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Comparison of binary and multi-point scales for monitoring damage by coconut rhinoceros beetle (O*ryctes rhinoceros*)

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Oryctes rhinoceros (L.) (Coleoptera: Scarabaeidae), most commonly known as the coconut rhinoceros beetle or CRB, is an invasive pest that affects palms, especially coconut (*Cocos nucifera*) and oil palm (*Elaeis guineensis*) and their effects can be detrimental. For the past 40 years, CRB populations have been controlled effectively by the *Oryctes nudivirus* (OrNV). A recent incursion causing adverse impacts in the Pacific has prompted researchers to re-evaluate the existing management approaches, so effective population control can be achieved once again. A vital component of the management process that is often overlooked is the need for a robust damage assessment and monitoring tool. Various damage assessment methods have been developed and the use of damage scales has been the most common among them; however, their accuracy and potential to monitor changes over time have not been explored. A study was carried out to 1) Compare and identify the capability of three damage scales: binary, 3-point and 5-point, in assessing damage severity levels and 2) To determine whether assessors affected the accuracy of the damage scales. To achieve these objectives, 20 individual standard palm pictures were selected that represented damage from none to palms killed by CRB activity and administered in an online survey across respondents with differing knowledge of CRB. The results indicated that damage intensity can be measured through the 5-point scale, but the accuracy within the scale is low compared with the binary and 3-point scales. The online survey revealed that experience increases the accuracy of damage scale use among assessors. Training remains a vital requirement in the use of the damage scales if accurate and reliable results are expected.

Understanding the drivers and barriers to preventing the spread of kauri dieback: An audience segmentation approach

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Audience segmentation and driver/barrier analysis provide a foundation for behavioural change interventions. This study assessed forest users' capabilities, opportunities, motivations and behaviour (COM-B) for two behaviours linked to the spread of kauri dieback: boot cleaning and responsible track use. COM-B drivers/barriers linked to best-practice boot cleaning and track use were assessed using an online survey administered to 451 New Zealand residents who had visited the Waitākere and/or Hunua Ranges within the past 4 years. Stepwise regression analysis was conducted to identify the strongest COM-B predictors of boot cleaning and track use compliance. Audience segmentation was conducted to identify distinct barrier/drive profile groupings within the sample, which were used to predict degree of compliance. Stepwise regression analysis revealed forest users were more likely to comply with boot cleaning guidelines if they were habitual boot cleaners; worried about spreading kauri dieback; were aware of the correct method of boot cleaning; and would not use track if there was no cleaning solution. Forest users were less likely to comply with boot cleaning guidelines if they considered boot cleaning to be inconvenient and were averse to the cleaning chemicals. A second stepwise regression found that forest users were less likely to comply with track use guidelines if they felt they were unlikely to spread kauri dieback; that mitigation efforts would be unsuccessful; that it is too time consuming to use alternative routes; and if they held the forest and its tracks as part of their identity. In contrast, forest users were more likely to comply with track use guidelines if they wished to protect kauri and followed rules habitually. Latent profile analysis of COM-B variables produced three-profile solutions for boot cleaning behaviours (named Conflicted, Receptive, and Engaged) and track use behaviours (named Non-Compliant Nature Lovers, Uncommitted, and Engaged). For boot cleaning, members of the Engaged segments were more likely to comply with guidelines than members of the Conflicted and Receptive segments. For track use, members of the Engaged segment were more likely to comply with track guidelines than Uncommitted respondents, who in turn were more like to comply than the Non-Compliant Nature Lovers. Conclusions: We employed two analysis strategies to demonstrate the utility of COM-B framework for identifying key drivers/barriers related to boot cleaning and track use compliance. The results can be used to tailor behaviour change interventions aimed at increasing the drivers and reducing the barriers for these two behaviours.

Badnaviruses in New Zealand

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Badnaviruses represent a significant threat to many plant species around the world. They are double-stranded DNA viruses with a circular genome that infect a wide range of hosts. This includes crops such as banana, citrus, taro, yam, sugarcane, and cacao. Badnavirus infections can cause significant yield losses and eventually lead to host death. Badnaviruses mutate rapidly as their reverse transcriptase, a vital enzyme required for replication, has a low proof-reading mechanism. They are seed-borne, vectored by phloem-sucking insects, and can be transmitted mechanically. Host jumps are also known to occur. In addition, they can be integrated in host genomes, and excise under stress conditions to cause disease. These factors make badnavirus diagnostics and risk assessments challenging. They also make the prediction and management of outbreaks difficult. Badnavirus sequences have been found in a variety of samples from indigenous New Zealand plant species, but little is known about whether they are integrated in the host genome or in a free, infective form, and what risk(s) they pose. In this study, DNA samples extracted from indigenous New Zealand plants were screened for badnavirus sequence using universal badnavirus primers. Positive results were found for a range of species, including those from the *Carex*, *Veronica*, and *Dacrycarpus* genera. Additional methods were used to further understand the identified badnavirus sequences and in which form they occurred, and to provide information to estimate the risk they could pose to the host plant species. DNA amplification methods that will amplify only circular forms of DNA were used to determine if the badnavirus sequence is in a free, infective form, while other methods to enrich viral DNA fragments prior to sequencing were used to determine if the sequence was integrated into the plant genome, and how much of the viral genome was present. These techniques were also applied to exotic plant species and will be useful in situations such as post-entry quarantine where the risk of a badnavirus sequence needs to be well understood before plants can be released.

Consequences of introduced Western honey bees (A*pis mellifera* L.) on the conservation status of Asian honey bees (*Apis cerana* F.) and its ecological system

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The anthropogenic introduction of *Apis mellifera* Linnaeus (Hymenoptera: Apidae, Apini) to the endemic range of Asian honey bees (*Apis cerana* Fabricius) began in the late 19th century. This human-induced movement of the non-native species to Asia has caused major repercussions. The number of managed colonies of *A. mellifera* in Asia has continued to increase. It is certainly a problem for the conservation of its Asian counterparts. The key concerns include abrupt displacement of *A. cerana* by *A. mellifera* in the apicultural industry, competition between *A. mellifera* and *A. cerana* for floral resources, and the introduction and exchange of pests and novel diseases. The field work for this research was conducted in Nepal to look at the resource overlap and competition between *A. mellifera* and *A. cerana* in the landscape of Nepal, where four species of honey bees are native and *A. mellifera* has been introduced for apicultural practices less than 30 years ago. Results from this study show that *A. cerana* collects significantly greater pollen loads and nectar volume when they are in a single-species apiary than in mixed-species apiary with *A. mellifera*. This indicates that *A. mellifera*'s introductions to many Asian countries have negative effects within the genus *Apis*, primarily interfering with beekeeping activities. Moreover, if *A. mellifera* become successful as feral colonies, it might able to cause local or small regional extinction of *A. cerana* from the Asian landscape.

Frankliniella panamensis (Insecta: Thysanoptera), an emerging global biosecurity pest?

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Frankliniella panamensis Hood 1925 (Insecta: Thysanoptera) is intercepted in fresh cut flower imports arriving at ports of entry in several countries. The known geographical distribution for *F. panamensis* is montane areas from Panama, Costa Rica, Colombia, Ecuador, and Peru where it is a flower inhabiting and polyphagous species. *F. panamensis* can be found in sympatry with *Frankliniella occidentalis*, a cosmopolitan and invasive thrips and pest of fruit, vegetable, and ornamental crops. *F. panamensis* and *F. occidentalis* adults have very similar morphology with only one distinguishing morphological character to distinguish them (presence/absence of hind-coxal microtrichia) that can only be observed in well-mounted slide specimens. *F. panamensis* has quarantine status in several countries, mainly for the importation of fresh cut flowers. However its status as a pest is unclear. For quarantine inspections, discrimination of *F. panamensis* and *F. occidentalis* adults and immatures is possible by sequencing the barcode region (mtDNA) and comparing it with available sequences in public databases. With the increasing global trade of horticultural and ornamental commodities and because thrips are found on these invasion pathways, international cooperation is crucial to establish knowledge on the biology, ecology and pest status of *F. panamensis*. Based on a review of *F. panamensis* from primary and secondary literature, we highlight knowledge gaps for relevant information needed for effective risk assessment, border detection and surveillance for this species.

R2M rapid biosecurity risk assessment and invasion mitigation: A toolbox for national programs

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The ability of national agricultural research and extension systems (NARES) and national plant protection organizations (NPPOs) to address emerging pathogen and pest risks is key to national food security. Many countries globally are in the process of improving these systems. R2M is a set of tools for rapid risk (R2) assessment and mitigation (M) planning for crop pathogens, pests and weeds at the national or regional level. These tools can help countries develop their strategies for effective management of current crop disease problems, as well as new invasive pathogens and pests. R2M tools act as building blocks in the development of a global surveillance and mitigation system for crop disease. The R2M ‘meta-tool’ for expert knowledge elicitation provides an automated method for leaders in national programs to create their own tools for evaluating risk and mitigation strategies. R2M also includes geographic risk analysis such as cropland connectivity analysis, and management scenario analysis such as impact network analysis. We provide examples of R2M applications in several countries and introduce the latest R2M options available for new applications. It is hoped that the R2M framework will provide the basis for a global community of practice trying to solve similar problems. We discuss examples involving the following: (1) Expert elicitation. (2) Crop connectivity. (3) Regional assessments of invasive species management scenarios.

Rapid and visual field diagnosis of tomato brown rugose fruit virus using reverse transcription-recombinase aided amplification (RT-RAA) combined with lateral flow strip (LFS)

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Tomato brown rugose fruit virus (ToBRFV) is causing significant disease outbreaks in tomato production areas worldwide. A simple, fast, and visual detection method for ToBRFV is critical for controlling the disease. Here a combination of Reverse transcription-recombinase aided amplification (RT-RAA) and lateral flow strip (LFS) was used to achieve this goal. The concentration of modified reward primer and magnesium acetate of the RT RAA- LFS assay were optimized after selecting the primers and probe. Also, the specificity and sensitivity rates of the assay were analyzed. The results showed that the RT RAA could amplify the target gene in just 16 min at a constant temperature of 39°C, and the amplification products could be visualized by LFS within 5 min. Importantly, there was no cross-reaction with other viruses that can infect tomatoes, such as Tobacco mosaic virus (TMV), Tomato mild mottle virus (ToMMV), Pepper mild mottle virus (PMMoV), Tobacco mild green mosaic virus (TMGMV), Broad bean wilt virus (BBWV), Pepper vein yellows viruses (PeVYV), or Chilli veinal mottle virus (CHiVMV). Furthermore, the RT RAA-LFD assay was highly sensitive, with a detection limit of 10 copies/ 50-µL reaction. A total of 146 field samples with suspected infection of ToBRFV were tested using both the RT RAA-LFS and RT-PCR methods, and the coincidence rate of the detection results was 100%, indicating that the RT RAA-LFS method is as reliable as the standard RT-PCR method for detecting ToBRFV. Overall, we here developed the RT RAA-LFS assay of ToBRFV that could be a valuable tool for the rapid and accurate detection of ToBRFV in tomato production areas, helping to prevent further disease outbreaks and reduce economic losses.

Genetic diversity and structure analysis of *Brontispa longissima* populations based on SSR markers

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A total of 468 samples were collected from 18 geographical populations in Hainan, Taiwan, Guangdong, Guangxi, Yunnan and Fujian province in China. The genetic diversity and structure of these 18 geographical populations were analyzed by using 6 SSR loci developed in this study. The results showed that all populations had genetic diversity, 16 of which deviated from Hardy-Weinberg equilibrium (HW P-Val) ($P < 0.001$), and 11 populations showed inbreeding phenomenon. The population differentiation index FST reflected the low degree of differentiation among different geographic populations of *Brontispa longissima* in China. The results of Bayesian clustering analysis based on SSR indicated that all populations were clustered into three branches; the Ruili Population was clustered separately, and the genetic structure of Hainan population (except Ledong and Sansha) was similar, and the remaining populations showed no obvious regularity. The correlation analysis of genetic distance and geographic distance based on SSR found that there was a correlation between the 18 geographic populations of *B. longissima* and their genetic distance ($r = 0.378$, $P < 0.05$).

The cellular immunological responses of *Brontispa longissima* larvae parasitized by *Asecodes hispinarum*

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Brontispa longissima is serious invasive pests of palm plants in Southern China, *Asecodes hispinarum* is an endoparasite of the *B. longissima* , which showed huge potential in biocontrol of *B. longissima*. In the present study, the early stage of parasitism (0-24 h after parasitism), *A. hispinarum* did not inhibit the encapsulation reaction of host larvae hemocytes, while in the late stage of parasitism (24-96 h after parasitism), *Brontispa longissima* larvae was significantly inhibited. The results that granulocytes and plasmatocytes could phagocytize bacteria from 2 to 48h and granulocytes, plasmatocytes and oenocytoids were prophenoloxidase/phenoloxidase positive hemocytes indicated that granulocytes, plasmatocytes and oenocytoids were the main immunocompetent hemocytes in *B. longissima* larvae. At 1, 2 and 4 days after *A. hispinarum* parasitism, the adhesion ability of host *Brontispa longissima* larvae blood cells decreased significantly. The results of this study would shed light on the understanding of cellular immune-disabling molecular mechanisms in *Brontispa longissima* larvae, parasitized by *A. hispinarum*, and may also open a new window to biological control of this pest.

