Insects associated with pecan trees, *Carya illinoinensis*: characterisation, impact and control

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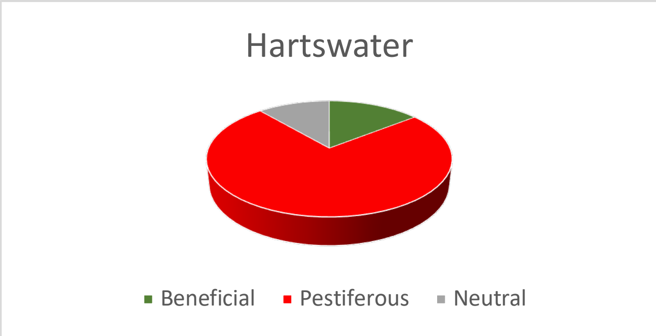
Project overview (1 October 2020 – 30 September 2021)

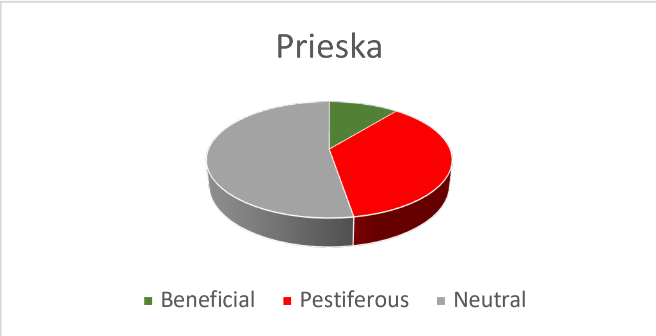
During this fiscal year, three principal objectives were pursued: (1) continue with insect surveys in the Hartswater, Prieska and Modimolle regions; (2) investigate the entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae,* as artificially-induced endophytes in pecan seedlings (establishment) and impact on pecan nut weight (trees); and (3) initiate pheromone-based surveillance and damage-correlation of African bollworm, *Helicoverpa armigera*, and false codling moth, *Thaumatotibia leucotreta*, in the Hartswater, Prieska and Jacobsdal regions. Following, a brief overview of each of the three research areas.

1. **Insect surveys**

Pecan nut trees, *Carya illinoinensis* (Juglandaceae), are native to North America and serve as host plant for at least 15 important insect pest species (listed in *Insects Associated with Pecan Cultivation in South Africa* by Hatting 2018), although only one of these 'true' pecan insect pests is associated with pecan cultivation in South Africa; *i.e*., yellow pecan aphid, *Monelliopsis pecanis*. However, as the local area under pecan cultivation expands, the dynamics of the host-pest relationship may also change, especially amidst a climate-changing environment. Ultimately, 'new' incursions are likely, with some exotic species posing a severe threat to the South African pecan industry. Another scenario entails 'host switching', where an indigenous insect moves from a particular vegetation type to now exploit a previously unaffected host plant, whether indigenous or exotic. A local example is the long-horned beetle, *Cacosceles newmannii* (Cerambycidae), moving onto sugarcane and causing economic losses for the unsuspecting growers. In addition to the abovementioned, development of resistance to chemical insecticides poses equal risks, but can be averted if sound pest control measures are practised; see "*Beginsels vir volhoubare insekbeheer by Pekanproduksie"* by Hatting 2020 (online publication on SAPPA website: https://www.sappa.za.org/wp-content/uploads/docs/2020/11/IPM-5-Nov-2020.pdf). The ongoing insect surveys thus serve as pre-emptive approach in alerting the local industry of new incursions and/or changes to the pest status of insects associated with pecan cultivation. Concurrently, grower awareness of beneficial insects is stimulated; an insect grouping which renders a valuable ecosystem service by suppressing pestiferous species.

During the 2020/21 reporting period, a total of 2 869 insects were collected at Hartswater, Prieska and Modimolle. The overall ratio (%) of pestiferous : beneficial : neutral insects was 54 : 19 : 27. The site-specific ratios are visually depicted in Figures 1-3. Note, above data exclude the large number of yellow pecan aphids and associated ladybird beetles (mainly *Hippodamia variegata*) recovered at Hartswater during April 2020. Typically, yellow pecan aphid populations peak during late summer into early autumn. Fortunately, no (new) exotic incursions were noted in this reporting period.





