RHODES UNIVERSITY
Where leaders learn

CBC

Centre for Biological Control

Annual Report 2020

RHODES UNIVERSITY
Where leaders learn

CBC

Centre for Biological Control

Annual Report 2020

Front cover photograph
Tapiwa Mushore sampling insects in a field of honeybush near Plettenberg Bay
Photo: Daniel Rogers

Back cover photograph
Aerial view of the Kariega mass-rearing facility, outside of Kariega (formerly Uitenhage)
Photo: Centre for Biological Control

Editors
Esther Mostert and Kim Weaver

Design and layout
Print Services Unit, Rhodes University

Contents

DIRECTOR'S REPORT

Professor Martin Hill providing some guidance on mass-rearing techniques to a community partner in the North West Province. **Photo:** Julie Coetzee

I write this report under very different circumstances to the one I wrote for the 2019 CBC Annual Report. What a year 2020 was. Very few of us would have imagined a year in which a pandemic gripped the world, causing untold hardship to people and certainly testing our resolve. We have all been affected by COVID-19. I do believe that the pandemic and associated lockdowns have afforded us an opportunity to reflect on what we do and how we do it and to be considerate in how we implement the positives that have come out of 2020. Without access to laboratories or fieldwork, we had a chance to breathe, to write, and to think of new ways of operating in the future. Despite the restrictions, I hope that you will agree from the report that follows that the CBC had a highly productive year.

One of the highlights of 2020 was the dramatic collapse of water hyacinth on Hartbeespoort Dam under the herbivore pressure from the planthopper, *Megamelus scutellaris*. Well done to Julie Coetzee and her team for persevering at this site where, given that it is highly eutrophic and has cold winters, I certainly was sceptical that we would ever achieve this level of management through biological control. This success has shown us the value of the mass-rearing and inundating releases of biological control agents, essentially using them as a green herbicide. Having a geography student on board who, through satellite imagery, has been able to show landscape changes in the water hyacinth infestation levels over time, has been important in showing landowners and water resource managers what can be achieved. The situation at Hartbeespoort has also been an eye-opener in terms of community engagement and has taught us some valuable lessons in how we communicate what we do.

Unfortunately, due to travel restrictions, our scientists were unable to conduct overseas surveys for new agents, but we are very fortunate to have some excellent collaborators scattered around the world, and thus we have still been able to identify and source potential agents for weeds of European and American origin. In return, we have projects surveying for insects on South African plants that have become weeds in other parts of the world, including Australia, New Zealand and the USA. The CBC was granted permission for the release of a new biological control agent, a cochineal *Dactylopius tomentosus* '*californica* var. *parkeri*', for the control of the thistle cactus, *Cylindropuntia pallida*, while our colleagues at the Agricultural Research Council were granted permission to release the moth, *Evippe* sp.#1, for the control of *Prosopis* in the Northern Cape. This represents the first release of an agent on *Prosopis* in 30 years. We are hopeful that these new agents will contribute significantly to the control of their respective weeds. Several years ago, we started collaborating with resource economists to quantify the benefit of biological control to justify the expenditure on biological control. Mary Maluleke's Master's showed that South Africa has saved more than R1 billion by not having to use herbicide on water lettuce, giant Salvinia, parrot's feather and red water fern for the last 20 years. Further, this is an argument for the multidisciplinary research conducted within the CBC; on the Rhodes University campus, we now collaborate with nine academic departments.

On the agricultural side, there has been considerable research over the last year, mainly in collaboration with Citrus Research International to develop entomopathogenic fungi and viruses for pests on citrus. A significant number of growers are now putting orchards under netting to reduce wind damage, and we have a number of studies on how netting might change pest complexes and their natural enemies. We have also completed a study on insects associated with honeybush tea in the southern Cape. It was another productive year in terms of outputs with about 50 peer reviewed papers, seven PhD students and 10 Master's students graduating.

