Centre for Biological Control

Annual Report 2023

Front cover photograph
Megan Reid (CBC PhD graduate now in the USA for a postdoc) and Kelby English (postdoc based in Gauteng) doing *Megamelus scutellaris* counts at Hartbeespoort Dam (December 2023).
Photo: Julie Coetzee

Back cover photograph
Michael Mathenge, Reyard Mutamiswa and Vershiyi Deric Tanka at Michael's field site where he has netted trees testing components of the sterile insect technique.
Photo: David Taylor

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Kim Weaver

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Heath White

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CBC

Centre for Biological Control

Annual Report 2023

Contents

DIRECTOR'S REPORT

Martin Hill with Bongiwe Gobongcwana and Nandipha Ngolothi at Waainek.
Photo: Kim Weaver

2023 was another very busy and productive year for the CBC. I would like to thank all of the students and staff of the consortium of institutions that comprise the CBC for all the hard work, long hours and blood, sweat and tears that have gone into the research presented in this Annual Report. In July, I attended the 19th Regular Session of the Commission on Genetic Resources for Food and Agriculture of the FAO in Rome in my position as president of the IOBC, and along with CABI we hosted a side event at this meeting. I was surprised that, despite our best efforts, there are still concerns around the safety of biological control, and a lack of acknowledgement of the benefits of this discipline. It is therefore important that we continue to produce rigorous science that is published in the international literature, and that we take every opportunity to disseminate information on the positive impact of biological control in reducing reliance on pesticides to the conservation and ecological sectors. To that end I am convening the 3rd International Conference on Biological Control in Costa Rica in June 2024 where we will once again have the audience of the environmental lobby of the some 183 member states of the FAO and we will discuss the risks and benefits of biological control.

There were some really significant events during 2023. Professor Iain Paterson was awarded the Vice Chancellor's Distinguished Research Award at the April graduation at Rhodes. In May the CBC was very well represented at the 16th International Symposium for the Biological Control of Weeds in Iguazu, Argentina. Well done to our colleagues at FuEDEI for hosting such a great conference. We managed to get the Vice Chancellor of Rhodes University, Dr Sizwe Mabizela to make the first release of the stem-boring weevil, *Listronotus appendiculatus* against *Sagittaria platyphylla* in the Botanical Gardens in Makhanda. Dave Kinsler played a significant role, along with our colleagues from Louisiana State University in plotting the decline of *Salvinia molesta* on Lake Ossa in Cameroon, which threatened the largest population of the African Manatee. It is these sorts of results that we need to convince the conservationists of the impact of biological control. 2023 marked the first sales of the novel nuclear polyhedrosis virus, formulated into the products Multimax and Codlmax by our industry partner River BioScience (RB). This commercialization, initially in South Africa, but soon to be worldwide, was the culmination of 10 years of research along with RB and CRI. Well done to Michael Jukes and Tamryn Marsberg for asking the question "Why?" when things didn't work out as expected. I would like to congratulate Professor Julie Coetzee on being awarded a prestigious Tier 1 SARChI in the ecology of freshwater invasive species and their control, which is a joint chair between Rhodes University and SAIAB. To put this into perspective, there are only 240 SARChIs

Left: Michael Jukes and Tamryn Marsberg are the faces behind the biopesticide products that were put onto the market in 2023 .
Photo: Michael Jukes

in South Africa, with less than half being Tier 1. Julie is in the process of having laboratories refurbished at an NRF facility in Observatory, Johannesburg, which will become the focal area for her research and another node of the CBC.

The CBC continues to attract excellent funding and it has been great to see an increase in international funding, which is a good indication of our standing in the international environment.

This year will also be remembered for the sad passing of Cliff Moran in late August. Cliff was the doyen of weed biological control in South Africa and there is no doubt that all of us within the CBC, and further afield, have felt the influence of his tremendous mentorship. Cliff was a dear friend and colleague, and I was fortunate enough to have his wise counsel whenever I needed it right up until he passed away. He will be sorely missed.

I am really fortunate within the CBC to be surrounded by some excellent people. I would like to thank Jeanne van der Merwe for everything that she does in the day to day finances of the CBC. Finally, a big thank you to Kim Weaver for once again producing an excellent Annual Report.

The CBC team that attended the XVI International Symposium on Biological Control of Weeds (ISBCW) in Puerto Iguazú, Misiones, Argentina. Standing, from left: Lenin Chari, Antonella Petruzzella, Grant Martin, Blaire Cowie, Nic Venter, Kim Canavan, Iain Paterson, Clarke van Steenderen, Martin Hill, Gianmarco Minuti, Rosali Moffat, Guy Sutton, Zezethu Mnqeta, Julie Coetzee, Samella Ngxande-Koza and Emma Sandenbergh. Seated, from left: Pippa Muskett, Kim Weaver, Megan Reid, Paula Gervazoni, Gerald Chikowore, Tamzin Griffith and Nompumelelo Baso. **Photo:** Kim Weaver

Staff, students and associates

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AQUATIC WEEDS

Releasing the water hyacinth planthopper onto Delta Park Dam. **Photo:** Kelby English

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. A highlight of the water weeds programme this year was the incredible effort the team put into establishing *Megamelus scutellaris* rearing stations at a number of water hyacinth-invaded systems in South Africa. Dr Kelby English, in particular, drove the winter maintenance and rearing of the bug for early Spring releases at Hartbeespoort Dam. Dr Daniella Egli also joined the water weeds team earlier this year and has made great strides in further implementing biological control in KwaZulu Natal, largely through collaboration with uMgeni Water. 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. Our Waainek Mass Rearing station released thousands of biocontrol agents across South Africa, as well as Zimbabwe. *Megamelus scutellaris* has officially established in Zimbabwe as a result, and is the first country in Africa outside South Africa where this has occurred.

The submerged and emergent weed research is progressing very well. This year we released *Listronotus appendiculatus*, against *Sagittaria platyphylla*, and are monitoring its establishment at release sites. We are waiting to hear whether permission to release the Florida biotype of *Cyrtobagous salviniae* will be granted. While host specificity testing of *Aphthona nonstriata* against *Iris pseudacorus* is complete, we will conduct multigeneration tests on *Iris* cultivars important to the Nursery Industry to ensure we understand any potential impacts to these cultivars. The *I. pseudacorus* programme is an incredible multinational effort between Belgium, Argentina, New Zealand and South Africa. We continue to monitor systems in southern Africa for new invasive macrophytes, and have initiated a study on Amazon frogbit (*Hydrocharis laevigata*) in Zimbabwe.

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 2023

- *Listronotus appendiculatus* was released against *Sagittaria platyphylla*.
- Nine community rearing stations are in operation at Hartbeespoort Dam.
- Rearing stations released ~200 000 *Megamelus scutellaris* between August and December 2023.
- *Megamelus scutellaris* has established at a number of sites in Zimbabwe.
- The decline of *Salvinia molesta* on Lake Ossa in Cameroon resulted in the return of the African manatee to this system.
- We initiated a research programme on Amazon frogbit (*Hydrocharis laevigata*) in Zimbabwe.

Water hyacinth

The augmentative biological control approach has continued against water hyacinth into 2023. This strategy involves frequently inundating water hyacinth with *Megamelus scutellaris*, reared at the CBC's Waainek Research Facility, and now at a number of new satellite rearing stations maintained by community members. In previous years, this approach has proved successful when implemented timeously, especially in the Highveld region, which experiences cold winters, focusing on springtime agent introductions when water hyacinth increases rapidly after winter dormancy. In July 2023, Johannesburg received its first bout of snow since 2012, a weather event that could severely decline agent populations.

Alongside the mass-rearing and release efforts, multiple research projects are focused on investigating varying aspects of water hyacinth and the interactions of its biological control agents. These include post-release evaluations, the effects of sub-lethal doses of herbicides on agent abundance, the impact of biological control on the rate of decomposition of water hyacinth, and the effects of acid mine drainage on agent survival and persistence.

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Satellite rearing stations

After successful results in previous years, there was an influx of community interest in setting up rearing stations

New rearing stations established in 2023 at Leloko Estate, Hartbeespoort Dam (left); and at Vaalkop Dam, North West Province (above). **Photos:** Kelby English and Julie Coetzee

Rearing station sites

System	Province	Number of stations
Blesbokspruit Wetland	Gauteng	1
Bronkhorstspruit	Gauteng	8
Benoni Lakes	Gauteng	1
Hartbeespoort Dam	North-West	9
Vaalkop Dam/Hex River	North-West	3
Inanda Dam	KwaZulu Natal	1
	TOTAL	**23**

The CBC hosted rearing workshops at Hartbeespoort Dam (above), attended by community stakeholders, and Inanda Dam (right), attended by uMngeni-uThukela Water colleagues, who have set up satellite rearing stations on rearing *Megamelus scutellaris* for water hyacinth biological control, in South Africa. **Photos:** Julie Coetzee

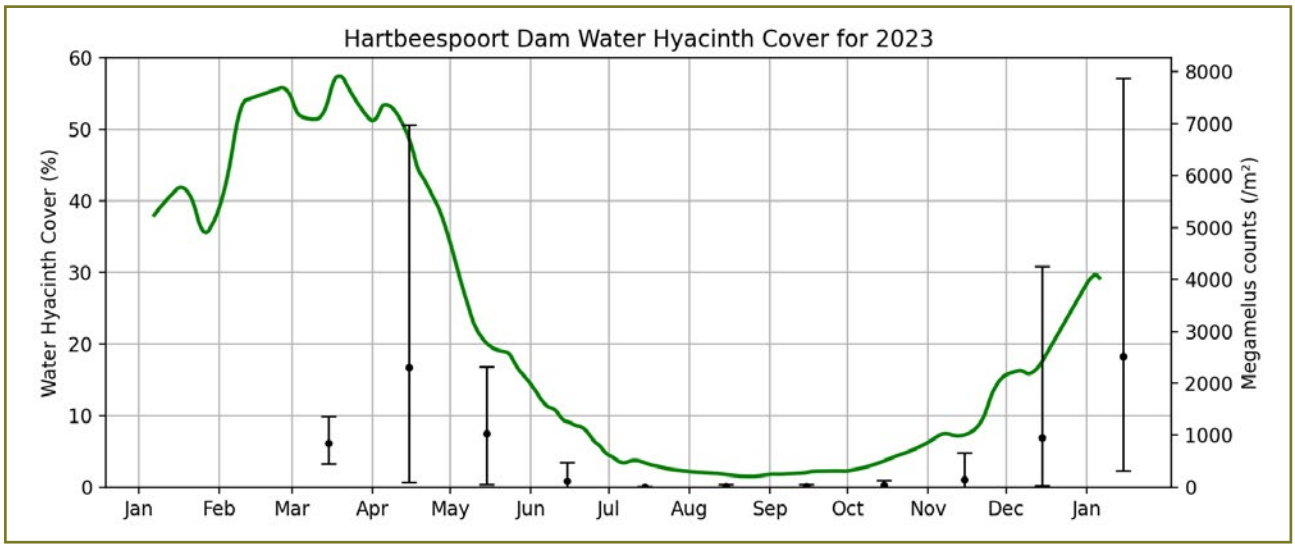
in the Highveld. An additional 13 stations were added: five at Hartbeespoort Dam, two at Vaalkop Dam, five at Bronkhorstspruit Dam, and one at Middle Lake in Benoni. Training sessions for community station managers were held at The Coves Estate in April and September. Engagement between these members was promoted to develop a community of practice where knowledge and experience can be shared.

Water hyacinth post-release evaluations

Dr Kelby English joined the waterweeds team as a Post Doctoral Fellow in January 2023. Her primary role is continuous post-release evaluations in the Highveld region, and community engagement with local stakeholders. A number of water bodies were monitored regularly across the Highveld. The severely hypertrophic Hartbeespoort Dam is important as understanding the impacts of biological control under extreme conditions can be useful as a case study for less impacted water bodies.

Hartbeespoort Dam is an artificial reservoir originally intended for irrigation, located in North West Province, opened in 1923 with a surface area of 2063 ha. It has become a recreational travel destination for water activities, and there are numerous golfing estates surrounding the dam, attracting holidaymakers. The Crocodile and Magalies Rivers lead into the dam, bringing an influx of nutrients from agricultural runoff and neighbouring cities, including Johannesburg and Pretoria. Water hyacinth was first recorded on the dam in the 1960s, and since eutrophication was never addressed, it has remained problematic.

Monthly surveys were carried out at five sites around the dam. At the beginning of the year, water hyacinth cover averaged around 50%, reaching a maximum of 60% in April. At the same time, *M. scutellaris* numbers peaked at 2312 ± 1255 per m^2, resulting in water hyacinth cover



Water hyacinth cover, and *Megamelus scutellaris* counts at Hartbeespoort Dam in 2023.

declining from 62% on 4 April to 21% on 4 May. Over the cold winter months, less than 5% cover was recorded and only began increasing in spring during October as plant growth conditions improved.

From August to November, the nine stations around the dam released over 250 000 M. scutellaris onto the dam. This incredible inundation resulted in M. scutellaris numbers increasing from 13.5 ± 9.9 per m^2 in August to 185.6 ± 140.5 per m^2 in November. Although the water hyacinth cover increased to 20% cover at the beginning of December, counts in early January 2024 were encouraging ~2500 insects/m^2. We are confident that the inundative release approach initiated at the beginning of the growing will ensure faster control than in previous years.

Pollution of dams and rivers in the Highveld by excess inorganic nutrients and metals has resulted in eutrophication of these systems. Water hyacinth is well recognised for its phytoremediation properties, whereby it can take up chemical pollutants (including metals) and lock them in plant biomass. In South Africa, mining activities continuously leave behind trials of sulphide mine tailings, such as the case of Blesbokspruit wetland, a RAMSAR wetland on the East Rand (figure below). The chemical breakdown of sulphide in the presence of oxygen and catalysing bacteria gives rise to acid mine drainage in the form of acid, metals and sulphates, which subsequently dissolve in water, becoming biologically available. Furthermore, sewage poses a direct threat to aquatic life. Heavy metal contaminants found in sewage, such as lead,

Water hyacinth infestation at Blesbokspruit Wetland on the East Rand of Gauteng. Note the gold mine tailings in the background. **Photo:** Julie Coetzee

cadmium and nickel have largely contributed to pollution of water reserves around South Africa. The ability of water hyacinth to take up contaminants in water may hinder biocontrol agent efficacy. The biocontrol agents are exposed to the accumulation of metals in the plant tissue through their feeding activity, for example, *M. scutellaris* is a phloem feeder, whilst *Neochetina* weevils are tissue feeders. There is, however, a paucity of information on the impacts of such environmental pollutants on the biology and physiology of herbivorous insects exposed to such contaminants, including water hyacinth biocontrol agents. In particular, such pollutants have the potential to alter insect–gut microbe communities. To investigate this, PhD student, Frank Bute, has taken a catchment approach

Frank Bute taking water hyacinth leaf and root samples, as well as water samples for heavy metal analyses, at Benoni Lakes.
Photo: Julie Coetzee

Raw sewage leak entry point from Brakpan informal settlement into Jan Smuts Dam. **Photo:** Tafara Frank Bute

around lakes in the East Rand region of the Gauteng Province to elucidate the impact of metal toxicity on insect-gut symbionts. His research aims to:

1. assess the level of environmental contaminants of targeted heavy metals in the sediment, water, and plants (leaf, roots).
2. evaluate the impact of metal pollution on the gut microbiome of the biocontrol agents by targeting the 16S gene using next-generation sequencing technology (NGS).
3. contrast and compare biological exposure dynamics of the biocontrol agents.

Integrated control

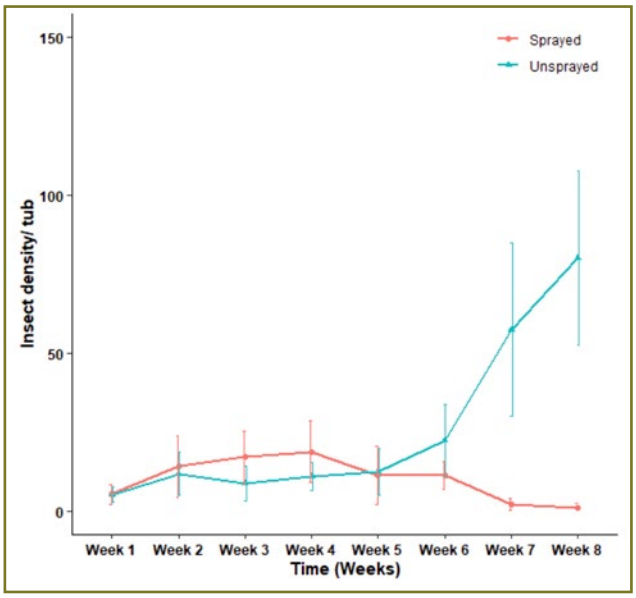
The combined utilisation of herbicides and biological control has proven effective in managing water hyacinth populations in South Africa. Careful application of herbicides in appropriate doses can effectively suppress the growth of water hyacinth while minimising adverse effects on biological control agents and the environment. While recommended-label doses of herbicides may be toxic to biocontrol agents, applying sub-lethal or retarded doses could minimize their impact.

Siyasanga Mnciva, a PhD student at the CBC, is working towards developing an integrated management system for water hyacinth control. This involves utilising the biological control agent *Megamelus scutellaris* along with a sub-lethal dose of glyphosate-based herbicide. Laboratory tests have identified sub-lethal doses of three registered glyphosate herbicides in South Africa—Kilo Max, Seismic, and Roundup.

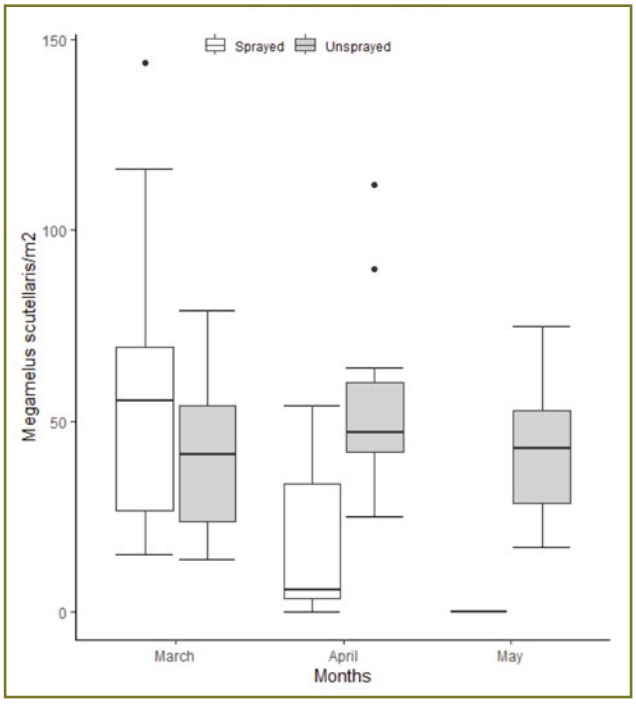
The study investigated the performance of *M. scutellaris* when exposed to herbicides and the combined impact of *M. scutellaris* herbivory and herbicide application on water hyacinth plants. Among the tested glyphosate herbicides, Roundup exhibited the highest toxicity to *M. scutellaris*, followed by Seismic and Kilo Max.

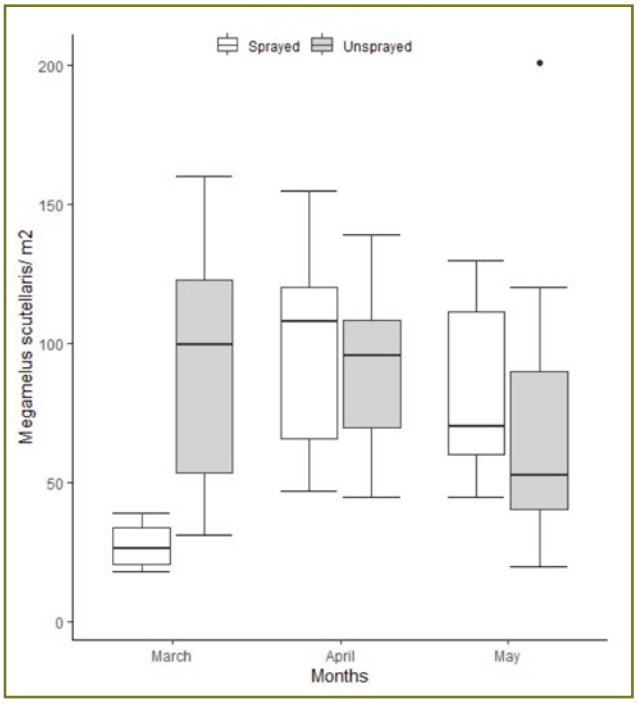
Indoor mesocosm studies and field studies at Bronkhorstspruit Dam were conducted to test the effectiveness of integrating identified sublethal/low concentrations of glyphosate herbicides with biocontrol. The integration approach of leaving unsprayed water hyacinth plants as refuge areas for insects to disperse during herbicide spray regimes was investigated. The mesocosm experiments showed that *M. scutellaris* was able to disperse from a large pool containing sprayed plants to neighbouring smaller containers of sprayed or unsprayed plants, while field results showed that the insect densities varied between sites ($\chi 2 = 26.4$, df = 1, P = 0.005) and treatments (Sprayed/Unsprayed) ($\chi 2 = 7.6$, df = 1, P < 0.050). At the Cloverhill site, more insects were recorded on the sprayed plot at the beginning of the experiment, but in April and May, there were more insects on the unsprayed plots as the plants from the sprayed plots were starting to die. Contrarily, at the Summerplace site, more insects were recovered from the sprayed plants in April and May.

These results suggest that glyphosate at low concentrations can be used along with biocontrol agents in the integrated management of water hyacinth in natural environments. Moreover, according to the results of this study, it is advisable to integrate the current herbicide management regimes of water hyacinth with a biocontrol agent refuge system. This way, a critical insect density of control agents will be preserved in the refuges, which will curb the regrowth of water hyacinth and limit re-infestations.



Left: Indoor mesocosm setup to investigate dispersal and population dynamics of *Megamelus scutellaris*, following a sublethal glyphosate-based herbicide application to water hyacinth, at Waainek Rearing Facility, Rhodes University, Grahamstown. **Photo:** Siya Mnciva.
Above: *Megamelus scutellaris* density following dispersal to sprayed and unsprayed water hyacinth plants.
Below: Density of *Megamelus scutellaris* on plots of water hyacinth plants that were sprayed with a sublethal herbicide dose, and on unsprayed plots at two sites at Bronkhorstpruit Dam, Cloverhill (A) and Summerplace (B), Bronkhorstpruit, Gauteng.





Giant Salvinia

Salvinia molesta, commonly referred to as Kariba weed or giant salvinia, is a South American weed with a broad distribution that thrives in tropical and subtropical areas globally. Recognised as one of the most problematic tropical aquatic weeds, it possesses the capacity to create thick layers on the surfaces of water bodies, displacing indigenous vegetation and causing harm to aquatic

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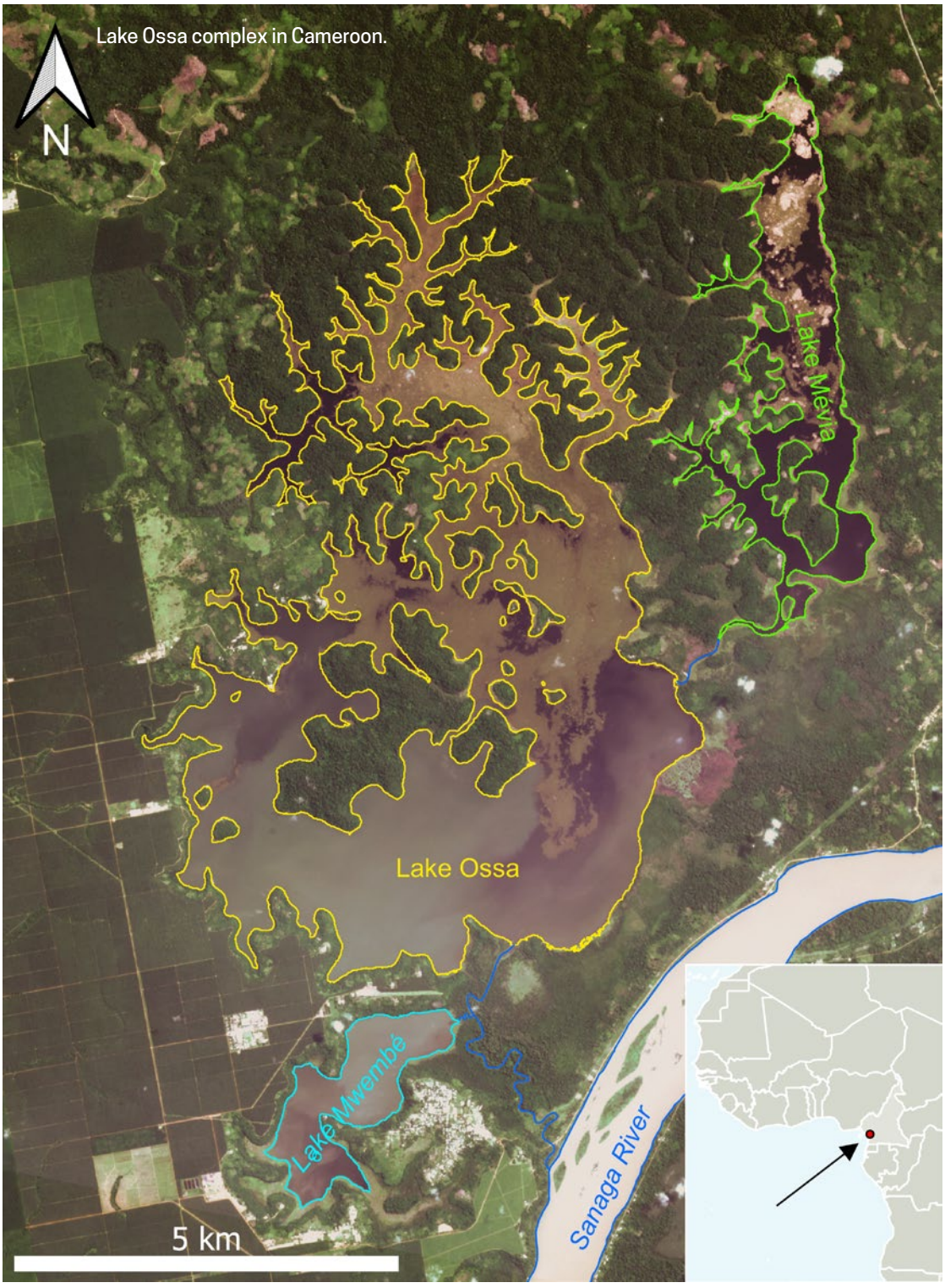
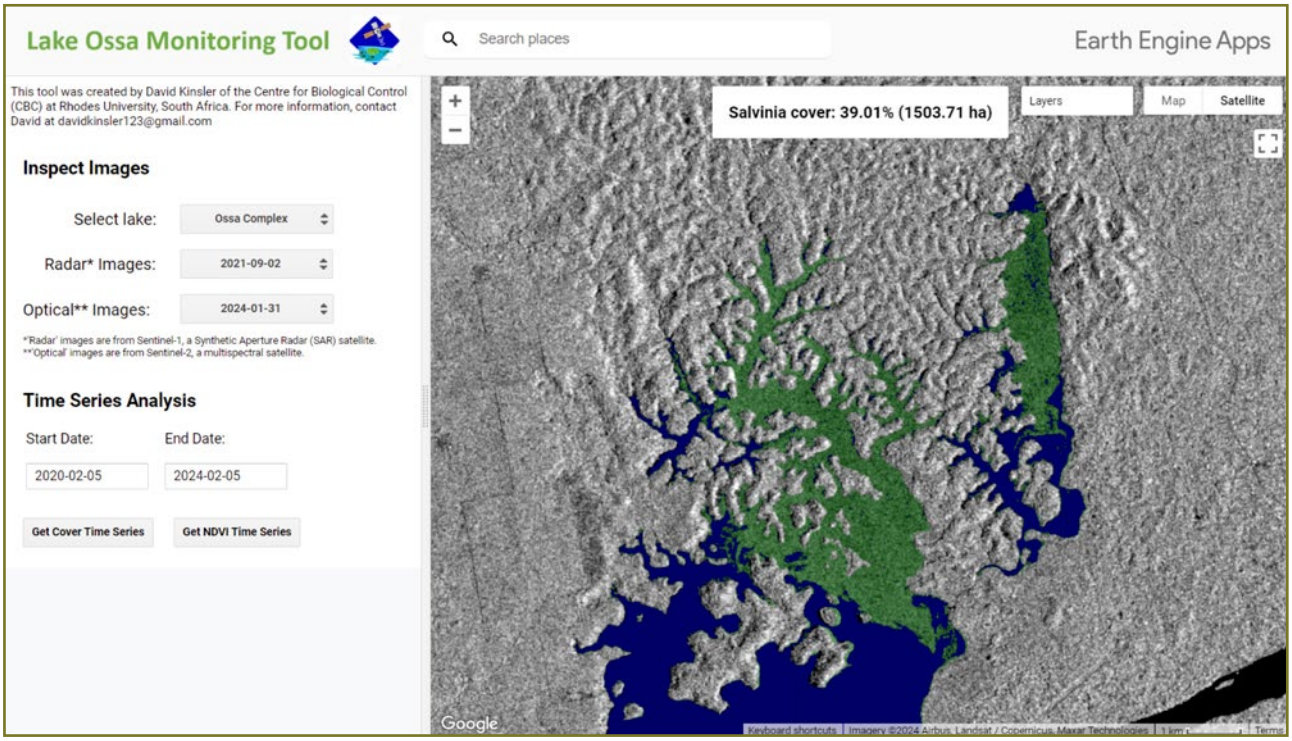


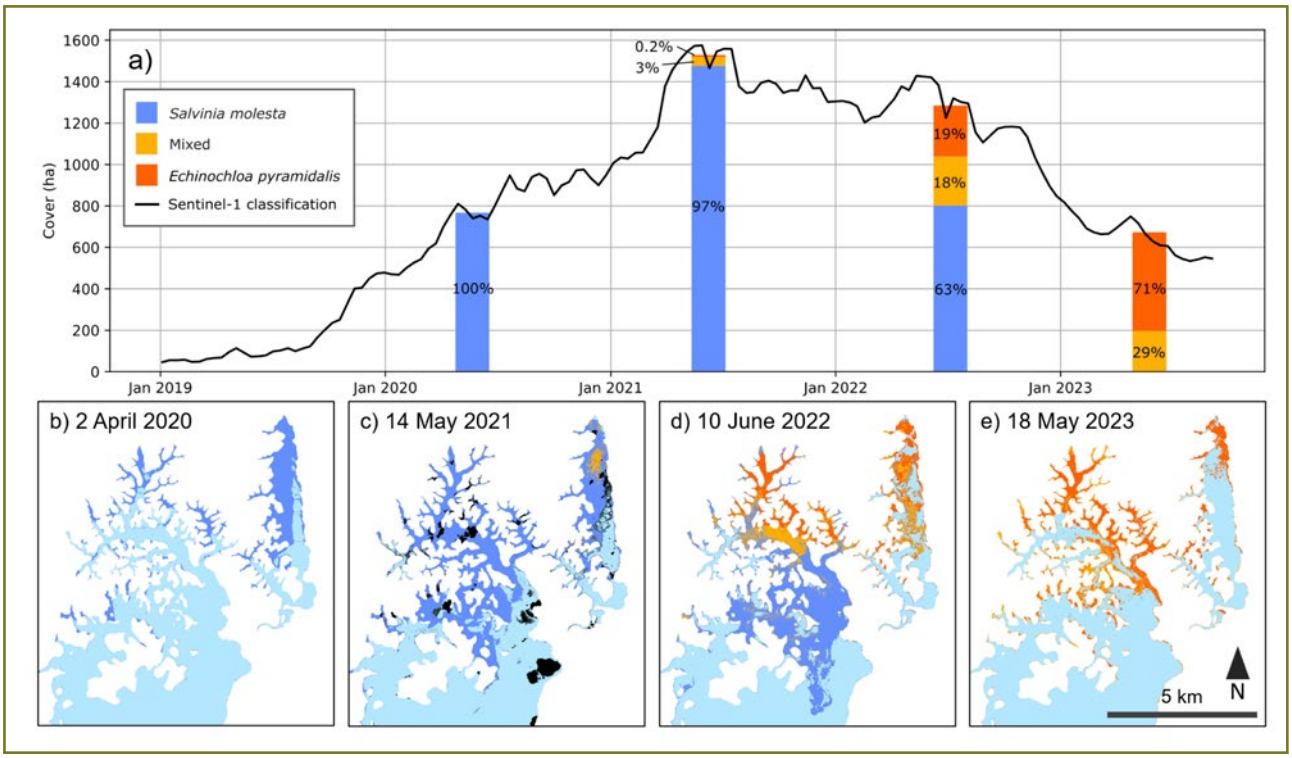
Lake Ossa complex in Cameroon.

ecosystems. Since 2019, Lake Ossa in Cameroon has become heavily invaded with *Salvinia molesta*, peaking to almost 50 % (1700 ha) coverage in 2021. The system is a biodiversity hotspot and provides important habitat to the vulnerable African manatee (*Trichechus senegalensis*), which lost significant grazing areas to the salvinia invasion.

In 2021, the Department of Entomology, Louisianna State University (LSU), in partnership with the African Marine Mammal Conservation Organisation (AMMCO), successfully released and established the biological control agent *Cyrtobagous salviniae* into the system in July 2021.

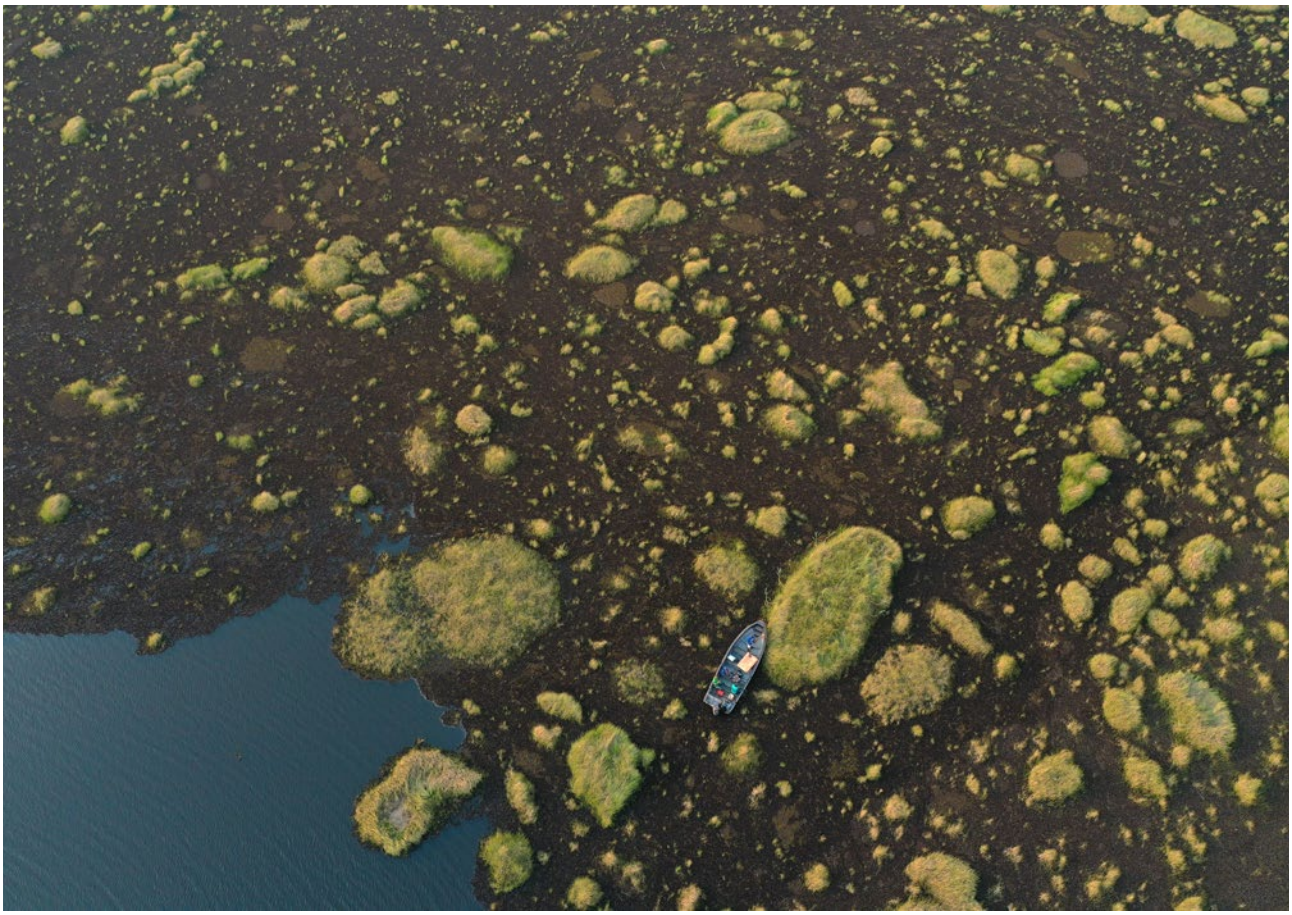


Above: A screenshot of the WebApp tool developed to detect the presence of *Salvinia molesta* on Lake Ossa, Cameroon.



Salvinia molesta cover on Lake Ossa. The biocontrol agent, *Cyrtobagous salviniae* was released in July 2021, which resulted in a significant decline, and by the May 2023, very little *S. molesta* remained on the system.

Due to the expansive and inaccessible nature of the lake, CBC was approached by LSU and AMMCO to develop a post-release satellite-based monitoring tool to quantify the changes and impact of the biological control programme across time. The tool, developed by Dave Kinsler, used radar satellite data (Sentinel-1) to penetrate the heavily clouded atmosphere and detect the presence of salvinia on the system. The near-real-time data from this tool, which was provided to stakeholders via a WebApp, showed a significant decline in salvinia over the last two years.



Brown patches of dying *Salvinia molesta* as the result of biological control by *Cyrtobagous salviniae* on Lake Ossa. The mats were used as a substrate by the native grass, *Echinochloa pyramidalis*. **Photo credit:** Aristide Takoukam

Common Salvinia

Salvinia minima, commonly known as common salvinia, is a recently introduced aquatic invader in the freshwater ecosystems of South Africa. Originally native to Central and South America, *S. minima* was initially documented in South Africa in December 2011, from Hartbeespoort Dam in the North-West province. The temporary absence of water hyacinth, controlled by *M. scutellaris* along with other biocontrol agents in 2021, led to the secondary invasion by common salvinia at Hartbeespoort Dam in the winter of that year. Since the invasion of common salvinia at the Dam, the population dynamics of water hyacinth and common salvinia have been interchanging.

MSc student Tressia Chikodza focused on examining the population dynamics between water hyacinth and common salvinia, using historical weather data, and plant cover data. In September 2019, (early autumn), water hyacinth coverage fluctuated. However, a substantial decline in water hyacinth as a result of *M. scutellaris* feeding occurred from October 2019- September 2020.

