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Centre for Biological Control

Annual Report 2022

Front cover photograph

Tapiwa Mushore monitoring fruit-piercing moth, *Serrodes partita*, in citrus orchards in the Kat River Valley, Eastern Cape.

Photo: David Taylor

Back cover photograph

Matthew Paper monitoring a water hyacinth invasion on the Nahoon River, Eastern Cape.

Photo: David Taylor

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Centre for Biological Control

Annual Report 2022

Contents

| | |
|---|----|
| DIRECTOR'S REPORT | 1 |
| STAFF, STUDENTS AND ASSOCIATES | 3 |
| AQUATIC WEEDS | 4 |
| Water hyacinth | 5 |
| Giant Salvinia | 9 |
| Common Salvinia | 11 |
| Brazilian Waterweed. | 13 |
| Delta Arrowhead | 14 |
| Yellow Flag Iris | 15 |
| Mexican Waterlily | 16 |
| Oxygen Weed | 18 |
| Ecological Restoration. | 20 |
| Mass Rearing | 22 |
| CACTUS WEEDS | 23 |
| Round-leaved Prickly Pear | 24 |
| Thistle Cholla | 24 |
| Orange-tuna Cactus | 25 |
| Round-leaf Tuna Cactus | 26 |
| Torch cactus | 27 |
| Mass Rearing and Quantifying the Benefits of Cactus Biological Control. | 28 |
| NORTHERN TEMPERATE WEEDS | 31 |
| Black Locust Tree. | 31 |
| Invasive Rosaceae. | 32 |
| Orange firethorn | 33 |
| Silver Leaf Cotoneaster | 34 |
| <i>Rosa rubiginosa</i> | 35 |
| Willows | 37 |
| Impact of the Invasive Alien Plant Species Clearing Programme on Socio-economic Benefits and Plant Biodiversity along the Northern Drakensberg | 37 |
| The Range X Project. | 38 |
| INVASIVE TREES PROGRAMME. | 40 |
| Australian acacias. | 40 |
| Prosopis | 42 |
| Pines | 43 |
| BUGWEED | 44 |
| TAMARIX. | 45 |
| INTERNATIONAL WEED PROJECTS | 47 |
| African boxthorn. | 47 |
| Crystalline Ice Plant | 49 |

| | |
|---|----|
| GRASSES. | 50 |
| Giant Rat's Tail Grass | 50 |
| African Lovegrass. | 51 |
| Guinea Grass | 52 |
| Gamba Grass | 54 |
| <i>Nassella</i> spp. | 55 |
| Pampas | 56 |
| <i>Tetramesa</i> as grass biological control agents | 57 |
| AGRICULTURAL ENTOMOLOGY | 59 |
| Selection for a UV-resistant isolate of a nucleopolyhedrovirus for improved field persistence and efficacy against FCM. | 60 |
| Evaluating mixtures of formulated baculovirus biopesticides against FCM | 61 |
| The expression and evaluation of baculovirus proteins as formulation additives for enhanced infectivity and improved FCM control. | 62 |
| The isolation, identification, and characterisation of novel baculoviruses for the control of emerging agricultural pests of potential concern for the SA citrus industry | 63 |
| Encapsulation of CrleGV in calcium alginate using a microfluidic device for improved UV resistance | 63 |
| An investigation into the biological and genetic stability of UV-tolerant baculoviruses for improved control of FCM. | 64 |
| Alternate hosts of the oriental fruit fly, <i>Bactrocera dorsalis</i> , in the Sundays River Valley | 64 |
| Augmentation of <i>Aphytis melinus</i> for control of California red scale <i>Aonidiella aurantii</i> on citrus | 65 |
| A comparison of control of key citrus pests in orchards under nets, in a biointensive IPM programme and a conventional programme | 66 |
| Integrated Pest Management (IPM) under nets in Mpumalanga Province | 66 |
| Biology and management of the fruit-piercing moth, <i>Serrododes partita</i> , in citrus orchards. | 67 |
| Bioprospecting for entomopathogenic fungi against foliar citrus pests. | 67 |
| Predicting the pathways and entry points to the southern African citrus production areas, for the Asian Citrus Psyllid, <i>Diaphorina citri</i> | 68 |
| Evaluation of potential repellents for false codling moth, <i>Thaumatotibia leucotreta</i> (Meyrick) (Lepidoptera: Tortricidae) | 68 |
| Regional differences in sex pheromones and sexual attractiveness in FCM | 70 |
| Investigating release ratios in an FCM SIT programme. | 70 |
| POLYPHAGOUS SHOT HOLE BORER. | 71 |
| Community Mass-rearing Projects | 73 |
| COMMUNITY ENGAGEMENT | 73 |
| Implementation and Monitoring Training. | 74 |
| Prosopis Engagement | 75 |
| School Engagement | 76 |
| Partners in the CBC's community engagement activities. | 76 |
| FUNDERS. | 77 |
| RESEARCH OUTPUTS. | 78 |
| CBC RESEARCH DAY | 85 |
| ACRONYMS AND ABBREVIATIONS. | 86 |

DIRECTOR'S REPORT



Professor Martin Hill and his family
Photo: Julie Coetzee

There is an often-used Chinese curse that states “May he live in interesting times” and 2022 was certainly interesting for the CBC. We had all just come through the Covid-19 crisis and were trying to work out how to interact with each other face to face again; fieldwork could once again be undertaken, and there was the opportunity for foreign travel and attending conferences in person. The CBC started the year facing some financial uncertainty, but by seeking new funding opportunities and increasing our cost efficiency, we were still able to make significant progress in the biological control of invasive alien plant species and several crop pests. It is a testament to the staff and students of the CBC that during the entire Covid pandemic and beyond, not only did we not lose a single insect culture, but we were able to continually supply high numbers of insects around the country. Well done to the people working in quarantine and the mass-rearing stations.

The research in this report is a wonderful balance of pre-release studies, quantitative post-release evaluation studies, and impact studies. Post-release evaluation has often been cited as the neglected science of the biological control discipline, so it is great to see so many of these sorts of studies being undertaken. Through the work of Samuel Motitsoe and Antonella Petruzzella, we are also starting to get an idea of how ecosystems recover after the impact of the weed is removed through biological control. The biological control programme against *Prosopis* spp. is gaining momentum, and we have restarted the programme on the possible biological control of Pines. The CBC has been approached by a number of different agencies in the USA, Australia, and New Zealand to assist in surveying for and developing biological control agents for weeds of South African origin.

The agricultural entomology section continues to grow and much of the research is now focused on field implementation of the entomopathogenic viruses and fungi that have been developed through laboratory studies over the last couple of years. New research includes the understanding of pest complexes and their respective biological controls under shade netting, which is fast becoming the preferred wind mitigation method.

In my opinion, one of the strengths of the CBC is its community engagement footprint. Large-scale biological control will only succeed if it has informed buy-in from the communities most affected by the pest. Not only does the CBC introduce biological control at the grassroots level through a number of different school initiatives, but also through interactions with land-users. In 2022, we made some excellent progress engaging with land-users affected by *Prosopis* in the Northern Cape, paving the way for uptake of new agents on this group of weeds through a Masters project.

Over the last year, the CBC convened the International Congress of Invertebrate Pathology and Microbial Control's annual meeting, the 54th Annual Meeting of the Society for Invertebrate Pathology, which was a virtual meeting in August. Well done to Michael Jukes and Sean Moore for pulling together an excellent conference. In October, the CBC hosted the weed biological control workshop in Golden Gate and we were all very excited to meet up again face to face and discuss biological control research in South Africa; thanks to Grant Martin and Kim Weaver for organizing a great meeting. At the end of the year, the CBC hosted the first of what is hoped to become an annual research day in which postgraduate students and researchers presented some of their work. Although this was focussed on the Rhodes University-registered students, next year we hope to expand it to include students from all of our consortium members. It was obvious that the world opened up in 2022, with the CBC contributing three plenary conference papers, 22 international conference papers and a staggering 54 local conference papers. Our students excelled at these

DIRECTOR'S REPORT

conferences, taking away a number of prizes. Added to this, we published 25 peer-reviewed papers and two book chapters. Capacity building remains one of the focus areas of the CBC and, once again, we had an excellent turnout of postgraduates during 2022. What is important is that all of these graduates are either employed in biological control, or have continued to study in this field.

During 2022, the CBC said goodbye to several research staff, including Ben Miller, who has taken up a PhD opportunity in the UK; Matt Paper, who is in the US; Rosali Smith, who has taken up a Postdoctoral Fellowship at FABI, and Emiel von Son, who has taken up a position with Citrus Research International in Stellenbosch. In addition, Candice Owen left Rhodes for a position at Chester University in the UK. We wish them well. Grant Martin was promoted to Senior Researcher, and Iain Paterson received a C1 rating from the National Research Foundation.

Just as we were all looking forward to winding down at the end of the year, the Department of Forestry, Fisheries and the Environment put out a call for tenders to conduct research and implementation of biological control of invasive alien plant species in South Africa. The timeline was tight, but I believe that the CBC put in three very competitive bids.

I thank my colleagues (staff and students) in the CBC, our consortium members, international and local collaborators and funders for their support during 2022. Further I would like to thank Rhodes University for creating the enabling environment in which we house the CBC. Finally, a huge thanks to Kim Weaver for single-handedly pulling together this excellent report.



Researchers at the Weed Biological Control Workshop in October at the Golden Gate National Park. **Photo:** Blair Cowie

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AQUATIC WEEDS



Sagittaria platyphylla invasion in the Makhanda Botanical Gardens. Photo: David Taylor

Our research into, and implementation of, biological control against aquatic weeds in South Africa continues to contribute to the fight against aquatic weeds that degrade our aquatic ecosystems, with knock-on socio-economic impacts. In 2022, we improved the implementation of floating aquatic weed biological control through increased mass-rearing efforts by including community partners as part of our implementation programme. This has not only resulted in enhanced biological control in shorter time frames but has exposed the public to the science of biological control, highlighting its safety and relative ease of application.

The submerged and emergent weed research is progressing very well, with host-specificity testing of *Aphthona nonstriata* against *Iris pseudacorus* complete, and permission to release *Listronotus appendiculatus* against *Sagittaria platyphylla* granted. Unfortunately, the promising *Megamelus toddii* against *Nymphaea mexicana* had to be rejected due to non-target feeding damage, but new agents will be evaluated. Restoration research is underway and adds an important component to biological control programmes that should be conducted as a matter of course.

PROGRAMME HIGHLIGHTS IN 2022

- Water hyacinth was reduced to less than 5% cover at Hartbeespoort Dam through biological control alone for the third year in a row.
- Nearly 400 000 *Megamelus scutellaris* were released from the Waainek Mass-rearing Facility for water hyacinth control around the country.
- Satellite rearing stations for water hyacinth and giant salvinia control agents have been established at a number of sites through stakeholder engagement with private, municipal, and government parties.
- *Neohydronomus affinis* was taken to Kenya and released against water lettuce, with dramatic results in six months.
- Permission to release the weevil, *Listronotus appendiculatus*, against *Sagittaria platyphylla* has been granted.
- Host-specificity testing of *Megamelus toddii* against *Nymphaea mexicana* was completed, but unfortunately indicated the hopper is not suitably specific for release in South Africa.
- Excellent international collaboration between South Africa, Argentina, Belgium, New Zealand and the USA will ensure a successful biocontrol programme against *Iris pseudacorus*.
- The international MadMacs project came to an end, forging collaboration with multiple partners from Europe and South America.

Water hyacinth

Our new approach to biological control of water hyacinth through frequent inundative releases, from both the CBC's Waainek Research Facility and satellite rearing stations, has changed the prospects for control of water hyacinth in South Africa. Feeding damage inflicted by the planthopper, *Megamelus scutellaris*, is reducing water hyacinth cover around South Africa, at sites where we have historically faced difficulty in getting agents established. Besides our mass-rearing and release efforts, the research on the biological control of water hyacinth includes a range of studies, from post-release evaluations to the effects of sub-lethal doses of herbicides on control agent efficacy.

Water hyacinth post-release evaluations

Our results show that adopting an augmentative approach to water hyacinth biological control, whereby control agents, in this case *M. scutellaris*, are released often and in high numbers to inundate and overwhelm the water hyacinth, is most likely to lead to success, as sites where augmentative releases have not been conducted are less likely to lead to successful reductions in the total cover of water hyacinth in the short term. Augmentative releases of *M. scutellaris* can significantly reduce water hyacinth infestations over the summer growing season as the control agent populations continue to increase. However, the management of these programmes at key points of the invasion must be implemented timeously, with emphasis on releases during springtime to reduce the lag time between plant and control agent population build-up, as significant reductions in the populations of *M. scutellaris* can be expected over winter or after floods. For this strategy to work, insect mass-rearing plays a pivotal role. Because *M. scutellaris* is easy to mass-rear due to its short developmental time and high reproductive output, improved techniques and infrastructural development in the rearing process have enabled these inundative approaches to be effective. Some of the worst water hyacinth infestations occur on the Highveld, so this is where we have focused

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most of our releases and post-release evaluations, as well as establishment of satellite rearing stations.

Rosali Smith monitored a number of Highveld sites on a monthly basis, and here we highlight Blesbokspruit wetland, the largest permanent wetland in the Highveld, and a Ramsar wetland. The wetland includes Marievale Bird Sanctuary, which contains about 230 bird species, some of which are threatened. Water hyacinth covers a large extent of the wetland's water surface. Because of its importance as a wetland, organisations that work to preserve the wetland include Blue Deal, City of Ekurhuleni, Blesbokspruit Trust, Gauteng Department of Agriculture and Rural Development (GDARD) and the Centre for Biological Control.

The CBC started releasing water hyacinth biological control agents in the wetland in the winter of 2020. Biological control agents that were released initially consisted of *Neochetina* spp. weevils and *M. scutellaris*. A satellite rearing station was set up at the Blesbokspruit Community Centre allowing *M. scutellaris* to be released in the wetland, as well as other water hyacinth sites in the Ekurhuleni Metro. The Blesbokspruit Community Centre is recognized as a biological control hub in the Metro.

Table B1: Rearing station sites

| System | Province | Number of stations |
|-----------------------|--------------|--------------------|
| Blesbokspruit Wetland | Gauteng | 1 |
| Bronkhorstspuit River | Gauteng | 4 |
| Roodeplaat Dam | Gauteng | 1 |
| Hartbeespoort Dam | North-West | 4 |
| Vaalkop Dam/Hex River | North-West | 2 |
| | TOTAL | 12 |

AQUATIC WEEDS

Initially, post-release evaluations of the wetland water hyacinth plants were not very encouraging. In 2021, *M. scutellaris* population counts were mostly 0, but by 2022, numbers had increased, with the highest number recorded in July at 58.1 ± 25.4 . This is quite significant as

it is one of the coldest months of the year. This increase can be attributed to monthly releases of *M. scutellaris* from the satellite rearing station by the caretaker, Patrick Ganda. In addition, other interested groups manually remove water hyacinth at the site, but owing to good communication, the wetland is divided into a manual removal zone and a biological control zone. Thus, control options do not interfere with each other.

Unfortunately, the wetland is situated in close proximity to a gold mine tailing, and receives heavy metal pollution. Because water hyacinth is an excellent phytoremediator, it is likely that heavy metals have accumulated in the plant tissues, which could be reducing the establishment of the control agents. This will be investigated in 2023.



Left: Ben Miller (CBC) and Patrick Ganda (Ekurhuleni Metro) inside one of the rearing tunnels at Blesbokspruit Wetland Reserve. Photo: Rosali Smith



Water hyacinth infestation at Blesbokspruit, February 2022. Note the gold mine tailing to the left. Photo: Julie Coetzee

MadMacs – Mass Development of Aquatic Macrophytes – Causes and consequences of macrophyte removal for ecosystem structure, function, and services

In 2019, Julie Coetzee was awarded funding by the Water Research Commission for a collaborative project with international stakeholders to determine the causes and consequences of macrophyte removal on ecosystem structure, function and services. Mass development of aquatic macrophytes in rivers and lakes is a global problem, and attempts to remove and control these annually consume substantial sums of money. Aquatic plant removal, however, does not address the causes of the mass development and is not sustainable. MadMacs aimed to determine the underlying causes of mass developments of aquatic plants by evaluating exactly what happens when the plant masses are removed, and analysing how people perceive the plants. Mass developments of aquatic plants were generally perceived as problematic, but it is largely unknown that they also deliver several ecosystem services.

While the results of MadMacs highlight the general findings from six case studies, the results from South Africa's case study at Hartbeespoort Dam stood out, largely due to the hypertrophic status of the system, and the nature of water hyacinth invasion. The results show that:

- The construction of Hartbeespoort Dam created a lake with limited flow and extremely high nutrient concentrations from urban waste. Because the water is deep and turbid, few submerged macrophytes grow, but conditions are ideal for the massive growth of free-floating macrophyte species.
- Free-floating water hyacinth in Hartbeespoort Dam was previously combated using herbicides. After spraying water hyacinth biomass, massive blooms of cyanobacteria occurred in Hartbeespoort Dam, an effect which we also observed in our mechanical macrophyte removal experiment. The cyanobacterial bloom has likely benefitted from a combination of high nutrient availability, removal of shading by free-floating aquatic plants, liberation from allelopathic substances which water hyacinth normally releases, turbid water preventing the growth of submerged plants and periphytic algae, and high water temperatures enabling fast cyanobacterial growth.
- Mass development of water hyacinth in Hartbeespoort Dam is currently combated by biocontrol, that is, by releasing insects that specifically target water hyacinth while leaving other plant species untouched. Recent observations indicate that another free-floating plant species, *Salvinia minima*, has increased in biomass, as water hyacinth declined. The other free-floating plant species probably benefits from high water-nutrient concentrations, decreased competition with water hyacinth for resources and space, and the fact that the released biocontrol agents specifically target water hyacinth, thereby favouring competing plant species.
- This indicates that the targeted removal of non-native plant species alone may only shift the problem of perceived nuisance growth to another species, rather than solve it.
- Removal of free-floating water hyacinth in Hartbeespoort Dam strongly increased CH₄ emissions. It is likely that before its removal, the free-floating vegetation acted as a barrier which captured CH₄ and stimulated CH₄ oxidation in the rhizosphere, thereby oxidising CH₄ that was produced in the anoxic sediment underneath the plants. The effect of removing the macrophyte barrier resulted in enhanced CH₄ emissions. This effect is likely to last until the macrophytes have re-grown.
- Plant removal showed no effects on diversity and abundance of sediment-dwelling macroinvertebrates in Hartbeespoort Dam, probably because the removal of free-floating water hyacinth only slightly disturbed the sediment.
- One week after macrophyte removal, the diversity of macroinvertebrates living within macrophyte beds was reduced in Hartbeespoort Dam, but we detected no effect six weeks after macrophyte removal. This indicates that, unsurprisingly, the removal of their habitat affects macroinvertebrates living within macrophytes, but that the remaining and re-growing plants are quickly recolonized.
- In contrast to the other study sites, removal of water hyacinth did not affect the zooplankton living underneath the free-floating plants in Hartbeespoort Dam, while diversity of phytoplankton tended to increase after macrophyte removal. This may be related to the decreased competition for light and nutrients after macrophyte removal, leading to improved conditions for phytoplankton.
- A very high percentage of both visitors and residents (more than 90%) perceived the mass development of water hyacinth as nuisance. People were most concerned about biodiversity, followed by boating, and the beauty of the landscape. Hartbeespoort Dam is one of few freshwater bodies which are available for recreation in South Africa, and water hyacinth at this site has been perceived as problematic for decades. The high perception as nuisance, and the absence

of a difference between residents and visitors might therefore be related to the fact that people across the entire country have been well aware of the continued struggle against water hyacinth for decades, combined with the high relevance of this water body for the entire country.

- In Hartbeespoort Dam, maximum plant removal would likely increase the estimated total economic value, because the value of boating, angling, and passive recreation would increase after plant removal. Mitigating the disadvantage of plant removal (increased risk of toxic cyanobacterial blooms) would cost less than the increase in recreational value. Furthermore, all forms of recreation declined under a do-nothing management regime where water hyacinth cover increased to 50% of the dam's surface, thereby reducing its economic value.

MadMacs combined basic science (ecosystem metabolism) with applied science (macrophyte management) and cross-disciplinary science (ecosystem services assessments), and communicated the results in an understandable way to relevant stakeholders, including the public, and water managers (e.g. DWS, Water Boards, Local Municipalities). MadMacs worked towards a change in attitude of these stakeholders, with the goal of improving the management of watercourses with dense aquatic vegetation. Dense macrophyte stands were generally perceived as a nuisance, and managers as well as the general public almost automatically thought that dense macrophyte stands were a sign that something was “wrong” and must therefore be removed.

In terms of Hartbeespoort Dam management, a **co-ordinated**, systems approach is needed to manage not only the mass developments, that is, water hyacinth and now common salvinia, but their cause – the excess nutrient load in the dam. As a top priority, research needs to focus on how nutrient loads upstream of the Dam can be reduced.

Focus on mass-removal can now rely on the very effective biological control programme against water hyacinth, but an effort into control of common salvinia is needed. However, if nutrient loading is not addressed, the removal of common salvinia will likely result in developments of cyanobacterial blooms which some local stakeholders regard as worse than the water hyacinth mass developments.



Samuel Motitsoe, Antonella Petruzzella and Julie Coetzee, in Oslo, during the final MadMacs workshop, October 2022. Photo: Julie Coetzee

Integrated control

The integrated control of water hyacinth, using both herbicides and biological control, has proved effective in managing water hyacinth populations in South Africa. When herbicides are applied carefully and in the correct doses, their application can suppress the plants' growth with minimal impact on biological control agents or on the environment. While exposure to herbicides at recommended-label doses has toxic effects on biocontrol agents, either by killing them directly or destroying their food source, applying herbicides at a sub-lethal or retarded dosage may only have minimal impacts on the biocontrol agent. Siyasanga Mnciva, a PhD student at the CBC, is attempting to develop an integrated management system for the control of water hyacinth using the biological control agent *Megamelus scutellaris* and a sub-lethal dose of glyphosate herbicide.

Laboratory tests have identified sub-lethal doses of three glyphosate herbicides that are currently registered in South Africa, namely Kilo Max, Seismic, and Roundup. The performance of *M. scutellaris* when exposed to herbicides was investigated, as was the combined impact of *M. scutellaris* herbivory and herbicide application on water hyacinth plants.

Of the three glyphosate herbicides tested, Roundup showed the highest toxicity to *M. scutellaris* ($LC_{50} = 0.1\text{mg/L}$, $X^2 = 55.5$, $P < 0.0001$), followed by Seismic ($LC_{50} = 1.13\text{mg/L}$, $X^2 = 32.25$, $P < 0.0001$) and, lastly, Kilo Max ($LC_{50} = 0.64\text{mg/L}$, $X^2 = 16.20$, $P < 0.0001$).

All the selected herbicide doses stunted the vegetative growth of water hyacinth plants, thereby limiting its proliferation. Interestingly, a dramatic increase in insect populations was observed in a low-dose herbicide application of Kilo Max (below left) and Seismic (below right) in the mesocosm experiments, even surpassing the population numbers in the herbicide-free control. Similar observations were reported in Roodeplaat Dam in December 2021, where substantial increases in *M. scutellaris* insects were observed after sub-lethal herbicide application by the

Department of Forestry, Fisheries and the Environment (DFFE).

The results of Siyasanga Mnciva's study recommend a 0.4 % concentration of Kilo Max as a sub-lethal dose in integration with the biological control agent *M. scutellaris* for sustainable management of water hyacinth. Field studies to investigate and confirm laboratory and mesocosm experiments are currently in progress.



Glyphosate herbicide treated water hyacinth plants with *Megamelus scutellaris* after six weeks; above left: 0.4 % Kilo Max; above right: 0.75% Seismic. Photo: Siya Mnciva

Giant Salvinia

Salvinia molesta, also known as Kariba weed or giant Salvinia, is a widely distributed South American weed that proliferates in tropical and subtropical regions across the world. It is one of the worst aquatic tropical weeds owing to its ability to form dense mats on the surface of water bodies, replacing native plants and damaging aquatic ecosystems.

Current state of *Salvinia molesta* in South Africa

Kariba weed remains under excellent biological control in South Africa due to suppression from *Cyrtobagous salviniae*, a host-specific weevil. However, infestations still occur at various sites across the country. For example, the Rheenendal Bridge in the Knysna area is currently at 100% coverage, despite the weevils being released in large numbers. As the waterbody is shaded by surrounding trees,

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the complete development of *C. salviniae* is restricted, leading to no biological control of the site. Poplar Dam in Jeffrey's Bay is also at 100% coverage, and infestations have been reported at several sites within the iXhobo River region in KwaZulu Natal. Additional infestations have been reported at sites along the Mooi River catchment, including Cato Ridge Golf Course.

AQUATIC WEEDS

Post-release evaluation

The CBC continues to quantify the success of biological control of *S. molesta* in South Africa through surveys and post-release evaluations. These surveys and evaluations are important tools in investigating the progress and status of biological control programmes, and provide insight into the population establishment of biological control agents, the level of weed suppression provided by the agents, and the level of biodiversity recovery evident in the invaded system. Post-release evaluations are crucial, as some sites

require augmentative releases as populations may crash or fail to develop under unfavourable conditions, such as cold winter temperatures or flooding events.

Post-release evaluations also allow for ongoing research projects which assess longer-term ecosystem recovery after the implementation of biological control. For example, the CBC has an ongoing project investigating the recovery of biodiversity in a system once biological control of *S. molesta* has been achieved across five sites in the Eastern and Western Cape provinces.



Before (above left) and after (above right) pictures of the success of the biological control weevil *Cyrtobagous salviniae* at The Craggs, Plettenberg Bay area (-33,94186; 23,46049). Photos: Antonella Petruzzella (1/12/2021) and Getrude Tshithukhe (30/11/2022)



Before (above left) and after (above right) pictures at Candlewood Farm, Western Cape (-33.96552; 22.97803). Photos: Antonella Petruzzella (left: 2/12/2021 and right: 01/12/2022)

Satellite mass-rearing facilities

The CBC set up four *C. salviniae* rearing facilities in 2022. These are located in George, Plettenberg Bay, Jeffrey's Bay, and Durban. Using these satellite rearing facilities, higher numbers of *C. salviniae* weevils are produced, with easy access to the weevils provided almost countrywide. The involvement and support of local stakeholders has been extremely valuable, with many community members expressing interest in the programme and in the biolog-

ical control of invasive weeds. This “buy-in” from stakeholders is of great importance for the sustainability of the programme, as well as for the reputation and sustainability of biological control as a whole.

The CBC has worked tirelessly over the years and has conducted extensive research into Kariba weed and the biological control thereof. The CBC has also consulted on projects outside of South Africa, where infestations are still problematic.



One of our efficient and cost-effective *Cyrtobagous salviniae* rearing facilities located in George. Photos: Getrude Tshithukhe (29/11/2022)

Common Salvinia

Common salvinia, *Salvinia minima*, is a relatively new aquatic invader in South Africa's freshwater ecosystems. Native to Central and South America, *S. minima* was first recorded in South Africa in December 2011, growing on the Hartbeespoort Dam in the North-West province. As is the case with its relative, *S. molesta*, common salvinia can form dense mats on the surfaces of water bodies, limiting their productivity and utility, and decreasing their aesthetic and recreational value. Common salvinia has been limited to Hartbeespoort Dam over the past few years, but has since spread rapidly, invading the Crocodile River in Roodekoppies Dam, as well as the Bon Accord and

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Roodeplaat Dams. As South Africa's freshwater systems provide favourable conditions for the growth and spread of *S. minima*, it is likely that the species will continue to

AQUATIC WEEDS

spread and become increasingly problematic across the country in the near future.

Competition

While *S. minima* was first observed in Hartbeespoort Dam in 2011, the rapid proliferation of the species in the dam is much more recent. With the success achieved in controlling water hyacinth on Hartbeespoort Dam through the inundative releases of biological control agents, the dam is no longer dominated by water hyacinth, and new niches and resources have been made available for secondary invasion. This appears to have been the case with common salvinia, which is now the dam's dominant species.

Tressia Chikodza, an MSc student at the CBC, is investigating the competitive interactions between water hyacinth and common salvinia on Hartbeespoort Dam, including the influence of herbivory by biocontrol agents on the competitive performance of water hyacinth. Tressia's study investigates the competitive ability of both plants with and without herbivory on water hyacinth by *M. scutellaris* to determine the impact of biological control when two species are competing for resources. This knowledge will allow for more accurate predictions regarding the population dynamics of the two invasive weeds at Hartbeespoort Dam. The competitive abilities of the two species were

Without biocontrol



With biocontrol



Damage on water hyacinth by *Megamelus scutellaris* when competing with *Salvinia minima*. Photo: Tressia Chikodza

determined using an inverse linear model with plant weight as the yield variable. In the absence of *M. scutellaris*, water hyacinth is more competitive than *S. minima*. However, when exposed to feeding by *M. scutellaris*, its competitive ability was reduced, and *S. minima* was the superior competitor. Tressia's results show that *M. scutellaris* destabilised water hyacinth's competitive abilities in favour of *S. minima*. Tressia will continue to explore aspects of the species' competitive abilities, including the potential use of allelopathy as a competitive strategy.



Salvinia minima invasion at Hartbeespoort Dam. Photo: Tressia Chikodza (27/10/22)

Host-specificity testing of *Cyrtobagous salviniae* Florida biotype

A new *C. salviniae* biotype was identified in 2001, which, unlike the well-known Brazil biotype used to control *S. molesta*, feeds and completes its development on *S. minima*. The Florida biotype is smaller in size than the Brazil biotype, and has successfully reduced mats of *S. minima* in Florida and Louisiana.

The weevil was imported into the CBC's quarantine facilities in July 2021, after which host-specificity testing was conducted. The test plants used in host-specificity

testing included *Salvinia hastata*, the only *Salvinia* species native to sub-Saharan Africa. The results show limited non-target effects on *S. hastata*. The survival rate of insects reared on *S. hastata* was very low compared to *S. minima*, while feeding damage was negligible. We have completed an application for release, which will be submitted to the relevant agencies for review. Owing to our success in controlling *S. molesta* with the Brazilian biotype, we are optimistic that the Florida biotype will meet the requirements for release, and that the Florida biotype will successfully control, or at least provide some relief from *S. minima* infestations before they become increasingly abundant.