Discovery of entomopathogenic fungi across geographical regions in southern China on pine sawyer beetle *Monochamus alternatus* and implication for multi-pathogen vectoring potential of this beetle

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Entomopathogen-based biocontrol is crucial for blocking the transmission of vector-borne diseases; however, few cross-latitudinal investigations of entomopathogens have been reported for vectors transmitting woody plant diseases in forest ecosystems. The pine sawyer beetle *Monochamus alternatus* is an important wood borer and a major vector transmitting pine wilt disease, facilitating invasion of the pinewood nematode *Bursaphelenchus xylophilus* (PWN) in China. Due to the limited geographical breadth of sampling regions, species diversity of fungal associates (especially entomopathogenic fungi) on *M. alternatus* adults and their potential ecological functions have been markedly underestimated. In this study, through traditional fungal isolation with morphological and molecular identification, 640 fungal strains (affiliated with 15 genera and 39 species) were isolated from 81 beetle cadavers covered by mycelia or those symptomatically alive across five regional populations of this pest in southern China. Multivariate analyses revealed significant differences in the fungal community composition among geographical populations of *M. alternatus*, presenting regionalized characteristics, whereas no significant differences were found in fungal composition between beetle genders or among body positions. Four region-representative fungi, namely, *Lecanicillium attenuatum* (Zhejiang), *Aspergillus austwickii* (Sichuan), *Scopulariopsis alboflavescens* (Fujian), and *A. ruber* (Guangxi), as well as the three fungal species *Beauveria bassiana*, *Penicillium citrinum*, and *Trichoderma dorotheae*, showed significantly stronger entomopathogenic activities than other fungi. Additionally, insect-parasitic entomopathogenic fungi (*A. austwickii*, *B. bassiana*, *L. attenuatum*, and *S. alboflavescens*) exhibited less to no obvious phytopathogenic activities on the host pine *Pinus massoniana*, whereas *P. citrinum*, *Purpureocillium lilacinum*, and certain species of *Fusarium* spp.—isolated from *M. alternatus* body surfaces—exhibited remarkably higher phytopathogenicity. Our results provide a broader view of the entomopathogenic fungal community on the vector beetle *M. alternatus*, some of which are reported for the first time on *Monochamus* spp. in China. Moreover, this beetle might be more highly-risk in pine forests than previously considered, as a potential multi-pathogen vector of both PWN and phytopathogenic fungi.

War on the invasive coconut rhinoceros beetle in Solomon Islands by Guadalcanal Plains Palm Oil Limited (GPPOL) through an Integrated Pest Management (IPM) approach

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ABSTRACT. Solomon Islands is currently experiencing a major agriculture threat to its productive palm industries due to the recent invasion of a new population of coconut rhinoceros beetle (CRB), a devastating pest of coconut and oil palms. Invasion of the CRB-G variant (clade I haplotype) in 2015 was first detected in Honiara. It rapidly spread eastwards towards GPPOL, a leading RSPO certified palm oil exporter (and major employer) in Solomon Islands. By 2016 it had reached both commercial and small holder plantations, leading to devastating palm damage (including death) for both coconut and oil palm producers. The aim of this presentation is to provide a case study from the Solomon Islands highlighting GPPOL's (ultimately) successful management efforts in responding to the invasive CRB-G pest populations. The financial losses due to significantly reduced yields were compounded by the enormous costs incurred while attempting to manage this invasive pest. This was achieved with the assistance of several national and international collaborators, including: Solomon Islands Ministry of Agriculture and Livestock, Pacific Community, Papua New Guinea Oil Palm Research Association, and AgResearch (New Zealand). Over 7 years (2016-2023), GPPOL has developed an integrated pest management strategy for plantations that currently incorporates two management streams as part of a response plan for controlling CRB. Currently these involve: Physical/Cultural control measures (insect trapping for monitoring, sanitation programs to sites of larval development through the use of palm trunk chipping and disc-harrowing), and Chemical Control applications (targeted spraying of young palms with and targeted trunk injection of mature palms). A third stream involving Biological Control is currently under development with lab and field testing of new isolates of viral and fungal (including native species) candidates for use as future biocontrol agents. Results obtained to date will be discussed within the context of the New Zealand Aid Programme that is supporting improved long-term sustainable CRB management solutions for the Pacific region.

An update on virus records for New Zealand plants with a focus on natives

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Over the past two centuries, exotic plants have established in New Zealand (NZ), bringing with them some of their viruses, as well as many of their invertebrate virus vectors. NZ records of plant viruses have more than doubled since 1989. Most plant virus records in NZ are from economically important plants including horticultural, ornamental, and unwanted invasive plant species. At least 16 viruses have invaded native plant species, likely jumping from crops, weeds, and garden ornamentals. Other newly found viruses are likely endemic to NZ. The increased number of plant viruses recorded over time reflects not only new incursions, but also changing diagnostic technologies increasing our ability to detect and identify viruses. There are still limited data available on the true extent of virus diseases in native plant ecosystems in NZ, and the trends of the current data will be discussed.

Coconut Rhinoceros Beetle (CRB) control and management in oil palm in the Solomon Islands

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Oryctes rhinoceros L. is an invasive and destructive pest of palm trees in Southeast Asia and the Pacific including Papua New Guinea and the Solomon Islands. The earlier beetle populations on the Solomon Islands known as the coconut rhinoceros beetle Pacific haplotype (CRBP) are controlled effectively by the biological control agent *Oryctes rhinoceros* nudivirus (OrNV). Another population of CRB was reported in 2015 on Solomon Island and it was associated with aggressive feeding behavior and huge yield damage to coconut and oil palms. The new CRB population is a novel haplotype (clade I) known as CRB-G and is not affected by the nudivirus. CRB-G progressively invaded Guadalcanal Oil Palm (GPPOL) plantation areas and the surrounding smallholder's oil palm blocks on Solomon Island, causing remarkable damage to all palms. With the incursion of CRB-G to Oil Palm fields on Solomon Island, a strategic IPM approach was implemented by GPPOL management team. The approach included cultural control by destroying all valuable breeding sites including old palm trunks and EFB decomposition sites. In addition to that, mechanical chipping of fresh palm trunks was implemented to help reduce palm trunk mass as well as increase the chipped matter decomposition rate within a short period. Disc harrow of chipped palm trunks and use of cover crop were also implemented. Moreover, mass trapping with pheromone lure baiting was carried out on a weekly basis. Chemical control was applied selectively, based on beetle population monitoring surveillance and palm damage surveys and assessment reports conducted weekly. Control efforts using this strategy have played an important role in controlling the beetle population and maintaining damage levels on all estates below 5% since early 2020. Ongoing research in collaboration with AgResearch NZ to develop long-term IPM strategies includes evaluating the efficacy of viral biocontrol through field transmission trials. Moreover, one fungus, *Metarhizium majus* strain, is being evaluated to determine its effectiveness in the field.

Where is it now? Assessing the presence of the granulate ambrosia beetle at a property in Kumeū, Auckland

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A native of tropical and subtropical east Asia, the granulate ambrosia beetle (GAB) *Xylosandrus crassiusculus* (Scolytinae) has previously invaded numerous countries including North, Central and South America, Europe, some Pacific Islands, South Africa and Queensland, Australia. This species poses a significant threat to New Zealand, due to its large host range (200+ tree and shrub species over 60 plant families) and the potential significant impacts. To date, at least eight native plants have been found to be hosts. GAB was first detected in Blockhouse Bay, Auckland, in 2019, and subsequently in other locations in Kumeū, Riverhead and Titirangi. Intensive searching for the presence of GAB in one property in Kumeū, which had previously experienced active GAB and tree loss, was conducted in January to March and late October to early November 2022. Ethanol lured flight intercept trapping were also used in both monitoring time-periods and although no active GAB was detected by searching on this property, three adult beetles were detected in January/March and 53 in October/November 2022 in the flight intercept traps. The location of activity of this beetle in plant species has not been detected and further research is required to determine the extent of the beetle in the Kumeū area.

Complete mitochondrial genome of *Schrankia costaestrigalis*

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The pinion-streaked snout *Schrankia costaestrigalis* is a new potato pest recorded in China. This paper analyzed the complete mitochondrial genome of *S. costaestrigalis*. Genomic DNA was isolated and subjected to paired-end sequencing (2 × 150 bp) of 300 bp inserts using the Illumina NovaSeq 6000 platform. The mitochondrial genome (mitogenome) was assembled de novo using NOVOPlasty v4.3.1 with the cox1 (GenBank: EF061755.1) of *S. costaestrigalis* isolate Scos02 as a seed sequence. Three pairs of primers were designed corresponding to the tRNA-Asn, rrnL, and control regions (CR, also an AT-rich region) to verify the accuracy of the mitogenome assembly and the close gap of mitogenome. The complete mitogenome (GenBank: OQ181231) occurred as a circular DNA molecule of 16,376 bp with 51.001% AT content, and it comprised 13 protein-coding genes (PCG), 22 tRNA genes, 2 rRNA genes, and 1 CR. All 13 PCGs had traditional ATN (Met) start codons: only nad6 starts with an ATC start codon; two PCGs (cox2 and nad5) start with an ATA start codon; six PCGs (cox1, atp6, cox3, nad4, nad4l, and cob) start with an ATG start codon; four PCGs (nad2, atp8, nad3, and nad1) start with an ATT start codon. All 13 PCGs using traditional TAA as stop codons: ten PCGs (nad2, cox1, atp8, atp6, cox3, nad3, nad4l, nad6, cob, and nad1) ended with TAA stop codons; three PCGs (cox2, nad5, and nad4) had an incomplete stop codon (T), consisting of a codon that was completed by the addition of A nucleotides at the 3′ end of the encoded mRNA. The traditional 22 tRNA genes were interspersed throughout the PCGs. Fourteen of the 22 tRNAs are encoded on the majority strand, and eight are on the minority strand. The 22 tRNA had a typical cloverleaf secondary structure, including tRNA-Ser. The lrRNA and srRNA were 1,400 and 790 bp in length, respectively. The CR of the mitogenome was 1,421 bp with 91.766% AT content, located between the srRNA and tRNA-Met genes.

Invasion Alert: The Coconut Rhinoceros Beetle Outbreak on Efate, Vanuatu

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An incursion of coconut rhinoceros beetle (CRB: *Oryctes rhinoceros*) was reported in Efate, Vanuatu in 2019. The beetle population was determined to belong to CRB-S (Clade II) similar to CRB populations found in the Bismark Archipelago, Papua New Guinea. A second incursion of CRB-G (Clade IA) was discovered in 2021. Damage mapping, beetle trapping, and genomic identification show that CRB-S has now spread around about half of the island from the site of the original incursion. CRB-G is mainly restricted to the port and surroundings of Port Vila with some outlying specimens. Through the efforts of Biosecurity Vanuatu, CRB is still contained in the Efate group of islands and has not spread to the major copra-producing islands in other parts of the country. Sanitation efforts appear to have reduced damage from CRB within the zone of the first arrival of the pest (Managaliliu, Efate). CRB damage, however, is severe in areas with old plantations and limited sanitation activities (e.g., Mele/Devil's point, Efate). A concerted effort is necessary to contain the beetle on Efate and to reduce its spread and impact on the island.

Botany in a biosecurity setting: Identification of invasive plants at MPI

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The Botanists of the Ministry for Primary Industries' Plant Health and Environment Laboratory are responsible for the identification of plant and invasive weed species to enhance the biosecurity of New Zealand. This includes verifying suspected exotic and new plants and seeds detected in New Zealand. Submissions come from a range of sources including the general public, interceptions at the border, incursion investigations, and surveillance programmes. We work in an applied diagnostic environment where results can have significant implications for New Zealand's biosecurity and primary industries. To identify the suspected exotic plant species, both the traditional morphology-based methods as well as DNA sequence-based identification tools are used to ensure accurate identifications. This is essential for providing timely and detailed diagnostic results to support biosecurity responses and long-term management actions of invasive plants. Here we present an overview of our diagnostic work with specific case studies from last few years.

Occurrence history of forest invasive pests in Korea over the last 50 years

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Understanding the occurrence patterns of invasive forest pests is fundamental for effective forest management from both economic and ecological perspectives. Here, we review the history of the occurrence patterns and causes of outbreaks and declines of invasive forest pests in Korean pine forests over the last 50 years. During this period, the major pests of pine forests in Korea have shifted from pine caterpillar (*Dendrolimus spectabilis* Butler), which is an indigenous species, to the pine needle gall midge (PNGM, *Thecodiplosis japonensis* (Uchida and Inouye)) and finally to pine wilt disease (PWD) caused by the pine wood nematode (*Bursaphelenchus xylophilus* (Steiner and Buhrer) Nickle). The PNGM is an invasive species, first discovered in Korea in 1929, that became widely distributed by 1992 and the major forest pest in the 1980s and 1990s. A suite of parasitic wasps attacking the PNGM contributed at least partially to the decline of PNGM densities. The PWD is also an invasive species, first discovered in Korea in 1988. Following the decline of the PNGM, damage from PWD has increased since 2003. These shifts in major forest pests might be related to changes in forest composition and interactions among forest pests.

