

It takes “guts” to understand stink bugs

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Stink bugs are one of the major pests in macadamia orchards and can cause significant damage to mature nuts, reducing the nut quality.

These pests are predominantly controlled with insecticides, but a more integrated pest management approach is needed. Knowledge of alternative food sources can assist in targeted pest control before the insects enter the orchard or indicate which plants can be used as trap crops around the orchards.

In this study, a detection assay relying on two plant DNA barcodes was developed to identify any plant material that the two-spotted stink bug (*Bathycorixa distincta*) feeds on. This should indicate any alternative food sources of the insect when not feeding on macadamia. An additional focus of this study was to identify the essential bacterial symbionts that stink bugs maintain in their intestines. These bacteria often contribute additional nutrition to the insects. Removal of this bacteria has resulted in mortality in other stink bug species. Species from the *Sodalis* and *Pantoea* genera were present in all the populations investigated. One species seems to be highly abundant and maintained in their gut. Investigations are currently

Trap crops are plants used to attract pests away from nearby crops

underway to determine how essential this gut bacterium of the two-spotted stink bug is for its survival. Knowledge in this regard can guide future alternative pest control measures.

Stink bugs as a pest of macadamia

Stink bugs contribute to more than half of the annual unsound kernel losses of macadamia, which is of great concern to the industry. The two-spotted stink bug is the most abundant species in orchards and one of the species that causes the most severe damage to mature nuts (Schoeman, 2020). These pests are predominantly controlled through insecticides, which are costly and which also have a negative impact on beneficial insects and the environment. We need to investigate alternative control measures to achieve

an integrated pest management approach. In this study we explored such approaches, firstly by developing a method to identify alternative food sources of the two-spotted stink bug, and secondly to determine if there are any essential gut bacteria that contribute to its survival.

A detection assay to identify stink bug plant diet

Two-spotted stink bugs are not present in macadamia orchards throughout the year, especially if nuts are not yet present. They thus also feed on other plants. Stink bug numbers could accumulate on alternative plant species before entering the macadamia orchards during nut set. If other prominent food sources of stink bugs can be identified, it could pinpoint locations where control measures can be implemented before they enter the orchards. Knowledge of alternative food sources can also guide the planting of trap crops around an orchard and, consequently, reduce the number of insects entering the orchard.

Plant DNA barcodes are used to identify plant species based on the DNA sequence of specific gene regions

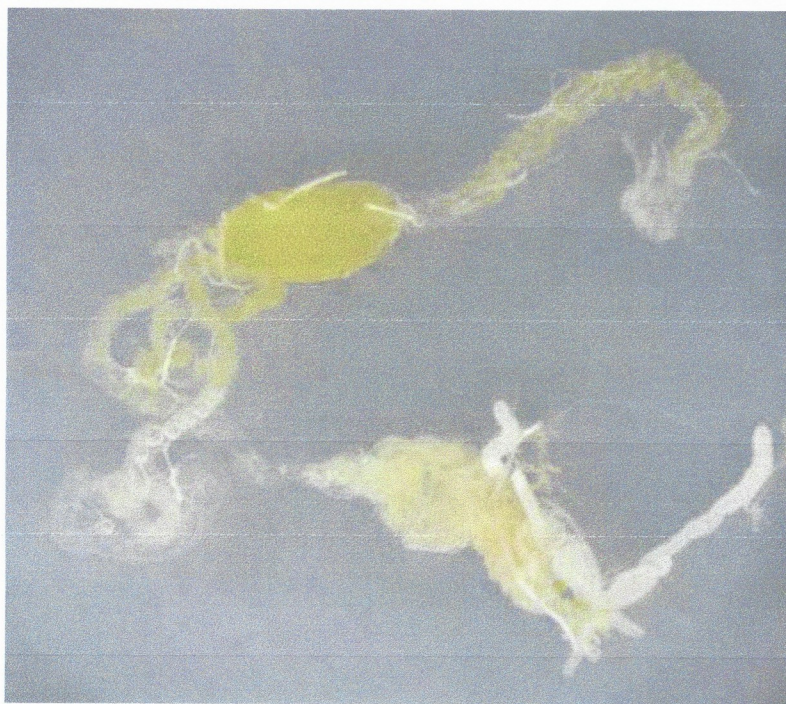


Figure 1. The stomach of the two-spotted stink bug from which DNA can be extracted to screen for plant DNA.

If an insect feeds on plant material, the DNA of the plant will be present in the stomach of the insect for a period of time. We developed a detection assay that can identify the food content of the stomach of the two-spotted stink bug. The assay is performed by first removing the stomach content from the insect (Fig 1) and then performing a DNA extraction. We further optimised two different plant DNA barcode markers (Taberlet et al., 1991) that can be used to detect any plant DNA present in the stomach.