The year 2020 saw the retirement of Dr Guy Preston, and I do not have the space here to do justice to the contribution that he has made to environmental protection in South Africa through founding the Working for Water Programme in 1995. Dr Preston understood the enormous threat posed by invasive alien species to South Africa's ecological infrastructure, agriculture, human livelihoods and human well-being. He forcefully presented the costs of invasive species, whether it be *Parthenium*, waterweeds, Australian trees, house crows or mice on South Atlantic islands; his bright yellow and dark green PowerPoint presentations always carried a clear message that inaction was not an option. By advocating for large-scale job creation in the environmental sector, Dr Preston ensured huge investment in South Africa's efforts to manage the spread of invasive plants, but he understands that the fight against plant invasions will only succeed with the help of targeted, effective biological control. Dr Preston ensured investment in biological control facilities and research. He has always supported weed biological control and I cannot thank him enough for his support and advice over the last 25 years.

Unfortunately, one of our CBC team members passed away this year. After a short illness, Lulama Poni, passed away on Saturday, 26 December 2020. Lulama was born in Salem in 1972, and spent his entire life in Makhanda. He came to work for the Biological Control Research Group (now the CBC) in 2009. Initially this was a half-day post as he worked for the SAEON Elwandle Node as a para-taxonomist in the mornings. Lulama was one of three people living and working with disabilities who we first employed in the mass-rearing unit when it was still located where the Life Science building currently stands. It is impossible to determine just how many waterweed biological control agents Lulama was responsible for releasing into South Africa in the years that he spent with us, but it would have been in the millions, and thus his contribution to the conservation of aquatic biodiversity in this country has been immense. For those of you who worked closely with Lulama, I am sure that you will remember him, as I do, as a thoroughly decent man with a wicked sense of humour. I was always impressed with his use of English and on being very up to date on current affairs. Lulama was the (self-appointed) spokesperson for the people working in the mass-rearing unit and always took me to task if there were issues (he argued logically and well, and always won).

Another huge loss was Pat Hulley's passing away in June. Pat took over the weed biological control baton at Rhodes University from Cliff Moran in the mid-1980s and will be remembered for his work on the biological control of *Solanum* weeds. Pat was a quintessential gentleman and a great mentor to me over the years, and we all miss his presence in the department. In November, we mourned the passing of Dr Olaf Weyl, one of our collaborators based at SAIAB. Olaf was recognised internationally for his work on freshwater fish invasion; we will miss him greatly.

The amount of research presented in this report from the consortium members that make up the CBC is truly impressive. I would like to thank Kim Weaver and Esther Mostert for pulling together the report. Further, I would like to really thank Ms Jeanne van der Merwe who gets through a mountain of work daily to ensure that the CBC runs smoothly and that we are constantly audit ready. Finally, I would like to thank Rhodes University who host the CBC and create the enabling environment for us to do the work we do.

The vision for the CBC is to conduct research to sustainably control both environmental and agricultural pests in order to protect ecosystems and the societies that depend on them, and to ensure that maximum benefits of biological control were realised through excellence in research, teaching and learning, implementation, and community engagement. The CBC is fortunate in that it has some excellent people, exciting projects, and an enabling environment within South Africa to achieve its vision.

Staff, students and associates

STAFF

Director
Prof. Martin Hill

Deputy Directors and Programme Leaders
Prof. Julie Coetzee
Dr Grant Martin
Prof. Sean Moore
Prof. Iain Paterson

Research Staff
Dr Candice Coombes
Philip Ivey
Hildegard Klein
Benjamin Miller
Esther Mostert
Samuel Motitsoe
Phillippa Muskett
Dr Candice Owen
Dr Guy Sutton
Kim Weaver

Centre Administrator
Jeanne van der Merwe

Support Staff
Slovo Dyira
Wandisile Mdiza
Mpilonhle Ndlovu
Samella Ngxande-Koza
Bongiswa Ngxanga
Matthew Paper

Mass-rearing facility staff

Waainek, Makhanda
Maretha Boshoff (Manager)
Landile Booi
Mlamli Mbangi
Lunga Ngeju
Vuyani Ntyinkala
Lulama Poni
Vusumzi Sukula
Lyle Titus

Kariega (Uitenhage)
Ruth Scholtz (Manager)
Carmen Peters
Karin Goliath
Lubabalo Malinga
Gugulethu Mkile
Ronel Roman
Arthur Scholtz
Daniel Scholtz
Byron Soetland

STUDENTS

Postdoctoral Fellows
Dr Kim Canavan
Dr Lenin Chari
Dr Blair Cowie
Dr Michael Jukes
Dr Samalesu Mayonde
Dr Antonella Petruzzella