Water hyacinth coverage began to increase in the following spring once again (September and October 2020) to a maximum of approximately 30% coverage in December

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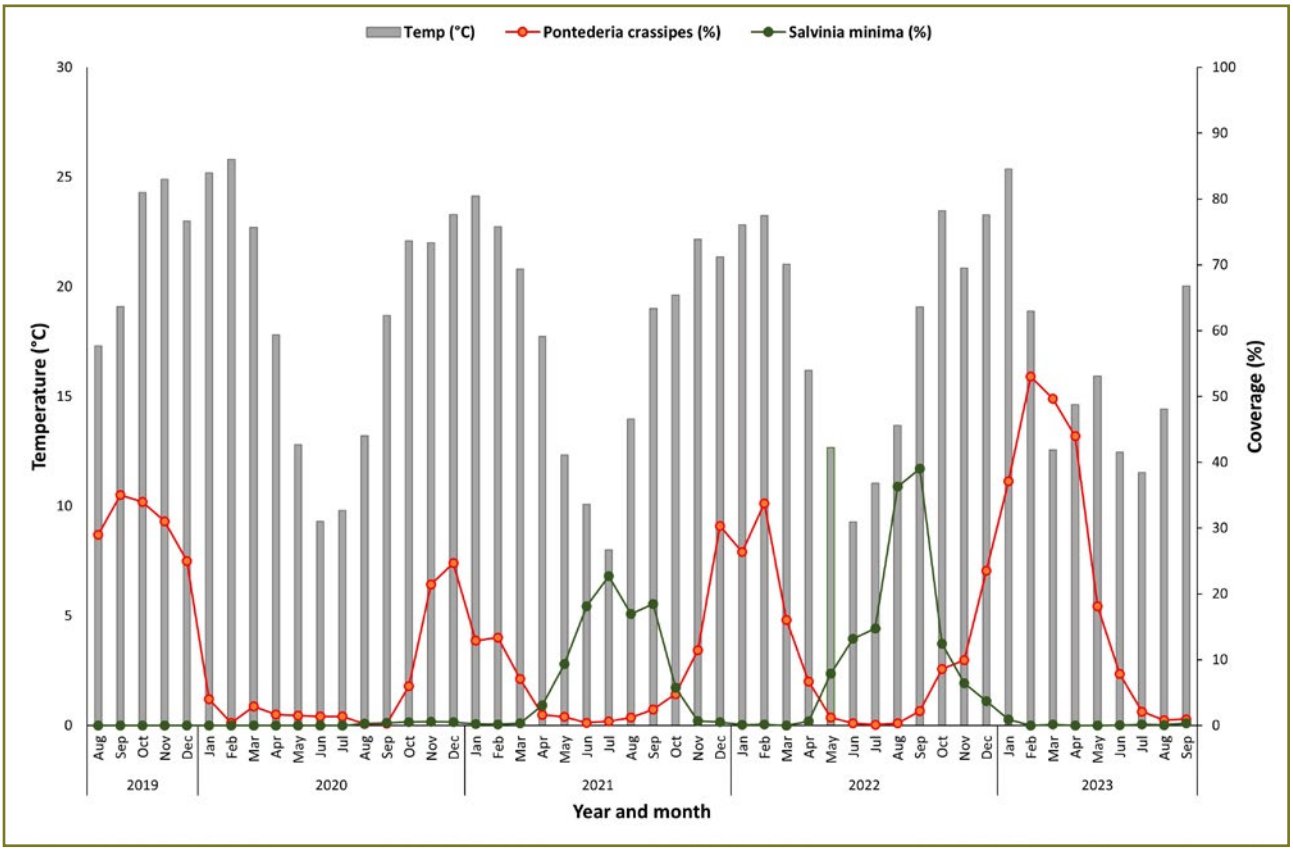
2020. However, cover declined over the height of summer from January 2021 to May 2021.

Following the decrease in water hyacinth in late Autumn (May 2021), common salvinia cover increased rapidly, reaching a maximum of 38% cover in midwinter (July 2021). Satellite data derived from a September 2021 Sentinel-2 image revealed that common salvinia covered more than 50% of the dam area, while water hyacinth cover was only 7.8%. During spring (September-November

Above: *Salvinia minima* invasion at Hartbeespoort Dam in 2022. **Photo:** Tressia Chikodza (27/10/22)

Below: *Pontederia crassipes* and *Salvinia minima* cover at Hartbeespoort Dam, from August 2019, until August 2023. Mean monthly temperature is included (error bars excluded to reduce clutter).

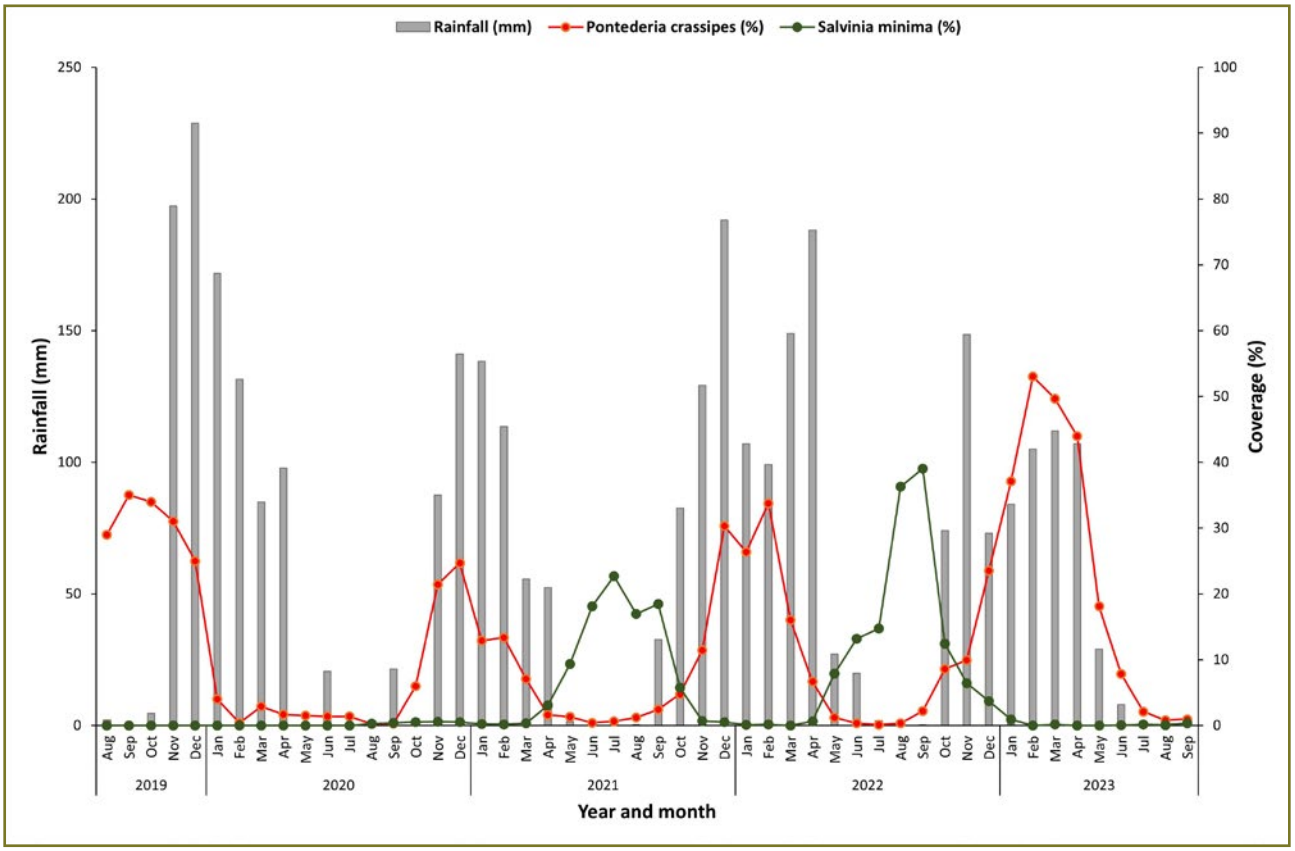


2021), water hyacinth reappeared once again as the dominant invader.

In late November 2022, following heavy rainfall, common salvinia cover was drastically reduced. In the absence of *M. scutellaris* and potential competition from common salvinia, water hyacinth dominated the water body by January 2022.

Above: *Pontederia crassipes* coverage in September 2021 at Hartbeespoort Dam in both hectares and percentages obtained from the macrophyte monitoring WebApp: *https://davidkinsler123.users.earthengine.app/view/macrophytemonitoring-tool* .

Below: *Pontederia crassipes* and *Salvinia minima* cover at Hartbeespoort Dam, from August 2019, until August 2023. Mean monthly rainfall is included (error bars excluded to reduce clutter).



As summer progressed at the dam, the biocontrol agent populations proliferated, resulting in water hyacinth populations crashing as a result of intense feeding damage. In the absence of water hyacinth, common salvinia dominated once again over the winter months. A similar cycle as the previous year was evident in the spring (September) of 2022, where common salvinia cover decreased while water hyacinth cover increased exponentially, reaching a maximum cover in February 2023. Interestingly, this trend was not evident in the spring of 2023; although P. crassipes cover decreased drastically by the end of summer as a result of biological control (April 2023), common salvinia did not increase as in previous years.

Brazilian Waterweed

Brazilian waterweed (*Egeria densa*) is the first submerged aquatic weed in South Africa to be targeted for biological control. In 2018, the first release of the leaf-mining fly, *Hydrellia egeriae*, took place. Mass rearing and release of *H. egeriae* has continued in 2023. A total of five releases have been made, one at Baviaanskloof on the 31st of January (5kg), and four at Midmar Dam by uMngeni-uThukela Water on the 7th of March (185g/182g), 18th of April (1.019kg), 16th of May (297g), and 6th of November (301g).

Post-release surveys in March 2023 found an average of 2559 mm/kg fresh weight, and the average weight of *E. densa* shoots was 3.04 ± 0.30 g per 15 cm shoot. However, in October and November, no fly larvae or pupae were recorded. In October, the average weight of the shoots was 2.53 ± 0.15 g per 15 cm shoot, and in November, the average weight of the shoots was 1.78 ± 0.15 g per 15 cm shoot. Although no flies were recorded in the spring surveys, the shoot weight has decreased compared to the autumn surveys. The water level at Midmar Dam is currently low (approximately 79% full), and the uMngeni-uThukela Water Catchment Management team, who monitor *Egeria* at Midmar monthly, have suggested that the low dam levels have resulted in a reduction of the *E. densa* infestation. uMngeni-uThukela Water will continue to release *H. egeriae* at Midmar Dam and continued post-release monitoring at this site is planned to determine the effectiveness of these releases.

RESEARCH AND TECHNICAL STAFF
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COLLABORATOR
FuEDEI

Delta Arrowhead

RESEARCH AND TECHNICAL STAFF
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COLLABORATORS
U.S. Army Engineer Research and Development Center's (ERDC) Environmental Laboratory (Nathan Harms), Australia's AgriBio Centre for AgriBioscience (Raylene Kwong)

Sagittaria platyphylla is one of the fastest-spreading invasive species in the country and was therefore prioritised by the CBC for biological control. Since 2014, the CBC have been assessing the suitability of four *Listronotus* spp. weevils, collected from the plant's native range in the USA, as candidate biological control agents. The fruit and flowers feeding *Listronotus appendiculatus* were selected due to their potential to reduce seed numbers and slow the growth of *S. platyphylla*. In 2021, a release application was submitted to the Department of Agriculture, Land Reform and Rural Development for permission to release the agent, and permission was granted in early 2022. In July 2023, Dr. Grant Martin travelled to the Lewisville Aquatic Ecosystem Research Facility, Texas, USA, to collect a new culture of the weevil to release. The trip was facilitated by Dr Nathan Harms, a research biologist with the U.S. Army Engineer Research and Development Center's (ERDC) Environmental Laboratory (EL), and Dr Ian Knight from the US Army Engineer Research and Development Centre, Vicksburg campus. Two hundred adults were collected and imported to CBC Quarantine facilities, where they were screened for any hitchhiking pests and/or parasitoids.

The culture was divided into two; one set was sent to CBC Postdoctoral Researcher, Dr Daniella Egli based at the University of Kwa-Zulu Natal, and the second was kept at CBC Makhanda. The official release of the agent was conducted at an invasion of *S. platyphylla* in Makhanda Botanical Garden, Makhanda, in September 2023. The release was supported by several members from the DFFE, including Specialist Programme Manager/Biodiversity Officer Ms Debbie Muir and Eastern Cape Biodiversity

Officer Abbie Heunis, residents from the city of Makhanda, staff and students from Rhodes University, and Universities VC Dr Sizwe Mabizela who made the ceremonial release.

Since the official release, Dr Egli has made additional releases in the river at Ezemvelo's Kranzkloof Nature Reserve in Kloof, a tributary of the Duzi River in KwaZulu-Natal Province. The release was attended by Futhi Shabalala of SANBI, Phindile Shangase of DFFE and the reserve manager, Waldo Bekker. An additional release was also made outside Lourensford Wine Estate in the Western Cape Province at the end of August 2023. We hope that *L. appendiculatus* will establish and make a significant contribution towards the management of one of South Africa's worst emergent aquatic weeds.

Right: Nathan Harms (ERDC) in the field collecting *Listronotus appendiculatus* weevils for Grant Martin to take back to SA. **Photo:** Grant Martin

Dr Sizwe Mabizela (RU VC), Debbie Muir (DFFE Official) and Grant Martin releasing the first *Listronotus appendiculatus* into the Makhanda Botanical gardens. **Photo:** Kim Weaver

Yellow Flag Iris

Exploration of management strategies implemented against yellow flag iris at a local scale

RESEARCH AND TECHNICAL TEAM
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STUDENTS
Gianmarco Minuti (PhD), Paula Gervazoni (PhD)

COLLABORATORS
VUB, FuEDEI, Conicet

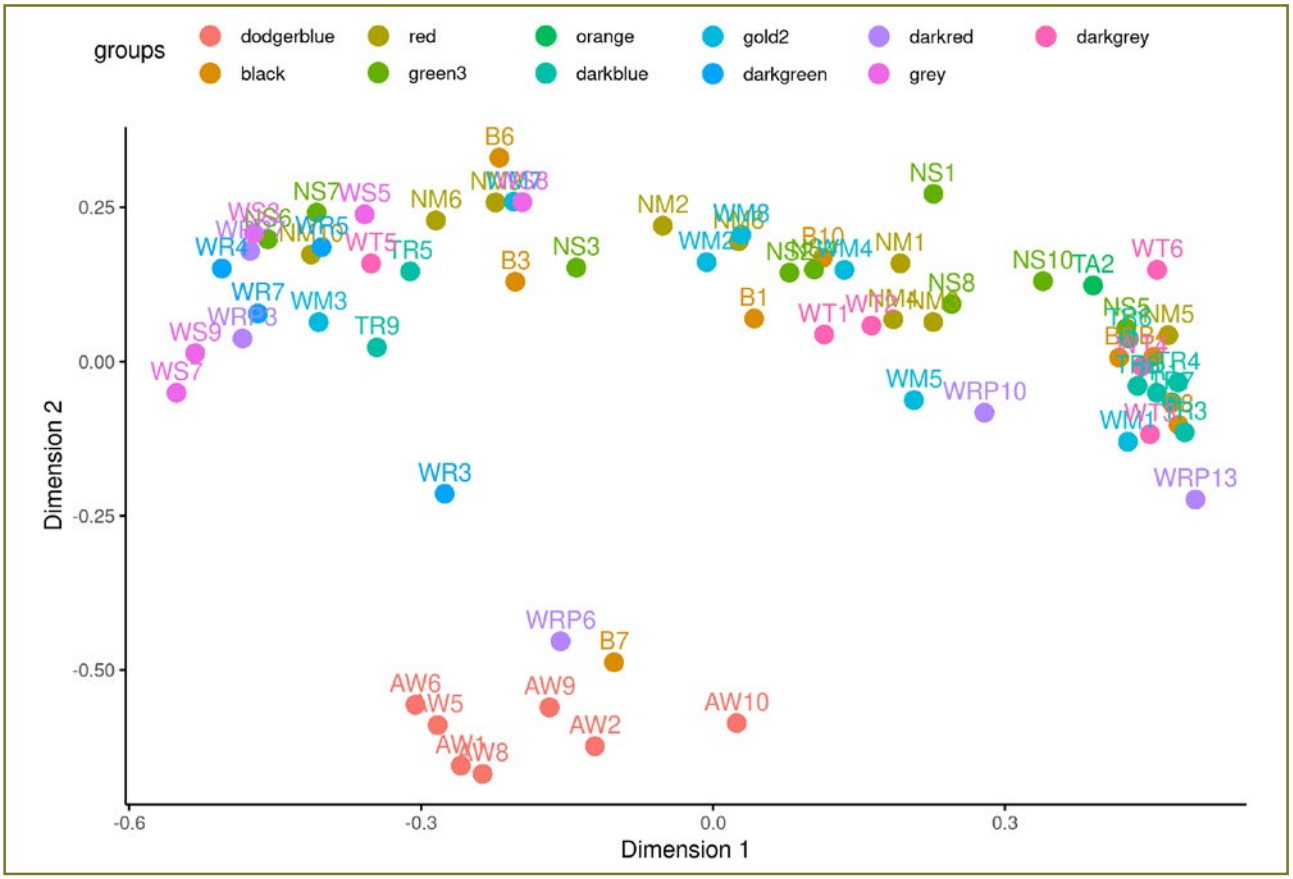
Surveys were conducted in protected natural areas invaded by *Iris pseudacorus* (Yellow Flag Iris) in Argentina and Uruguay within the scope of international collaboration global-south for the study of *I. pseudacorus*. The aim was to consult park rangers regarding local-scale strategies used to combat this invasion. Two methods were applied in these areas: periodic mechanical removal with soil coverage to block sunlight and removal coupled with the planting of native species. However, the gathered information revealed the inefficacy of these approaches, as they necessitate recurring application. This underscores the considerable challenge, high costs, and ongoing efforts involved in managing

Mechanical control effort applied against *Iris pseudacorus* invasions in Uruguay. **Photo:** Giannina Orcasberro

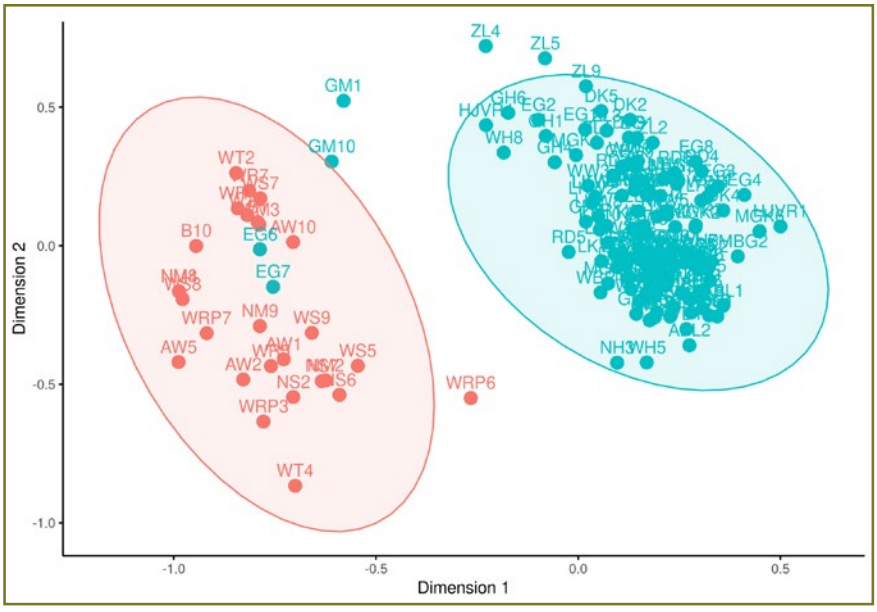
invasions by this species, even on a small scale. It reaffirms the urgent need to explore and implement more effective biological control methods like biological control.

Population genetics of *Iris pseudacorus* in New Zealand

The CBC has collaborated with researchers at Manaaki Whenua (Landcare Research) to investigate the population genetics of invasive *Iris pseudacorus* populations in New Zealand. Preliminary analyses show that, as is the case in South Africa, *I. pseudacorus* populations in New Zealand reproduce primarily through sexual means, i.e. through the production of sexually produced seed. A large amount of gene transfer was evident between and within populations, as indicated by the lack of clear clustering of samples from the same populations, with the exception of population AW (Auckland Waiatarua Reserve) (figure below). While the samples grouped quite clearly based on the country in



Above: Non-metric MDS for *Iris pseudacorus* individuals from various populations in New Zealand, based on Jaccard's similarity index. Different colours represent different populations.



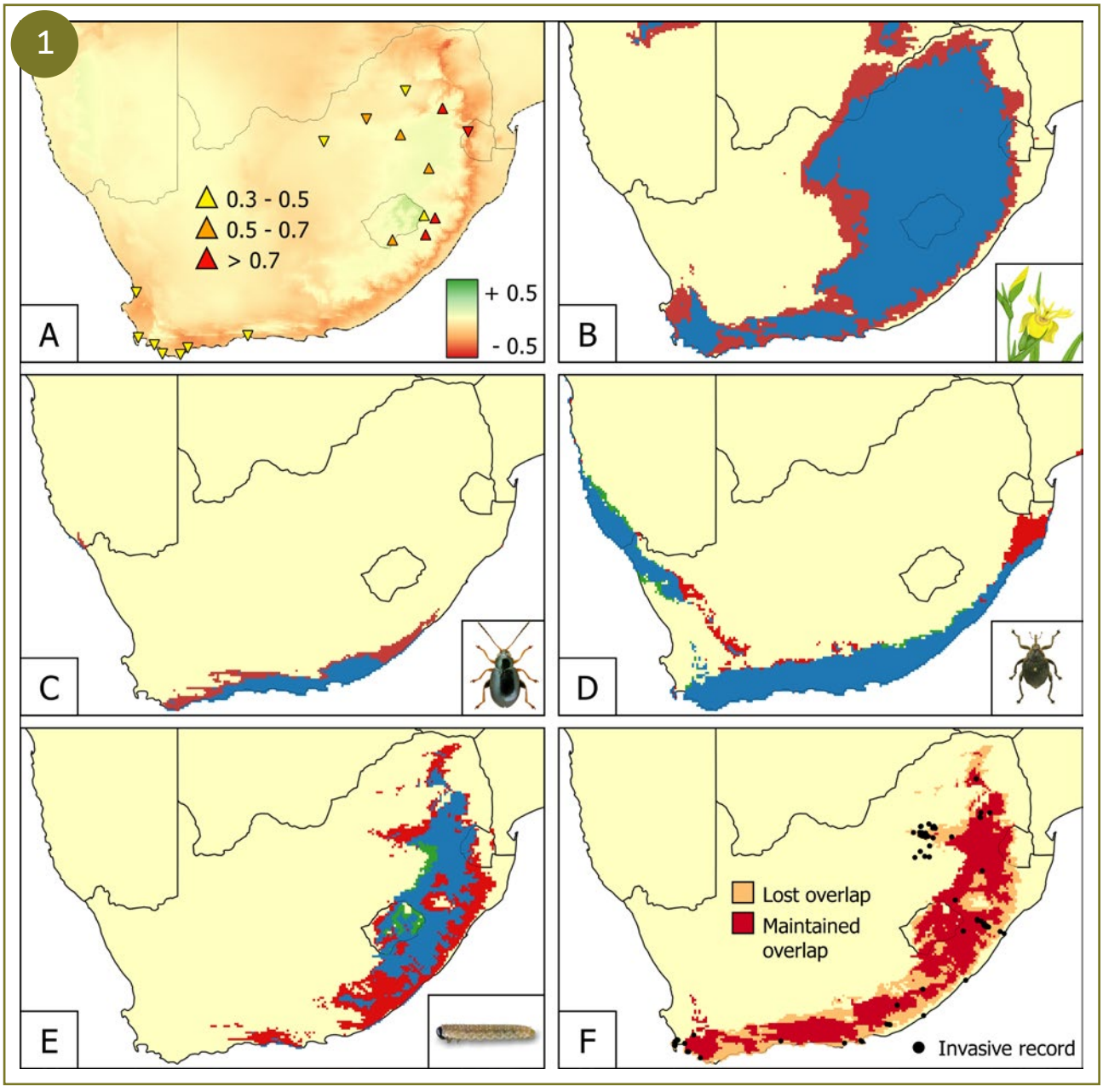
Right: Non-metric MDS for *Iris pseudacorus* individuals from different populations in New Zealand and South Africa based on Jaccard's similarity index. Red dots represent samples from New Zealand, and blue dots represent samples from South Africa.

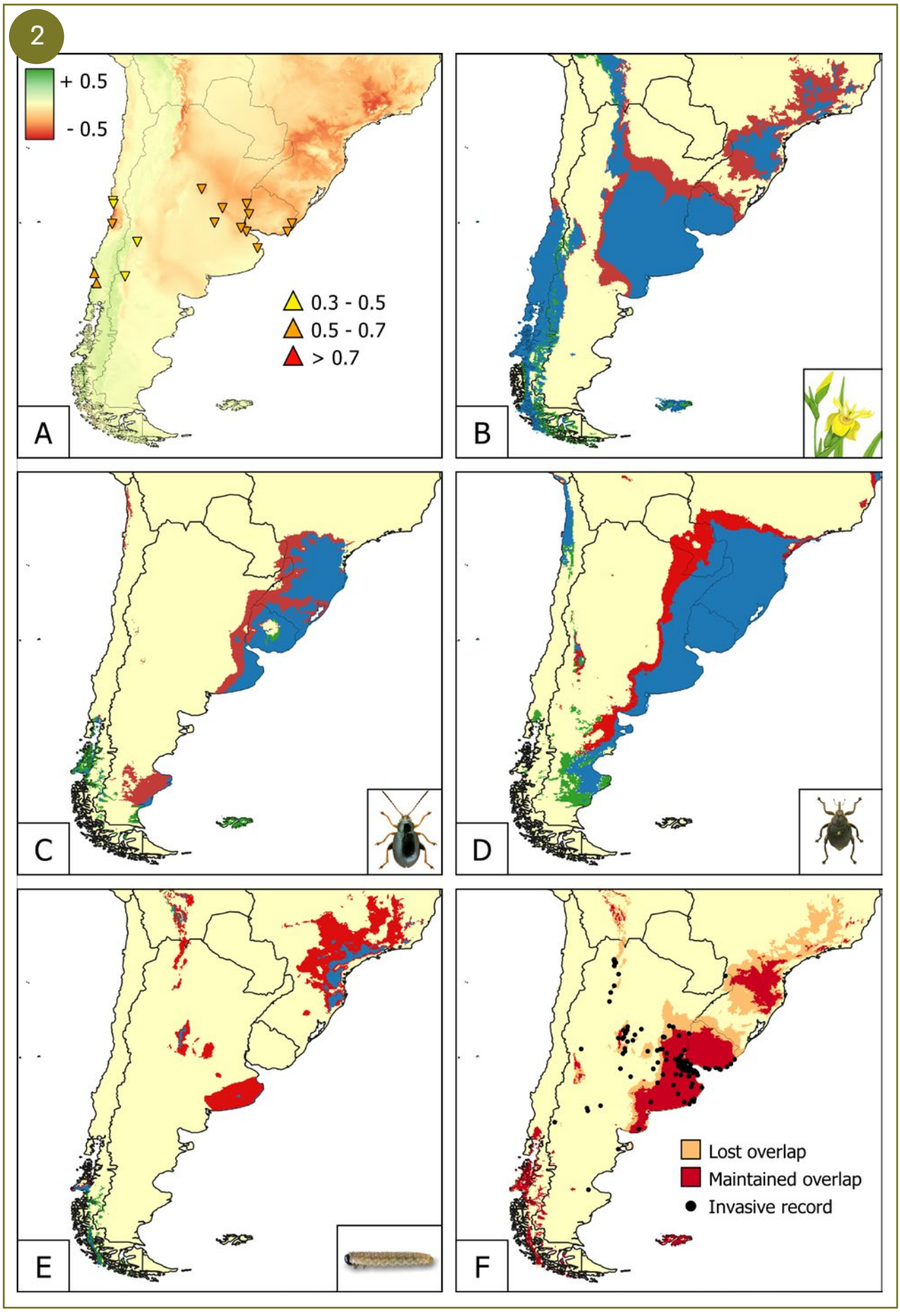
which they were collected, some overlap is evident, with three (almost four) South African *I. pseudacorus* samples falling into the New Zealand group. Two of these samples were collected from Emmarentia Botanical Gardens in Johannesburg, and the other two from a Guest House in Groot Marico, North West Province.

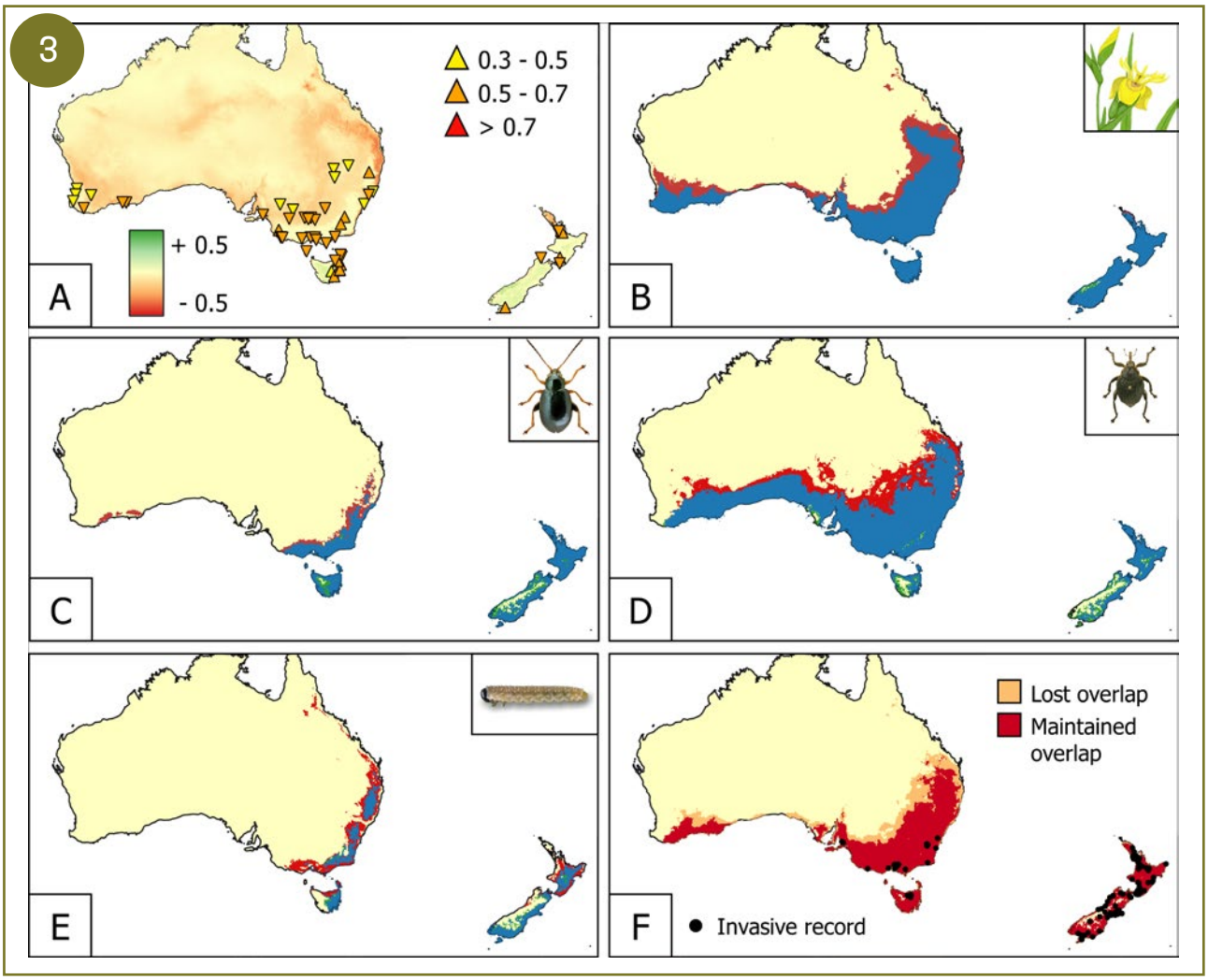
Species distribution modeling

PhD student, Gianmarco Minute used MaxEnt to model and compare the present and future climatic suitability of *Iris 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

Invasion risk, climatic suitability and biocontrol potential for *Iris pseudacorus* in 1. South Africa, 2 southern South America, and 3. Australia and New Zealand. (A) Net change in suitability for *I. pseudacorus* between present and future climatic conditions (green indicates an increase in suitability, red a decrease). Ramsar sites at risk of invasion are represented by triangles: colours represent their current suitability score; upward and downward direction indicate if suitability is predicted to increase or decrease in the future. Binary suitability maps for (B) *I. pseudacorus*, (C) *Aphthona nonstriata*, (D) *Mononychus punctumalbum*, and (E) *Rhadinoceraea micans* show areas where suitability will be gained (green), lost (red), or maintained (blue) under future climatic conditions. (F) Climatic niche overlap between *I. pseudacorus* and its candidate biocontrol agents based on present and predicted future climatic conditions. Black dots indicate present occurrence records of the weed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.




2
+ 0.5
- 0.5
0.3 - 0.5
0.5 - 0.7
> 0.7
A
B
C
D
E
F
Lost overlap
Maintained overlap
Invasive record



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 prioritising areas for their release.

Aphthona nonstriata

While host-specificity testing of indigenous Iridaceae is complete, the candidate biological control agent *Aphthona nonstriata* remains in the CBC's quarantine facilities to undergo further multi generational host-specificity testing in the new year. The flea beetle is specific to the Iris genus, of which no species are native to South Africa. As such, its release would not pose threats to native biodiversity. However, exotic irises, which may support the development of *A. nonstriata*, are of economic importance in the horticultural industry. Multi-generational host-specificity tests will be conducted using the Iris species previously shown to support the development of first-generation *A. nonstriata* beetles. Once these trials are complete, a release application for *A. nonstriata* will be sent to the DFFE. If approval is granted, we will begin releasing the flea beetle at invaded sites across the country.

In addition, Gianmarco tested the effect of various insect densities on adult *Iris pseudacorus* plants. Preliminary results show that even low densities (n = 5) are enough to stunt the growth of *I. pseudacorus* aboveground biomass. However, the insects were not able to kill the plants even at the highest tested density (n = 20). Our hypothesis is that the resilience of *I. pseudacorus* to *A. nonstriata* herbivory could be due to the energy reserves stored within the rhizomes of adult plants. To test this, Gianmarco conducted a second experiment, where young plants (germinated from seeds) were exposed to low (n = 4) and high (n = 8) densities of the insect. The results showed high plant mortality even at the lowest insect density, confirming our hypothesis. This information can be used to tailor releases to target vulnerable stages of the plant and also means that *A. nonstriata* could be able to control the sexual reproduction of *I. pseudacorus*.

Red water fern

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Azolla filiculoides biological control elicits ecosystem recovery – A case study from Belgium

Field sampling and mesocosm experiments were conducted to assess the recovery of aquatic ecosystems upon the biological control of *Azolla filiculoides* by the weevil *Stenopelmus rufinasus* (which is considered naturalised in Belgium). Water parameters, macroinvertebrate assemblages and zooplankton communities were analysed before, during and after Azolla invasion and compared to systems that were biologically controlled by *S. rufinasus* and used nearby uninvaded ponds/mesocosms as references. Our results show that the presence of the weevil not only accelerated the disappearance of water fern from the systems but also elicited the recovery of favourable water conditions. However, changes in macroinvertebrates and zooplankton communities were long-lived, and full recovery was not observed during the experiment. This is probably due to the gap observed between Azolla's invasion and the build-up of the weevil population. It is therefore advisable to facilitate *S. rufinasus* timely introduction/redistribution in order to accelerate water fern control and minimise its impacts on native aquatic ecosystems.

Below: Gianmarco Minuti sampling *Azolla filiculoides* in Belgium. **Photo:** Michael Sean Staniszewski

Amazon Frogbit

RESEARCH AND TECHNICAL TEAM
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STUDENT
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COLLABORATORS
CABI - Switzerland, FuEDEI

Hydrocharis (=Limnobium) laevigata (Amazon Frogbit) is a floating aquatic macrophyte native to South America and invasive in parts of Asia, Australia, North America and Central America. This weed forms dense mats, potentially reducing aquatic biodiversity, disrupting food webs and limiting access to dams, rivers and lakes. Recently, the weed was observed in central and southern African countries, including the Democratic Republic of Congo, Zambia and Zimbabwe. However, its actual distribution and impacts are yet to be quantified.

Janet Ncube, a PhD student, is collecting baseline data on the ecology and distribution, as well as socio-economic impacts of the weed in Zimbabwe. A predictive distribution model indicates a wide distribution of potential habitats spread across southern Africa. In Zimbabwe, Amazon frogbit has been observed at 16 of the 20 sites surveyed (table on page 24). These sites, which constitute both lentic and lotic waters, are distributed across four catchments which flow northwards towards the Zambezi River and ultimately into Mozambique. There are reports, particularly along the Zambezi River, of the transient nature of the infestations due to the rapid flow of the water. This further increases threats of invasion of downstream water bodies. Future surveys will be targeted towards catchments which flow towards the south to determine the southern limit of the distribution.

Hydrocharis laevigata infestation in Harava Dam in Chitungwiza, Zimbabwe. **Photo:** Gerald Chikowore

Catchment area	Water body	*H. laevigata*	Other weed species	Remarks
Mazowe	Nyadire dam	present	*Potamogeton crispus* *Lagarosiphon major*	Large mats of *H. laevigata* along edges of the dam
	Suswe dam	absent	—	Dam silted and drying up
	Chinyika dam 1	present	*Pontederia crassipes*	*P. crassipes* dominant
	Chinyika dam 2	present	*Salvinia molesta* *Ludwigia* spp.	Kariba weed dominant
Manyame	Harava dam	present	—	Infested with *H. laevigata*
	Damview dam	present	*Lagarosiphon major; Nymphaea* spp.	*Nymphaea* spp more dominant
	Manyame river	present	*Hydrocotyle ranunculoides*	*H. ranunculoides* dominant
	Gwebi river	present	*Azolla* spp.	Invaded by *H. laevigata*
	Darwendale	present	*Pontederia crassipes*	Large mats of *H. laevigata* floating
	Rain field dam	present	—	*H. laevigata* is dominant
	Lake Chivero	present	*Pontederia crassipes*	Water hyacinth is dominant
	Zambezi river	present	*Pontederia crassipes*	*H. laevigata* is dominant
	Mana Pools National park	present	*Pontederia crassipes*	The pool is 100% covered with water weeds
Mazowe	Mazowe dam	absent	—	No infestation observed
Gwayi	Shangani dam	absent	*Salvinia molesta*	Dam infested by *S. molesta*
Umzingwane	Umguza dam	absent	*Pontederia crassipes*	Dam heavily infested with P. crassipes
	Umguza river	absent	*Pontederia crassipes*	dominated by P. crassipes
Save	Rusape dam	present	*Pistia stratiotes*	Juvenile plants of *H. laevigata* observed
	Muchekeranwa dam	present	*Lagarosiphon major* *Nymphaea* spp.	Large mats of *H. laevigata* on the edges of the dam
Sanyati	Lake Kariba	present	Pontederia crassipes	Few plants observed due to low water levels

Oxygen Weed

Lagarosiphon major, also known as the African curly-leaved waterweed, African elodea, or oxygen weed, is a submerged macrophyte native to southern Africa and is invasive in places like New Zealand, Ireland, the UK, and Australia. This weed can produce dense floating mats that readily colonise infested habitats up to 6 meters in clear water waters. The success of this weed outside of its native range may be explained by the Enemy Release Hypothesis (ERH), which states that escape from specialist herbivores allows plants to grow and reproduce more successfully.