Brazilian Waterweed

Efforts for the biological control of *Egeria densa* are focussed on mass-rearing the control agent, *Hydrellia egeriae*, followed by post-release evaluations. The two biggest infestations occur in the Groot River, in the Baviaanskloof, and in Midmar Dam, KZN. Floods earlier in the year washed away much of the *E. densa* in the Groot River, but by July, the plant and insect populations had

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Egeria densa in the Groot River, Baviaanskloof, July 2022. Photo: Antonella Petruzzella

increased. By comparison, in July 2021, the field population of *H. egeriae* was 395 imm/kg fresh weight, while *E. densa* shoots weighed 2.32 ± 0.28 g (per 15 cm shoot); while in July 2022, we estimated 252 imm/kg fresh weight, with 15 cm shoots weighing 1.39 ± 0.13 g. Even though parasitoids were present at the site, inundative releases at the site have proved effective in establishing the agent.

In March 2022, survey results revealed no immature flies at Midmar Dam. We therefore met with Umgeni Water in

August to assist with preparing a management plan for the dam, which included implementing biological control. Experience has shown that frequent releases are necessary to sustain field populations of the biological control agent, so in collaboration with Umgeni Water, we released ~8000 *H. egeriae* in Midmar Dam in late November 2022.

We continue to monitor *E. densa* sites, and provide feedback to the water managers.

Delta Arrowhead

Sagittaria platyphylla is regarded as one of the fastest spreading invasive species in the country owing to its ability to reproduce both sexually and asexually. Since 2014, we have assessed the suitability of four *Listronotus* spp. weevils as potential biological control agents, and in 2020, permission to release *L. appendiculatus* was requested. Permission was not granted because the independent reviewers felt that while the weevil showed great promise as a biological control agent, they suggested that in order to confirm that the weevil is completely safe for release in South Africa, paired choice or multiple generation tests should be conducted on the closely related, but rare aquatic macrophyte, *Limnophyton obtusifolium*. After a number of failed attempts at obtaining sufficient numbers of this plant, which is restricted to northern KwaZulu Natal in South Africa, we reapplied for permission to release the weevil based on the main premise that leaving *S. platyphylla*

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uncontrolled would have more of a negative impact on South Africa's biodiversity than the potential non-target impact on a species that does not occur where *S. platyphylla* has invaded.

On 14 December 2022, permission was granted by the Department of Agriculture, Land Reform and Rural Development to release the weevil after three independent reviewers assessed the application, stating that the revised application provided adequate argument and motivation for the safe release of *L. appendiculatus* in South Africa. The CBC will release the weevil in 2023, after rearing significant numbers to improve the chances of establishment.

Geographical considerations:

- The distance between *S. platyphylla* and *L. obtusifolium* populations is great.
- The distance between *L. obtusifolium* sites is also vast.
- *L. obtusifolium* populations are seasonal and rain dependent.
- The weevil, *L. appendiculatus* flies infrequently, preferring swimming and crawling, therefore will not be able to cover large distances.

Insect physiological considerations:

- *L. obtusifolium* does not have suitable oviposition sites for *L. appendiculatus*.
- Only large plants with large petioles are suitable for larval survival, and this rarely occurs in *L. obtusifolium*.
- Fitness of *L. appendiculatus* is reduced when reared on petioles only.



Adult *Listronotus appendiculatus*. Photo: Dave Taylor.

Plant ecological considerations:

- *L. obtusifolium* is a facultative annual, producing flowers and fruits before it could potentially be found by *L. appendiculatus*.
- Achenes are the primary mechanism of dispersal and survival.
- Achenes survive extended dry periods in the soil.
- *L. appendiculatus* is unable to survive extended dry periods, and therefore will not be able to build up sustainable populations under dry conditions.

Yellow Flag Iris

Yellow flag records in South Africa

Records of *Iris pseudacorus* infestations in South Africa continue to increase, with over 130 populations recorded as of November 2022. The use of social media and citizen science platforms has been extremely useful for record acquisition, as well as for creating increased public awareness and engagement between researchers and citizen scientists. The increase in infestation records is alarming but not surprising, as *I. pseudacorus* is known to reproduce and spread prolifically via both seed and rhizome fragmentation.

Yellow flag population genetics

Genetic analyses performed by Emma Sandenbergh in 2021 showed high levels of gene flow occurring between and within South African *I. pseudacorus* populations, suggesting that sexual reproduction may be the primary mode of spread. Emma is conducting similar analyses for *I. pseudacorus* populations in New Zealand, and preliminary results suggest that these populations also experience high levels of gene flow and demonstrate high genetic variability.

Sexual reproductive output

Paula Gervazoni and Gianmarco Minuti, both PhD students, are currently assessing the sexual reproductive capacity of yellow flag iris in the invaded (Argentina) and native (Belgium) range of the species. Paula and Gianmarco tested the presence of dormancy, seedling survival and seedling growth in Argentina and Belgium, providing new insights into the reproductive characteristics of yellow flag iris. While the results have yet to be analysed, preliminary results suggest that introduced yellow flag populations produce seeds with a higher germinability than native populations.

Paula also assessed the production of reproductive organs (flowers and seed capsules) in Argentina, adding to the information obtained by Emma in South Africa in 2019. These results show a better performance in terms of flower and capsule production, as well as the number of seeds produced per capsule, in the species' introduced range. This could be explained by different hypotheses, one being that invasive species utilize resources more effectively in their introduced range, owing to the reduced pressure exerted by natural enemies in the species' native range. Paula also conducted surveys in parts of the species' native European range, including Switzerland and Italy.

RESEARCH AND TECHNICAL TEAM

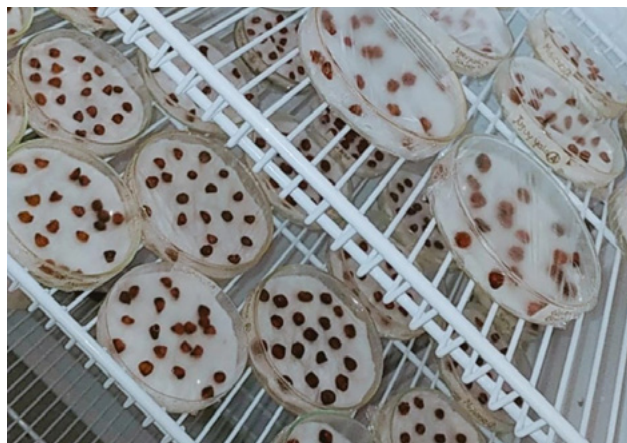
Prof. Julie Coetzee, Emma Sandenbergh, Samella Ngxande-Koza

STUDENTS

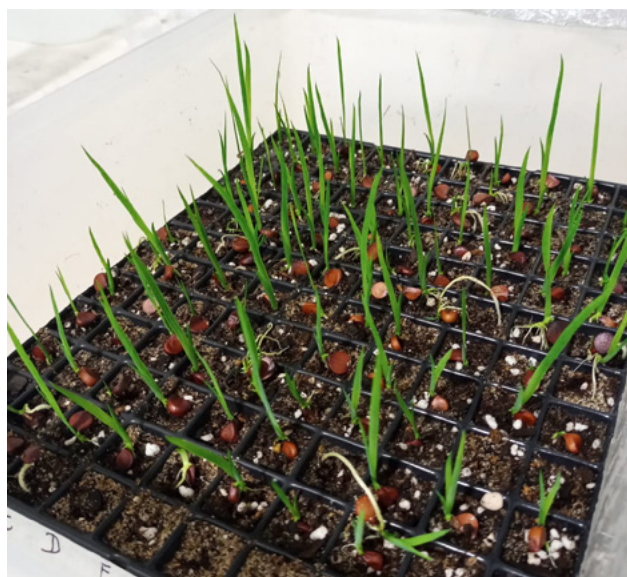
Gianmarco Minuti (PhD), Paula Gervazoni (PhD), Erin Boon (Hons)

COLLABORATORS

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Above and below: Argentinian *Iris pseudacorus* seeds germinating experiment. **Photos:** Paula Gervazoni



Above: PhD student Paula Gervazoni sampling *Iris pseudacorus* plants in the native European range

Public knowledge on yellow flag invasions in Argentina and South Africa

Paula gathered information regarding the distribution of *Iris pseudacorus* in Argentina and South Africa through a Survey Monkey questionnaire which collected citizen science reports, adding to the existing knowledge of the weed's distribution. The survey also provided information regarding the general public's knowledge of the yellow flag problem, their awareness of biological control, and their feelings around applying biological control as an invasion management tool for this weed. Paula's results showed a greater degree of knowledge on yellow flag invasions in South Africa than in Argentina, as well as a higher level of agreement with the application of biological control as a management tool.

Species distribution modelling

Gianmarco used MaxEnt to model and compare the present and future climatic suitability of *I. pseudacorus* and its three candidate biological control agents (*Aphthona nonstriata*, *Mononychus punctumalbum*, and *Rhadinoceraea micans*). The models predict a net decrease in climatic suitability for the plant in the southern hemisphere, whereas the overlap between the climatic niche of the plant and that of its candidate biological control agents is expected to remain more or less stable, even increasing in some regions, as a result of climate change. These results are promising for the success of biological control of *I. pseudacorus* in the southern hemisphere, and could be used to select the most suitable agents for a specific invaded range, as well as prioritizing areas for their release.

Yellow flag biological control

A flea beetle, *Aphthona nonstriata*, has been identified through host-range studies as a potential biological control

agent for *I. pseudacorus* in South Africa. Host-specificity tests show that the flea beetle is specific to the *Iris* genus, of which South Africa has no native representatives. As such, in the unlikely event that non-target effects occur, these would not pose any threats to indigenous biodiversity.

Erin Boon, an honours student at the CBC, has shown that *A. nonstriata* can cause substantial damage to *I. pseudacorus* individuals under quarantine conditions. Feeding by *A. nonstriata* was shown to significantly affect both the morphology and physiology of *I. pseudacorus*, and resulted in decreased leaf growth and increased physiological stress.

A similar study was conducted by Gianmarco, who tested the effect of various insect densities on *I. pseudacorus* plant growth. His preliminary results show that even at low densities ($n = 5$), feeding by *A. nonstriata* can stunt the growth of yellow flag iris. However, even at high densities ($n = 20$), the insects were not able to kill the plants. Further testing is needed to assess whether higher insect densities or longer exposure to herbivory can lead to successful management of the plant. Gianmarco hypothesizes that the resilience of yellow flag iris to herbivory by *A. nonstriata* may be due to the energy reserves stored within the rhizomes of adult plants. To test this, he will conduct a second experiment, whereby seedlings will be exposed to the same protocol. Seedlings used will have a similar aboveground biomass to the adult plants tested in the first experiment, but with no developed rhizome to use as a storage organ.

A release application for *A. nonstriata* will be sent to the Department of Agriculture, Land Reform and Rural Development in the near future. If approval is granted, we will begin releasing the flea beetle at invaded sites across South Africa in 2023.

Mexican Waterlily

Nymphaea mexicana Zuccarini (Nymphaeaceae) originates from southern USA and Mexico and has become a problematic invader in South Africa. In 2016, research into the development of a biological control programme for Mexican waterlily was initiated, and has since been the focus of Megan Reid's MSc and PhD theses.

Waterlily genetics

Research into the genetic structure of *N. mexicana* revealed that multiple hybrids exist across the country, and that these can be separated into four groups, based on their

similarity to other species. One group is genetically similar to a European waterlily, *Nymphaea alba* L., another group is similar to the American *Nymphaea odorata* Aiton, the

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Above: Damage from *Bagous longulus* on *Nymphaea mexicana* leaf. Photo: Megan Reid

third group has a mix of genetic material from various unknown sources, including *Nymphaea tetragona* Georgi, and the remaining group is *N. mexicana* that is most similar to *N. mexicana* from the native range. Understanding the genetic origins of these groups provides clarity on which additional species can be surveyed for biological control agents for hybrid groups.

Biological control of Mexican waterlily

Pre-introductory surveys carried out from 2020 to 2021 across invaded sites in South Africa revealed that a native weevil, *Bagous longulus* Gyllenhal (Coleoptera: Curculionidae), has expanded its host range and is feeding and surviving on *N. mexicana* at three sites: one in Gqeberha/Port Elizabeth, EC; another in Knysna, WC; and the third in Wartburg, KZN. Further surveys for this species nationwide revealed that it is broadly distributed and that it is specific to *Nymphaea* species, including the South African *Nymphaea nouchali* Burm. f. and *Nymphaea lotus* L. in the field. Further host-specificity tests were conducted to compare feeding and survival on *N. nouchali* and *N. mexicana* from two invaded populations, and a hybrid, *Nymphaea marliacea chromatella* Latour-Marliac.



Above: *Megamelus toddi* nymphs on *Nymphaea mexicana* in quarantine. Photo: Megan Reid



Above: Side view of *Bagous longulus*. Photo: David Taylor

These tests showed that there were no differences in feeding or preferences between the weevil populations tested, but that there was lower preference for the tested hybrid, confirming observations in the field.

In 2018, two biological control agents, a weevil and a planthopper, were identified during surveys in the native range. The weevil was imported into quarantine conditions in 2018 and 2020, but a culture was not successfully established. The planthopper, *Megamelus toddi* (Hemiptera: Delphacidae), was successfully reared in quarantine in December 2021, and host-specificity tests were conducted using the same test plants that were investigated for host specificity of *B. longulus*. Multi-generational trials showed that *M. toddi* can survive equally well on *N. mexicana* and the South African *N. nouchali*, but that it cannot survive for long on the hybrid. Owing to the lack of its host specificity, *M. toddi* was rejected as a potential biological control agent.

Currently, *B. longulus* is being investigated for use in augmentative biological control of *N. mexicana* while preparations are made for other agents from the native range to be investigated for management of *N. mexicana*.



Above: Closeup of *Megamelus toddi*. Photo: Joseph Sigmon.

Oxygen Weed

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Lagarosiphon major, commonly known as African elodea or oxygen weed, is a submerged perennial aquatic plant native to South Africa. Oxygen weed is a problematic invasive species in New Zealand, Ireland, the United Kingdom, and other European countries.

PhD student Nompumelelo Baso (below) and Wandisile Mdiza (right) sampling *Lagarosiphon major* in a farm dam south of the town of Cala, Eastern Cape (site typical of *L. major* in the native range). **Photos:** Nompumelelo Baso and Wandisile Mdiza



The enemy release hypothesis (ERH) explains the success of invasion by alien plant species outside of their native range. It posits that natural enemies are top-down stressors that regulate plant populations in their native range, and that in the absence of these stressors, exotic plants are able to redirect resources to growth and reproduction, without the added expense of defence. Classical biological control is seen as evidence for this hypothesis. Comparing the biomass and species richness between oxygen weed's native (SA) and invaded (NZ) ranges, there is empirical evidence for the ERH in the case of New Zealand where climate modelling has shown that more than 90% of the country is vulnerable to invasion by *L. major* (Figure page 19).

Biological control of oxygen weed

A biological control programme for *L. major* is currently being established, with two promising potential biological control agents (*Polypedilum tubercinatum* and *Hydrellia*

lagarosiphon) under investigation.

The *H. lagarosiphon* fly has proved to be host-specific and damaging enough to control *L. major*, but stakeholders expressed concern over its efficacy in areas where *L. major* plants do not reach the surface of the water in New Zealand. However, Nompumelelo Baso, a PhD student at the CBC, has found through field observations and preliminary laboratory studies that the early larvae are able to search for and find the host plant even when it is not topping out. Further investigation is underway to quantify this, and upon confirming efficiency under fully submerged conditions, Landcare will seek permission to release the fly in New Zealand.

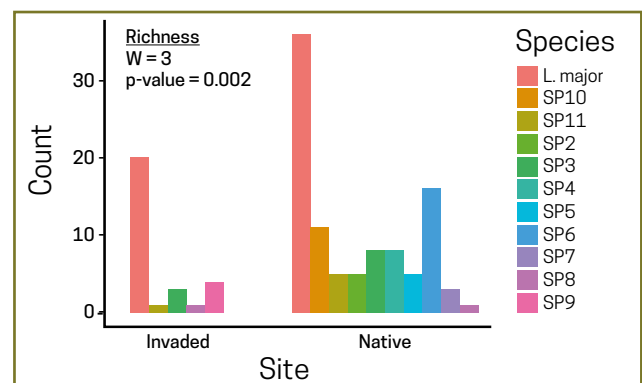
The *P. tubercinatum* midge underwent host-specificity testing by Earle et al. (2013) and showed promising results for the biological control of *L. major* in Ireland. While the midge proved promising for Irish *L. major* populations,



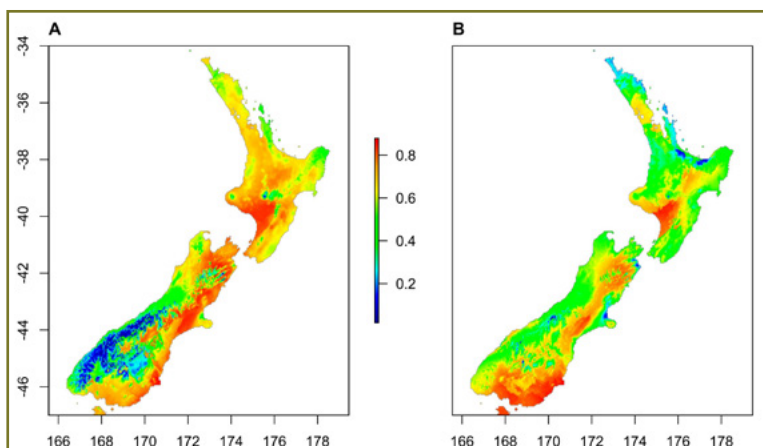
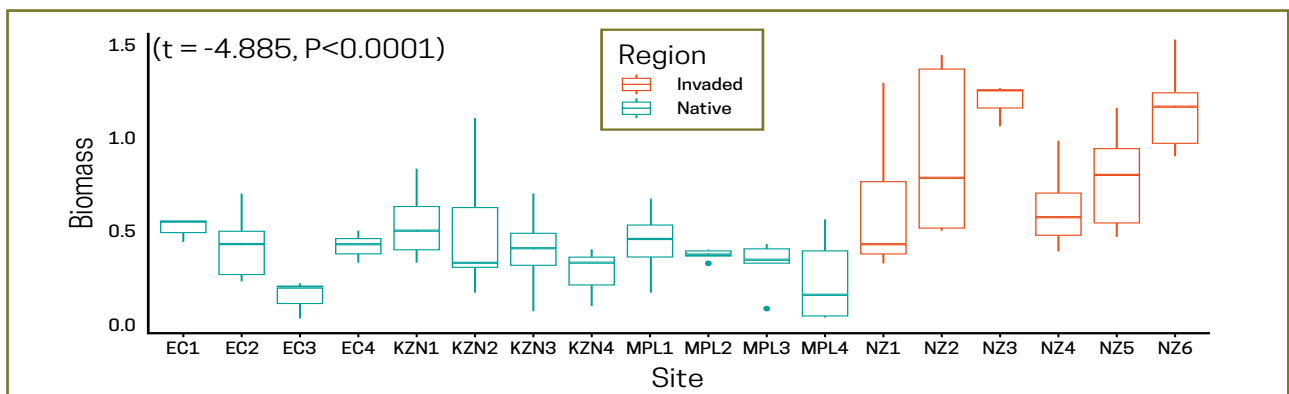
Above and below: Typical *Lagarosiphon major* infestation outside its native range in New Zealand. Photos: Angela Bownes



testing for suitability of the agent for New Zealand populations is still necessary. As such, Nompumelelo compiled a test plant list using centrifugal phylogenetic methodology. As there are no representatives of the Hydrocharitaceae family (to which *L. major* belongs) in New Zealand's native flora (De Lange & Rolfe, 2010), exotic Hydrocharitaceae species present in New Zealand will be included in host-specificity tests. Species selection was widened to include four families in the order Alismatales (namely, Potamogetonaceae, Haloragaceae, Alismataceae, and Hippuridaceae), which include some key New Zealand natives. The risk of non-target effects of introduced biological control agents is significantly reduced owing to the absence of native Hydrocharitaceae species.



Above: Species frequency per plot (5 m²) according to region (native and invasive), SP1 = *L. major*.



Above: Wet *Lagarosiphon major* biomass (kg/0.02 m²) per site, in both the native and invasive range.

Left: Maxent ecological niche modelling for *Lagarosiphon major* in New Zealand. (A) Current suitability, (B) Future suitability.

Ecological Restoration

Exotic invasive plants present one of the greatest challenges to natural resource management. The removal of invasive plant species through chemical, mechanical and/or biological methods is often seen as beneficial, but following removal, the native plant communities do not always re-establish nor do ecosystems recover. Instead, these systems are highly susceptible to re-invasion or establishment of a novel invader (secondary invasion) taking advantage of newly available resources and habitat disturbance. This is a common situation faced by managers and restoration ecologists, not only in South African freshwater systems, but in several systems around the world. To prevent this from occurring, active restoration, which involves re-introducing native plant communities after invasive plant control, has been recognized as a relevant strategy to limit invasions and support the recovered aquatic biodiversity processes and functions. However, several studies have indicated that plant management efforts have had only moderate restoration success. These unsatisfying results have increasingly been attributed to a failure to account for priority effects, that is, the effect of species on the survival and performance of other species, depending on the order and timing in which they arrive at a site. However, priority effects have only recently been considered for restoration practices and remain little explored, especially in freshwater systems.

Restoration experiment

Since 2021, the CBC has continued to expand its research agenda into the role of ecosystem restoration after aquatic weed control to ensure ecosystem recovery. The restora-

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tion team has set up an ongoing whole-pond manipulation experiment at the Waainek Research Facility. The aim is to mimic the situation of a highly invasive floating plant-invaded (*Pistia stratiotes*) system at first, followed by successful invasive plant control by the biological control agent *Neohydronomus affinis* (Coleoptera: Curculionidae). After achieving complete control, native vegetation was actively re-established and then a secondary invader, the South American rooted submerged plant, *Egeria densa*, invaded the system.

The native species were established following three native plant species (*Lagarosiphon major*, *Stuckenia pectinata* and *Vallisneria spiralis*) priority effects: native plants were planted (1) simultaneously, (2) six weeks earlier and (3) six weeks later relative to the secondary invader arrival, the rooted submerged plant *E. densa*. Other biotic communities were also monitored: phytoplankton, periphyton, zooplankton and macroinvertebrates, together with water quality parameters. The experiment has been running for seven months and will allow us to assess how the biota and water quality change and/or recover during invasive plant control management and native vegetation re-establishment. We hope to guide future restoration efforts in a way that maximizes the likelihood of desired species establishment by strengthening native species priority effects to curb future re-invasions and/or secondary invasions.



Aerial photo of the whole-pond manipulation experiment at Waainek Research Facility showing 100% cover of the floating invasive plant *Pistia stratiotes* (water lettuce). Photo taken 12/04/2022. Photo: David Kinsler



Above: Experimental enclosure unit 100% covered by water lettuce.
Photo: Antonella Petruzzella



Above: Water lettuce heavily damaged by the biological control agent *Neohydronomus affinis*. Photos: Antonella Petruzzella 27/06/2022



Above: Experimental pond cleared of the water lettuce.
Photo: Antonella Petruzzella 11/10/2022



Above: Active planting of native submerged plants. Photo: Blaine Camons



The Waainek Team in Tunnel B- Samella Ngxande-Koza, Landile Booie, Siyaxolisi Mini, Martin Hill, Mthobeli Mvandaba, Lyle Titus, Vuyani Ntyinkala, Siyanda Ntamo, Bongwiwe Gobongcwana, Nandipha Ngolothi (missing Nokuthula Kom). Photo: Kim Weaver

Mass Rearing

The year 2022 started out with uncertainties regarding funding and delays from the Department of Forestry, Fisheries and the Environment. The delay and lack of funds resulted in the retrenchment of two of the Waainek Research Facility employees. The facility had to be operated by two employees who mainly maintained the insect cultures and kept the plants in good condition. This was done successfully with the full support of the CBC team.

In July 2022, the CBC was granted emergency funding from the Department of Forestry, Fisheries and the Environment that will run until the end of March 2023. Through Rhodes University's Human Resource Division and the Association for Persons with Disabilities (APD), the CBC was able to employ seven new staff, all living with disabilities. Three of the seven new employees had previously worked for the CBC, and we were able to employ a senior laboratory assistant.

At the beginning of the spring season, the insect populations were thriving and ready to be released, with the exception of *Cyrtobagous salviniae*. Requests for all agents other than *C. salviniae* were attended to without fail. Increasing the population of *C. salviniae* has been a challenge owing to cold temperatures. However, the CBC is putting various measures in place to increase numbers to respond to the requests. For example, plants are being fertilized regularly to improve feeding material, and tubs have been moved to a warmer growing tunnel.

This year, agents have been sent to many different provinces for release on populations of *Pontederia crassipes*, *Pistia stratiotes*, *Myriophyllum aquaticum*, a few populations of *Salvinia molesta* and, one population of *Egeria densa*.

Number of biological control agents released on four aquatic invasive weeds

| Weed | Agent | Numbers of releases made | Total number of insects or immature larvae released |
|-------------------------------|------------------------------|--------------------------|---|
| <i>Pontederia crassipes</i> | <i>Megamelus scutellaris</i> | 61 | 393 740 |
| <i>Myriophyllum aquaticum</i> | <i>Lysathia</i> sp. | 18 | 6170 |
| <i>Egeria densa</i> | <i>Hydrellia egeriae</i> | 1 | 4397 |
| <i>Pistia stratiotes</i> | <i>Neohydronomus affinis</i> | 23 | 7493 |
| <i>Salvinia molesta</i> | <i>Cyrtobagous salviniae</i> | 8 | 2750 |
| TOTAL | | 111 | 414 550 |

CACTUS WEEDS



Invasive cacti have been targeted for biological control in South Africa for nearly 120 years. The first biocontrol agent ever released against a cactus weed globally was released very near to the central hub of the CBC in the Eastern Cape against the drooping prickly-pear, *Opuntia monacantha*. The agent was a cochineal insect, *Dactylopius ceylonicus*, and it was very successful at controlling the weed where it was released. The CBC still mass-rears and releases this agent in order to ensure that any new infestations of the weed are quickly controlled.

The CBC is working on 20 cactus weeds, five of which are pre-release targets, and 15 post-release. Pre-release targets are those that have had no effective biocontrol agents released in South Africa and require host-specificity testing and efficacy studies under quarantine conditions. These include *Opuntia elata*, *Opuntia megapotamica*, *Opuntia engelmannii* and *Cylindropuntia pallida*, all of which have cochineal insects as potential agents, and *Trichocereus spachianus*, for which both *Hypogeococcus* mealy bugs and cochineal insects are being considered as agents. Post-release targets are those that the CBC mass-rears and releases agents for, and conducts post-release evaluations to quantify the success of the biocontrol intervention.

Cactus biocontrol is the most effective way to sustainably control cactus weeds. A very high proportion of the cactus weeds that have been targeted for biocontrol in the past are now considered under complete control. There is therefore a good chance that the pre-release species targeted for biological control will be effectively controlled in future. However, one of the constraints to cactus biocontrol is the taxonomic confusion within the family Cactaceae, and within the cochineals and *Hypogeococcus* which are used as biocontrol agents. These constraints are overcome using genetic studies and bioassays, which are essential in order to select the most effective biocontrol agents for each of the cactus weeds.

Although cactus biocontrol is effective, the agents are generally very poor dispersers, so mass-rearing and releasing large numbers of agents is important for maximizing the impact of the agents we have released in the country. It is also important to quantify the success of biocontrol in order to motivate for its continued use and to motivate for future investments in the science and practice. The CBC has collected important data that show how effective cactus biocontrol is, and the many benefits it provides to the environment and societies of South Africa.

PROGRAMME HIGHLIGHTS IN 2022

- The CBC has released agents at hundreds of sites all over South Africa, and has shown that these releases resulted in the control of invasive alien cactus weeds on a national scale.
- New agents for four cactus weeds that do not yet have effective biocontrol agents released on them have been imported into quarantine and are under evaluation for efficacy and host specificity. These include the cochineal, *Dactylopius confertus*, which is the first of the many agents that have been tested against *Trichocereus spachianus* that appears to be damaging to the target weed.
- The cochineal lineage developed by the Wits University CBC group for the control of the Kenyan form of *O. engelmannii* has been released in Kenya where it is being mass-reared and released against this highly problematic invasive species.

Round-leaved Prickly Pear

The biological control programme against round-leaved prickly pear has been hampered by taxonomic confusion since it was first initiated. The species has several intraspecific taxa, and the species delimitations are very blurred, so there are many disagreements among taxonomists about what *Opuntia engelmannii* actually is, and which cacti fall within the species. The CBC has taken some important steps towards better understanding this species through the use of genetics and bioassay studies. One important discovery is that what we thought was a single species, is actually at least two species, one of which is *Opuntia megapota*, which is a plant of South American origin, as opposed to North America where *O. engelmannii* is indigenous.

The CBC teams at the ARC Roodeplaat and Wits University have tested at least 15 lineages of cochineal from North America against *O. engelmannii* in South Africa. There are at least three forms of this plant in South Africa and a fourth that is very problematic in Kenya. The most problematic form of the plant in South Africa is often called the Northern Cape variety, but in fact, is found at many sites in the Eastern Cape, the Western Cape, and Namibia. None of the agents that has been tested in the quarantine facilities at Wits or ARC Roodeplaat has been sufficiently damaging to warrant release against this form of *O. engelmannii*, but the *Opuntia stricta* agent, *Dactylopius opuntiae* 'stricta', has been effective at controlling it at a site near Douglas in the Northern Cape, and the agent for *Opuntia ficus-indica*, *D. opuntiae* 'ficus', has been effective at controlling it in Namibia. At most sites in South Africa, this form of *O. engelmannii* is still highly problematic and is a serious agricultural and environmental pest. The reasons for the variable success may be genetic variability and taxonomic

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FUEDEI, CABI

confusion within the entity that we call the Northern Cape variety (for example, this variety may actually comprise multiple species of various origins), or it could be due to climatic variability across the distribution where the species is invasive, with some areas being more suited for biological control, or it could be a combination of both factors. The CBC will be working to understand the reasons for the variable success against the Northern Cape variety so that the level of control against this species can be improved across its entire invaded distribution. One of the key questions that still needs to be answered is where in the USA the Northern Cape variety originated, as this would be the best place to look for biological control agents.