Doctoral Students
Pascal Osa Aigbedion-Atalor
Nompumelelo Baso
Gerald Chikowore
Ernst de Beer
Paula Gervazoni
Tamzin Griffith
Karlien Grobler
Wayne Kirkman
Adolphe Lehavana
Anthony Mapaura
Gianmarco Minuti
Zezethu Mnqeta
Tshililo Mphephu
Phillippa Muskett
Keneilwe Sebola
Rosali Smith
Guy Sutton
Megan Reid
Marcel van der Merwe
Clarke van Steenderen
Abigail Wolmarans

Master's Students
Sara Salgado Astudillo
Tahnee Bennett
Benjamin Coetzer
Luke Cousins
Petrus Iita
David Kinsler
Zanele Machimane
Batandwa Masiko
Brett Mason
Thuthula Mela
Siyasanga Mnciva
Karabo Moloi
Wayne Mommsen
Siphokazi Msengana
Tapiwa Mushore
Matthew Paper
Samantha Prinsloo
Daniel Rogers
Emma Sandenbergh
David Taylor
Garyn Townsend
Sage Wansell
Ekhona Zozo

ASSOCIATES

CBC Consortium Partners

University of Witwatersrand
Prof. Marcus Byrne
Nic Venter

University of Cape Town
Associate Prof. John Hoffmann
Fiona Impson
Catharina Kleinjan

University of KwaZulu-Natal
Prof. Colleen Downs

University of Free State
Dr Frank Chidawanyika

Research Associates
Prof. Gavin Fraser
Prof. Caroline Knox
Prof. Cliff Moran
Prof. Terry Olckers
Dr Costas Zachariades
Dr Helmuth Zimmermann

AQUATIC WEEDS

In 2020 the CBC continued research into the biological and integrated control of a number of invasive aquatic weed species in South Africa. This research included the five major floating aquatic weeds (water hyacinth, parrot's feather, salvinia, water lettuce, and red water fern), as well as new studies on the biological control of common salvinia (*Salvinia minima*). Biological control continues to be the most successful method of control for the majority of these floating aquatic weeds, and more recently, the CBC has experienced unanticipated success with the control of water hyacinth, which has always proved the most difficult floating macrophyte to control. This success is largely due to a focused mass-rearing and release campaign of the water hyacinth hopper, *Megamelus scutellaris* at Hartbeespoort Dam.

Using expertise gained from our success with the biological control of floating water weeds, which provides the best return on investment, the CBC has shifted the emphasis of the aquatic weed programme to submerged and emerging aquatic weed projects, which have been termed the 'low-hanging fruit' projects. These projects encompass a far more holistic approach to biological and integrated control of invasive aquatic plants in South Africa and its neighbouring countries. A biological control programme against the submerged aquatic weed, Brazilian waterweed (*Egeria densa*), was initiated with the release of a hydrellia fly in October 2018, while the release of an agent against the emergent Delta arrowhead (*Sagittaria platyphylla*) is imminent. Programmes on Mexican waterlily (*Nymphaea mexicana*) and yellow flag iris (*Iris pseudacorus*) have identified potential agents whose host-specificity trials are underway.

The aquatic weeds programme also keeps a number of potentially serious aquatic invaders, some of which already occur in South Africa, on a 'watch list', with the aim of conducting feasibility studies into their biological control. These include pickerelweed (*Pontederia cordata*), watercress (*Nasturtium officinale*), yellow heart (*Nymphoides peltata*), water poppy (*Hydrocleys nymphoides*), Amazon frogbit (*Limnobium laevigatum*) and Canadian waterweed (*Elodea canadensis*). This programme will also be on the lookout for additional species such as alligator weed (*Alternanthera philoxeroides*), and fanwort (*Cabomba caroliniana*) which are serious invaders elsewhere, but are yet to be recorded as established in South Africa.

The aquatic weeds programme includes a number of activities involving identification, screening, release and, most importantly, evaluation of biological control agents, as well as integrating biological control into management through the transfer of appropriate technology to water managers. This programme made a significant contribution to capacity building and knowledge transfer in the field of biological control of weeds, and results were published in top international journals, placing this research in an international context.