Beyond soil baiting: developing an eRNA protocol for the rapid detection of *Phytophthora* species

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Phytophthora, the ‘plant destroyer’ genus, continues to threaten plantation and horticultural sectors, and urban and native forests, globally. In New Zealand, several *Phytophthora* species affect commercial and native forestry, e.g., *Phytophthora pluvialis* and *P. cinnamomi* on *Pinus* spp., *P. agathidicida* on kauri (*Agathis australis*), and the recently described *P. podocarpi* on tōtara (*Podocarpus* totara). To manage the spread of soil-borne Phytophthora species, such as *P. agathidicida*, soil and leachate samples are collected from nurseries and forests to determine presence/absence. Identifying these pathogens rapidly is essential to limiting their spread and impact. Soil or leachate baiting is a proven and preferred method for isolating *Phytophthora* species. However, this process can take weeks to complete and requires trained experts to isolate and identify these species. We propose the use of a molecular tool to identify these pathogens rapidly and more accurately. Here we outline an RNA extraction approach that will form the basis for an eRNA tool that can reduce the time to identify *Phytophthora* species from 4–6 weeks to 1–2 weeks.

Genomic identification of *Oryctes rhinoceros* Nudivirus, a biocontrol agent of Coconut Rhinoceros Beetle

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Coconut Rhinoceros Beetle (CRB, *Oryctes rhinoceros*) has its native range in Asia but is an introduced pest species of coconut palms in the Pacific. In an attempt to limit the damage and control the spread of CRB, *Oryctes rhinoceros* Nudivirus (OrNV) was introduced in the 1970s as a biocontrol agent. Following a period of very effective biocontrol there are now variants of CRB that are no longer affected by the OrNV biocontrol. We have initiated a study on the genetic diversity of CRB across the Pacific to address the question: are there CRB genotypes that are more susceptible to OrNV and/or more successful in establishing and spreading? In parallel we are studying OrNV isolates, originally identified in the CRB native range to determine the efficacy of different OrNV isolates as biocontrol agents. Here we describe the development of an assay for the rapid identification of different OrNV isolates. We have generated whole genome sequences of six OrNV isolates and, combined with OrNV genome sequences from the public domain, used this to design multiple PCR primer sets across the OrNV genome targeting regions of known variability. Currently, seven OrNV amplicons are generated for each sample. Individual samples are tagged with a unique barcode and combined for multiplex analysis using Oxford Nanopore Technologies sequencing. This assay allows us to detect and identify OrNV isolates in field samples from across the Pacific in order to understand and follow the presence and spread of strains across the region. In addition it assists in determining the efficacy of different OrNV isolates used in biocontrol assays where OrNV preparations are fed to CRB in the laboratory and in field settings. A detailed understanding of the diversity of OrNV isolates in the Pacific can contribute to improving management of CRB.

Catch a free ride with me ─ Ant hitchhiking on vehicles in Taiwan

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Human transportation can facilitate long-distance dispersal of organisms, allowing them to colonize new areas and thus increasing the probability of biological invasions. Here, we report on 41 cases of ants hitchhiking on vehicles (cars and scooters) in Taiwan, collected from social media platforms between 2017 and 2022. Among the eight hitchhiking ant species, six were invasive and constituted 93% (n = 38) of the cases. Moreover, about half of the cases (n = 22) were from the invasive species *Dolichoderus thoracicus*. The ants colonized the vehicles within a day in 60% of the cases (n = 25), while a few colonizations took over a month (n = 4). The hitchhiking events occurred more frequently during summer (June to September, n = 26) than spring (March to May, n = 10) or fall/winter (October to December, n = 5). To our knowledge, this is among the first studies of ant hitchhiking on vehicles. Further research on the factors underlying ant hitchhiking behavior and colonization attempts will provide useful implications for ant invasion management.

Controlling damaging invasive pests by learning from successful biocontrol

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Biocontrol acts as an effective, pesticide free and non-GMO method of controlling invasive species. However, decline in efficacy of a successful biocontrol system can have devastating consequences for native ecosystems, agriculture, and the economy. Argentine stem weevil (ASW), a significant rye grass pest that was controlled by the effective biocontrol agent *Microctonous hyperodae*, used to have overwinter parasitism rates of 70%; however, 7 years after its original release, there was a significant decline in parasitism rates. Researchers hypothesised that this decline was due to the evolution of resistance by the ASW to *M. hyperodae*, likely caused by the strong selective pressure imposed by high initial parasitism rates. The clover root weevil (CRW) is currently controlled by *M. aethiopoides* and, with multiple similarities in seasonality, biotype and ecology to the ASW system, *M. aethiopoides* could similarly fail as a biocontrol agent for CRW in the future. Investigating the potential for such failure is critical, since CRW represents an exceptional threat to the New Zealand dairy industry. My PhD project will use population genomics tools to understand how specific genetic differences within/between populations may be associated with biocontrol failure and success. In addition, the microbiomes of insect species have been shown to influence their behavior, reproduction, and interactions with other species, thus examination of microbial communities, including endosymbionts, will help determine differences among parasitoid wasp populations. I will therefore perform metagenomic studies to understand microbial diversity and abundance across different populations of *M. aethiopoides* to examine the potential role of the microbiome in biocontrol success. Finally, temperature plays a crucial role in influencing the behavior of both wasps and their host weevils, which in turn may impact parasitism effectiveness. Thus, I will test the impact of different temperatures on host-parasitoid interactions at the transcriptomic level to elucidate the wasp's biology and its mechanisms of response to changing environments, including via changes in gene expression and metabolic pathways.

The mixed voices regarding using invasive species in schools

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This study identified the characteristics of ecological experts' and biology teachers' attitudes toward invasive species flowing into Korean schools for educational purposes and explored opinions on the post-processing of invasive species after education. Sixteen Korean ecological experts and twenty biology teachers participated in the study, and an open questionnaire using invasive rabbits and reptiles as examples was organized and presented. The collected qualitative data were analyzed through constant comparative method. As a result, ecological experts showed a relatively positive perception of the use of invasive species for educational purposes in schools, but on the contrary, biology teachers showed a relatively negative perception. In addition, regarding post-processing, ecological experts expressed their opinion on euthanasia treatment through specialized institutions, but biology teachers prominently presented the idea of transferring animals to educational administrative institutions or animal-related institutions or sending animals for adoption to the students who wanted. Although the introduction of invasive species to schools for educational purposes is becoming more active, confusion continues because there are no institutional guidelines. In addition, confusion in the educational field is expected in the future as ecological experts and biology teachers present different opinions on the attitude and post-processing of invasive species upon their introduction to schools. Therefore, based on the opinions of ecological experts, it is necessary to prepare a legal and institutional management system for the management of invasive species at school and provide ecological education for teachers. Key words: secondary schools, invasive species, ecological education, post-processing, ecological experts, biology teachers.

Strawberry guava (*Psidium cattleianum*) detection from satellite imagery using machine learning and species distribution modeling on Hawai‘i Island

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Strawberry guava (*Psidium cattleianum*) trees have invaded tropical forests and degraded native ecosystems around the world. Because populations are dense and widespread, field crews are unable to accurately survey all current locations of strawberry guava. Remote sensing technologies and deep learning methods allow researchers to increase the spatial scale that invasive species can be detected. This project aims to use satellite imagery and supervised machine learning methods, such as random forests and convolutional neural networks, to identify strawberry guava trees on Hawai‘i Island and map their present distributions. Habitat suitability modeling for strawberry guava will also allow us to predict where trees may spread and highlight areas that are high priority to manage. The climate and topographical variables that are used for these models, including air temperature, precipitation, slope, and elevation, influence strawberry guava population distributions on the island and these relationships help predict how their niche may shift due to climate change. Three potential carbon emissions scenarios estimated by the Intergovernmental Panel on Climate Change (IPCC) will determine the magnitude of how climate variables will change in these models. Awareness of the existing distributions of strawberry guava trees, and other invasive species, and where they are likely to spread are important for informing land management decisions and protecting native ecosystems in Hawai‘i and around the world.

Management option in controlling the outbreak of Coconut Rhinoceros Beetle Guam biotype in Kananam, Madang Province, Papua New Guinea

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Coconut is the fourth most significant agriculture cash crop in terms of its economic importance to PNG. According to the most recent census figures, 35% of total households in PNG or an estimated 2.6 million people are engaged in coconut activities to sustain their livelihoods and for income generation. On average the coconut industry contributes over K126.5 million per year to the PNG economy through the export revenue it generates (Medium Term Development Plan III Vol 2). Coconut Rhinoceros Beetle Guam biotype (CRB-G) was first reported in PNG in 2009 and in 2019 it was confirmed to be present in NCD, parts of Central and Gulf Provinces through a delimiting survey by Kokonas Indastri Koporesen (KIK) and other stakeholders (NAQIA and PNG OPRA). Towards the end of 2019, CRB-G was first identified in Kananam Village, Madang Province. The infestation site is approximately 17km away from the only Coconut Research Station in PNG. Hence, the management approach taken was to carry out sanitation in the infested area. The objective was to completely destroy all breeding sites, infested host plants of CRB, and identify, record and destroy all CRB samples collected. This is important to minimize CRB-G population below the economic threshold and prevent further spread of the pest. The total infested area is about 400Ha. The sanitation took a period of 13 weeks, in which a total of 6528 breeding sites were destroyed, with 24,051 CRB samples collected, recorded and destroyed. From the sanitation exercise, it was observed that CRB favors rotten standing coconut poles and stumps more than coconut logs laying on the ground. The other rotten logs (both standing and, on the ground,) are not so favoured by CRB. During the sanitation exercise, it was noted that CRB adults also favors rotten *Hydriastele costata* more than rotten coconut logs, especially the ones lying on the ground. According to the results, it is concluded that the intervention was effective in reducing the buildup of beetle population and minimized the further spread for about 6 months. Furthermore, from this exercise, knowledge of preference and abundance of beetle population in different hosts provides direction for appropriate and adequate management strategies to be applied in a Coconut Rhinoceros Beetle Guam infested area.

Exploring the nature of *Arhopalus ferus* (Coleoptera: Cerambycidae, Spondylidinae) pheromone attraction

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Cerambycid species of the Spondylidinae subfamily are distributed worldwide and are known for being prolific invaders that infest conifers. They are no strangers to NZ where *Arhopalus ferus*, the Burnt pine longhorn beetle, is well-established and requires monitoring at high-risk sites such as ports, airports, and sawmills. This monitoring is required as part of the requirements to meet pine log export standards set by the Ministry of Primary Industries (MPI), and its surveillance is highly reliant on trapping using a lure composed only of host volatiles. Over the past 15 years, research on cerambycid pheromones has expanded worldwide through the identification of more than 400 cerambycid pheromone attractants. Recent advances in the area of cerambycid pheromones have allowed for pheromones to be synthesized for use as lures in traps. More recently, the identification of the male-produced aggregation pheromone in *Arhopalus rusticus* has enabled investigations into the nature of *A. ferus* pheromone attraction. Here we present the initial progress of our research, using laboratory and field bioassays to identify the specific chemical components responsible for *A. ferus* attraction, which may be used to help develop pheromone-based monitoring tools in NZ.

First investigation into the seed-borne microbial communities of *Metrosideros excelsa*

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Recent studies have disclosed that bacteria and fungi can inhabit plant seeds, and some microbial species are likely founders of the endophytic community in developing seedlings. As successful plant establishment during the early stage of development is crucial for plant fitness and resistance to environmental stresses, the effects of seed microbiome on the seedling performance are of particular interest in protection against plant pathogens. The fungal pathogen *Austropuccinia psidii* is the causal agent of myrtle rust, which infects more than 480 species of the Myrtaceae family globally and poses a severe threat to New Zealand endemics. Individual juvenile plants of *Metrosideros excelsa* demonstrate different susceptibility to the pandemic strain of *A. psidii*, but the origin of such variation is unclear. We hypothesise that seedlings contain seed-derived microbial communities, which may contribute to pathogen resistance or susceptibility. We isolated 24 species of fungi and 17 bacterial taxa from *M. excelsa* seeds. Our results suggest that seeds of *M. excelsa* harbour diverse microbial assemblies. The frequently isolated fungi belonged to *Aureobasidium*, *Cladosporium*, *Penicillium*, and *Pestalotiopsis*, and were often isolated with bacteria *Bacillus*, *Erwinia*, and *Methylobacterium*. Each tree demonstrated individual isolation frequency and species composition of seed-borne microorganisms, which may contribute to the different levels of induced resistance to phytopathogens.