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It is essential to have an indication of how long after the stink bugs have entered macadamia orchards one can still detect other plant material in their stomach. We thus performed a feeding time trial where stink bugs were fed on maize, then transferred to macadamia and collected at different timepoints (6 hours, 1 day, 3 days, 5 days and 7 days) after transfer (Fig 2). Preliminary analysis suggests that even after one week, the maize DNA can still be detected in their stomach. This suggests that even if the insects have started feeding on macadamia, they can still be collected to detect other food material.

Insect samples collected from farms in Mpumalanga and Limpopo, at different times of the season, are currently being tested with the two DNA barcodes to identify the plant material in their stomachs. Data from these analyses can help to identify other plants that the stink bugs regularly feed on when not in macadamia orchards, and guide future control measures.

An essential bacterial symbiont for the two-spotted stink bug

Bacteria present in the intestinal tracts of insects contribute to their overall health and survival (Engel & Moran, 2013). They can either provide them with additional essential nutrients, improve their immunity or help them to resist the defence responses from plants. Stink bugs have a specialised section in their gut (M4 section)

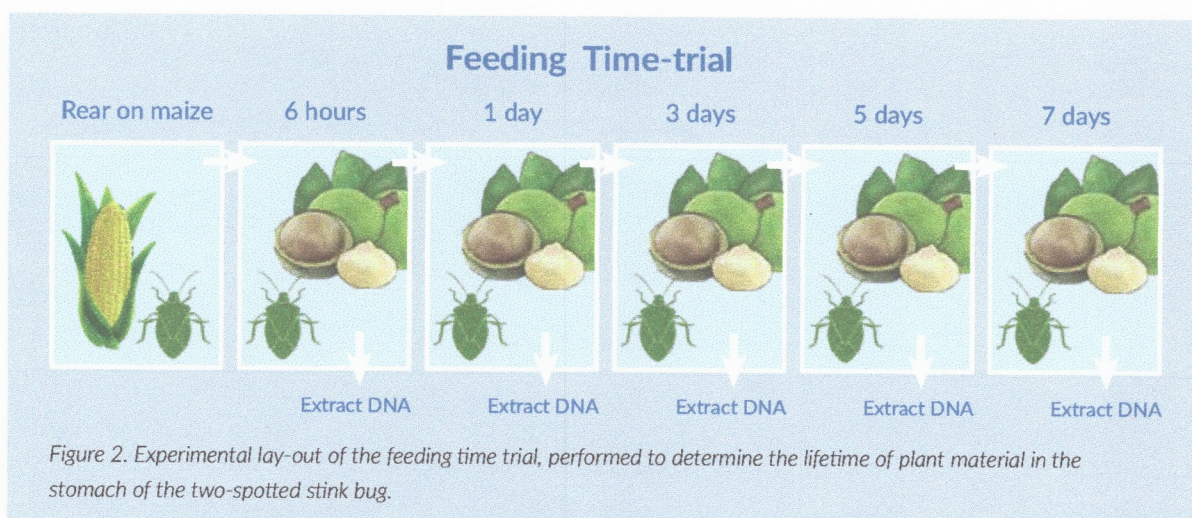


Figure 2. Experimental lay-out of the feeding time trial, performed to determine the lifetime of plant material in the stomach of the two-spotted stink bug.

where a single, essential bacterium is maintained. For many stink bug species, if this essential gut bacterium is removed, they either have restricted growth or do not survive up to adulthood (Tada et al., 2011, Taylor et al., 2014). Nothing is currently known about the bacteria present in the two-spotted stink bug gut and if the insect can survive without its bacteria.

A survey was performed to identify the bacteria present in the gut of adult two-spotted stink bugs in two provinces (Limpopo and Mpumalanga) and in a lab-reared population, maintained at the FABI Biological Control Centre, University of Pretoria. This data was also used to determine if any of these bacteria are present on the egg surface and early developmental stages of the insect, to see if it is transferred from the mother to the offspring on the egg surface. In the survey, 11 adults were collected from three farms in Limpopo and two

in Mpumalanga. From the lab-reared population, 11 samples of adults, egg patches and second instars were collected. The guts were removed from the adult insects and the specialised M4 section was separated from the rest of the gut and DNA was extracted from each section. The eggs and second instars were used as a whole for DNA extraction.

A bacterial-specific barcode marker (16S) was used to amplify any bacterial DNA in the samples (PCR and high-throughput sequencing). This information was used to identify any bacteria that were conserved in all the geographic locations and bacteria that were maintained in both the early instars and the adults.