PROGRAMME HIGHLIGHTS IN 2020

- Successful biological control of water hyacinth on the Hartbeestpoort Dam.
- Establishment of remote rearing stations for the water hyacinth planthopper, *Megamelus scutellaris,* around South Africa.
- Development of a remote sensing application for monitoring water hyacinth.
- Importation of five populations of the water hyacinth grasshopper, *Cornops aquaticum,* from Argentina into CBC quarantine.
- Submission of the release application for the Delta arrowhead weevil, *Listronotus appendiculatus.*

Water hyacinth

Despite efforts to control water hyacinth (*Pontederia crassipes*), it remains South Africa's most problematic aquatic macrophyte. There are currently nine species of biological control agent released for its management in South Africa, with *Megamelus scutellaris*, the water hyacinth planthopper, the most recently released agent. The research on biological control of water hyacinth includes a range of studies, from post-release evaluations, to interactions between different guilds of agents, effects of elevated CO_2 on water hyacinth biological control, as well as research into mass-rearing and inundating release efforts.

RESEARCH TEAM
Prof. Julie Coetzee, Prof. Martin Hill, Maretha Boshoff, Dr Candice Owen, Kim Weaver, Matthew Paper, Esther Mostert, Philip Ivey, Benjamin Miller, Dr Samuel Motitose, Dr Antonella Petruzella (Postdoc)

STUDENTS
Keneilwe Sebola (PhD), Siyasanga Mnciva (MSc), Matthew Paper (MSc), David Kinsler (MSc), Clarke van Steenderen (PhD), Benjamin Coetzer (MSc), Perryn Richardson (BSc Hons.), Batandwa Masiko (MSc), Khalipha Dambuza (BSC Hons.)

COLLABORATORS
FuEDEI, University of Corrientes, Harties Foundation, MadMacs, Prof. John Terblanche and Henrieka Bosua (Stellenbosch University)

Hartbeespoort Dam Biological Control – post-release evaluations

At the beginning of 2020, the CBC witnessed the spectacular collapse of water hyacinth on Hartbeespoort Dam as the result of an intensive mass-rearing and release campaign of water hyacinth biological control agents. The CBC initiated this campaign in 2018 and increased efforts in 2019 when the team released hundreds of thousands of the agent, *Megamelus scutellaris*. In the absence of herbicide spraying of the water hyacinth on the dam since 2017, the *Neochetina* weevils had the opportunity to increase their population size, which inflicted damage to the plants that we have not seen before in the field. This has been a true success story of biological control.

Julie Coetzee being interviewed by SABC in February 2020 about the impact of biological control on water hyacinth at Hartbeespoort Dam. **Photo:** Benjamin Miller

Benjamin Miller and Zolile Maseko with pilot Nico Jacobs from Rhino911 in February 2020 – on an aerial survey of water hyacinth of the Bospoort and Vaalkop dams, as well as the Hex River near Rustenburg. **Photo:** Nico Jacobs

Neochetina weevil adults – characteristic feeding scars on the leaves alert us to their presence, as they are nocturnal. **Photo:** Benjamin Miller

Neochetina weevil larvae in the crown of the plant –damage caused by the larvae results in the waterlogged plants sinking with wind movement. **Photo:** Benjamin Miller

Megamelus scutellaris adults and nymphs on the stem of a water hyacinth plant. **Photo:** Benjamin Miller

By July 2020, the water hyacinth cover on the dam was less than 2.5%. Cold winter temperatures affected the remaining plants and the control agents, resulting in die-off of the insects, as well as a reduction in their food quality. However, as soon as it began to warm up, the water hyacinth plants began to grow again from seeds in the seed bank, while the insect populations lagged behind. Seed bank analysis showed that approximately 1 kg of sediment contained over 450 viable water hyacinth seeds.

The control agent populations take a long time to increase on their own as it starts to warm up. Therefore, the CBC has facilitated setting up mass-rearing facilities around the dam; the biggest with the Harties Foundation. The purpose of the facilities is to rear thousands of the control agents again for regular release onto the remaining plants, so that the water hyacinth does not reach problematic proportions once more. We continued our mass release campaign throughout the remainder of 2020.

Matthew Paper inspecting mass-rearing tubs at the Redstone housing estate - a CBC community partner in the North West. **Photo:** Benjamin Miller

Remote sensing of water hyacinth levels

David Kinsler is developing a remote sensing method to measure past and current water hyacinth occurrence on Hartbeespoort Dam in the North West Province. This is vital research, which aims to provide better insight into the biological control-induced collapse of water hyacinth on the Hartbeespoort Dam during the summer of 2019/20.

