium* are not very similar with each other. This calls into doubt whether *T. variabile* can be used as a behavioral surrogate species.

#### **Future Directions**

Because of low sample size for the small larvae comparisons, further replicates should be accumulated before a final interpretation is made. Moreover, the dual choice assay used here only tests behavioral responses under still-air conditions. As a result, future work should explore the orientation of larvae in a wind tunnel with air movement, as well as other more practical assays, such as the ability of these stimuli to trap larvae in commonly used traps. Finally, other closely related, non-quarantine dermestids should be tested as well, including *Trogoderma inclusum*.

#### Literature Cited

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