The Wits team has also been working on the control of the Kenyan form of *O. engelmannii*, which is problematic in wildlife reserves in Kenya, but is not present in South Africa. The Kenyan form appears to have originated in Texas, USA, and the most effective cochineal lineage tested against this form of the plant was collected by the CBC in Texas. After conducting efficacy trials and host-specificity testing in quarantine at Wits, the cochineal was released in Kenya. Kenyan wildlife reserves, such as Loisaba in the Laikipia district, are mass-rearing and releasing the agent, as well as conducting post-release evaluations.

Thistle Cholla

The thistle cholla, *Cylindropuntia pallida*, is indigenous to Mexico and is a serious invasive alien pest in South Africa and Australia. Biological control, using a cochineal insect originally collected from Mexico, has been very effective in Australia, and South African biocontrol researchers were hoping that the same success could be repeated in South Africa. The cochineal insect controlling *C. pallida* in Australia has been imported into South Africa on three occasions, but in each case the population of the cochineal has slowly diminished until it has petered out. This is in stark contrast to the situation in Australia, where the cochineal thrives and reaches very high population densities in a short period of time. The *C. pallida* plants in Australia

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have a few subtle morphological differences to the South African plants. Both are *C. pallida*, but each population has a different origin, and it appears that the cochineal that was collected in Mexico is effective against the Australian *C. pallida* but not the South African *C. pallida*. To confirm this, Pippa Muskett travelled to Australia to import a

new consignment of the cochineal and some cladodes of Australian *C. pallida* to do experiments within quarantine. A bioassay to quantify the efficacy of the Australian cochineal on South Africa *C. pallida* and Australian *C. pallida* (as a control) will be conducted to determine whether the suspected genetic mismatch is the reason for

the limited damage inflicted by the cochineal on South Africa *C. pallida* plants. If the bioassay indicates that the South African plants are inferior hosts, then a new cochineal that is adapted to South African plants should be collected from the native distribution of Mexico.

Orange-tuna Cactus

RESEARCH TEAM

Phillippa Muskett, Prof. Iain Paterson

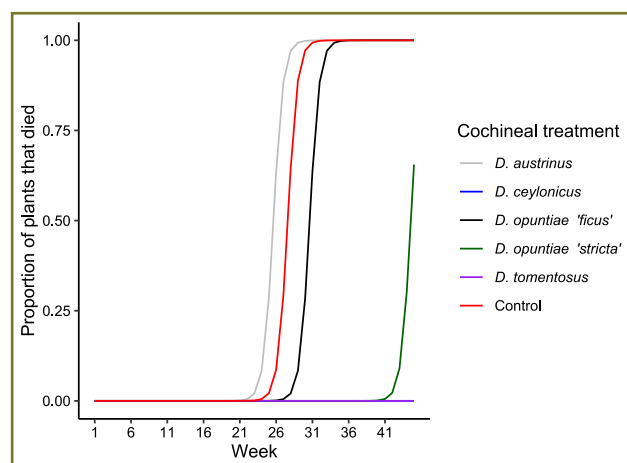
COLLABORATOR

FUEDEI

Opuntia elata, known as Orange-tuna cactus, is an emerging invasive Cactaceae which has a negative impact on the carrying capacity of rangelands in South Africa. It is closely related to other damaging invasive *Opuntia* species, and the number of locations where it is found in the country has grown rapidly over the last few years, so it is a species that is likely to become very problematic in future if left unmanaged. *Opuntia elata* is considered a good target for biological control because many of its close relatives, such as *Opuntia monacantha*, are effectively controlled by host specific cochineal species.

In 2019, a survey of the South African distribution confirmed that *O. elata* was not being controlled by any of the cochineal species currently released in South Africa, and in early 2020 a new cochineal lineage was collected from the native distribution in Argentina and brought back to the CBC quarantine facility for testing.

A trial was set up to compare the ability of all the cochineals used for biological control in South Africa to establish and kill *O. elata*. This was done to make sure that none of the biocontrol agents for other cactus weeds would be effective against *O. elata*, and to make sure that the new agent was more damaging than any available agents that have already been released. Surprisingly, *Dactylopius austrinus*, the biocontrol agent for jointed cactus (*Opuntia aurantiaca*), was able to kill the *O. elata* plants faster than



The proportion of *Opuntia elata* plants killed each week by the different species of cochineal released in South Africa, compared to the rate at which the *Opuntia monacantha* plants were killed by *Dactylopius ceylonicus*. *Opuntia monacantha* is very effectively controlled by *D. ceylonicus* in the field, so this treatment is the control.

D. ceylonicus killed *O. monacantha* (above). This suggests that *D. austrinus* could potentially be an effective biological control agent for *O. elata*.

We then conducted an insect life history trial on loose cladodes (joints of the cactus) of *O. elata* in quarantine. This study compared the new cochineal from Argentina to the cochineals we already have in South Africa. It was confirmed that *D. opuntiae 'stricta'* could establish but was not particularly successful on *O. elata*, producing an average of only 2.3 mature females per replicate and an average of 260.8 (± 98.6 S.E.) crawlers per female. *Dactylopius austrinus* was significantly more successful than other South African cochineals, and equally successful as the new cochineal species and also produced a similar number of mature females 17.3 (± 5.8 S.E.) females/rep with an average of 394.9 (± 45.6 S.E.) crawlers/female for *D. austrinus*, and 14.3 (± 4.5 S.E.) mature females/replicate and 392.9 (± 50.2 S.E.) crawlers/female for the Argentinean cochineal. Given these results, it is strange that *D. austrinus* has not already started to control *O. elata* in the field. To investigate the impact that the cochineals have on *O. elata* further, we are now conducting a damage trial using whole potted plants in quarantine to determine whether the new cochineal, or *D. austrinus*, is more effective as a control agent for *O. elata*.



Opuntia elata at a site in Makhanda, South Africa. Photo: Pippa Muskett



Opuntia megapotamica in flower in the Eastern Cape. Photo: Phillippa Muskett

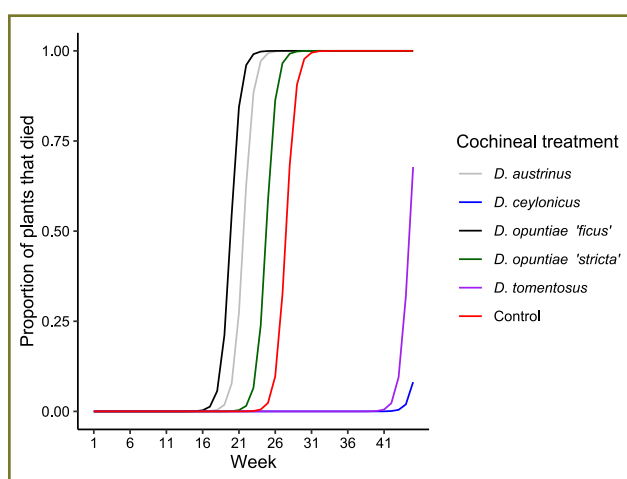
Round-leaf Tuna Cactus

The round-leaf tuna cactus, *Opuntia megapotamica*, is a widely distributed and very problematic invasive cactus in the Eastern Cape, but until 2021, it was incorrectly thought to be a variety of *Opuntia engelmannii*. Now that *O. megapotamica* is correctly identified, we are hoping to find an effective cochineal species to use as a biological control agent against it.

Opuntia megapotamica was included in trials for a new biological control agent for *Opuntia engelmannii* run by the CBC team at Wits University and ARC Roodeplaat. Fifteen cochineal species (six lineages of *Dactylopius confusus* and nine lineages of *Dactylopius opuntiae*) from North America were able to survive and thrive on *O. megapotamica*. The *D. opuntiae* lineage collected in Flagstaff, Arizona, was the most damaging and could be considered for release as a biocontrol agent for this species. In South Africa, we have already released two lineages of *Dactylopius opuntiae* for the control of *Opuntia stricta* and *O. ficus-indica* but hybridization between these lineages can reduce their efficacy as biological control agents, so further studies into their potential to hybridize with the new lineage would be necessary before release.

Since *O. megapotamica* is of South American origin, it is important to determine whether cochineals from South America (that have had old associations with the target plant) are more effective than the North American cochineals that were tested against it when it was thought to be *O. engelmannii*, a North American cactus species. A cochineal lineage collected off a close relative of *O. megapotamica* in South America (the same lineage that is being

tested for *O. elata*) has been imported into quarantine at the CBC and studies are underway to determine its efficacy against *O. megapotamica* compared to the efficacy of all the cochineals that are used for the control of other cacti in the country. The new South American cochineal performed better than South Africa cochineals, producing an average of 14.7 (± 3.8 S.E.) mature females/replicate and 531.52 (± 41.85 S.E.) crawlers/female (pictured below). The impact of the new cochineal will now be compared with the Flagstaff cochineal to determine which is most effective so that only the most damaging cochineal is released.



The proportion of *Opuntia megapotamica* plants killed each week by the different species of cochineal released in South Africa, compared to the rate at which the *Opuntia monacantha* plants were killed by *Dactylopius ceylonicus*. *Opuntia monacantha* is very effectively controlled by *D. ceylonicus* in the field and is therefore the control in this experiment.

Torch cactus

Torch cactus, *Trichocereus spachianus*, has become increasingly problematic in the arid regions of South Africa, forming dense thickets of spiny, upright columns that grow in an 'organ-pipe' like formation. It has very large, showy, white flowers which open on overcast days and at night. While the flowers are beautiful, the negative impacts of this cactus weed on rangelands, and the dramatic increase in its range and abundance in recent years, has resulted in the initiation of a biological control programme.

As no wild populations of *T. spachianus* were found during surveys in what is thought to be the indigenous distribution

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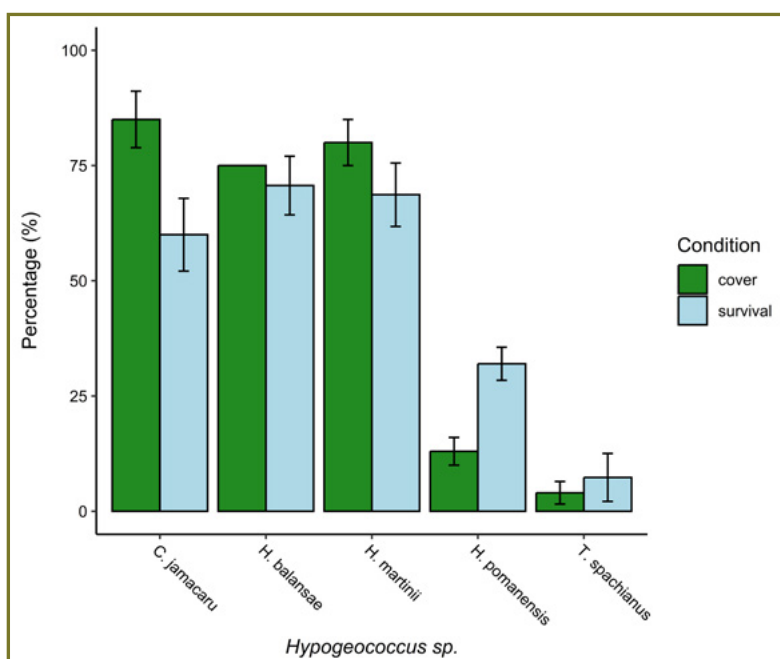
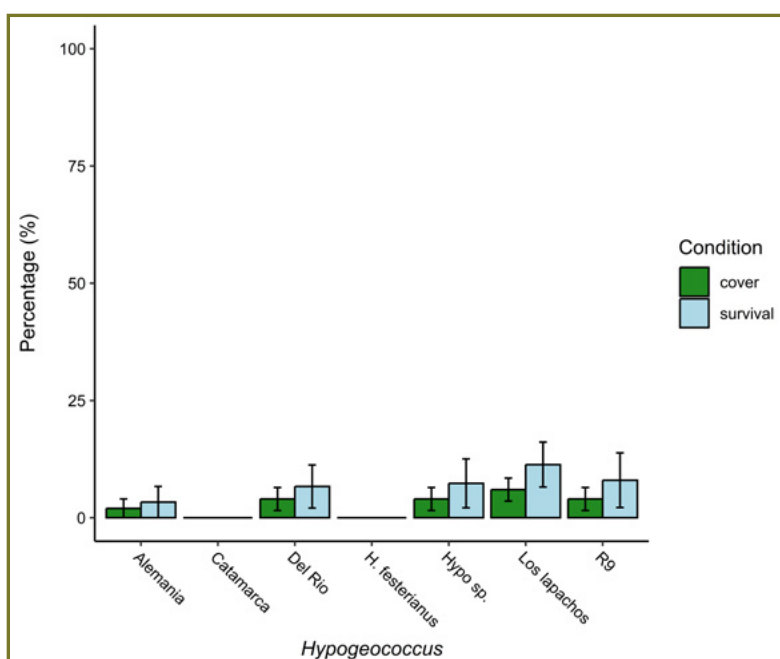
STUDENT

Tamzin Griffith (PhD)

of the plant in Argentina, closely related cactus species were instead searched for potential biocontrol agents. Argentina is home to various galling mealybugs in the genus *Hypogeococcus* and owing to the success of these insects as biocontrol agents of closely related invasive alien cactus species, they were selected for further testing.

Hypogeococcus festerianus and five lineages of the species complex, *Hypogeococcus pungens*, were imported into the quarantine facility at Rhodes University. Laboratory trials revealed that, in most cases, crawler establishment for these *Hypogeococcus* was zero on *T. spachianus*; however, they did survive and establish on the other cactus species used in the experiment. Where establishment did occur, it was extremely low (top figure) compared to what is expected of a successful biocontrol agent (bottom figure) and would therefore not permit their use as biocontrol agents in South Africa.

As none of the *Hypogeococcus* were damaging enough to be considered as biocontrol agents, another possible solution to the *T. spachianus* problem in South Africa, *Dactylopius confertus*, a cochineal, was investigated. This cochineal insect differs from most other cochineal species in that it feeds on *Trichocereus* and *Harrisia* species cacti, not the usual *Opuntia* hosts. *Dactylopius confertus* was introduced into Namibia accidentally by an unknown pathway and is now present on *Harrisia* species that are invasive around the capital city of Windhoek. Since this cochineal is known to be damaging to

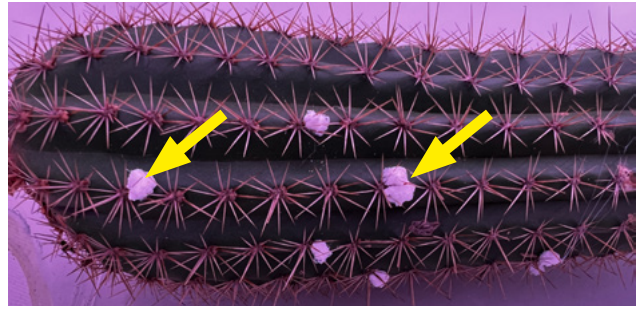


Above left : Initial survival of crawlers and percentage cover four months post-establishment on *Trichocereus spachianus* for six *Hypogeococcus* lineages/species.

Left: Initial survival of crawlers and percentage cover four months post-establishment of *Hypogeococcus* sp., a successful biocontrol agent, on its target weeds in South Africa.

other *Trichocereus* species, efficacy testing against torch cactus was warranted. *Dactylopius confertus* was recently collected in Namibia and imported into the quarantine facility at Rhodes University. Preliminary studies have shown that the cochineal does establish on *T. spachianus* after only a few days of being introduced (figure on the right). Compared to the *Hypogeococcus* tested previously, these results are already more promising and, hopefully, the cochineal will prove to be sufficiently damaging. If the cochineal does prove to be damaging enough to be considered as a biocontrol agent, then host-specificity testing will be initiated.

This pic doesn't have a photographer credit. Is that OK?



Settled *Dactylopius confertus*, as indicated by the yellow arrows, on a section of cut *Trichocereus spachianus*. Photo: Tamzin Griffith

Mass Rearing and Quantifying the Benefits of Cactus Biological Control

Post-release evaluations are one of the most important stages in biological control programmes, but this stage is often neglected. Data collected during post-release evaluations can be used to improve the release strategies of existing agents and determine whether additional releases are required to achieve the desired level of control. Moreover, evaluating success influences the continuous support of biological control research and implementation.

The CBC Kariega cactus team mass-rears and releases cactus agents throughout South Africa, and conducts post-release evaluations to determine the success of these releases. This includes the comparison of areas where the agents have been released to the distribution of target weeds to ensure that releases have been conducted throughout the weed's distribution, and measuring the biomass and reproductive output of cactus infestations before and after release.

The CBC has released six agents on ten target weeds at 247 sites in the country. Biological control agents have been released in every province in the country, covering 51% of the quarter degree squares (an area of about 27 km²) where the target weeds are present. This suggests that the release efforts by the CBC could be at least doubled, and that other large mass-rearing facilities are required to ensure that the agents are provided for releases throughout the country. The CBC is working with the Association for People with Disabilities in Upington, Northern Cape Province, to start mass-rearing cactus agents in that previously neglected part of the country. Limpopo Province has also been identified as a part of the country where the CBC has conducted fewer releases than elsewhere. Zezethu Mnqeta will be based at the University of Limpopo in 2023, and she is eager to work with the CBC to mass-rear and release

RESEARCH TEAM

Prof Iain Paterson, Zezethu Mnqeta, Ruth Scholtz and the Kariega Team

biological control agents against cactus invasive alien plants occurring within the province.

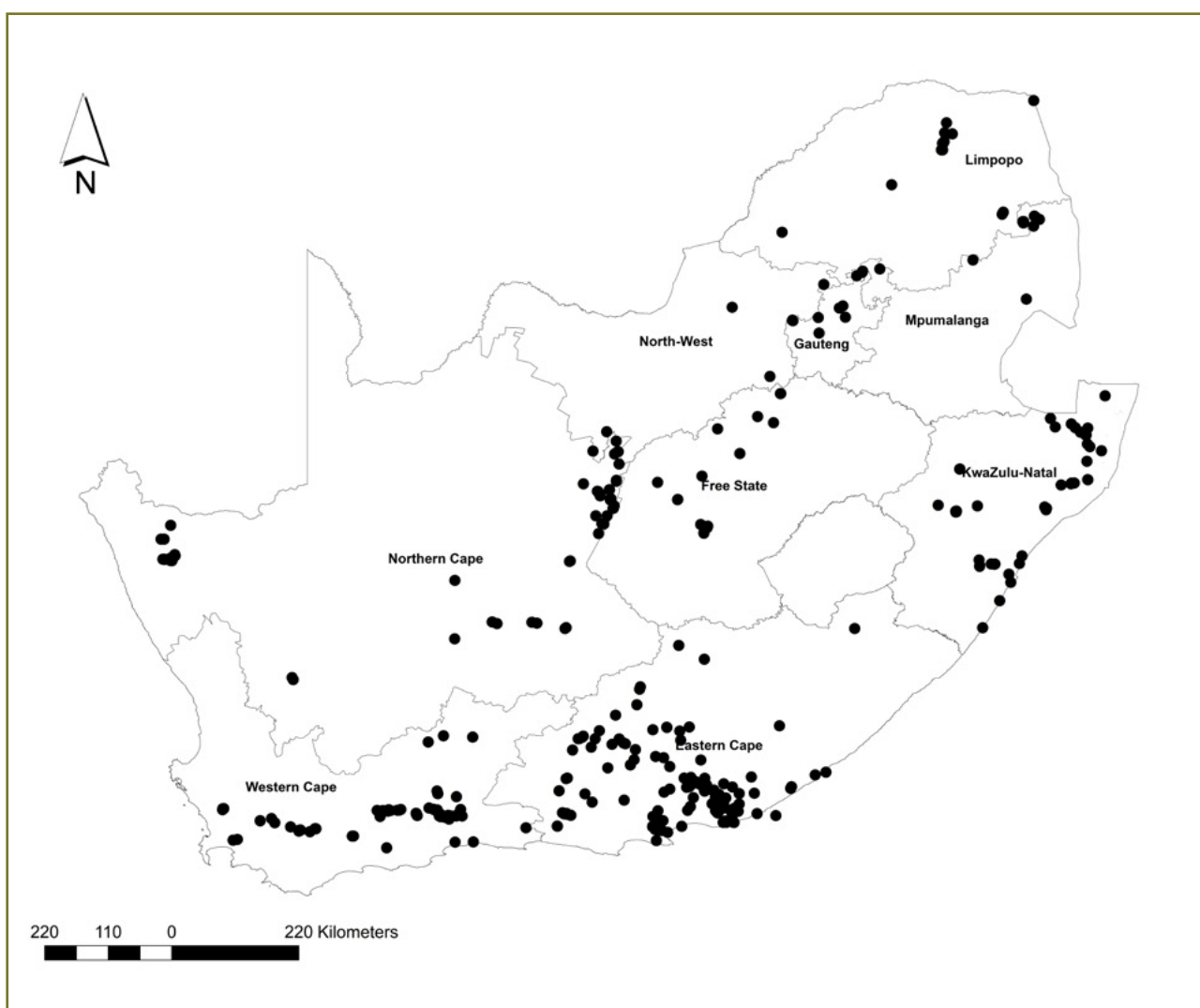
Post-release evaluations at sites where the CBC have released agents indicated that, for most species, there was a significant increase in the density of the agent after release, followed by a decline in plant biomass and reproductive output. For example, the number of jointed cactus cladodes (stem segments of a cactus) per/m² decreased from 30 (± 0.75 S.E.) to 17 (± 1.4 S.E.) over a four-year monitoring period; for drooping prickly pear, the number of fruits declined from 135 (± 15.6 S.E.) to 25 (± 7.9 S.E.), and cladodes declined from 89 (± 12.7 S.E.) to 36 (± 8.9 S.E.); for Australian pest pear, the number of cladodes reduced from 141 (± 19.3 S.E.) to 37 (± 14.2 S.E.), and the number of fruits decreased from 125 (± 20.1 S.E.) to 40 (± 26.1 S.E.).

The Kariega team is made up of 15 mass-rearing technicians led by the facility manager, Ruth Scholtz, assisted by the Postdoctoral Research Fellow, Zezethu Mnqeta.

This year, 91 releases were conducted, with a total of 8 127 galls/infected cladodes being released (Table 1). We have released in all nine provinces in South Africa, on 11 target weed species using six agents. This brings the total number of releases of biocontrol agents by the Cactus Team since the project was started in 2015 to 541 releases, totalling 402 707 galls/infected cladodes, covering every province of the country.

Releases of cactus biological control agents from the CBC mass-rearing facility this year (2022)

| Weed | Agent | # releases | # cladodes/galls |
|-----------------------------|-----------------------------------|------------|------------------|
| <i>O. aurantiaca</i> | <i>D. austrinus</i> | 7 | 3 050 |
| <i>O. stricta</i> | <i>D. opuntiae 'stricta'</i> | 16 | 701 |
| <i>O. cespitosa</i> | <i>D. opuntiae 'stricta'</i> | 8 | 428 |
| <i>O. monacantha</i> | <i>D. ceylonicus</i> | 7 | 1 071 |
| <i>O. ficus-indica</i> | <i>D. opuntiae 'ficus-indica'</i> | 2 | 38 |
| <i>O. engelmannii</i> | <i>D. opuntiae 'stricta'</i> | 1 | 22 |
| <i>C. imbricata</i> | <i>D. tomentosus 'imbricata'</i> | 36 | 1 447 |
| <i>C. fulgida mamillata</i> | <i>D. tomentosus 'imbricata'</i> | 1 | 78 |
| <i>C. jamacaru</i> | <i>Hypogeococcus sp.</i> | 5 | 641 |
| <i>H. martinii</i> | <i>Hypogeococcus sp.</i> | 3 | 293 |
| <i>H. pomanensis</i> | <i>Hypogeococcus sp.</i> | 5 | 358 |
| TOTAL | | 91 | 8 127 |



Sites where the CBC has released cactus biocontrol agents in South Africa



Above: The Kariega team at the mass-rearing facility. Gugulethu Mkile (top left), Kenton Toontjies, Lubabalo Malinga, Byron Soetland, Arthur Scholtz, Denise Mwethudana, Carmen Peters, Karin Goliath, Daniel Scholtz, Yanga Mgxoteni (bottom left), Ronel Roman, Ziyanda Simayile, Heloise Jacobs, Ruth Scholtz, Jazmyn Rossouw, Chaals Vena. **All photos:** Ruth Scholtz



Above: Gugulethu Mkile and Arthur Scholtz measuring width of *Cylindropuntia imbricata* near Kariega Hospital



Above: Gugulethu Mkile and Arthur Scholtz measuring distance from the tagged plant to the nearest plant for *Cylindropuntia imbricata* infestation near Kariega Hospital



Above: Lubalalo Malinga, Arthur Scholtz, Byron Soetland, Ruth Scholtz, Carmen Peters and Ronel Roman measuring the total number of cladodes and cochineal-infested cladodes of *Opuntia aurantiaca* in the Kwandwe Nature Reserve
Right: Karen Goliath and Lubabalo Malinga counting the number of *Cylindropuntia imbricata* cladodes at the Allison Farm outside Klipplaat.

NORTHERN TEMPERATE WEEDS

Leaves and flowers of female
crack willow, *Salix fragilis*.
Photo: Grant Martin

The Northern Temperate Weeds (NTW) programme aims to bring the benefits of biological control to the mountain grasslands of South Africa. The high elevation grasslands of South Africa are important systems for water security, as they provide nearly half of all the country's water run-off and are a key biodiversity hotspot with high plant endemism. The biome is also an invaluable resource, supporting livestock central to the livelihoods and economies of commercial, small-scale, communal farming/agriculture, but under severe threat from invasive alien plants, particularly species deriving from the cooler Northern Temperate regions of the globe.

This programme is multi-faceted and has involved conducting feasibility studies on several alien tree species invading the highland regions of South Africa to determine which species should be prioritized for biological control. The programme made excellent progress over the last year. Specifically, the programme has continued to conduct pre-release studies on *Robinia pseudoacacia* and *Gleditsia triacanthos*; it has also now prioritized a pair of *Rosaceae* species, *Pyracantha angustifolia* and *Cotoneaster pannosus*, for biological control and has continued assessing additional invasive species as candidates for biological control.

This programme has benefited from the support and collaboration of both local and international entities. In South Africa, close collaboration with the Plant Sciences

Department and the AfriMontane Research Unit based at the University of the Free State, QwaQwa campus, as well as the ARC-PPRI. International collaboration has been developed between the CBC and Yunnan University (China), Virginia Tech Blacksburg (USA), the US Forestry Science Laboratory (Morgantown, USA), the Centre of the Region Haná for Biotechnological and Agricultural Research (Czech Republic), and CABI (Switzerland).

The programme has recently also combined with Range X, an ambitious project aimed at investigating the ecological drivers of range-expanding plant species at high altitudes. The overall aim of the Range X programme is to contribute to efforts to mitigate the effect of climate change on the environment and the communities reliant on those environments. The NTW programme aims to investigate how future climate scenarios may affect the growth parameters of a number of the species being considered for biological control.

The programme has pioneered the development of the Southern African Mountains Invasive Alien Plants Working Group. This working group seeks to increase collaboration among researchers and conservation managers to facilitate the best management and research practices for IAPs in southern Africa with the ultimate goal of reducing the impact of plant invasions.

Black Locust Tree

PhD candidate Abigail Wolmarans continued investigating *Robinia pseudoacacia* invasions in South Africa. Her work confirmed that, unlike many other countries, no host-specific insects (potential biocontrol agents) are yet associated with the tree in South Africa. In addition, these studies have highlighted that the foliage of the tree should be the primary guild targeted for biological control.

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STUDENT

Abigail Wolmarans (PhD)

STUDENT

Virginia Tech University, USA and CABI Switzerland

A modified prioritization system originally suggested by Harris (1973) has been used to refine the known available candidate agents from over 50 to three, namely: *Obolodiplosis robiniae* (Cecidomyiidae) - locust gall midge; *Odontota dorsalis* (Chrysomelidae) – locust leafminer; and *Parectopa robinella* (Giacillariidae) – locust digitate leaf miner.

The programme is still working with researchers from Virginia Tech University, Blacksburg, Virginia USA as well as CABI, Delémont Switzerland. Researchers at Virginia Tech continue to investigate the impact, abundance and specificity of *Obolodiplosis robiniae* Haldeman (Diptera: Cecidomyiidae), *Odontota dorsalis* Thunberg (Coleoptera: Chrysomelidae) (Chrysomelidae), and *Agrilus diffilis* Gory (Coleoptera) in Virginia. CABI

continues to investigate the abundance impact and specificity of two leaf-mining moths, *Phyllonorycter robinella* Clemens and *Parectopa robinella* Clemens (Lepidoptera: Gracillariidae), the acacia gall midge, *Obolodiplosis robiniae* Haldeman (Diptera: Cecidomyiidae), and a leaf-cutting locust sawfly, *Nematus tibialis* (Hymenoptera: Tenthredinidae), in Europe. These studies have also identified several parasitoids associated with these candidate agents.

In August 2022, a sampling and collection trip was conducted in Switzerland, Austria, and France to determine the impact of *O. robiniae* and *P. robinella* in the introduced range. The agents were collected and transported back into the CBC's quarantine facility for final host-specificity testing.

Invasive Rosaceae

Invasive Rosaceae are becoming dominant and problematic in the Grassland Biome of South Africa. Plant reproductive ecology experiments have been conducted over the last five years on a number of these problematic invasive Rosaceae family species. Excessive fruit and seed numbers are produced by the species; high seed soil bank numbers and seed viability are all factors facilitating their invasion. In addition, owing to the fleshy nature of the fruits, these seeds are dispersed by birds and mammals into novel and inaccessible environments.



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STUDENT

Gcinile Blessing Carvalho (Hons)

Management of these invasive Rosaceae is particularly challenging; however, through careful consideration of each species, certain stages of the plant life cycle could be identified which can be targeted for management. For example, *Pyracantha angustifolia* seeds have a short lifespan in the soil seed bank, whereas seed dispersal of *Cotoneaster pannosus* is not facilitated by birds. Thus, even though the species are highly productive, there are different solutions to be explored for controlling or mitigating the spread of these species. At this stage, biological control is the only feasible large-scale control option for these species, and the findings will help guide their management.

Impact of invasive Rosaceae on soils

In an attempt to determine if these invasive Rosaceae species impact the soil's chemical and mechanical characteristics, an Honours student, Gcinile Blessing Carvalho, investigated the impact of three IAP Rosaceae species – *Cotoneaster pannosus*, *Pyracantha angustifolia*, and *Rosa rubiginosa* on soil properties. She was able to show that some of the essential elements, such as Calcium, as well as pH, increased with the introduction of an invasive Rosaceae on the open grassland soils. Gcinile also checked if seed germination of invasive Rosaceae is facilitated in these transformed soils. The germination trials of *P. angustifolia* revealed higher germination in all soils other than from the parent plant.