Currently only the lab-reared and Limpopo samples have been analysed. Based on this data, two abundant bacterial genera were identified, namely *Sodalis* and *Pantoea*. Related

bacterial species have been identified in other stink bug species, such as the green vegetable bug (*Nezara viridula*), suggesting that these are important bacterial symbionts for stink bugs.

The *Pantoea* species was highly abundant in the specialised M4 section of the gut in all adults investigated as well as on the eggs and second instars (Fig 3). This suggests that this bacterial species is obtained by the young hatchlings, from the egg surface, and then maintained in their guts (Fig 4). Ongoing studies are exploring options to remove this bacterial symbiont from the insect egg surface and evaluate the effect this has on the survival of the two-spotted stinkbug. The genome sequence of this *Pantoea* symbiont is also being analysed and will provide further insights into the functional role of this symbiont. The removal of this symbiont can likely be considered as an alternative pest control approach in the future.

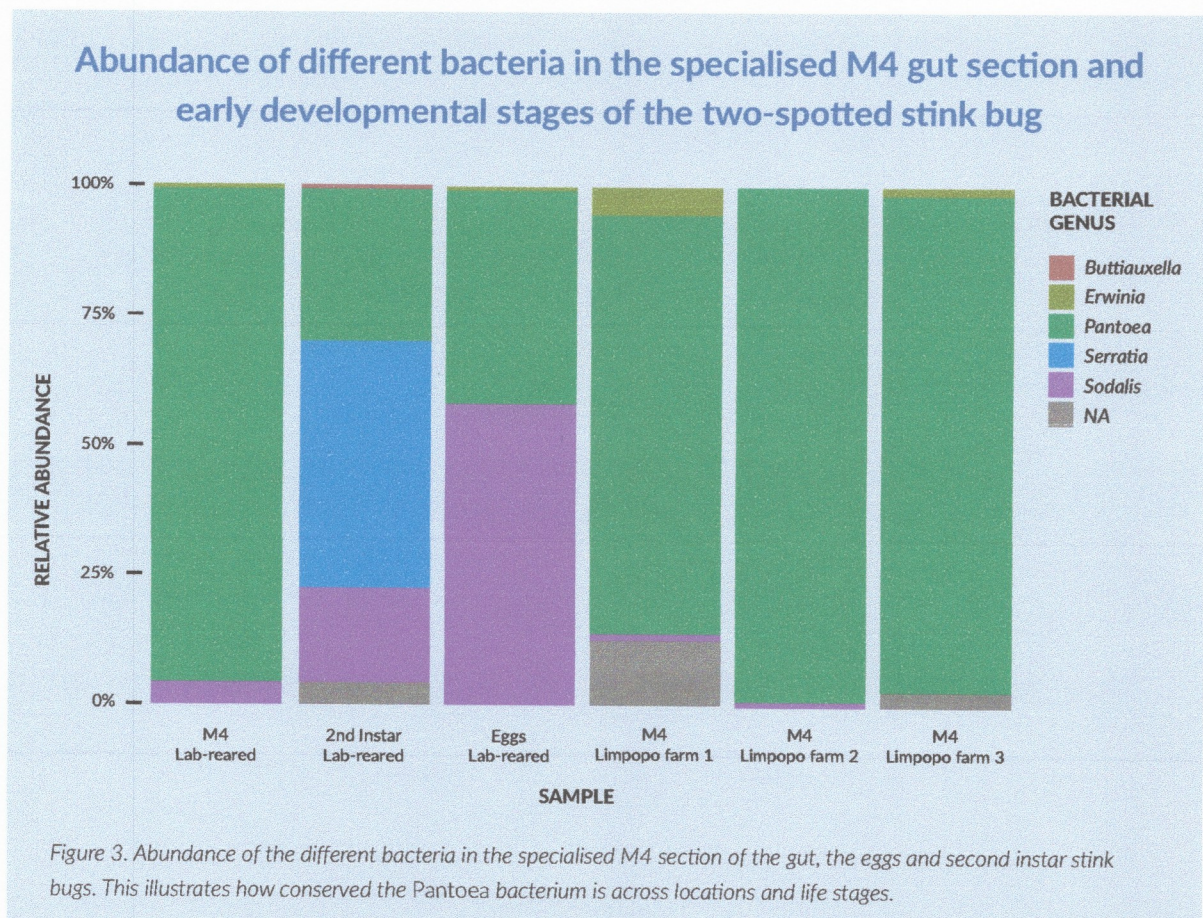




Figure 4. Newly hatched stink bugs remain on the egg surface after hatching and likely obtain their bacterial symbiont from this surface. It is then maintained in their gut up to adulthood.

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