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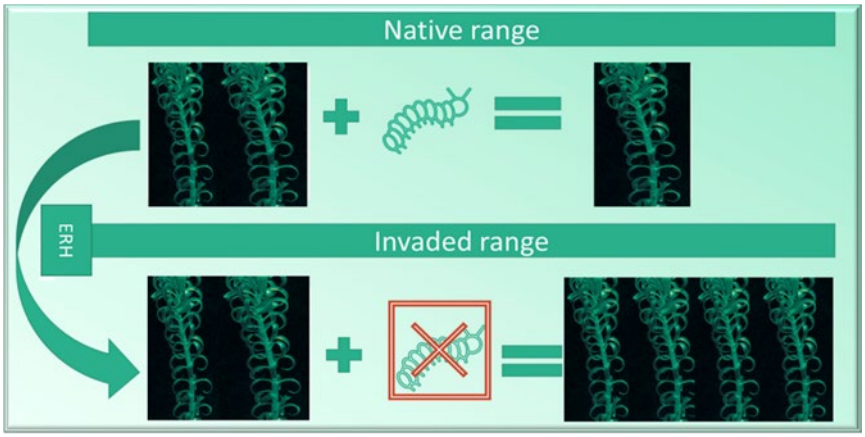
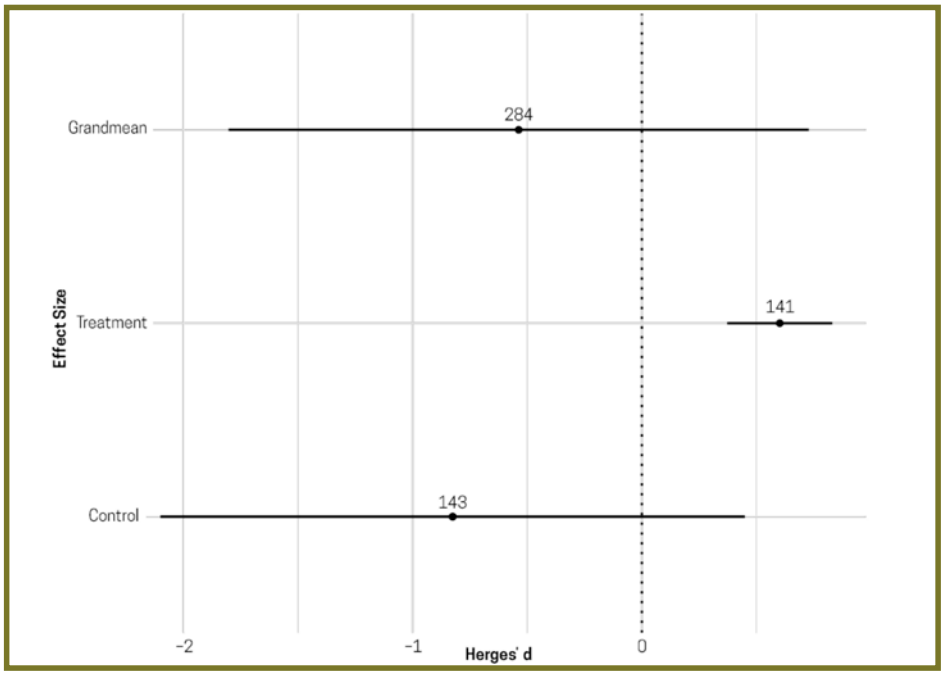


Left: Simplified visual representations of the Enemy Release Hypothesis and possible implications for *Lagarosiphon major*.

Below: Forest plot clustered by type of manipulation. Hedge's d estimates ±95% CI. Numbers represent k=number of outcomes per category.



The first part of this research investigated the possible general applicability of this hypothesis in freshwater ecosystems. Although the evidence for the general applicability of the ERH in aquatic macrophytes was found to be limited in this study, the introduction of a stress factor (e.g., temperature increase, competition, herbivory, etc.) during manipulative experimentation was found to lead to better performance of invasive macrophytes relative to their native counterparts.

Specific investigation of applicability of this hypothesis to *L. major* in New Zealand revealed that this species does, in fact, benefit from enemy release compared to plants in the native range of South Africa, where populations of this weed are subject to herbivory pressure from various natural enemies. As a result, New Zealand had higher L. major cover and abundance, as well as almost double the biomass of this weed compared to South Africa. Thus, management of this weed would benefit from the incorporation of biological control into the existing control methods. Degree-day modelling showed that *Hydrellia lagarosiphon*, a specialist herbivore of this weed previously found to be a suitable candidate for L. major biocontrol, could survive and maintain viable populations at various sites in New Zealand.

Biomass in the native range. **Photo:** Wandisile Mdiza

Biomass in the invaded range. **Photo:** Angela Bownes

Invasive Aquatic Plants and Mosquito Proliferation in Water Ecosystems

Invasive aquatic plants thrive in water ecosystems, outcompeting native vegetation and disrupting ecological balance. Concurrently, mosquitoes, particularly species like Aedes aegypti and Anopheles spp., are vectors for various diseases, including malaria, dengue, and Zika virus. Understanding the linkages between invasive aquatic plants and mosquito proliferation is crucial for the effective management of both invasive plants and mosquitoes. Invasive aquatic plants alter aquatic ecosystems by outcompeting native flora, depleting oxygen levels, and reducing biodiversity. These changes create breeding grounds for mosquitoes as stagnant water becomes more prevalent, increasing the risk of disease transmission. Therefore, by studying the relationship between invasive aquatic plants and mosquitoes, we can protect ecosystems and enhance public health on a broader scale. The aim of this study is to investigate if there is a relationship between the presence of aquatic invasive plants and mosquito invasions. To do this, mesocosms with the five worst invasive species in South Africa were set up and assessed weekly for mosquito activity. Mesocosms with invasive plants seemed to have more mosquito individuals as compared to the control tubs, with water lettuce in particular supporting more mosquitoes. More studies are to be done next year to understand why this was the case.

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STUDENT
Thoriso Masalesa (MSc)

COLLABORATOR
Leiden University (Emily Strange)

Mosquito larvae collected from the mesocosms, awaiting identification. **Photo:** Thoriso Masalesa

Mesocosms established to determine oviposition preference of local mosquito species in the presence of five aquatic invasive plant species. **Photo:** Thoriso Masalesa

Ecological Restoration

RESEARCH AND TECHNICAL TEAM

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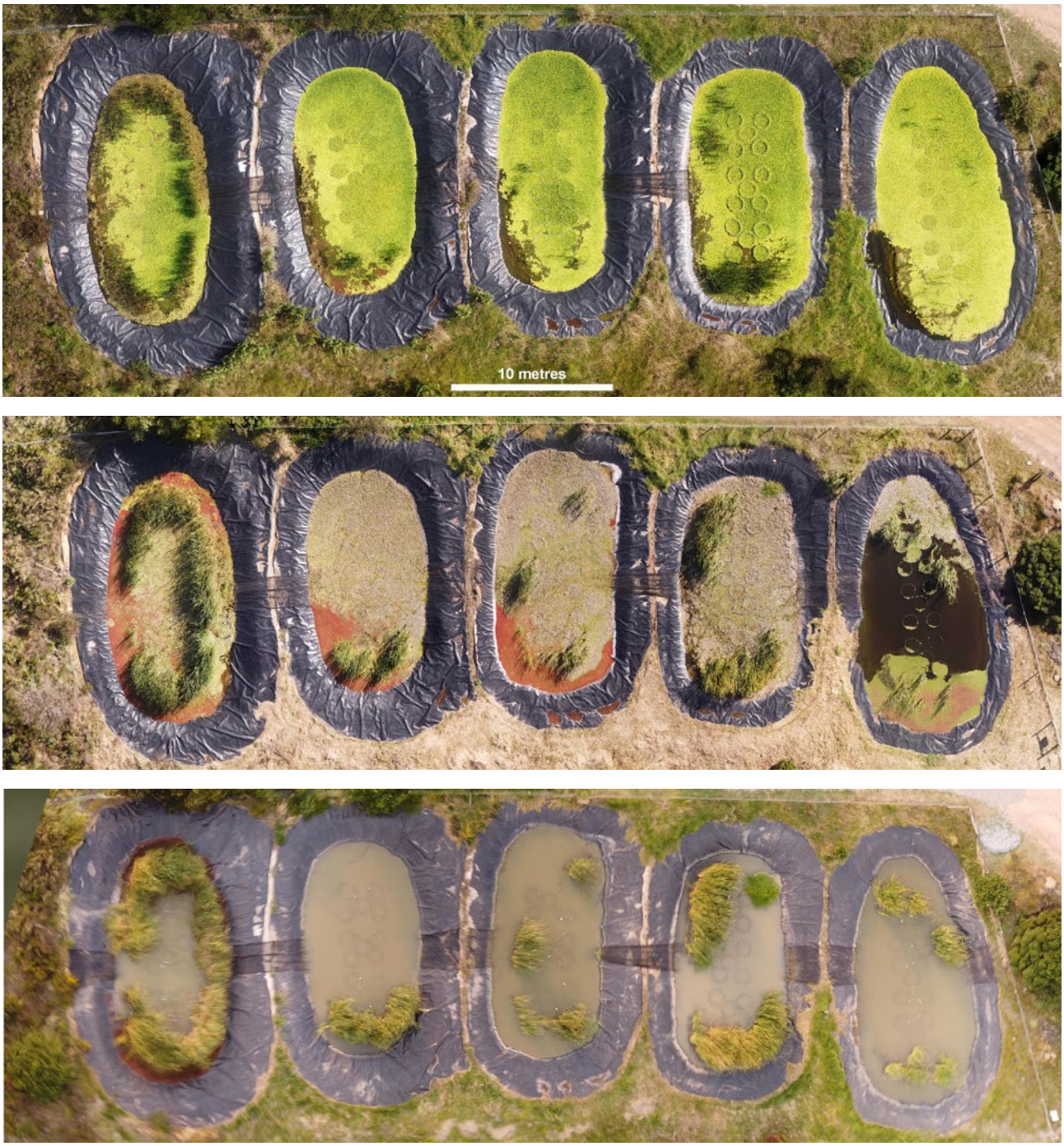
COLLABORATORS

RU, DZE (Ryan Wasserman and Chad Keates), CBC (Tafara Frank Bute), Albany Museum (Musa Mlambo)

Invasive alien plants present one of the greatest challenges to natural resource management. Their control through chemical, mechanical and/or biological methods is often seen as beneficial, but following control, the native plant communities do not always re-establish, nor do ecosystems recover. Instead, these systems are highly susceptible to re-invasion or secondary invasion (proliferation of non-target alien species following efforts to suppress dominant target invaders), taking advantage of newly available resources and habitat disturbance. This is a common challenge 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 recognised as a relevant strategy to limit invasions and support the recovery of 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, i.e., effect of species on the survival, establishment and/or growth 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 yet remain little explored, especially in freshwater systems.

Since 2021, the CBC has continued to expand its research agenda, considering the role of ecosystem restoration after aquatic weed control to ensure ecosystem recovery. The restoration team has set up a whole-pond manipulation

experiment at the Waainek mass-rearing facility. The aim was to mimic a situation of a highly invasive free-floating plant (*Pistia stratiotes*) invaded system at first, followed by successful invasive plant control using the biological control agent *Neohydronomus affinis* (Coleoptera: Curculionidae). After achieving complete control, native vegetation was actively re-established, and then a new invader, the South American rooted submerged plant *Egeria densa*, invaded the system. Five treatments manipulating native plant community composition (unplanted, three monocultures: *Lagarosiphon major*, *Stuckenia pectinata* and *Vallisneria spiralis*, and mixture) were established for each timing of arrival of native plants (priority effect): native plants were planted (1) six weeks before, (2) same time and (3) six weeks after the submerged plant invader *E. densa* arrival. The experiment ran for thirteen months in total. The performance (e.g., productivity) of the submerged invader was assessed by harvesting its biomass by the end of the experiment.



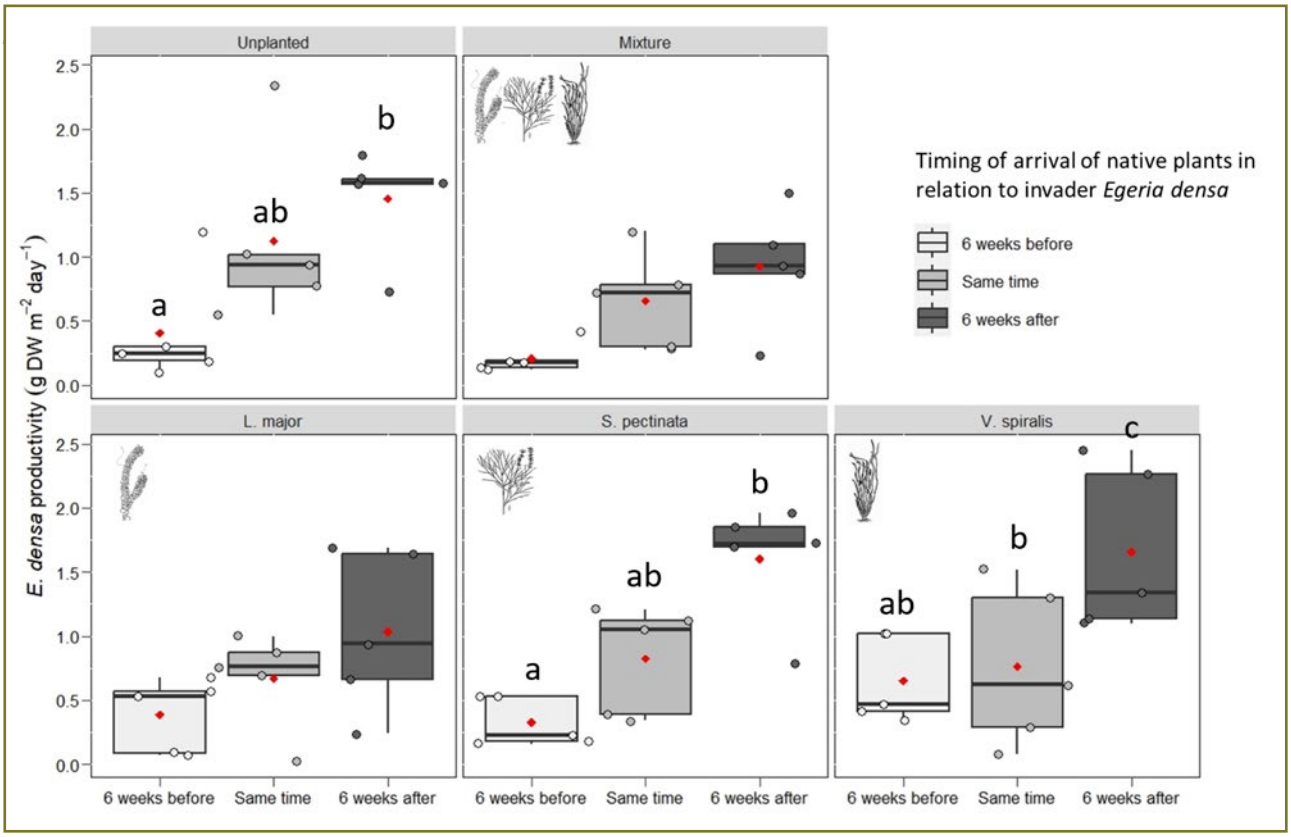
Aerial photos of the whole-pond manipulation experiment at Waainek Mass-Rearing facility showing 100% cover (invaded state), control phase and 0% cover (clear state) of the invasive free-floating plant *Pistia stratiotes* (water lettuce). Photos taken 12/04/2022, 22/07/2022 and 11/10/2022. **Photos:** David Kinsler

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

Above and below: Final plant biomass harvest. Photos taken 12/06/2022.
Photos: David Taylor, Tafara Frank Bute

Preliminary results showed that timing of the arrival of the native plant *L. major* did not affect *E. densa* productivity. However, timing of the arrival of *S. pectinata* and *V. spiralis* did affect *E. densa* performance. Planting *S. pectinata* and *V. spiralis* six weeks before or at the same time as the arrival of *E. densa* led to a 50 to 80% reduction in invader productivity. Even though we observed the lowest *E. densa* productivity when planted in combination with three native plant species (mixture), it was not due to the timing of the arrival of native plants. It is important to highlight that the unplanted treatment, i.e. invader *E. densa* growing alone, showed evidence of biotic resistance. We believe that is caused by the competition with the plants growing from the seed bank. Further analysis will be performed to disentangle the effects of the plant biomass from planting and seed bank on invader productivity.

In addition, other aquatic communities, including phytoplankton, periphyton, zooplankton, macroinvertebrates, tadpoles and microbials, together with water quality parameters, were monitored. This 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. Therefore, we hope to guide future restoration efforts in a way to maximise the likelihood of desired species establishment by strengthening native species priority effects to curb future re-invasions and/or secondary invasions.



Effects of plant composition (unplanted, *Lagarosiphon major*, *Stuckenia pectinata*, *Vallisneria spiralis* and their mixture) on the submerged invader Egeria densa productivity in three 'timing of arrival of native plants' treatments: native plants arriving 6 weeks before, at the same time and 6 weeks after Egeria densa planting. The box shows the median in the middle, and is bounded by the first and third quartiles. The whiskers extending from the box represent the range of the data. Red diamonds indicate the mean. Different lowercase letters denote statistically significant differences between treatments. Significance level at $p<0.05$.

Does freshwater biodiversity recover after invasive free-floating plant control?

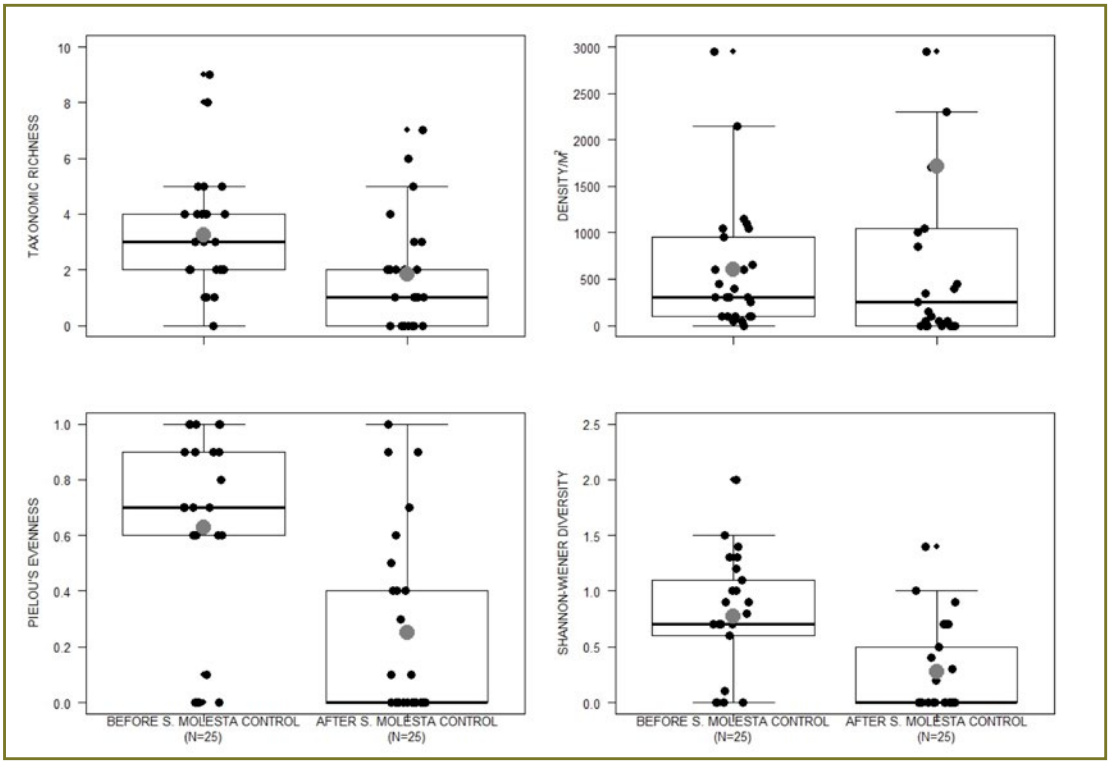
Invasions of freshwater bodies by floating vegetation, including giant salvinia (*Salvinia molesta*), threatens biodiversity globally. The large, dense mats formed by these plants can reduce light and nutrient availability, and change pH and other water quality parameters. These plants are also able to produce and release allelopathic compounds, gaining a competitive advantage over other primary producers. The inhibition of aquatic plants, for example, decreases habitat complexity, and complex habitats promote the diversity of species and availability of resources, hence negatively affecting higher trophic levels. Invasive plant control through classical biological control by the weevil *Cyrtobagous salviniae* and/or mechanical control has successfully reduced many of these invasions, and it is often considered one of the first steps in enabling recovery of invader-dominated sites. After successful control, native assemblages are expected to recover on their own, assuming that ecological communities are resilient to invaders, and such removal will allow natural communities to recover to a pre-invaded condition. However, management success is often limited to assessing whether the plant invader has been successfully controlled. Thus, little is known about the responses of aquatic communities following this practice. To address this deficit, honours student Hlumelo Mantshi investigated macroinvertebrate community responses to control of *S. molesta* in five freshwater systems located in the Western and Eastern Cape regions of South Africa.

RESEARCH AND TECHNICAL TEAM
Dr Antonella Petruzzella, Dr Samuel Motitsoe, Prof. Julie Coetzee, Prof. Martin Hill, Hlumelo Mantshi, Eva Rekkers (Leiden University, NL)

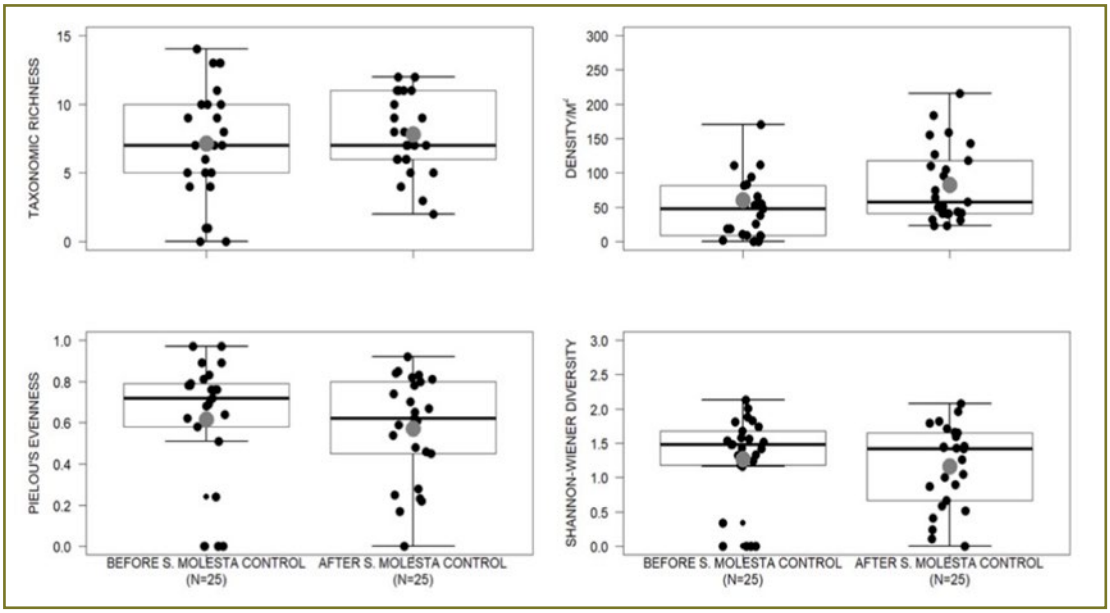
COLLABORATORS
Leiden University (Emily Strange), Albany Museum (Musa Mlambo)

Five field sites invaded by *S. molesta* (>60% coverage) were considered for both benthic and pelagic macroinvertebrate

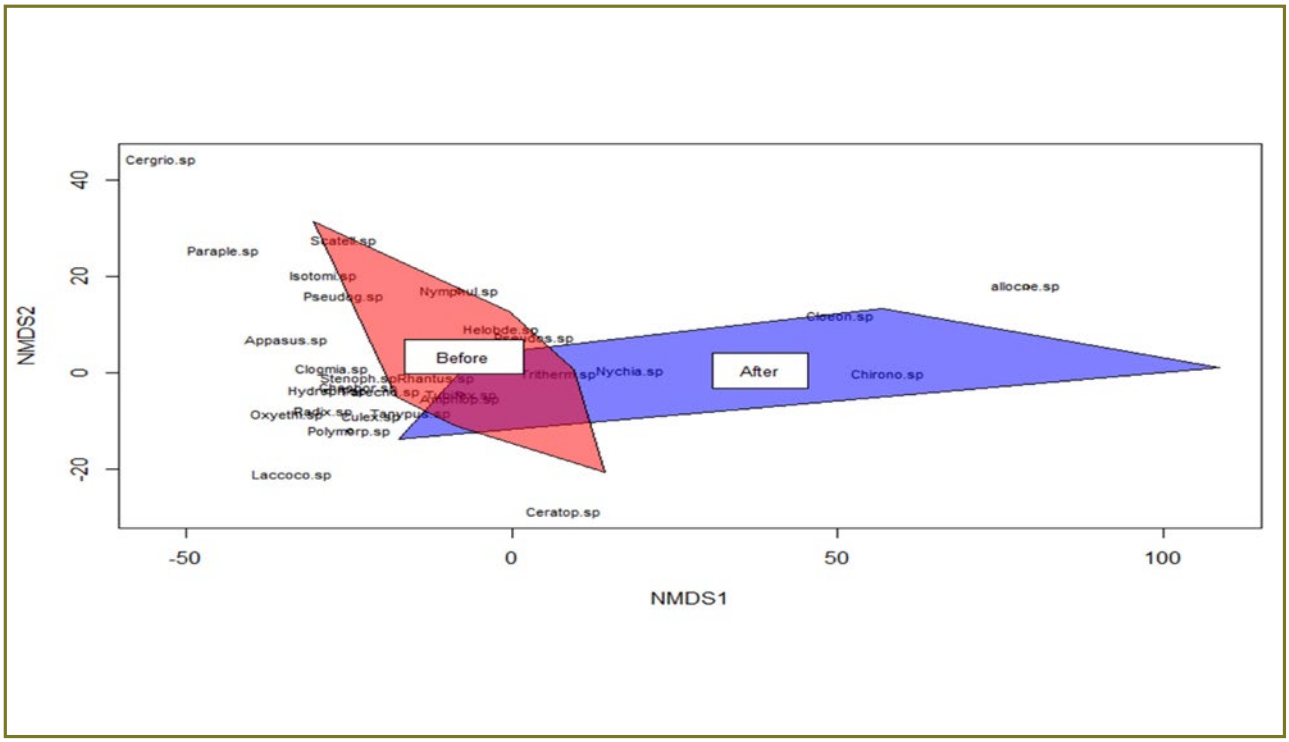
sampling before and after *S. molesta* control. Water quality parameters i.e. DO and temperature , showed that invasive macrophytes have a significant impact on the system, however these changes can be reversed by controlling the weed. Benthic macroinvertebrate taxa richness, diversity, and evenness significantly decreased after *S. molesta* control, while density did not change. Similarly, no change was observed between measures of pelagic macroinvertebrates. Macroinvertebrate community structure showed that even though the indices might not be significantly different, new organisms such as mayflies colonized the systems after weed control . This study provides valuable evidence of the impacts of *S. molesta* on aquatic biodiversity and how biological control facilitates biodiversity recovery with the use of relevant biological indicators, and further suggests that successful control of invasive aquatic weeds should be measured based on the return of biological and functionality of important aquatic biota, not just clearing the target weed biomass.



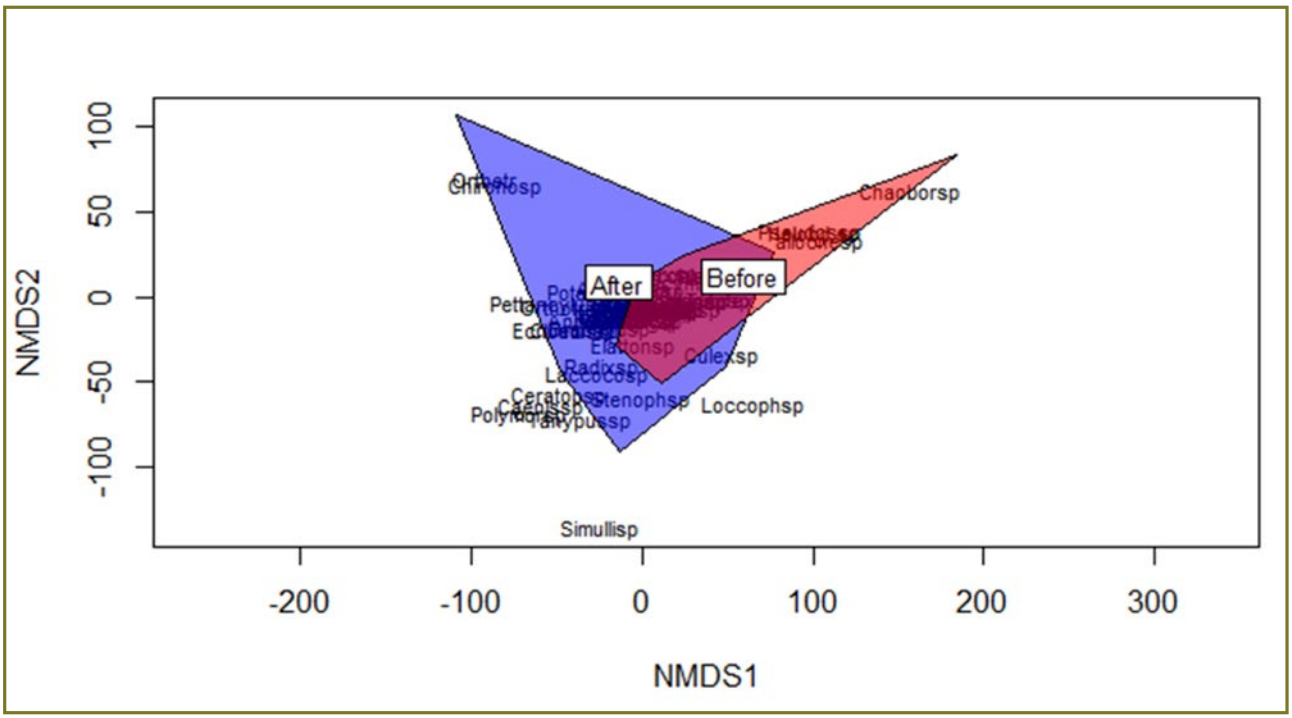
Benthic macroinvertebrate community parameters before and after the control of *Salvinia molesta*.



Pelagic macroinvertebrates community parameters before and after the control of *Salvinia molesta*.

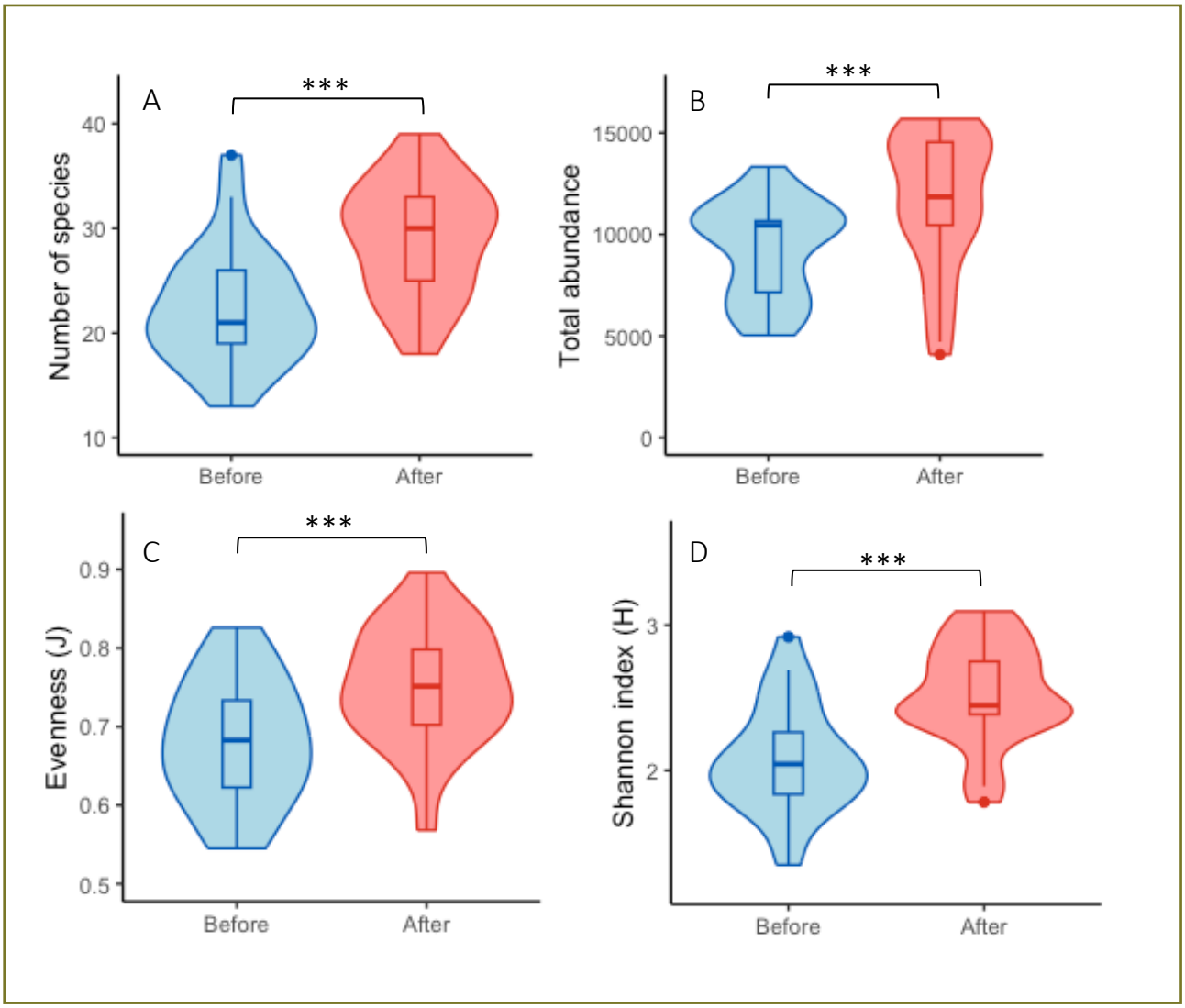


NMDS for benthic macroinvertebrates before and after control of *Salvinia molesta*.



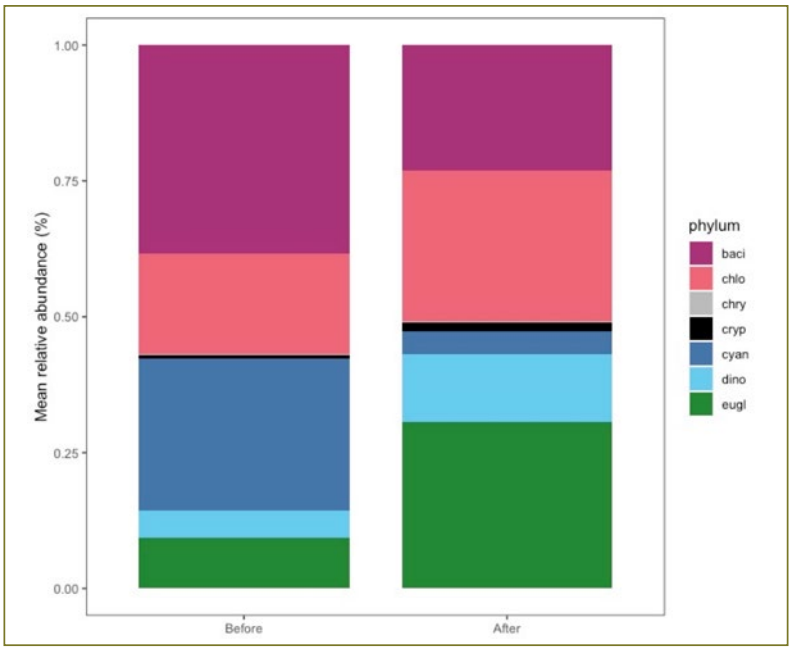
NMDS for pelagic macroinvertebrates before and after control of *Salvinia molesta*.

Leiden University MSc student, Eva Rekkers, in collaboration with the CBC, quantified freshwater phytoplankton biodiversity before (Summer 2021-2022) and after *S. molesta* control (Summer 2022-2023). The number of species of phytoplankton increased significantly by 29.3% with an average of 22 species observed before and 29 species observed after *S. molesta* control (Z=4.62) across five sampling sites. The total abundance before the control of *S. molesta* was on average 9277 individuals, whereas after control was on average 11,645 individuals (25.5% increase) (Z=4.352). Pielou's Evenness and Shannon diversity index of phytoplankton also significantly increased on average by 12% (Z=4.045) and 23.3% (Z=5.659) respectively. The mean evenness increased from 0.67 to 0.75 and diversity from 2.06 to 2.54 after invasive plant control.



Phytoplankton (A) number of species, (B) total abundance, (C) Pielou's evenness (J) and (D) Shannon diversity index before (blue) and after (orange) *Salvinia molesta* control. Vertical lines indicate the interquartile range and medians are indicated by a solid horizontal line. Statistical significance between the two sampling periods is indicated at p<0.001 level (***).

Before the control of *S. molesta*, the three most abundant groups were Bacillariophyceae (38.3%), Cyanobacteria (28.0%), and Chlorophyceae (18.6%). After the removal of the free-floating plants, Euglenophyceae became the dominant group (30.7%), closely followed by Chlorophyceae (27.8%), and Bacillariophyceae (23.1%). Bacillariophyceae was found among the dominant groups both before and after IAAP control. However, the degree of dominance departs from the data collected in healthy systems.



Relative abundance of groups before and after *Salvinia molesta* control (baci = Bacillariophyceae, chlo = Chlorophyceae, chry = Chrysophyceae, chryp = Cryptophyceae, cyan = Cyanobacteria, dino = Dinophyceae and eugl = Euglenophyceae).

Mass Rearing

The Waainek Mass-Rearing Facility's primary goal is to maintain ongoing reproduction of healthy water weed biocontrol agents to guarantee a steady supply of agents to invaded sites in South Africa and beyond. Large number of agents were dispatched for release at both satellite mass-rearing stations and invaded areas. In particular, *Cyrtobagous salviniae* mass rearing for release against *S. molesta* has been productive this year, with three times as many being sent for release than in 2022. Each year, we get more requests from around the country, mostly for *Pontederia crassipes*, *Pistia stratiotes*, and *Salvinia molesta*. Most of these requests are from North West, Gauteng, and KwaZulu Natal, indicating an increasing interest in the biological control of invasive plants. Mid-year, we supplied a new *Megamelus scutellaris* mass-rearing facility in Zimbabwe, and shipped a consignment of *C. salviniae* for release there too.

RESEARCH TEAM
Samella Nxgande-Koza and team

FUNDERS
DFFE

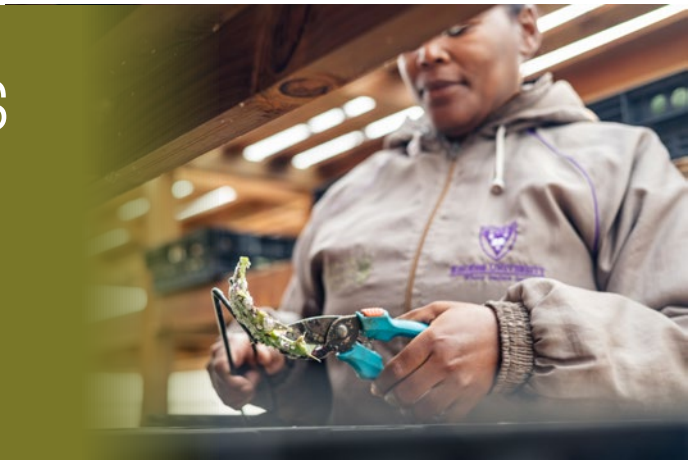
In 2022, we set up satellite rearing stations in the Garden Route (George and Plettenberg Bay) to reduce the pressure on our facility and allow us to focus on other areas of the country. However, these two stations experienced challenges as a result of strong weather conditions, so we are still supplying them with agents for release.