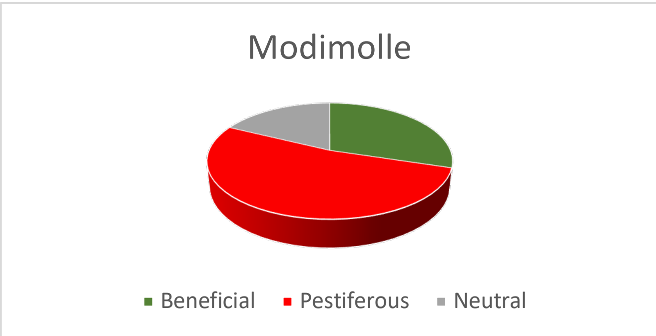


Fig. 1-3 Comparative number of insect 'types' recorded in each of the three regions from 1 October 2020 to 30 September 2021 (note: neutral insects are characterised as those species of which the activity/role on pecan trees remain unknown)

Beneficials at the three sites comprised mainly ladybird beetles, spiders, lacewings and assassin bugs. The most prevalent pest insects recorded at Hartswater and Prieska were sap-sucking insects in the order Hemiptera; *i.e.*, plant bugs (Miridae), seed bugs (Rhyparochromidae), stinkbugs (Pentatomidae) and leafhoppers (Cicadellidae), as well as snout weevils (Coleoptera: Curculionidae) with biting-chewing mouthparts. An annual sighting at Prieska, around December every year, is that of the green citrus leafhopper, *Empoasca distinguenda.* Feeding occurs on the younger flush, causing a characteristic yellowing of the leaves (Fig. 4). Sap-sucking insects are often associated with the transmission of viruses to plants. In the case of *E. distinguenda* on pecan, preliminary tests during 2020/21 could not confirm such association, although more advanced testing is probably justified. This will be explored during the next cycle.

Fig. 4. Yellowing of leaves caused by the green citrus leafhopper

Similar to the previous two seasons, the Modimolle surveys revealed high numbers of snout beetles (also called weevils), notably *Dereodus schoenherri* (Coleoptera: Curculionidae). This species and other snout beetles recorded on pecan trees are mainly foliage-feeders and not necessarily serious pests. However, pecan producers should be on the lookout for the exotic pecan weevil, *Curculio caryae* (see photos on page 39-40 of the *Pecan Insect Guide*) and hickory nut weevil, *Conotrachelus hicoriae*. These insects affect the nuts and are considered primary pecan pests in the United States (US), but are still absent from South Africa. Ongoing surveillance for these species is needed across the South African pecan industry.

2. Endophytes

Endophytes are defined as microorganisms that live asymptotically within a plant without causing any harm to the host. In these symbioses, the endophyte acquires nutrition and shelter from the host plant, while in exchange, some endophytes can facilitate nutrient uptake, increase host-plant resistance to insects and/or diseases, enhance tolerance to drought stress and/or improve host competitive ability. The use of endophytic fungi for induced host-plant resistance against insect pests (and other biotic stresses) holds potential value also for the South African pecan industry. Collaborative research among researchers at ARC-Small Grain and scientists in the US, has confirmed endophytism of a strain of the entomopathogenic fungus (EPF), *Beauveria bassiana*, in pecan seedlings through artificial inoculation. Two field trials were subsequently initiated to (1) verify the establishment/non-establishment of endophytic EPF in pecan seedlings (inoculation of seedlings and/or during grafting of nursey plants) and (2) to measure the effect of topical EPF applications on pecan nut weight, following adult tree inoculations.

2.1 Nursery plants

Treatments comprised four EPFs, *viz*., *Metarhizium anisopliae* (strain M8), *Metarhizium anisopliae* (strain V5), *Beauveria bassiana* (strain R495) and *Beauveria bassiana* (strain R444). Treatments were either a seed-treatment (powder formulation) + topical spray (fungal spores in suspension) of seed and seedlings, respectively, or inoculation (dry spore powder or spore suspension) at the time of grafting, *i.e*., during September/October 2020 (Fig. 5). All plants were maintained as per standard nursery practices.