Orange firethorn

Pyracantha angustifolia is a medium-sized, evergreen shrub, native to northeastern China, which is increasingly becoming invasive in warm temperate climates worldwide. The species' ability to survive in areas affected by frost and its attractive orange to red berries have contributed to its horticultural success. Once planted, the shrubs' berries are eaten and distributed by birds and mammals. The species has been identified as one of the most damaging and fastest spreading species in the grasslands of South Africa, and is therefore a candidate for biological control. PhD candidate, Lehlohonolo Adams, has continued to work on the species post his MSc. Thus far, Lehlohonolo has investigated the integration of the species into rural communities. He has also been investigating the importance of larger mammals in the long-distance dispersal of the seeds, showing species such as baboons and eland may be able to transport the seeds over long distances, in addition to increasing their viability.

RESEARCH TEAM

Dr Grant Martin, Prof. Sandy Steenhuisen (UFS-ARU)

STUDENT

Lehlohonolo Adams (PhD) and Gcinile Blessing Carvalho (Hons)

COLLABORATOR

Yunnan University, China

To start the process of identifying potential biological control agents, we are collaborating with Yunnan University in China, where the full suite of potential biological control agents for *P. angustifolia* in China will be identified. Initial host-specificity testing, starting in 2023, will be done on closely related species in China; however, additional testing will have to be conducted on prioritized agents in South Africa, given the number of indigenous Rosaceae species.



The invasive pyracantha (red) in the Clarens Conservancy area in the eastern Free State. Photo: Alexandra Howard

Silver Leaf Cotoneaster

One of the most abundant and widespread invasive Rosaceae in the grassland biome is *Cotoneaster pannosus*. MSc student, Karabo Moloi, is investigating the species' impact in the grassland biome, its modes of invasion, and its suitability for biological control. *Cotoneaster pannosus* has an aggressive root system which aids competition, and access to water and enables survival in unfavourable conditions. Additionally, *C. pannosus* forms dense monocultures, creating a canopy which shades out indigenous plants and presenting a major risk to rangeland quality and associated livelihoods, to water provision in strategic water source areas, and to local biodiversity. Research within this programme has now shown that *C. pannosus* has an extended fruiting season that persists through winter into spring and produces up to six million seeds per shrub, with a significant positive relationship between shrub volume and seed production. Additionally, the high densities of seed rain at 160 000 seeds per m² directly below the shrub canopy was significantly higher than seed rain away from parent *C. pannosus* shrubs. This trend was similar for the soil seed bank. Furthermore, seeds demonstrated high viability of approximately 60% after being buried for a year. Opportunistic observations and remote camera footage revealed that a variety of indigenous frugivorous birds and mammals consumed *C. pannosus* fruits that had fallen underneath shrub canopies, and while perched on tree branches. *Cotoneaster pannosus* fruit consumers included speckled mousebirds (*Colius striatus*), Cape white-eyes (*Zosterops pallidus*), common duiker (*Sylvicapra grimmia*) and Namaqua rock mice (*Micaelamys namaquensis*).

RESEARCH TEAM

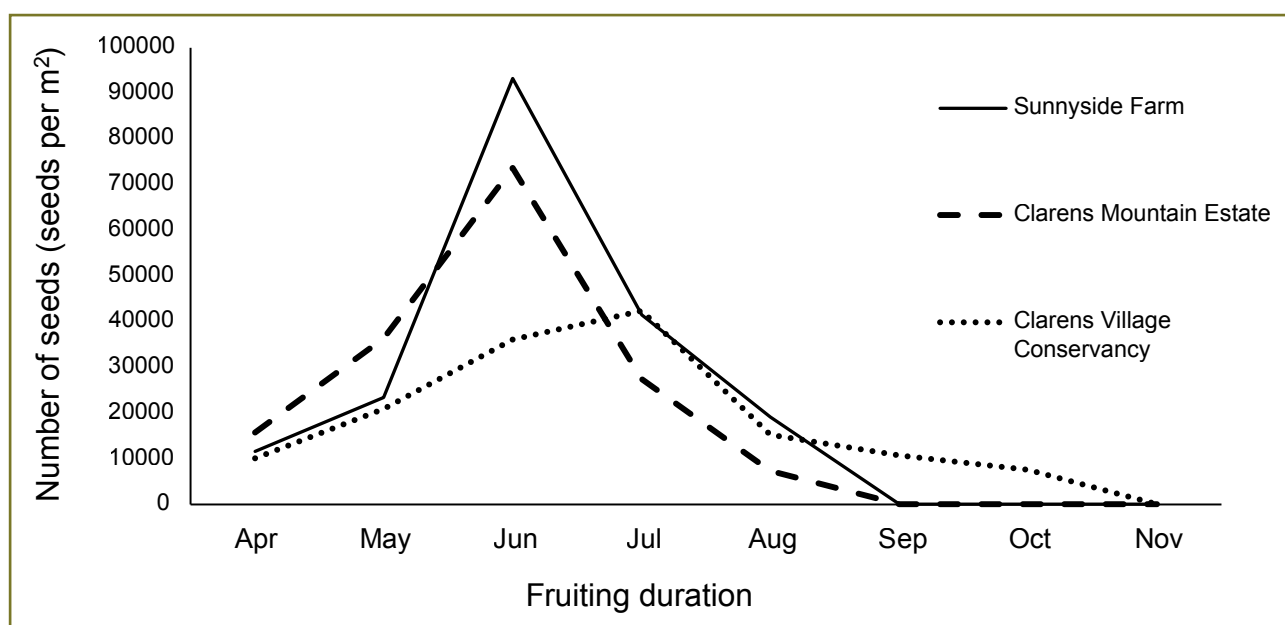
Dr Grant Martin, Prof. Sandy Steenhuisen (UFS-ARU)

STUDENT

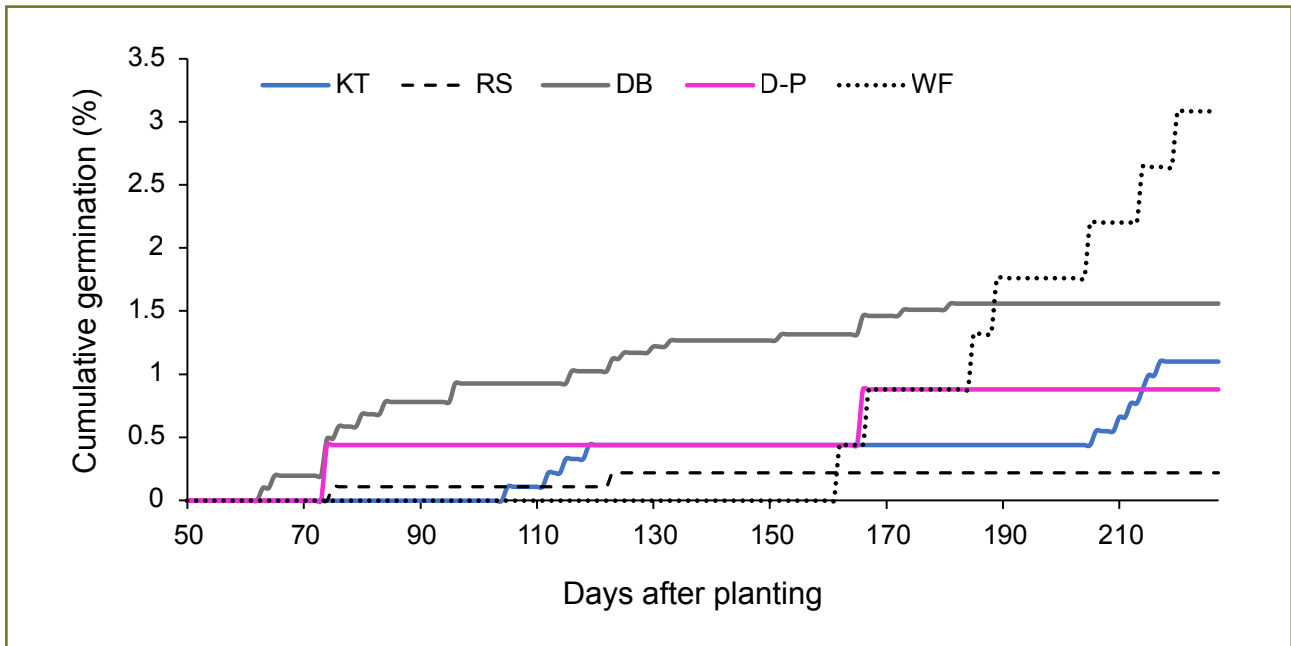
Karabo Moloi (MSc)

In addition, the germination rate and success of *C. pannosus* seeds were explored through bird-feeding trials conducted in aviaries and germination experiments consisting of three treatments: seeds buried as whole fruits, manually de-pulped seeds, and seeds excreted by birds. *Cotoneaster pannosus* seeds that were ingested by birds had an average retention time of fifteen minutes, depending on the bird species. The birds swallowed *C. pannosus* fruits whole, except for rose-ringed parakeets which broke seeds open with their beaks, and Cape turtle doves which did not eat the fruits presented to them. Seeds that were buried as whole fruits had the longest germination time (over 160 days), while manually de-pulped seeds and seeds that were ingested by birds took 80 days to germinate. Germination success for *C. pannosus* seeds was below 5% for all treatments. Many ingested seeds had not germinated after 300 days.

This research has shown that birds are *C. pannosus* fruit consumers and therefore seed dispersers, but unlike other invasive Rosaceae species such as *Pyracantha angustifolia*, ingestion by birds is not as important for breaking seed dormancy. Several results, including large fruit production,



Fruiting period of *Cotoneaster pannosus* over a fruiting season in three study sites located in the Clarens area, Free State Province, as determined by seed rain.



Cumulative *Cotoneaster pannosus* germination percentage for the period of the study for seeds ingested by Knysna Turaco (KT), Red-winged Starlings (RS) and Dark-capped Bulbuls (DB) together with seeds from manually de-pulped fruits (D-P) and seeds planted as whole fruits (WF)

dense seed rain and a persistent soil seed bank underneath shrub canopies, suggest that invasion success of *C. pannosus* relies mainly on the recruitment of seedlings near parent shrubs which results in thick monospecific stands. Furthermore, the seed dormancy and low germination ability of *C. pannosus* seeds mean that seeds dispersed into hostile environments remain viable until conditions are ideal for successful germination.

These findings suggest that if left uncontrolled, *C. pannosus*

will be a formidable invader, with the ability to spread within and into new ecosystems in South Africa. It is hoped that these findings will help facilitate the management of the species, especially if populations are small and detected at an early stage, and will indicate that biological control should be pursued.

For her PhD, Ms Moloi will pursue various ecological questions regarding the impacts of invasive Rosaceae in the grassland biome.

PROGRAMME HIGHLIGHTS IN 2022

- Ms Karabo Moloi received a distinction for her MSc entitled “Reproductive ecology of an invasive *Cotoneaster* species in Afromontane grasslands of the eastern Free State, South Africa”.
- The CBC started an International collaboration with Yunnan University, China to continue work on *Cotoneaster pannosus*.

Rosa rubiginosa

Despite the economic importance of *Rosa rubiginosa*, there have been ongoing studies investigating whether the species may be driving bush encroachment into the Grassland biome, as well as the seed ecology of the species, in an attempt to determine what might be limiting the spread of the species.

MSc candidate Patricia Masole has shown that *R. rubiginosa* seeds have very low seed germination (below 1% over

a year), but can survive long periods in the soil. In addition, the introduced rose seed gall wasp, *Megastigmus aculeatus*

RESEARCH TEAM

Dr Grant Martin, Prof. Sandy Steenhuisen (UFS-ARU)

STUDENT

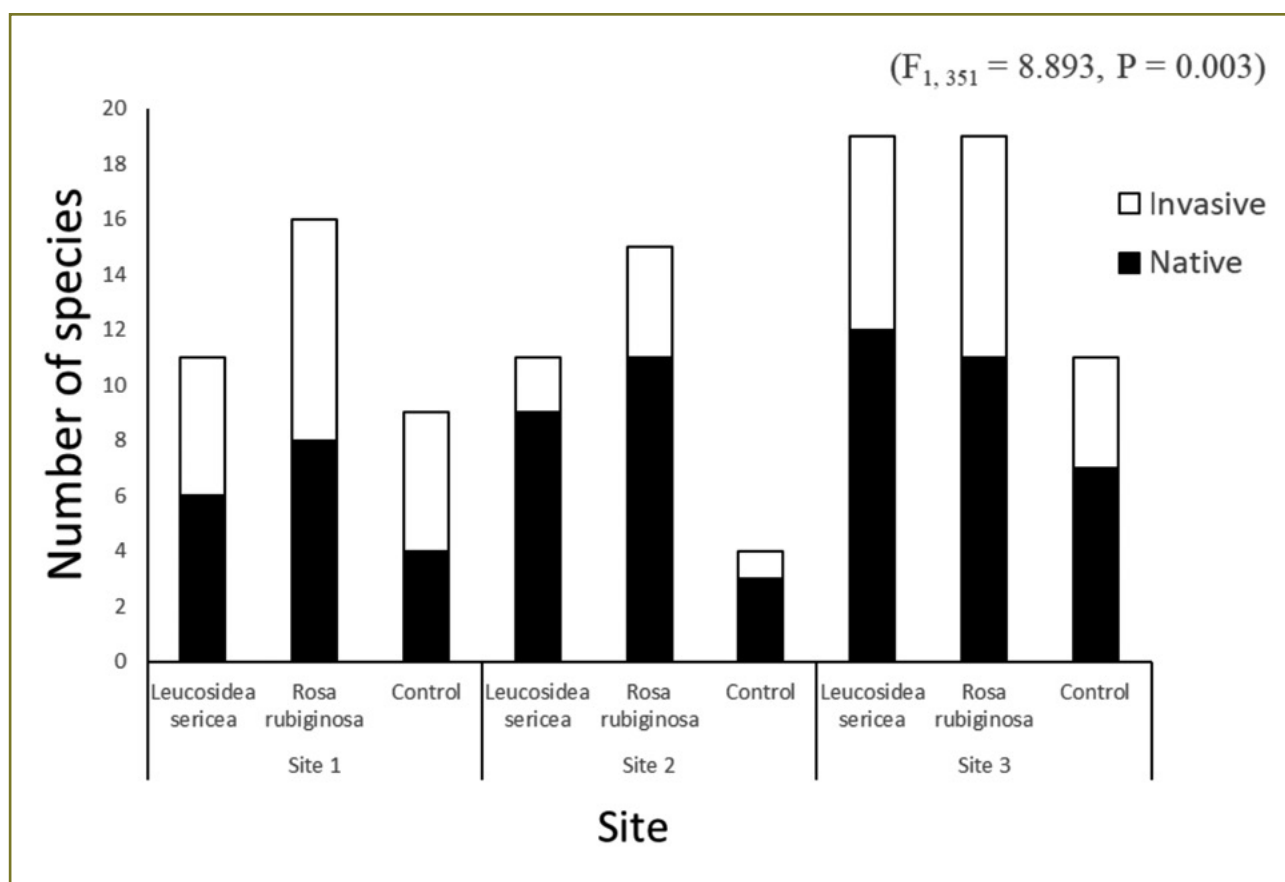
Patricia Masole (MSc)



Patricia Masole sieving soils searching for *Rosa rubiginosa* seeds. Photo: Grant Martin

Swederus (Hymenoptera: Torymidae), is widespread and abundant in South Africa, but is insufficiently damaging to impact the number of seeds entering the environment.

In a study investigating the ‘nurse plant effect’ of *R. rubiginosa* in the grassland biome, we were able to show woody species richness and abundance of invasive woody species were higher under *R. rubiginosa* than under the native *Leucosidea sericea* or in control plots of open grassland. The positive interaction observed conformed to the nurse plant hypothesis and is probably related to the mechanical protection of native and invasive saplings by *R. rubiginosa* thorny stems against grazing ungulates. This interaction and resulting protection facilitate rapid woody species encroachment into the South African grassland biome, a concern for agricultural and conservation sectors



Total richness of native and invasive species (number of species recorded) under each treatment (native Rosaceae - *Leucosidea sericea*, invasive Rosaceae - *Rosa rubiginosa* and the Control - open grassland) at each site

Willows

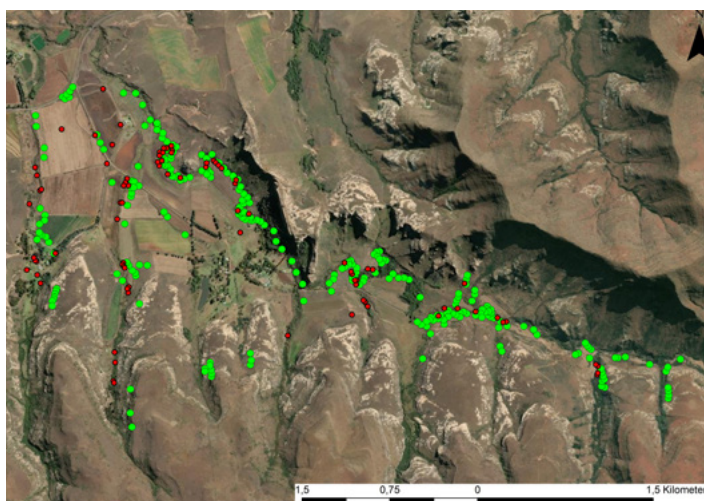
Salix species, commonly known as Willows, are a diverse group of dioecious trees and shrubs, native to Northern Temperate regions, but are invasive in many parts of the world, including the grassland biome of South Africa. *Salix babylonica*, the weeping willow, and *Salix fragilis*, the crack willow, were found to be the most prominent invasive species in South Africa's grassland biome in the 1970–1980s. The two species remain abundant and are currently classified as CARA regulations. An MSc study, conducted by Tapiwanashe Mashamba, is currently reassessing the population demographics of these two willow species in the Free State and northern Eastern Cape to determine if biological control should be pursued. Preliminary results reveal that ~85% of *S. babylonica* populations are dominated by adult mature trees showing signs of decreased vigour. In addition, analysis of historical imagery at select sample sites shows a decline in populations. Gender ratios suggest that the scarcity of saplings may be a result of the absence of male trees. In contrast, populations of *S. fragilis* are dominated by younger trees indicating a spreading population. Although *S. fragilis* should be considered for biological control, *Salix babylonica* should not.

RESEARCH TEAM

Dr Grant Martin, Prof. Sandy Steenhuisen (UFS-ARU)

STUDENT

Tapiwanashe Mashamba (MSc)



Records of *Salix babylonica* in 1970 (green occurrence records) compared to 2018 (red occurrence records) in the study site in eastern Free State

Impact of the Invasive Alien Plant Species Clearing Programme on Socio-economic Benefits and Plant Biodiversity along the Northern Drakensberg

Attempts to manage alien plant invasions in South Africa have a long history, going back well over a century. Since 1995, the South African Government has aimed to support both private and state landowners through the “Working for Water” (WfW) programme. The programme intentionally offered employment opportunities to historically disadvantaged people from rural areas, where unemployment is a significant problem. It also aimed to preferentially employ women and people living with disabilities. The programme has received both local and international recognition for its endeavours; however, it has also received its fair share of criticism.

In this study, conducted by MSc Candidate Nthableni Bologo, we aimed to determine the impact of historical WfW clearing programmes on the native plant diversity, the socio-economic benefits experienced by communities,

RESEARCH TEAM

Dr Grant Martin, Prof. Sandy Steenhuisen (UFS-ARU)

STUDENT

Nthambeleni Bologo (MSc)

and the perceived effectiveness of Invasive Alien Plant Management interventions by landowners along the northern Drakensberg, Mpumalanga Province, South Africa.

Vegetation surveys were conducted from January to April 2022 in grassland sites previously cleared by WfW teams and in paired nearby, uncleared control sites. The study showed that despite the clearing (20 years prior), biodiversity has not returned to its original levels as there were

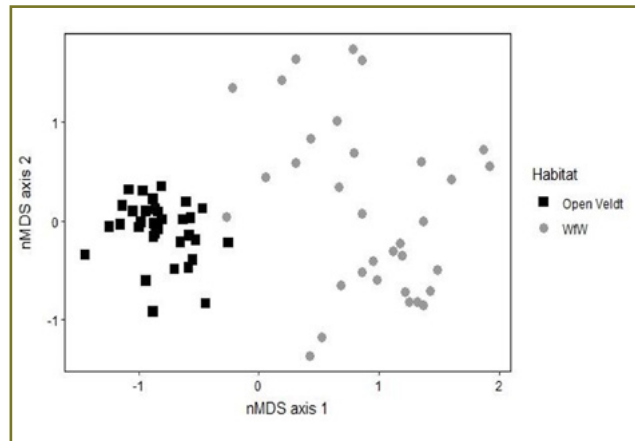
NORTHERN TEMPERATE WEEDS

significant differences in plant communities between cleared and uncleared sites with lower species diversity in cleared plots.

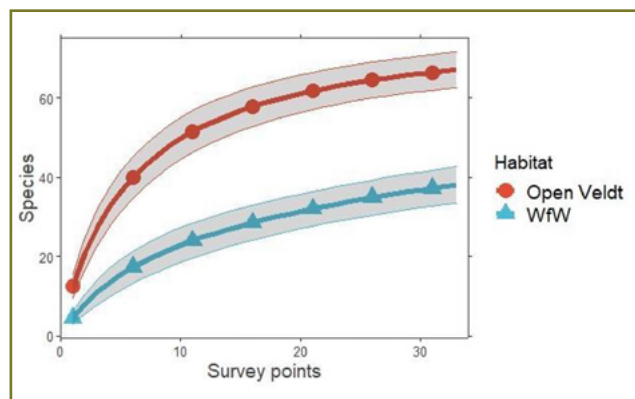
Questionnaires completed by over 70 WfW participants indicated that employees who have benefited from the programme are aware of invasive alien plant species and their impacts and would want to see improvement in the implementation of the project in future. The long-term implementation and impact of the programme on the employed community needs to be further assessed.



MSc candidate Nthableni Bologo collecting plant biodiversity data in a conservation area near the town of Graskop, Mpumalanga. Photo: Grant Martin



Above and below: Different vegetation communities between cleared and open veld (pristine grasslands) and the species richness between open veld and cleared sites



The Range X Project

The high elevation regions of South Africa have unique, specialised, and endemic plant species as the environment is harsh. However, predicted global climate change may open up this unique niche to both native and invasive “lowland” plants which will have a major impact on ecology, livelihoods, endemic alpine species, and water production in these high areas. To inform and guide policy, as well as management within these regions, it is important to understand the process of species migrating upwards as a result of climate warming. The Afromontane Research Unit (ARU) has partnered with the European Union’s Horizon 2020 initiative called ‘The Range X project’ which aims to investigate mechanisms underlying the success of and impacts on biodiversity and ecosystem functioning of range-expanding species under climate change, across the globe. The ARU component of Range X is funded by the Department of Science and Innovation through BiodivERsA. The CBC’s Northern Temperate Weeds programme is collaborating with ARU in this initiative, investigating how invasive *Rosacea* species might perform

RESEARCH TEAM

Dr Grant Martin, Prof. Sandy Steenhuisen (UFS-ARU), Prof. Ralph Clark (UFS-ARU), Dr Stephanie Payne (UFS-ARU), Dr Ona Gwate (UFS-ARU), Lesego Malekana

at different elevations under changed climate conditions.

A full-factorial warming experiment is underway using open top chambers (OTCs) placed on the top of Sentinel Peak (3100 m) and comparing them to OTCs positioned at lower altitudes down below near the Witsieshoek (2180 m). The OTCs are widely used to mimic global warming as they are very effective in elevating ambient temperatures by 1-2 °C. The CBC aims to determine how the invasive species and the native biodiversity respond to standard management practices at different altitudes by using matched plot designs and clearing large areas of woody invasives over an increasing altitude gradient. Post-doctoral candidate, Ona Gwate, has completed the first year of sampling with some very interesting results, one of which shows that the entire altitudinal range of the Drakensberg Mountain system is suitable for the establishment of a number of invasive *Rosacea*.

MSc candidate, Lesego Malekana, has completed the first season of sampling and clearing at three sites of different altitudes in the Maluti Mountains of South Africa, targeting a complex of woody invasive Rosaceae species, namely *Cotoneaster pannosus*, *Rosa rubiginosa*, *Pyracantha angustifolia*, and the native species, *Leucosidea sericea*. Time and continued monitoring will now tell if management will be increasingly needed at high elevation and if managing these populations will become harder (i.e., less effective) at high elevation, relative to lower elevation sites; and if the removal of plants causes a disturbance. Will native plants fill the gap, or will other aliens take advantage of this?

Being part of this global initiative should contribute to an understanding of the impact of climate change on invasive species in our mountain systems and provide insights on how it may affect management.

Right: Lesego Malekana completing the first season of field work at the lowest altitude site (1500 m.a.s.l.) in the Maluti Mountains of South Africa. Photo: Grant Martin



Above from left to right- Dr Grant Martin, Dr Ralph Clark (UFS-ARU), Dr Sandy Steenhuisen (UFS-ARU), Evelin Iseli (ETH Zurich), Georg Flückiger (ETH Zurich), Stephanie Payne (UFS-ARU), Dr Ona Gwate (UFS-ARU), , Alexander Jake (ETH Zurich) and Jamie Allison (ETH Zurich) at the open top chambers in Switzerland. Photo: Grant Martin

PROGRAMME HIGHLIGHTS IN 2022

- The whole ARU-Range X team was able to replicate sites in Switzerland while attending the International Mountain Conference 11–15 September 2022, Innsbruck, Austria.
- The first year of plant trait measurements was completed at all altitudes.
- All sites at all altitudes were cleared of invasive Rosaceae species.

INVASIVE TREES PROGRAMME



Biocontrol Agent
*Coelocephalapion
gandolfoi*

The Invasive Trees Programme has expanded this year to include Pines, along with the Australian *Acacias* and *Prosopis*.

The UCT Invasive Trees Programme team, Emeritus Associate Professor John Hoffmann, Fiona Impson, Catharina Kleinjan, and Emeritus Professor Cliff Moran have been part of the CBC Consortium since 2017. This team has many years of experience in the field of biological control of invasive trees and has developed strong collaborations with several international institutions.

The focus for the UCT team's research is on the biological control of several Australian *Acacia* (wattle) species, and also *Prosopis* (mesquite) originating from both South and North America. Australian *Acacias* and *Prosopis* are destructive invaders across large parts of South Africa. Both groups of trees are of some benefit as agroforestry plants, providing timber, fuel, fodder, and shade, but where they have become invasive, their negative impacts on biodiversity and water resources outweigh any benefits, and management and control of further spread of these species is necessary.

The commercial utilization of several *Acacia* species has largely restricted biological control to the use of agents that have no impact on the vegetative growth of these trees (i.e., agents that attack the buds, flowers or seeds). Despite this constraint, several agents are now well established

and curbing the invasiveness of the acacias, and efforts are underway to introduce additional, different species of agents.

While there has been some success with biological control of *Prosopis* to date, using two seed-feeding beetles, levels of control have not been enough to alleviate the problem. Engagement with stakeholders in the farming community created an incentive to expand the biological control programme to include agents that damage other parts of the trees. The Department of Agriculture, Land Reform and Rural Development granted permission for release of two additional agent species, which were released in 2021.

The project to target the cones of *Pinus pinaster* (pines) was reinitiated this year with support from all the affected stakeholders. The research team plans to build on the data collected in the early 2000s and develop potential biological control agents that target the cones of the invasive *Pinus pinaster*.

Despite severe funding challenges during 2022, progress has been made on the invasive tree projects. Collaboration with the Instituto Politécnico de Coimbra in Portugal, Tel Aviv University in Israel and FuEDEI in Argentina has continued, and a new contract with colleagues at Manaaki Whenua Landcare Research in New Zealand has been initiated.

Australian acacias

Following the successful releases of the flower galling midge, *Dasineura pilifera*, on *Acacia baileyana* and *Acacia dealbata* in 2021, sites were re-visited in September 2022 to assess the status of this agent. The persistence of *D. pilifera* was confirmed at two of the Western Cape sites, with one of the sites having a particularly well-established

RESEARCH TEAM

Emeritus Associate Prof. John Hoffmann, Fiona Impson, Catharina Kleinjan

COLLABORATORS

University of Tel Aviv, Israel; Instituto Politécnico de Coimbra and Centre for Functional Ecology, University of Coimbra, Portugal; FuEDEI, Manaaki Whenua - Landcare Research New Zealand

population, which is promising for future collection and re-distribution of *D. pilifera*. Unfortunately, establishment could not be confirmed at Eastern Cape release sites.

The application requesting permission to release the gall-forming wasp, *Perilampella hecataeus*, for biological control of *Acacia dealbata*, *A. baileyana* and *A. decurrens* which was submitted to the Directorate of Plant Health in the Department of Agriculture Land Reform and Rural Development in 2021 was approved. Dr Robin Adair (Australis Biological) collected a consignment of 10 kg of *P. hecataeus* galls in Australia in late November for shipping to the quarantine facility in Stellenbosch, South Africa. Unfortunately, the shipment of galls arrived in poor condition and no female wasps emerged for releases, so progress on this project will continue in the new year.

The collaborative project on *Acacia longifolia* with Manaaki Whenua – Landcare Research New Zealand progressed well during 2022. Following consultation and public participation, an application to import the bud-galling wasp, *Trichilogaster acaciaelongifoliae*, into New Zealand was approved, and a collection (10 kg) of *A. longifolia* galls containing larvae and pupae of *T. acaciaelongifoliae* was made in the Banhoek Valley, Stellenbosch for export. The galls were hand-carried to Christchurch, New Zealand, to the quarantine facility on 13 November. Direct release of the gall wasps depended upon approval from both the Environmental Protection Agency and Ministry of Primary Industries following the outcome of the disease testing. Despite some unexpected delays, release permission was finally granted and the first releases of *T. acaciaelongifoliae* took place on the 8 December 2022.



From top left (anti-clockwise): Fiona Impson on arrival with shipment in quarantine, New Zealand; Arnaud Cartier (quarantine manager) at Manaaki Whenua – Landcare Research quarantine facility in Lincoln; *Trichilogaster acaciaelongifoliae* galls within rearing cages; Richard Hill doing first releases of *T. acaciaelongifoliae* on North Island, New Zealand. Photos: Fiona Impson

Prosopis

During 2021, two additional biological agents were released on *Prosopis* spp. to supplement the activities of the seed-feeding beetles already present in South Africa.