Using cloud-based remote sensing software, David can process large volumes of high-resolution satellite image data (approximately 600 images) efficiently and accurately. The result is a detailed time-series of water hyacinth cover and health, as well as the presence of algae and cyanobacteria, spanning 2015 to present.

The time series shows water hyacinth decreased from 40% dam coverage in October 2017 down to 2% over the early summer months of 2020 (January to March). It remained low throughout 2020 until the end of 2020, and beginning of the next summer, since when the water hyacinth has recovered. David's work also serves to inform stakeholders about the status of water hyacinth on the dam, and to monitor the efficacy of efforts to curb its growth. The CBC is currently using the data to develop an app for easy access to this information, which will be displayed on the CBC website in 2021.

Satellite image of the Hartbeespoort Dam on 9 September 2017 (top) and 20 July 2020 where there is less than 2.5% cover. **Photo:** David Kinsler

Waterweed collaborations – MadMacs project

The year 2020 kicked off with a collaborative project, coordinated by the Water-JPI, called 'MadMacs' – Mass development of aquatic macrophytes, and the causes and consequences of macrophyte removal for ecosystem structure, function, and services. The team included researchers from Norway (Norwegian Institute for Water Research (NIVA)), Germany (Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB)), France (Rennes University), Brazil (Universidade Federal do Paraná (UFPR)), and South Africa (CBC, Rhodes University). Each country identified a system invaded by aquatic macrophytes in order to determine the effects on ecosystem function of large mats of macrophytes and the effect of their removal.

Christophe Piscart from Université de Rennes 1, France and CBC PhD student, Nompumelelo Baso were part of the MadMacs collaborative team. **Photo:** Benjamin Mistelli

Problems with macrophyte mass development occur across the world, but managers do not generally exchange experiences across countries, particularly not across continents. Many water managers feel isolated with their particular problem of nuisance growth in their particular system, and their experiences often cannot be compared owing to the lack of a harmonised study design. The MadMacs case studies have quite different management histories while, at the same time, applying an homogenised BACI (Before-After, Control-Impact) study design, such that the involved stakeholders and scientists benefit from the trans-national exchange of experiences. This collaboration will be the first to provide such data on a trans-national basis and use them to provide internationally applicable guidelines for new, knowledge-based management of watercourses with dense aquatic vegetation.

Keneilwe Sebola sampling some large water hyacinth on Hartbeespoort Dam. **Photo:** Benjamin Miller

The MadMacs team selected Hartbeespoort Dam, a hotspot of water hyacinth invasion, for the South African case study. The dam is subject to serious anthropogenic pollution, climate change, and hydromorphological alterations. Water hyacinth has been present on the system since the 1960s and was successfully controlled in the 1980s using herbicides. The *Neochetina* weevils; the mite, *Orthogalumna terebrentis;* the moth, *Nipograpta albiguttalis,* and the mirid, *Eccritotarsus catarinensis*, have all been released on the dam since the 90s, and have all established, but remained at low numbers due to the herbicide campaign. In 2016, however, herbicidal control was halted, resulting in massive plant growth, to the point that in mid-2018, more than 50% of the dam was covered by the plant.

The Hartbeespoort Dam field campaign commenced in early January 2020, with team members from Norway, Germany and France. We began the 'before removal' sampling on 10 January. The sampling was conducted at Kurper Oord, a site that belongs to Department of Water and Sanitation (DWS). The sampling approach followed a 'BACI' design – 'Before-After, Control-Impact' whereby two sites were selected and allocated either 'control' or 'impact'. The core of sampling in the BACI design is one week before and one week after macrophyte removal in order to make sure that the case studies are comparable. At the Hartbeespoort Dam site, water hyacinth had been manually removed through collaboration with the Harties Foundation, a non-profit company operating around the Dam.

Photographs of the 'before' and 'after' water hyacinth removal at the Hartbeespoort Dam site. Photo: Warren Aker, Golding Consulting

A number of biodiversity measurements were made at both the control and impact sites, before removal of water hyacinth, and one week after removal. These measurements included macroinvertebrate and fish diversity assessments as well as water chemistry measures, fluxes of CO_2, CH_4 and N_2O, sedimentation rates, and periphyton development.