Fig. 5. (Left) nursery plants inoculated with *M. anisopliae* strain V5 and close-up (right) of graft

During October 2020, plant material (leaves and stems) was collected and transferred to brown paper bags for drying until molecular test could be performed. At the time of this publication, DNA results were still pending.

2.2 Adult trees

Field trials were performed at two sites, Hartswater and Prieska. The cultivars sprayed at Hartswater comprised Wichita (with controls), Choctaw and Western; while those at Prieska included Wichita (with controls), Pawnee and Western. Treatments were *M. anisopliae* (strain M8), *M. anisopliae* (strain V5) and *B. bassiana* (strain R495), all applied as fungal suspensions through a Stihl mist blower during October 2020 (average 6 x 10^5 spores/L) and December 2020 (average 2.4 x 10^5/L). At harvesting, 30 nuts were randomly collected from 4 quadrants under each tree (*ca*. 120 nuts per tree) and weighed for comparison among treatments and controls.

At Hartswater, the nuts collected from fungus-treated Wichita trees were significantly heavier than those from untreated controls. In particular, the nuts from trees sprayed with *M. anisopliae* (strain M8) weighed, on average, 8.31g versus 7.46g, 7.42g and 7.34g for nuts from controls, *B. bassiana* (R495) and *M. anisopliae* (V5), respectively ($LSD_{0.05}$ = 0.354); see Fig. 6. When comparing the three cultivars at this site, the Choctaw nuts (averaging 8.85g each) significantly outweighed both the Wichita (7.52g) and Western (5.84g) nuts ($LSD_{0.05}$ = 0.3097). Although positive in terms of nut weight-response, the actual effect of endophyte sprays to Choctaw remains unknown as controls were not included for this cultivar and thus warrant further investigation.

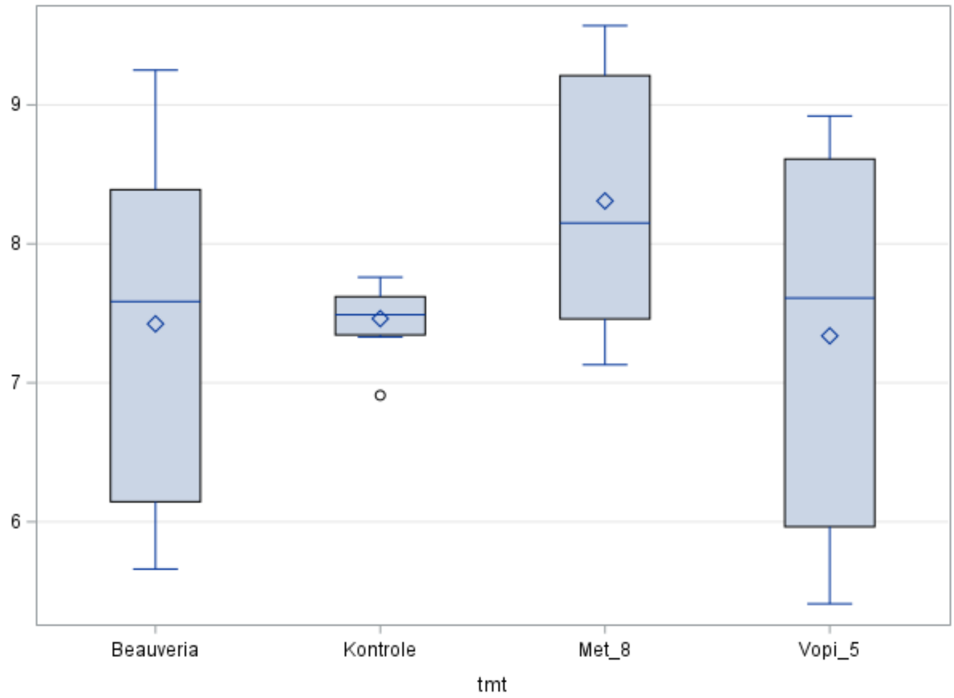


Fig. 6. Nut weight (Y-axis) in response to fungal applications on Wichita during October and December 2020