Modelling abundance and risk impact of the Asian yellow-legged hornet, *Vespa velutina nigrithorax* (Hymenoptera: Vespidae) in Korea: Application of a species abundance model

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The Asian yellow-legged hornet, *Vespa velutina nigrithorax*, is native to Southeast Asia. However, it has invaded many countries in temperate regions, causing serious threats to apiary and human health in non-native counties. With a growing demand for estimating the potential distribution of this species, multiple studies have reported occurrence-based models (SDM). Unfortunately, they are less informative for predicting local abundance patterns associated with its impact. The aim of this study was to develop an abundance model (SAM) for *V. velutina* in Korea to support the forecast of its impact and management strategies. The abundance of *V. velutina* was investigated across the study area and used for model development with bioclimatic and land composition variables. Along with the SAM, the classical SDM was tested to determine whether it could provide a reasonable prediction in the estimate of local abundance. As a result, the SAM showed better performance than the SDM to evaluate the impacts caused by *V. velutina* with higher discriminative power and accuracy. On the other hand, the SDM was not able to discriminate abundance in the occupied area of *V. velutina*, indicating an unclear occurrence-abundance relationship or oversimplification of the estimated niche. Based on the final SAM, risk indices for human health and honeybee loss were suggested. These results could provide support for risk management of *V. velutina* in Korea and give biological information to other countries where this species has already established or with possible invasion in the future.

Hybrid performance and host plant adaptation in fall armyworm

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Spodoptera frugiperda, also known as fall armyworm (FAW), is an agricultural polyphagous pest that can adapt to more than 350 host plants and inflict significant economic losses, posing a serious threat to food security. There are two FAW strains that are anatomically identical but associated with different food preferences, the “rice strain” (SfR) that prefers rice and grass, and the “corn strain” (SfC) that prefers corn, cotton and sorghum. These two strains are genetically distinct for a few known genetic loci, with mitochondrial and Z-linked haplotypes the most commonly used, while the rest of the genome displays few polymorphisms between strains that could explain their adaptation to plants. Besides, constitutive transcriptional variations between strains have been found at the mitochondrial level. Hence, mitochondria are thought to play an important role in host plant adaptation of FAW. Furthermore, despite the strain differences, a high prevalence of FAW hybrids in the field has been reported. In this study, we investigated whether the mitochondrial genome can influence transcriptional patterns at the nuclear level in FAW. To test this hypothesis, we compared the performance and gene expression of FAW strains and reciprocal hybrids. We first maintained the two inbred laboratory strains (RR: SfR♀ × SfR♂, CC: SfC♀ × SfC♂) and produced 2 F_1 hybrid generations (RC: SfR♀ × SfC♂, CR: SfC♀ × SfR♂). The hybrids are heterozygous for all chromosomes except for the mitochondrial and sexual chromosomes. We then cultivated these four genotypes in triplicate on both artificial diet (Poitout) and corn plant leaves in parallel, and recorded phenotypic traits such as weight over time, survival, and sex ratio to evaluate the performance differences. During the rearing, we collected the stage 4 larvae from each genotype for RNA-seq to compare the gene transcriptional differences. Surprisingly, we observed a strong directionality of maternal effects and a strong interaction of genotypes on performance when reared on corn plant leaves. Moreover, for both diets, the two hybrid genotypes outperformed the parental strains in larval weight, and CR got the highest survival rate with corn diet, which suggested heterosis effects and might help explain the fact that most FAW invasive populations are interstrain hybrids in the wild. Further RNA-seq analysis will help determine if mitochondrial genome is at play in transcriptional patterns for relating genotypes to performance in FAW. This study can help shed light on the invasive nature and the genomic basis of host plant preference in FAW.

An analysis of the activity of freshwater turtles as invasive species in South Korea using a satellite tracking system

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Invasive Alien Species (IAS) are a raising global concern due to their potential challenges to native ecosystems and biodiversity. South Korea is also suffering from IAS with regular reports on non-native species from the wild. Thus, the Ministry of Environment has designated many alien species as “Ecosystem Disturbance Species (EDS)” based on their negative impacts on the native ecosystem. Among many other organisms, this list includes six freshwater turtles, Red-eared Sliders (*Trachemys* sp.), River-Cooter (*Pseudemys concinna*), Florida Red-bellied Cooter (*P. nelsoni*), Chinese Stripe-necked Turtle (*Mauremys sinensis*), Alligator Snapping Turtle (*Macrochelys temminckii*), and Common Snapping turtle (*Chelydra serpentina*). For better management of these EDS, basic ecological studies such as the home range and activity are crucial. Although a few studies on *T. scripta elegans* were also insufficient, other IAS turtles remained unstudied. Hence, we studied home ranges and daily activities of three IAS turtle species, *T. s. elegans*, *P. concinna*, and *P. nelsoni*, in the reservoir at Chonnam National University, South Korea, between August 2021 and June 2022. We used the satellite tracking method for our study instead of using radiotelemetry. Although radio tracking is a well-practiced method for studying home ranges and daily activities of turtles, it is time-consuming, requires a large amount of manpower, and involves the investigator’s subjective views. Whereas, satellite tracking facilitates having many coordinates in a short period with less effort. We recorded coordinates by attaching tackers (WT-300 Mallard, KoEco, KR) to the studied individuals eight times a day at an interval of three hours. Home ranges were estimated by creating maps from the coordinates in ArcMap 10.8.2. following MCP (90%) and KDE (95%) methods. We found 1566 of a total of 2425 coordinates during the study period. Our study revealed that all of the species were most active between 12:00 ~ 15:00. Almost 58.0% of coordinates for the *T. s. elegans*, 38.3% for the *P. concinna*, and 53.7% for the *P. nelsoni* were from this time period. The average home ranges were 1.69 ha, 2.28 ha for the *T. s. elegans*, 1.44 ha, 1.95 ha for the *P. concinna*, and 1.31 ha, 1.88 ha for *P. nelsoni*. This study is significant to have knowledge of ecology, home range, and daily activity patterns of IAS in South Korea, which in turn could be helpful for their better management. Thus, our study could be useful in preparing conservation plans for native species in light of challenges from IAS.

Reproduction system development of *Ceracris kiangsu* Tsai and its host preference

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The yellow-spined bamboo locust (YSBL), Tsai (Orthoptera: Arcypteridae) has historically been regarded as the second worst pest species affecting bamboo. It has been recorded in East Asia and Southeast Asia, including China, Laos and Vietnam, with the scale and frequency of YSBL population outbreaks increasing. Since 2014, there have been a large number of outbreaks of YSBL in Laos, with subsequent establishment in South China in 2019. YSBL could be a major threat to food and nutrition security where it has established. In this study, we validated and graded the ovarian development of YSBL from both indoor and outdoor populations. Their ovarian development was graded into five stages. Four ovarian developmental stages before the oviposition were identified, and the fundamental ovarian structure of YSBL displayed red dots on the matured eggs inside the calyx at ovarian developmental stage V. Body weight had a significant effect on ovarian developmental stage. This may provide a potentially rapid way to identify the population source as either ‘native’ or ‘immigrant’ from the phenotypic traits without dissection. In addition, the laboratory host preferences demonstrated that wheat and rice were more suitable for nymphs’ growth of YSBL than growth on maize from the life table aspect. However, there is no wheat damage reported from field sites.

Comparative genomic analysis of *Erwinia amylovora* strains reveals population structure, single-nucleotide polymorphisms, and plasmid diversity

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The enterobacterium *Erwinia amylovora* is the causal agent of fire blight, a devastating disease affecting apple, pear and other Rosaceae species. Because of the highly spreadable and infectious nature of fire blight, *E. amylovora* has been listed as a quarantine organism in China. Fire blight was first observed in Xinjiang Province of China in 2016, and only emerged in Xinjiang and Gansu in China to date. The complete genomes of three strains isolated from infected pears in Xinjiang and Gansu were sequenced, annotated, and compared with the genomes of other *Erwinia* spp. strains. Genomes synteny was analyzed, and sequence variations including nucleotide substitutions, inversions, insertions, and deletions were detected. Several singleton and shared features of the genome of *E. amylovora* detected in China were identified that offered a first view into evolutionary aspects within the genus *Erwinia*. Of these, KL17-17 and KL20-28 contains a conserved 28 kb plasmid pEA29, but no plasmid was identified in ZY20-3-2. Remarkably, a novel plasmid pEA3 was identified in KL17-17, and has not been reported previously. This might be part of the pathophysiological mechanism resulting in the weak pathogenicity of KL17-17. The genomes were compared with those of other 127 *E. amylovora* strains isolated from different hosts or geographical regions. Analysis of the population genomic structure revealed that the three strains from China formed a distinct structural group. Phylogenomic analysis was performed to infer the evolutionary relationships among *E. amylovora* strains, which indicated that the Chinese isolates, all descended from a common ancestor, are closely related to a lineage of Western Asia strains. These results provide useful information for understanding the genomic dynamics and propagation path of *E. amylovora* strains including those in China, developing genetic markers for surveillance of the pathogen or diagnosis of the disease, and eventually developing measures to eradicate it.

Cost-effective portfolio allocation across quarantine, surveillance and eradication using info-gap theory

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Invasive species can lead to community-level damage to the invaded ecosystem and extinction of native species. Ecological systems, and the species within, are highly complex and variable. Uncertainties need to be considered in bio-economic modelling to assist in decision making and evaluate the robustness of designed policy. In our research, info-gap decision theory (IGDT) is applied to model and manage such uncertainty. IGDT is a non-probabilistic theory to enable robust decision making. Such robust decision making methods are often desirable in ecological systems characterized by Knightian uncertainty, without considering the probability or frequency of policy outcomes. This research provides a novel method for applying IGDT to determine the robust population threshold estimate and the allocation of funds in a biosecurity context, in particular the cost of pre-border prevention versus post-border surveillance and eradication. We use the risk of incursion of the Asian house gecko, *Hemidactylus frenatus* Duméril and Bibron, 1836 onto Barrow Island as a case study. Our work provides guidance for decision makers to balance the robustness against parameter estimate errors and specific total budget limit. We demonstrate that, allocating budget to both quarantine and surveillance results in a more robust option, irrespective of the risk of incursion. Increasing investment in either quarantine or surveillance increases the annual budget, but also decreases the total budget limit (i.e., the maximum total budget that decision makers may allocate to all three biosecurity activities). Budget allocated to quarantine should outweigh that to surveillance. A higher estimated population threshold for post-border surveillance detection could increase robustness against unacceptable total management costs. The method outlined here can be used to assist in robust portfolio allocation of limited budget to manage invasive species in a wider context, and to better tackle uncertainty in protection of biodiversity and native species in a cost-effective manner.

Insecticidal activity of new *Metarhizium* isolates against different populations of CRB larvae from Papua New Guinea and Solomon Islands

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The coconut rhinoceros beetle (CRB) (*Oryctes rhinoceros*) is one of the most destructive insect pests of coconut and oil palm in tropical Asia and the Pacific Islands. A new variant, known as CRB-G, has spread into the Pacific Islands causing significant levels of damage. Biopesticides containing *Metarhizium* spp. are the strongest candidates for fungal biological control against the emerging CRB threat and selection of the most virulent and robust isolate could be the determining factor for success. In this work, CRB specimens with natural fungal infection were collected in Papua New Guinea (PNG) and Solomon Islands (SI). Putative entomopathogenic fungi were isolated, identified and multiplied on rice. These new isolates and some previously obtained from other Pacific countries were tested for virulence against CRB larvae populations at PNG and SI in lab bioassays. Four isolates identified as *Metarhizium majus* (conidia length ~10-15 µm) were obtained from SI and five isolates identified as *Metarhizium pinghaense* (conidia length ~4-6 µm) were obtained from PNG. Conidia yield on rice was 10 times higher for *M. pinghaense* (10_9 conidia/g) in comparison with *M. majus* , which could be related with the differences in conidia size. The most virulent isolate was *M. majus* SI4 that caused 100% mortality in 20-23 days against the old variant (CRB-S) in bioassays carried out in PNG, whereas isolates of *M. pinghaense* did not show pathogenicity against larvae. *M. majus* SI4 isolate was also the most virulent against the mixed population (CRB-S and CRB-G) at SI and was selected for further evaluation under field conditions.