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
Pontederia crassipes	*Megamelus scutellaris*	94	392 500
Myriophyllum aquaticum	*Lysathia* sp..	15	5 987
Pistia stratiotes	*Neohydronomous affinis*	25	11 866
Salvinia molesta	*Cyrtobagous salviniae*	14	9 230
Egeria densa	*Hydrellia egeriae*	5	6 966
Sagittaria platyphylla	*Listronotus appendiculatus*	7	740
TOTAL		**167**	**427 289**

The Waainek Team: Nokuthula Kom, Samella Ngxande-Koza (Manager), Lyle Titus, Siyaxolisa Mini, Vuyani Ntyinkala, Nandipha Ngolothi, Siyanda Ntamo, Mthobeli Mvandaba, Bongiwe Gobongcwana and Landile Booi. **Photo:** David Taylor

CACTACEAE WEEDS



Ronel Roman working at the Kariega facility.
Photo: David Taylor

Invasive alien cactus species are some of the worst invaders of South Africa's arid semi-deserts and savannas. The dense thickets that are formed in infested areas result in a loss of grazing capacity and reduced access to shade and water, which can mean the difference between life and death for livestock and wildlife in the hot, arid parts of South Africa. In the Americas, where cactus are indigenous, there is a suite of highly specialized herbivorous insects that regulate cactus populations. There are very few cactus species that are considered indigenous or beneficial outside of the Americas, so the majority of these specialist herbivores can be used as biological control agents.

There are six cochineals and a mealybug that have been released in South Africa for the biological control of invasive alien Cactaceae. All are very successful agents if they are used against the correct target weed, and result in declines in cactus reproduction and densities. This results in increased benefits to land users, including increased carrying capacity for grazing, reductions in the number of injured livestock and improved access to natural resources, such as water.

The CBC works on the development of new biocontrol agents for cactus weeds that do not have effective agents yet, as well as the implementation and post-release monitoring of effective agents that are already available for use in the country. We currently have three different cochineal species under investigation in quarantine that will hopefully be safe and effective agents for four target cactus weeds. The mass-rearing facility at Kariega produces six agents that are effective against 12 cactus weeds, producing large numbers of agents to augment field populations and make sure that the correct agents are established at every site in the country where they are needed. The Kariega team also monitors the success of biocontrol releases through feedback from land users and long-term monitoring of cactus and agent densities at selected sites. All three of the agents in quarantine are likely to be suitable for release in South Africa, so hopefully in the next year the Kariega facility will have new agent species to mass-rear and release.

PROGRAMME HIGHLIGHTS IN 2023

- A new biocontrol agent for thistle cholla, *Cylindropuntia pallida*, has been imported into quarantine from Mexico. It is a lineage of *Dactylopius tomentosus* that is particularly damaging to South African thistle cholla, and is likely to be an effective agent.
- Efficacy trial in quarantine have indicated that a lineage of *Dactylopius ceylonicus* collected in Argentina, is damaging to *Opuntia megapotamica*, while *O. elata* proved to be susceptible to the jointed cactus biocontrol agent, *D. austrinus*. These efficacy trials have therefore identified suitable agents for two emerging weeds in the country.
- After testing six lineages of the mealybug *Hypogeococcus* that were collected in Argentina, none proved effective against torch cactus, *Trichocereus spachianus*. But a cochineal insect of South American origin, *Dactylopius confertus*, is proving to be damaging and suitably host specific against this target weed.
- Almost six-hundred releases of cactus agents have now been conducted by the CBC in every province of the country. These releases have had significant benefits for land users and natural ecosystems.

Thistle Cholla

The thistle cholla, *Cylindropuntia pallida*, is invasive in many arid areas around the world. It is particularly invasive in Australia and South Africa, affecting local farming and disrupting livestock productivity. Furthermore, the long and sharp spines often impale small animals and damage the quality of wool, thus lowering its commercial value. Infestations of this species form spiny thickets that can restrict human and animal movement, reducing access to grazing, shade, and water sources. Further to these issues, *C. pallida* can outcompete native species and is a threat to indigenous biodiversity and ecosystem functioning. In South Africa, *C. pallida* has invaded many areas of agricultural land in the Eastern Cape and initially was controlled with herbicides, which successfully reduced plant numbers at most sites that were sprayed. However, chemical control proved to be ineffective in the long-term, as regrowth was seen continually after follow-up treatments were applied. Given the success of cochineal in controlling other *Cylindropuntia* species, biological control was considered a promising strategy.

Cylindropuntia pallida is also invasive in Australia, and Australian biological control researchers have been working to develop a successful agent for the species. A cochineal biotype called *Dactylopius tomentosus* 'bernardina' was collected from Baja California, Mexico from the cactus species *Cylindropuntia bernardina* (formerly *Cylindropuntia californica* var. *parkeri*). Host specificity testing conducted in Australia showed that it was a suitable and effective agent for *C. pallida* in Australia. This resulted in the agent's release in Australia, where it has become an extremely effective and damaging agent, reducing *C. pallida* infestations. Due to the success of the agent in Australia, the release of *D. tomentosus* 'bernardina' was approved in South Africa. However, after three unsuccessful attempts to rear the agent on South African plants in quarantine at the CBC, the possibility of the South African plants being unsuitable hosts was considered.

Given the unsuccessful attempts to rear *D. tomentosus* "bernardina" on South African *C. pallida* , the fitness, and fecundity of the cochineal was tested on both Australian and South African *C. pallida* plants in order to determine if the South African plants were inferior hosts. The results indicated that the cochineal that was successfully controlling the *C. pallida* in Australia was incompatible with South African plants, and would not make a good biological control agent in South Africa. This is evident

RESEARCH TEAM
Phillippa Muskett, Prof. Iain Paterson

STUDENT
Wade Sexton (MSc)

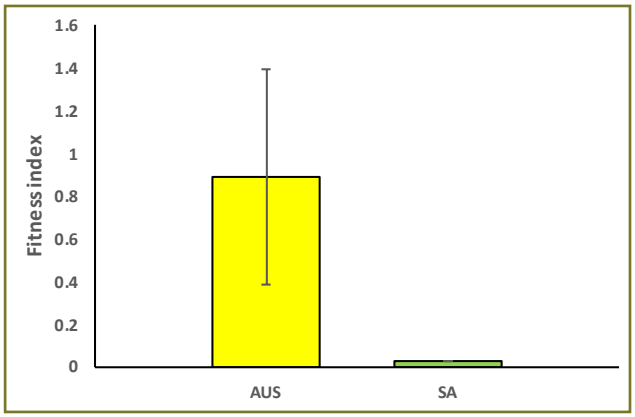
COLLABORATORS
University of Guadalajara (Liberato Portilla and Ana-Lilia Vigueras); NSW DPI (Andrew McConnachie)

A large *Cylindropuntia pallida* infestation near Gariep Dam, Gauteng. **Photo:** Chris Wahlberg

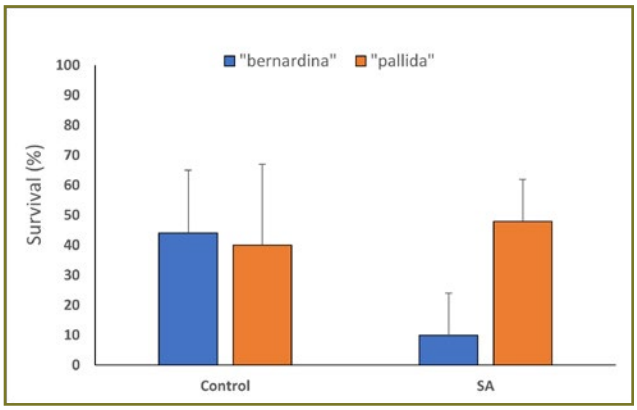
from the much lower survival rate of the crawlers and the overall lower fitness of the cochineal on South African plants.

Since the cochineal from Australia was ineffective against South African plants, Wade Sexton and Iain Paterson travelled to the native distribution of *C. pallida* in Mexico to look for other potential agents. In collaboration with Liberato Portilla and Ana-Lilia Vigueras from the University of Guadalajara, they were able to survey multiple sites in Hidalgo, Mexico in search of cochineal on *Cylindropuntia pallida*. On the final day of the trip, a good consignment of cochineal *D. tomentosus* 'pallida' was collected and imported successfully into quarantine in South Africa.

The new cochineal from Mexico is now under evaluation in quarantine. Fitness and fecundity testing was conducted in the same manner to how the Australian cochineal was tested, and results showed that the Mexican "pallida" cochineal had a much higher survival on South African plants than that of the Australian "bernardina" cochineal. This is excellent news for *C. pallida* biological control because it means that the 'pallida' cochineal is likely to be an effective agent in South Africa. Studies are now underway to determine the impact of the agent on whole potted plants and to conduct host specificity testing to determine if the cochineal is safe to release in South Africa. Hopefully, this agent will bring about control of South Africa's top priority weed for eradication.



The fitness index of the cochineal *Dactylopius tomentosus* "bernardina" on Australian and South African *Cylindropuntia pallida* plants.



The average survival (%) of first-generation crawlers on control and South African (SA) *Cylindropuntia pallida* plants for the "bernardina" biotype used in Australia and the "pallida" biotype imported from Mexico.

Above: Wade Sexton inspecting the cochineal imported from Mexico in quarantine. **Photo:** David Taylor

Right: A happy team after collecting cochineal in Hidalgo, Mexico. **Photo:** Iain Paterson

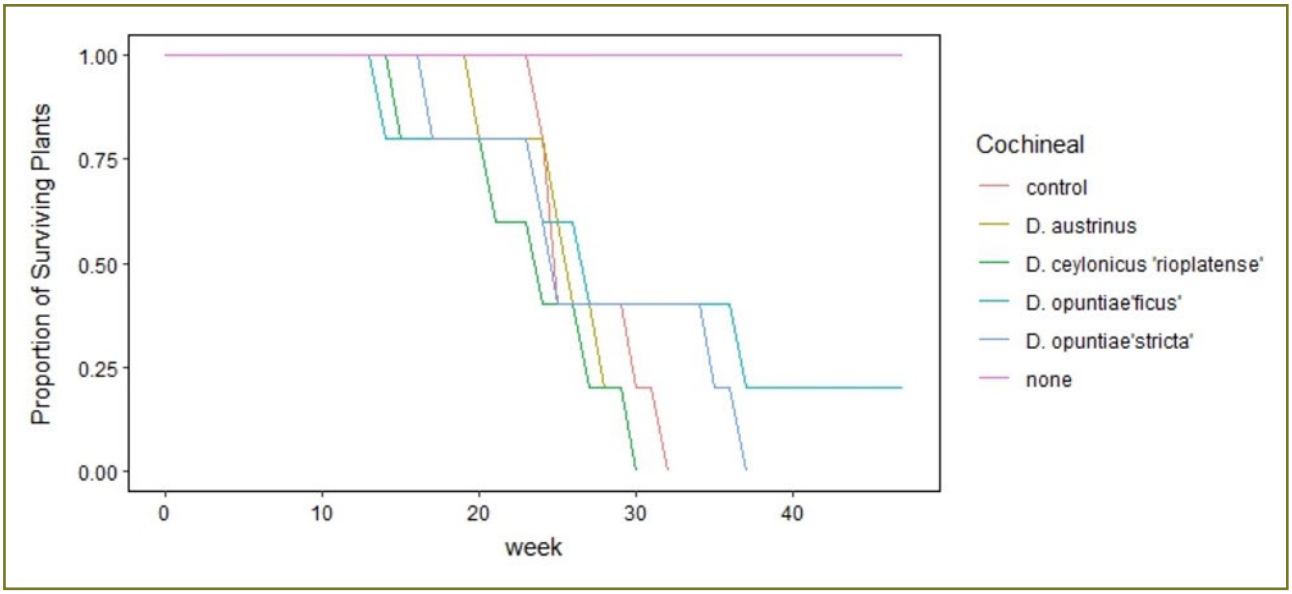
Round-leaf Tuna Cactus

RESEARCH TEAM
Prof. Iain Paterson, Phillippa Muskett

COLLABORATORS
Wits University (Nic Venter and Samalesu Mayonde), ARC- PPRI (Kedibone Mofokeng)

Opuntia megapotamica has been invasive and problematic throughout rangelands in the Eastern Cape of South Africa since at least the 1930s but until 2021 it was incorrectly identified as a subspecies of *O. engelmanii*. *Opuntia megapotamica* is a South American species of Cactaceae with a similar native distribution to *O. elata* and *O. Rioplatense*, while *O. engelmanii* is a North American species. Before the misidentification was corrected, *O. megapotamica* was included in trials for a new cochineal species to control *O. engelmanii*. A cochineal known as *D. opuntiae* 'Flagstaff' collected off *O. engelmannii* in the USA showed great promise as a biological control agent for *O. megapotamica*, and has been in quarantine in Pretoria at the Agricultural Research Council since then.

Opuntia megapotamica was included in the trials for a new biological control agent for *O. elata*, considering that it originates from a similar distribution and is a very close relative. In a quarantine based trial that compared insect fitness when the different cochineals fed on *O. megapotamica*, *D. ceylonicus 'Rioplatense'*, the new cochineal from South America, performed better than all of the cochineal lineages already released in South Africa, producing an average of 14.7 (± 3.8 S.E.) mature females/replicate and 531.52(± 41.85 S.E.) crawlers/female. *D. ceylonicus 'Rioplatense'* was also the most effective cochineal lineage for *O. megapotamica* in terms of how quickly and effectively it killed the plant, but *D. austrinus* was also effective (figure below). The *D. opuntiae 'Flagstaff'* cochineal has now been moved into CBC quarantine in Makhanda in order to compare the impact of the *'Flagstaff'* cochineal and the *'Rioplatense'* cochineal. The more damaging of the two cochineals will be prioritised for release.



The proportion of surviving *Opuntia megapotamica* plants when exposed to cochineals already present in South Africa and the new potential agent, *D. ceylonicus 'Rioplatense'*. 'None' indicates plants without cochineal, and 'control' indicates the successful agent *D. ceylonicus* on the target weed *O. monacantha*.

Orange-tuna Cactus

RESEARCH TEAM
Phillippa Muskett, Prof. Iain Paterson

COLLABORATOR
FUEDEI

ACKNOWLEDGMENT
Red Meat Research and Development South Africa for funding & DFFE

Orange Tuna Cactus, *Opuntia elata*, has been on the list of early intervention targets since 2017 when the number of recorded infestations started to increase rapidly. Large infestations reduce the carrying capacity of rangelands in the Karoo and other semi-arid regions of South Africa. Many of its close relatives are serious invasive weeds but are kept under control using biological control agents. This

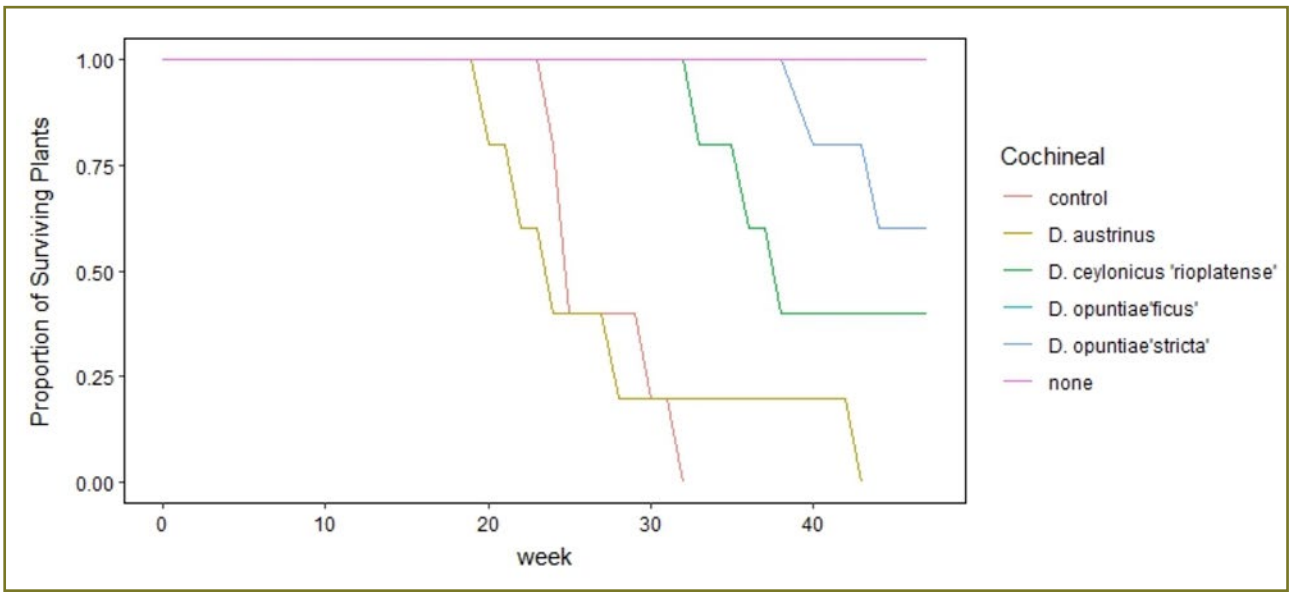
suggests that *O. elata* is very likely to become increasingly problematic, but also that it is likely to be an excellent target for biological control. As with all *Opuntia* weeds, cochineal insects are the most promising potential agents.

Some cochineal species are used to control more than one species of cactus, such as the cochineal *Dactylopius opuntiae 'stricta'* which was introduced to control *O. stricta*, but also controls *O. cespitosa*. A trial was conducted to confirm that none of the six cochineal species already released in South Africa could control *O. elata*. Utilizing a cochineal species that is already released in South Africa would be the safest and most economical option if the cochineal is sufficiently damaging. Damage trials indicated that *Opuntia elata* was susceptible to *Dactylopius austrinus*, which has been used for the control of *O. aurantiaca* in South Africa since the early 1900s.

At the same time, a new species of cochineal, *D. ceylonicus 'Rioplatense'* was imported into quarantine from Argentina. It was collected off a close relative of *O. elata* (*O. Rioplatense*) in the native distribution and has the potential to be an effective biological control agent. *D. ceylonicus 'Rioplatense'* successfully reproduced on *O. elata* but *D. austrinus* also thrived on it. There was however a lot of variation in the data and there was no significant difference between the fitness of *D. austrnus* and *D. ceylonicus 'Rioplatense'* on *O. elata*.

Insect fitness may not directly indicate the insects' ability to damage the target weed, therefore, a trial using whole potted plants was set up in quarantine to compare the agents' efficacies by calculating the time it took each cochineal to kill a plant. Thirty 1st instar crawlers were released onto each plant. Five cochineals, including *D. austrinus*, *D. ceylonicus*, *D. opuntia 'ficus'* and *D. opuntia 'stricta'* which have already been released in South Africa for the control of other cactus weeds, and the new agent, *D. ceylonicus 'Rioplatense'*, were tested with five replicates. Five plants that did not have cochineal on them were included as a negative control and five *O. monocantha* were exposed to *D. ceylonicus*, because *O. monacantha* is a similarly sized cactus species to *O. elata* and is successfully controlled by *D. ceylonicus* in the field, and could therefore be used as a positive control. *Dactyloipus austrinus* killed *O. elata* far more effectively in the whole plant trial than *D. ceylonicus 'Rioplatense'* from Argentina and, besides one outlier, was able to kill *O. elata* at a similar rate to the positive control (figure below). These results suggest that *D. austrinus* is an effective agent for *O. elata*. Field trials will now be conducted to see if *D. austrinus* is effective against *O. elata* populations in the field. If there are promising results from the field trials, then *D. austrinus* can be promoted as an agent for *O. elata*, and no new biocontrol agent will need to be released for the control of this rapidly increasing weed.

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



The proportion of surviving *Opuntia elata* plants when they were exposed to cochineals already released in South Africa for other cactus weeds and the new cochineal, *D. ceylonicus 'Rioplatense'*. Plants without any cochineal (none) were included as negative controls, while the successfully controlled *Opuntia monacantha* and its agent were included as positive controls. If a cochineal can kill its target weed at a similar rate and efficacy as this positive control then it is likely to be a damaging agent in the field.

Torch cactus

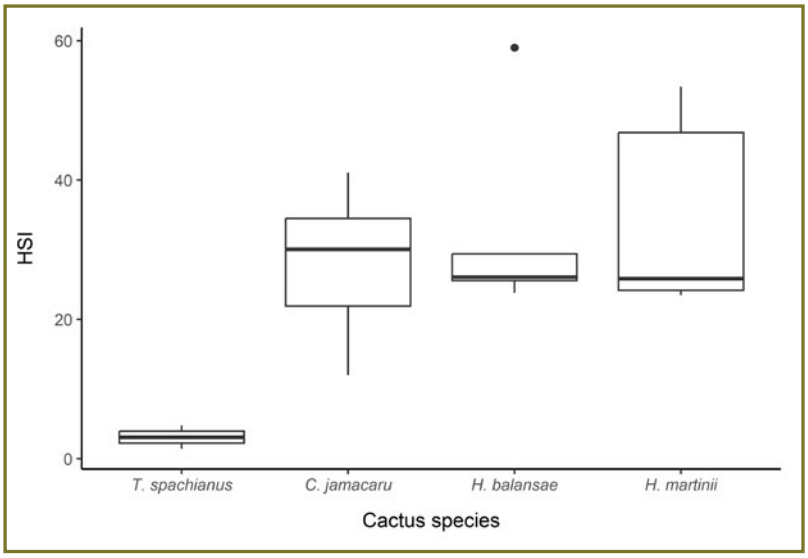
Torch cactus, *Trichocereus spachianus*, has become increasingly problematic in the arid regions of South Africa, forming dense thickets of spiny, upright columns that grow in an 'organ-pipe' like formation. It has very large, showy, white flowers, which open on overcast days and at night. While the 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.

RESEARCH TEAM
Prof. Iain Paterson

STUDENT
Tamzin Griffith (PhD)

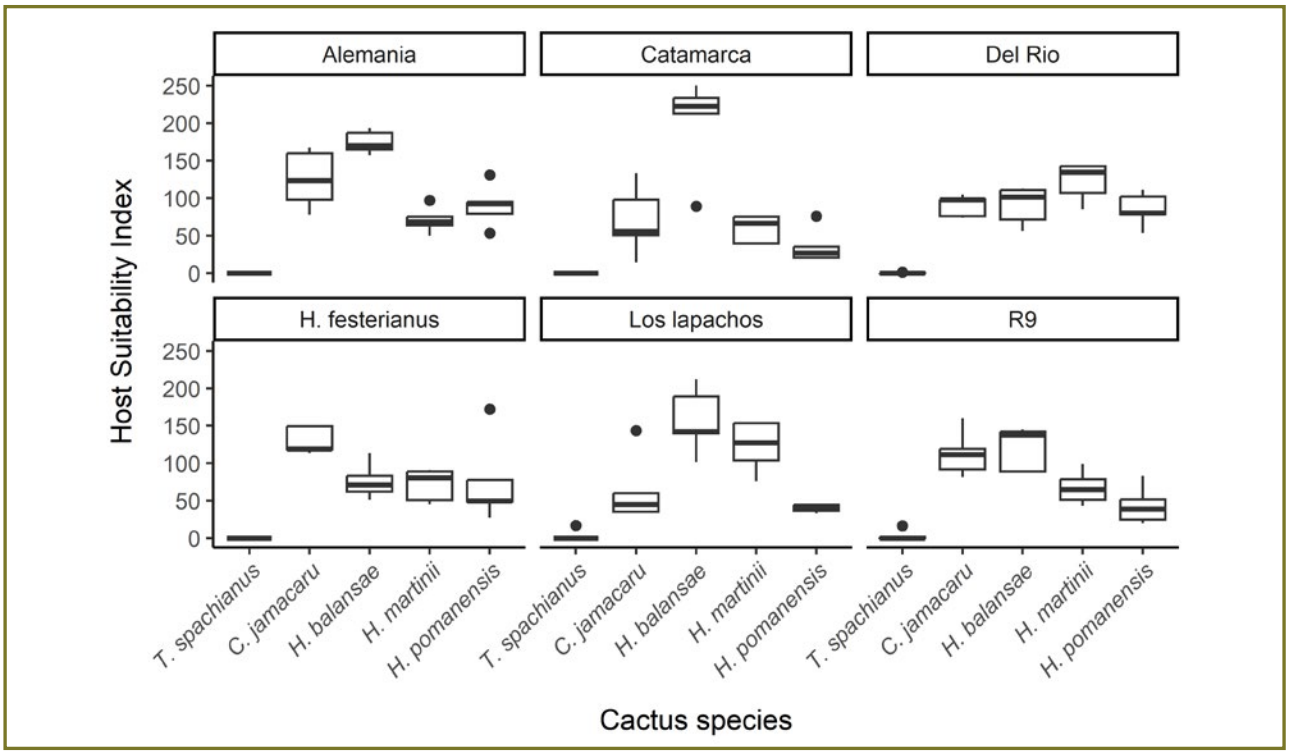
COLLABORATORS
FuEDEI

Hypogeococcus sp., currently used as a biocontrol agent in South Africa for closely related cactus weeds, was tested against *T. spachianus*, but failed to establish on the cactus and was not damaging. A new agent from the indigenous distribution was therefore required, but there are no known wild populations of *T. spachianus* in the indigenous distribution, so other closely related cactus weeds had to be used as a source for potential agents. Several



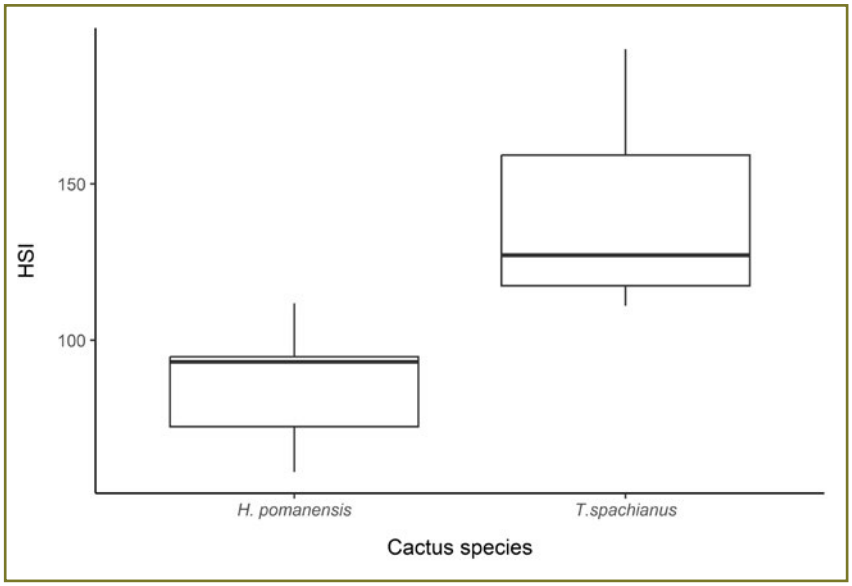
Left: The suitability of *Hypogeococcus* sp., the agent used for control of *Cereus jamacaru*, as well as *Harrisia balansae* and *H. martnii*, on *Trichocereus spachianus* and its three target weed species. The low suitability of the insect on *T. spachinaus* compared to the other plants indicates that it is not a suitable agent for that weed.

Below: The suitability of six lineages of *Hypogeococcus* collected in Argentina. Each lineage was tested on five South African cactus weed species, and in all cases *Trichocereus spachinaus* was an unsuitable host.



Hypogeococcus species and lineages, collected from closely related species in Argentina were evaluated. For each species/lineage, a Host Suitability Index (HSI) was calculated. For all species/lineages, the HSI score was very low for *T. spachianus* (figures on page 40) meaning that, compared to other cacti species, *T. spachianus* was not a suitable host. It could therefore be concluded that *Hypogeococcus* was not a suitable biocontrol agent for *T. spachianus*. Since none of the *Hypogeococcus* proved suitable candidates, an alternative approach to address the *T. spachianus* issue in South Africa involved investigating *Dactylopius confertus*, a cochineal insect. This cochineal, unlike other cochineal species that feed on Opuntiaeae hosts, feeds on *Trichocereus* and *Harrisia* species within the subtribe Cereanae. Since this cochineal is known to be damaging to other *Trichocereus* species, efficacy testing against torch cactus was warranted.

Dactylopius confertus was collected in Namibia, where populations of the insect have naturalised after an unknown, accidental introduction. Experiments comparing the performance of *D. confertus* on *H. pomanensis* (the plant that it occurs on in Namibia) and *T. spachianus*, revealed that *T. spachianus* had a higher suitability for *D. confertus* (figure above). Further efficacy assessments are currently in progress but the preliminary data suggests that *D. confertus* may be sufficiently damaging to *T. spachianus* to result in plant death (figure alongside). Preliminary host specificity testing has also shown that *D. confertus* is suitably host specific; however, the test plant list needs to be expanded. *Dactylopius confertus* is therefore a very promising agent for *T. spachinaus* that appears to be both damaging to its host plant and sufficiently host specific.



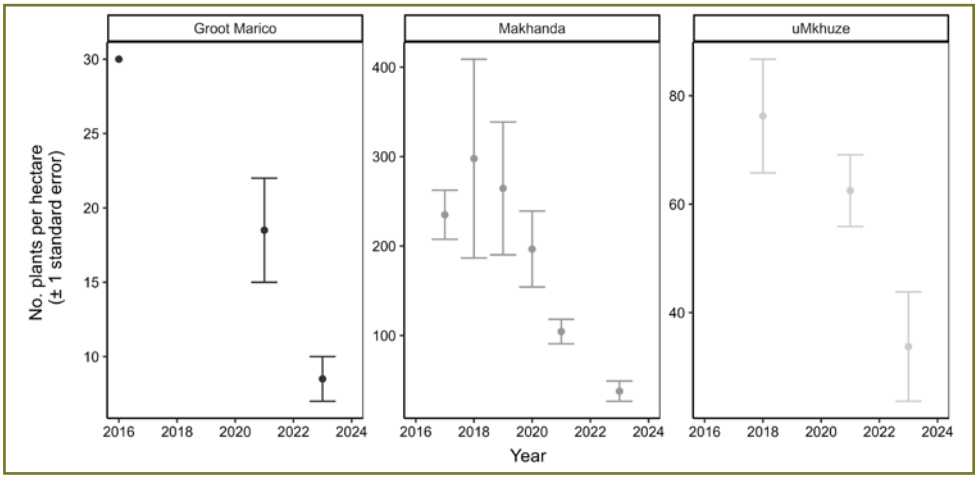
The suitability of *Dactylopis confertus* on *Harrisia pomanensis* (the host plant it was collected off in Namibia) and the target weed *Trichocereus spachianus*. The higher host suitability indicates that *D. confertus* is a promising candidate agent for *T. spachinanus*.

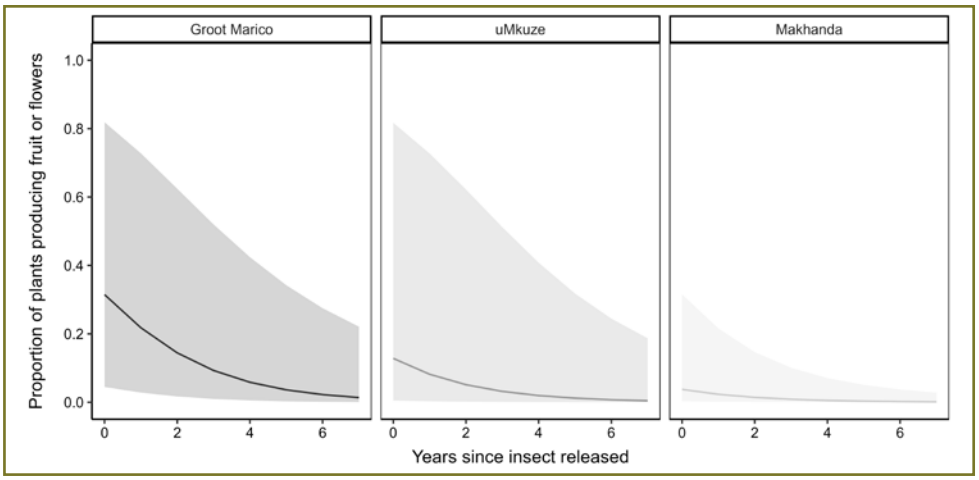
Dactylopius confertus thriving on *Trichocereus spachianus* in quarantine.
Photo: Tamzin Griffith

Queen of the Night

RESEARCH TEAM
Phillippa Muskett, Dr Guy Sutton, Liam Yell, Prof. Iain Paterson

Cereus jamacaru De Candolle is a very tall, columnar cactus that can reach heights of six or seven meters. It has large, white flowers that open at night or in overcast conditions which is why it is commonly referred to as Queen of the Night. It produces large dark pink, red, or orange fruit and reproduces primarily through the large number of seeds found within the fruits. It is indigenous in South America, but by 1999 it was recorded in all nine provinces of South Africa and was forming dense infestations. At one site, densities of 40 000 plants per hectare were recorded. In 1983, a biological control agent called *Hypogeococcus* sp. was released to control another cactus weed, *Harrisia martinii*, in South Africa. *Hypogeoccocus* sp. (Hemiptera: Pseudococcidae) is a galling mealy bug that was known to also establish on *C. jamacaru* when it was released for *H. martinii* and was then intentionally released on *C. jamacaru* in 1999. In 2018, the first post-release study was conducted where Sutton et al. were able to confirm that where the agent had been established for over ten years the plant could be classified as being under complete control. The Sutton et al. study compared sites with the agent and without the agent and used plant demographics to estimate changes in plant populations over time. In the current study, we released the agent at sites where it was not present and monitored it over time. We now have excellent data to show the number of plants declining over time as they are killed by the agent (figure below). At these long-term monitoring sites, plant density decreased significantly in relation to the number of years since *Hypogeococcous* sp. was released ($X2 = 1188.1$, $df = 1$, $P < 0.001$) and there was an average decrease in plant density of 18 % each year after *Hypogeococcous* sp. was released ($\beta = -0.19$). There was also a statistically significant decline in the proportion of plants producing either fruit or flowers ($X2 = 8.67$, $df = 1$, $P = 0.003$) across all three sites, with an average decrease of 40 % in the proportion of plants producing either fruits or flowers each year after *Hypogeococcous* sp. was released ($\beta = -0.50$). Three years after releasing *Hypogeococcous* sp., there were no fruits or flowers found on the transects at Mkuze and Makhanda, while no fruits and flowers were found on the transects at Groot Marico seven years after release. These data indicate that the plant is actually brought under control even quicker than estimated in Sutton et al. (2018), and also confirms the conclusions of that study which were that *C. jamacaru* should be considered under complete biological control in South Africa.





Above: The mean density of Queen of the Night plants per hectare, at three long-term monitoring sites in South Africa, from the initial release of *Hypogeococcus sp.* at each site until the end of 2023.

Left: The proportion of Queen of the Night plants with flowers or fruits over time at the three long-term monitoring sites in South Africa from the initial release of *Hypogeococcus sp.* at each site until the end of 2023.

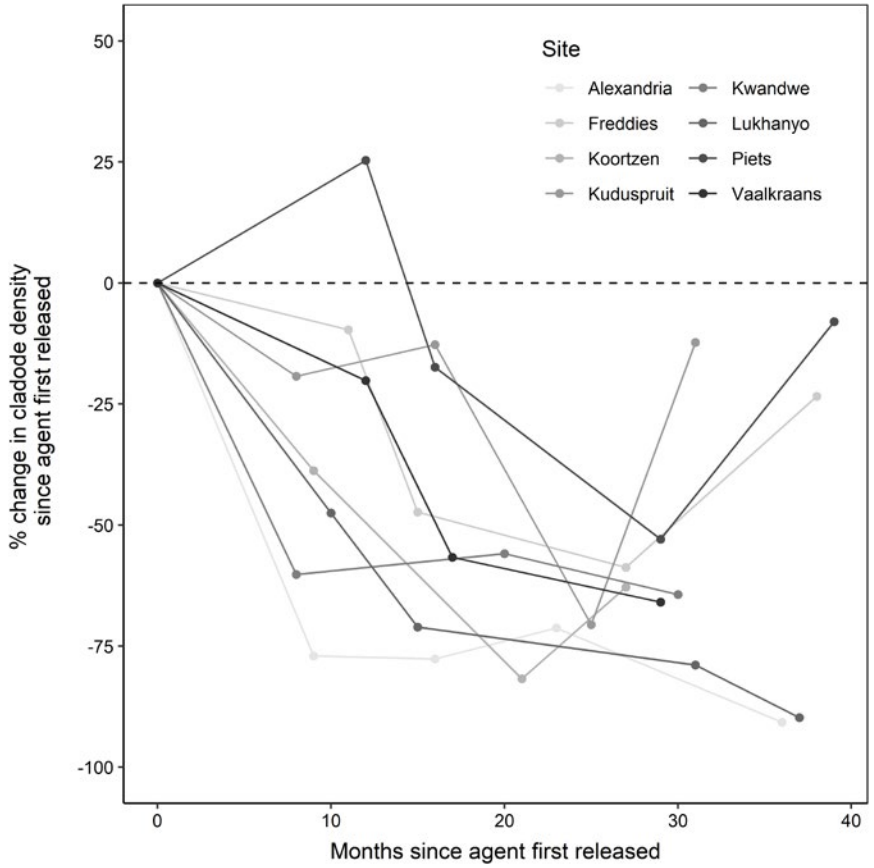
Mass-rearing, releasing and monitoring the success of cactus biological control agents

The team based at the Kariega Cactus Biocontrol Mass-rearing Facility is responsible for producing cactus agents for the whole of South Africa. Since the initiation of this project in 2015, over 600 releases have been conducted at sites in every province in the country. This year was a productive year for the cactus team, having released five species of cochineal and the galling mealybug *Hypogeococcus* sp. at 57 release events and in very impressive numbers (table on page 44). Releases have been made in National Parks, Provincial Nature Reserves, private farms and commonage. The vast majority of releases result in a reduction in the invasive alien cactus, and land users have indicated that they have benefited from the reduction in negative impacts that were caused by the cactus.

Biological control success is affected by climate, and climatic conditions at sites can vary significantly, even within a relatively small geographic area. Although this results in variability in success, on average, the density of all target cactus species is reduced after the release of the agents. For example, biocontrol of jointed cactus is impacted by heat, humidity and rainfall, with better control in dry and hot conditions than in cool, wet conditions. After monitoring for between 30 and 40 months at eight sites, the average number of cladodes (cactus stem joints) reduced from 31 to 18 per m^2, but some sites were more successful than others. Three of the sites experienced increases in cactus density about 25 months after the release, probably associated with high levels of rainfall. These data are important, as they can be used to indicate where and when more releases are required. Despite the variability in success, all the sites have less cactus after the release of the agent, so cactus populations have been reduced at all sites. All of the sites presented in the figure below are in the Eastern Cape Province, where winters are relatively cool. Releases conducted in Hluhluwe-Mfolozi Nature Reserve and in Pafuri in the Kruger National Park virtually eliminated the weed with 24 months (figures on page 44).

RESEARCH TEAM
Ruth Scholtz and team

FUNDERS
DFFE and Commonland (Netherlands)



The change in jointed cactus density after the release of *Dactylopius austrinus* cochineal at eight sites in the Eastern Cape.