Fish diversity was assessed using electrofishing. The sites covered with dense water hyacinth (before clearing), had less diverse and abundant marginal / littoral zone dependent fish species. This may have been exaggerated by the limited access within the dense coverage, as well as the dam's depth. However, it is also likely that the dense coverage of water hyacinth provides an homogenous (and plentiful) habitat with too little open water and light for many species, so only species such as carp (*Cyprinus carpio*) and sharptooth catfish (*Clarias gariepinus*) inhabit the benthos, and mosquitofish (*Gambusia affinis*) inhabit the roots. Although a snapshot in time, these results indicate that fish species that rely on the marginal/littoral zones require a combination of open water habitat and sufficient cover for protection. Fish were observed moving out into the open water, and only when threatened, did they take refuge in the vegetation cover (both marginal and floating water hyacinth).

The results of the Hartbeespoort study will form part of the whole MadMacs programme; there are still a number of tests and analyses, including data analyses, that need to be finalised, but a basic summary of results shows that:

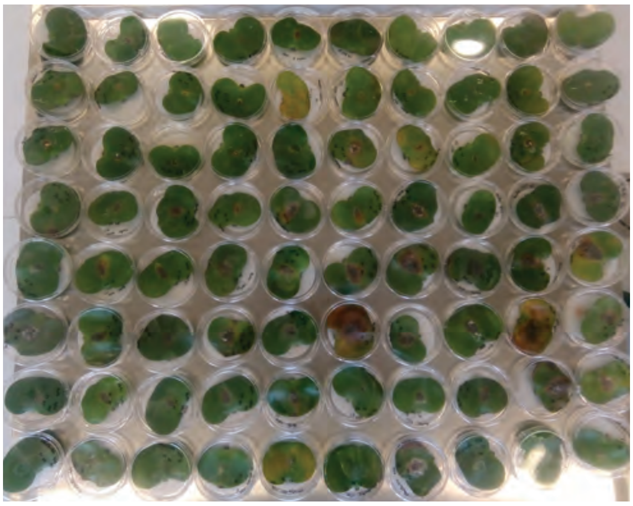
- Water quality in terms of phosphates is poor – the tests showed that phosphates in the water at the test site ranged between 2.5 and 4 mg P/l. The international standard is 0.1 mg P/l, and the SA standard is 0.2 mg P/l, 20 times less than we measured. These values were found in both the cleared and control sites.
- Biodiversity beneath the water hyacinth mat was lower than in the open water; this has consequences for ecosystem structure and functioning.
- Following the removal of water hyacinth from the impact site, within less than a week, the cyanobacteria (blue-green algae) bloomed (most likely *Microcystis aeruginosa*, but this needs to be confirmed at the laboratory). It was present in the water hyacinth control site before the removal, but was not able to bloom due to lack of sunlight, a requirement for photosynthesis. Water hyacinth prevents the build-up of blue-green algae through its shading effect, and not through its uptake of nutrients.
- The perceptions of residents and tourists around the presence of water hyacinth are mixed, although most agreed the weed was a nuisance, affecting property values, tourism, and health. Some respondents perceived its presence to be more beneficial than the presence of blue-green algae. The questionnaire still needs in-depth analysis.

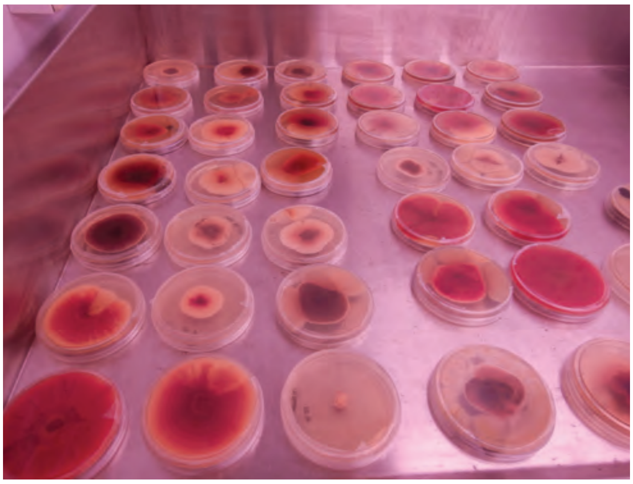
Natural pathogens used to aid control of water hyacinth

The focus of Siyasanga Mnciva's MSc is to enhance the effectiveness of the leafhopper, *Megamelus scutellaris,* with naturally occurring phytopathogens for biological control of water hyacinth in South Africa. Her research highlighted that *Megamelus scutellaris* and a naturally occurring pathogenic fungi associated with water hyacinth in South Africa are compatible.