The first of these is a leaf-tying moth, *Agnippe* sp. (formerly *Evippe* sp. #1), which was evaluated and deployed by colleagues at the ARC-PHP facility at Roodeplaat. This moth originates from Argentina but had already been deployed in Australia and its development as a biological control agent in South Africa was facilitated through collaboration with Dr Rieks van Klinken, CSIRO, Australia. Additional releases and monitoring occurred during 2022 with the involvement of CBC consortium members.

The second new agent is a weevil, *Coelocephalapion gandolfoi*, which feeds and oviposits in immature pods where larval development takes place. These beetles were originally released at the start of summer in 2021. Evidence of their presence was observed at the end of the 2021/22 summer, but persistence across winter has not yet been confirmed.

MSc student, Gretha van Staden, researched a community of practice (CoP) to promote the use of biological control, and the development and implementation of integrated management plans for *Prosopis*. Her study site was in the Northern Cape where she reached out to farmers in various

RESEARCH TEAM

Emeritus Associate Prof. John Hoffmann, Fiona Impson, Catharina Kleinjan, Philip Ivey

COLLABORATORS

Gretha van Staden (MSc)

COLLABORATORS

Dr Fernando McKay, FuEDEI

ways, sharing information with them about *Prosopis* and ways to utilize and manage the plant. Gretha's research concluded that establishing a CoP with the aid of a local champion to both coordinate the flow of information and initiatives (such as the initial release of biological control agents) sustains the interest in integrated *Prosopis* management. The CoP structure also fosters trust and cooperation between diverse stakeholders and researchers. Having a local champion to coordinate this provides the project with a recognisable figurehead and increases stakeholders' will and ability to communicate concerns and ideas. The champion also ensures that communication from the project and researchers reaches the stakeholders and broader community. Continuing with projects such as this will not only improve the implementation of biological control science, but also improve agricultural practices and food security. Addressing *Prosopis* in the Northern Cape is an action in support of future generations, in accordance with stewardship and practices that support biodiversity in natural rangelands.



Carien Kleinjan, Elmarie Hoft, Ben de la Fontaine and Hennie le Roux (manager of the facility) accepting an *Agnippe* consignment from the ARC in November 2022. The consignment was released at the Witsand site where evidence of persistence of *Agnippe* from previous release was present. Photo: Elmarie Hoft.

Pines

RESEARCH TEAM

Dr Grant Martin, Dr Gerald Chikowore,
Jufter Musedeli

COLLABORATOR

FABI

Of the many pine species introduced in the early 1800s, nine are recognized as invasive alien plants (IAPs) in South Africa. These invasive pines have been shown to decrease native biodiversity, driving endemic species to extinction; deplete valuable water resources; cause extensive environmental degradation, and exacerbate the impact of wildfires. The current approach to tackling invasive pines involves intensive training and deployment of implementers, grouped into teams across the landscape to control these plants. These teams access lower elevations, while specialised high-angle, high-elevation and helicopter teams clear remote mountain tops and steep cliffs. Unfortunately, these extensive manual and mechanical control methods are costly, time-consuming and insufficient to prevent the spread of the pines. The CBC is re-investigating biological control as a cost-effective, sustainable and effective method for the control of European pine species in South Africa. Research will focus on the seed production of the species.

Suggestions that one or more of several species of cone-feeding insects might be introduced into South Africa for biological control of invasive pine species were first considered in the 1980s. However, nothing was done to pursue this approach in South Africa until funding was made available through the Working for Water Programme (WfW) of the South African Department of Water Affairs in 1997. Four research organizations collaborated on the programme: in South Africa, the University of Cape Town, and the Agricultural Research Council Plant Protection Research Institute; and, in France, the Institut National de la Recherche Agronomique (INRA), and the University of Orleans. This grouping provided the facilities and skills needed to: (i) undertake surveys of the appropriate species of pines throughout Europe; (ii) resolve the taxonomic



Researchers from the The Nature Conservancy monitoring the impacts of invasive European Pines in the Western Cape Province. Photo: Richard Bugan

status of potential agents, and (iii) conduct host-specificity and pathogenicity tests on potential agents in the field in Europe and in quarantine in South Africa.

Significant progress was made to show that the cone weevil, *Pissodes validirostris*, is specific to only three hosts, *P. pinaster*, *P. halepensis* and *P. pinea* and that it may prove to be a valuable biological control agent. Unfortunately, in 2009, a decision was taken by the biocontrol researchers in South Africa to suspend work on biological control on pines due to concerns that the weevil might facilitate the spread of the recently discovered presence in South Africa of pine pitch canker, *Fusarium circinatum*. The negative impact of *Pinus pinaster* invasions and the change in its status as a commercial forest species, as well as new information on spread of pine pitch canker, suggests that biological control of pine cones requires renewed attention.

The CBC is hoping to work with affected parties to try find a suitable solution to assist in the management of the seeds of European pines in South Africa.

BUGWEED



Bugweed (*Solanum mauritianum*), which is originally from subtropical South America, is invasive over large parts of South Africa. Two insect biological control agents, the lace bug, *Gargaphia decoris*, and the flower-feeding weevil, *Anthonomus santacruzi*, were released in 1999 and 2008, respectively. Both agents have established in the field where they can be damaging, but this is mainly limited to low altitude sites. Prominent bugweed invasions also occur in high-altitude regions within South Africa (> 1000 m) that experience cold winters; however, owing to climatic incompatibility, the agents have not established in these regions. Consequently, during 2020, an additional agent, the flower-feeding weevil, *Anthonomus morticinus*, was collected from Uruguay's temperate regions that are climatically similar to the cooler high-altitude regions of South Africa where bugweed remains a problem. Laboratory thermal assessments indicate that *A. morticinus* appears better adapted to lower temperatures than its congener, *A. santacruzi*. Unfortunately, humidity trials indicate that its lower humidity tolerance is not dissimilar to its congener, *A. santacruzi*. Nevertheless, *A. morticinus*' ability to withstand lower temperatures is encouraging.

Host-range testing of *A. morticinus* is ongoing with promising results. Relative to *A. santacruzi*, *A. morticinus* appears to exhibit an equal or narrower host range on commercially grown Solanaceae. Taxa from the Solanaceae family are important agricultural plants grown for human consumption, thus it is important that any potential bugweed biocontrol agents do not exhibit non-target effects on these plants. Research continues to test *A. morticinus*' host range on Solanaceae species that are native to South Africa, and results thus far show promise.

In addition, Manaaki Whenua – Landcare Research in New Zealand is collaborating with Wits University to conduct host-range testing of *A. morticinus* on the two Solanaceae species indigenous to New Zealand. Testing is underway at the Wits Quarantine Facility.

RESEARCH TEAM (WITS):

Prof. Marcus Byrne, Nic Venter, Dr Blair Cowie and Dr Samalesu Mayonde (Postdocs)

STUDENTS:

Vusumuzi Mkhomazi (MSc, Wits), Yaron Keizan (MSc, Wits)

COLLABORATORS:

Prof. Terry Olckers, UKZN, Dr Angela Bownes, Landcare Research New Zealand, Dr Simon Fowler Landcare, NZ, Hugh Gourlay Landcare, NZ, Dr Lindley Hayes, Landcare, NZ



Yaron Keizan (MSc candidate) surveying bugweed plants in George, Western Cape for *Anthonomus santacruzi* establishment in August 2022. Photo: Nic Venter

TAMARIX



Coniatus tamarisci weevil feeding on *Tamarix ramosissima* foliage in the Wits Quarantine facility, Johannesburg.
Photo: Nic Venter

Tamarix chinensis and *T. ramosissima* (Tamaricaceae), native to Eurasia, are Category 1b invaders that become dominant shrubs or trees in riparian ecosystems, mainly in the central to western half of South Africa. A research programme was initiated in 2014 at Wits University to explore the feasibility of using biological control against these species. However, *Tamarix usneoides*, which is native to South Africa, has created additional challenges in finding host-specific biocontrol agents. While the invasive *Tamarix* and the indigenous *T. usneoides* have slightly different distributions, they do co-occur. The invasive *Tamarix* are largely distributed in the cooler, wetter interior of the Eastern and Western Cape Provinces while *T. usneoides* grows in semi-arid regions with low annual rainfall and occurs widely in the Northern Cape (and Namibia), and in some parts of the Western Cape Province. Additionally, the two invasive species and the indigenous *T. usneoides* readily hybridize to form three additional hybrid genotypes. Interestingly, the Swart River in Prince Albert/Western Cape and the Groot River/Eastern Cape are the only localities known where all six *Tamarix* genotypes co-occur (the three parental species and their respective hybrids). Abundance measurements of the invasive *Tamarix* in the Eastern and Western Cape provinces show that the weed continues to spread. Hence, management interventions are required, of which biological control offers the best long-term prospects.

The Wits' research has to date rejected the leaf-feeding beetle, *Diorhabda carinulata*, and the scale insect, *Trabutina mannipara*, as potential biological control agents of alien *Tamarix* owing to non-target feeding on native *T. usneoides*. In collaboration with BBICA Onlus in Rome, Italy, the leaf-feeding weevil, *Coniatus tamarisci*, was imported into the Wits Quarantine in October 2020. Research is currently underway at the Wits University Quarantine Facility to test the host-specificity of *C. tamarisci*. Thus far, the weevils have been tested in small-scale, petri-dish trials against the range of *Tamarix* genotypes they will encounter in the field in South Africa. The

RESEARCH TEAM (WITS):

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results so far are encouraging, suggesting some level of feeding discrimination against the indigenous *T. usneoides*. Currently, the weevils are being tested using whole trees in quarantine, by tracking the weevils' behaviour through from oviposition to complete larval development. In addition, differences in secondary metabolites between the *Tamarix* genotypes are being investigated to explore the chemical signals that herbivorous insects use to select between potential host plants. Studies on other weeds have shown that insect host-plant selection can be influenced by the secondary metabolites of the potential host plant. Being from different taxonomic clades suggests that the indigenous and alien species should differ in their secondary metabolite profile and help guide the selection of potential biocontrol agents.

An allied project, in collaboration with the Centre for Invasion Biology at Stellenbosch University, is exploring the value of satellite-based remote sensing to map the national distribution of the different *Tamarix* genotypes. If successful, it will provide a valuable management tool to monitor the progress of any biocontrol agent eventually released against the weed. Results to date have shown that the different genotypes can be distinguished from their hyperspectral signatures, using an array of colours reflected from the trees' foliage. The objective is to now train analysis programmes to extend that differentiation to satellite images of whole stands of the trees.



Above: Dr Blair Cowie (Wits University) (right) and Dr Flaminia Mariani (BBCA, Italy) surveying insects found on *Tamarix* species near Rome, Italy in 2022. **Photo:** Dr Massimo Cristofaro



Above: Blair Cowie with 100s of *Coniatus* collected in Talamone, Tuscany. **Photo:** Massimo Cristofaro



Above: An aspirator full of *Coniatus* weevils collected in Rome. **Photo:** Massimo Cristofaro



Right: Blair Cowie sampling *Tamarix* trees for weevils along the coastal dunes of Borgo Grappa. **Photo:** Massimo Cristofaro

INTERNATIONAL WEED PROJECTS



Crystalline ice plant showing enlarged bladder cells filled with hypersaline solution

African boxthorn

African boxthorn (*Lycium ferocissimum*) is a noxious weed in Australia that is native to southern Africa. In collaboration with CSIRO, Australia, the CBC investigated potential biological control agents for the plants with the ultimate goal of reducing the negative consequences of this weed in Australia. Since 2017, the CBC staff and students at Rhodes University have conducted native-range surveys for potential biological control agents in the Eastern and Western Cape provinces of South Africa. Over this period, a number of herbivorous arthropods were collected and identified. Four insect species (two ladybird beetle species, *Cleta eckloni* and *Cleta sp1*; a tortoise beetle, *Cassida distinguenda*; and a flea weevil, *Neoplatygaster serietuberculata*) and one pathogen species (rust fungus, *Puccinia rapipes*) showed the most potential as biological control agents owing to their impact and specificity to their host, *L. ferocissimum*.

In addition to field observations, host-specificity testing was also conducted at both the CBC and CSIRO (Australia) research facilities. No-choice experiments showed that *Lycium australe*, an Australian native plant that grows in places where *L. ferocissimum* has invaded in Australia, is only weakly to moderately fed upon and reproduced by all four insect candidate agents. Choice trials, however, showed that these insects preferred to feed on their original host, and there was very little oviposition

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and reproduction on *L. australe* when *L. ferocissimum*. Unfortunately, this minimal risk was still deemed too high and the programmes investigating *C. eckloni*, *Cleta sp1* and *N. serietuberculata* were halted in 2021. Over the last year (2022), there has been a greater emphasis on plant host-specificity testing for *C. distinguenda*. Multi-choice and multi-generational tests are currently being carried out at CSIRO Australia to determine if this non-target feeding on other *Lycium* species is incidental, exploratory, or forced, or if the beetle can successfully live for multiple generations on the non-target *Lycium* species.

In contrast to the insect agents, there were more promising results for the rust fungus which appears to be suitably host specific. Comprehensive tests have been done in Australia, and *L. australe* (and several plants of economic value) has been shown to be resistant/immune to infection by the rust fungus. The fungus has now been approved for release in Australia and initial post-release evaluations are being carried out to assess establishment. The funding

PROGRAMME HIGHLIGHTS IN 2022

- Three shipments of *Cassida distinguenda* were exported to Australia
- An Australian indigenous *Lycium* species, *Lycium australe*, was imported into South Africa, in the CBC quarantine for multi-choice host-specificity testing
- One survey for stem-mining insects was conducted in the Western Cape Province
- Rust fungus released in Australia

INTERNATIONAL WEED PROJECTS

of this project in South Africa has now come to an end, but the CBC remains in collaboration with CSIRO in the further development and testing of candidate agents already imported into Australia.



Above: Fruits and flower of *Lycium ferocissimum*



Above: Rust fungus (*Puccinia rapipes*) collected from the native range of *Lycium ferocissimum*. The rust has been tested, approved for release, and released in Australia to control the plant. Credit: Alan Wood



Above: Paired choice experiment to investigate host preference of one of the candidate biological control agents, *Neoplatygaster serietuberculata*, between *Lycium ferocissimum* and *Lycium australe*.

Crystalline Ice Plant

Crystalline ice-plant, *Cryophytum crystallinum*, is one of the over 1700 species of vygies (plants in the family Aizoaceae) that are endemic to southern Africa. There are a few species from the family that are indigenous elsewhere in the world, but the vast majority (over 96%) of the family only occur naturally in southern Africa, and diversity is particularly high on the west coast of South Africa. Crystalline ice-plant is an invasive alien plant in California (USA) and in Mexico. It forms dense monocultures in coastal areas and is very damaging on offshore islands, which are vulnerable to invasions and have unique biodiversity. Crystalline ice-plant appears to be covered in crystals because it is covered by large bladder cells that hold a hypersaline solution that the plant accumulates from its immediate surroundings. When the plant dies, this leaches into the topsoil, making it too salty for any other plant species besides *C. crystallinum* to germinate. Indigenous plants and animals in the USA and Mexico are therefore excluded from areas where crystalline ice-plant has invaded, and several rare indigenous plants and animals are threatened by the infestations.

The CBC has been working in collaboration with the United States Department of Agriculture to develop a biological control programme against this invasive alien species. The first step of the programme was to determine where the weed populations found in North America originated. Crystalline ice-plant is abundant in parts of Europe, and it was not clear whether South Africa or Europe was the correct place to look for potential agents. A genetic study was conducted by Caitlin Webb, an MSc student at the CBC, to elucidate the invasion history. She discovered that the most likely pathway of introduction to California was from the west coast of South Africa, through Europe where *C. crystallinum* is an abundant alien species, and then to North America. Caitlin has also conducted preliminary host-specificity testing on one of the promising potential agents, a stem-mining weevil called *Lixus carinerostris*. The weevil feeds on crystalline ice-plant and

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close relatives within the same sub-tribe, but is clearly a *Cryophytum* specialist. There are so few Aizoaceae that are indigenous or of economic importance in North America that it is very likely that the stem-mining weevil is suitably host specific to be released as a biocontrol agent in the USA.

Lixus carinerostris will be exported to the USDA-ARS quarantine facility in San Francisco where it will undergo further host-specificity testing. While these tests are being conducted, the CBC will continue to look for new agents and conduct preliminary host-specificity tests on those that are promising. A root-girdling weevil, *Calodemas prolixus*, is the next potential agent that will be subjected to host-specificity testing. Hopefully these agents can be released in North America and will reduce *C. crystallinum* densities to protect the indigenous diversity along the south-west coast of the continent.



Above: The stem-mining weevil on a flower.

Inset: Lenin Chari collecting Ice Plant on the West Coast. Photos: Iain Paterson

GRASSES



Biological control of invasive alien grasses has only been implemented on four species globally. The limited resource allocation to grass biocontrol projects largely stemmed from concerns over feasibility and the likelihood of success. However, in recent years, there has been an increasing awareness of the need to target alien grasses, given their major environmental and socio-economic impacts. This has resulted in greater investment in grass biocontrol research.

The CBC established an alien grass team that has initiated several novel grass biocontrol projects. Many of the most problematic invasive grasses are native to South Africa, and the CBC has been in an excellent position to develop biocontrol programmes against these species. Evidence from these projects is increasingly showing that prospects for grass biocontrol are good, whereby target grasses have been found to support damaging and host-specific natural enemies. Continued assessments are anticipated to lead to the roll-out of biocontrol on a number of new alien grass targets.

PROGRAMME HIGHLIGHTS IN 2022

- Two *Tetramesa* species that are prospective biocontrol agents for *Sporobolus pyramidalis* and *S. natalensis* were sent to quarantine in Brisbane in November 2022 for final host-specificity testing.
- Native range surveys for *Megathyrsus maximus* in Kenya and South Africa have identified several prospective biocontrol agents.
- *Tetramesa* wasps were found on *Andropogon gayanus* from the Great Dyke in Zimbabwe, in 2022. These insects are known to be monophagous across a variety of grass species and therefore are promising as biocontrol agents.
- The potential *Tetramesa* biocontrol agents found in each grass project are all undescribed species. Consignments of the wasps have been sent to an expert taxonomist in Iran, Prof. Hossein Lotfalizadeh, to obtain a formal description of the wasps. These will represent the first new *Tetramesa* species described from Africa since the 1950s, and the first representatives of this genus from southern Africa.

Giant Rat's Tail Grasses

The giant rat's tail grasses, *Sporobolus pyramidalis* and *Sporobolus natalensis*, are two African grasses that invade rangelands and pastures in eastern Australia, costing the livestock industry approximately AUS\$ 60 million per annum in grazing losses. The CBC has been evaluating prospects for biocontrol of these species since 2017. Surveys across South Africa have identified three stem-boring *Tetramesa* wasps (Hymenoptera: Eurytomidae)

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Queensland Department of Agriculture and Fisheries, Australia (Tamara Taylor)

that appear to attack only *S. pyramidalis* and *S. natalensis*, despite surveying more than 70 other non-target grass species. Two of these wasps are also damaging, reducing seed production and plant survival. The two damaging species, *Tetramesa* sp. 1 and *Tetramesa* sp. 2, have been tested against >20 non-target grasses in a greenhouse using no-choice tests, which confirmed that the wasps are host specific. The Covid-19 pandemic prevented the importation of both species into quarantine in Australia in 2020 and 2021. However, in September 2022, both wasps were imported into quarantine at the Queensland Department of Agriculture and Fisheries in Brisbane, Australia. Despite

an initial flush of wasps emerging, there were insufficient numbers of wasps that emerged from this consignment to establish a viable culture. Several valuable lessons were learnt with regard to the conditions required to rear these wasps under quarantine conditions. A second consignment of wasps was sent to Brisbane in November 2022. The team at Queensland DAFF are currently working on this consignment to establish viable cultures of the two *Tetramesa* species. Once these wasps are established in quarantine, they will be tested for their ability to utilise native Australian *Sporobolus* species to further scrutinise their potential as biocontrol agents.

African Lovegrass

African lovegrass, *Eragrostis curvula*, is another African grass that has become highly problematic in Australia, particularly in New South Wales (NSW). Dense infestations can reduce grazing capacity by over 50% and farm profitability by 25%. In 2019, the CBC initiated preliminary surveys across southern Africa in search of potential biocontrol agents, in a collaboration with NSW Department of Primary Industries. Surveys conducted in 2021 on *E. curvula* and other closely related grasses (particularly other *Eragrostis* species) identified two promising candidate control agents, both undescribed *Tetramesa* spp., whose larvae feed within the grass culm. Subsequent surveys, however, have recorded both *Tetramesa* spp. from other native South African *Eragrostis* species growing sympatrically with *E. curvula*.

Both *Tetramesa* spp. have been host-tested on more than 20 non-target grasses under greenhouse conditions in South Africa. These tests confirmed that both wasp species were able to complete development and produce viable progeny on at least two native South African *Eragrostis* species. The non-target grasses used by both *Tetramesa* species in the greenhouse trials are very closely phylogenetically related

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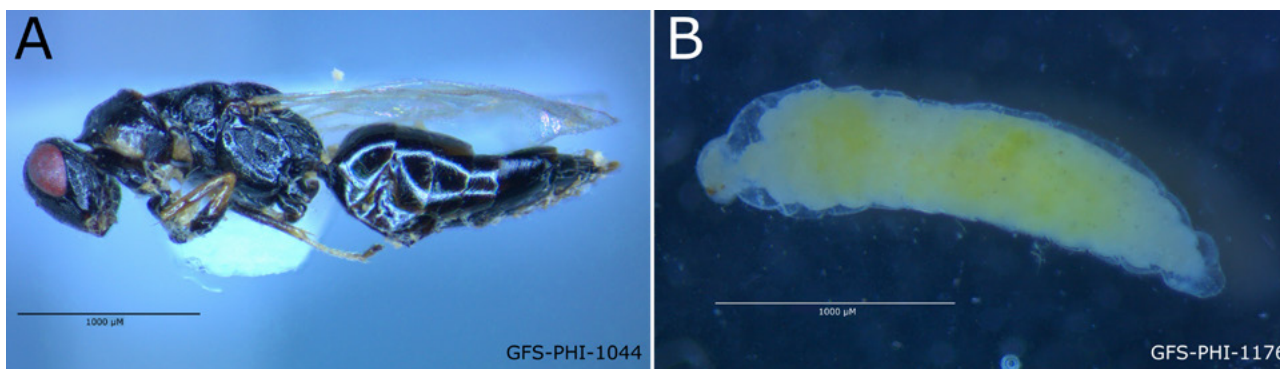
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to *E. curvula*. As such, additional host-specificity testing is currently being performed under quarantine conditions in South Africa to screen the wasp's ability to utilize native Australian *Eragrostis* species.

Guy Sutton and collaborators from NSW-DPI (Dr Andrew McConnachie and Mr Muhammed Nawaz) carried out surveys across the invaded distribution of *E. curvula* in New South Wales, South Australia, and Victoria in September 2022. Surprisingly, an abundant and widespread stem-boring wasp was recorded on *E. curvula* from every field site visited in New South Wales and Victoria. The wasp has been preliminarily identified as a *Tetramesa*



A) Adult stem mining insect, *Tetramesa* sp. and B) larvae of the *Tetramesa* sp. collected on *Eragrostis curvula* from field surveys in New South Wales, Australia. Photo: Clarke van Steenderen

sp., however, genetic barcoding is currently underway to confirm the identification of this wasp species. This raises an interesting question: is the wasp we found in Australia the same species that attacks *E. curvula* in South Africa, or is this wasp native to Australia? The answer to this question has important implications for the biocontrol programme going forward. If it is the South African wasp, additional

host-specificity testing in South Africa is probably unnecessary. An assessment of the distribution, density and impact of the wasp found in Australia is an important next step for this project, as the wasp appears to be particularly damaging in the field and could potentially be mass-reared and inundative releases performed to improve the control of *E. curvula* invasions in Australia.

Guinea Grass

Megathyrus maximus is a perennial African grass that has been introduced in many regions outside of its native distribution for pasture development. It has become a serious environmental pest in Texas, USA, where it reduces grazing capacity, alters natural fire regimes and negatively affects native wildlife populations. Previous biocontrol surveys had been performed in West Africa and Kenya, but DNA samples provided by the CBC indicated that Guinea grass populations in Texas probably originated from southern Africa. This resulted in the CBC initiating a collaboration with the University of Texas in 2019 to identify potential biocontrol agents for Guinea grass from southern Africa and continue surveys in Kenya (below). These surveys have identified several possible biocontrol agents, including two stem-galling midges (Cecidomyiidae) and a stem-boring

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wasp (*Tetramesa* sp.; Eurytomidae). All three species are currently undescribed species, so we are working with expert taxonomists in Iran (Hossein Lotfalizadeh) and Australia (Peter Kolesik) to get these insects formally described.



The CBC team (Guy Sutton and Iain Paterson) and University of Texas collaborators (Aaron Rhodes, Ivy Ngiru, Aimee Gaitho, Godfrey Gitimu) surveying for insects on *M. maximus* with the research team at Mpala Research Station in Kenya. **Photo:** Rob Plowes.



(Above left) Adult female cecid, (above right) Stem gall on *M. maximus* with emergence hole. Photo: Iain Paterson

The first midge species, which is currently undescribed, has been recorded in abundance across many field sites in both South Africa and Kenya, and forms conspicuous galls on the stems of *M. maximus* (photos above). Preliminary field surveys indicated that this midge species appears to utilize only *M. maximus* under field conditions in both Kenya and South Africa, despite surveying a range of closely related and structurally similar grasses in both regions, so indicating a high degree of specificity. The galls appear to act as a nutrient sink, depleting the plant of its resources and preventing it from producing seed. We are currently trying to establish cultures of this midge in the laboratory at the CBC facilities, and under greenhouse conditions in the KwaZulu-Natal Province for host-specificity testing. Surveys in KZN at various points throughout the 2021/2022 summer have shown that this midge is heavily parasitised, with parasitism rates increasing over the summer season, from approximately 80% parasitism in early summer, rising to 99% parasitism in late summer. The high parasitism rate is a positive sign for biocontrol because the parasitoids are unlikely to be present outside the native distribution of the midge. This bodes well for its use as an agent outside its native distribution, such as in Texas, where it may escape parasitism pressure and reach higher population densities.

The second midge, which will soon be described as a new genus, was collected by Iain Paterson, Rob Plowes and Aaron Rhodes in the Arabuko Sokoke State Forest in Kenya in June 2022. The midge forms dense clusters of galls (photo right) at the base of the plant, which appear to prevent the plant from growing new stems. Searches of sympatric non-target grasses at Arabuko Sokoke State Forest indicated that this midge is likely

host-specific to *M. maximus*, as it was not recorded on any closely related grass species in the area. The galls are heavily parasitised by solitary and gregarious parasitoids alike, which again, bodes well for biocontrol, because the parasitoids are unlikely to be present outside the native distribution. If it is used as an agent outside of the native distribution, such as possible release in Texas, it may escape parasitism pressure and reach higher population densities.

The third and final candidate agent is a stem-boring wasp belonging to the *Tetramesa* genus. *Tetramesa* wasps are grass-feeding specialists that are typically host-specific

and very damaging to their host grasses. The undescribed *Tetramesa* species has only been recorded from three field sites in South Africa to date, two sites in KwaZulu-Natal Province (Durban and Hluhluwe) and one site in the Eastern Cape Province near East London. Wasp densities on *M. maximus* are much lower than the *Tetramesa* found on other South Africa grasses to date, and the wasp is not as widely distributed. Three unsuccessful attempts have been made to establish a culture of the wasp under greenhouse conditions in KZN. Additional surveys and field collections across South Africa are currently being performed to understand more about the distribution and biology of the wasp, which will assist in establishing a viable culture and assessing this species as a biocontrol agent. Surveys for additional candidate agents in both Kenya and South Africa are planned for 2023, as well as host-specificity testing on all three candidate agents.



Typical clustered gall morphology of the second gall-midge collected at Arabuko Sokoke State Forest in Kenya. Photo: Iain Paterson.

Gamba Grass

Gamba grass (*Andropogon gayanus*), a noxious weed in Australia, is native to tropical and subtropical savannas of Africa. Although the grass is highly invasive in northern Australia, it remains an economically important foraging grass for livestock. The negative impacts of the grass in Australia include reducing indigenous biodiversity and disrupting fire regimes, and therefore, ecosystem functioning.

Since January 2018, the CBC has been conducting surveys for phytophagous insects associated with gamba grass across a portion of the grass's native distribution in South Africa. To increase the likelihood of identifying host-specific insects, a wide range of sites in protected areas, disturbed roadside habitats, and across different climatic zones and during different seasons was investigated. The aim of these surveys was to determine the feasibility of biocontrol as a strategy for managing the grass. It was anticipated that the damage inflicted by stem-mining insect species could weaken the stem of gamba grass and reduce the environmental risk from fires in Australia but still allow the grass to be used for grazing.

Numerous sites surveyed between 2018 and 2021 showed evidence of insect exit holes in the stems of the grass, which suggested the existence of insects that could damage the stems. More than 65 insect species associated with the grass were identified, but none of them showed any biocontrol potential. In Zimbabwe in 2022, four members of the

RESEARCH TEAM

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Tetramesa genus — known to be monophagous across a variety of grass species — were collected from a single location along the Great Dyke, a huge mineral deposit that is important to the nation's economy. The identity of these specimens was confirmed using genetic techniques.

Going forward, the project aims to collect infested grass from more areas within the grass's native range, including Benin, Kenya, Nigeria and Zimbabwe. South Africa appears to be on the periphery of the plant's indigenous distribution, and is therefore probably not the ideal area to survey for promising potential agents. The presence of *Tetramesa* at the Zimbabwean site is a strong indication that Zimbabwe could produce a greater abundance of stem-mining insects such as *Tetramesa*, and any future surveys should target this unexplored geographic region, that is well within the grass' native range, rather than on the periphery of it. The identification of a *Tetramesa* species associated with gamba grass in Zimbabwe is promising for the prospects for biocontrol of this species. The CBC hopes to conduct work in Zimbabwe and could collaborate with the University of Zimbabwe on this project in the future.

(Inset)
Herbivorous,
stem-mining
insect, *Tetramesa*
sp., collected
from *Andropogon*
gayanus growing in
Mvurwi, Zimbabwe.
Photo: Clarke van
Steenderen



Gamba grass
tussock in Mkhuze
Nature Reserve,
KwaZulu-Natal,
South Africa.
(right) Photo: Lenin
Chari

Nassella spp.