Jointed cactus at Pafuri before releasing the agents. **Photo:** Nic Venter

The same site 5 months after release. **Photo:** Nic Venter

Releases of cactus biological control agents from the CBC mass-rearing facility this year (2023)

Weed	Agent	# releases	# cladodes/galls
Opuntia aurantiaca	*Dactylopius austrinus*	10	10300
O. stricta	*D. opuntiae 'stricta'*	12	1994
O. humifusa	*D. opuntiae 'stricta'*	1	50
O. monacantha	*D. ceylonicus*	5	731
O. ficus-indica	*D. opuntiae 'ficus-indica'*	6	162
Cylindropuntia imbricata	*D. tomentosus 'imbricata'*	10	3071
Cereus jamacaru	*Hypogeococcus* sp.	9	579
Harrisia martinii	*Hypogeococcus* sp.	2	297
Solanum elaeagnifolium	*Leptinotarsa texana*	2	122
TOTAL		**57**	**17 306**

NORTHERN TEMPERATE WEEDS


Photo: Michael Sochor

The Northern Temperate Weeds (NTW) programme aims to bring the benefits of biological control to the mountain grasslands of South Africa. These high-elevation grasslands are crucial for water security, as they account for nearly half of the country's water runoff. They also serve as a significant biodiversity hotspot, with high plant endemism. Furthermore, these grasslands are a valuable resource for livestock, which are central to the livelihoods and economies of commercial, small-scale, and communal farming and agriculture. However, these ecosystems are currently under threat from invasive alien plants, particularly those originating from the cooler Northern Temperate regions of the world.

The NTW initiative is multifaceted and has included conducting feasibility studies on several invasive tree species in South Africa's highland regions. The goal of these studies is to identify which species should be prioritized for biological control. Over the past year, the initiative has made significant progress. It has continued to conduct pre-release studies on *Robinia pseudoacacia* and *Gleditsia triacanthos*. It has also prioritized *Pyracantha angustifolia* and *Cotoneaster pannosus*, two Rosaceae species, for biological control. Many years of work on trying to untangle the complexities of invasive Rubus species are starting to bear fruit, suggesting biological control may be an option for their management. *Salix babylonica* has been highlighted as a poor option for classical weed biological control. The programme is also continuing to assess additional invasive species as potential candidates for biological control.

The NTW initiative has benefited from the support and collaboration of both local and international entities. In South Africa, it has worked closely with the Plant Sciences Department and the Afromontane Research Unit at the University of the Free State, QwaQwa & Bloemfontein, as well as the ARC-PPRI. Internationally, it has continued a strong collaboration with CABI (Switzerland). It is also continuing relations with the US Forestry Science Laboratory (Morgantown, USA), the Centre of the Region Haná for Biotechnological and Agricultural Research (Czech Republic). A new collaboration is also developing with Department of Horticulture at Iowa State University investigation the phylogenies of South African and American Rubus species

The NTW programme has also continued its contributions towards the globally implemented Range X, a project aimed at investigating the ecological drivers of range-expanding plant species at high altitudes. The primary goal of the Range X initiative is to contribute to efforts to mitigate the impact of climate change on the environment and the communities that rely on those environments. The NTW initiative aims to investigate how future climate scenarios may affect the growth parameters of several species being considered for biological control.

The NTW initiative has also pioneered the development of the Southern African Mountains Invasive Alien Plants Working Group. This group aims to enhance collaboration among researchers and conservation managers to facilitate the best management and research practices for Invasive Alien Plants (IAPs) in southern Africa. The ultimate goal of this working group is to reduce the impact of plant invasions.

PROGRAMME HIGHLIGHTS IN 2023

- The leafmining beetle *Odonata dorsalis*, a candidate biological control agent for the invasive tree *Robinia pseudoacacia*, was introduced from the USA into the CBC quarantine facility.
- Caged, multi-choice, and infield host specificity testing for the candidate leaf galling midge *Obolodiplosis robiniae* are near completion. The testing has been conducted in Switzerland by CABI, Switzerland

Multi-choice cage tests at CABI, Switzerland. **Photos:** Lauréline Humair

Black Locust Tree

RESEARCH TEAM
Dr Grant Martin , Dr Lenin Chari

COLLABORATORS
CABI Switzerland (Dr Philip Weyl and Lauréline Humair) and U.S. Forest Service's Northern Research Station, West Virginia (Prof. Andrew Liebhold)

The North American Black locust, *Robinia pseudoacacia* L. (Fabaceae) is distributed in virtually every temperate and subtropical region of the world. An associated gall midge, *Obolodiplosis robiniae* Haldeman (Diptera Cecidomyiidae) has unintentionally followed the tree on its global spread and is often regarded as a pest. As the midge has not yet been recorded in South Africa, it is being considered as a candidate biological control agent, however, despite several attempts, culturing the species under quarantine conditions has not been successful. Therefore, a three-fold method has been implemented by researchers from the CBC to demonstrate the agent's suitability as a safe biological control agent for South Africa.

Firstly, the phylogenetic trees of closely related Fabaceae that share the same distribution (native and invaded) as *R. pseudoacacia* were determined. Then information from experts in the field of galling insects, literature surveys, agricultural pest lists, social science platforms from both the native and invaded range were used to determine if any damage (non target attack) from the midge had ever been recorded. Experts and literature suggest that *O. robiniae* does not use any species, other than those from the *Robinoide* clade. In addition, the midge has also never been recorded on several closely related leguminous fodder and horticultural species growing in close proximity at high densities to *R. pseudoacacia*, suggesting negligible risk to South African growers of the same species.

Damage by leaf galling gall midge, *Obolodiplosis robiniae*. **Photo:** Lauréline Humair

Second, host specificity assessments through field surveys (native and invaded range) have been conducted. These

surveys are regarded as one of the best indicators of the ecological host range, however, this information is difficult to quantify and infrequently available, thus seldom used when determining the safety of a candidate biocontrol agent. Over 40 closely related species were considered growing in close proximity to *R. pseudoacacia*. No non-target attacks were recorded on any surveyed species other than the known host, again suggesting *O. robiniae* would be potentially safe for release in South Africa.

Finally, host range testing has been conducted by CABI in Europe. Black locust was introduced in Europe in the 17th century and is now naturally found in most of the continent, from southern Italy to southern Norway. Since its introduction, many of its natural enemies native to North America, including *O. robiniae* have found their way over and many cause considerable damage to the *R. pseudoacacia* and are abundant in the environment making for a perfect setup for open field trials. To date, no galls have been found on any test plants, but damage has been recorded on the host *R. pseudoacacia*. The larvae have been collected for molecular identification. In addition, large multi-choice cage tests have been conducted, seven non-targets and two controls were set up in three outdoor cages in the CABI garden. Again, no damage galling was recorded on any non-target plants confirming the specificity of the agent.

In addition to work on *O. robiniae*, a survey was conducted with the assistance of Andrew 'Sandy' Liebhold, a research entomologist at the U.S. Forest Service's Northern Research Station in Blackburg, West Virginia. The survey was aimed at collecting and culturing the Locust leafminer (*Odonata dorsalis*). In addition to the collection, surveys for non-target attack were also conducted on native fabaceae growing in close proximity to *R. pseudoacacia*. A culture was successfully imported into the quarantine facility at Rhodes University. The culture is currently small but stable, and it is hoped that host specificity testing can be conducted shortly.

Honey Locust

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

STUDENT
Lehlohonolo Adams (PhD) and Gcinile Blessing Carlvalho (Hons)

COLLABORATOR
Yunnan University, China

Gleditsia triacanthos L (Fabaceae), commonly known as honey locust, is a fast-growing deciduous tree from the Central and East United States of America and has become one of the fastest-spreading alien trees in South Africa, threatening the functioning of grasslands in the country. In South Africa, the Asian seed-feeding bruchid, *Megabruchidius tonkineus* Pic (Coleoptera: Chrysomelidae: Bruchinae), is a natural enemy of *G. triacanthos* and has been found to cause minimal damage to the plant's seeds. To address this issue and aid in prioritising the tree for biological control, PhD candidate Thandeka Mahlobo is investigating the impacts of the tree in the grassland of the Free State Province, the phytochemical profile of *G. triacanthos*, and the thermal tolerance of *M. tonkineus*.

In order to determine the impact of the plant on soil microbiota and associated insect communities samples have been collected from five study sites in invaded and uninvaded areas around the Free State. i.e., two sites in Bloemfontein, one in Holfontein, about 22 km south of Kroonstad, and two others in Clarens. Thus far thermal biology studies have shown that the productivity and survival of *M. tonkineus* are affected by temperature, especially fluctuating temperature, possibly explaining its limited impact as an agent.

Asian seed-feeding bruchid, *Megabruchidius tonkineus* Pic (Coleoptera: Chrysomelidae: Bruchinae), is a natural enemy of *Gleditsia triacanthos*.

Damage caused by the agent to the plant's seeds.
Photos: David Taylor

Willows

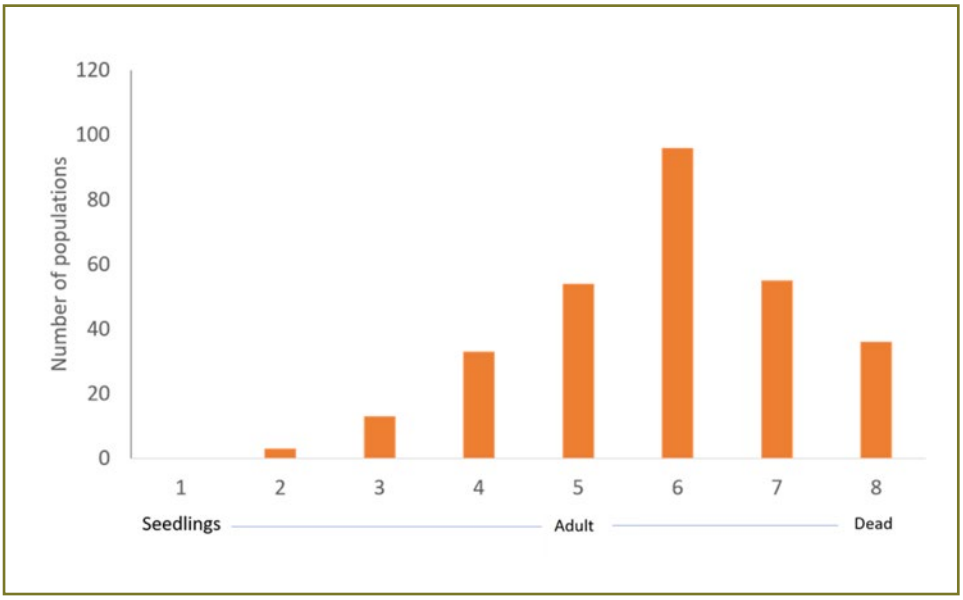
RESEARCH TEAM
Dr Grant Martin, Prof. Sandy Steenhuisen (UFS-ARU)

STUDENT
Tapiwanashe Mashamba (MSc)

The Salix species, commonly referred to as Willows, are a diverse group of dioecious trees and shrubs that originate from the Northern Temperate regions. However, these species have become invasive in many parts of the world, including the grassland biome of South Africa. Willows can have significant effects on the environment, including altering the biodiversity of a region, disrupting ecosystems, and causing economic losses. The two most prominent invasive species in South Africa's grassland are *Salix babylonica*, known as the weeping willow, and *Salix fragilis*, the crack willow. These two species remain abundant and are currently classified under the CARA regulations.

An MSc study, led by Tapiwanashe Mashamba, is currently reassessing the population demographics of these two willow species in the Free State and northern Eastern Cape provinces. The objective is to determine if biological control should be pursued. Preliminary results reveal that approximately 85% of *S. babylonica* populations are dominated by adult mature trees showing signs of decreased vigour. Analysis of historical imagery at select sample sites also shows a decline in populations. Gender ratios suggest that the scarcity of saplings may be due to the absence of male trees.

In contrast, populations of *S. fragilis* are dominated by younger trees, indicating a spreading population.



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.

River invaded by *Salix fragilis* with a few *S. babylonica*, near the town of Clarens, Free State South Africa. **Photo:** Tapiwanashe Mashamba

Invasive Rosaceae

RESEARCH TEAM
Dr Grant Martin, Prof. Sandy Steenhuisen (UFS-ARU), Dr Stephanie Payne (UFS Dept. Plant Science)

STUDENT
Karabo Moloi (PhD)

Invasive Rosaceae species are increasingly becoming dominant and problematic in the grassland biome of South Africa. Over the past five years, the Plant Sciences Department and the Afromontane Research Unit at the University of the Free State, in collaboration with the CBC, have been conducting a series of plant reproductive ecology experiments on several problematic invasive Rosaceae species. These species produce an excessive number of fruits and seeds, contributing to their rapid spread and dominance in the environment. High seed soil bank numbers and seed viability are all factors that facilitate their invasion. Furthermore, due to the fleshy nature of the fruits, these seeds are dispersed by birds and mammals into novel and inaccessible environments.

Managing these invasive Rosaceae species is a complex task. However, through a meticulous analysis of each species, certain stages of the plant life cycle can be identified for targeted management. For instance, the seeds of Pyracantha angustifolia have a short lifespan in the soil seed bank. In contrast, the seed dispersal of *Cotoneaster pannosus* is not facilitated by birds. Therefore, despite their high productivity, different solutions can be explored to control or mitigate the spread of these species. At this stage, biological control is the only feasible large-scale control option for these species. The findings from this research will help guide their management.

Rosaceae complex

PhD candidate, Karabo Moloi, is currently exploring the transformative impacts of invasive Rosaceae species on grassland ecosystems, as well as the potential for ecosystem resilience. The research is based on the hypothesis that the introduction of winter fruiting invasive Rosaceae species has created a surplus of resources that were previously absent within the ecosystem. These resources are particularly attractive to native insects, rodents, birds, and mammals, leading to significant changes in the community composition of species that benefit from the additional resources during harsh winters

The Rosaceae species selected for the study include *Rubus argutti*, *Cotoneaster pannosus*, *Rosa rubiginosa*, *Pyracantha angustofolia*, and the native species *Leucosidea sericea*. The study aims to document and compare the species traits that facilitate their invasion, determine whether ecosystem biotic resistance plays a role in preventing these species from completely overwhelming the invaded area and invading new regions, and investigate the role of the selected invasive Rosaceae species as ecosystem engineers.

In addition, the study will also determine the distribution of the selected Rosaceae species in South Africa, and their potential spread with climate change. This research is crucial as it can provide valuable insights into the effects of invasive species on grassland ecosystems and the potential for ecosystem resilience. The findings of this study could inform strategies for managing invasive species and conserving grassland ecosystems.

Orange firethorn

RESEARCH TEAM
Dr Grant Martin, Prof. Sandy Steenhuisen (UFS-ARU), Prof. Colleen Downes (UKZN)

STUDENT
Lehlohonolo Adams (PhD)

Pyracantha angustifolia is a medium-sized, evergreen shrub, native to north-eastern 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

Pyracantha angustifolia seeds left from baboon dung after the dung were filtered through a sieve. **Photo:** Lehlohonolo Adams

Common duiker eating *Pyracantha angustifolia* seeds. **Photo:** camera trap

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. Interviews have revealed that landowners tried different methods including chemical and mechanical applications to control the species but none was successful and the species keeps on becoming more invasive. Although the communities use the species for various purposes, it is not of subsistence use thus less conflicts are expected should the species be controlled. 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.

Repeat photography shows that there is facilitation of invasion and the species has been using perching structures to increase its invasion. Historical photographs show the species to mostly be under other trees, fences and electric lines. Invasion by this species and other fleshy-fruited invasive species are changing the vegetation structure in the grassland.

Rosa rubiginosa

RESEARCH TEAM
Dr Grant Martin, Prof. Sandy Steenhuisen (UFS-ARU), Dr Stephanie Payne (UFS Dept. Plant Science)

STUDENT
Patricia Masole (MSc), Zinhle Sithole (Hons.)

Despite the economic significance of *Rosa rubiginosa*, which has led to the discontinuation of biological control programs, ongoing research is being conducted to investigate whether the species is driving bush encroachment into the grassland biome and to study the seed ecology of the species. MSc candidate, Patricia Masole, has been able to show that while the seeds of the invading *R. rubiginosa* population exhibit low germination percentages, they remain viable in the soil for over two years. However, a large number of seeds are produced by the species. Patricia showed that on average, *R. rubiginosa* shrubs produce an average of 480 ± 57 fruits per shrub, with an average of 18 seeds per fruit.

To determine whether the seed predator *Megastigmus aculeatus* (rose seed chalcid) associated with the hips of the species is limiting or at least reducing the spread of *R. rubiginosa* species, she randomly collected 100 rosehips every month from March until September 2021. They were divided into five emergence jars (100 ml) where each jar was covered with gauze tightened with a rubber band and stored in an insect tent (bug dorm) at ambient temperature. 148 *M. aculeatus* emerged from some of the 700 *R. rubiginosa* fruits, giving an overall percentage of 1.28% of seeds predated. This low percentage of damaged seeds suggests that the chalcid is not significantly impacting the rose populations.

Therefore, to understand what was happening to the seeds of *R. rubiginosa*, Honours student Zinhle Sithole conducted an investigation into the seed dispersal and seed predators of the plant. Camera traps were used to record the behaviour of visitors at caches of rosehips on the ground under shrubs in invaded grasslands at two study sites. The results showed that seed caches were rapidly depleted by both birds and rodents. Birds primarily removed fruits, while most of the rodents consumed fruits at the caches. Furthermore, it was observed that *Otomys auratus* and *Rhabdomys dilectus* were seed predators rather than dispersers. This study will provide valuable information regarding the seed dispersal and predation of *R. rubigi-*

nosa, and potential drivers of its spread in the Free State rangelands.

Finally to determine whether *R. rubiginosa* acts as a nurse plant and facilitates the recruitment of woody plant species in the grasslands, we assessed the differences under three treatments: *R. rubiginosa* canopy, *Leucosidea sericea* Eckl. & Zeyh. (native) canopy, and open plots of herbaceous grassland (control) all different woody plant species (10 cm and above) found were counted and identified. This study showed *R. rubiginosa* had a greater nurse plant effect when compared to both *L. sericea* and open grassland (control), facilitating both native and invasive species. There were significantly more native species than invasive species under all three treatments. Furthermore, *R. rubiginosa* had more invasive species underneath it (13 species) compared to both *L. sericea* (11 species) and the control plots (11 species. To determine if *R. rubiginosa* is creating unique microclimates she used two temperature and light loggers, one placed under *R. rubiginosa* shrubs and the second placed in open grassland. This showed *R. rubiginosa* had both low average temperature and light (14.6 °C, 3940.5 lux respectively) while open grassland had both high temperature and light (17.7 °C, 17544.36 lux respectively).

A pair of *Rhabdomys dilectus* feeding on fallen *Rosa rubiginosa* seeds. **Photo:** camera trap

Rubus spp.

RESEARCH TEAM
Dr Grant Martin, Dr Costas Zachariades (ARC-PHP)

Research into the biological control options for *Rubus* species in South Africa has been *ad hoc* over the last five years as it has not been directly funded. However, due to the recognized significant impact of the species, research towards developing a biological control programme has been conducted by the Centre for Biological Control, in collaboration with the Agricultural Research Council – Plant Health and Protection (ARC-PHP), Cedara. Fortuitously, while research in biological control has been conducted, there has also been significant progress made towards untangling the complexities of the *Rubus* genus in South Africa. This has been facilitated by researchers from South African National Biodiversity Institute (SANBI) and The Centre of the Region Haná for Biotechnological and Agricultural Research, Crop Research Institute, Czech Republic. These two streams of research have managed to clarify several aspects which have previously hampered and limited biological control efforts.

Two taxa, members of *Rubus section Cuneifolii* and *Rubus section Arguti*, previously referred to, respectively, as the upright and the sprawling forms of *Rubus cuneifolius*, are the most abundant and widespread species in higher- and mid-elevations of KZN. A preliminary field survey in South Africa observed few natural enemies on these two invasive taxa, in contrast to those on sympatric native *Rubus* spp., indicating that the reverse may also be true, namely that natural enemies of *R. sect. Cuneifolii* and *R. sect. Arguti* in North America may be host specific. A molecular study using microsatellites is currently underway to determine genetic diversity of these two taxa in South Africa, and to match them with taxa in North America. A final stumbling block is our relatively poor understanding of the biosystematics of *Rubus* in eastern North America, which includes a large number of species. Two possible strategies to resolve this are: (1) to undertake molecular studies on a subset of the species within these two sections in North America, and (2) to expose South African trap plants at selected sites in North America in order to attract

and harvest natural enemies as candidates for importation into South African quarantine. The programme is currently working with researchers in the USA to assist in determining the closest matches to the dominant invasive *Rubus* from South Africa.

Flowers of *Rubus section Cuneifolii*.
Photo: Michael Sochor

The long-term success of WfW clearing in the northern Drakensberg escarpment, Mpumalanga province

RESEARCH TEAM
Dr Grant Martin, Dr Gerald Chikowore, Prof. Sandy Steenhuisen (UFS-ARU)

STUDENT
Nthambeleni Bologo (MSc)

This year saw the completion of a MSc study conducted by Nthambeleni Bologo. Nthambeleni is the Assistant Director responsible for Lowveld North and Escarpment Management area, under the sub-directorate Environmental Programmes Non-Infrastructure Implementation (Working for Water) Mpumalanga Region, Department of Forestry, Fisheries and the Environment. Nthambeleni's project aimed to determine the effectiveness of the WfW programme in the northern Drakensberg escarpment, Mpumalanga Province.

The study surveyed 80 community members employed in the WfW programme in the study area to determine if they had benefited from the programme. The surveyed family sizes ranged from one to eleven persons per household, with an average of five people per household. All indicated that their main source of income was from the WfW programme. The study found that 46% of the participants had a good knowledge of invasive alien plants (IAPs) while 86% of the respondents believe IAPs are a major problem. 91% of the participants believed that WfW had benefited communities living in the northern Drakensberg escarpment, with 97% indicating that the programme had improved their living conditions.

The study revealed that the programme has significantly contributed to rural communities' livelihoods. However, short-term contracts and intermittent funding have significant implications for the communities now relying on the funding. In addition, limited landowners (10) were interviewed in this study. Most (60%) of the landowners who responded believed the programme was beneficial, and 70% of the landowners indicated that clearing of IAPs had increased stream flow and water availability. In addition, 90% of the landowners indicated that they had seen a reduction of IAPs of between 20% to 80% on their properties due to clearing by WfW.

The study showed that despite areas being cleared for over ~25 years, many important native plant species have not returned, and the communities remain different. Presumably, species recovery might not occur without some form of additional management, therefore restoration actions including monitoring of cleared sites should be considered to accelerate native vegetation recovery.

This study concludes that there is a need for quantitative, long-term, post management evaluations and assessments of the impacts of the funding models on communities used

in broad scale initiatives. This will ensure that both the benefits and the failures of the project can be accurately determined to ensure its continued development and success. In addition, WfW should have dedicated funding for research to provide valuable long-term capacity. Finally, WfW should develop long-term clearing management plans with clear and realistic goals for all priority areas, and progress towards those should be routinely monitored to ensure continued improvement in the implementation of the programme.

Cuneifolii invading in kwaZulu Natal. **Photo:** Michael Sochor

The Range X project

RESEARCH TEAM
Dr Grant Martin, Dr Gerald Chikowore, Prof. Sandy Steenhuisen (UFS-ARU), Prof. Ralph Clark (UFS-ARU), Dr Stephanie Payne (UFS-ARU), Dr Ona Gwate (UFS-ARU)

STUDENT
Lesego Malekana (MSc)

STUDENT
ETH Zurich, Zürich (Jake Alexander)

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 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 change. 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 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. ARU Post-doctoral candidate, Ona Gwate, has now completed two years 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 Rosaceae.

Lesego Malekana, an MSc candidate, has successfully completed two seasons of sampling and clearing at three

sites of varying altitudes in the Maluti Mountains of South Africa. The sites were targeted for a complex of woody invasive Rosaceae species, including *Cotoneaster pannosus*, *Rosa rubiginosa*, *Pyracantha angustifolia*, and the native species, *Leucosidea sericea*. The initial analysis of the impact data has been intriguing, revealing a significant impact on biodiversity at all sites. This impact is likely due to reduced temperature and light caused by invasions. However, the impact seems to be more severe at lower altitudes, while native plant communities appear more intact at higher altitudes. Experiments conducted to remove the invasive species have shown rapid recovery of plant richness. However, pioneer and alien species seem to be dominating the initial recovery, indicating that more analysis is required. Lesego has successfully completed all fieldwork and aims to finalize the study by mid-2024. Following this, he plans to continue similar studies in Europe with the Alexander Lab, ETH University, Zurich, Switzerland to look at competition and biotic interactions behind the invasiveness of herbaceous plants in South African, Maluti-Drakensberg and the Alps in Switzerland.

Lesego Malekana, assisting in the manual clearing of invasive Rosaceae sp. in mid altitude study sites in the eastern Free State. **Photo:** Grant Martin

INVASIVE TREES

Gall sorting in Australia. **Photo:** Fiona Impson

Despite severe funding challenges during 2023 and the sad passing of the CBC's long-term colleague Cliff Moran, progress has been made on the invasive tree projects.

Australian acacias

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

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

A 20-year study looking at the impacts of fire, clearing and biological control on seed banks and densities of *Acacia cyclops* has now been completed. The study shows that, with diminishing seed production by the plants due to the two biological control agents, the seed banks in the soil have reached levels that are too low for *A. cyclops* to fully replace plants that are destroyed by fire or wood harvesting. The outcome is that the density of A. cyclops has declined substantially (typically by >90%) in several areas and is continuing to do so. The effect of the agents together with fire has exceeded all expectations and is providing another example of a highly successful biological control programme.

Perilampella hecataeus galls on *Acacia baileyana* in Australia. **Photo:** Fiona Impson

Mature galls of the flower galling midge, *Dasineura pilifera* were collected in Stellenbosch from *Acacia baileyana* in 2022 and released in Mpumalanga during April 2023 on *A. decurrens*. Additional galls were collected from the site of establishment in Stellenbosch (October 2023) and these will be held under outdoor ambient conditions for further releases to be made in the 2024 season. There has been no confirmed establishment of *D. pilifera* at Eastern Cape release sites.

The collaborative project on *Acacia longifolia* with Manaaki Whenua – LandCare Research New Zealand progressed well during 2023. The shipment of *Trichilogaster acacieaelongifoliae* from South Africa in 2022, resulted in several releases of the wasp in New Zealand in December

2022 and establishment was confirmed in August 2023 and the site was revisited in November 2023 to check on gall development. A second shipment of 8kg *T. acacieaelongifoliae* galls was hand carried to New Zealand in November 2023. Unfortunately, there were some delays with disease testing (using larval body contents for slide smears) and awaiting final permissions for field releases but despite this,179 females were released into two field sites on North Island.

Following the visit to New Zealand, F. Impson also visited Australia to make a collection of the gall wasp *Perilampella hecataeus*, for biological control of *Acacia baileyana*, *A. dealbata* and *A. decurrens*. The collection focused on areas in Victoria (within a 3-4 hour driving distance both east and west of Melbourne) and Tasmania where good populations of the wasp had previously been encountered. Whilst very low populations of *Perilampella hecataeus* were found at many of the 70 *A. baileyana* and *A. dealbata sites* visited, only 7 sites yielded enough galls with mature larvae and pupae within them for collection. A small consignment of the gall midge *Dasineura pilifera* was also collected.

Perilampella hecataeus release on *Acacia baileyana*. **Photo:** Fiona Impson

The shipment was handled in Stellenbosch at the ARC, PHP quarantine facility at Vredenburg, and during December approximately 2000 *P. hecataeus* females emerged from the gall material. This resulted in the first field releases of *P. hecataeus* in South Africa, mostly into local sites, and a single release was made onto *A. dealbata* in Mpumalanga.

Prosopis

During 2021 two additional biological agents (a leaf-tying moth Agnippe sp. and a seed-feeding weevil *Coelocephalapion gandolfoi*), were released against Prosopis to supplement the activities of the seed-feeding beetles already present in South Africa.

A field trip to the Northern Cape province in December 2022, confirmed the persistence of *"Agnippe"* at two sites and an additional release was conducted at one of these sites. In March 2023, the persistence of *C. gandolfoi* was confirmed at one of the release sites. Only one *Agnippe* site was re-visited, but the moth was found to be persisting at this site, particularly favouring the young plants.

RESEARCH TEAM
Emeritus Associate Prof. John Hoffmann, Fiona Impson, Catharina Kleinjan, Philip Ivey, Dr Blaire Cowie

STUDENT
Gretha van Staden (MSc)

COLLABORATORS
FuEDEI (Fernando McKay)

Subsequent evaluation in December 2023, failed to find evidence of *C. gandolfoi* at any of the release sites, but further evaluation is needed particularly as drought conditions appear to be having an impact. The persistence of *"Agnippe"* across two winters was confirmed at one site, the moth however remains scarce at this locality.

Pines

RESEARCH TEAM
Dr Grant Martin, Dr Gerald Chikowore, Dr Kim Canavan, Prof. Martin Hill, Jufter Musedeli

COLLABORATORS
Prof. Brett Hurley (FABI), Prof. Bernard Slippers (FABI), Prof. Heila Marchante (University of Coimbra), Dr Elizabete Marchante (University of Coimbra), Louise Stafford (TNC), Prof. John Hoffmann (UCT)

Invasion of the fynbos biome in South Africa by wilding pines poses serious threats to biodiversity, water provision and endangers surrounding settlements due to recurrent fires. Cognisant of these challenges, The Nature Conservancy (TNC) in conjunction with the Centre for Biological Control (CBC), the Forestry, Agricultural Biotech Inst (FABI) are in the process of exploring sustainable management options including the once shelved biological control programme against wilding pines. During 2023, significant stakeholder engagement was undertaken involving the scientific community and the forestry industry and there was consensus that wilding pines need to be managed. To this end, several trials including further host-specificity tests for the potential biological control agent, the pine cone weevil (*Pissodes validirostris*) have been initiated. Novel technologies such as chemical ecology are being considered to confirm the specificity of the weevil against pine species originating from the Iberian Peninsula.

In addition, partnerships were forged with researchers in institutions in the native range such as the University of Coimbra in Portuga and Dr. Alain Roques at INRAE-Zoologie Forestiere Centre de recherche d'Orléans in France. These partnerships will result in studies to optimize the rearing of the cone weevil in Portugal, the centre of origin of the Iberian strain. These studies will also include thermal physiology of the weevil as a determinant of its potential range. Since the climate in the Iberian Peninsula closely matches the Mediterranean climate in the Western Cape Province, this study is expected to confirm that the weevil will not establish beyond the intended region. Coupled with the low dispersal capacity of the weevil, this will further reduce risks associated with the release of the weevil in South Africa. Most importantly, this will hopefully give us access to the weevils and develop protocols on culturing the weevil.

Dense stand of Invasive Mediterranean pine seedling returning after management, Western Cape. **Photo:** Richard Bugan

BUGWEED

Solanum mauritianum being visited by a native *Apis mellifera* bee. **Photo:** Nic Venter

Bugweed (*Solanum mauritianum*), 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 populations that can be damaging, but this is mainly limited to warmer low altitude sites. However, prominent bugweed invasions also occur in high altitude regions within South Africa (> 1000 m) that experience cold winters, but due to climatic incompatibility, these agents have not established in these regions. Consequently, an additional agent, the flower-feeding weevil *Anthonomus morticinus* was collected from temperate regions of Uruguay, during 2020, 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 *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 in the Solanaceae family are important agricultural plants grown for human consumption, thus it is essential that any potential bugweed biocontrol agents don't exhibit non-target effects on these plants. Work is ongoing to test *A. morticinus*' host range on Solanaceae species that are native to South Africa and results thus far are showing promise. Moreover, work is underway to test new, additional candidate biocontrol agents.

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

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

STUDENT
Yaron Keizan (MSc, Wits)

COLLABORATORS
UKZN (Terry Olckers), LandCare Research New Zealand (Angela Bownes, Simon Fowler, Hugh Gourlay Lynley Hayes)

Dr Blair Cowie (postdoc, Wits) and Ms Holly Cox (Auckland Council, NZ) surveying bugweed plants in Uruguay for potential biocontrol agents in November 2023. **Photo:** Nic Venter

TAMARIX

Corimalia sp. imported into Wits Quarantine for host range testing on invasive *Tamarix*. **Photo:** Nic Venter

Tamarix chinensis and *T. ramosissima* (Tamaricaceae), native to Eurasia, are Category 1b invaders that have 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 for the target weeds. 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 semiarid regions with low annual rainfall and occurs widely in the Northern Cape (and in Namibia) and some parts of the Western Cape Province. Additionally, the two invasive species and the indigenous *T. usenoides* 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* due to non-target feeding on native *T. usneoides*. In collaboration with BBCA Onlus in Rome, Italy, the leaf feeding weevil *Coniatus tamarisci* was imported into the Wits Quarantine in October 2020 and again in late 2022 to supplement the quarantine culture. Whole plant no-choice and multi-choice trials unfortunately indicated that *C. tamarisci* showed no preference for the invasive *Tamarix* over the native *T. usneoides*. During no-choice trials, feeding, oviposition, and larval development did not differ between the invasive and native *Tamarix*. Multi-choice trails supported the no-choice results, whereby the weevils did not show a preference for the invasive *Tamarix* over the native *Tamarix*. Based upon these results and those from previous candidate agent testing, it is recommended that *C. tamarisci* be rejected as a potential biocontrol agent and that native range surveys of *T. chinensis* and *T. ramosissima* focus on finding Eriophyid mites which are more likely to be host specific.

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 spread of the weed and the progress of any biocontrol agent eventually released against invasive *Tamarix*. 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.

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

STUDENTS:
Sivenathi Hatile (MSc, Wits), Alekzandra Szewczuk (PhD, Wits and CIB, Stellenbosch University).

COLLABORATORS:
Biotechnology and Biological Control Agency (BBCA), Rome, Italy (Massimo Cristofaro and Francesa Marini)

INTERNATIONAL WEED PROJECTS

Iain Paterson at a site in Struisbaai. **Photo:** Emma Sandenbergh

Crystalline Ice Plant

Crystalline ice-plant, *Cryophytum crystallinum*, is a species of 'vygie' (Aizoaceae) endemic to southern Africa. The species is a problematic invasive alien plant in California (USA) and Mexico, where it forms dense monocultures in the absence of natural enemies. These monocultures prove highly damaging to coastal and offshore island ecosystems, threatening their unique biodiversity. Crystalline ice-plant was named as such as a result of the plant being covered in bladder cells which resemble crystals. These cells hold a hypersaline solution which leaches into the topsoil when the plant dies, preventing seed germination and making the environment uninhabitable to any plants other than *C. crystallinum*. This allows *C. crystallinum* to outcompete co-occurring plant species and ultimately become dominant in an invaded system, altering ecological processes, and threatening indigenous biota. The CBC continues to collaborate with the United States Department of Agriculture to develop a biological control programme against *C. crystallinum* in the USA and Mexico.

As crystalline ice-plant is also abundant in various parts of Europe, genetic analyses have been conducted by MSc student Cailtin Webb and researchers at the CBC to investigate the potential origin of the invasive *C. crystallinum* populations in the USA and Mexico. While these analyses are ongoing, the results so far suggest that *C. crystallinum* was likely transported from the Western Cape of South Africa to Europe, and subsequently transported to the United States and Mexico. As such, South Africa's west coast was identified as an appropriate location to conduct native-range surveys to identify potential biological control agents.

RESEARCH TEAM
Prof. Iain Paterson, Emma Sandenbergh

STUDENTS
Caitlin Webb (MSc), Jackey Mukhawana (BSc Honours)

COLLABORATORS
United States Department of Agriculture, San Francisco (Dr Patrick Moran); BBCA Onlus, Rome (Dr Massimo Cristofaro)

Multiple native range surveys have been conducted by researchers, students, and collaborators of the CBC, through which a stem-mining weevil, *Lixus carinerostris*, has been identified as a potential biological control agent to target *C. crystallinum*. During these surveys, the weevil was observed both feeding and ovipositing on *C. crystallinum* and the close relative, *Cryophytum guerichianum*.

Preliminary host-specificity testing conducted by MSc student Caitlin Webb has shown that while *L. carinerostris* is not necessarily species-specific, it is certainly a *Cryophytum* specialist. As the Americas have very few indigenous Aizoaceae, with no native *Cryophytum* species, *L. carinerostris* should prove safe to release as a biological control agent in this region. However, to ensure the safety and efficacy of the candidate biological control agent, further host-specificity testing will be conducted in both the native South African range and in the USDA-ARS quarantine facility in San Francisco. The first consignment of *L. carinerostris* was collected in the Western Cape and exported into quarantine at the USDA labs in November 2023.

Jackey Mukhawana investigating an ice plant at a site in Oudsthoorn. **Photo:** Wade Sexton

Jackey Mukhawana and Emma Sandenbergh at a site near Agulhas. **Photo:** Wade Sexton

GRASSES

Megathyrsus maximus seed head. **Photo:** D.Taylor

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 2023

- Two *Tetramesa* species that are prospective biocontrol agents for *Sporobolus pyramidalis* and *S. natalensis* were sent to quarantine in Brisbane in November 2023 for final host-specificity testing.
- Native range surveys for *Megathyrsus maximus* in Kenya and South Africa have identified several prospective biocontrol agents.
- Host-specificity assessments have allowed us to rule out a stem-boring *Tetramesa* sp. as a possible agent for the *M. maximus* project.
- Host-specificity assessments have commenced for a stem-galling midge and a seed-galling midge on *M. maximus* following promising field assessments of their host-specificity in South Africa and Kenya.
- The *Tetramesa* and midges found in each grass project are all undescribed species. Consignments of the insects have been sent to an expert taxonomist in Iran, Prof. Hossein Lotfalizadeh (Tetramesa) and Dr. Peter Kolesik in Australia (midges) to obtain formal descriptions.

Giant rat's tail grass, *Sporobolus* spp.

RESEARCH TEAM
Dr Guy Sutton, Prof. Iain Paterson, Dr Kim Canavan (postdoc)

COLLABORATORS
Queensland Department of Agriculture and Fisheries (QDAF) (Tamara Taylor, Lauren Kelk, David Comben)

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 biological control of these species since 2017. Surveys across South Africa have identified three stem-boring *Tetramesa* wasps (Hymenoptera: Eurytomidae) that appear to attack only *S. pyramidalis* and *S. natalensis*, despite surveying more than 70 other non-target grass species, and reduce seed production and plant survival. The two most damaging species, *Tetramesa* sp. 1 and *Tetramesa* sp. 2, have been tested against >20 non-target grasses in the greenhouse, which confirmed that the wasps are likely host specific. The covid-19 pandemic prevented the importation of both species into quarantine in Australia in 2020 and 2021.

Above: Field collections of *Tetramesa* spp. on *Sporobolus pyramidalis* in northern KwaZulu-Natal Province, South Africa.

Below: David Comben (QDAF), Pippa Muskett, Liam Yell and Guy Sutton (CBC). **Photos:** David Comben

Two consignments of wasps were imported into quarantine at the Queensland Department of Agriculture and Fisheries (QDAF) in Brisbane, Australia, in late 2022. 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 regards to the conditions required to rear these wasps in quarantine. A third consignment of wasps were collected in northern KwaZulu-Natal in November 2023. The team at QDAF are currently working on this consignment to establish viable cultures of the two *Tetramesa* species. Once these wasps are established in quarantine in Brisbane, they will be tested for their ability to utilise native Australian *Sporobolus* species to further scrutinise their potential as biological control agents.