A human-in-the-loop approach for annotation of sparse audio events for bioacoustic monitoring

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The collection and annotation of bioacoustic data presents a number of challenges to researchers. Focus is often constrained to highly vocal species and limits the study of less vocal species. In Aotearoa New Zealand, invasive species such as possums, mustelids (ferrets, stoats, weasels), and rats pose a significant threat to our native biodiversity, yet little bioacoustic data related to these species currently exists. This data is critical in the analysis of species behaviour and the development of improved conservation tools such as audio lures. We present a human-in-the-loop annotation tool designed to aid in the analysis of sparse audio events within long-term field recordings. This tool combines a wavelet-based segmentation method that automatically extracts transient features from within field recordings. We present a number of novel annotation interface methods that present segments to users for efficient annotation such as grid view and point mapping for outlier detection. This tool combines fine-tuning of a pre-trained ResNet-based model, which learns to identify target features over time and can provide the user with segment predictions improving annotation in a human-in-the-loop process. This process combines the accuracy of human classification and the speed of machine annotation to greatly reduce the empty-space in sparse field recordings with up to a 90% reduction in audio and a 4.0x improvement in annotation efficiency compared with manual annotation. Training on 200 5-minute field recordings resulted in a classification accuracy of 95%. This tool allows users to train custom audio classification models for any application with sparse features and this classification pipeline can be easily exported for use in the field making real-time bioacoustic monitoring of less-vocal species a possibility.

Finding the next biological control agent for coconut rhinoceros beetle in the Pacific

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A new haplotype (CRB Clade I) of coconut rhinoceros beetle, *Oryctes rhinoceros,* is invading the Pacific, threatening coconut and oil palm production. Historically, coconut rhinoceros beetle has been controlled successfully by its co-evolved pathogen, Oryctes rhinoceros nudivirus (OrNV), which was introduced to the Pacific in the late 1960s. However, these new invaders are resistant to the original biocontrol strain of OrNV and are causing intense damage to coconut palms in the affected countries. Screening new strains of OrNV against invasive CRB populations is a first step to finding a solution for this serious pest. Here we present results from bioassays conducted in the Solomon Islands and Papua New Guinea using two new OrNV strains: V23B and Dug42. Adult CRB were dosed orally with droplets of OrNV solution diluted with sugar water then kept individually for up to 6 weeks. Beetles in the control group were fed droplets of sugar water. All beetles were fed pieces of sugar cane and checked regularly, at least every 2-3 days. Deaths were recorded as they occurred and dead beetles dissected to collect gut samples. At the end of each bioassay, all surviving beetles were killed and dissected to collect gut samples. All gut samples were preserved for PCR analysis and histology to determine beetle haplotype and presence/absence of OrNV infection. Strain V23B showed strong virulence (50% mortality) against CRB from Guadalcanal but did not have the same impact on CRB from Port Moresby (0% mortality). Strain Dug42 showed some virulence (37% mortality) against CRB from Guadacanal and is being tested against CRB from Port Moresby. Implications from these bioassay results for biological control efforts against new CRB invasions are discussed.

CRB arrival and spread on the Papua New Guinea mainland in Madang and Morobe provinces

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Despite the presence of *Oryctes rhinoceros* (coconut rhinoceros beetle; CRB) in the New Guinea Islands, Papua New Guinea (PNG) since the 1940s, the mainland of PNG remained free from the pest until recently. While CRB was reported from Port Moresby in 2009, the first report of CRB from the Northern provinces was recorded from the Markham valley in 2018. In September 2019, CRB was reported from coconut groves at Kananam Village on the northern coast of Madang Province. Haplotype analysis showed that the earliest records were CRB-S (clade II), similar to beetles found in the New Guinea Islands. CRB-S has now been found from Madang to Malala along the coast. More recently, CRB-G (clade 1b) has also been encountered in populations around Kananam village. A complicating factor of the outbreak is the presence of large numbers of dead, standing, coconut palm trunks, resulting from BCS (Bogia coconut syndrome), which act as breeding sites for the developing larvae. While the impact of beetle feeding can be observed in some pockets, damage is less than expected. OrNV is naturally widespread among CRB-S in the zone which may be contributing to limit the impact of the pest.

Management Options for controlling the outbreak of Coconut Rhinoceros Beetle Guam Biotype in Kananam village, Madang Province, Papua New Guinea

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Coconut is the fourth most significant agriculture cash crop in terms of its economic importance to Papua New Guinea (PNG). According to the recent census figures, 35% of total households in PNG or an estimated 2.6 million people are currently engaged in coconut activities for income generation and as food to sustain their livelihoods. On average, the coconut industry contributes over K126.5 million per year to the PNG economy through the export revenue it generates. Coconut Rhinoceros Beetle Guam biotype (CRB-G) was first reported in PNG in 2009. A delimiting survey conducted by Kokonas Indastri Koporesen (KIK) and other partner organizations (NAQIA and PNG OPRA) in August 2019 confirmed CRB-G to be present in National Capital District (NCD), parts of Central and Gulf provinces. Apart from NCD, Central and Gulf provinces, the rest of PNG was still free of CRB-G. However, in November 2019, CRB-G was detected and identified to be present in Kananam village, Madang Province. The infestation site is situated approximately 17 km away from the Coconut Research Station. Hence, the management approach taken was to carry out sanitation in the infested area. The objective of the approach was to completely destroy all breeding sites, reservoirs, and infested host plants of CRB, and identify, record and destroy all CRB samples collected. The approach also aims to minimize CRB-G population below the economic threshold and prevent further spread of the pest. The total infested area is about 400 hectares. The sanitation took a period of 13 weeks, in which a total of 6528 breeding sites were destroyed, with 24,051 CRB samples collected, recorded and destroyed. During the sanitation exercise, it was observed that CRB favors rotten standing coconut poles and stumps more than coconut logs lying on the ground. The other rotten logs (both standing and lying on the ground) were not so favored by CRB. It was also noted that CRB adults also favors the rotten palm *Hydriastele costata* more than rotten coconut logs, especially the ones lying on the ground. Based on the results of the sanitation, it is concluded that the intervention was effective in reducing the buildup of beetle population and minimized the further spread for about 6 months. Furthermore, from this exercise, knowledge of highly favored hosts and abundance of beetle population in different hosts provides direction for appropriate and adequate management strategies to be applied in an area infested by CRB-G.

The invasion of coconut rhinoceros beetle, *Oryctes rhinoceros* (L) Scarabaeidae: Dynastinae, in Solomon Islands

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The coconut rhinoceros beetle (CRB) (*Oryctes rhinoceros*) was accidentally introduced into Samoa with planting material more than a century ago. During the last century, CRB has spread to several other Pacific and Indian Ocean islands and is considered to be a major pest of coconut and other palms. In 2015 CRB was discovered in a small outbreak in Honiara, Guadalcanal, Solomon Islands. This was the first record of CRB from Solomon Islands. Following delimitation surveys and a public awareness programme CRB presence was confirmed beyond Honiara. Later in 2015, CRB was also reported from the Shortland Islands in the Western Province with other islands surveyed from 2015 to 2020. Where CRB presence was confirmed, beetles were collected and analysed for haplotype and presence of the classical biological control agent, Oryctes rhinoceros nudivirus (OrNV) with a distribution map and timeline of invasion subsequently developed. The initial populations belonged to two distinct variants: CRB-G (clade IA) in Honiara and CRB-S (clade II) in Shortland Islands; no OrNV was originally detected in either population. Despite control efforts, by 2020 CRB-G had spread to islands in eight provinces and CRB-S had spread to islands in seven provinces. Additionally, CRB-S and CRB-G have been found to co-occur on several islands. In 2019, OrNV was detected from field collected CRB from Guadalcanal and has since spread to Malaita Island. In both cases the virus was detected where CRB-G and CRB-S co-occur. The two haplotypes appear to have spread following patterns of air and sea movement between the point of origin (CRB-G from Honiara and CRB-S from the Shortland Islands) to other islands/provinces. The monitoring of CRB dispersal, coupled with local observations during field surveys, has allowed tracking of invasion pathways and allowed biosecurity measures and pest management recommendations to be tailored to specific outbreaks.

Effect of chilling temperature on survival and post-diapause development of Korean population of *Lymantria dispar asiatica* (Lepidoptera: Erebidae) eggs

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Due to the economic and ecological impact of European subspecies *Lymantria dispar* L. in the invaded area, such as in North America, *L. dispar asiatica* is also considered an important quarantine pest in non-native regions. *L. d. asiatica* is also considered an important quarantine pest in non-native regions because the flight ability of females and the broad host range of Asian strains may pose a serious threat to the ecosystem of such areas and result in costly management programs. To prevent the introduction and local establishment of *L. d. asiatica* in the port area, North American countries have demanded a certificate of inspection of freedom for vessels passing through regions where the Asian strains are present. The winter chilling temperature could be a key factor in determining the initial population size and occurrence time of larvae for the year in terms of an outbreak. The response of eggs relative to the chilling temperature would provide foreknowledge for the determination of the potential geographic range of *L. d. asiatica*. Thus, in this study, we examined the effect of chilling temperatures (−12, −6, 0, 6, and 12°C) during the diapause phase on the survival and post-diapause development of *L. d. asiatica* eggs, collected before winter, in order to characterize their thermal response. The eggs hatched in all the treatments, indicating that all the examined conditions could partly or sufficiently satisfy the thermal requirement for eggs to enter post-diapause development. However, exposure to chilling temperatures significantly affected both the survival and development times of overwintering eggs in a given temperature range. The survival rates declined at −12°C, and the development rates accelerated as the chilling temperature increased. This information could offer clues for the assessment of the outbreak potential in native regions and the possibility of range expansion in non-native regions through the consideration of winter conditions that favor *L. d. asiatica* egg hatching and their subsequent development.

A comparative analysis of monitoring methods for Rapid ‘Ōhi‘a Death in Hawai‘i: high-resolution satellite imagery and digital mobile sketch mapping surveys

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Dramatic and widespread mortality of ‘ōhi‘a lehua (*Metrosideros polymorpha*) caused by the fungal pathogen *Ceratocystis lukuohia*, commonly referred to as Rapid ‘Ōhi‘a Death (ROD), has resulted in increased monitoring via remote sensing of native forests on the islands of Kaua‘i and Hawai‘i. High resolution (< 0.5m) satellite imagery, obtained with assistance from federal partners and funding from the National Geospatial Agency and National Reconnaissance Office, has been used to expand monitoring areas and complement aerial imagery to identify new outbreaks. We present a visualization and quantification of active (red crown) ROD mortality progression identified from high-resolution satellite imagery across 304,817 hectares on East Hawai‘i from 2013 to 2021. Beginning in 2013, satellite-identified new mortality occurred within 1,630 hectares. The affected area increased annually, eventually peaking in 2017 at 44,078 hectares. Following 2017, the annual affected area has declined, though limited cloud-free satellite imagery in 2018 may mask a peak in that year. Affected active ROD mortality reached a low in 2021 at 12,878 hectares. Although available annual satellite imagery coverage is not consistent, these data are useful for viewing overall forest mortality trends and can be compared to weather events and other disturbances to examine mortality patterns. Satellite-identified active mortality was compared with existing helicopter-based digital mobile sketch mapping (DMSM) surveys completed biannually (2016 to 2021). Areas experiencing a high incidence of active mortality were largely detected by both methods, though there were significant differences in outlying areas. Approximately 45.3% of DMSM suspect areas were also identified by satellite surveys, while 24.9% of satellite suspect areas were also identified in the DMSM dataset. While imagery availability and cloud coverage are ongoing limitations, high-resolution satellite imagery is a cost-effective way to supplement DMSM surveys and is valuable for ongoing ROD monitoring efforts. We are currently developing an automated identification methodology to reduce strain on individual analysts and expedite the identification process. The implementation of an automated system for suspect identification will allow new satellite imagery to be immediately processed and updates of mortality outbreaks be promptly available.

The status of non-native species in the iconic Jellyfish Lake, Palau: Are we doing enough?

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Ongeim’l Tketau (also known as Jellyfish Lake) is a stratified marine lake found in Palau’s “Rock Islands Southern Lagoon” World Heritage Site. The marine lake has limited connection to the surrounding ocean with its native diversity evolving over millennia of isolation. With its normal population of millions of endemic Golden jellyfish (*Mastigias papua etpisoni*), the lake is an iconic tourism site generating (pre-covid) millions of dollars yearly from visitor permit fees. The influx of visitors (up to 50,000 per year) over the last decade has also seen the introduction of non-native species into the lake, with tourists believed to be the primary vector. To date, seven known non-native species have been introduced. These are, with date of discovery, an orange sponge (*Haliclona* sp. - 2001), an invasive brown sea anemone (*Exaiptasia diaphana* with its three endosymbionts - 2003), and a recently discovered stinging colonial (SC) polyp (*Linuche* sp. - 2014) with an unidentified endosymbiont. Four quantitative surveys of *E. diaphana* occurrence (2009–2012) showed a weak correlation between *E. diaphana* and other native species; however, its abundance was also decreasing by 2012. Both non-native cnidarian species with endosymbionts appear to “bleach” (lose endosymbionts) at high water temperatures. Over the last six years lake temperatures (0–12m depth) have generally been warmer than average (above 31.15°C) and these non-native cnidarians have bleached and had reduced abundance. Introduced species threaten the economic benefits of the lake for tourism. Though we have not observed any adult *Linuche* medusae in the lake, there is still a potential for the SC polyp to impact the tourist experience through its stinging *Linuche* medusae, albeit the potential may be miniscule considering the environmental barriers the medusa must overcome. Other introductions could threaten to disrupt the Golden jellyfish polyp-medusae life cycle, and subsequently their population. While non-native introductions are the greatest threat to the Golden jellyfish population, efforts to address this risk (an awareness campaign 13 years ago, a rinse station) have been minimal. There is only one entry point into the lake, so effective management of tourist numbers and their behavior in the lake is feasible. There is a definite need for awareness by tour guides and educational briefings to reduce risks of introductions. Effective long-term monitoring and research on non-native species in Jellyfish Lake is needed to detect introductions quickly and address prevention methods and effective management of tourism in the lake.