The CBC, in collaboration with the Afromontane Research Unit and the Centre for Invasion Biology, supervised PhD student, Anthony Mapaura who investigated three alien grass species, *Nassella trichotoma*, *N. tenuissima* and *N. neesiana* that are invasive in South Africa's montane grasslands. The status of the *Nassella* species was explored by assessing their current distributions, potential spread under predicted climatic changes, and impacts on soil and plant diversity. The *Nassella* species were found to be more widely distributed than previously known and are predicted to continue expanding their ranges. The species were also found to negatively impact native plant diversity

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Anthony Mapaura (UFS)

and soil conditions. All three *Nassella* species present a serious threat to South African grasslands and this work played an important role in guiding future management strategies, including biocontrol.



Pasture lands invaded by *Nassella trichotoma* on the Boschberg near Somerset East, Eastern Cape province, South Africa.
Photo: Anthony Mapaura



Top left: Thembelihle Mbele with a *Cortaderia jubata* plant in Daleyreville, North West Province, South Africa. Below left: *Cortaderia selloana* in Khathu, Northern Cape Province, South Africa. Right: *Cortaderia jubata* in North West Province, South Africa. Photos: Thembelihle Mbele and Kim Canavan

Pampas

The CBC, in collaboration with the University of the Free State and SANBI, will supervise an MSc project on *Cortaderia* spp. in South Africa, led by Thembelihle Mbele. The project will assess the current status of the two alien invasive pampas grasses in South Africa, *Cortaderia selloana* and *C. jubata*. These pampas grasses are both Category 1b in the National Environmental Management: Biodiversity Act (NEMBA) and cultivating or trading them are prohibited. However, they have become increasingly popular in horticultural trading, particularly as ornamental plants for décor. This project will assess their phylogeography to confirm the distribution of the species and potential source areas of introduction. Seed germination trials will also be conducted to explore the seed viability in traded

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SANBI

inflorescences and naturalised populations. Thembelihle has completed a Project Development Internship through SANBI and plans to commence her Master's degree in 2023.

Tetramesa as grass biological control agents

Prior to the start of the grass biocontrol programmes initiated by the CBC in 2017, no *Tetramesa* species were formally described from sub-Saharan Africa. This made developing *Tetramesa* as possible grass biocontrol agents very difficult, as the adults and larvae are morphologically extremely similar, making telling different species apart, and thus assessing their host-range and biocontrol potential, almost impossible. PhD student, Clarke van Steenderen, has worked on developing molecular techniques to help distinguish between more than 120 *Tetramesa* specimens collected from grasses across South Africa and Zimbabwe. To date, Clarke has generated mitochondrial COI and nuclear 28S genetic sequences from 19 grass species across South Africa (and one from *Andropogon gayanus* in Zimbabwe). The nuclear gene (28S) has proved to be very useful for confirming whether these wasps belong to the *Tetramesa* genus (Figures below).

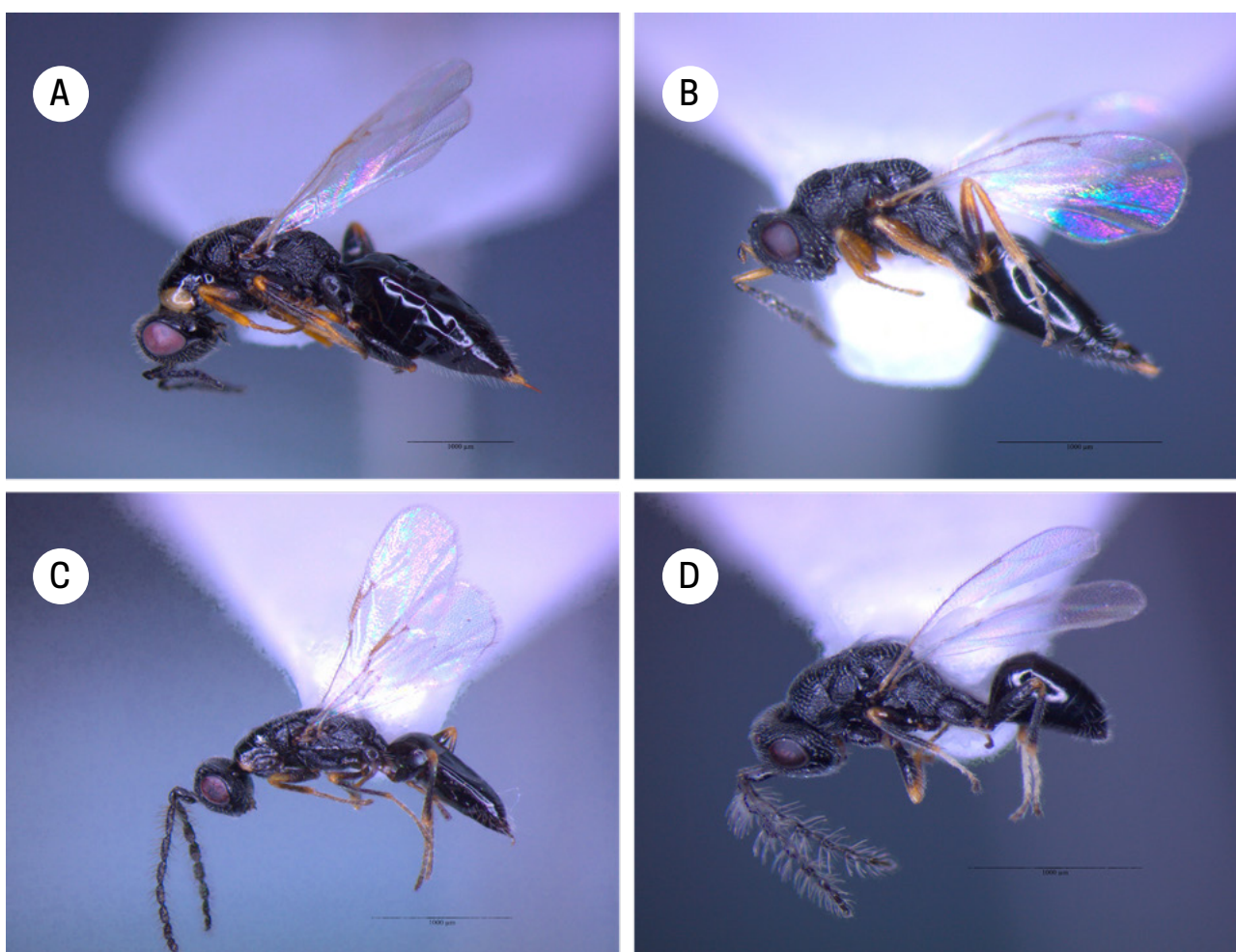
RESEARCH TEAM

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COLLABORATORS

Dr Hossein Lotfalizadeh (Iranian Research Institute of Plant Protection), Queensland Department of Agriculture and Fisheries, Australia; Department of Primary Industries (New South Wales), Australia; CSIRO, Australia.

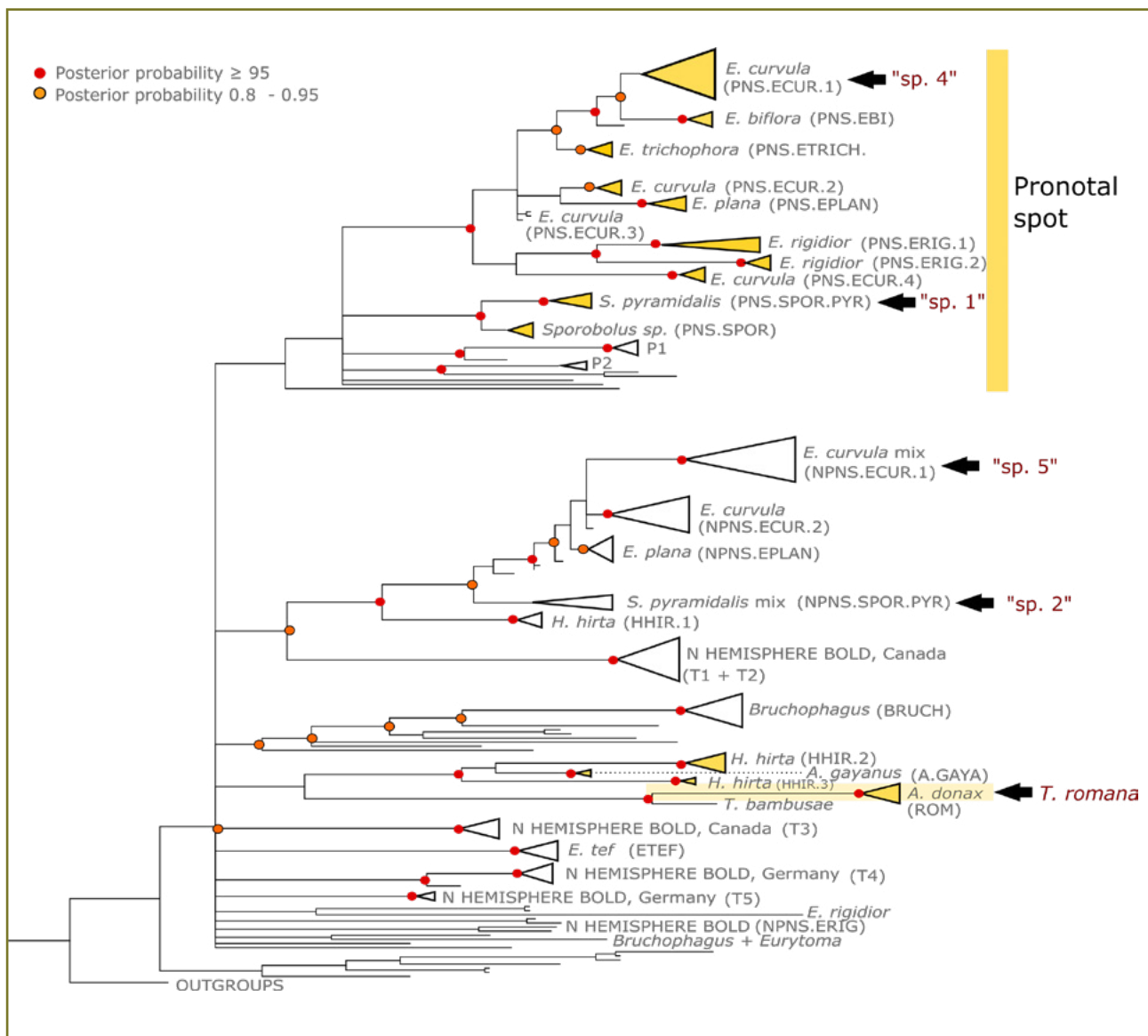
DNA barcoding using the COI region has identified at least 11 well-supported genetic groups, with many groups likely to represent previously undescribed *Tetramesa* species (Figures below). There are two major clades that have one clear morphological difference, one clade has a golden shoulder (pronotal spot), while the other does not. By cross-referencing the DNA sequences with host-plant



Typical morphology of *Tetramesa* species. (A) Pronotal spot morphotype (female); (B) Non-pronotal spot morphotype (female); (C) Pronotal spot morphotype (male); and (D) Non-pronotal spot morphotype (male). Examples of pronotal spot species are *Tetramesa* sp. 1 and *Tetramesa* sp. 4, and non-pronotal spot species are *Tetramesa* sp. 2 and *Tetramesa* sp. 5. Scale bars are shown below each image. Note that the terms “pronotal spot” and “golden-shouldered”, and “non-pronotal spot” and “non-golden shouldered” are sometimes used interchangeably in this text. **Photos:** Clarke van Steenderen

records, we have been able to determine the host-range and specificity of these wasps. Many of the *Tetramesa* appear to be host specific, being recorded only from a single, or a few very closely related grass hosts, which has helped us prioritise *Tetramesa* as possible biocontrol agents for grass weeds. DNA barcoding has been integral in assessing the host-specificity of the *Tetramesa* (*Tetramesa* sp. 1, 2, 4 and 5) on *Sporobolus* spp. and *E. curvula* and allowed for prioritisation of the species for further testing.

We are currently in the process of sequencing a number of wasp specimens collected on *E. curvula* in Australia, which might be native Australian *Tetramesa*. This would add a very new and exciting dimension to the project. Our genetic work to date has been integral in untangling the taxonomy of the *Tetramesa* genus, identifying promising potential grass agents, and determining their host ranges.



Condensed Bayesian COI phylogeny. Notice the higher clade resolution in the golden-shouldered section of the tree.

AGRICULTURAL ENTOMOLOGY



Apart from historical collaborations, Rhodes University Entomology has been working actively and consistently with the agricultural sector for the last 20 years. This collaboration has grown sharply since the formation of the CBC. Although the CBC has worked on crops such as apples, litchis, macadamias, pecans, potatoes and crucifers, the overwhelming bulk of the research has been on citrus-related issues, mainly in collaboration with Citrus Research International (CRI). This stands to reason, not only owing to the extent and importance of the industry in the Eastern Cape, but also to the fact that citrus is the number one export-revenue-generating agricultural industry in the country, valued at around R30 billion annually. When the Covid-19 pandemic struck in 2020, many commercial sectors in the country and around the world suffered tremendously. However, agriculture stood out as an exception, even flourishing during 2020. In stark contrast, 2021 and 2022 were two of the most difficult years for agriculture in South Africa, with export citrus suffering a plethora of blows, most prominently, dramatically elevated shipping costs, which also led indirectly to the increase in costs of numerous other essential inputs. Currently, an estimated 80% of farmers are operating at a loss.

Despite this, the citrus industry has continued to show complete confidence in research, by fully approving proposals and budgets submitted for the coming year, including the CBC's contributions. In the number of projects and volume of funding, the CBC is now the southern African citrus industry's number one research partner. However, the expectations and the needs of the southern African agricultural industry remain high and thus the CBC's responsibility to continue producing high-quality research and usable outputs remains high. With the Covid-19 pandemic restrictions now lifted, we are unfettered in our access to laboratories, rearing facilities and orchards (despite the frustration of loadshedding once we are there).

Outputs during the 2022 research year have been diverse and exciting. As has been the case for the last 20 years, there was a strong emphasis on baculovirology, confirming the CBC as the centre for expertise on insect viruses in South Africa. Novel approaches are being explored, targeted at producing more effective biopesticides for important agricultural pests. However, the importance of Integrated Pest Management (IPM) and biological control in general (beyond just insect viruses) is growing,

PROGRAMME HIGHLIGHTS IN 2022

- Organised and hosted the 54th Annual Meeting of the Society of Invertebrate Pathology, at which eight oral talks were presented by members of the CBC.
- Attended and presented at the 11th Citrus Research Symposium at which sixteen oral talks and eight posters were presented by members of the CBC.
- Attended workshops hosted by our commercial and research partners, River Bioscience and Citrus Research International, to present our latest research and discuss current and future projects and opportunities.
- In collaboration with River Bioscience and Citrus Research International, members at the CBC assisted in defending a joint European patent application for use of CrpeNPV as a biopesticide, which was successful resulting in the patent being granted as filed.
- Published several research articles in peer-reviewed journals.

with vital export markets becoming more intolerant of chemical residues, even when proven safe, and becoming more demanding that their suppliers implement sustainable IPM programmes that will help to reduce carbon footprints. Consequently, we are focussing more of our projects on such priorities, something which the citrus industry has always been committed to. This includes studies on the use of parasitoids for the biological control

of the key pests, California red scale and citrus mealybug, and the influence of protective netting over citrus orchards in the implementation of IPM. The results and progress on all of the research projects in the Agricultural Entomology Research Programme are listed below, providing an overview of the interesting and important work being conducted here at the CBC.

Selection for a UV-resistant isolate of a nucleopolyhedrovirus for improved field persistence and efficacy against FCM

An integrated pest management programme is utilized to control *Thaumatotibia leucotreta*, the false codling moth, one of the most important pests of the South African citrus industry. One key component of this programme is the utilization of entomopathogenic viruses, most notably the baculovirus *Cryptophlebia leucotreta* granulovirus (CrleGV-SA). Baculoviruses have several key characteristics which make them ideal for use as a biological control agent, such as their narrow host range and non-target effect on beneficial insects or humans. However, they have some drawbacks, the most notable of which is their sensitivity to ultraviolet (UV) radiation, leading to their rapid degradation in the field. A UV-tolerant isolate, CrleGV-UVT, was previously selected by exposing the virus to UV irradiation in an environmental chamber mimicking a typical

RESEARCH TEAM

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summer day in the Sundays River Valley. The UV-tolerant isolate was selected after five exposure cycles and had a 100-fold improvement in virulence relative to the wild-type isolate (CrleGV-SA) after an exposure cycle. Although CrleGV-SA is an effective biological control agent, its repeated use can lead to the development of resistance. Recently a novel baculovirus, *Cryptophlebia peltastica* nucleopolyhedrovirus (CrpeNPV), was discovered in *Cryptophlebia peltastica*, commonly known as litchi moth. This novel isolate holds excellent potential

for use as a biocontrol agent owing to its broad host range, and it is highly virulent against *T. leucotreta* and other important lepidopteran pests. We propose to select a UV-tolerant isolate of CrpeNPV, using a similar approach to that of CrleGV-UVT. The development of a UV-tolerant CrpeNPV isolate would provide an effective alternative to CrleGV-SA. Upon selecting a tolerant isolate, field trials will be conducted to ensure that the UV tolerance observed in the laboratory is carried into the field. The first round of UV exposures has been completed, and artificial diet bioassays are due to start soon.



Marcel van der Merwe conducting field trials in the Addo region. Photo: Marcel van der Merwe

Evaluating mixtures of formulated baculovirus biopesticides against FCM

The baculovirus *Cryptophlebia leucotreta* granulovirus (CrleGV) is currently used for the control of *T. leucotreta*, while a second virus *Cryptophlebia peltastica* nucleopolyhedrovirus (CrpeNPV) is currently undergoing registration for commercial use against this pest. Two other baculoviruses are also used in South Africa, these being *Helicoverpa armigera* nucleopolyhedrovirus (HearNPV) and *Cydia pomonella* granulovirus (CpGV), which are either applied on or near citrus orchards for the control of other important pests.

Research is required to better understand the interactions which may occur between these viruses on the same host, as very few cases of synergistic or antagonistic interactions have been documented. Baculovirus mixtures between CrleGV and CrpeNPV were evaluated by Jukes (2018) and Taylor (2021) with possible synergistic interactions observed whereby improved lethal concentrations were reported. However, an antagonistic effect as a result of an increased lethal time was also observed. CrleGV-SA, CrpeNPV and CpGV-M are known to infect *T. leucotreta*, while HearNPV infects African bollworm. Although HearNPV is not known to affect *T. leucotreta*, studies have indicated synergistic interactions between two baculoviruses where one was non-infectious to the target host.

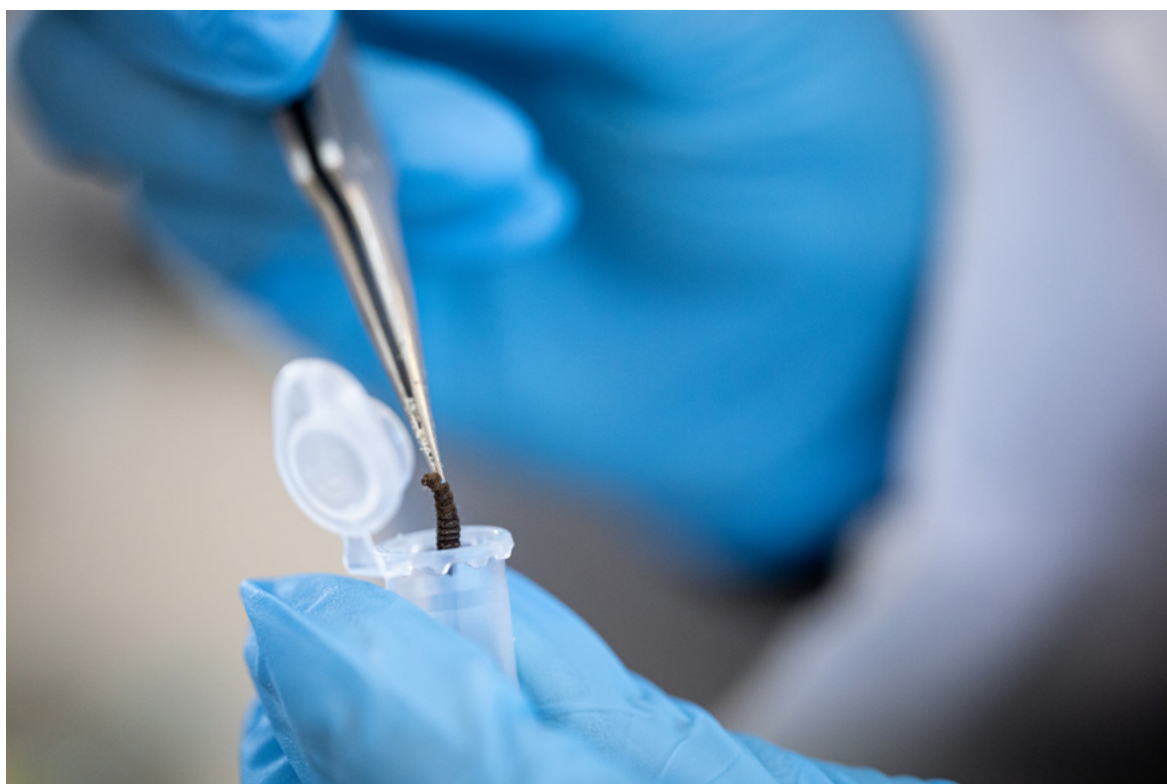
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Siviwe Tole

In this study, interactions between the four viruses, CrleGV-SA, CrpeNPV, CrleGV, and HearNPV in various combinations will be evaluated against the key citrus pest, *T. leucotreta*. The identification of any synergistic interactions between these viruses could assist in developing improved biopesticides, while the identification of any antagonistic interactions could help inform better IPM procedures regarding the application of these viruses in the field. Current results include the successful extraction of virus from larval cadavers infected with CrleGV-SA, CpGV-M, CrpeNPV and HearNPV. From these samples, DNA has been successfully extracted with target regions amplified by PCR and sequenced. Biological assays evaluating the virulence of CrleGV-SA against *T. leucotreta* larvae are currently underway in preparation for mixed virus bioassays to evaluate potential interactions.



The collection of baculovirus infected insects. Photo: David Taylor

The expression and evaluation of baculovirus proteins as formulation additives for enhanced infectivity and improved FCM control

To date, the most effective method for managing *T. leucotreta* and other pests in this industry has been to use an integrated pest management (IPM) programme, which aims to combine chemical pesticides, cultural practices, biological pesticides, and other control options into a single strategic approach to combat *T. leucotreta* and other pests in this industry. Baculovirus-based biological pesticides formulated with baculoviruses such as *Cryptophlebia leucotreta* granulovirus (CrleGV) are key components of this IPM programme. However, these control choices have limitations, such as the virus's sensitivity to UV light and slow speed of kill. Specific baculovirus proteins, such as enhancin and gp37 have been found to enhance the infectivity of other baculoviruses. Existing South African baculoviruses provide a diverse bioresource library, with at least two isolates harbouring the gp37 gene: *Cydia pomonella* granulovirus (CpGV-SA) and *Cryptophlebia peltastica* nucleopolyhedrovirus (CrpeNPV). The incorporation of these proteins in baculovirus formulations could result in increased CrleGV infection and improved *T. leucotreta* control in the field.

The overall aim of this study is to express CrpeNPV gp37 gene in bacterial cells. To date, the CrpeNPV gp37 gene has been successfully cloned into the protein expression vector, pCA-528. Subsequently, the gp37 protein was expressed at 25°C in *E. coli* Rosetta cells and resolved at approximately 42 kDa using 12% SDS-PAGE. Western blot analysis was used to validate authenticity of gp37 using anti-His antibodies, confirming the successful expression of the target

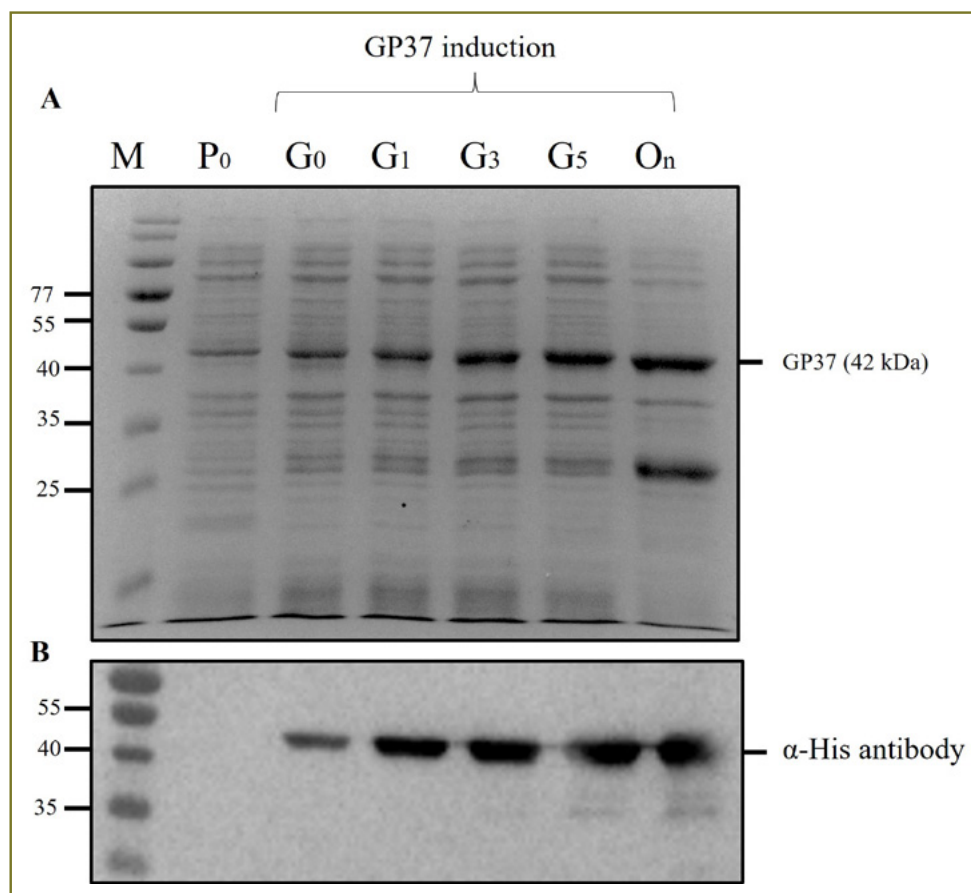
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protein. Future objectives include a solubility analysis to determine if the protein is soluble or insoluble, which will then be followed by purification of the protein, dialysis, and Bradford assay to concentrate and quantify expressed protein. Lastly, biological assays will be performed to investigate whether the purified protein can enhance the effectiveness of CrleGV in terms of lethal concentration and lethal time when used as an additive.



Expression of gp37 at 25 °C. SDS-PAGE analysis (A) Lane M- Protein ladder, Lane P0- PCA528 (GP37 absent), Lane G0- non-induced cells, Lane G1- On hours post-induction with 1 mM IPTG and Western blot analysis (B) using anti-His antibody, respectively.

The isolation, identification, and characterisation of novel baculoviruses for the control of emerging agricultural pests of potential concern for the SA citrus industry

The South African citrus industry faces several challenges, of which emerging pests are of great importance, particularly given the absence of control options which can readily be implemented for their immediate management. This project aims to collect, isolate, identify, and characterise novel baculoviruses from emerging pests such as *Serrodus partita* (Fabricius) (Lepidoptera: Erebididae), *Archips occidentalis* (Walsingham) (Lepidoptera: Tortricidae) and *Ectomyelois ceratoniae* (Zeller) (Lepidoptera: Pyralidae), among others. To date, samples of *S. partita* have been obtained from which baculovirus occlusion bodies have been purified, enabling the successful extraction of genomic DNA for use in downstream PCR analysis. Amplicons have been successfully generated using three sets of universal primers which target specific baculoviral genes, with preliminary sequencing results suggesting the isolation of a novel baculovirus, matching most closely to other known Noctuidae alphabaculoviruses. The genetic

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characterisation of this novel virus has now moved onto the next stage, with sequencing and assembly of the complete genome underway. Host-range evaluation of this novel baculovirus is underway, with preliminary data indicating that neither *Thaumatotibia leucotreta* nor *Spodoptera frugiperda* are susceptible hosts. Evaluation of additional key pests such as *Helicoverpa armigera*, *Cryptophlebia peltastica*, and *S. partita* as potential hosts are currently being investigated. Bioprospecting for other potential baculovirus isolates will continue during this period of evaluation.

Encapsulation of CrleGV in calcium alginate using a microfluidic device for improved UV resistance

Ultraviolet (UV) radiation remains a major challenge for baculovirus-based biological pesticides, capable of causing rapid inactivation of occlusion bodies (OBs) when exposed. This project aims to investigate the effect of encapsulation of CrleGV in UV protective reagents using a microfluidic device. The design and 3D printing of a microfluidic device is currently underway and will be used to evaluate the encapsulation of baculovirus OBs in various UV protectants. Encapsulated OBs will be exposed to UV radiation and evaluated using microscopy and/or qPCR to determine which offers the greatest amount of UV protection. Additionally, virus samples will be tested against *Thaumatotibia leucotreta* larvae to assess viability.

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An investigation into the biological and genetic stability of UV-tolerant baculoviruses for improved control of FCM

There remain various challenges when using baculoviruses, one of which is the high level of sensitivity to UV irradiation, with direct exposure to sunlight shown to decrease pathogenicity. To mitigate this, isolates of CrleGV with increased UV tolerance (UVT) have been developed via successive UV exposure. However, the successful implementation of these novel isolates as biopesticides will require mass production in suitable hosts and field evaluation. The mass production process could lead to the contamination of these isolates with covert wild type (WT) isolates which have previously been shown to be present within host populations. Alternatively, repeated infection in the host population without any form of UV selection pressure could lead to reversion and a loss of UV tolerance.