We have also performed a field-study across the KwaZulu-Natal province between 2017 and 2019 which has recently been published in Biocontrol Science and Technology showing that anthropogenic disturbance (e.g. mowing, prescribed burns) negatively impact both *Tetramesa* species in their native range. Mowing and prescribed burns resulted in *Sporobolus* patches being unoccupied by both wasps for more than 6-9 months post-disturbance, and once the wasps did recolonize the affected sites, their abundances were significantly lower than unaffected sites. These results have important implications for the possible biological control of *S. pyramidalis* and *S. natalensis*, as our results indicate that integrating biological control with other management techniques will require careful consideration, allowing sufficient habitat for the wasps to avoid any significant disturbance created by mowing, herbicide application and/or prescribed burns.

Guinea Grass

RESEARCH TEAM
Prof. Iain Paterson, Dr Guy Sutton, Liam Yell

STUDENT
Benjamin de la Fontaine (MSc)

COLLABORATORS
University of Texas (Rob Plowes, Aaron Rhodes, Colin Morrison), Mpala Research Centre (Aimee Gaitho), USDA, BBCA (Massimo Cristofaro, Francesca Marini)

Megathyrsus 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. However, DNA samples provided by the CBC indicated that Guinea Grass populations in Texas likely originated from southern Africa. This resulted in the CBC initiating a collaboration with the USDA, BBCA and the University of Texas in 2019 to identify potential biocontrol agents for Guinea Grass from southern Africa, and continue surveys in Kenya. These surveys have identified several possible biological control agents, including two stem-galling midges (Cecidomyiidae), a stem-boring wasp (*Tetramesa* sp.; Eurytomidae) and a suite of seed-galling midges (Cecidomyiidae). All of these species are currently undescribed, so we are working with expert taxonomists in Iran (Hossein Lotfalizadeh) and Australia (Peter Kolesik) to get these insects formally described.

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*. Preliminary field surveys have indicated that this midge species appears to only utilise *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. This indicates a high degree of specificity. The galls appear to act as a nutrient sink, depleting the plant of its resources and stopping the plant from producing seed. Our first few attempts to establish cultures of this midge in 2022 and early 2023 were not successful, but it did allow us to learn important aspects of the fly's biology, which allowed us to optimise our rearing protocols. A viable culture has now been established under greenhouse conditions in the KZN province, which has already passed through two complete generations. A small experiment was performed which indicated that Guinea Grass plant size was an important determinant for gall initiation. Younger *M. maximus* plants that were still short (about 30 cm in height) and about to produce their first panicle were found to produce three times more adult midges compared to larger plants. Now that we have a viable fly culture, no-choice host-range tests are being performed to evaluate the host-specificity and thus suitability as a biological control agent of this species under controlled conditions.

Adult specimen of the blister-galling midge and the conspicuous galls formed on *Megathyrus maximus* (Guinea Grass) (note the pupal casings left behind on the galls after flies have emerged). **Photos:** David Taylor, Iain Paterson

The second midge will soon be described as the first species in a new genus. It was collected in the Arabuko-Sokoke State Forest in Kenya in June 2022. The midge forms dense clusters of galls at the base of the plant, which appears to prevent the plant from growing new stems. Extensive searches of sympatric non-target grasses at Arabuko-Sokoke State Forest indicated that the midge is likely host-specific to *M. maximus*, as it wasn't recorded on any closely related grass species in the area. The galls are heavily parasitized by solitary and gregarious parasitoids alike, which bodes well for biological control, because the parasitoids are unlikely to be present outside the native distribution. As such, if it is used as an agent outside of the native distribution, such as possibly being released in Texas, it may escape parasitism pressure and reach higher population densities.

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

The third candidate agent is a stem-boring wasp belonging to the *Tetramesa* genus. *Tetramesa* wasps are grass-feeding specialists (see sections on *Sporobolus* and *Eragrostis* for more details) 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* species found on other South Africa grasses to date, and the wasp is not as widely distributed. Despite several unsuccessful attempts to establish a culture of the wasp under greenhouse conditions in KZN in 2022 and early 2023, a small culture was established mid-way through 2023. While establishing this culture, we collected an abundant *Tetramesa* species from a closely-related grass, *Setaria sphacelata* (African bristlegrass), at the field sites where we collecting *Tetramesa* on Guinea Grass, which made us worry if the *Tetramesa* on Guinea Grass may be spill-over from *S. sphacelata*. As such, we performed no-choice cross-inoculation trials in the greenhouse with *Tetramesa* collected from both plant species, which indicated that both wasp populations were able to feed and complete development on both plant species. More importantly, the *Tetramesa* collected on Guinea Grass produced three-fold more progeny on *S. sphacelata* than on Guinea Grass. This result clearly indicated that the *Tetramesa* recorded on Guinea Grass

Adult specimen of the seed-attacking gall midge on *Megathyrus maximus*. *Photo:* Benjamin de la Fontaine

is not suitably host-specific to be considered further for biological control.

The fourth and final candidate agents are a potential suite of seed-attacking gall midges (Diptera: Cecidomyiidae). At least one seed-attacking midge has been recorded in abundance from Guinea Grass seeds from a range of sites across South Africa. We are currently exploring the possibility that these morphologically similar seed-feeders may be a number of cryptic species using genetic barcoding techniques. The seed-feeders appear to be able to tolerate drier climatic conditions than the aforementioned stem cecids, which may be important for biological control endeavours in the USA. More recently, we have reared morphologically similar seed-attacking cecids from eight sympatric grasses across the *Panicum*, *Setaria*, *Urochloa*, *Melinis* and *Cenchrus* genera. Our current research is aimed at determining whether these cecids are a single generalist species found on all the grasses sampled or host-specific entities associated with a single or a few host grasses, using a combination of genetic barcoding and oviposition trials. This work will be invaluable for assessing the taxonomy and host-specificity of these seed cecids and their potential as biological control agents.

Cogon Grass

RESEARCH TEAM
Dr Guy Sutton, Prof. Iain Paterson, Liam Yell, Dr Kim Canavan

COLLABORATOR
United States Department of Agriculture [USDA] (Dr Gregory Wheeler)

Cogon Grass (*Imperata cylindrica* (L.) Beauv.) is one of the ten worst weeds in the world, a federally listed noxious weed country-wide in USA, and one of the most problematic invasive weeds in the south-eastern USA. Cogon Grass negatively impacts native plant communities, aggressively competes with other vegetation for soil nutrients, alters the rates of decomposition of native grasses, and alters natural fire cycles. An exciting new biological control project has been initiated as a collaboration between the CBC and the United States Department of Agriculture (USDA) in 2023.

In early 2023, a two-week survey of the natural enemies associated with Cogon Grass across South Africa was performed (figure below). More than 17 sites were sampled across the Eastern Cape and KwaZulu-Natal Provinces, however, very few natural enemies were collected from the Cogon Grass samples. This result likely points to the fact that Cogon Grass may itself be an alien in South Africa, although the origin and history of the grass in South Africa is unknown. Future work will focus on surveying the insect communities associated with Cogon Grass in East Africa (e.g. Kenya, Uganda, and Tanzania) as preliminary surveys in these areas between 2013-2015 found a number of insects associated with Cogon Grass that could potentially be developed as biological control agents.

Greg Wheeler (USDA) and Liam Yell (CBC) conducting field collections of Cogon Grass (*Imperata cylindrica*) in northern KwaZulu-Natal Province, South Africa. **Photo:** Guy Sutton

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

Nassella spp.

RESEARCH TEAM
Dr Kim Canavan, Dr Anthony Mapaura, Prof. Vincent Ralph Clark, Prof. Sandy-Lynn Steenhuisen (University of the Free State (UFS) and Afromontane Research Unit (ARU))

STUDENT
Lumko Mboyi (M.Sc. student, UFS and ARU)

The CBC in collaboration with the ARU and the UFS are continuing research on three alien grass species that are problematic invasives in South Africa, *Nassella trichotoma*, *N. tenuissima* and *N. neesiana*. Previous research led by Dr. Mapaura found that the grasses are impacting native biodiversity in montane grasslands and are more widely distributed than previously realised. Lumko Mboyi's project will extend this work and look at *Nassella* spp. invasive patterns, distribution and impacts in the Eastern Cape Drakensberg. This work has found that all three species present a major threat to these important ecosystems and management interventions are needed. The outcomes of these projects will allow insight into which areas to prioritise control efforts.

Pampas

RESEARCH TEAM
Dr Kim Canavan, Prof. Sandy-Lynn Steenhuisen (UFS and ARU)

STUDENT
Thembelihle Mbele (M.Sc., SANBI, UFS)

COLLABORATORS
SANBI; University of Oviedo, Spain

The CBC in collaboration with UFS and SANBI is supervising a project on *Cortaderia* spp. in South Africa, led by M.Sc. student Thembelihle Mbele. The project is looking into 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 (NEM:BA) and are prohibited from being traded and cultivated. However, they have become increasingly popular in horticultural trading, particularly as ornamental plants for décor (figure on page 68).

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 inflorescences and naturalised populations.

The team are also collaborating on a global project on *C. selloana* led by Dr. Adrián Lázaro-Lobo and Dr. Borja Jiménez-Alfaro from the University of Oviedo, Spain.

The project will look into biological patterns in the global invasion of *C. selloana*. The research is intended to provide information to better manage the species in the future. Populations were collected across three provinces in South Africa and will be sent to Spain for further analysis.

This work will also produce a monograph on *C. selloana* to provide a comprehensive resource on all aspects of the plants including taxonomy, environmental impacts and management options.

Left: *Cortaderia jubata* in Gauteng province. **Photo:** Thembelihle Mbele

Right: *Cortaderia selloana* inflorescence. **Photo:** Kim Canavan

Below: Thembelilhe Mbele collecting *C. selloana* in Ladybrand, South Africa. **Photo:** Kim Canavan

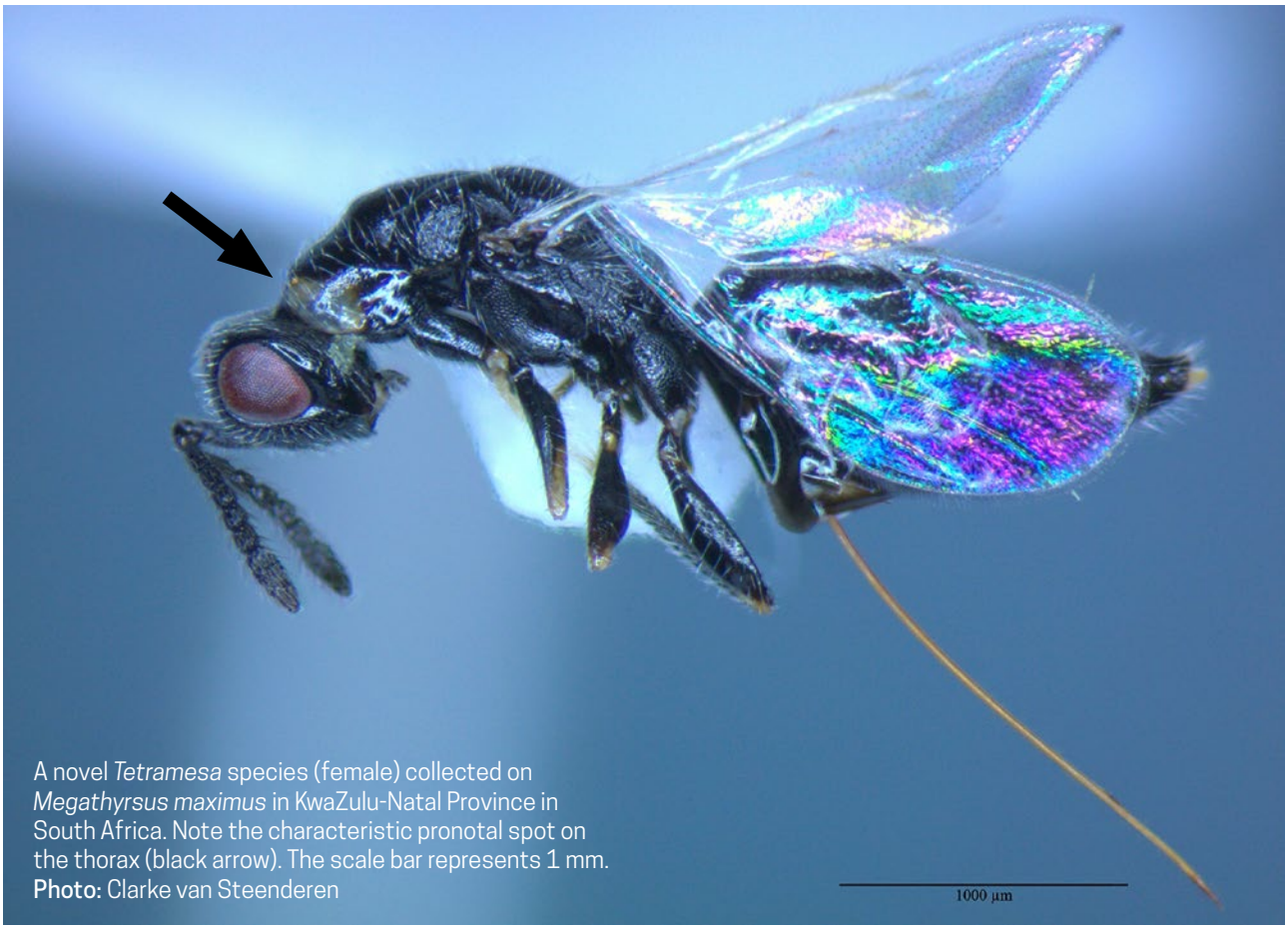
Discovery of novel *Tetramesa* spp.

RESEARCH TEAM
Dr Clarke van Steenderen, Dr Guy Sutton, Prof. Iain Paterson, Liam Yell, Dr Kim Canavan

The genetic barcoding of the *Tetramesa* Walker wasps (Hymenoptera: Eurytomidae) has been an integral part of the grass biocontrol programme at the CBC, where we have now identified at least 16 putative novel southern African *Tetramesa* taxa. Ten of these were associated with grasses native to South Africa that have become invasive weeds elsewhere in the world, including: *Eragrostis curvula*, *Sporobolus pyramidalis*, *S. natalensis*, and *S. africanus*. Eight of these species showed evidence of specificity to a single host plant, and two showed specificity to multiple grass species in the same genus. Based on molecular and field-based evidence, the *Tetramesa* collected on *E. curvula* (*Tetramesa* sp. 4) are likely not suitably host-specific for release in regions with a high diversity of native *Eragrostis* species, such as Australia where *E. curvula* is a problematic alien invasive. The *Tetramesa* collected on *Sporobolus* species, however, showed strong evidence of host-specificity and are likely good candidates for the biological control of *S. pyramidalis* and *S. natalensis* in Australia. As part of our genetic work, we have also developed a BLAST-like application for the quick identification of *Tetramesa* sequence data. The app can be accessed here: https://github.com/clarkevansteenderen/TetramesaID

In our last report, we reported the discovery of unidentified *Tetramesa* wasps on *E. curvula* in Australia. We have now confirmed that these are identical to the South African *Tetramesa* that we have collected on *E. curvula* in the Free State (*Tetramesa* sp. 4) and which the CBC were assessing as a possible biological control agent to export to Australia. Given that our previous work demonstrated that this wasp can feed and complete development on a number of South Africa native *Eragrostis* species, this project will focus on assessing how widespread the wasp is in Australia, whether it is posing a risk to native Australian *Eragrostis* grasses and if it is curtailing the spread of the weed in Australia, going forward.

We are continuing to add to our database, and are likely to find numerous additional eurytomid taxa that are new to science. We also aim to combine our molecular results with morphological lines of evidence in order to better understand this complex taxonomic group and unravel the co-evolutionary patterns with their host plants.



A novel *Tetramesa* species (female) collected on *Megathyrsus maximus* in KwaZulu-Natal Province in South Africa. Note the characteristic pronotal spot on the thorax (black arrow). The scale bar represents 1 mm. **Photo:** Clarke van Steenderen

AGRICULTURAL ENTOMOLOGY

The commercially sold bottles of MultiMax and CodlMax. They are used on a range of different crops.
Photo: Michael Jukes

After nearly a decade of development, the Centre for Biological Control's Agricultural Entomology research group saw the commercialisation of two new biological pesticides, developed in close collaboration with River BioScience (Pty) Ltd and Citrus Research International. The new products, MultiMax and CodlMax, are formulated with the patented virus Cryptophlebia peltastica nucleoployhedrovirus (CrpeNPV) and represent a significant milestone in the centre's efforts to develop environmentally friendly and effective pest control systems. These new products have entered the South African market to help control the false codling moth (*Thaumatotibia leucotreta*) and the codling moth (*Cydia pomonella*) in high value crops such as Litchi and Apple. Registration of these products for use against the codling moth in Europe is well underway, with its entry into the European market anticipated during 2024. With many countries facing increased resistance to chemical insecticides and an ever greater need to manage agricultural pests, these products aim to further support the agricultural industry by providing new control options which are highly effective.

The CBC's Agricultural Entomology research group further enjoyed a positive year filled with important findings and outcomes from the diverse range of projects, each of which aims to tackle various challenges and the wide range of pest complexes which are faced by the South African agricultural industry. From the collection and assessment of psylloid species (Hemiptera: Psylloidea) which may serve as possible vectors of citrus greening in South Africa, to the search for novel entomopathogenic fungi which could be developed into biopesticides for the control of foliar citrus pests, the group is immensely proud of the outstanding research being conducted at the CBC.

PROGRAMME HIGHLIGHTS IN 2023

- Presented oral and poster presentations at ESSA, SIP, and the RU postgraduate conference.
- Two new products based on CrpeNPV, MultiMax and CodlMax, entered the market
- Have seven peer reviewed publications.

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

RESEARCH TEAM
Dr Candice Coombes, Prof. Martin Hill, Prof. Sean Moore

STUDENT
Sifundo Ngxekisa (MSc)

The management of false codling moth (FCM) is important for the South African citrus industry given its status as a quarantine pest. This involves a variety of pre-harvest control techniques including the use of semiochemicals, organic compounds that convey chemical messages between individuals, which induce behavioural or physiological modifications such as insect repellents. Semiochemical pest management strategies are preferred over other techniques because they are host-specific, residue-free, and less toxic. This renders them safe for the environment and humans. Although there has been an

extensive amount of research on the use of repellents in integrated pest management (IPM) programmes, much of this research has been focused on pest species of medical and veterinary importance, such as the *Anopheles* mosquitoes (Meigen) (Diptera: Culicidae). Thus, there is scope to increase the use of repellents against insect pests of agricultural importance. Recently, the deterrent effects of several plant extracts, essential oils, insecticides and fruit, on reducing FCM oviposition on oranges under laboratory conditions has been investigated. Eight compounds were identified as having significant deterrent effects: two essential oils (lavender and peppermint), two plant crude extracts (garlic and marigold), three chemicals (Delegate, Coragen, and Warlock), and a mango. Moving forward, this research is now aimed to firstly establish whether these compounds are true repellents or simply mask the attractive odour of the oranges and secondly, to determine whether the findings of the laboratory trials are reflected under more realistic conditions using individually potted young fruit-bearing trees housed in a shade tunnel at the Waainek Research Facility, Makhanda. Our preliminary results show that FCM does oviposit on the artificial fruit and that the number of eggs oviposited on fruit treated with peppermint and lavender oil is greatly reduced. Further trials are still in progress with cage trials expected to be initiated later this year when outside night temperatures are more suitable for oviposition.

Artificial oranges used for true repellency trials. **Photo:** David Taylor

False codling moth, Thaumatotibia leucotreta (Meyrick) eggs laid on an artificial orange. **Photo:** David Taylor

Effect of netting on entomopathogens for the management of *Thaumatotibia leucotreta* in citrus orchards in South Africa

Entomopathogenic viruses are a very significant part of the integrated pest management (IPM) programme, and they are employed as biopesticides in South Africa to combat *Thaumatotibia leucotreta* and other important citrus and stone fruit pests. The Cryptophlebia leucotreta granulovirus (CrleGV) is a commercially available viral biopesticide for *T. leucotreta* management in the field. Despite its favourable characteristics such as high host specificity and being environmentally friendly, one of the most important shortcomings of CrleGV is its sensitivity to ultraviolet (UV) radiation from the sun, which causes degradation and loss of efficacy in the field. As a result, farmers must spray the biopesticide at dusk and more frequently, which is labour intensive and costly. It is critical to constantly seek new methods or modify existing strategies of pest control for a better future for the citrus industry. The use of shade netting is becoming a very prominent aspect of citrus farming in South Africa. Netting has many advantages, such as acting as a buffer of temperature extremes and increasing relative humidity, which promotes plant growth. Shade netting also protects the crops from pests including birds, bats and insects such as aphids, whiteflies and perhaps *T. leucotreta*. Most importantly, shade netting could be a solution to the many effects of a more concerning

RESEARCH TEAM
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STUDENT
Kurhula Luphondo (MSc)

problem, climate change. Although the use of shade netting is increasing in citrus orchards across the country, little research is done on shade nets and therefore, there are many important questions that require answers. One such question is how shade netting affects entomopathogens, which is the aim of this study. The identification of a positive impact of netting on CrleGV's lifespan or persistence could be important as shade nets can be included in more citrus orchards for the control of many pests including *T. leucotreta*. Currently, virus is being used successfully, multiplied from *T. leucotreta* larval cadavers infected with CrleGV and a small-scale trial is in progress to allow for the alteration of methodology if required. Semi-field exposure of CrleGV both under and without netting and surface dose biological assays to assess virulence/persistence of CrleGV under and without netting will be performed in the future.

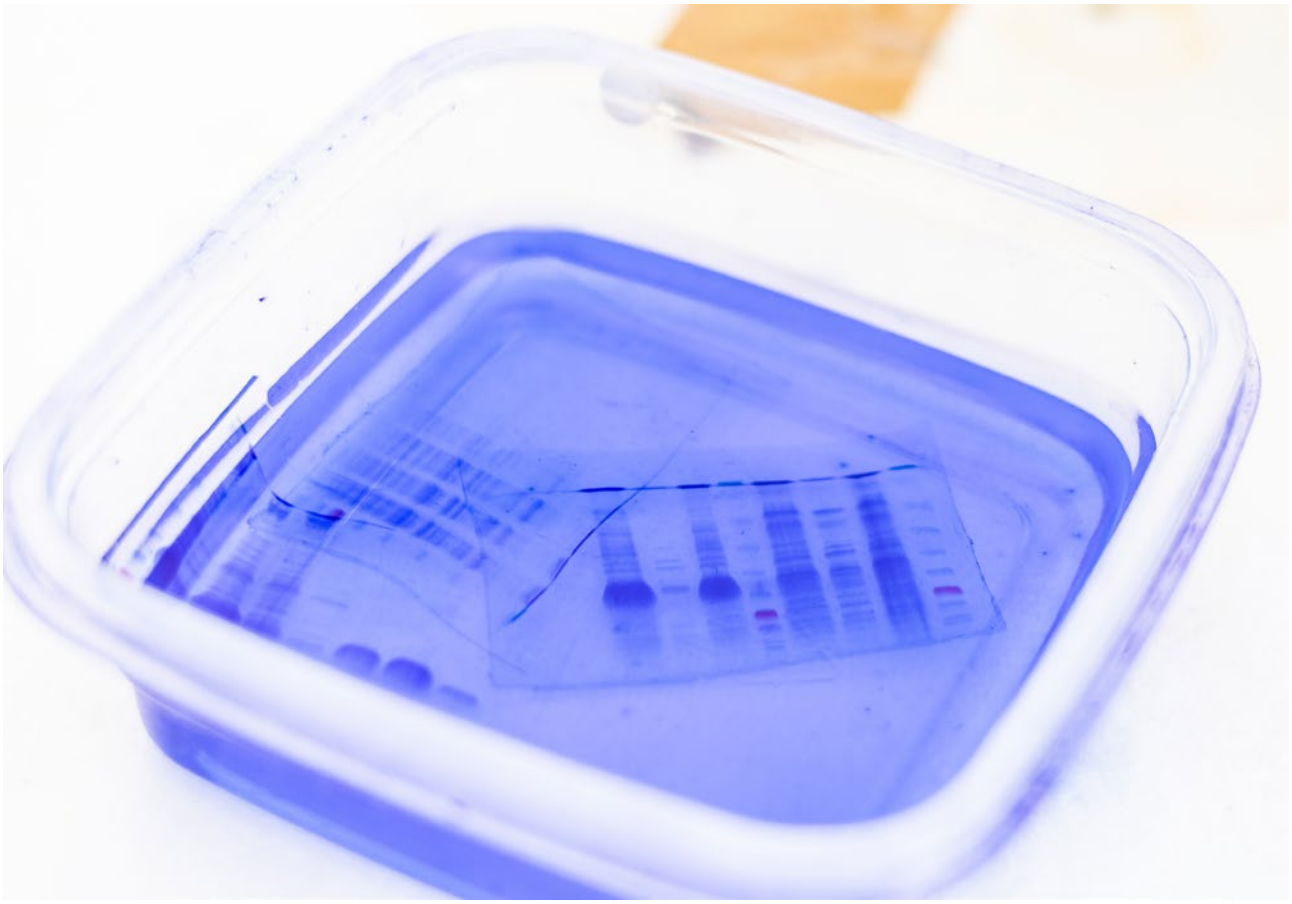
The expression and evaluation of Cryptophlebia peltastica Nucleopolyhedrovirus gp37 as a formulation additive for enhanced infectivity with Cryptophlebia leucotreta granulovirus and improved *Thaumatotibia leucotreta* control

RESEARCH TEAM
Prof. Martin Hill, Prof. Sean Moore, Dr Michael Jukes, Prof. Caroline Knox

STUDENT
Naho Muleya (MSc)

False Codling Moth, *Thaumatotibia leucotreta*, is a serious agricultural pest causing damage to fruit crops in South Africa. Furthermore, *T. leucotreta* poses a significant risk to South African citrus export markets due to strict phytosanitary requirements. Two baculoviruses, Cryptophlebia leucotreta granulovirus (CrleGV) and Cryptophlebia peltastica nucleopolyhedrovirus (CrpeNPV) infect *T. leucotreta*, causing larval mortality. Both viruses are utilised in integrated pest management (IPM) programmes to reduce fruit damage in agricultural fields. However, these control options have limitations, including sensitivity of the virus to UV radiation and slow speed of kill. An interesting observation reported in the literature is that specific baculovirus proteins, such as enhancin and gp37, can enhance the infection of another baculovirus. To initiate primary infection, baculovirus proteins such as gp37 facilitate the penetration of virions through the peritrophic membrane. The genome of CrpeNPV encodes gp37, while that of CrleGV-SA does not. A previous study reported that bacterial-expressed gp37 encoded by Cydia pomonella granulovirus (CpGV) resulted in a 13.98- and 20.20- fold improvement in LC_{50} when combined with Spodoptera exigua NPV or Autographa californica NPV respectively, indicating that gp37 significantly enhanced the infectivity of NPVs and the lethality of *Bacillus thuringiensis* in *S. exigua* larvae. The aim of this study was to express CrpeNPV gp37 in a bacterial system and then evaluate its effect on larval mortality when combined with CrleGV-SA in laboratory bioassays. Oligonucleotides targeting the gp37 gene from CrpeNPV genomic DNA were designed, enabling the subsequent amplification and cloning of the gene into the pCA528 vector (kindly provided by Matthias Mayer) for expression in *E. coli* Rosetta cells. A time course induction study was performed in which transformed cells were induced at 25 °C and 18 °C for 0-, 3-, 5- and 24-hours post-induction (hpi). The expressed protein was named 6×His-SUMO-gp37. SDS-PAGE and Western blotting of samples collected at various time points showed that 6×His-SUMO-gp37 was visible at 3 hpi, with maximal expression at 24 hpi. Solubility analysis showed that the native 6×His-SUMO-gp37 was predominantly in the insoluble fraction at both temperatures. The 6×His-SUMO-gp37 was successfully purified under denaturing conditions. However, the native 6×His-SUMO-gp37 was found to be insoluble, resulting in the pellet of insoluble protein being used for biological assay analysis. Surface dose-response biological assays were performed to evaluate the mortality of LC_{50} and LC90 CrleGV-SA alone and in combinations with either 6×His-SUMO-gp37 or the induced empty vector as a control against *T. leucotreta* neonate larvae. The results were statistically analysed using a one-way ANOVA in R Studio. The insoluble 6×His-SUMO-gp37, when combined with CrleGV-SA, showed no effect on *T. leucotreta* larval mortality. Continued

development and optimisation of the expression system is required to improve protein solubility, which would enable downstream experiments such as protein purification, quantification, and evaluation of its effect on the biological activity of baculovirus-based biopesticides.



SDS-PAGE gels run by Naho showing the expression of the baculovirus protein gp37 in *E. coli* cells. **Photo:** David Taylor

Evaluating baculovirus mixtures against false codling moth, *Thaumatotibia leucotreta* Meyrick

RESEARCH TEAM
Prof. Martin Hill, Prof. Sean Moore, Dr Michael Jukes, Prof. Caroline Knox

STUDENT
Siviwe Tole (MSc)

The false codling moth (FCM), *Thaumatotibia leucotreta*, is an important pest of citrus, stone fruit, avocados, peppers, and other important agricultural crops in southern Africa. Baculovirus based biopesticides are components in an integrated pest management (IPM) programme to manage the pest in the field. Despite these viruses being relatively host-specific and safe to humans and animals in comparison to chemical insecticides, their application is hindered by their slow speed of kill, sensitivity to UV light, and the potential for insect resistance. The interaction of two viruses in mixed baculovirus interactions against the same larval host offers the potential in improving the application of baculoviruses as biopesticides in the field. Previous studies using a combination of two baculoviruses infecting *T. leucotreta* demonstrated a reduction in lethal concentration in laboratory bioassays, indicating that such mixtures may have the potential for application in the field. The aim of this study is to evaluate baculovirus mixtures of CrleGV-SA, CrpeNPV, CpGV-M and HearNPV, to determine whether synergistic or antagonistic interactions occur against T. leucotreta neonate larvae, measured by lethal concentrations. Preceding this, genome analysis was conducted. A set of oligonucleotides targeting unique regions in each viral genome were designed to distinguish

between the viruses present in the samples. Occlusion bodies were extracted from larval cadavers infected with CrleGV, CrpeNPV, CpGV and HearNPV and the genomic DNA was extracted from purified occlusion bodies from each sample. The genomic specific oligonucleotides were used together with universal oligonucleotides targeting regions of the *polh* in PCR assays. The results of the universal oligonucleotides *(polh)* confirmed the presence of baculovirus in the viral samples. The genomic specific oligonucleotides confirmed the target viruses in the samples with the expected amplicon sizes generated. The sequencing results also confirmed the presence of the target viruses in the viral samples. Surface-dose response biological assays were performed to evaluate the lethal concentrations of each virus alone and in combinations against *T. leucotreta* neonate larvae. Statistical analysis is underway to compare lethal concentrations in the single and mixed infections. In addition, DNA extractions and PCRs were performed on *T. leucotreta* larval cadavers that were collected from biological assays in both single and mixed infections. Analysis of the data is underway where the PCR amplicons will be sent for sequencing to determine the cause of larval mortality.

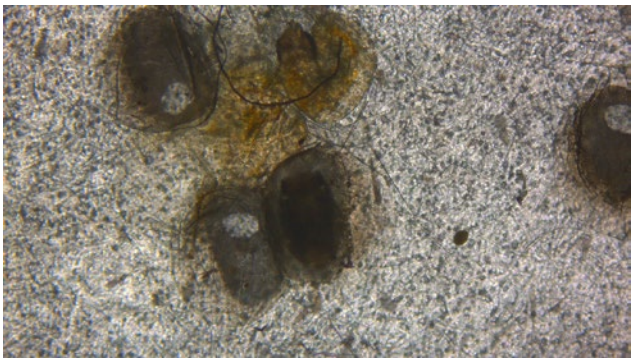
Siviwe Tole preparing virus samples for surface dose response assays against the false codling moth. **Photo:** David Taylor

Trichogrammatoidea cryptophlebiae (Nagaraja) efficacy against a range of economically important tortricid pests in South Africa

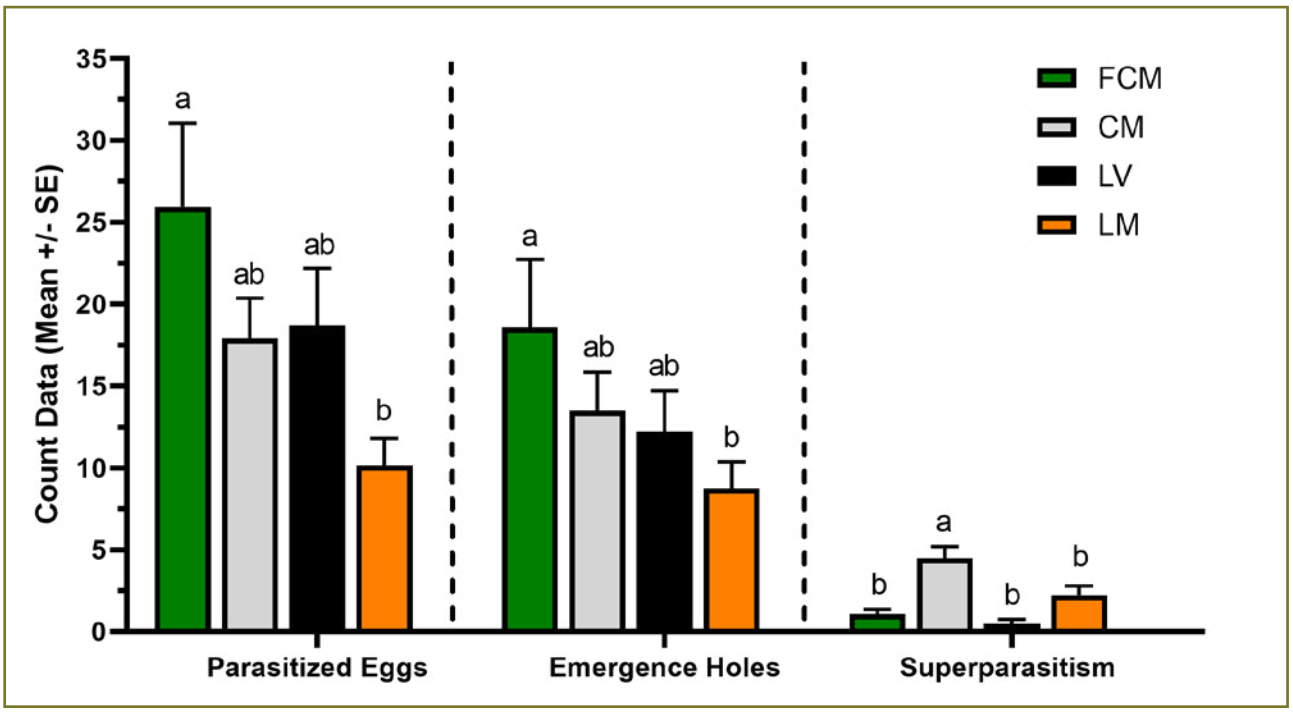
RESEARCH TEAM
Prof. Martin Hill, Prof. Sean Moore, Dr Megan Mulcahy

STUDENTS
Emma Stirk (MSc)

In agriculture today there are chemical regulations imposed on farmers to make use of fewer insecticides for the control of pests. Due to these regulations, alternative methods for pest control need to be developed and then assessed to determine their efficacy in the control of their target pest. One of the most widely studied alternative control options are parasitoid wasps from the family Trichogrammatidae. Trichogrammatidae species have been mass-reared globally and have successfully controlled an array of agricultural pests. The mass production of parasitoids ensures high-quality insects are released into the field through the implementation of quality assurance measures. This research will determine important rearing parameters that affect the successful mass-production of the parasitoid *Trichogrammatoidea cryptophlebiae*. *Trichogrammatoidea cryptophlebiae* targets the egg stage of tortricid life cycles, thereby preventing larval damage to fruit and reducing the pest population. Additionally, this research will determine the parasitoid's efficacy in controlling five tortricid moth species, *Thaumatotibia leucotreta*, *Thaumatotibia batrachopa*, *Lobesia vanillana*, *Cryptophlebiae peltastica*, and *Cydia pomonella*, in five different tree crops, citrus, macadamias, table grapes, litchis, and apples respectively. If *T. cryptophlebiae* proves to effectively control these four pest species, it could result in the product being registered for commercial sale and lead to a reduction in chemical usage.



Lobesia vanillana eggs parasitized by *Trichogrammatoidea cryptophlebiae*. **Photo:** Emma Stirk



The count data of four different host species, *Thaumatotibia leucotreta* (FCM), *Cydia pomonella* (CM), *Cryptophlebiae peltastica* (LM) and *Lobesia vanillana* (LV), eggs that were parasitized and superparasitized by *Trichogrammatoidea cryptophlebiae* and the total parasitoids that emerged.

Progress has been made in this research through conducting a series of no-choice trials that have shown that *T. cryptophlebiae* has successfully parasitized eggs of four of the five tortricid species being researched in this project. *Thaumatotibia leucotreta*, *C. pomonella*, *C. peltastica* and *L. vanillana* were successfully parasitized. Unfortunately, due to the difficulties in rearing *T. batrachopa*, this species was unable to be tested for parasitism in the laboratory. It has proven to be a very interesting trial, with this being the first recording of *T. cryptophlebiae* parasitizing *L. vanillana* eggs (K5). The no-choice trials have provided insight into the effects of different host species on parasitism ability of *T. cryptophlebiae*. The data collected indicated that *T. leucotreta* is the most suitable host for mass rearing of *T. cryptophlebiae*, as it had the highest number of eggs parasitized, the most offspring to successfully emerge and a low occurrence of superparasitism (K6). Through visual observations it was seen that one *C. pomonella* egg was able to support the full development of up to three *T. cryptophlebiae*, whereas the other three tortricid hosts were only able to support the full development and emergence of two parasitoids per egg. It was very interesting to see that in one case *T. cryptophlebiae* oviposited five eggs into one *C. peltastica* egg.

Investigating release ratios in an FCM SIT programme

RESEARCH TEAM
Dr Candice Coombes, Prof. Sean Moore, Prof. Martin Hill

STUDENT
Michael Mathenge (PhD)

The Sterile Insect Technique (SIT) for false codling moth (FCM), an important quarantine pest of citrus in South Africa, was commercially implemented in 2007. This involves mass-rearing, sterilisation and mating of released sterile FCM males with native females, resulting in production of non-viable eggs, hence no progeny is produced aiding in pest suppression. The technology is now practised in several different regions of the country, where it is highly recommended as a foundation of area-wide integrated pest management (AW-IPM). The success of SIT relies on achieving an overflooding ratio of sterile to wild males in the targeted release sites. The current benchmark 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 the rate of population growth of FCM in field cage studies. Insect-proof cages were erected around several individual trees in a citrus orchard located in the Sunday's River Valley, Eastern Cape in May 2023. Various ratios of sterile and wild moths (male and females) were subsequently released. Unfortunately, due to the winter season, the released insects were adversely affected by cold conditions resulting in the rescheduling of the trial until next year February, during the summer. In the interim, a similar trial is being evaluated under laboratory conditions. Fruit infestation has been recorded in certain crosses of sterile and wild FCM. 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. This identified the need to examine the interaction between the egg parasitoid, *Trichogrammatoidea cryptophlebiae* and SIT. A laboratory study assessed the acceptability and suitability of FCM eggs from higher release ratios and the egg parasitoids. The egg parasitoids were found to parasitise FCM eggs from higher release ratios of sterile to fertile FCM. This suggests that these infertile eggs can provide additional host material for egg parasitoids, aiding in parasitoid population build-up, which may translate into greater suppression of FCM in citrus orchards.

