# Can Internal Parasites Affect Wound-healing Rates in Insects?

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## Abstract

**By definition, parasites depend on the resources of their host to survive. This relationship can result in a decrease of energy and fitness for the host. The parasitic nematode *Chondronema passali* resides in the hemocoel cavity of the bess beetle, *Odontotaenius disjunctus*. Although this parasite is non-lethal, a single beetle can harbor thousands of nematodes. Previous research has demonstrated this parasite affects the stress reaction of beetles, but very little research has investigated the effect parasites have on the host's ability to heal a wound. Wound healing can be thought of as an indicator of the effectiveness of the immune system and by studying healing we can infer the effect this parasite has on the fitness of its host. We conducted a series of experiments where beetles were wounded with a dremel rotary drill and observed every hour for 12 hours after initial wounding. Each hour beetles were given a value from 1-5 to measure their status in the wound healing process, and values were summed to generate a "wound healing score" for each beetle. Beetles were killed and dissected following the experiment to define gender and parasite abundance. Out of 188 beetles, 83% were infected with *C.passali.* Wound healing scores were not significantly predicted by parasite status. Beetle weight was a predictor of wound healing scores where heavier beetles had higher scores. Oxygen consumption was also measured in a subset of beetles after wounding, and we found parasitized beetles tended to have higher respiration (10% higher) than non-infected beetles.**

## Background

- **The bess beetle (left) is found in rotting logs throughout forests in the Eastern United States**
- **Beetles form cavities in logs and rear up to 10 young during summer**
- **The beetle is commonly infected with a naturally-occurring nematode parasite *C. passali* (see figure on left)**
- **Beetles can have as many as 4000 nematodes each!**
- **How can this beetle exist with such a heavy parasite burden?**

## Methods

**We applied a uniform-sized wound to each beetle by drilling a circular hole in their dorsal pronotum using a dremel rotary drill (pictured below left). This led to bleeding and then healing, which we quantified using a scoring system we developed for the project (Fig. 1), whereby each distinct stage of wound closure was noted and assigned a score. This was done every hour, for 12 hours. At the end of the 12 hours, we tallied all scores for each beetle to establish a "wound healing score" (Table 1). Beetles were then dissected to determine gender and parasite status (pictured below right). Parasite abundance received a value from 1 to 3, with 1 being 10 or fewer nematodes and 3 being 100 or more. We also measured the respiration rates of the beetles after wounding using a respirometer. Beetles were placed in a simple tube sealed with an oxygen sensor which collected respiration data for 5 minutes.**

**Figure 1.** Scoring system for the stages in the beetle wound healing process. Stages were established by us in a preliminary experiment by observing wounded beetles until complete wound closure.

Stages were classified based on distinguishable physical characteristics as well as clotting progression of the blood (hemolymph).

These stages were easily distinguished by us as well as by outside observers

Most beetles healed after 12-24 hours

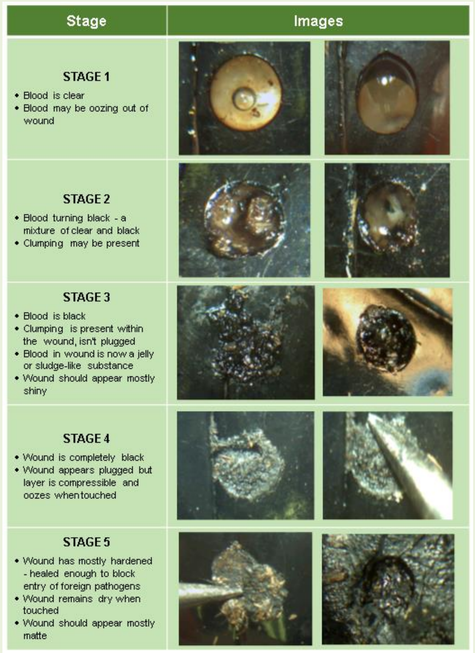


**Table 1.** Example wound stage data from 6 beetles. The sum of scores on the right is the overall wound healing score

| Beetle # | 1hr | 2hr | 3hr | 4hr | 5hr | … | 12hr | Sum of Scores |
|---|---|---|---|---|---|---|---|---|
| 1 | 1 | 1 | 1 | 2 | 2 | … | 4 | 30 |
| 2 | 2 | 2 | 3 | 3 | 3 | … | 4 | 38 |
| 3 | 1 | 1 | 1 | 2 | 2 | … | 3 | 27 |
| 4 | 2 | 2 | 2 | 2 | 2 | … | 2 | 24 |
| 5 | 2 | 2 | 2 | 2 | 3 | … | 5 | 37 |
| 6 | 1 | 2 | 2 | 2 | 2 | … | 5 | 32 |

## Results

**188 beetles were examined over the summer across 5 experiments. Prevalence of the nematode was 83%. After accounting for sex, body weight and expt., there was no significant effect of the nematode parasite on wound healing (Table 2, Fig. 2). Larger beetles had faster healing rates (Fig. 3). The nematode was associated with higher respiration rates during wound-healing (about 10% higher), suggesting more energy is required to heal wounds when parasitized (Fig.4).**

**Table 2. Summary of statistical analysis (general linear model) examining predictors of healing rates in beetles**

| Predictor | df | MS | F | p |
|---|---|---|---|---|
| Expt # | 4 | 387.3 | 7.08 | 0.0000 |
| Sex | 1 | 41.0 | 0.75 | 0.3874 |
| Parasite Score | 3 | 34.8 | 0.64 | 0.5925 |
| Body Weight | 1 | 554.2 | 10.13 | 0.0017 |
| Sex*Parasite | 3 | 86.6 | 1.58 | 0.1951 |
| Error | 175 | 54.7 | | |

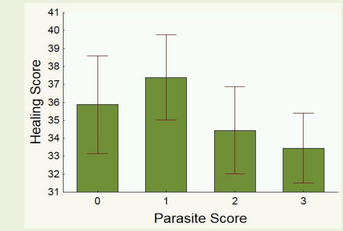


**Figure 2. Mean wound healing scores across parasite infection groupings.** Beetles with the highest nematode burdens had slightly lower healing scores but overall parasite status was not a significant predictor of wound healing rates.

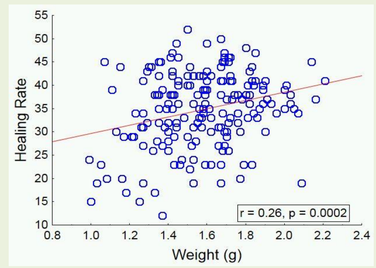


**Figure 3. Wound healing rates and weights of beetles (n=188).**Wound healing rate was positively correlated with weight of beetles. Heavier beetles tended to heal their wounds faster.

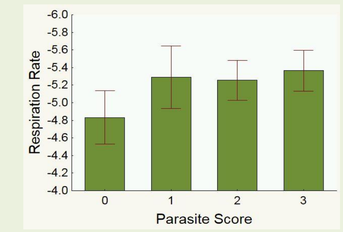


**Figure 4. Mean respiration rates in relation to parasite status.** Respiration rates of infected beetles were 10% higher than uninfected beetles.

## Conclusions

- **The effect of the nematode parasite, *C. passali*, appears to be minimal to the healing process of its host. There was no detectable reduction in overall wound healing rate, though parasitized beetles did require more energy to heal than did those without the parasite.**
- **If wound healing rates are unaffected, by extension, parasitized hosts' immune systems appear fully-functional**
- **The wound-staging system we created is the first numerical evaluation of wound-closure rates in insects, and may be useful for future investigations.**

## Acknowledgements

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