Studying additive or synergistic effects among introduced insects and native phytopathogens is necessary to achieve biological control of water hyacinth. This study explored an integrated approach to using *M. scutellaris* in combination with native indigenous fungi to control this problematic weed. Indigenous fungi associated with water hyacinth in South Africa were surveyed, isolated and identified to determine their prevalence in the country. Genetic profiling using PCR and sequencing also confirmed morphological identification of the fungal isolates. Bioassays to assess the pathogenicity of the isolated fungi were conducted and pathogenic indigenous fungi were selected for integration studies.

During this study, 42 fungal isolates belonging to four genera were collected. Of all the fungal pathogens isolated, the most virulent were confirmed to be *Fusarium incarnatum*, *Fusarium oxysporum* and *Fusarium equiseti*. By the end of six weeks, the bioassays showed these fungi caused 68%, 63% and 54% disease infection on the plants, respectively, suggesting the potential of these isolates as effective biological control agents to manage water hyacinth. As far as is known, this is the first study to report *Fusarium incarnatum* as a candidate biological control agent on water hyacinth.





Siyasanga's experimental setup for in vitro leaf pathogenicity tests (above) and fungi morphology (below). **Photo:** Siyasanga Mncinva

The results showed that the combined effect of *Megamelus scutellaris* and *Fusarium* species caused significant plant damage to water hyacinth, with the combination of *Fusarium incarnatum* and *Megamelus scutellaris* showing the highest plant damage of 87% at the end of nine weeks.

Water hyacinth grasshopper – *Cornops aquaticum*

The water hyacinth grasshopper, *Cornops aquaticum,* was first introduced into South Africa from Brazil in 1995, with subsequent collections made in Trinidad and Venezuela in 1996, and Mexico in 1997. Detailed pre-release studies were conducted prior to the release of *C. aquaticum* to assess its host range and impact on water hyacinth. *Cornops aquaticum* was considered to be sufficiently host specific with limited potential to establish permanent populations on native Pontederiaceae species. Laboratory trials further demonstrated significant impacts of the grasshopper herbivory on the growth, biomass accumulation and competitive ability of water hyacinth. After a considerable stay in quarantine, *C. aquaticum* was approved for release in South Africa in late 2010. Initial field releases were made between 2011 and 2016; however, these populations failed to overwinter successfully, and never established permanent populations.

Because establishment success could possibly be assisted by acclimation of individuals prior to release, or through the re-collection of *C. aquaticum* populations from colder regions within the native range, populations from five different sites were collected from Argentina in December 2019, and brought back to the CBC quarantine. The genetic relatedness of these populations, and their similarity to the original South African quarantine population is currently being assessed. This assessment will be followed by experiments on the thermal tolerance and suitability of *C. aquaticum* to South African climates.

Neochetina sp. population genetics

Neochetina eichhorniae and *Neochetina bruchi* are weevils that both feed exclusively on water hyacinth and display similar life cycles and biology. Adults feed on the leaves, while larvae tunnel into the petioles and crown of the host plant, resulting in the plant decaying, becoming waterlogged, and sinking. Impact studies have shown that *Neochetina* weevils' feeding reduces plant biomass, reproduction, and shoot development. *Neochetina bruchi* can be distinguished from *N. eichhorniae* by the presence of a tan-coloured, v-shaped chevron on the lower region of the elytra, and by dark sutural ridges that are slightly shorter, and positioned further towards the posterior end of the elytra.