Michael Mathenge releasing false codling moth in the field cages in Sundays River Valley. **Photo:** David Taylor

Bolstering biopesticide effectiveness: Baculovirus advancements through field trials, bioprospecting, and qPCR quantification

RESEARCH TEAM
Dr Marcel van der Merwe, Dr Michael Jukes, Prof. Caroline Knox, Prof. Martin Hill, Prof. Sean Moore

This research project aims to further develop baculoviruses into effective biopesticides via several distinct objectives, including field trials utilising baculoviruses both alone and in novel combinations, bioprospecting for novel baculoviruses in cruciferous pests, and developing a quantitative polymerase chain reaction (qPCR) protocol to precisely quantify baculovirus samples.

Plutella xylostella (Diamondback moth, DBM) and *Helicoverpa armigera* (African Bollworm) pose significant threats to cruciferous vegetables, lacking effective biological control agents in the market. Diseased larvae exhibiting typical baculoviral symptoms were gathered from cabbages on a farm in the Belmont Valley, Makhanda, from which the baculovirus Plutella xylostella granulovirus (PlxyGV) was isolated. This baculovirus is currently undergoing development into a commercial product for the control of DBM on various leafy crops. Similarly, the baculovirus Helicoverpa armigera nucleopolyhedrovirus (HearNPV) has undergone formulation into the commercial product HeliMax (River Bioscience, South Africa) for use against bollworm on various crops. This project will conduct field trials to assess their efficacy against these target pests, both as individual spray treatments and in combination, to assess the potential for future development of a product with a dual-formulation of these two viruses.

Baculovirus efficacy can also be enhanced by incorporating symbiotic microbes associated with the target pest. Notably, yeasts have demonstrated a substantial ability to improve the effectiveness of baculoviruses when used in tandem. The yeast *Pichia kudriavzevii*, isolated from the gut of *Thaumatotibia leucotreta* (false codling moth) larvae, has previously been shown to influence the oviposition preference of adult females and the feeding behaviour of neonates. Combining this yeast with the baculovirus Cryptophlebia leucotreta granulovirus (CrleGV) resulted in heightened efficacy in laboratory assays. Field trials are underway to assess whether this enhanced efficacy is replicated in real-world conditions. Initial data suggests positive outcomes; however, further trials are essential to substantiate and validate this observation.

Field surveys have been conducted, resulting in the identification and collection of *Hellula undalis* (Cabbage webworm) in the field. A laboratory culture of this pest is currently being established. This would enable the induction of potential covert baculovirus infections via exposure to high stress conditions, such as increased population density, a technique that has been previously demonstrated as an effective means of investigating the presence of potential novel baculoviruses. Currently, no virus-based biopesticides are available for the control of this specific pest.

Another challenge facing the production and use of baculovirus-based biopesticides is the precise quantification of viral occlusion bodies, which is paramount in both laboratory and commercial environments. The existing methodologies for quantifying baculovirus samples rely on labour-intensive and time-consuming dark field microscopy, a process susceptible to human error. A quantitative polymerase chain reaction (qPCR) protocol for accurately quantifying granuloviruses is currently under development. This protocol aims to provide a more efficient and accurate means of quantifying baculovirus samples, addressing the limitations associated with current methodologies.

Marcel van der Merwe looking for insect damage on cabbage plants. **Photo:** David Taylor

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

RESEARCH TEAM
Prof. Martin Hill, Prof. Sean Moore, Dr Michael Jukes and Prof. Caroline Knox

STUDENT
Tahnee Bennett (PhD)

In South Africa there are several agriculturally important insect pests, such as *Phthorimaea operculella*, *Plutella xylostella* and *Thaumatotibia leucotreta*. The 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. Several studies have shown that viruses that are harboured by these insect pests can be isolated and biologically characterised, and some are now commercially available as biopesticides, e.g., Cryptogran® used against *T. leucotreta*. The status of certain pests on various crops may change because of changes in farming practices or changing climatic conditions. Examples of pests whose status is or may be on the rise on certain agricultural crops in South Africa, include *Serrodes partita* (fruit piercing moth), *Ectomyelois ceratoniae* (carob moth), *Archips occidentalis* (citrus leafroller) and *Lobesia vanillana* (grapevine moth). To safeguard crop production in South Africa, it is of great importance to continuously bioprospect for novel biological control options, particularly viruses, against major, minor, and emerging insect pests. This project aims to isolate, identify, and characterise novel viruses from various insect pests that already impact, or have the potential to impact, one or more important agricultural crops. To date, infected larval cadavers were obtained. Baculovirus occlusion bodies have been purified, enabling the successful extraction of template DNA. Extracted DNA was used for the PCR amplification of the targeted *polh/gran*, *lef-8*, and *lef-9* genes. Sanger sequencing of the target regions was conducted, followed by phylogenetic analysis. Illumina sequencing and de novo assembly was performed to reassemble the complete genome, with sequence analysis currently underway.

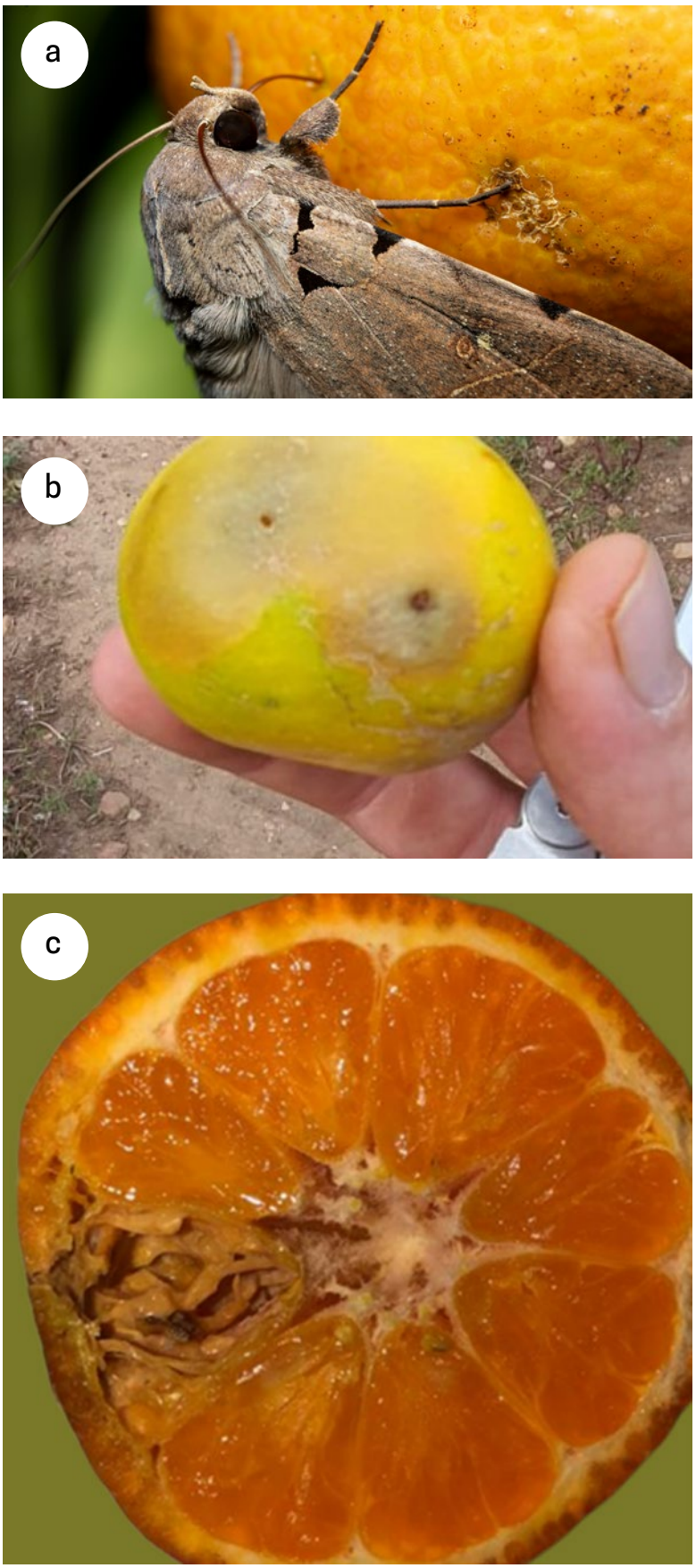
Extraction of DNA from Lepidopteran pests in search of novel baculoviruses. **Photo:** David Taylor

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

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

STUDENT
Tapiwa Mushore (PhD)

The fruit-piercing moth, *Serrodes partita* (Fabricius) (Lepidoptera: Erebidae), is a polyphagous, multivoltine pest of citrus in South Africa. The adult moth uses its sclerotised proboscis to pierce the skin of ripening or ripe fruit, from which it extracts the juices. This piercing action initiates a fermentation process within the fruit, which in turn attracts other secondary feeding moths, commonly referred to as fruit-sucking moths. Because of this feeding activity,



Serrodes partita damage on soft citrus — a) piercing action with its specialised proboscis, b) damaged fruit exhibiting rotting symptoms and distinct puncture holes, c) damaged fruit with an emptied sac. **Photo:** David Taylor and Wayne Kirkman

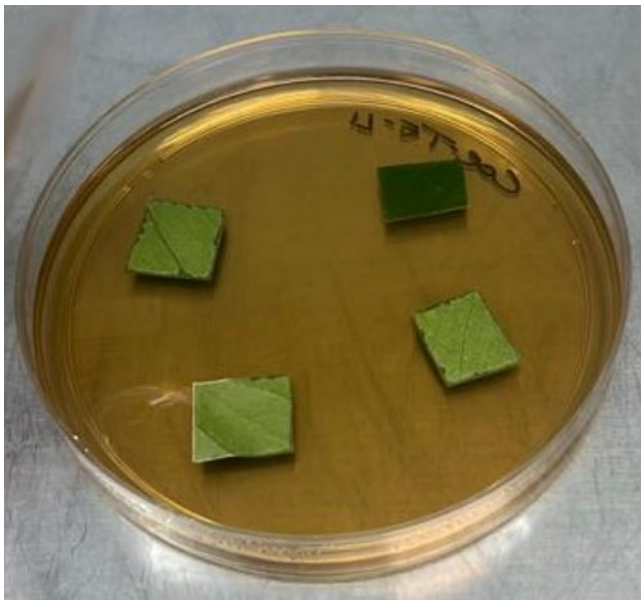
the affected fruits eventually rot, drop to the ground, and become unsuitable for the market. *Serrodes partita* exhibits an outbreak occurrence pattern, recurring every five to 10 years. In South Africa's Eastern Cape province, specifically in the Upper Kat River Valley, citrus growers have expressed growing concerns about the impact of this moth on Satsumas and Clementines. This raises the possibility of a shift in the population dynamics of *S. partita*, where these occurrences have become more frequent and less sporadic. Such a trend poses a significant threat to fruit arboriculture in the Eastern Cape region. Given the limited range of management options, combined with the moth's propensity for sudden outbreaks, citrus growers find themselves without effective means to manage this pest. The objective of this study was therefore, to investigate the biology of *S. partita* and explore various management practices. The study established that weather parameters, particularly rainfall, temperature and humidity, are significant in governing the timing and magnitude of *S. partita* outbreaks. Higher moth infestations were observed at the orchard's periphery in closest proximity to the natural vegetation and the moths were observed to display a strong preference for damaged fruit (85%) over undamaged fruit. In terms of management, this research highlighted the potential of the funnel trap, with the incorporation of an electric zapper offering a promising solution for mass trapping adults. However, striking a balance between performance and cost-effectiveness remains a critical objective. In addition an attractant bait with more longevity needs to be formulated. Currently fresh mashed banana was still found to be the most effective in luring moths to the traps. The synthetic proprietary Australian baits evaluated were found to be ineffective, along with banana essence. A novel baculovirus was also identified during failed *S. partita* rearing attempts, that may hold promise against the larvae. Continual investigation into these and more potential solutions along with a more thorough, long-term, understanding of *S. partita* population dynamics, is essential to mitigate the impact of outbreaks in citrus orchards.

Bioprospecting for entomopathogenic fungi against foliar citrus pests

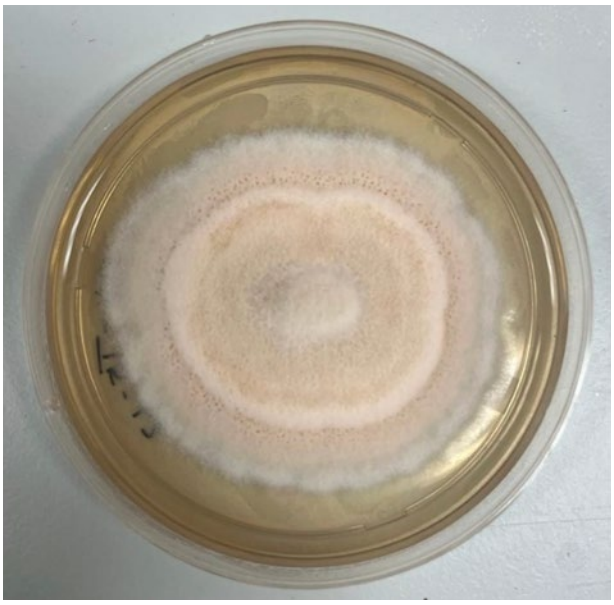
RESEARCH TEAM
Dr Candice Coombes, Prof. Martin Hill, Prof. Sean Moore

STUDENT
Erin Boon (MSc)

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 false codling moth (FCM). However, against foliar citrus pests, these same isolates that performed well under laboratory conditions, performed poorly under field conditions. This was attributed, most likely, to 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. Bioprospecting for these EPF was thus initiated. Currently, 15 strains have been isolated from infected foliar pests and once identified, their virulence toward citrus mealybug, a foliar citrus pest, will be evaluated under laboratory conditions. The top-performing isolates will then be further investigated for UV tolerance. In addition, EPF may exist as endophytes within plant parts e.g., leaves, roots and stems. Endophytes are described as fungi or bacteria that exist within the plant tissue without causing harm to the host. Thus, further monthly bioprospecting of plant material has been initiated in three conventional and three organic citrus orchards in the Sunday's River Valley, Eastern Cape. Identified EPF will also be included in the virulence bioassays, whilst other potentially useful fungi, e.g., *Muscodor* spp., for which several beneficial uses have been reported in agriculture through the release of volatile organic compounds (VOCs), such as plant pathogen antagonists, bio-fumigants and as bioinsecticides, will be stored appropriately for future research.



Inoculation of leaf samples onto agar growth medium for isolation of endophytic fungi. **Photo:** Erin Boon



Endophytic fungus isolated from the leaves of citrus trees. **Photo:** Erin Boon

Mealybug pests of citrus: rearing, parasitism, and cold treatment efficacy

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

STUDENT
Deric Vershiyi Tanka (PhD)

Seven mealybug (Hemiptera: Pseudococcidae) species have been identified to occur as pests on citrus in South Africa. These species include *Delottococcus aberiae* (De lotto), the oleander mealybug *Paracoccus burnerae* (Brain), the longtailed mealybug *Pseudococcus longispinus* (Targioni-Tozzetti), the Karoo-thorn mealybug *Nipaecoccus viridis* (Newstead), striped mealybug *Ferrissia virgata* (Cockerell), and the citrophilous mealybug *Pseudococcus calceolariae* (Maskell). All these species except citrus mealybugs Planococcus citri (Risso), are phytosanitary pests for some citrus importing countries. Consequently, there is often zero tolerance for any presence on exported fruit. This is particularly true for the South Korean market. Due to stringent regulations and a zero tolerance for pesticide residue and pests in exported fruits, managing mealybugs has become more challenging for farmers. Control usually relies on both chemical and biological methods. Despite this, postharvest infestation levels on citrus fruit can still be problematic, especially when considering the species' phytosanitary status. Therefore, there is a need for further research into postharvest management options. This study aims to investigate the efficacy of cold treatments on the immature and mature stages of these mealybug species to determine the most cold tolerant stage and species. This requires the successful establishment of a laboratory culture for each species from field collected populations. In addition, since parasitoids are well known for managing mealybug populations in the field, the presence of parasitoids from these field collected populations will also be monitored. With the exception of a few of these aforementioned species, very little is known about the parasitoids associated with the less studied species.

The influence of phenology on the efficacy of *Anagyrus vladimiri* augmentation for mealybug control

RESEARCH TEAM
Prof. Sean Moore and Prof. Martin Hill

STUDENTS
Wayne Mommsen (MSc)

In May 2019 South Korean inspectors rejected numerous grapefruit consignments from Letsitele, Hoedspruit and Onderberg because of live mealybug found on fruit. Growers expressed deep concern as mealybug management on a phytosanitary level was not easily attainable. Regular spray interventions for CBS and thrips control is known to cause repercussions of mealybug because it undermines the naturally occurring biocontrol complex. Releases of commercially produced parasitoids is common practice to augment beneficial insect populations and knowledge of the harmful effects of insecticides on parasitoids is necessary to ensure success. Timing of augmentative releases with the phenology of citrus and mealybug is considered important for the successful establishment of parasitoids to improve mealybug control. Consequently, field trials were conducted to compare efficacy of early vs. late releases of *Anagyrus vladimiri* (Triapitsyn), an effective parasitoid of the citrus mealybug, *Planococcus citri* (Risso). Semi-field bioassays were conducted concurrently to determine the impact of various thripicides on *A. vladimiri* and results assisted with IPM planning. October and November releases of *Anagyrus vladimiri* ensured early parasitism and reduced mealybug infestation. January releases were not too late to augment biocontrol. However, at harvest, the difference in efficacy between treatments was unclear. This could be explained by high levels of natural parasitism observed in the treated and untreated orchards, which highlights the importance of conservation biocontrol. The discovery of hyperparasitoids, and a new discovery of *Pseudaphycus* sp. in citrus orchards, could explain peculiar trends observed in mealybug infestation and the uncontrollable levels of mealybug experienced during the second season of trials. The accumulated proportion of hyper-

parasitoids found in samples of mealybug infested fruit was comparatively larger than the primary parasitoids, *Anagyrus vladimiri*, *Coccidoxenoides perminutus* (Girault) and *Leptomastix dactylopii* (Howard). It is therefore imperative to investigate the impact of other factors, including hyperparasitism, on the success of augmentative releases.

Development of parasitism-related intervention thresholds for the management of California red scale (*Aonidiella aurantii*) on citrus

RESEARCH TEAM
Prof. Martin Hill and Prof. Sean Moore

STUDENT
Vhuawelo Simba (PhD)

The South African citrus industry faces a challenge for market access, particularly to Europe, due to inadequate Integrated Pest Management (IPM) compliance. Key pests affecting citrus production include thrips, mealybug, and red scale. Although IPM implementation has been researched and recommended for over 50 years, many growers have not adopted it due to its complexity and lack of enforcement. The situation is now urgent as effective control measures for red scale are diminishing or being banned. It is imperative that the citrus industry swiftly adopts necessary IPM practices to ensure continued market access and sustainable production. The current project aims to monitor and analyse red scale infestation in various citrus orchards to understand relationships between factors such as parasitism, parasitoid host-feeding, weather, pest management, and soil vegetation on the infestation levels at harvest. Throughout the study, we will evaluate red scale parasitism and live or dead red scale infestations. Orchards of different citrus cultivars have been selected from the Eastern Cape citrus production region and will be monitored using standard methods, including fruit examination and yellow sticky traps. All relevant environmental conditions will be recorded during the study period. Data collected over three years will be statistically analysed to identify any meaningful relationships between measured factors and red scale infestation on fruit at harvest.

Augmentation of *Aphytis melinus* DeBach (Hymenoptera: Aphelinidae) for the control of California red scale Maskell (Hemiptera: Diaspididae) in citrus

RESEARCH TEAM
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STUDENT
Ernst de Beer (PhD)

Aphytis lingnanensis was reared and tested in South Africa in the early 2000s for augmentation against red scale on citrus and was found to be ineffective. *Aphytis melinus* is now available and it is important that the efficacy of augmentation thereof on red scale is determined locally. Field trials, quality assessments and molecular identification on *A. melinus* from two insectaries were done. For field trials, wasps were released during three seasons in three orchards in each of the Eastern and Western Cape, while three comparable orchards were studied as untreated control orchards. Wasps were released over a five- to 10-week period. Red scale infestation was monitored and a sample of 20 infested fruit from each orchard was collected randomly every 4 weeks. Aphytis species responsible for parasitism were identified and percentage parasitism recorded. Results of this study of field trials suggest that the augmentation of *A. melinus* did not significantly increase the level of parasitism above that of the untreated control. Five repetitions with six replicates of longevity and flight tests were performed. Wasps from two insectaries were kept at 23°C and 65% RH with honey.

Flight tests were performed in tubes of 16 by 30 cm, with a light above a clear sticky ceiling. On average in five replicates, 65%, 33% and 17% *A. melinus* wasps were alive on days one, five and 10 respectively. The overall sex ratio was 1.58 for females to males but 1.05, 2.19 and 2.66 for non-flyers, non-crawlers, crawlers, and flyers respectively. In flight tests, only 42% of wasps could initiate flight in 24 hours while 52% remained on the tube floor and 6% attempted to crawl upwards. COI genes were sequenced and compared against Genbank sequences using BLAST. Molecular identifications did not confirm morphological identifications for all species, indicating unexpected genetic complexity.

Citrus fruit infested with red scale. *Photo:* Ernst de Beer

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. 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). ACP will reach the southern African regions in the future. The successful introduction of a 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 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 (max and min parameters, flight activity), wind, barometric pressure, humidity, altitude, max flight distances, visual cues, and solar radiation.

RESEARCH TEAM
Dr Clarke van Steenderen, Dr Guy Sutton, Mr Wayne Kirkman, Dr Tim Grout, Dr Evans Mauda, Prof. Aruna Manrakhan, Prof. Paul Fourie, Prof. Sean Moore, Prof. Hano Maree, Prof. Martin Hill, Dr Katelyn Faulkner

This study generated 102 MaxEnt climatic models using different combinations of environmental variables to predict the suitability of ACP in Africa under current and future climate conditions. These models were validated using occurrence data in the invaded regions of the USA and Brazil, which showed a high overall prediction accuracy. We therefore had high confidence in the predictions for Africa. The findings to date suggest a potential pathway for ACP into South Africa through Mozambique, where the eastern regions of the country are the most climatically suitable for the psyllid. The Limpopo, Mpumalanga, KwaZulu-Natal, and Eastern Cape provinces are at the highest risk, with the Durban and Maputo harbours being high-risk entry points. Future climate scenarios for 2050 and 2070 revealed a moderate increase in ACP suitability in the interior regions of South Africa. Additionally, the central regions of Africa may become more suitable and facilitate dispersal from west to east, linking up to the potential southward route through Mozambique

An assessment of the status of psylloid species (Hemiptera: Psylloidea), as possible vectors of *Candidatus* Liberibacter species, causative of citrus greening in South Africa

RESEARCH TEAM
Prof. Martin Hill, Dr Evans Mauda, Mr Ludzula Mukwevho

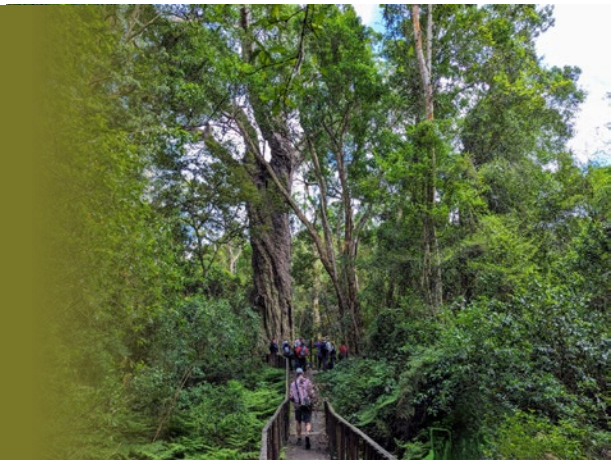
STUDENT
Raynold Moagi (MSc)

Psylloids (Hemiptera: Psylloidea), commonly referred to as "jumping plant-lice", constitute a significant group of small phytophagous, phloem-feeding insects known for their high host-plant specificity. Certain species within this group are economically significant pests, particularly in agriculture, with the potential to act as vectors for plant-pathogenic bacteria, causing devastating diseases in various crops. In the citrus industry, two well-known psyllids, the African citrus triozid (ACT), *Trioza erytreae* and the Asian citrus psyllid (ACP), *Diaphorina citri*, are recognized vectors of the Gram-negative bacteria *Candidatus* Liberibacter spp., associated with citrus greening disease. The ACP has a global economic concern with its potential to vector "*Candidatus* Liberibacter asiaticus" (CLas), a bacterium capable of rapidly killing citrus trees.

Collection of psylloid species in commercial citrus environments by Raynold Moagi. **Photo:** Tshepang Makitla

Both ACP and CLas are currently not present in southern Africa. Recent discoveries have identified indigenous psylloid species colonising commercial citrus environments in South Africa. However, the ecological role of these indigenous psylloid species within citrus environments remains unknown. The aim of this study was to: (i) determine the diversity and community structure of indigenous psylloid species in citrus environments; and (ii) determine the presence of citrus DNA in the guts of indigenous psylloid species residing in citrus environments. Field surveys were conducted across 12 distinct commercial citrus environments in Limpopo and Mpumalanga provinces, utilising double-sided yellow sticky traps and sweep-nets to capture psylloid species. All collected specimens were taxonomically identified using both published and unpublished keys, and thereafter preserved in 70% ethanol for subsequent DNA analysis. A total of 47 species were recorded, with 45 classified under 19 genera and five families. The most diverse genera were *Diaphorina* (13 species), *Acizzia* and *Trioza* (7 species each), followed by *Euphyllura* and *Psylla* (2 species each). The results further indicated significant variations in the community structure of psylloids based on the plant species sampled and collection methods used in citrus environments. Furthermore, laboratory studies are currently underway to determine the citrus-feeding potential of these indigenous psylloid species collected in citrus environments. Genomic DNA (gDNA) extraction from both collected psylloid specimens and citrus leaf samples utilising two distinct extraction methods, is underway. Molecular analysis, involving polymerase chain reaction (PCR) and Sanger sequencing of the extracted gDNA, will follow to ascertain the presence of citrus DNA in the guts of these indigenous psylloid species. The outcomes of this research will contribute valuable insights to the effective management of the citrus industry, establishing a foundation for proactive measures to mitigate potential threats that might arise from these indigenous psylloid species colonising commercial citrus environments.

POLYPHAGOUS SHOT HOLE BORER



A 600+ year old yellowwood tree (Afrocsrpus falcatus) in Woodville Forest, Wilderness, Western Cape being admired by a group of researchers from the bark beetle mycobiome (BBM) workshop. October 2023. **Photo:** Garyn Townsend

The polyphagous shot hole borer or PSHB (*Euwallacea fornicatus*) is an ambrosia beetle native to Southeast Asia. It has become a highly invasive tree pest in various countries and was discovered in South Africa in 2017. It is now established in eight of the nine provinces of the country, making it the largest outbreak of this beetle worldwide. Female PSHB beetles locate host trees, bore into them, and release a symbiotic fungus, *Fusarium euwallaceaea*. This pathogenic fungus grows in the vascular tissue of the tree, inhibiting water and nutrient uptake and causing a disease known as Fusarium dieback which in many cases kills the host tree. The impacts of PSHB are still primarily seen in urban forests where it preferentially attacks non-indigenous, ornamental trees. To date, the beetle has been recorded attacking 162 tree species in South Africa. Monitoring in indigenous forests, which has been ongoing for five years, has shown a consistent increase in the number of infested trees with the beetle being recorded attacking 78 native species, 42 of which are potential reproductive hosts. Various projects investigating the impacts of the beetle and its fungus in botanical gardens, commercial plantations and on agricultural crops such as avocado, macadamia, pecans and deciduous fruits in South Africa are also ongoing.

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

STUDENT
Garyn Townsend (PhD, UP)

COLLABORATORS
FABI, Vietnamese Academy of Forest Sciences (VAFS)

Garyn Townsend and Wilma Nel of the university of Pretoria looking at a PSHB infested tree in an Acacia plantation in the Phu Tho province in Vietnam. **Photo:** Garyn Townsend

Garyn Townsend's PhD, based at the Forestry and Agricultural Biotechnology Institute (FABI), focuses on monitoring the impacts of PSHB in native forests and investigating the potential for biological control of the beetle and/or its fungus. A scouting trip to Vietnam, where the beetle is native to, took place in April 2023 in collaboration with the Vietnamese Academy of Forest Sciences. Over 100kgs of PSHB infested material was brought back to South Africa and all emerging insects were collected to identify and characterize any potential natural enemies of PSHB. Various wasp species were collected with some in the family Mymaridae being possible parasitoids of PSHB. Rearing trials of these wasps on PSHB colonies failed and

further scouting trips to Vietnam and Japan are planned for 2024. Collections of bark and ambrosia beetle natural enemies in South Africa are also continuing. The 2023 Bark Beetle Mycobiome Workshop took place in the Garden Route, where students and researchers from four countries met to discuss bark and ambrosia beetle invasions, fungal symbioses and how to strengthen international collaborations and research. This workshop also focused on collecting and describing the diversity of indigenous bark and ambrosia beetles in South African forests, a very important and understudied topic that will contribute to our knowledge of these cryptic insects.

All work on PSHB 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 this pest which poses a significant threat to agricultural crops, urban and native forests throughout the world. In 2023, PSHB was detected in Australia and Argentina where it is attacking a variety of tree species. This, once again, shows how easily these pests can travel between nations and emphasises the importance of collaborative research.

Bark and ambrosia beetle collections taking place at the Bark beetle Mycobiome workshop in the Garden Route **Photo:** Unknown

COMMUNITY ENGAGEMENT

The CBC includes community engagement as one of their main mandates and this involves engaging with communities around South Africa about the topics of invasive alien species and biological control. 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 2023.

Waterweed engagement

The CBC continued to show support and guidance with various stakeholders around different invaded systems. Kelby English drove many different training sessions and workshops with community partners. There has been an increase in partners around Hartbeespoort Dam, they all want to contribute to the biological control efforts on the dam as discussed above in the aquatic weed section. Below are the dates of the training workshops that Kelby facilitated and held with our partners that rear agents for invasive aquatic weeds:

- 13 April at The Coves with the new and old partners at Hartbeespoort Dam.
- 26-27 July at Inanda Dam with uMngeni-uThukela Water.
- 5 September at The Coves with partners from North West, Gauteng and KwaZulu Natal.
- 9 October at the Westlake Conservation Centre with the City of Cape Town mass rearing team.
- 21 November at Jake's Gerwel Technical School with community members from Bonnievale.

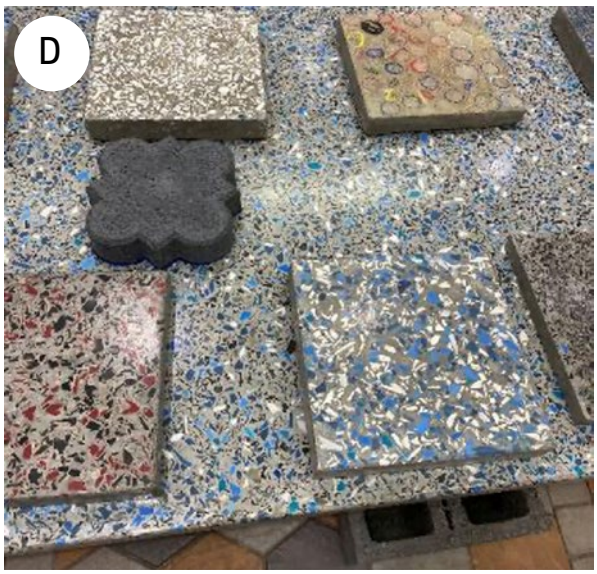
The CBC will continue to engage with communities who assist in implementing biological control as part of the management plans. We would like to thank all our partners around the country for fighting this fight with us!

Dries Botha and Kelby English at The Coves during a training workshop. **Photo:** Daniella Egli

Prosopis engagement

Prosopis poses a serious threat to livelihoods of livestock farmers as it destroys access to grazing and competes for scarce but vital groundwater resources. The CBC along with other stakeholders are working with farming communities to implement biocontrol of *Prosopis*, create meaningful employment for people living with disabilities and developing business opportunities to deal with biomass. This work is ongoing and the centre thrives on being involved in community engagement as we work towards the sustainable development goals.

Dr Graham Harding of Invader Plant Specialists,



A: *Prosopis* invasion illustrating absence of grass and shrubs for grazing.
B: Felled *Prosopis* biomass to be chopped into chunks.
C: *Prosopis* biomass chipped ready for construction.
D: Different aggregates in "cement".
E: *Prosopis* biomass store and toilet at facility in Cape Town.
F: Completed *Prosopis* biomass insulated concrete construction at the Upington biological control agent mass rearing facility.
Photos: Philip Ivey

Potted *Prosopis* in mass rearing facility ready for biological control agents. *Photo:* Philip Ivey

Right: Cactus biological control storage boxes under construction by Rebecca Saulse and Vuyani Ntlanganiso. *Photo:* Philip Ivey

on contract with the Centre for Biological Control, has worked with farmers to develop farm scale management plans for *Prosopis*. Since the November 2022 roadshow, eight farmers in the Brandvlei area have worked with Graham to develop plans. Werner Olivier, a local farmer and champion of *Prosopis* management gave strong support to the development of management plans. Farmers are currently reviewing the plans.

In order to prove that we can use *Prosopis* biomass insulated concrete in construction, the team worked with chemical engineers at Natural Engineering Solutions. Farmers from the Williston district of the Northern Cape delivered two truckloads of *Prosopis* trunks and stems to the construction facility in Cape Town. The engineers had previously tested small samples of *Prosopis* biomass but had to test their formula at scale. Natural Engineering Solutions constructed two buildings (Figure F), a store with toilet facility and office, using *Prosopis* biomass insulated concrete. Both of these buildings were subject to the longest vibration test as we transported them over 843 km to Upington. We have proven that *Prosopis* biomass insulated concrete in construction is possible. We now need to encourage construction companies to adopt these technologies alongside their conventional construction.

In order to prepare for the rearing of cactus biological control agents, team members at the Association of and for Persons living with Disabilities, Oranje-Benade, manufactured boxes for storage and attended a training event to introduce them to biological control. Hennie le Roux and Elmarie Hoft facilitated the repair of the nursery tunnel after storm damage and it now houses *Prosopis* plants ready for the mass rearing of the leaf tying moth.

Training day, "An introduction to biological control" including: Hennie le Roux, Montell Solomon, Anika Coetzee, Steven Sifumba, Johnie Jafta, Rebecca Saulse, Geraldine du Raan, Erin-Pearl Tyers, Corné le Roux, Christopher Strauss, Vuyani Ntlanganiso, Katrina Maklaro, Thabelo Khoele and Philip Ivey. **Photo:** Philip Ivey

School engagement

The longest standing school engagement is the Science Internship Programme, this programme exposes learners to different departments within the Science Faculty. School learners are selected for the programme through GADRA, an education NGO based in Makhanda. In 2023, we welcomed fifteen Grade 10 and 11 learners to Rhodes University. The CBC coordinates this programme and the learners are exposed to our research and facilities along with other departments who have joined.

Partners in the CBC's community engagement activities

Agri SA, U3A Grahamstown, Red Meat Research and Development South Africa, Grahamstown Horticultural Society, 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, Red Meat Producers Roadshow, The Coves, Lakland, Leloko, Harties Harbour, Harties HBK, Kosmos, Ifafi Senior Club, Estate DÁfrique, Pecanwood, uMngeni-uThukela Water, Ezemvelo KZN Wildlife, City of Cape Town.

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

Funders

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National Funders

Department of Forestry and Fisheries and the Environment (DFFE)

Citrus Research International (CRI)

Research for Citrus Export (RCE) Sector Innovation Fund of the Department of Science and Technology (DST)

National Research Foundation (NRF).

Department of Science and Technology – National Research Foundation – The South African Research Chairs Initiative (DST-NRF SARChI)

Red Meat Research and Development South Africa

River BioScience

Drakenstein Trust

South African Water Research Commission (WRC)

International Funders

AgriFutures Australia

Australian Government Department of Agriculture and Water Resources (Rural R&D for Profit programme).

Biosecurity South Australia (Prima Industries and Regions, South Australia)

Shire of Ravensthorpe, Western Australia

Queensland Department of Agriculture, Australia

New South Wales Department of Primary Industry; Australia

Manaaki Whenua –LandCare Research, New Zealand

Research Outputs

Graduates

PhD

1. MAPAURA, Anthony, in Botany. Degree by thesis. Thesis: Determining the trajectory of graminoid invasions in southern Africa's mountains: the case of *Nassella*. University of the Free State, Qwaqwa. Supervisors: Prof. Ralph Clarke (UFS), Prof. Sandy Lyn-Steenhuisen (UFS), Kim Canavan and Prof. David Richardson (US).
2. REID, Megan Kim, in Entomology, in the Department of Zoology and Entomology. Degree by thesis. Thesis: A native weevil and an exotic planthopper: investigating potential biological control agents for Nymphaea mexicana zuccarini (Nymphaeaceae) and its hybrids in South Africa. Supervisor: Professor JA Coetzee. Co-supervisor: Distinguished Professor MP Hill.
3. VAN STEENDEREN, Clarke Julian Mignon, in Entomology, in the Department of Zoology and Entomology. Degree by thesis. Thesis: A molecular investigation of stem-galling *Tetramesa* Walker (Hymenoptera: Eurytomidae) on African grasses: applications to biological control. Supervisor: Dr G Sutton. Co-supervisor: Professor ID Paterson.

Clarke van Steenderen with his supervisors

MSc

1. DAMBUZA, Khalipha, in Entomology, in the Department of Zoology and Entomology. Degree by thesis. Thesis: Evaluation of potential repellents for false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae). Supervisor: Dr C Coombes. Co-supervisors: Distinguished Professor MP Hill and Professor S Moore (CRI).

Liam Yell and supervisors

Khalipa Dambuza

2. David Louis KINSLER, in Geography, in the Department of Geography. Degree by thesis. Thesis: Remote sensing as a monitoring solution for water hyacinth (*Pontederia crassipes*) in the context of the biological control programme at Hartbeespoort Dam. Supervisor: Ms GK McGregor. Co-supervisor: Professor JA Coetzee.
3. MABUZA, Mefika Michael, in Entomology, in the Department of Zoology and Entomology. Degree by thesis. Thesis: The possible effects of insecticide drift from citrus orchards, and acute toxicity of insecticides on the biocontrol agents of *Pontederia crassipes* (Mart.) Solms-Laub (Pontederiaceae) established along citrus orchards in the Lowveld region of Mpumalanga Province, South Africa. Supervisor: Distinguished Professor MP Hill. Co-supervisor: Mr L Mukwevho (UMP).
4. RICHARDSON, Perryn Heather, in Entomology, **with distinction**, in the Department of Zoology and Entomology. Degree by thesis. Thesis: Characterisation of the False Codling Moth (*Thaumatotibia leucotreta*) gut microbiome and its host-microbe physiological interactions. Supervisor: Dr CA Owen. Co-supervisor: Professor JS Terblanche (Stell).
5. YELL, Liam Dougal, in Entomology, **with distinction**, in the Department of Zoology and Entomology. Degree by thesis. Thesis: Native-range studies on insect herbivores associated with African Lovegrass (*Eragrostis curvula*) in South Africa: prospects for biological control in Australia. Supervisor: Professor ID Paterson. Co-supervisor: Dr G Sutton.