At Prieska, none of the fungal applications to Wichita yielded heavier nuts compared to controls. In terms of cultivar performance, all cultivars differed significantly from each other, with Wichita averaging 8.79g, Pawnee 8.09g and Western 6.45g ($LSD_{0.05}$ = 0.316). By pooling the two sites and considering a treatment x cultivar interaction, the *M. anisopliae* (M8) spray on Choctaw yielded the heaviest nuts, averaging 9.15g (Fig. 7). Notably, two of the top three treatments comprised a *Metarhizium* x Choctaw combination, while *Metarhizium* was also involved in the three overall top performers.

For cultivar Western, the *Beauveria* treatment yielded an average nut weight of 6.32g, which was significantly heavier than the 5.7g measured with *Metarhizium* (V5) (Fig. 7). These trials are the first ever conducted to investigate the potential benefits of endophytic EPFs in pecan trees and the positive trends, noted so far, do support the notion.

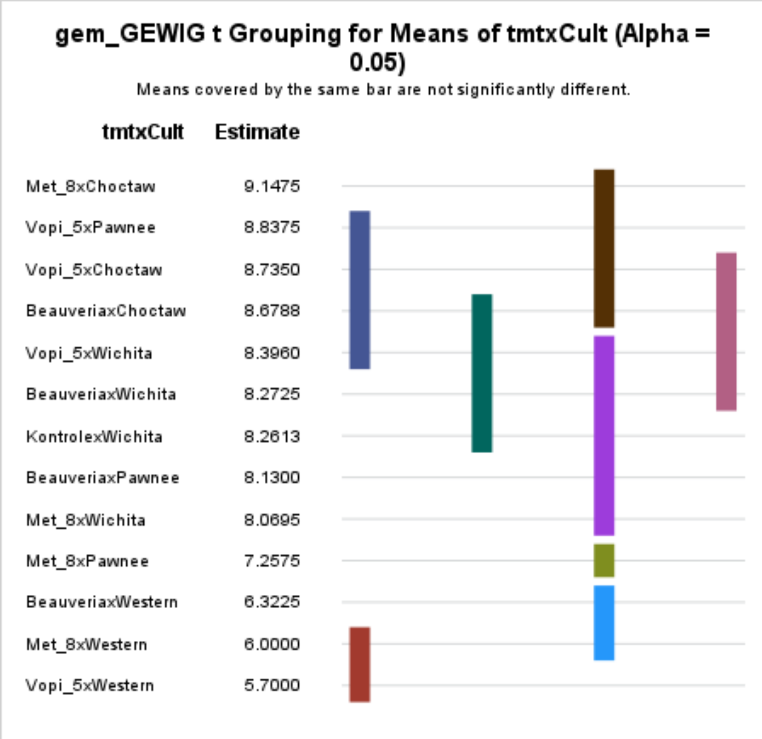


Fig. 7. Average nut weight of pooled site data for all treatment x cultivar interactions

3. Pheromone-based surveillance and damage-correlation of false codling moth, *Thaumatotibia leucotreta* and African bollworm, *Helicoverpa armigera*

The South African Pecan Nut Producers Association has expressed the desire to gather more 'pecan-specific' data on the flight patterns and damage potential of the false codling moth (FCM) (see pages 46-48, *Pecan Insect Guide*) and African bollworm (see pages 49-50, *Pecan Insect Guide*). Both pests have an adult moth stage, enabling monitoring of male moths by pheromone trapping. Monitoring of moth flights (using bucket funnel traps and yellow sticky traps, loaded with a sex pheromone) was initiated during September 2020 at the farm level. A total of 27 traps were commissioned in the Vaalharts (19 traps), Prieska (4) and Jacobsdal (4) regions. Traps are being serviced every two weeks by technical staff from SAPPA and/or selected pecan farmers and sent to the author for sorting and counting. Moth flights will be monitored over three seasons to account for seasonal variation. The first-year dataset (September 2020 to September 2021) for bollworm flights in the 'Hartsvallei' area is presented in Fig. 8.