The need for a coordinated international development effort to improve plant protection and strengthen resilience in the Pacific

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Pacific Island Countries and Territories (PICTs), face common challenges in plant protection due to their relatively fragile environments and susceptibility to invasion by exotic pests, diseases, and weeds. The introduction and spread of weeds, pests and diseases is having a significant impact on the ecosystem, economy, food security, and human health of these islands. Despite successful past and ongoing efforts led by Aotearoa New Zealand, and other international and local scientists and practitioners, to manage and control pests of economic importance within PICTs, the issue remains a significant challenge. Many government ministries and departments, crown enterprises, universities and other agencies in New Zealand contribute to the development effort in PICTs. The current harm and risks arising from invasive species are growing due to increased trade and travel and the impacts of climate change. With the limited resources, including people, in PICTs, a coordinated international development effort is necessary to address these challenges in the Pacific region. Greater collaboration from Aotearoa New Zealand is critical to ensure our work is synchronised and streamlined. Donors must also provide consistency and continuity in funding to sustain these efforts. By working together, New Zealand partners and donors can support Pacific nations to better manage and control existing and emerging pests, to strengthen resilience, and safeguard their environment, biodiversity, food security, and human health.

Tracking biosecurity threats with publicly available photos: A case of Coconut Rhinoceros Beetle

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The coconut rhinoceros beetle, *Oryctes rhinoceros*, (CRB) is a major pest of coconut and oil palms that causes significant damage in the Asia/Pacific region. Traditional methods for surveillance and detection of CRB involve in-person inspections, but these are expensive to carry out and were made impossible by travel restrictions during the COVID-19 pandemic. Here, a method to remotely detect possible CRB incursions and assess damage levels using publicly available photos from the Google search engine is described. The method involves searching for pictures of palms and identifying those with the distinctive V-shaped cuts in fronds characteristic of CRB feeding. The method was validated by examining palm photos from known outbreak areas and those free from the pest. In addition, we found evidence of CRB damage in the previously unreported sites of Timor Leste and Mexico; and possible evidence of CRB in the Solomon Islands even before it was first reported in 2015. Potential limitations and broader applications of this approach are also discussed.

Indiscriminate disregard of spatial and temporal ecology lead to spurious predictions of species' invasion success

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Understanding the determinants of invading alien species establishment success is pivotal in predicting incursions and developing effective early response strategies. Propagule pressure has been implicated as the primary factor determining establishment success and even as the "null model of biological invasions". However, this premise has been highly contentious because of its deceptively simple concept (i.e., introducing more individuals increases establishment success) that dismisses other factors, such as the environment. Moreover, individuals' dynamic behavioural responses to their spatial and temporal environments are complex factors rarely considered. Using virToad — a hyper-realistic life-history simulator of the cane toad (*Rhinella marina*) for predicting spatio-temporal population dynamics and management outcomes (Pili et al. 2022), we simulated 18,000 incursion scenarios to understand the interacting effects of (i) propagule size (2–20 individuals), (ii) propagule number (1–3 introductions), (iii) spatial environment (different landscapes; airport vs. wharf), and (iv) temporal environment (changing daily rainfall; introduced between day 1–730) on establishment success. Running-window binomial regression models revealed that the importance of propagule size would significantly and substantially vary in different landscapes and fluctuate with changing environmental conditions — signifying changes in regimes and interaction among factors. Generally, propagule size swamps the effects of the spatial and temporal environment when ≥ 12 individuals were introduced (≥75% probability of establishment); however, this was undermined when individuals were introduced in separate events (> 1 propagule number). But In realistic scenarios (at most, propagule size = 3; propagule number = 3 per year), disregarding the effects of temporal and spatial changes in the environment led to false predictions of establishment success. Given the recent prominence of accidental transport and introduction of alien species — characterised by few individuals and infrequent introduction events, accounting for spatiotemporal differences and changes in the environment is requisite in assessing establishment success and predicting incursions for decision support.

Remote sensing to support surveillance, response and eradication

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Introduction: When preparing for and responding to incursions by organisms such as insects, it would be useful to know the distributions of their host plants to inform surveillance and management. We are investigating the use of satellite data for identifying the spatial distributions of several plant species that are hosts for some important biosecurity hazards. The main challenge is to discriminate plants with similar seasonal and trait characteristics at the landscape scale. *Methods*: This study developed a new method for differentiating maize−a plant threatened by numerous biosecurity hazards−from other New Zealand crops based on the Sentinel−2 satellite time series (TSs) method. Combining phenological information from multiple temporal, spatial and spectral bands and the vegetation indexes contained in the open and free images acquired by Sentinel−2 enables seasonal trend analysis, which is extremely useful for crop type mapping. However, a lack of large-scale training datasets hampers development and testing of advanced methods for agricultural phenology-based classifications. To improve the training data, we used the TimeSen2Crop dataset captured in Europe. To capture crop phenology, Sentinel−2 imagery dating from 1st of September to 31st of April for five growing seasons was used. Surface reflectance data was filtered to be cloud−and shadow−free. Different adjustments to the temporal component of the training data were tested to match Southern Hemisphere seasons. Phenological curves were extracted from time series by fitting harmonic regressions, which decomposes signals over time into their constituent frequencies (i.e. cosines and sines). Subsequently, various Machine Learning (ML) models were compared, and Random Forest (RF) was selected for optimal accuracy over a number of seasons and regions. Finally, and for the latest growing season, an opportunity to further improve classification accuracy was explored by incorporating New Zealand data from previous seasons. After semi-automatic maize paddocks vectorization from aerial imagery, matching Sentinel−2 images were transformed into a training data and used for augmentation of the main training dataset. *Results and Discussion*: The Green Chlorophyll Vegetation Index computed as harmonic regression accurately depicts phenological curves. The overall accuracy of classifying maize crop areas reached about 92% based on c. 1400 field samples. The results demonstrate that: (1) landuse and crop type classification based on Sentinel−2 time series can be highly effective for identifying crop phenological information to help optimise surveillance and incursion responses over large areas; (2) surveillance resources can be allocated to the most appropriate locations after suitable sampling sites have been identified.

Natural enemies of the invasive myrtle rust in Aotearoa New Zealand

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Myrtle rust (*Austropuccinia psidii*) was first detected in New Zealand in 2017. Since then, at least two natural enemies have been found to occur on myrtle rust infestations on native plants. Both of these may have the potential to help regulate myrtle rust. One of these natural enemies belongs to the gall midge family (Cecidomyiidae), is new to science, and recently formally described as *Mycodiplosis constricta*. The other natural enemy, a fungal mycoparasite (*Sphaerellopsis macroconidialis*), has been found for the first time in New Zealand, and is the first time globally it has been found on myrtle rust. We are investigating the spatial and temporal occurrence of these two natural enemies by hand searching myrtle rust infested plants as part of a larger research program. To date we have found the presence of these natural enemies in the Auckland region, Central North Island, and Taranaki region during and following the peak infection period between January and April. We are also investigating the host range of these gall midge flies in New Zealand by inspecting historic rust specimens in the New Zealand Fungarium (Manaaki Whenua Landcare Research) and National Forest Mycological Herbarium (Scion) collections for *Mycodiplosis* spp. larvae. This information is critical to inform the potential of both species as biological control agents and to understand indirect impacts of the myrtle rust invasion on New Zealand ecosystems.

Emerging biosecurity risks in South Korea: Higher occupancy and detection probability of invasive turtles in the Korean environment indicate potential conservation threat to native species

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A biosecurity threat is anything that may pose challenges to the economy, environment, or community through altering inter-species interactions and intensifying impacts of diseases and contaminants. Among many other means, invasive species may create multidimensional biosecurity threats by changing native ecosystems and their functioning. However, it is believed that the pet trade plays an important role in the introduction and spread of invasive species beyond their native range. Being one of the global pet trade epicenters, South Korea has always been considered vulnerable. For instance, at least 13 alien turtles, six of which have been announced as ‘ecosystem disturbance species’ based on their potential biosecurity threats, are already reported from Korean nature. Still, we know very little about their occupancy and detection probability, and subsequent impacts on native turtles and ecosystems. Thus, we explored reservoirs in Seoul, Daejeon, Gwangju, and Jeju Island in South Korea to estimate the relative occupancy and detection probability of invasive and native turtles. We checked 30,917 reservoirs and selected 120 (30 from each study site), half of which were from forest and half from the urban category (based on turtle’s core water habitat), for our study. We surveyed reservoirs between April and June 2021, from 8 am to 6 pm, and collected data on turtle species, observation time, air temperature, water temperature, humidity, open water area, etc. The study followed the visual survey method. We found a higher occupancy and detection probability of invasive species than the native species in both forest and urban reservoirs. The detection probability of invasive and native species was increased with larger open water areas and higher water temperatures, whereas it decreased with the time of the day. However, invasive species seemed to be adapted to the low water temperature and shifted activity to the whole day. In contrast, the native species showed low detection probability during the afternoon and evening and in the low water temperature. Furthermore, the detection probability of invasive species was consistently increased with the dates, while it decreased for native species. Our study indicated a higher relative dominance of invasive turtles in the Korean environment and a forthcoming potential conservation threat to native species. The results also suggested negative impacts on native ecosystems and their functioning by possible alteration of species composition. Thus, we urge immediate purification

of existing policies and formulation and implementation of stricter control and eradication measures for invasive species.

Biosecurity risk calculator: Tracking biosecurity risk along the supply chain for continuous assurance

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Phytosanitary requirements for imports and exports are typically very prescriptive, with a set of entry requirements needing to be met before trade can proceed. However, this can result in overtreatment, does not reward good industry practice, and may be costly for regulators to oversee. Therefore, there is a growing interest in establishing a more risk-based approach that relies upon continuous assurance, where digital technologies are used to automate and streamline compliance. To support this transition, we have prototyped a 'Biosecurity Risk Calculator' that uses data-driven analytics to track infestation risk in consignments as they pass through production and the supply chain. Our initial focus was on the effect of cold storage on infestation rate in horticultural produce as cold temperatures are used as an end point treatment as well as a storage option along most of the supply chain. We quantified the mortality effect of exposure to variable temperatures across the supply chain and show that in some cases residual risks can be very low without the need for additional treatment. The calculator could be used by regulators to make decisions based on real-time risk analysis, thereby making more efficient use of limited inspection resources. It can also assist commercial entities maintain quality and reduce costs related to pest detection and management. Ultimately, the Biosecurity Risk Calculators could be developed for a wide variety of uses beyond fresh produce, such as to calculate the biosecurity risks associated with shipping vessels, grains, and seeds for planting.

Euwallacea fornicatus in Western Australia: host range, distribution and response activities

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Polyphagous shot-hole borer (PSHB, *Euwallacea fornicatus*) was first detected in WA in August 2021, in a box elder maple (*Acer negundo*) on a residential property in Perth, Western Australia. In association with its symbiotic ambrosia clade *Fusarium* fungus it is one of the most significant global threats to urban forests, impacting amenity, horticulture and plantation trees in California, South Africa, Israel and Vietnam. The symbiotic complex of the PSHB haplotype 38 (H38) and its symbiotic fungus *Fusarium* sp. [AF-18] in Western Australia is different to other invasive populations of PSHB, which may influence host use and impacts. To date PSHB has only been detected in a restricted distribution around the Perth metropolitan area. A quarantine area and movement restrictions are in place to restrict further spread. PSHB impacts in WA range from localised shot holes with no lesions and little impact on tree health in non-reproductive hosts, to limb failure and tree death in reproductive hosts that PSHB can breed in. The host list is updated regularly on the DPIRD PSHB website at agric.wa.gov.au/borer. More than 85 species from 64 genera have been found to be hosts of PSHB in WA, at least 20 of which are new global host records. At least 45 of these hosts are reproductive in WA. The most significant host in WA has been the box elder maple, *Acer negundo,* with robinia/black locust (*Robinia pseudoacacia*), poinciana (*Delonix regia*), Moreton Bay and Port Jackson figs (*Ficus macrophylla* and *F. rubiginosa*) and coral trees (primarily *Erythrina* x *sykesii*) also impacted significantly. This poster will summarise DPIRD response activities, including management of infested trees, and what we know about PSHB in WA.