Peer-reviewed articles

Rhodes University

1. Verlaque, R., Hardion, L., Lambertini, C., Canavan, K., Verlaque, M., & Vila, B. (2023). New highlights on Old World giant Phragmites (Poaceae) using leaf and floral bract microscopic characters. *Aquatic Botany,* 184, 103591. https://doi.org/10.1016/j.aquabot.2022.103591
2. Chikowore, G., Martin, G. D., Chidawanyika, F., Hill, M., Neser, S., Day, M., Grice, T., Chikwenhere, G., Mangosho, E. & Sheppard, A. (2023). Weed biological control in Zimbabwe: Challenges and future prospects. *South African Journal of Botany,* 154, 336-345.
3. Cabrera Walsh, G., Sosa, A.J., Mc Kay, F., Maestro, M., Hill, M., Hinz, H.L., Paynter, Q., Pratt, P.D., Raghu, S., Shaw, R., Tipping, P.W. and Winston, R.L. 2023. Is Biological Control of Weeds Conservation's Blind Spot? *The Quarterly Review of Biology, The University of Chicago Press* 98:1, 1-28. doi.org/10.1086/723930
4. Buitenhuis, R., Cock, M.J.W., Colmenarez, Y.C., De Clercq, P., Edgington, S., Gadaleta, P., Gwynn, R., Heimpel, G., Hill, M., Hinz, H.L., Hoddle, M.S., Jäkel, T., Klapwijk, J.N., Leung, K., Mc Kay, F., Messelink, G.J., Silvestri, L., Smith, D., Sosa, A., Wäckers, F.L., Cabrera Walsh, G., Wyckhuys, K.A.G. & Zaviezo, T. 2023. Sustainable use and conservation of microbial and invertebrate biological control agents and microbial biostimulants. Background Study Paper No. 71. Commission on Genetic Resources for Food and Agriculture. Rome, FAO. https://doi.org/10.4060/cc3571en
5. Mason, P. G., Hill, M., Smith, D., Silvestri, L. C., Weyl, P., Brodeur, J. & Vitorino, M.D. (2023). Best practices in the use and exchange of microorganism biological control genetic resources. *BioControl* 68: 311-327. https://doi.org/10.1007/s10526-023-10197-3
6. Mason, P. G., Barratt, B. I., Mc Kay, F., Klapwijk, J. N., Silvestri, L. C., Hill, M., Hinz, H.L., Sheppard, A., Brodeur, J., Vitorino, M.D., Weyl, P. & Hoelmer, K. A. (2023). Impact of Access and Benefit Sharing implementation on biological control genetic resources. *BioControl* 68: 235-251. https://doi.org/10.1007/s10526-023-10176-8
7. Mason, P.G., Mc Kay, F., Silvestri,· L.C., Hill M., Weyl, P., Hinz · H.L., Brodeur, J.· Diniz VitorinoM. & Barratt B.I.P. 2023. International agreement for the use and exchange of classical biological control genetic resources: a practical proposal. *BioControl* 68:329–339. https://doi.org/10.1007/s10526-023-10177-7
8. Britton, J. R., Lynch, A. J., Bardal, H., Bradbeer, S. J., Coetzee, J. A., Coughlan, N. E., Dalu, T., Tricarico, E., Gallardo, B., Lintermans, M., Lucy, L., Liu, C., Olden, J.D., Raghavan, R. & Pritchard, E. G. 2023. Preventing and controlling non-native species invasions to bend the curve of global freshwater biodiversity loss. *Environmental Reviews*, (ja). https://doi.org/10.1139/er-2022-0103
9. Gervazoni, P., Minuti, G., Fuentes-Rodriguez, D., Coetzee, J., Sosa, A., Sabater, L., & Franceschini, C. 2023. Citizen Science Improves the Known and Potential Distribution of a Strong Wetland Invader: Implications for Niche Modeling and Invasion Management. *Environmental Management*, 71: 1176-1187. https://doi.org/10.1007/s10530-011-0092-x

10. Nqayi, S.B., Zachariades, C., Coetzee, J., Hill, M., Chidawanyika, F., Uyi, O.O. & McConnachie, A.J. 2023. Do thermal requirements of *Dichrorampha odorata*, a shoot-boring moth for the biological control of *Chromolaena odorata*, explain its failure to establish in South Africa? *African Entomology* 31, 1-10. http://dx.doi.org/10.17159/2254-8854/2023/a13597

11. Misteli, B., Pannard, A., Aasland, E., Harpenslager, S. F., Motitsoe, S., Thiemer, K., Llopis, S., Coetzee, J., Hitl, S., Köhler, J., Schneider, S.C., Piscart, C. & Thiébaut, G. 2023. Short-term effects of macrophyte removal on aquatic biodiversity in rivers and lakes. *Journal of Environmental Management*, 325, 116442. https://doi.org/10.1016/j.jenvman.2022.116442

12. Thiemer, K., Immerzeel, B., Schneider, S., Sebola, K., Coetzee, J., Baldo, M., Thiebaut, G., Hilt, S., Köhler, J., Harpenslager, S.F. & Vermaat, J.E. 2023. Drivers of Perceived Nuisance Growth by Aquatic Plants. *Environmental Management*, pp.1-13. https://doi.org/10.1007/s00267-022-01781-x

13. Mwanza, P., Dealtry, G., Lee, M., & Moore, S. 2023. Transmission Electron Microscopy Observation of Morphological Changes to Cryptophlebia Leucotreta Granulovirus following Ultraviolet Irradiation. *Pathogens*, 12(4), 590. https://doi.org/10.3390/pathogens12040590

14. Burckhardt, D., Moagi, R. & Mauda, E. (2023) *Anoeconeossa exsul* Burckhardt, a new eucalypt psyllid from Australia and South Africa (Hemiptera: Psylloidea). *Entomologische Zeitschrift · Schwanfeld* · 133 (1)

15. Magengelele N, Martin G. 2023. Distribution and impact of the native South African wasp, *Megastigmus transvaalensis* (Hussey, 1956) (Hymenoptera: Torymidae) on the invasive *Schinus terebinthifolia Raddi* (Anacardiaceae) in South Africa. *African Entomology* 31 https://doi.org/10.17159/2254-8854/2023/a13583

16. Reid, K.K., Hill, M.P. & Coetzee, J.A. 2023. Love at first bite? Pre-release surveys reveal a novel association between a native weevil and the invasive *Nymphaea Mexicana* Zuccarini (Nymphaeaceae) in South Africa. *African Entomology* 31 https://doi.org//10.17159/2254-8854/2022/a10298

17. Dixon, E., Paterson, I., Muskett, P. & McConnachie, A. 2023. Host-specificity testing of the leaf-feeding flea beetle, *Phenrica guerini*, a biological control agent for the invasive alien cactus, *Pereskia aculeata*. *Biocontrol Science and Technology* 19: 1-13. https://doi.org/10.1080/09583157.2023.2214342

18. Ivey, P.J., Hill, M.P., Kenfack Voukeng, S.N. & Weaver, K.N. 2023. Nagoya Protocol and Africa's willingness to share biological control agents, are we deterred by barriers instead of using opportunities to work together? *BioControl* 68:253–259. https://doi.org/10.1007/s10526-023-10184-8

19. Mushore, T., Coombes, C., & Hill, M. 2023. Entomopathogenic fungi associated with cultivated honeybush, Cyclopia spp., in South Africa and their pathogenicity towards a leafhopper pest, *Molopopterus* sp. (Hemiptera: Cicadellidae). *African Entomology,* 31. https://doi.org/10.17159/2254-8854/2023/a14183

20. Reid, M. K., Paterson, I. D., Coetzee, J. A., Gettys, L. A., & Hill, M. P. (2023). Know thy enemy: investigating genetic contributions from putative parents of invasive Nymphaea mexicana hybrids in South Africa as part of efforts to develop biological control. *Biological Control*, 184, 105291. https://doi.org/10.1016/j.biocontrol.2023.105291

21. Moore, S.; Jukes, M. The History of Baculovirology in Africa. *Viruses* 2023, 15, 1519. https://doi.org/10.3390/v15071519

22. Canavan, K. & Paterson, I.D. (2023). Prioritization of alien plant targets for biological control in South Africa's offshore sub-Antarctic islands. *Antarctic Science* 35: 235-242. https://doi.org/10.1017/S0954102023000135

23. Goolsby, J.A., Moran, P.J., Martínez Jiménez, M., Yang, C., Canavan, K., Paynter, Q., Ota, N. & Kriticos, D.J. (2023). Biology of Invasive Plants 4. *Arundo donax* L. *Invasive Plant Science Management* 16, 2: 81-109.

24. Van Steenderen, C.J., Sutton, G.F., Owen, C.A., Martin, G.D. & Coetzee, J.A. (2023). Sample size assessments for thermal physiology studies: An R package and R Shiny application. *Physiological Entomolgy* 1-9.

25. White, V. G., Raath, P. J., Hardie, A. G., van der Berg, V., & Sutton, G. F. (2023). Calculating soil titratable acidity from routine soil analyses of cultivated South African soils using various model structures. *South African Journal of Plant and Soil* 40(2): 1-9.

26. Manrakhan, A., Daneel, J. H., Serfontein, L., Mauda, E., Sutton, G. F., & Hattingh, V. (2023). Efficacy of an existing *Ceratitis capitata* (Diptera: Tephritidae) cold disinfestation treatment schedule for *Ceratitis cosyra*. *Journal of Economic Entomology* 116 (3): 779-789.

27. Sutton, G. F., van Steenderen, C. J. M., Yell, L. D., Canavan, K., McConnachie, A., & Paterson, I. D. (2023). First record of an African grass-feeding wasp (*Tetramesa*; Eurytomidae) on the invasive grass *Eragrostis curvula* (African lovegrass; Poaceae) in Australia. *BioInvasions Records*, 12: 673-680.

28. Miller, B., Coetzee, J. & Hill, M. 2023. Evaluating the establishment of a new water hyacinth biological control agent in South Africa. *African Entomology* 31(2023).

29. Mnciva, S.T., Coombes, C. & Coetzee, J.A. (2023) Morphological and molecular characterisation of naturally occurring pathogenic fungi for *Pontederia crassipes* (water hyacinth) in South Africa. *Biocontrol Science and Technology* 33:9, 869-884. https://doi.org/10.1080/09583157.2023.2245987

30. van der Merwe, M., Jukes, M.D., Knox, C., Moore, S.D., Hill, M.P. Naturally Occurring Yeasts Associated with Thaumatotibia leucotreta Can Enhance the Efficacy of the Cryptophlebia Leucotreta Granulovirus. *Pathogens* 2023, 12, 1237.

31. Sutton GF, Day MD, Canavan K, Paterson ID (2023) Anthropogenic disturbance affects specialist, but not generalist, endophagous insects associated with two African grasses: implications for biological control. *Biocontrol Science and Technology*. 33: 1051-1064

32. Faltlhauser, A. C., Jiménez, N. L., Righetti, T., Visintin, A. M., Torrens, J., Salinas, N. A., McKay, F., Hill, M.P., Cordo, H.A. & Sosa, A. J. (2023). The importance of long-term post-release studies in classical biological control: Insect–plant monitoring and public awareness of water hyacinth management (Pontederia crassipes) in Dique Los Sauces, Argentina. *Entomologia Experimentalis et Applicata* 171 (12): 965-977. https://doi.org/10.1111/eea.13355

33. Franceschini, M.C., Hill, M.P., Fuentes-Rodríguez, D., Gervazoni, P.B., Sabater, L.M. and Coetzee, J.A. 2023. Performance and field host range of the different life stages of *Cornops aquaticum*, a biological control agent of water hyacinth. *Entomologia Experimentalis et Applicata*. 171 (12): 954-964. https://doi.org/10.1111/eea.13354

34. Paper, M.K., Raubenheimer, S.L., Righetti, T., Coetzee, J.A., Sosa, A., and Hill, M.P. 2023. Effects of elevated CO_2 on feeding responses of biological control agents of Pontederia crassipes Mart. *Entomologia Experimentalis et Applicata*. 171 (12): 998-1008. https://doi.org/10.1111/eea.13289

35. Van Steenderen, CJ.M., Sutton, G.F., Yell, L.D., Canavan, K., McConnachie, A.J., Day, M.D. and Paterson, I.D. 2023. Phylogenetic analyses reveal multiple new stem boring Tetramesa taxa (Hymenoptera: Eurytomidae): implications for the biological control of invasive African grasses. *BioControl* 68: 697-708. https://doi.org/10.1007/s10526-023-10231-4

36. Cowie, B.W, Heystek, F. and Paterson, I.D. 2023. Will climate affect the establishment and efficacy of *Agnippe* sp. #1 (Lepidoptera: Gelechiidae), a promising biological control agent of Mesquite in South Africa? *Biocontrol* 68: 681-695.

37. Mulcahy, M., Conlong, D., and Hill, M. 2023. Determining the efficacy of push-pull for management of *Eldana saccharina* (Walker (Lepidoptera: Pyralidae) in sugarcane through on-farm field trials in KwaZulu-Natal.: Push-pull in sugarcane. *African Entomology,* 31.

Book chapters

1. Moran, P.J., De Clerck-Floate, R., Hill, M.P., Raghu, S., Paynter, Q. and Goolsby, J.A. 2023. Chapter 6: Mass-Production of Arthropods for Biological Control of Weeds: A Global Perspective. In: Morales-Ramos, J.A., Rojas, G.M. and Shapiro-Ilan, D.I. *Mass-production of beneficial organisms. Invertebrates and Entomopathogens*. Second Edition. Elsevier, Academic Press, London, UK.

2. Madzivanzira, T.C., Coetzee, J.A. and Dalu, T. 2023. Factors Structuring Aquatic Macrophytes. In: Kumar, S., Bauddh, K., Singh, R., Kumar, N., Kumar, R. (eds) Aquatic Macrophytes: Ecology, Functions and Services. Springer, Singapore.

3. Impson, F.A.C., Marchante, H., Marchante, E., López-Núñez, F.A., Hill, R. & Minteer, C. 2023. Biological control of Acacia species: History, progress, and prospects. In: Richardson, D., Le Roux J. Marchante E. (eds) (2023) Wattles – Australian Acacia species around the world. CABI Wallingford, pp. 327-341. ISBN 978-1-80062-217-3.

Conference proceedings

National Conference Presentations

1. Baso, N., Bownes, A., Hill, M. & Coetzee, J. (2023). The battle of aquatic plants: *Lagarosiphon major* vs *Egeria densa* with *Hydrellia* fly referees! *National Symposium on Biological Invasions*. 4-6 July 2023. Houw Hoek Hotel, Western Cape.
2. Canavan, K. Canavan, S., Clark, V.R., Gwate, O., Mapaura, A., Richardson, D.M., Steenhuisen, S.-L., Sutton, G., Martin, G.D.(2023). Invasive alien plants in South Africa's mountains. *National Symposium on Biological Invasions*. 4-6 July 2023. Houw Hoek Hotel, Western Cape.
3. Ivey, P. (2023). Banking on biological control and biomass use to beat back *Prosopis* invasion. *National Symposium on Biological Invasions*. 4-6 July 2023. Houw Hoek Hotel, Western Cape.
4. Martin, G., Steenhuisen, S., Bolongo N.(2023). 25 Years of Working for Water – Successful? *National Symposium on Biological Invasions*. 4-6 July 2023. Houw Hoek Hotel, Western Cape.
5. Paterson, I., Mnqeta, Z., Griffith, T., Sexton, W., Muskett, P. (2023). Biological control of cactus weeds in South Africa: an evaluation of success and an update on new targets. National Symposium on *Biological Invasions*. 4-6 July 2023. Houw Hoek Hotel, Western Cape.
6. Sandenbergh, E. Petruzzella, A. & Coetzee, J. (2023). Insight into the distribution, genetic diversity, and sexual reproductive capacity of *Iris pseudacorus* in South Africa: Implications for management. *National Symposium on Biological Invasions*. 4-6 July 2023. Houw Hoek Hotel, Western Cape.
7. Cowie, B., Heystek, F., Paterson, I.D., Byrne, M.J. (2023). The influence of climate on the *Prosopis* biocontrol agent, *Evippe* sp. #1 in South Africa. *National Symposium on Biological Invasions*. 4-6 July 2023. Houw Hoek Hotel, Western Cape.
8. Kolisi, S., Mlambo, M. & Motitsoe, S. (2023). Predicting the spread of a notorious thairdae invader in South Africa using ensemble models. *National Symposium on Biological Invasions*. 4-6 July 2023. Houw Hoek Hotel, Western Cape.
9. Bennett T., Hill M.P., Moore S.D., Jukes M.D., Knox C., Dealtry G. (2023). Genetic analysis and semi-field assay of a UV-tolerant strain of CrleGV for improved control of *Thaumatotibia leucotreta*. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
10. Chikodza, T., Hill, M., Coetzee, J. (2023). The effect of biological control agents on the population dynamics of *Pontederia crassipes* (C. Mart) Solms (Pontederiaceae) and *Salvinia minima* Baker (Salviniaceae). *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
11. Cousins, L., Moore, S., Peyper, M., Marsberg, T., Hill, M. (2023). A comparison of false codling moth infestation in organic versus conventional citrus orchards. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
12. Dambuza, K., Coombes, C., Hill, M., Moore, S. (2023). Evaluation of potential repellents for false codling moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae). *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
13. De Beer, E., Hill, M., Marsberg, T., Peyper, M., Moore, S., Bester, R., Maree, H. (2023). Augmentation of *Aphytis melinus* for the control of red scale in citrus. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
14. Githae, M., Coombes, C., Moore, S., Hill, M. (2023). Susceptibility of false codling moth eggs from different sterile: fertile release ratios to parasitism. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
15. Griffith, T., Paterson, I.D. (2023). Developing a biological control programme for a challenging target weed, *Trichocereus spachianus*. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
16. Kirkman, W., Gebeyehu, S., Carstens, E., Fourie, P., Manrakhan, A., Mauda, E., Cook, G., Hattingh, V. (2023). The current status of Asian citrus greening and its primary vector the Asian citrus psyllid in Africa, and preparation by the Southern African citrus industry for their imminent arrival. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.

17. Mela T., Jukes M., Knox C., Moore S., Hill M. (2023). Development and optimisation of a qPCR assay for the enumeration of Cryptophlebia leucotreta granulovirus (CrleGV) used for commercial applications. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
18. Mnciva, S., Hill, M., Coetzee, J. (2023). Effect of sublethal doses of glyphosate herbicide on water hyacinth (*Pontederia crassipes* Mart.) (Pontederiaceae) and its biocontrol agent, *Megamelus scutellaris* Berg. (Delphacidae: Hemiptera). *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
19. Moagi, R., Mauda, E., Mukwevho, L., Hill, M. (2023). Psylloid community structure and diversity in and adjacent to citrus orchards. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
20. Moore, S., Marsberg, T., Jukes, M., Opoku-Debrah, J., Mwanza, P., Taylor, D., Knox, C., Hill, M., Dealtry, G., Lee, M., Grobler, A., Booyens, W. (2023). Building a better baculovirus biopesticide: improving control of tree fruit Lepidoptera. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
21. Muleya N., Jukes M., Hill, M., Moore, S., Knox C. (2023). The expression and evaluation of CrpeNPV GP37 as a formulation additive for enhanced infectivity with CrleGV and improved *Thaumatotibia leucotreta* control. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
22. Mushore, T., Coombes, C., Hill, M., Moore, S. (2023). Bait and bait presentation in the management of the fruit-piercing moth, *Serrodes partita*, in Citrus orchards. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
23. Muskett, P., Dixon, E., McConnachie, A., Paterson, I.D. (2023). Host-specificity testing of the leaf-feeding flea beetle, *Phenrica guerini*, a biological control agent for the invasive alien cactus, *Pereskia aculeata*. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
24. Mommsen, W., Moore, S., Hill, M. (2023). Managing augmentative releases of *Anagyrus vladimiri* (Triapitsyn) for successful biocontrol of the citrus mealybug, *Plannococcus citri* (Risso). *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
25. Richardson, P., Terblanche, J., Owen, C. (2023). Impact of the gut microbiome on life history, body composition and stress resistance in False Codling Moth (*Thaumatotibia leucotreta*). *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
26. Sexton, W., Paterson, I.D., Muskett, P. (2023). *Dactyloipus tomenstosus* 'californica var parkeri' (Dactylopiidae): A successful biocontrol agent against *Cylindropuntia pallida* (Cactaceae) in Australia, but unsuccessful in South Africa. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
27. Sutton, G., van Steenderen, C., Martin, G., Coetzee, J., Owen, C. (2023). 'ThermalSampleR': An R package and Shiny GUI to perform sample size calculations for thermal physiology studies. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
28. Tole S., Jukes M., Hill, M., Moore, S., Knox C. (2023). Evaluating baculovirus mixtures against false codling moth *Thaumatotibia leucotreta* Meyrick. (Lepidoptera: Tortricidae). *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
29. Townsend, G., Hurley, B., Hill, M., Roets, F. (2023). Assessment of the spread, host range and drivers of the polyphagous shot hole borer (PSHB) in indigenous South African forests. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
30. van Son, E., Manrakhan, A., Kirkman, W., Coetzee, J. (2023). A survey of fruit fly hosts in the Eastern Cape thicket. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
31. van Steenderen, C., Mauda, E., Kirkman, W., Faulkner, K., Sutton, G. (2023). Predicting the possible establishment and spread of the Asian citrus psyllid *Diaphorina citri* Kuwayama in Southern Africa using species distribution modelling. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.

32. Yell, L., Sutton, G., van Steenderen, C., Canavan, K., McConnachie, A., Muskett, P., Paterson, I.D. (2023). Native-range studies on insect herbivores associated with African Lovegrass (*Eragrostis curvula*) in South Africa: prospects for biological control in Australia. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
33. Moagi, R., Mauda, E., Mukwevho, L. & Hill, M. 2023. Psylloid community structure and diversity in and adjacent to citrus orchards. *Rhodes University's Annual Postgraduate Conference*. 8-9 September 2023. Rhodes University, Makhanda, Eastern Cape.
34. De la Fontaine, B. 2023. Insects versus Invaders: Hunting a biocontrol agent for Guinea grass. *Rhodes University's Annual Postgraduate Conference*. 8-9 September 2023. Rhodes University, Makhanda, Eastern Cape.
35. Bennett, T.T., Hill, M.P., Moore, S.D., Jukes, M.D., & Knox, C. 2023. The isolation, identification, and characterisation of novel insect viruses for the control of emerging agricultural pests. *Rhodes University's Annual Postgraduate Conference*. 8-9 September 2023. Rhodes University, Makhanda, Eastern Cape.
36. Tole, S., Knox, C., Jukes, M., Moore, S. & Hill, M. 2023. Evaluating baculovirus mixtures against false codling moth *Thaumatotibia leucotreta* Meyrick. (Lepidoptera: Tortricidae). *Rhodes University's Annual Postgraduate Conference*. 8-9 September 2023. Rhodes University, Makhanda, Eastern Cape.
37. Ngxekisa, S., Coombes, C. A. & Jukes, M. 2023. Semi-field efficacy of the UV-tolerant Cryptophlebia leucotreta granulovirus isolate. *Rhodes University's Annual Postgraduate Conference*. 8-9 September 2023. Rhodes University, Makhanda, Eastern Cape.

National Conference Posters

1. Mbele, T., Canavan, K., Steenhuisen, S.-L. (2023). The invasive status of *Cortaderia* species in South Africa. *National Symposium on Biological Invasions*. 4-6 July 2 023. Houw Hoek Hotel, Western Cape.
2. Bessinger, R., Coetzee, J. (2023). How the decay of water hyacinth, *Pontederia crassipes*, impacts the water column. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
3. Boon, E., Coetzee, J., Hill, M. (2023). Assessing the impact of the potential biocontrol, *Aphthona nonstriata* Goeze (Coleoptera: Chrysomelidae), on the invasive *Iris pseudacorus* L. (Iridaceae) in South Africa. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
4. Luphondo, K., Jukes, M., Coombes, C., Hill, M., Moore, S. (2023). Evaluation of stability and maintenance of ultraviolet tolerant strain of the Cryptophlebia leucotreta granulovirus- SA for the management of false codling moth. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
5. Maneli, S., Paterson, I.D., Muskett, P. (2023). The potential for biocontrol of the Brazilian cactus (*Brasiliopuntia brasiliensis*). *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
6. Musedeli, J., Hurley, B., Chikowore, G., Martin, G. (2023). Suitability of *Pissodes validirostris* as a biological control agent for European pines in South Africa. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.
7. Ngxekisa, S., Coombes, C., Jukes, M., Moore, S. (2023). Semi-field efficacy of the UV-tolerant Cryptophlebia leucotreta granulovirus isolate. *23rd Congress of the Entomological Society of Southern Africa*. 11-14 July 2023. Stellenbosch University, Western Cape.

International Conference Presentations

1. Canavan, K., Downey, P.O., Hill, M.P., Ivey, P., Sutton, G.F. & Paterson, I.D. (2023). The South African Biological Control Target Selection System. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
2. Chikowore, G., Dube, K. & Martin G. (2023). Re-initiating classical weed biological control in Zimbabwe-where to start? *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
3. Coetzee J.A., Kinsler D., Miller B.E., Sebola K., Smith R. & Hill M.P. (2023). Is inundative classical biocontrol the future for the most difficult weeds?: the water hyacinth saga. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
4. Cowie, B.W., Kleinjan, C.A., Heysteck, F., Kistensamy, Y., Mc Kay, F., Bowers, K.E., Thompson, D.C., Paterson, I.D. & Ivey P. (2023). Managing Prosopis:

an update on biological control efforts made in South Africa. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.

5. Diaz, R., Wahl, C., Woodley, S. & Pham, K., Kinsler, D. (2023). Time factor: Biological control of giant salvinia, *Salvinia molesta* (Salviniales: Salviniaceae), leads to restoration of ecological functions in subtropical wetlands. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
6. Faltlhauser, A.C., Jiménez, N.L., Righetti, T., Salinas, N.A., Mc Kay, F., Cordo, H.A., Hill, M. & Sosa, A.J. (2023). The importance of long-term post-release studies: insect-plant monitoring and public awareness of water hyacinth management in Dique Los Sauces, Argentina. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
7. Gervazoni, P.B., Sosa, A.J., Coetzee, J., Bertucci, S.E. & Franceschini M.C. (2023). Public knowledge on Invasive Alien Plants and Biological Control in the Southern Hemisphere. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
8. Hill, M. (2023). **Keynote address**. Classical weed biological control and the Nagoya Protocol – it's time to put it to the test. *XVI International Symposium on Biological Control of Weeds.* 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
9. Martin, G.D., Weyl, P.S.R., Humair, L.F.I., Chikowore, G. & Wolmarns, A. (2023). Determining ecological host range from native and invaded range phylogenies. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
10. Minuti, G., Coetzee, J.A. & Stiers, I. (2023). Present and future climatic suitability of the aquatic invasive plant *Iris pseudacorus* (Iridaceae) and its candidate biocontrol agents. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
11. Mnqeta, Z. & Paterson, I.D. (2023). Perceptions of land-users on the efficacy, benefits and safety of biological control of three invasive alien Cactaceae in South Africa. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
12. Muskett P.C. & Paterson, I.D. (2023). Identifying the most effective cochineal species to control *Opuntia elata* and *Opuntia megapotamica* in South Africa. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
13. Ngxande-Koza, S.W., Coetzee J.A., & Hill M. (2023). The impact of mass-rearing biological control agents on invasive aquatic plant control. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
14. Paterson I.D., Webb, C., Cristofaro, M. & Moran, P. (2023). Prospects for the biological control of crystalline ice-plant (*Cryophytum crystallinum*). *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
15. Paterson, I. (2023). IOBC Global Cactus Working Group Workshop. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
16. Petruzzella, A., Motitsoe S.N., Tshithukhe, G., Hill, M.P. & Coetzee J.A. (2023). Does native aquatic plant diversity recover after biological control of invasive floating plants? *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
17. Reid, M.K., Hill, M.P. & Coetzee, J.A. (2023). Comparing the host specificity of a South African weevil and North American planthopper: the challenge of host specificity that is "just right" for management of *Nymphaea mexicana* (Nymphaeaceae) and its hybrids in South Africa. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
18. Stiers, I, Meert, P., Minuti, G. & De Boeck, S. (2023). Bugs 2 the Rescue: a citizen science project. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
19. Sutton G.F., van Steenderen C.J.M., Canavan K., Yell L., Day M.D., Taylor T.K., McConnachie A.J., Chari L.D., Plowes R., Rhodes A.C., Goolsby J.A., & Paterson I.D. (2023). Biological control of invasive African grasses: progress and prospects. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
20. van Steenderen, C.J.M., Sutton G.F., Yell, L., Canavan, K., Chari, L D., McConnachie, A., & Paterson, I.D. (2023). The *Tetramesa* (Hymenoptera: Eurytomidae) as biological control agents of invasive African grasses: A molecular investigation. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.

21. Weaver, K.N., Moffat, R., Hill, M.P., Petruzzella, A., Ngxande-Koza, S.W. & Coetzee J.A. (2023). Community mass-rearing of waterweed biocontrol agents reaps rewards. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
22. Weyl, P., Martin, G. & Coetzee, J. (2023). Aquatic Weeds Workshop. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
23. Zachariades, C., Moran, C., Hoffmann, J. & Hill, M.P. (2023). Assessing the outcome of biological control on weed populations. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
24. Jukes, M., Marsberg, T., Moore, S., Knox, C., Hill, M., Wennmann, J., Jehle, J., Lopez-Ferber, M., Rabalski, L. (2023). Cryptophlebia peltastica nucleopolyhedrovirus: A single approach for multiple pests. *International Congress on Invertebrate Pathology and Microbial Control & 55th Annual Meeting of the Society for Invertebrate Pathology*. 30 July- 3 August 2023. University of Maryland, College Park, MD, USA.
25. Coetzee, J.A. 2023. **Keynote address**. Recent developments in control of aquatic macrophytes: insights from Sub-Saharan African. *16th International Symposium on Aquatic Plants*. 13-17 November 2023. University of Antwerp, Antwerp, Belgium.
26. Baso, N., Coetzee, J., Bownes, A. & Paynter, Q. 2023. Investigating the Enemy Release Hypothesis on the invasiveness of *Lagarosiphon major* in New Zealand: Implications for management. *16th International Symposium on Aquatic Plants*. 13-17 November 2023. University of Antwerp, Antwerp, Belgium.
27. Strange, E., Schrama, M., Don, I., Coetzee, J., Wasserman, R. and Boerlijst, S. 2023. Mosquitoes and Macrophytes: a dangerous mix? *16th International Symposium on Aquatic Plants*. 13-17 November 2023. University of Antwerp, Antwerp, Belgium.
28. Petruzzella, A., Motitsoe, S., Hill, M., Mancunga, L., Kolisi, S., Maneli, S., Mantshi, H., Mlungu, Z., Nkibi, E. & Coetzee, J. 2023. Achieving the goal "more natives, less invasives": Priority effects as a potential tool for restoration. *16th International Symposium on Aquatic Plants*. 13-17 November 2023. University of Antwerp, Antwerp, Belgium.
29. Schneider, S., Coetzee, J., Harpenslager, S., Immerzeel, B., Köhler, J., Misteli, B., Padial, A., Thiébaut, G., Thiemer, K. & Vermaat, J. 2023. Mass development of aquatic macrophytes-causes and consequences of macrophyte removal for ecosystem structure, function and services. *16th International Symposium on Aquatic Plants*. 13-17 November 2023. University of Antwerp, Antwerp, Belgium.
30. Stiers, I., Meert, P., Minuti, G. & De Boeck, S. 2023. Bugs 2 the Rescue: a citizen science project. *16th International Symposium on Aquatic Plants*. 13-17 November 2023. University of Antwerp, Antwerp, Belgium.
31. Rekkers, E., Petruzzella, A., Motitsoe, S., Mantshi, H., Coetzee, J., Hill, M. & Strange, E. 2023. Early phytoplankton responses to invasive floating plant control in subtropical freshwater systems. *16th International Symposium on Aquatic Plants*. 13-17 November 2023. University of Antwerp, Antwerp, Belgium.
32. Chikodza, T., Hill, M. & Coetzee. 2023. The effect of biological control on the population dynamics of *Pontederia crassipes* (C. Mart) Solms (Pontederiaceae) and *Salvinia minima* Baker (Salviniaceae). *16th International Symposium on Aquatic Plants*. 13-17 November 2023. University of Antwerp, Antwerp, Belgium.

International Conference Posters

1. Baso, N.C., Coetzee, J.A., Bownes, A. & Paynter, Q. (2023). Investigating the enemy release hypothesis: a case study of *Lagarosiphon major* in New Zealand. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
2. Bowers, K.E., DuBois, J.W., Cowie, B.W., Ivey, P. & Thompson, D.C. (2023). Native range exploration in the southwest United States reveals potential biological control agents for *Prosopis* (Fabaceae) for South Africa. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
3. Chari, L.D., Mauda, E.V., Martin, G.D. & Rafter, M.A. (2023). Biological control prospects for African Boxthorn (*Lycium ferocissimum*; Solanaceae), a noxious weed in Australia. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
4. Chikowore, G., Wolmarans, A. & Martin, G.D. (2023). Pre-release studies on *Robinia pseudoacacia* in South Africa. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
5. Cowie, B.W., Heysteck, F., Kistensamy, Y., Byrne, M.J. & Paterson, I.D. (2023). The influence of climate on

the establishment and efficacy of *Evippe* sp. #1 in South Africa. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.

6. Gervazoni, P.B., Sosa, A., Coetzee, J.A., Orcasberro, G., Nin, M. & Franceschini, M.C. (2023). Yellow flag iris invasion in South America: is biological control needed? *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
7. Griffith, T.C. & Paterson, I.D. (2023). Selection of potential agents for an invasive cactus weed, *Trichocereus spachianus*. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
8. Jimenéz, N., Faltlhauser, A., Franceschini, C., Cecere, C., Daddario, F., Villamil, S., Fernández, S.A., Hill, M., Mc Kay, F., Cabrera Walsh, G. & Sosa, A. (2023). Education, outreach activities and training of human resources to promote Weed Biological Control in Argentina. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
9. Lehavana, A., Hill, M.P., Coetzee, J.A., Zachariades, C., Birkinshaw, C. (2023). Biological control using *Cyrtobagous salviniae* as a potential strategy for managing the invasion of *Salvinia molesta* in Madagascar. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
10. Martin, G.D., Weaver, K.N., Chikowore, G., Steenhuisen, S. & Venter, N. (2023). Northern temperate weeds programme in South Africa. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
11. Moffat, R., Coetzee, J.A. & Hill, M.P. (2023). *Egeria densa* (Hydrocharitaceae): The first submerged aquatic weed targeted for biological control in South Africa. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
12. Musedeli, J., Hurley, B., Chikowore, G., Hill, M.P. & Martin, G.D. (2023). The pine cone weevil, *Pissodes validirostris* as a potential biological control agent for European pines in South Africa. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
13. Ncube, J., Coetzee, J.A., Faltlhauser, A., Martin, G. D., Miller, B., Sosa, A. & Weyl, P.S.R. (2023). Collaborative efforts to manage the cryptic invader, *Limnobium laevigatum*, in southern African water-ways. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
14. Righetti, T., de la Fuente, D., Paper, M.K., Coetzee, J.A., Hill, M., Brentasi, E., Salinas, N. & Sosa, A.J. (2023). Effects of elevated CO_2 on *Megamelus scutellaris* (Hemiptera: Delphacidae) and its yeast-like endosymbionts (YLS). *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
15. Salinas, N.A., Smith, M., Coetzee, J.A., Hill, M.P., Brentassi, M.E. & Sosa, A.J. (2023). Population genetic structure of *Megamelus scutellaris* (Hemiptera: Delphacidae) and its implications for the biological control of water hyacinth (*Pontederia crassipes*). *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
16. Sandenbergh, E. & Coetzee, J.A. (2023). Targeting *Iris pseudacorus* (Iridaceae) for biological control in South Africa, and beyond. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
17. Venter, N., Cowie, B.W., Mkhomazi, V.L., Keizan, Y.Y., Mayonde, S.G. & Byrne, M.J. (2023). Update on *Solanum mauritianum* (Solanaceae) biocontrol in South Africa. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
18. Venter, N., Hatile, S.L., Mayonde, S.G., Cowie, B.W. & Byrne, M.J. (2023). Biocontrol efforts against invasive *Tamarix* (Tamaricaceae) in South Africa: 2014 – 2023. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
19. Zachariades, C., Martin, G.D., Sochor, M., Mason, B. & Widrlechner, M.P. (2023). Towards biological control of invasive North American *Rubus* species in South Africa: species identities, genetic diversity and origins. *XVI International Symposium on Biological Control of Weeds*. 7-12 May 2023. Puerto Iguazú, Misiones, Argentina.
20. Minuti, G., Staniszewski, M., Kanika, S. & Stiers, I. 2023. Assessing the recovery of aquatic ecosystems after the biological control of the invasive water fern *Azolla filiculoides*- A case study from Belgium. *16th International Symposium on Aquatic Plants*. 13-17 November 2023. University of Antwerp, Antwerp, Belgium.

CBC Research Day

This year the CBC hosted their second CBC Research Day where students and staff had the opportunity to share their research. Most of the not in attendance students came to Rhodes University to meet the rest of the team. Thanks to all of the presenters and to the rest of the team for attending.

Back:
Caroline Knox, Vhuawelo Simba, Tahnee Bennett, Tafara Franke Bute, Landile Booi, Reyard Mutamiswa, Marcel van der Merwe, Sean Moore, Ernst de Beer, Michael Mathenge, Jackey Mukhawana, Martin Hill, Michale Jukes, Wade Sexton, Liam Yell, Mthobeli Mvandabe, David Kinsler, Siviwe Tole, Philip Ivey, Emma Stirk, Grant Martin, Lyle Titus, Julie Coetzee, Lubabalo Malinga, Samuel Motitsoe, Lungile Bhungane, Gugulethu Mkile, Arthur Scholtz, Vershiyi Deric Tanka, Tapiwanashe Mashamba, Sive Kolisi, Evans Mauda, Hlumelo Mantshi, Lenin Chari, Kurhula Luphondo, Tapiwa Mushore, Lesego Malekana, Nwabisa Magengelele, Siyaxolisa Mini, Raynold Moagi, Bongiwe Gobongcwana, Wayne Kirkman.

Middle:
Nandipha Ngolothi, Siyanda Ntamo, Patricia Masole, Tamzin Griffith, Daniela Egli, Nthambeleni Bolongo, Rochelle Bessinger, Antonella Petruzzella, Abigail Wolmarans, Phillipa Muskkett, Karin Goliath, Carmen Peters, Simnikiwe Hambile, Lulutho Mancunga, Sonwabise Maneli, Siya Mnciva, Nokuthula Kom, Zizile Mlungu, Esethu Nkibi, Alungile Jeme, Vuyani Ntyinkala.

Bottom:
Byron Soetland, Thoriso Masalesa, Clarke van Steenderen, Kelby English, Emma Sandenbergh, Kim Canavan, Ticia Swanepoel, Jaqui Van Dyk, Ndalo Koza, Samella Ngxande-Koza, Kim Weaver, Karabo Moloi, Lehlohonolo Adams, Ben de la Fonteine, Nompumelelo Baso.

Online or Absent:
Gerald Chikowore, Iain Paterson, Guy Sutton.

Acronyms and Abbreviations

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
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
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
PSHB	Polyphagous Shot Hole Borer
SAAB	South African Association of Botanists
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
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
WfW	Working for Water
Wits	University of Witwatersrand

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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 keep growing in South Africa and expanding our reach and presence in Africa, and to consolidate international partnerships to strengthen research and implementation globally.