Damage quantification was performed during December 2020 by collecting all aborted nutlets on the soil surface within a 2m radius around selected trees. A total of 30 trees (*i.e.*, 12 Wichita, 7 Choctaw, 5 Western, 3 Pawnee, 2 Mohawk and 1 Ukulinga) were inspected at Bull Hill (3 trees), Jan Kempdorp (6), Tadcaster (3), Hartswater (3), Magogong (3), Prieska (7), Modderrivier (3) and Jacobsdal (2). Nutlets were sorted according to size (5 categories: XS, S, M, L, XL) and inspected for bollworm feeding damage (Fig. 9) or topical fungal growth. These data will later be used to investigate possible correlations/interactions among the parameters: insect species, flight patterns, nut abortion, cultivar, damage and/or area. Initial observations from Year 1 data show significant nutlet abortion, ranging from 13 to 440 nutlets per tree. However, from a total of 1 800 nutlets collected, only 2.8% showed feeding damage associated with bollworm. The data also showed a clear grouping of bollworm damage with the extra small and small nutlets, while fungal growth (19.6% of nutlets collected) was associated with the medium, large and extra large nutlets. Future data from Year 2 and 3 will enable a 3-year average for interpretation and, hence, a better understanding of the bollworm and its impact on pecan production.

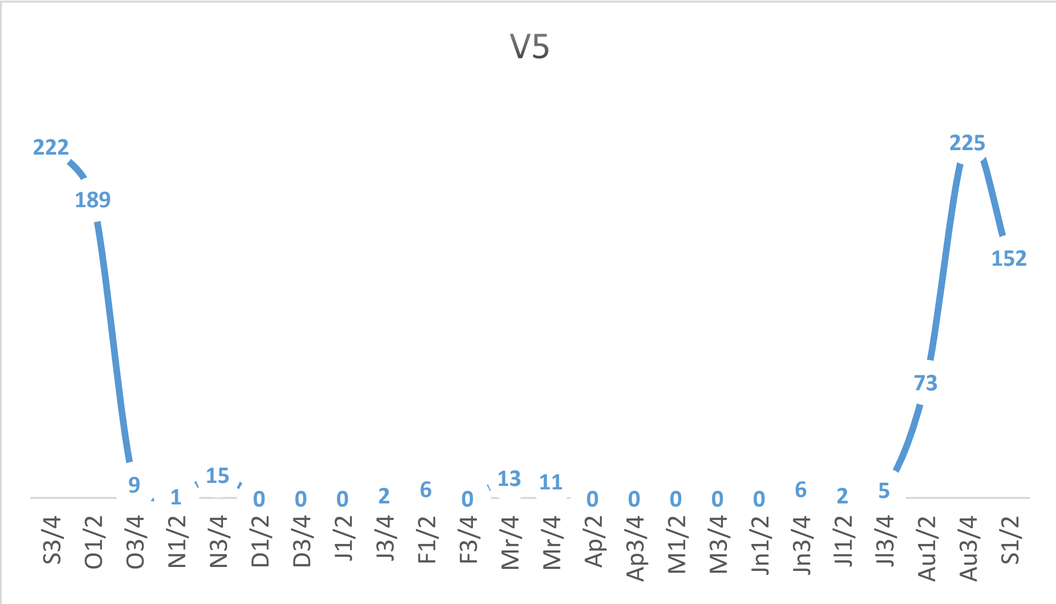


Fig. 8. Number of bollworm moths (Y-axis) recorded in the 'Hartsvallei' area during Year 1 of the monitoring sub-project. Peaks in flight activity occurred during week 3 and 4 of September 2020 and during week 3 and 4 of August 2021

Fig. 9. Nutlets showing characteristic feeding damage by African bollworm

All of the above objectives will continue in 2021/22. Results of the endophyte DNA-tests on nursery plants (seedlings and grafting) will be shared in a future article in SA Pecan. Farmers collaborating on the insect project are thanked for their valuable inputs.