A collaborative study to assess the biogeography of *Lophodermium* species on non-natives pines in the Southern Hemisphere

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An increase in the prevalence of pine needle pathogens globally has prompted research focusing on the identification and taxonomy of these species to help mitigate future outbreaks. Approximately 38 species of *Lophodermium* have been described infecting the needles and cones of pine trees. Apart from *L. seditiosum*, which is considered the primary pathogen of Lophodermium needle cast, the majority of these species are considered endophytes of healthy trees. However, identification of these species has historically been based on the differences observed in a limited number of morphological characters. When re-evaluated with DNA sequence data, the presence of cryptic taxa were revealed. *Pinus* spp. were established as non-natives in the Southern Hemisphere in the late 19th century and concurrent with this, several pathogens were accidentally introduced. Thus, *L. seditiosum* and several endophytic species have been reported from countries in the Southern Hemisphere, but only a few reports have been verified using DNA sequence data. This study aimed to collect and identify a large collection of *Lophodermium* isolates from *Pinus* spp. that were introduced into Colombia, Chile, South Africa, Australia and New Zealand, using DNA sequence and morphological analyses. A multi-locus phylogenetic analysis revealed the presence of five *Lophodermium* taxa. A species belonging to the *L. conigenum-australe* complex was collected from all countries, except Chile. The species, *L. indianum* and *L. molitoris*, were collected only in Colombia and New Zealand, respectively. Two lineages of *L. pinastri* emerged from the isolates collected in Chile, New Zealand and Australia. Most importantly, the pathogenic *L. seditiosum* was not identified amongst any of the collections obtained from the countries considered. Morphological analyses revealed high morphological variation in isolates from the *L. conigenum-australe* complex and overlapping features for the two distinct lineages of *L. pinastri*. Apart from the *L. conigenum-australe* complex, there was little overlap in the *Lophodermium* spp. present in each of the countries, suggesting that several independent introductions of these species has occurred into the Southern Hemisphere countries over time. This emphasizes the need not only to identify forest pathogens, but also to share the responsibility of biosecurity across borders to protect planted forests.

Multiplex PCR improves efficiency for determining coconut rhinoceros beetle haplotype and presence of its biocontrol virus

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The re-emergence of coconut rhinoceros beetle (CRB; *Oryctes rhinoceros*) as an invasive pest in the Pacific was unexpected after more than 30 years of successful biological control through the introduction of the biocontrol virus Oryctes rhinoceros nudivirus (OrNV) in the 1970s. OrNV was originally isolated from the beetle's native range in Malaysia. An explanation for the recent wave of Pacific invasions by CRB emerged on discovering that most of the invasive populations belonged to a new haplotype grouping; CRB-G (Clade I). CRB-G is less affected by the commonly used biocontrol OrNV isolates. Hence, determining CRB haplotype and presence of its biocontrol virus are key steps that assist timely management decisions pertaining to outbreaks of this major palm tree pest. Until recently, three separate PCR reactions (using two different DNA dilutions), followed by a restriction digest, have been used to distinguish between invasive CRB populations and to determine presence of OrNV. This study describes a multiplex PCR targeting CRB and OrNV DNA in the same PCR, followed by a restriction digest. Method validation using gut samples collected from more than 250 CRB, representing OrNV infected and uninfected samples and key haplotypes, showed multiplex PCR virus results closely correlated with single PCR-based results. Weak positive virus bands in the multiplex PCR were followed up with a single PCR for OrNV using more dilute DNA, to rule out sample cross contamination and confirm establishment of infection. Multiplex PCR followed by restriction digest was effective at distinguishing the invasive beetle haplotype and determining the presence of OrNV. The multiplex PCR method has significantly reduced processing time and laboratory resources. We will present details of our progress and discuss the opportunity that multiplex assays present within the context of efforts to improve CRB management.

The known unknowns in international border interceptions of insects

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Propagule pressure (the number of individuals arriving in new areas) is one of the key drivers of establishment success of invasive species, including insects. However, border interception data, which have the potential to act as a proxy for true arrival rates of insects into a country, are seldom used in this way. In large part this is due to the limited amount of interception data in many countries, difficulties accessing such data, and when these data are available, difficulties in addressing their biases caused by variation in interception probability for different taxa due to policy changes and operational influences. The type of interception data required to fully quantify arrival probabilities are rarely available. To improve the use of interception data as a proxy for propagule pressure, we investigated the relative number of established species and intercepted species by taxonomic group and by biological characteristics, using several international datasets. We identified higher ratios of intercepted to established species for plant feeding insect groups relative to ratios for non-plant feeding groups, even in countries with broad-spectrum border inspection strategies. This could be due to higher interception probabilities for plant feeding insect groups, caused by phytosanitary preferences in inspections, or lower establishment probabilities for plant feeding insect groups. To address this variation and improve establishment predictions based on interception frequency, we developed a methodology to account for the ratio of established species to intercepted species per taxonomic grouping.

Performance of *Metarhizium majus* applied in artificial breeding sites to control larvae of coconut rhinoceros beetle (CRB) in Solomon Islands

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The fungi *Metarhizium* spp. are well known as pathogens of CRB (*Oryctes rhinoceros*) but natural infection in the field is relatively low. This has led several affected countries to adopt the incorporation of *Metarhizium* spores into artificial breeding sites (ABS) as a release strategy. ABS are piles of decomposing palm logs and associated plant waste created artificially in the field and treated with the fungus. Female CRB are attracted to the decomposing material to lay eggs with commercial aggregation pheromone. CRB larvae, pupae and emerging adults may get infected and die. Although this strategy has been used for many years, the real impact on CRB population reduction and fungus dispersal has not been determined. In this work, a strain of *Metarhizium majus* was isolated from one adult CRB cadaver found in an oil palm plantation at Tetere, Guadalcanal. This strain was tested by applying nil, low (100g/ABS), moderate (200g/ABS) and high (400g/ABS) doses of sporulated rice to four 2x2m ABS arranged in a grid. 50 CRB larvae were added to each ABS. This design was repeated at three oil palm blocks: Tetere, Mberande and Nekama. CRB numbers inside the ABS were assessed every 2 months for 6 months. Before applying the fungus, soil samples were taken at 10m, 50m and 100m from each ABS. The soil samples were 'baited' with CRB larvae to detect *Metarhizium* sp. Soil baiting was repeated at 2-monthly intervals. Between 5 and 20 CRB larvae/ABS were found during the 2-monthly evaluations. *M. majus* was detected in Tetere soil samples from all distances/treatments before and after the inoculation of the ABS, suggesting the fungus was already established in this block after a *M. majus*-based commercial product was applied in 2017. In contrast, *Metarhizium* sp. was not detected in soil samples collected from Mberande and Nekama before ABS inoculation, but the fungus was detected later in soil samples from all treatments, even at 100m away from the site. This suggests that *M. majus* can spread up to 100m from treated ABS and persist for at least 4 months. *M. majus* may contribute to CRB control if high levels of inoculum can persist in breeding sites under field conditions.

CRISPR/Cas12a technology combined with RPA for rapid and sensitive detection of *Claviceps purpurea*

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Eating cereals contaminated by ergot sclerotium could lead to ergotism, also called “Saint Anthony’s fire”, which results in burning sensations in the arms and legs. The most prominent member of the causal agents is *Claviceps purpurea,* which could infect multiple hosts including wheat, barley, rye, Gramineae weeds, etc. Recently, the notorious disease has emerged or re-emerged in several places all over the world. Therefore, accurate and sensitive detection of *C. purpurea* from wheat seeds is required for food safety and control of the pathogen spread. We developed a rapid, highly sensitive and portable *C. purpurea* detection system based on recombinase polymerase amplification (RPA) and CRISPR/Cas12a technologies. A highly efficient and specific CRISPR RNA (crRNA) was designed for a core ergot alkaloid biosynthetic gene. With the extracted DNA, the whole reaction could be completed within 1 h under a constant temperature (37℃). The assay showed no cross-reactivity with other cereal pathogens including *Fusarium* spp., *Alternaria* spp. and *Puccinia* spp. In addition, our immunochromatographic stripe detection system had a high sensitivity and showed positive reactions with at least ten genomic copies in a single reaction. The detection results for 10 field samples using the RPA-CRISPR/Cas12a system was consistent with those using qPCR and PCR. Taken together, the RPA-CRISPR/Cas12a system is an efficient and powerful tool for on-site detection, contributing to rapid and sensitive diagnosis of *C. purpurea*.

Using fluorescence in situ hybridisation to detect Oryctes rhinoceros nudivirus infection in coconut rhinoceros beetle (*Oryctes rhinoceros*)

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Coconut rhinoceros beetle (CRB; *Oryctes rhinoceros*) has been an invasive pest species of coconut and oil palm across the Asia/Pacific region since the early 20th Century. These invading CRB populations were successfully limited by a virus, Oryctes rhinoceros nudivirus (OrNV), in what is now a landmark case for classical biocontrol. The recent re-emergence of CRB as an invasive pest and discovery of the virus-tolerant CRB haplotype (CRB-G) has led to the need to explore additional options for biocontrol, including unique OrNV isolates capable of establishing and maintaining infection in CRB-G. CRB infection status is currently determined using a combination of PCR assays and histology from midgut tissue. While the combination of the two methods provides a conclusive answer to whether an individual is infected, there are limitations to both. PCR provides limited information on the viral locality and the severity of infection, while positive histology assessments can only be made in mid to late-stage infection. To expand our toolkit for assessing infection status and improve our knowledge of infection dynamics, we recently developed an assay using fluorescence in situ hybridisation (FISH). FISH allows us to directly detect OrNV at earlier stages of infection and better understand the extent of infection. By improving our understanding of infection progression, we can better evaluate distinct OrNV isolates in the context of finding an isolate capable of causing disease in CRB-G.

Attention Learning strategy of CLIP guided by Counterfactual causality applied to insect identification

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Biological invasion is one of the important global problems of ecology and environment that all countries in the world are facing, among which the invasion of pests — foreign invasive insects as a major part — is the focus of prevention and control. Pest invasion threatens the ecological security and biodiversity of a country or region as well as the development of agriculture and forestry. To intercept biological invasion, improving the quarantine quality of agriculture and forestry imports and exports is essential. With the continuing increase in global trade transactions, manual quarantine used in most countries now has failed to meet the new requirements of efficient and intelligent processes. We apply deep learning to the task of insect identification. Considering the problems of current research on simplex models, unmatched datasets, and poor generalization, we propose for the first time to apply Transferable Visual Models Learned from Natural Language Supervision (CLIP) to insect identification. Based on CLIP with its excellent capability of feature extraction and working efficiency, we propose an attention learning strategy of CLIP guided by Counterfactual causality (CAL-CLIP), which incorporates a new supervisory signal defined by counterfactual causality to optimize attention learning. We studied the performance of our approach through experiments on the challenging insect benchmark dataset IP102, showing that the Top-1 accuracy of CLIP transfer learning and our proposed model CAL-CLIP reached 54.62% and 71.58%,both of which far exceeded the result of 46.79% of the ResNet50 baseline model, and the accuracy of CAL-CLIP is higher than those of the existing studies. Furthermore, we apply CAL-CLIP on another benchmark dataset D0 that contains insect images of high quality. The experiment shows that over 95% of images can be identified correctly. The results demonstrate that our approach achieves excellent performance on the challenging benchmark dataset IP102, and indicates that dataset D0 is suitable to be regarded as the standard of constructing foreign invasive insect datasets in future practical applications. Our proposed approach provides a feasible solution to rapid and effective intelligent detection of invasive insects.

How do commodity imports and climatic filtering drive compositional changes in non-native insect establishments?

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Non-native insect invasions substantially impact agriculture, human health and biodiversity around the world with considerable economic consequences. It is important to quantify the relative importance of commodity imports vs climatic matching as drivers of global variation in invasions. To evaluate their relative contributions to explaining patterns of non-native insect species establishments in different world regions, we conducted direct ordination analyses to quantify temporal changes in family-level composition of non-native species and also the changes in native range composition of non-native species. We analysed data for the five most data-rich world regions, i.e., North America, Europe , Australia, Japan and New Zealand. Establishment records were grouped into 20-year intervals to quantify dynamical compositional changes. Using these ordination analyses, we quantified the relative importance of imports from various world regions vs. similarities in climatic conditions with our five regions. When characterising composition at the family level, we found that there were significant temporal changes in composition especially in Australia, Japan and New Zealand. In these regions, composition was dominated by scale insects prior to 1900 but then shifted to a more diverse set of species. Neither North America nor Europe exhibited drastic compositional changes during the entire 140 year period. Changes in the composition of established species were not fully explained by either imports (12.3%) or climatic factors (10.6%). In contrast, temporal variation in the native range of invading species was low during the 140 year period. Established species in New Zealand were predominantly from Australasia, while established insects in North America originated predominantly from Europe and those in Europe may have had association with Nearctic, Indomalaya, and Afrotropic regions. Climatic factors in the destination regions had the greatest effect (55.1%) on the total variation in the native range of established species but this effect cannot be segregated from that of imports. Based on these results, we conclude that climatic filtering acts as a dominant driver of establishment success for non-native insects in all five regions while imports still have minor but substantial effects. We will discuss implication of these results to biosecurity policies.