A series of preliminary biological assays have been conducted to determine the activity of the WT and UVT isolates against neonate FCM larvae. The results from these assays were used to determine the concentration of virus which was applied during the passage assay, while also providing a point of comparison for future biological assays. A trial run of the passage assay has been completed

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Kurhula Lufhondo (Hons) and Sifundo Ngxekisa (Hons)

using the UVT isolate. Recovered samples will be re-evaluated with and without exposure to UV to determine whether the increased tolerance remains stable following passage in FCM larvae. Additionally, detached fruit assays were conducted for both isolates alongside a control, with the experiment replicated three times. The detached fruit assays included indoor and outdoor treatments to investigate the effect of UV radiation on these CrleGV isolates. An immediate assay was also included to assess the viability of CrleGV-UVT and CrleGV-WT. Several important methodological challenges were identified for both the passage and detached fruit assays which are now being addressed. These results will assist in developing procedures for the mass production of the CrleGV-UVT isolate for use in the field.

Alternate hosts of the oriental fruit fly, *Bactrocera dorsalis*, in the Sundays River Valley

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In 2020 a *Bactrocera dorsalis* individual was detected in the Sundays River Valley (SRV) in a trap 5 km from any cultivated host, leading to speculation that *Bactrocera dorsalis* had established a low-level population on indigenous thicket species. Twenty-four Methyl Eugenol and 24 Biolure traps were set up in citrus orchards as well as in adjacent native thicket to monitor populations of fruit fly species in the region. In May and June of 2021, a total of three individual *B. dorsalis* were detected in the traps. Since June 2021, no other individuals have been caught, presumably due to the declaration of establishment of *B. dorsalis* in the SRV and the accompanying regulated management



Emiel van Son examining field traps for fruit fly in the Addo region. Photo: David Taylor

practices. Monthly surveys of the traps continue as well as ongoing emergence experiments of indigenous fruit collected from around trap sites.

Ten thicket species have been investigated for suitability as alternate hosts through emergence experiments, based on fruit phenology. *Lycium ferocissimum*, *Azima tetracantha* and *Capparis sepiaria* have been shown to host *C. capitata*, with infestation rates ranging from 33.8 flies/kg fruit to

114.1 flies/kg fruit. Twenty-seven individuals of a *Neoceratitis* species, an indigenous Tephritid, have been reared from *Lycium ferocissimum*. Two previously confirmed hosts of oriental fruit fly, *Capparis sepiaria* and *Opuntia ficus-indica*, have to date not produced any *B. dorsalis*. No new thicket species have been investigated over the winter months of 2022. New data and results should commence with the monthly trap-monitoring and fruit collections in the summer months of 2022/2023.

Augmentation of *Aphytis melinus* for control of California red scale *Aonidiella aurantii* on citrus

Aphytis lingnanensis was reared and tested in South Africa in the early 2000s for augmentation against red scale on citrus. No evidence was found that the augmentation of *A. lingnanensis* influenced the population of red scale. Imported *A. melinus* is now available and hence it is important that the efficacy of augmentation of *A. melinus* on red scale is determined locally. *Aphytis melinus* was released during the last three seasons in three orchards in both the Eastern and Western Cape, while three comparable orchards were studied as untreated control orchards. *Aphytis melinus* wasps were released at 240 000 per ha, allocated over a five- to 10-week period. Releases started in October in organic orchards and in January in conventional orchards. Fortnightly, levels of red scale infestation were determined by scouting 10 fruit on each of 10 trees per orchard. A sample of 20 infested fruit from each orchard was collected randomly every four weeks. Red scale on collected fruit was inspected and classified as either alive,

RESEARCH TEAM

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dead, or parasitized, using 20 red scale individuals per fruit. *Aphytis* species responsible for parasitism were identified and percentage parasitism recorded. Results of this study during three seasons of field trials suggest that the augmentation of *A. melinus* did not significantly increase the level of parasitism above that of the untreated control. This may be because the natural occurrence of *A. africanus* dominated parasitism in all orchards. *Marietta* spp., the hyperparasitoid of *Aphytis* spp., was recorded in the organic orchards at unprecedentedly high levels.



'Nadorcott' Mandarin infested with red scale. Photo: Ernst de Beer



Aphytis lingnanensis pupa ventral view from an inverted female red scale. Photo: Ernst de Beer

A comparison of control of key citrus pests in orchards under nets, in a biointensive IPM programme and a conventional programme

Around the world, commodity markets demand that citrus should be produced with less chemical intervention to reduce risks to humans and the environment. The aim of this study is to determine if it is possible to obtain the same yield and export percentage in citrus orchards where an integrated pest management (IPM) programme, heavily based on biological control, is applied, in comparison to orchards that rely primarily on chemical pest control. We focussed particularly on the following pests and their natural enemies: citrus mealybug, *Planococcus citri*, citrus thrips, *Scirtothrips aurantii*, red scale, *Aonidiella aurantii*, and citrus red mite, *Panonychus citri*.

During the first season, different control strategies were applied. Scouting initiated intervention, both spraying and natural enemy augmentation. However, in December we had an outbreak of citrus mealybug and different interventions had to be applied in order to protect the crop. More chemicals were used within the 16 ha, commercial chemical treatment block and the 10 ha, IPM trial site, than within the 4 ha, strong biological trial site. The biological management relied strongly on more beneficial insects, including the release of predators such as *Cryptolaemus montrouzieri* and *Nephus bipunctatus*.

Thrips, red scale, red mite, and even an outbreak of citrus mealybug were controlled by harvest time and most of the sooty mould had been washed off by the winter rain. All four key species were managed in such a way as to protect the crop and to protect the natural habitat of beneficial insects that started to boom. Certain fruit export problems included cosmetic damage from mealybug rind damage, and cosmetic damage from dead and alive red scale on the rind. Owing to the mealybug outbreak, interventions were made with specifically chosen chemical sprays and/

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or increased releases of parasitoids and predators. This increased the cost/ha considerably. To farm sustainably, it should also be economical. It was found after the first season that switching from chemical treatment to biological in one season can be done, but it is too expensive.

The lessons learnt in the first season of 2020/21 led to certain changes in the control strategy within the IPM programme. Certain chemicals were replaced with others and increased numbers of beneficial insects were added early in the season. During the second season of 2021/22 considerable focus was placed on controlling mealybug. To date mealybug is under good control due to the presence of beneficial insects. Red mite remained low through the season. Some thrips damage occurred on fruit, and could be related to the infestation levels recorded during the season, which were never zero, but always very low. Late season red scale is present on some outside fruit, although some parasitism is evident. Final damage and infestation analyses will be conducted at harvest. Production costs were reduced substantially for both the chemical and biological programmes, from the first to the second season. However, these will have to be weighed up against losses to thrips and red scale damage.

Through this study we hope to find a commercially viable option to farm sustainably and economically using an IPM programme.

Integrated Pest Management (IPM) under nets in Mpumalanga Province

The incidence and severity of citrus thrips throughout three citrus growing seasons (August 2018–April 2021) were found to be less under the 20% enclosed shade net than in the open citrus orchard. These scouting results are further supported by decreased thrips damage in the enclosed orchards as compared to the open orchards..

RESEARCH TEAM

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Mealybug populations were higher and more concentrated during the 2018/2019 season in the open orchard. During the following two seasons, the mealybug populations were higher under netting than in the open orchard. Total FCM trap catches were higher in the first two seasons under netting, but in the last season, the FCM moth numbers were lower than in the open orchard. Fruit fly species were caught in higher quantities in the open orchard for three consecutive seasons than in the netted orchard. Red scale (crawlers, white caps, male scales and adult females) were found in higher populations under the netted orchard than in the open orchard. Citrus fruit sampled for residue tests resulted in higher concentrations of active ingredients for three consecutive seasons under the enclosed netting than

in the open orchard. Seychelles scale and pink wax scale were present at higher populations under litchi shade net than the control, for three growing seasons. Mango scale seemed to be similar in population densities at both litchi sites. Greater numbers of false codling moth were trapped under netted orchards for two consecutive seasons, with the last season being lower under the netted orchard than in the open orchard. Higher trap catches of litchi moth were recorded in the open orchards for two seasons, while higher counts were recorded under nets during the last season. Fruit fly species were trapped in higher numbers in the open orchard for three consecutive seasons. There was a higher percentage of Class 1 fruit under the netted orchard than in the open orchard.

Biology and management of the fruit-piercing moth, *Serrododes partita*, in citrus orchards

Fruit-piercing moth, *Serrododes partita* (Fabricius) (Lepidoptera: Erebididae), is a polyphagous, multi-voltine pest of citrus. The adult moth pierces the skin of ripening or ripe citrus fruit and sucks the juices, facilitating the entry of secondary fungal pathogens and premature fruit fall. In the Eastern Cape, (southernmost Kat River Valley) the population dynamics of *S. partita* have changed over the past two decades, with recent observations showing recurring infestations as opposed to seasonal outbreaks every five to 10 years. Current pest management practices are limited and only practical in small orchards. The aim of this study is to develop an effective trapping system for monitoring and managing *S. partita* through development of an effective bait. The use of attractive baits (e.g., banana) has shown to be promising, but impractical for long-term use. Bait presentation trials have been conducted to determine the best trap design for monitoring adult populations in the field, with the funnel trap showing promising results. Bait

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presentation trials and development of a synthetic lure are still ongoing. Pest populations and damage assessments are being conducted to establish intervention thresholds for control as well as to establish population peaks in citrus orchards, and the effect of weather on pest outbreaks. Pest activity has been observed to coincide with fruit ripening of the most susceptible cultivars, Satsumas and Clementines. Efforts are still being made towards laboratory rearing to facilitate future studies, and bioprospecting for pathogens aimed at controlling larval stages.

Bioprospecting for entomopathogenic fungi against foliar citrus pests

Entomopathogenic fungi (EPF) have been shown to play important roles in the management of insect pest populations under natural conditions. Previous research has shown that several isolates are effective at managing the soil-dwelling life stages of FCM. However, against foliar citrus pests, these same isolates, which performed relatively well under laboratory conditions, performed poorly under field conditions. This can most probably be attributed to

RESEARCH TEAM

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UV radiation. As these isolates were obtained from the soil environment, it stands to reason that EPF isolated from the foliar environment may be more suited for foliar application and thus management of foliar pests. Biopros-

pecting for these EPF was thus initiated. Currently, one isolate (identity/efficacy still unknown) has been recovered. This research is ongoing and should be expanded to include the UV tolerance of the top-performing isolates. In addition, EPF may exist as endophytes within plant parts, for example, leaves, roots, and stems. Thus, further bioprospecting of plant material should be expanded. The identification of these endophytes will also be beneficial in

identifying potential *Muscodor* spp., which are reported to have several beneficial uses in agriculture through the release of volatile organic compounds, which have been reported as plant pathogen antagonists and as bioinsecticides. To date, one fungal isolate of the species *Fusarium equiseti* has been recovered from the cadaver of California red scale. Its pathogenicity has not yet been tested. Field surveys for infected insects will occur early 2023.

Predicting the pathways and entry points to the southern African citrus production areas, for the Asian Citrus Psyllid, *Diaphorina citri*

The presence of the Asian Citrus Psyllid (ACP), *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae), in parts of Africa is currently putting citrus production in southern Africa at risk. The ACP is an effective vector of the destructive citrus greening disease known as Huanglongbing (HLB), which is associated with the gram negative α -Proteobacteria *Candidatus Liberibacter asiaticus* (Las).

The ACP will reach the southern African regions in the future. The successful incursion of a new pest would be determined by the density of invading propagules (arrival) and the optimal conditions for establishment once the pest arrives. In the case of ACP, the vector could arrive either by natural dispersal from infested areas using Rutaceous trees as host corridors aided by wind currents, or by human mediated dispersal (trafficking of Rutaceous plants from infested areas). Currently, sampling techniques available for the vector are not sensitive. The southern African

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citrus production region is also large in size, being located between central Zimbabwe and south-western areas of South Africa. The establishment of pathways of introduction of the vector would help in determining where to focus detection efforts and rapid response preparations. The findings of this study will be used to validate, refine or refute the current Risk Heat Map developed by the CRI Biosecurity division. Parameters to be considered include pest occurrence, host abundance (citrus), temperature (maximum and minimum parameters), flight activity, wind, barometric pressure, humidity, altitude, maximum flight distances, visual cues, and solar radiation.

Evaluation of potential repellents for false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae)

There has been extensive research on the use of semiochemicals as repellents in insect pest management, particularly in push-pull strategies. No study has been conducted on repellents for false codling moth (FCM). This study assessed FCM oviposition repellence in plants, essential oils, and some pesticides used commercially for FCM control. Choice and no-choice repellence trials were conducted in complete darkness in a controlled environment room. Oranges treated with solutions/suspensions of potential repellents were placed into a cage with gravid FCM females

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for four hours, with oviposition being recorded every hour. Of the 33 tested products, only eight significantly repelled FCM oviposition when compared to the control in the initial repellence trials; that is, two essential oils (lavender

and peppermint), two plant crude extracts (garlic and marigold), one fruit (mango), and three chemicals (Delegate, Coragen, and Warlock). All identified repellents, except for mango, were further investigated for their repellent and ovicidal properties in dose-response bioassays at five different concentrations. All tested botanicals were identified to have dual action (both repellent and ovicidal properties), as they significantly reduced FCM oviposition and hatchability. Garlic was identified as the best performing botanical. Warlock was the only chemical insecticide that did not show ovicidal activity when egg hatchability was

compared to oviposition. Larval penetration was less than egg hatchability for all tested repellents, and oviposition was affected by concentration, with the higher concentrations being the most effective. The efficacy of these repellents can be further tested in semi-field and/or field trials, and they may have potential for dispensing in an orchard or for spraying onto trees. Moreover, they can be implemented in push-pull strategies where they can be used in conjunction with FCM attractants. Results from this study have the potential to improve FCM control in current integrated pest management programmes.



Khalipha Dambuza preparing fruit for the evaluation of insect repellents. Photo: David Taylor

Regional differences in sex pheromones and sexual attractiveness in FCM

The rationale of this project is based on reports that FCM males in South Africa prefer mating with females from their own populations, resulting in speculations or the hypothesis that there are sub-species of the moth, which may compromise pheromone control tools such as attract-and-kill, mating disruption, monitoring, and sterile insect technique. However, all previous reports on this topic have been incomplete, and there is evidence that new species may arise through the evolution or intraspecific variation of sex pheromones. This project specifically aims to analyse and compare the relative blend of the isomers in female FCM pheromones in populations from different regions, determine whether male moths' preferences for females from their own populations are retained when not given a choice, and devise solutions (universally attractive pheromone blend and universally attracted FCM culture for SIT) in the event that these preferences are

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retained. Results, so far, have shown that females from all five populations attract males from Addo, Marble Hall, and Nelspruit, indicating no potential chemical signalling barriers that may trigger speciation. However, males from Nelspruit were not attracted to females from Citrusdal. These findings are still preliminary, and further repetitions will provide more insights. Optimisation for the methodology of pheromone collection from female moths during scotophase has been established and validated for Gas chromatography and Mass spectrometry.

Investigating release ratios in an FCM SIT programme

The Sterile Insect Technique (SIT) for FCM was commercially implemented in South Africa in 2007. The technology is now practised in several different regions of the country where it is greatly recommended as a foundation of area-wide integrated pest management (AW-IPM) in these regions of the country. The success of SIT is achieving an overflooding ratio of sterile males to wild males in the targeted release sites. The current target minimum ratio is 10:1; however, higher ratios are often achieved and may be more successful. Therefore, this study focuses on examining the efficacy of higher release ratios and rate of population growth of FCM in field cage studies. Similarly, different crosses of treated and untreated FCM have resulted in some fruit infestations when conducted in the laboratory. The trial will be conducted in a field cage to test the impact of these different crossings on the fruit. The field studies will commence in March 2023, twelve weeks before the start

RESEARCH TEAM

Prof. Martin Hill, Prof. Sean Moore, and Dr Candice Coombes

STUDENTS

Michael Mathenge (PhD)

of harvest season at Sundays River Valley. With SIT being an AW-IPM strategy, different field situations suggest that SIT combined with parasitoid releases achieves a better control than either of the methods used alone. Therefore, there would be a need to examine the synergistic relationship between the egg parasitoids, *Trichogrammatoidea cryptophlebiae* and SIT. A laboratory study to assess the acceptability and suitability of FCM eggs from higher release ratios and the egg parasitoids is in progress.

POLYPHAGOUS SHOT HOLE BORER

Garyn Townsend inspecting dead branches for signs of bark and ambrosia beetle activity in Woodville Forest, Western Cape. Picture: Abby Gilson



Emiel van Son admiring a 400 year old yellowwood tree (*Afrocarpus falcatus*) that is part of a permanent monitoring plot in the Garden of Eden, Western Cape. Picture: Garyn Townsend

RESEARCH TEAM

Prof. Brett Hurley (FABI, University of Pretoria), Prof. Martin Hill, Dr Francois Roets (Stellenbosch University)

STUDENT

Garyn Townsend (PhD, UP)

COLLABORATOR

FABI

The polyphagous shot hole borer or PSHB (*Euwallacea fornicatus*) is an invasive ambrosia beetle native to South-east Asia. It has become a highly invasive tree pest in various countries and in 2017 was discovered in South Africa. It is now well established in eight of the nine provinces of the country, making it the largest outbreak of this beetle worldwide. The PSHB releases its symbiotic fungus, *Fusarium euwallaceae*, into infested trees. The fungus grows in the vascular tissue of the tree, inhibiting water and nutrient uptake which causes dieback and, in many cases, death. It has been found attacking various ornamental trees and more importantly, several native tree species. It poses a major threat not only to agricultural crops and urban trees, but to native forests throughout Africa. Various projects investigating the impacts of PSHB on agricultural crops such as pecan nut, grapevine and various deciduous fruits are ongoing in South Africa. Nectarines and plums have both been found to be susceptible to the *F. euwallaceae* pathogen.

The primary impact of PSHB is still mostly seen in urban forests where it usually attacks non-indigenous trees. Monitoring in indigenous forests is ongoing and to date the beetle has been recorded on 67 indigenous tree species, 27 of which are reproductive hosts of the beetle. Data from permanent monitoring plots in indigenous forests in the Western Cape and Kwa-Zulu Natal have shown that the spread of the beetle in these natural areas is slow, but consistent. Beetle populations and tree attacks are highest near urban areas, where source populations are present and

POLYPHAGOUS SHOT HOLE BORER

certain tree species are more susceptible to attack by PSHB.

As part of Garyn Townsend's PhD, based at the Forestry and Agricultural Biotechnology Institute (FABI), investigations into the potential for biological control of the beetle and/or its fungus are ongoing. In addition to monitoring the impacts and spread of PSHB in indigenous forests, this project aims to identify and characterise any potential natural enemies of PSHB. Collaboration with the Vietnamese Academy of Forest Sciences has been established. Vietnam is one of the countries PSHB is native to, and scouting trips for natural enemies are set to take place in 2023. The project is also beginning to describe the diversity and abundances of indigenous bark and ambrosia beetles in our native forests as well as characterising the diversity and prevalence of these beetles' natural enemies and identifying their host specificity. Various parasitoid wasps of bark and ambrosia beetles have been identified, including a *Cerocephala* species previously unknown to science. However, their ability to attack PSHB is still unknown.

This work is being done in collaboration with nine institutions throughout South Africa, as well as overseas, forming a research network that is taking a holistic approach to understanding the biology of PSHB and its



Garyn Townsend looking for bark beetles at an infested site in Florida, USA where a bark beetle workshop was held. Photo: Unknown

ecological, urban and agricultural impacts. This research will contribute to protocols and policies aiming to control the spread and impacts of PSHB both in South Africa and throughout its entire invaded range.



A *Polemistus* wasp, parasitoids of bark and ambrosia beetles, discovered emerging from a beetle infested log. Photo: Simon van Noort

COMMUNITY ENGAGEMENT

Centre for Biological Control

Our valuable water systems are under threat from pollution and alien invasive plants. Both of these reduce our ability to use the water. Invasive species are threats to our landscape as well as our agricultural crops. Discover how insects can help save our environment. Learn how YOU can be an environmental steward.

A key aspect of the work the CBC does is engaging with communities around South Africa about the topics of invasive alien species and biological control. The aim of these engaged activities is to interact with the public so that they can learn about invasive species and that there is a sustainable control option which should be integrated into their management plans. Our team also interacts with school learners to teach them about the field of entomology and applied entomology through sharing our research in agricultural entomology and biological control. Below we highlight our activities over the course of 2022.

Community Mass-rearing Projects

The CBC started engaging with community groups and concerned citizens to participate in mass-rearing projects after the success of biological control on the Hartbeespoort

Dam over the summer of 2019/2020. The aim was to increase the number of insects released onto the systems as the CBC has only one facility that cannot meet the high demand. Since 2019, the CBC team has set up 15 rearing facilities and provided scientific consultation on three others that were funded by the stakeholders themselves. These facilities have released thousands of the biological control agent *Megamelus scutellaris* on water hyacinth invasions around the country, which has contributed to the biological control effort. The people involved in this are also increasing the awareness of the benefits of biological control. Having Rosali Smith based in Gauteng has been great for community members to have the presence and support needed.



Antonella Petruzzella and Getrude Tshithukhe monitoring the water quality at the rearing facility set up by the local municipality in Jeffreys Bay to help manage the invasion on Poplar Dam. Photo: Kim Weaver

In addition to the water hyacinth rearing projects, there has been scope to start rearing projects for *Cyrtobagous salviniae* in areas on the Garden Route such as George, Jeffreys Bay and Plettenberg Bay, where *Salvinia molesta* is prevalent. Community members and local government have acted together to assist in controlling the weed with the support of the CBC team led by Getrude Tshithukhe. The CBC has supported three satellite mass-rearing stations in partnership with a local school and the local municipalities. This is still ongoing, and we hope to see progress in 2023. Since 2021, the CBC has also been assisting a school in Zimbabwe with mass-rearing and release of *Cyrtobagous salviniae* for the control of *Salvinia molesta* on a dam on the school's property.

Implementation and Monitoring Training

Dr Rosali Smith, based in Gauteng, works closely with active municipalities in fighting against the waterweeds invading aquatic systems in the Province. The Ekurhuleni Municipality have severe water hyacinth infestations on many of the water bodies they manage, and Rosali has been working with them to integrate biological control into their management plan to improve implementation of control measures. In December 2022, Rosali, Samella Nxgande-Koza and Kim Weaver gave two-day training workshops on invasive plants and biological control, which included sharing information about invasive species and how biological control works. The participants were then assisted to integrate biological control into the management plans of systems, and shown how to monitor the process. They were taught how to monitor biological control in the field, too, which will be an advantage in



The Ekurhuleni team at Belsbokspruit surveying water hyacinth. Photo: Kim Weaver

their workplace. There is a very productive mass-rearing facility that they oversee and manage at the Blesbokspruit Centre which has released many *Megamelus scutellaris* in the area. We trust that this partnership will grow, resulting in the successful integration of biological control into their management plan.



Ekurhuleni staff and the CBC team at Blesbokspruit Research Centre. Photo: Kim Weaver

Prosopis Engagement

This year with Gretha van Staden's research, the CBC made progress in engaging with stakeholders around integrated implementation plans for their properties. This was done through online surveys and a follow-up meeting that the CBC hosted in July in Groblershoop. A roadshow followed where the CBC team visited Brandvlei, Kenhardt, Upington, Prieska and Williston in October–November. Additional to these in-person meetings, the CBC had a campaign where awareness was raised through a large WhatsApp group and Instagram.

The CBC hopes to take this forward through the Community of Practice project and will continue to work with

farmers to develop plans and invest in projects to make use of *Prosopis* biomass, and biological control in order to better manage the invasion. Graham Harding is contracted to work with up to twenty farmers to develop farm-scale management plans to address the threat of *Prosopis*. We have a project in Brandvlei that will include the whole community. The offices and store at APD Upington mass-rearing facility will be manufactured using *Prosopis* biomass in cement construction techniques. We will mass rear the *Prosopis* leaf-tying moth, *Agnippe* sp. (formerly, *Evippe* sp. #1), in Upington and make new releases in early summer 2023. The working group will also be resuscitated.



Kenhardt meeting as part of the roadshow. Photo: Philip Ivey

School Engagement

The CBC is involved in a number of school engagement activities, the longest standing of which is the Science Internship Programme, which exposes learners to different departments within the Science Faculty. School learners are selected for the programme through GADRA, an education NGO based in Makhanda. This year, we welcomed twelve Grade 10 and 11 learners to Rhodes University who were exposed to a several participating Departments.

SciFest Africa was back after two years of being an online event. The CBC team did a great job in putting together an interactive exhibition for the learners and general public. The festival was a great success and it was wonderful for the students and staff to interact with learners again.

The CBC hosted a few school groups up at the Waainek Research Facility which is a great space to teach them about invasive species and biological control. Interactions with learners in Makhanda is so important to make the schools aware of the research that is going on right under their noses.



Above:
Nompumelelo
Baso with a group
of learners at the
CBC's exhibition.
Photo: Kim Weaver

Left: Prof. Julie
Coetzee with a
group of Grade
6 learners at the
Waainek. Photo:
Pippa Muskett.

Partners in the CBC's community engagement activities

Agri SA, U3A Grahamstown, Red Meat Research and Development South Africa, Grahamstown Horticultural Society, SciFest Africa, Eastern Cape Game Management Association, Wildlife Ranching South Africa EC, Professional Hunters Association of South Africa EC, SANParks, Addo Elephant National Park, Camdeboo National Park, Thomas Baines Nature Reserve, Sibuya Game Reserve, Wool Growers Association, and Red Meat Producers Roadshow

Schools: Ntsika Secondary School, Nombulelo Secondary School, Khutliso Daniels School, Victoria Girls High School, Graeme College, Merrifield College, Stirling High School.

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Red Meat Research and Development South Africa

River BioScience

Drakenstein Trust

South African Water Research Commission (WRC)

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Queensland Department of Agriculture, Australia

New South Wales Department of Primary Industry; Australia

Manaaki Whenua – Landcare Research, New Zealand

Research Outputs

Graduates

PhD

1. Tshililo Mphephu
2. Zezethu Mnqeta

MSc

1. Kedibone Mofokeng
2. Ekhona Zozo
3. Matthew Paper
4. Emma Sandenburgh
5. Caitlyn Webb
6. Tahnee Bennett

Peer-reviewed articles

Rhodes University

1. Adams, L. D., Martin, G. D., Downs, C. T., Clark, V. R., Thabethe, V., Raji, I. A., & Steenhuisen, S. L. (2022). Seed dispersal by frugivores and germination of the invasive alien shrub *Pyracantha angustifolia* (Franch.) CK Schneid. in Free State Province, South Africa. *Biological Invasions*, 1–11.
2. Aigbedion-Atalor, P. O., Hill, M. P., Azrag, A. G., Zalucki, M. P., & Mohamed, S. A. (2022). Disentangling thermal effects using life cycle simulation modelling on the biology and demographic parameters of *Dolichogenidea gelechiidivoris*, a parasitoid of *Tuta absoluta*. *Journal of Thermal Biology*, 103260.
3. Canavan, K., Magengelele, N. L., Paterson, I. D., Williams, D. A., & Martin, G. D. (2022). Uncovering the phylogeography of *Schinus terebinthifolia* in South Africa to guide biological control. *AoB Plants*, 14(1), plab078.
4. Coetzee, J. A., Paper, M. K., Miller, B. E., Kinsler, D., Cilliers, C. J., Hill, M. P. (2022) Into Africa: *Salvinia minima* Baker (Salviniaceae) invades South Africa. *BioInvasions Records* 11(4), 1011–1018.
5. Coetzee, J. A., Miller, B. E., Kinsler, D., Sebola, K., & Hill, M. P. (2022). It's a numbers game: inundative biological control of water hyacinth (*Pontederia crassipes*), using *Megamelus scutellaris* (Hemiptera: Delphacidae) yields success at a high elevation, hypertrophic reservoir in South Africa. *Biocontrol Science and Technology*, 1–10.
6. Cornette, R., Motitsoe, S. N., & Mlambo, M. C. (2022). A new desiccation-resistant midge from ephemeral rock pools in South Africa, *Polypedilum* (*Pentapedilum*) *cranstoni* sp. nov. (Diptera: Chironomidae). *Zootaxa*, 5128(3), 397–410.
7. Goddard, M., Owen, C. A., Martin, G., & Coetzee, J. A. (2022). The thermal physiology of *Lysathia* sp. (Coleoptera: Chrysomelidae), a biocontrol agent of parrot's feather in South Africa, supports its success. *Biocontrol Science and Technology*, 1–10.
8. Masole, P., Steenhuisen, S. L., & Martin, G. D. (2022). Current status of the invasive shrub *Berberis julianae* CK Schneid. (Berberidaceae) in Golden Gate Highlands National Park (Free State Province, South Africa). *South African Journal of Botany*, 150, 99–105.
9. Minuti, G., Stiers, I., & Coetzee, J. A. (2022). Climatic suitability and compatibility of the invasive *Iris pseudacorus* L. (Iridaceae) in the Southern Hemisphere: considerations for biocontrol. *Biological Control*, 104886.
10. Misteli, B., Pannard, A., Labat, F., Fosso, L. K., Baso, N. C., Harpenslager, S. F., & Piscart, C. (2022). How invasive macrophytes affect macroinvertebrate assemblages and sampling efficiency: Results from a multinational survey. *Limnologia*, 96, 125998.
11. Moore, S. D., Peyper, M., Kirkman, W., Marsberg, T., Albertyn, S., Stephen, P. R., & Hattinigh, V. (2022). Efficacy of various low temperature and exposure time combinations for *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) larvae. *Journal of Economic Entomology*.
12. Moore, S., & Manrakhan, A. (2022). Postharvest Disinfestation Treatments for False Codling Moth and Fruit Flies in Citrus from South Africa. *Horticulturae*, 8(3), 221.

13. Motitsoe, S. N., Hill, J. M., Coetzee, J. A., & Hill, M. P. (2022). Invasive alien aquatic plant species management drives aquatic ecosystem community recovery: An exploration using stable isotope analysis. *Biological Control*, 173, 104995.
14. Mwanza, P., Jukes, M., Dealtry, G., Lee, M., & Moore, S. (2022). Selection for and Analysis of UV-Resistant *Cryptophlebia leucotreta* Granulovirus-SA as a Biopesticide for *Thaumatotibia leucotreta*. *Viruses*, 14(1), 28.
15. Paterson, I.D., Witt, A. 2022. Biological control of cactus pests and pest cactus in Africa. *Acta Horticulturae*. 1343: 563–568.
16. Prinsloo, S., Hill, M. P., Moore, S. D., Malan, A. P., & Coombes, C. A. (2022). Interaction between an entomopathogenic fungus and entomopathogenic nematodes for increased mortality of *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae). *Biocontrol Science and Technology*, 1–14.
17. Smith, R., Coetzee, J.A., & Hill, M.P. (2022). Best of both worlds: The thermal physiology of *Hydrellia egeriae*, a biological control agent for the submerged aquatic weed, *Egeria densa* in South Africa. *BioControl* 67:365–374.
18. Sutton, G.F., & Martin, G.D. 2022. Testing MaxEnt model performance in a novel geographic region using an intentionally introduced insect. *Ecological Modelling* 473.
19. van der Merwe, M., Jukes, M. D., Knox, C., Moore, S. D., & Hill, M. P. (2022). Mutualism between Gut-Borne Yeasts and Their Host, *Thaumatotibia leucotreta*, and Potential Usefulness in Pest Management. *Insects*, 13(3), 243.
20. van Steenderen, C. (2022). BinMat: A molecular genetics tool for processing binary data obtained from fragment analysis in R. *Biodiversity data journal*, 10.
21. van Steenderen, C. J. M., & Sutton, G. F. (2022). SPEDE-sampler: an R Shiny application to assess how methodological choices and taxon-sampling can affect Generalised Mixed Yule Coalescent (GMYC) output and interpretation. *Molecular Ecology Resources*.
22. Venter, N., Cowie, B.W., Paterson, I.D., Witkowski, E.T.F., Byrne, M.J. (2022). The Interactive Effects of CO₂ and Water on the Growth and Physiology of the Invasive Alien Vine *Pereskia aculeata* (Cactaceae): Implications for its Future Invasion and Management. *Environmental and Experimental Botany*. 194: 104737.
23. Wansell, S. N. L., Geerts, S., & Coetzee, J. A. (2022). Where are the seeds? Lack of floral morphs prevent seed production by the tristylous *Pontederia cordata* in South Africa. *Ecology and Evolution*, 12, e9366.
24. Zachariades, C., Uyi, O., Hill, M. P., Mersie, W., & Molo, R. (2022). The benefits to sub-Saharan Africa of the biological control of weeds: already considerable, but could be far greater. *Current Opinion in Insect Science*, 100932.

Book chapters

1. Canavan, K., Canavan, S., Clark, V. R., Gwate, O., Mapaura, A., Richardson, D. M., Steenhuisen, S. & Martin, G. D. (2022). Invasive Alien Plants in the Montane Areas of South Africa: Impacts and Management Options. In Misiune, I., Depellegrin, D., & Egarter Vigl, L. (Eds) *Human-Nature Interactions: Exploring Nature's Values Across Landscapes*, 169–180. Springer-Nature, London, UK.
2. Novoa A., Canavan K., Canavan S. (2022). Ch. 9. The role of second homes in non-native plant invasions. In: Barros A., Shackleton R., Rew L.J., Pizarro C., Pauchard A. (eds.) *Tourism, Recreation and Biological Invasions*, CABI, Gloucester, UK.

Conference proceedings

Plenary lectures, Keynote addresses and invited talks

1. Hill, M.P. (2022). The current status of biological control of arthropods. *Proceedings of ISBCA 6* – D.C. Weber, T.D. Gariepy, and W.R. Morrison III, eds. *International Symposium on Biological Control of Arthropods*. Online from British Columbia, Canada March 15–17 and 22–25, 2022.
2. Hill, M. (2022). Measuring success in weed biological control in South Africa: improved, but could be better. *National Symposium on Biological Invasions*. 6–8 July 2022. University of Fort Hare, Alice, Eastern Cape.
3. Hill, M.P. & Coetzee, J.A. (2022). Current status of biological control of weeds and future perspectives. *3rd International Weed Conference Weed problems and management challenges: Future perspectives* 20–23 December, 2022, Anand Agriculture University, Anand, Gujarat, India

National

1. Ivey, P., van Staden, G., Hill, M., & Weaver, K. (2022). Towards a National Programme for Prosopis Management in South Africa, biological control, biomass use and everything in between! *Invasive Species Forum*. 16 March 2022. Kimberley, Northern Cape.
2. Adams, L.D., Martin, G.D., Clark, V.R., & Steenhuisen, S. (2022). Reproductive ecology of an invasive alien plant *Pyracantha angustifolia* in the eastern Free State grasslands. *Southern African Mountain Conference*. 14–17 March 2022. Champagne Sports Resort, KwaZulu- Natal.
3. Chikowore, G., Chidawanyika, F., & Martin, G.D. (2022). The impact of *Robina pseudoacacia* L. (Fabaceae) invasion on montane grassland ecosystems in South Africa. *Southern African Mountain Conference*. 14–17 March 2022. Champagne Sports Resort, KwaZulu- Natal.
4. Gwate, O., Canavan, K., Martin G.D., Canavan, S., Sutton, G., Richardson, D.M., Carbutt, C., & Clark, V.R. (2022). An inventory of non-native plant species in African mountains: Towards the development of database to help address global change. *Southern African Mountain Conference*. 14–17 March 2022. Champagne Sports Resort, KwaZulu- Natal.
5. Gwate, O., Canavan, K., Martin, G.D., Canavan, S., Sutton, G., Richardson, D.M., & Clark, V.R. (2022). Assessing habitat suitability for four plant species invading southern African mountains under climate change. *Southern African Mountain Conference*. 14–17 March 2022. Champagne Sports Resort, KwaZulu- Natal.
6. Canavan, K., Canavan, S., Clark, V.R., Gwate, O., Richardson, D.M., Sutton, G., & Martin, G.D. (2022). The non-native plants that threaten biodiversity in South Africa's mountains. *Southern African Mountain Conference*. 14–17 March 2022. Champagne Sports Resort, KwaZulu- Natal.
7. Masole, P., Steenhuisen, S. & Martin, G.D. (2022). Is the thorny invasive *Rosa rubiginosa* facilitating the recruitment of native and exotic woody species in the mountain grasslands of the Eastern Free State. *Southern African Mountain Conference*. 14–17 March 2022. Champagne Sports Resort, KwaZulu- Natal.
8. Moloi, K., Martin, G.D., Clark, V.R., & Steenhuisen, S. (2022). Reproductive ecology of *Cotoneaster panossus* in montane grasslands of Eastern Free State. *Southern African Mountain Conference*. 14–17 March 2022. Champagne Sports Resort, KwaZulu- Natal.
9. Wolmarans, A., Martin, G.D., Paterson, I.D., & Weyl, P.S.R. (2022). Controlling the spread of the black locust. *Southern African Mountain Conference*. 14–17 March 2022. Champagne Sports Resort, KwaZulu- Natal.
10. Mapaura, A., Canavan, K., Richardson, D.M., Clark, V.R., & Steenhuisen, S. (2022). Climate change and predicted changes in distribution of invasive *Nasella* species in South Africa and Lesotho. *Southern African Mountain Conference*. 14–17 March 2022. Champagne Sports Resort, KwaZulu- Natal.
11. Adams, L.D., Martin, D.D., Steenhuisen, S., & Downs, C.T. (2022). Fleshy-fruited invasive alien plants in grasslands: preliminary results from a systemic review. *National Symposium on Biological Invasions*. 6–8 July 2022. University of Fort Hare, Alice, Eastern Cape.
12. Baso, N.C., Bownes, A., & Coetzee, J.A. (2022). Investigating the enemy release Hypothesis: A Case Study of *Lagarosiphon major* In New Zealand. *National Symposium on Biological Invasions*. 6–8 July 2022. University of Fort Hare, Alice, Eastern Cape. Poster presentation.
13. Canavan, K., Paterson, I.D., Ivey, P., Sutton, G.F., Bownes, A., Visser, V., Mapaura, A., & Hill, M.P. (2022). The South African Biological Control Target Selection System. *National Symposium on Biological Invasions*. 6–8 July 2022. University of Fort Hare, Alice, Eastern Cape.
14. Chikodza, T., Hill, M., & Coetzee, J. (2022). The effect of biological control agents on the population dynamics of *Pontederia crassipes* and *Salvinia minima*. *National Symposium on Biological Invasions*. 6–8 July 2022. University of Fort Hare, Alice, Eastern Cape.
15. Coetzee, J.A., Miller, B.E., Smith, R., & Hill, M.P. (2022). Beyond Hartbeespoort: towards the successful biological control of water hyacinth in South Africa. *National Symposium on Biological Invasions*. 6–8 July 2022. University of Fort Hare, Alice, Eastern Cape.
16. Griffith, T.C., & Paterson, I.D. (2022). Investigating biological control options against torch cactus in South Africa. *National Symposium on Biological Invasions*. 6–8 July 2022. University of Fort Hare, Alice, Eastern Cape.
17. Hill, M. (2022). Measuring success in weed biological control in South Africa: improved, but could be better (Keynote address). *National Symposium on Biological Invasions*. 6–8 July 2022. University of Fort Hare, Alice, Eastern Cape.

18. Kolisi, S., Motitsoe, S.N., Mlambo, M., & Zengeya, T. (2022). Invasive freshwater snails (Gastropoda: Mollusca) in South Africa: patterns, trends and impacts. *National Symposium on Biological Invasions*. 6–8 July 2022. University of Fort Hare, Alice, Eastern Cape.
19. Martin, G., Adams, L., Masole, P., & Maloi, K. (2022). Impacts of Northern Temperate Weeds on the Grassland Biome of South Africa. *National Symposium on Biological Invasions*. 6–8 July 2022. University of Fort Hare, Alice, Eastern Cape.
20. Mayonde, S., Paterson, I.D., & Byrne, M.J. (2022). Tracking the origin of invasive *Opuntia engelmannii* lineages in South Africa. *National Symposium on Biological Invasions*. 6–8 July 2022. University of Fort Hare, Alice, Eastern Cape.
21. Miller, B.E., Coetzee, J.A., Martin, G.D., Weyl, P., & Hill, M.P. (2022). Amazon frogbit (*Limnobium laevigatum*): prevention is better than cure. *National Symposium on Biological Invasions*. 6–8 July 2022. University of Fort Hare, Alice, Eastern Cape.
22. Motitsoe, S.M., Petruzzella, A., Hill, M.P., & Coetzee, J.A. (2022). Secondary invasion, the “new normal” for aquatic systems in South Africa. *National Symposium on Biological Invasions*. 6–8 July 2022. University of Fort Hare, Alice, Eastern Cape.
23. Muskett, P., & Paterson, I.D. (2022). Potential for biological control of two invasive *Opuntia* species in South Africa. *National Symposium on Biological Invasions*. 6–8 July 2022. University of Fort Hare, Alice, Eastern Cape.
24. Petruzzella, A., Motitsoe, S.N., Hill, M.P., & Coetzee, J.A. (2022). Achieving the goal “more natives, less invasives”: Priority effects as a potential tool for restoration. *National Symposium on Biological Invasions*. 6–8 July 2022. University of Fort Hare, Alice, Eastern Cape.
25. Reid, M.K., Hill, M.P., & Coetzee, J.A. (2022). A native weevil and a foreign planthopper: could they control *Nymphaea mexicana* in South Africa? *National Symposium on Biological Invasions*. 6–8 July 2022. University of Fort Hare, Alice, Eastern Cape.
26. Townsend, G., Hurley, B., Hill, M.P., Roets, F., & de Beer, Z.W. (2022). Initial assessment of the polyphagous shot hole borer (PSHB) in indigenous South African forests. *National Symposium on Biological Invasions*. 6–8 July 2022. University of Fort Hare, Alice, Eastern Cape.
27. Webb, C., & Paterson, I.D. (2022). Potential biological control agents for Crystalline ice plant: a plant indigenous to South African but an invasive pest in U.S.A. *National Symposium on Biological Invasions*. 6–8 July 2022. University of Fort Hare, Alice, Eastern Cape.
28. Yell, L., Sutton, G., Canavan, K., van Steenderen, C., & Paterson, I. (2022). Utilising field-based methods for host-specificity testing prior to quarantine testing in weed biological control. *National Symposium on Biological Invasions*. 6–8 July 2022. University of Fort Hare, Alice, Eastern Cape. Poster presentation.
29. van der Merwe, M., Jukes, M., Knox, C., Moore, S., & Hill, M. (2022). An investigation into yeast-baculovirus synergism for the improved control of false codling moth. *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal.
30. Dambuza, K., Coombes, C., Hill, M., & Moore, S. (2022). Evaluation of potential repellents for false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae). *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal.
31. van Steenderen, C., Marsberg, T., Peyper, M., Kirkman, W., Hill, M., & Moore, S. (2022). The role of sterile females in the false codling moth sterile insect technique programme - *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal.
32. Aigbedion-Atalor, P., Upfold, J., Coombes, C., Moore, S., & Hill, M. (2022). Regional attractiveness between *Thaumatotibia leucotreta* (Meyrick) males and females from different geographical regions of South Africa: implications for semiochemical-dependent control. *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal.
33. Bennett, T., Mwanza, P., Hill, M., Moore, S., Jukes, M., Knox, C., & Dealtry, G. (2022). Genetic and biological characterisation of a UV-tolerant strain of the *Cryptophlebia leucotreta* granulovirus for improved control of false codling moth. *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal.
34. Makitla, T., Mukwevho, L., Manrakhan, A., Hill, M., & Moore, S. (2022). The effects of orchard sanitation on fruit flies and false codling moth in citrus. *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal.

RESEARCH OUTPUTS

35. Prinsloo, S., Coombes, C., Moore, S., Malan, A., & Hill, M. (2022). Interaction between indigenous entomopathogenic nematodes and the fungus *Metarhizium anisopliae* against late instar false codling moth larvae. *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal.
36. Muller, K., Hill, M., & Moore, S. (2022). A comparison of IPM under nets and in open citrus orchards in Mpumalanga Province. *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal.
37. de Beer, E., Hill, M., Marsberg, T., Peyper, M., & Moore, S. (2022). Augmentation of *Aphytis melinus* for the control of red scale in citrus. *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal.
38. Mommsen, W., Moore, S., & Hill, M. (2022). The influence of timing, insecticide residues, hyperparasitism and interspecific competition on *Anagyrus vladimiri* augmentation for mealybug control. *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal.
39. Mushore, T., Coombes, C., Hill, M., & Moore, S. (2022). Biology and management of the fruit-piercing moth, *Serrodes partita*, in citrus orchards. *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal.
40. Marsberg, T., Peyper, M., Cousins, L., & Moore, S. (2022). The interaction between a granulovirus and two insect growth regulators: a possibility for improved control of false codling moth. *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal.
41. Mulcahy, M., Thackery, S., De Araujo, L., Stirk, E., & Moore, S. (2022). From pest control to area-wide integrated pest management: Repositioning of the false codling moth (FCM) SIT programme in citrus. *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal.
42. Peyper, M., Moore, S., Kirkman, W., Marsberg, T., & Cousins, L. (2022). The use of an anthranilic diamide insecticide for mating disruption of false codling moth. *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal.
43. Cousins, L., Moore, S., Peyper, M., Marsberg, T., & Hill, M. (2022). A comparison of false codling moth infestation in organic versus conventional citrus orchards. *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal.
44. Moore, S., Marsberg, T., Peyper, M., Cousins, van der Merwe, M., & Kirkman, W. (2022). Pre-harvest monitoring of false codling moth infestation: assessment of accuracy and development of a new system. *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal.
45. Moagi, R., Mauda, E., Mukwevho, L., & Hill, M. (2022). An assessment of the potential pest status of *psylloid* species, as possible vectors of *Candidatus Liberibacter* species, causative of citrus greening in South Africa. *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal. Poster presentation.
46. Richardson, P., Terblanche, J., & Owen, C. (2022). Of moths and microbes: Insights into pest thermal physiology. *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal. Poster presentation.
47. van Son, E., Kirkman, W., Coetzee, J., & Manrakhan, A. (2022). Alternate hosts of the oriental fruit fly, *Bactrocera dorsalis*, in the Sundays River Valley. *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal. Poster presentation.
48. Mela, T., Jukes, M., Knox, C., Moore, S., & Hill, M. (2022). Development and optimisation of a qPCR assay for the enumeration of *Cryptophlebia leucotreta* granulovirus (CrLeGV) used for commercial applications. *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal. Poster presentation.
49. Lufhondo, K., Jukes, M., & Coombes, C. (2022). Evaluation of stability and maintenance of ultraviolet tolerant strain of *Cryptophlebia leucotreta* granulovirus-SA for the management of false codling moth. *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal. Poster presentation.
50. Manqele, S., Coetzee, J., & Owen, C. (2022). Does diet quality influence false codling moth thermal limits? *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal. Poster presentation.
51. Shuttleworth, A., Johnson, S., Heiduk, A., Peyper, M., Cousins, L., & Moore, S. (2022). Environmental SEM imaging of male false codling moth androconia and coremata – *11th Citrus Research Symposium*. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal. Poster presentation.

52. Shuttleworth, A., Johnson, S., Heiduk, A., Peyper, M., Cousins, L., & Moore, S. (2022). Chemical ecology of male false codling moth (*Thaumatotibia leucotreta*; Tortricidae) and the FCM larval parasitoid *Agathis bishop* (Braconidae) – 11th Citrus Research Symposium. 21–24 August 2022. Champagne Sports Resort, KwaZulu Natal. Poster presentation.
53. Reid, M.K., Coetzee, J.A., Hill, M.P. (2022). A native weevil exploring exotic cuisine: The host range expansion of *Bagous longulus* on an invasive waterlily in South Africa. *Sixth research symposium at Fountainhill Estate, Wartburg* (Managing and Rehabilitating the Environment for Biodiversity Optimisation, 19 & 20 October 2022).
54. Chikodza, T., Hill, M. & Coetzee, J. (2022). The effect of biological control agents on the population dynamics of *Pontederia crassipes* and *Salvinia minima*. *The Conservation Symposium*. 30 October–4 November 2022. Premier Resort Hotel and Blue Marlin Hotel, KwaZulu Natal
5. Motitsoe, S.N., Hill, J.M., Coetzee, J.A., & Hill, M.P. (2022). Invasive alien aquatic plant species management drives aquatic ecosystem community recovery: An exploration using stable isotope analysis. *22nd International Conference of Aquatic Invasive Species (ICAIS22)*. 18–22 April 2022. Hybrid conference in Oostende, Belgium (Virtual).
6. Coetzee, J.A., Motitsoe, S.N., Sebola, K., & Petruzzella, A. (2022). MadMacs: Mass development of aquatic macrophytes – causes and consequences of macrophyte removal for ecosystem structure, function, and services. *22nd International Conference of Aquatic Invasive Species (ICAIS22)*. 18–22 April 2022. Hybrid conference in Oostende, Belgium (Virtual).
7. van Steenderen, C.J.M., & Sutton, G.F. (2022). SPEDE-sampler: An R Shiny application to assess how methodological choices and taxon-sampling may affect DNA-based Generalised Mixed Yule Coalescent (GMYC) species delimitation. *International Statistical Ecology Congress (ISEC)*. 27 June–1 July 2022. Cape Town, South Africa. Poster presentation.
8. Paterson, I.D. (2022). Local adaptations or genetic diversity: which is more beneficial in a biocontrol agent population? *XXVI International Congress of Entomology (ICE)*. 17–22 July 2022. Helsinki, Finland.
9. Ivey, P., & Hill, M.P. (2022). Willingness to share biological control agents: how accessible is Africa? *XXVI International Congress of Entomology (ICE)*. 17–22 July 2022. Helsinki, Finland.
10. Motitsoe, S., Paterson, I.D., Coetzee, J.A., Martin, G.D., & Hill, M.P. (2022). Measuring success of aquatic weed biological control in Africa. *XXVI International Congress of Entomology (ICE)*. 17–22 July 2022. Helsinki, Finland.
11. Owen, C. A., Bosua, H., & Terblanche, J. S. (2022). Can insights into the metabolic costs of digestion in biocontrol insects help practitioners with post-release efficacy predictions? *XXVI International Congress of Entomology (ICE)*. 17–22 July 2022. Helsinki, Finland.
12. Thackeray, S., & Moore, S. (2022). Biological control in South Africa – successes, challenges and opportunities. *International Congress of Invertebrate Pathology and Microbial Control and the 54th Annual Meeting of the Society for Invertebrate Pathology*. 1–4 August 2022. Nelson Mandela Bay, South Africa (Virtual).

International

1. Acheampong, M.A., Hill, M.P., Moore, S.D., & Coombes, C.A. (2022). UV sensitivity of *Beauveria bassiana* and *Metarhizium anisopliae* isolates under investigation as potential biological control agents in South African citrus orchards. *The IV International Symposium on Fungal Stress - ISFUS & International Fungal Biology Conference – IFBC*, Brazil.
2. Hill, M. (2022). The current status of biological control of arthropods (Keynote address). *The 6th International Symposium on Biological Control of Arthropods (ISBCA)*. 15–17 and 22–24 March 2022. Online from British Columbia, Canada.
3. Mnqeta, Z., & Paterson, I. D. (2022). Perceptions of land-users on efficacy and safety of biological control of invasive alien Cactacea in South Africa. *6th International Symposium on Biological Control of Arthropods*. 15–17 and 22–25 March 2022. Online from British Columbia, Canada.
4. Minuti, G., Coetzee, J.A., & Stiers, I. (2022). Present and future climatic suitability of the invasive aquatic plant *Iris pseudacorus* L. and its candidate biocontrol agents. *22nd International Conference on Aquatic Invasive Species (ICAIS22)*. 18–22 April 2022. Hybrid conference in Oostende, Belgium.

13. Bennett, T.T., Hill, M.P., Moore, S.D., Jukes, M.D., Knox, C., & Dealtry, G. (2022). Genetic analysis and field application of a UV-tolerant strain of CrleGV for improved control of *Thaumatotibia leucotreta*. *International Congress of Invertebrate Pathology and Microbial Control and the 54th Annual Meeting of the Society for Invertebrate Pathology*. 1–4 August 2022. Nelson Mandela Bay, South Africa (Virtual).
14. Mela, T., Jukes, M., Knox, C., Moore, S., & Hill, M. (2022). Development and optimisation of a qPCR assay for the enumeration of Cryptophlebia leucotreta granulovirus (CrleGV) used for commercial applications. *International Congress of Invertebrate Pathology and Microbial Control and the 54th Annual Meeting of the Society for Invertebrate Pathology*. 1–4 August 2022. Nelson Mandela Bay, South Africa (Virtual).
15. Taylor, T.G., Hill, M.P., Moore, S.D., Knox, C., & Jukes, M.D. (2022). Investigating baculovirus synergism for improved management of false codling moth, *Thaumatotibia leucotreta*, in the field. *International Congress of Invertebrate Pathology and Microbial Control and the 54th Annual Meeting of the Society for Invertebrate Pathology*. 1–4 August 2022. Nelson Mandela Bay, South Africa (Virtual).
16. Mushore, T., Coombes, C., & Hill, M. (2022). Entomopathogenic fungi associated with cultivated Honeybush fields in South Africa and their pathogenicity towards the leaf hopper *Molopopterus* sp. *International Congress of Invertebrate Pathology and Microbial Control and the 54th Annual Meeting of the Society for Invertebrate Pathology*. 1–4 August 2022. Nelson Mandela Bay, South Africa (Virtual).
17. van der Merwe, M., Jukes, M.D., Moore, S.D., & Hill, M.P. (2022). Selection of a UV-resistant isolate of Cryptophlebia peltastica nucleopolyhedrovirus for improved field persistence and efficacy against *Thaumatotibia leucotreta*. *International Congress of Invertebrate Pathology and Microbial Control and the 54th Annual Meeting of the Society for Invertebrate Pathology*. 1–4 August 2022. Nelson Mandela Bay, South Africa (Virtual).
18. Coombes, C., Hill, M., & Moore, S. (2022). Entomopathogenic fungi and South African citrus pests: past, present, and future. *International Congress of Invertebrate Pathology and Microbial Control and the 54th Annual Meeting of the Society for Invertebrate Pathology*. 1–4 August 2022. Nelson Mandela Bay, South Africa (Virtual).
19. Marsberg, T., & Moore, S.D. (2022). The interaction between a granulovirus and Neem oil for improved control of *Thaumatotibia leucotreta*. *International Congress of Invertebrate Pathology and Microbial Control and the 54th Annual Meeting of the Society for Invertebrate Pathology*. 1–4 August 2022. Nelson Mandela Bay, South Africa (Virtual).
20. Sutton, G. F., Yell, L. D., van Steenderen, C., Canavan, K., McConnachie, A., & Paterson, I.D. (2022). Biological control of *Eragrostis curvula* (African lovegrass): native-range surveys in Africa. *22nd Australasian Weeds Conference*. 22–26 September 2022. Adelaide, Australia.
21. Kirkman, W., Gebeyehu, S., Carstens, E., Fourie, P.H.F., Manrakhan, A., & Hattingh, V. (2022). Preparation by the South African Citrus Industry for the imminent arrival of Asian citrus greening (HLB) and its primary vector Asian citrus psyllid (ACP). *International Citrus Congress (ICC)*. 06–11 November 2022. Mersin, Turkey.
22. Mommsen, W., Hill, M.P., & Moore, S.D. (2022). Factors influencing successful biocontrol of mealybug in South Africa using augmentative releases of *Anagyrus vladimiri*. *XIV International Citrus Congress (ICC)*. 06–11 November 2022. Mersin, Turkey.

CBC Research Day

This year the CBC hosted their very first CBC Research Day where students and staff had the opportunity to give a speed talk on their research. The day presented an opportunity for not-in-attendance students to present their work and meet the team at Rhodes University. Some of the team joined and presented virtually. Thanks to all of the presenters.



Back:

Arthur Scholtz, Sean Moore, Guy Sutton, Kenton Toontjies, Wayne Kirkman, Tahnee Bennett, Caitlin Webb, Luke Cousins, Sam Motimele, Siviwe Tole, Danie Grabe, Grant Martin, Chaals Vena, Natanah Gusha, Khalipha Dambuza, Michael Mathenge, Ronel Roman, Marcel van der Merwe, Iain Paterson, Liam Yell, David Kinsler, Martin Hill, Michael Jukes, Tapiwa Mushore, Sam Motitsoe, Pippa Muskett, Rosali Smith, Yanga Mgxoteni, Evans Mauda, Philip Ivey, Mthobeli Mvandaba, Lenin Chari, Landile Boo.

Top Middle:

Bongiwe Gobongwana, Siyanda Ntamo, Ziyanda Simayile, Siyaxolisi Mini, Jazmyn Rossouw, Nandipha Ngolothi, Heloise Jacobs, Megan Reid, Ruth Scholtz, Sive Kolisi, Lulutho Mancunga, Soso Maneli, Naho Muleya, Jeanne van der Merwe, Daniel Scholtz, Wayne Mommsen, Byron Soetland

Bottom Middle:

Clarke van Steenderen, Zezethu Mnqeta, Tressia Chikodza, Nokuthula Kom, Tamzin Griffith, Candice Coombes, Thabisa Mdlangu, Lesedi Setsiba, Kim Weaver

Bottom:

Caroline Knox, Emma Sandenbergh, Samella Ngxande-Koza, Ndalo Koza, Nompumelelo Baso, Julie Coetzee, Nonkazimulo Mdimba, Siya Mnciva, Getrude Tshithukhe, Wandisile Mdiza, Raynold Moagi,

Online or Absent:

Ernst de Beer, Gretha van Staden, Kim Canavan, Karlien Muller, Karabo Moloi, Antonella Petruzzella, Carien Kleinjan, Emiel van Son, Garyn Townsend, Siviwe Tole, Stef Botha, Thembelihle Mbhele, Thuthula Mela, Ntando Mkhwanazi, Nthambeleni Bologu, Patricia Masole.

Acronyms and Abbreviations

| | |
|----------|---|
| ACP | Asian Citrus Psyllid |
| AFLP | Amplified Fragment Length Polymorphism |
| AgriSA | Agricultural Research Council of South Africa |
| ARC-PPRI | Agricultural Research Council – Plant Protection Research Institute |
| ARU | Afromontane Research Unit |
| BBCA | Biotechnology and Biocontrol Agency |
| CBA | Cost Benefit Analysis |
| CBC | Centre for Biological Control |
| CpGV | Cydia pomonella granulovirus |
| CPUT | Cape Peninsula University of Technology |
| CRI | Citrus Research International |
| CrleGV | Cryptophlebia leucotreta granulovirus |
| CrpeNPV | Cryptophlebia peltastica nucleopolyhedrovirus |
| CSIRO | Commonwealth Scientific and Industrial Research Organisation |
| DEA | Department of Environmental Affairs |
| DEFF | Department of Environment, Forestry and Fisheries |
| DALRRD | Department of Agriculture, Land Reform and Rural Development |
| DPI | Department of Primary Industries |
| DST | Department of Science and Technology |
| EPF | Entomopathogenic fungi |
| EPN | Entomopathogenic nematodes |
| FABI | Forestry and Agricultural Biotechnology Institute |
| FCM | False Codling Moth |
| FuEDEI | Fundación para el Estudio de Especies Invasivas |
| FURB | Fundação Universidade Regional de Blumenau |
| HearNPV | Helicoverpa armigera nucleopolyhedrovirus |
| ICWG | International Cactus Working Group |
| IPM | Integrated Pest Management |
| NCE | Namibian Chamber for Environment |
| NEMBA | National Environmental Management: Biodiversity Act |
| NRF | National Research Foundation |
| NRM | Natural Resource Management |
| OB | Occlusion Body |
| PSHB | Polyphagous Shot Hole Borer |
| SAAB | South African Association of Botanists |

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| SAHTA | South African Honeybush Tea Association |
| SANBI | South African National Biodiversity Institute |
| SANParks | South African National Parks |
| SAPIA | Southern African Plant Invaders Atlas |
| SARChI | South African Research Chairs Initiative |
| SASRI | South African Sugarcane Research Institute |
| SIT | Sterile Insect Technique |
| SRV | Sundays River Valley |
| U3A | University of the Third Age |
| UCT | University of Cape Town |
| UFS | University of the Free State |
| UKZN | University of KwaZulu-Natal |
| UMP | University of Mpumalanga |
| USACE | United States Army Corps of Engineers |
| USDA | United States Department of Agriculture |
| UV | Ultraviolet |
| UVT | UV Tolerance |
| WfW | Working for Water |
| Wits | University of Witwatersrand |
| WT: | Wild Type |



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CBC Vision

The CBC seeks to:

- (i) sustainably control environmental and agricultural pests for the protection of ecosystems and the societies that depend on them, and
- (ii) ensure that the maximum benefits of biological control are realised through excellence in research, implementation and community engagement.

CBC Mission

The CBC's Mission is to make the Rhodes University Centre for Biological Control an internationally recognised research institute and a leading Research Centre.