Validation for PCR Detection of *Sorghum halepense* - Ring Test method

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Johnsongrass (*Sorghum halepense*), an Invasive weed that poses a challenge for traditional morphology-based identification due to its multiple geographic types. Developing a novel diagnostic technique is essential to address this issue. In this study, we validated three existing molecular diagnostic assays for *Sorghum halepense* using the Ring Test method and experimental material of Sorghum seeds. The assays included: (I) conversion of the RAPD marker to the SCAR marker, (II) RFLP digestion reaction based on rDNA-ITS (Internal Transcribed Spacer) sequence PCR product, and (III) Real-time PCR method based on SNP technology. Our analysis using kappa statistics revealed that method III had the highest true positive rate, followed by method I and method II, while method I had the highest true negative rate, followed by method II and method III.

New derivatives of tenvermectins against Pine Wood Nematode, *Bursaphelenchus xylophilus* as potential nematicide

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The pine wood nematode (PWN), *Bursaphelenchus xylophilus*, causes significant damage to pine trees in China since from 1982. And now it is still the most serious forest disaster in China, which posed a serious threat to China's ecological safety. Because this disease involves very complicated interactions between a pathogenic nematode, its vector beetle, host pine species, and fungi in dead hosts. There are few effective control measures available for PWD. The injection of chemical agent exerted a preventative effect against the pine wilt disease, which brings about a direct effect on the nematode and is environmentally friendly, the authors aimed to develop a novel trunk-injection agent against the disease. Sixteen-membered macrocyclic lactone antibiotics are used extensively in veterinary to control parasitic infections, emamectin benzoate (EB), avermectins(AVMs) and ivermectins have been considered as the typical representatives, and have recently been successfully applied as a trunk injection for preventative control of the pine wilt disease (PWD). In previous papers, the authors reported that many insecticidal 16-membered macrocyclic lactone compounds, were potent nematicidal activity and thus a candidate for the ingredient of a trunk-injection agent. In further work to explore the trunk-injection agent, using domain swapping of avermectin PKS, two hybrid compounds named tenvermectins A and B, which possess partial structural features of avermectin and milbemycin, were obtained from two genetically engineered strains *Streptomyces avermitilis* MHJ1011 and *Streptomyces avermitilis* AVE-H39. TVMs A and B showed enhanced insecticidal activity against *Bursaphelenchus xylophilus*. In order to discover more potent TVM derivatives, microbial transformation of TVM-A and TVM-B by *Mycolicibacterium* sp. Huz021 and *Bacillus subtilis* were investigated and offered us eight metabolites with regioselectivity, especially rare glycosuccinylated products. Their chemical structures were well characterized via HR-ESI-MS and 1D/2D NMR spectra. All these metabolites exhibited good nematicidal activities against *Bursaphelenchus xylophilus*. These results indicated that the metabolites can serve as potential trunk-injection agents for future.

The morphological thermal adaptation of an invasive beetle

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Phenotypic plasticity is observed in many animal species; it is effective for them to cope with many types of environmental threats. The multicolored Asian ladybird *Harmonia axyridis* shows a cuticular pigmentation plasticity that can be rapidly induced by temperature changes, in the form of changeable melanin spot patterns to adjust heat-absorbtion. We also observed *H. axyridis* displays thermal phenotypic plasticity at different developmental stages. It is unknown whether these superficially similar temperature-induced specializations share similar physiological mechanisms. In this study we confirmed the melanin level changes of *H. axyridis* pupa could be induced by temperature, and then screened the efficient time window for thermal sensing of *H. axyridis* pre-pupa; it is suggested that the late stage of pre-pupa (late stage of 4th instar larva) is the critical period to sense thermal signals and adjust its pupal melanin spot area size to adapt to upcoming thermal conditions. The *HaADC*(aspartate decarboxylase) and *Ha-ebony* (NBAD synthase) of the aspartate-β-alanine-NBAD pathway were then proved in regulation of cuticular melanization for pupa through RNA interference experiments; knockdown of these two genes enlarged the melanin spot size. Finally, we designed a random injection of *Ha–ADC* at different pre-pupal stages, to further study the regulation window during this process. Combined with all evidence observed, we suggested the spot size determination can be regulated very close to the time point of pupation, and genes of the aspartate-β-alanine-NBAD pathway play an important role at the molecular level. However, the aspartate-β-alanine pathway controls spot size and number only in the pupae. An upstream regulation analysis revealed the roles of *Hox* genes and elytral veins in pupal and adult spot formation. Both the pupae and the adults exhibited similar morphological responses to temperatures. They occurred in different body parts and were regulated by different pathways. These phenotypic adaptations are indicative of an effective thermoregulatory system in *H. axyridis* and explains how insects contend with certain environmental pressure based on various control mechanisms.

Isolation, identification, and biocontrol potential of *Leuconostoc mesenteroides* WZ-44 from the surface of pear young fruit against *Erwinia amylovora*

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Erwinia amylovora causes fire blight in Rosaceae which is a highly destructive disease, especially to pears and apples, and has spread to more than 60 countries and regions worldwide. In this study, a bacterial strain WZ-44 was isolated from the surface of Korla pear young fruit, which has strong antagonistic activity against *E. amylovora* and *E. pyrifoliae*, and the antibacterial circle diameters were 1.77 cm and 2.47 cm, respectively. WZ-44 also has some inhibition against *Dickeya fangzhongdai, Valsa pyri, Alternaria alternata* and *Colletotrichum gloeosporioides*. Based on the colonial and cellular morphology, physiological and biochemical characteristics, partial nucleotide sequence of 16S rRNA gene, the isolate was identified as *Leuconostoc mesenteroides*. WZ-44 grew between 10°C and 40°C, and its optimum growth temperature was 25-35°C. The sterile filtrate of WZ-44 cultured in MRS liquid medium had strong antagonistic activity against *E. amylovora*, and its antagonistic activity was 3.51 times that of 500 µg/mL zinc thiazole solution and 1.87 times that of 100 µg/mL benziothiazolinone solution respectively. The secondary antibacterial substances of WZ-44 showed strong tolerance against high temperature, protease, acid conditions (pH less than 5.0) and ultraviolet light treatment. When the pH value was more than 6.0, the antagonistic activity of the sterile filtrate was completely lost, but could be entirely restored when the pH value was regulated to 4.0. When *E. amylovora* cells were treated by the sterile filtrate of WZ-44 for more than 5 minutes， the integrity of the cell membrane was damaged, the proteins and nucleic acid began to leak, and most of the cells collapsed. But WZ-44 showed stronger tolerance to the bactericide zhongshengmycin and kasugamycin than the pathogen *E. amylovora*. In addition, the greenhouse experiments showed that when the WZ-44 biocontrol agent was applied twice continuously at 1.0×10^8 cfu/mL, 2.5×10^8 cfu/mL and 5.0×10^8 cfu/mL respectively, the control efficacy against pear fire blight was 68.7%, 78.2% and 84.3%. To sum up, *L. mesenteroides* WZ-44 is a promising biocontrol bacterium for the control of fire blight.

Study on competitive mechanism of *Tetranychus truncatus* replacing *Tetranychus turkestani* from toxicology perspective

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Xinjiang is the largest cotton planting area in China and the world, and *Tetranychus turkestani* is the dominant population of cotton field pests in Xinjiang, which is only reported to occur in Xinjiang in China. *Tetranychus truncatus* is a similar species to *T. turkestani*. The harm, host range, living habits and mating behavior of the two species of spider mites are very similar, and overlap in niche. Before the 21st century, *T. truncatus* was a minor pest of cotton in Xinjiang, which only occurred in a small number of cotton fields. In recent years it has gradually replaced the dominant species, *T. turkestani*. The competitive expansion of *T. truncatus* in cotton fields of Xinjiang relative to *T. turkestani* is a typical biological invasion event. However, in the existing studies the mechanism of *T. truncatus* replacing *T. turkestani* is generally discussed from the perspective of macro-ecology by studying the interference of environmental factors on the reproduction, growth, population diffusion of these two species, as well as interspecific competition and competition for cotton resources. In the study, a unique perspective of toxicology was used to explore the molecular mechanism of *T. truncatus* replacing *T. turkestani*. 16S rRNA and Illumina technique were performed to compare the microbial communities of two harmful mites. The microbial diversity index (Ace index, Chao1 index, Simpson index) of the flora showed that the species diversity and richness of the microbial community of *T. truncatus* were higher than those of *T. turkestani*. *T. truncatus* is abundant in ochrobactrum, which is related to cellulose degradation indicating that *T. truncatus* may prefer crops with high fiber content such as cotton. *T. turkestani* is abundant in agrobacterium, which is related to plant pathogenicity. Enzyme activity tests showed that three detoxification enzymes (P450s, GSTs and CCEs) of *T. truncatus* was significantly higher than that of *T. turkestani*; Three different types of acaricides, spirodiclofen, fenpropathrin and abamectin, were selected to induce mite response, and the activity of the three detoxification enzymes of *T. truncatus* increased significantly more than that of *T. turkestani*. The stronger adaptability of *T. truncatus* to chemical stress is an important reason for its competitive expansion advantage and gradually becoming the dominant population of mite pest. The study provides a unique toxicological perspective for explaining the expansion of *T. truncatus* instead of *T. turkestani* in Xinjiang cotton fields, and also enriches the content of invasive biology research.

Identification and function analysis of olfactory genes from *Bactrocera minax* (Diptera: Tephritidae)

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Bactrocera minax (Diptera: Tephritidae) is an important pest that causes serious damage to citrus plants, which are found in China, northern India, Nepal and Bhutan. The larvae parasitize citrus fruits, pupate in the soil, and are exposed only in the adult stage. Therefore, it is an important measure to trap this citrus fruit fly with the attraction of odour-baited substances. Due to less research on the olfactory recognition mechanism of this insect pest so far, we studied the olfactory system of *B. minax*. A total of 126 chemosensory genes have been identified in the antennal and leg transcriptomes of adults, including 59 odorant receptors (ORs), 16 ionotropic receptors (IRs), 39 odorant binding proteins (OBPs), five chemosensory proteins (CSPs) and three sensory neuron membrane proteins (SNMPs). The expression pattern and function of ORs, a key protein in the recognition of environmental odors, were studied. The results showed that all the *BminORs* were highly expressed in both male and female antennae. Among them, the functions of four ORs (OR3, OR12, OR16, OR24) were studied and had significant electrophysiological responses to volatiles of the citrus host plants, including methyl eugenol, which was attractive to a variety of fruit flies, and linalool, which was attractive to female *B. minax*. These results indicate that these four ORs play an important role in the location host plants and oviposition of *B. minax*. At the same time, we also carried out functional studies on the CSPs of *B. minax*, and found that CSP3 can bind the host volatiles of *B. minax*, among which the binding ability to citral is the strongest, and through behavioral bioassays, we found that citral has a significant attractant effect on female *B .minax*. These results indicate that CSP3 is also involved in the host localization process of female *B .minax*. In conclusion, the identification and functional analysis of olfactory genes of *B. minax* lay a foundation for further research on the encoding mechanism of olfactory sense of the fruit fly, and also provided a new ideas and strategy for the control of the insect pest.

Ovarian development and oogenesis of *Tuta absoluta* (Lepidoptera: Gelechiidae) adults

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Tuta absoluta (Meyrick) is a quarantine invasive pest in the world, which has spread widely from its origin in South America to the main tomato producing areas on all continents of the world, and has become a major threat factor affecting the development of tomato industry. The pest was introduced into Xinjiang in 2017, causing serious losses to local tomato production, and spread to Yunnan, Gansu and other regions in a short time, greatly threatening the healthy development of China's tomato industry and other related industries. A clear understanding of the ovarian development and oogenesis of female adult of this pest is conducive to accurate prediction of the occurrence period and quantity of the pests, so as to determine the appropriate control period. Based on the observation of the reproductive system structure and ovarian development level of the female adults of *T. absoluta*, this study further compared its oogenesis dynamics under the conditions of unmated + water, unmated + 20% glucose solution, mating + water, and mating + 20% glucose solution. The results showed that the reproductive system of female adult mainly includes ovary, ovariole, accessory gland, bursa copulatrix, vaginal diverticulum, spermatotheca and spermathecal gland, etc. Left and right ovaries mostly contain four ovarioles. The progress of ovarian development can be divided into five stages, pre yolk deposition phase (stage I), yolk deposition phase (stage II), egg maturation phase (stage III), peak phase of oviposition (stage IV) and terminal phase of oviposition (stage V). Feeding 20% glucose solution significantly promoted the development of oocytes, and the number of the mature oocytes and total oocytes were significantly higher than those fed on water at 24 h after eclosion. After oosorption of females, the number of various types of oocytes in the ovaries increased significantly when the females supplemented with 20% glucose solution. Mating can significantly promote the oogenesis of female adults. The mating females fed on 20% glucose solution or clean water had significantly higher number of mature oocytes than unmated females 96 h after eclosion. The research results can provide a basis for the field control of this invasive pest.