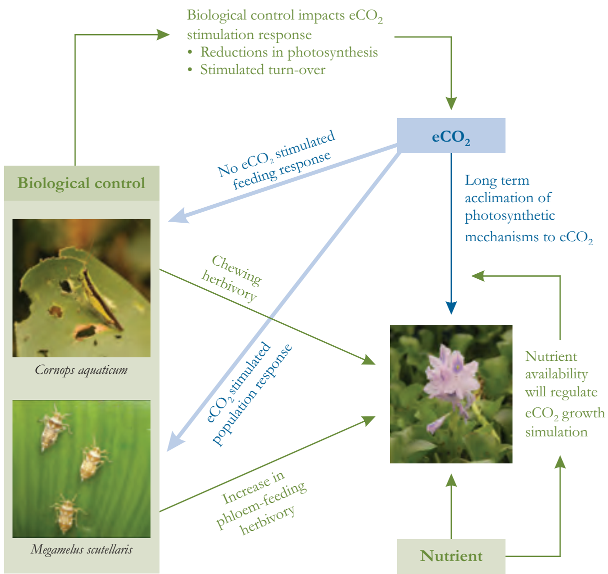
To avoid interspecific competition, *N. eichhorniae* oviposits in the younger, central leaves of the host plant, while *N. bruchi* prefers the bulbous older petioles. *Neochetina eichhorniae* has a longer generation time (approximately 120 days) than *N. bruchi* (approximately 96 days), which has been linked to the latter species causing greater damage. *Neochetina bruchi* also causes a greater reduction in plant growth under high nutrient levels than *N. eichhorniae*. The two species have displayed differences in thermal tolerance, with *N. bruchi* being able to withstand slightly lower temperatures than *N. eichhorniae,* which is able to survive at higher temperatures than *N. bruchi*.

The first populations of *N. eichhorniae* and *N. bruchi* were released in South Africa in 1974 and 1990, respectively. They were originally collected in Argentina and imported into the country via Florida, United States of America. Owing to low establishment rates after the first release of both agents, a second population of *N. eichhorniae* and *N. bruchi* from the same region was released in 1985 (via Australia) and 1996 (via Zimbabwe), respectively. The weevils are two of the most effective biological control agents used across the world for the control of water hyacinth, with releases in 41 countries. Notable cases of effective control include those from the United States of America, Papua New Guinea, Mexico, West Africa, Lake Victoria, and the warmer regions of South Africa.

Recently microsatellite markers have shown the two *Neochetina* species can hybridise although hybrid performance and biology (thermal and cold tolerance, oviposition preference, and generation time, for example) may differ from each of its parent species. Because such differences could have important implications for the efficacy of biological control programmes, 186 of 200 adults collected across South Africa, (77 *N. bruchi* and 109 *N. eichhorniae*) were identified to species. Three legs from one side of each weevil's body were used for DNA extraction. Eighteen primers (10 for *N. eichhorniae* and eight for *N. bruchi*) were used for multiplex PCR reactions. One multiplex PCR combination out of three successfully amplified all target fragments for *N. bruchi*: the Nb5 (VIC), Nb27 (FAM), and Nb43 (NED) combination. Once the genetics foundation has been laid, the project can be expanded by an Honours or Master's student to incorporate cross-species breeding, and assessments of hybrid performance.

Effects of elevated CO_2 on biological control of water hyacinth

Global climate change and the current rise of human-driven atmospheric CO_2 has prompted increased research on the influence of this rapid change on ecological relationships across the globe. The impact of elevated CO_2 (eCO_2) affects plant-insect interactions, changing plant quality and resource allocation, which has cascading effects on herbivores. This, in turn, has significant effects on insect responses, particularly on how feeding guilds that feed exclusively on specific plant species respond to such changes. Alterations in plant physiology due to future predicted eCO_2 could affect the success of biological control. Therefore, the response of water hyacinth grown at eCO_2, and how this may affect the feeding response of two control agents, *Cornops aquaticum* and *Megamelus scutellaris,* was investigated.



A schematic illustrating the influence of elevated CO_2 on plant-insect interactions for *P. crassipes* and two of its biological control agents *Cornops aquaticum* and *Megamelus scutellaris,* which represent chewing and phloem-feeding herbivory, respectively. **Credit:** Matthew Paper

The exposure of water hyacinth plants to long-term eCO_2 conditions resulted in significant growth over time. However, water hyacinth grown at eCO_2 underwent an acclimation of leaf CO_2 assimilation rates to levels equal to or slightly lower than those seen under eCO_2 conditions. Insect herbivory by both agents varied under eCO_2 conditions as the response differed between the different feeding guilds. The chewing herbivory of C. aquaticum did not improve under eCO2, with no compensatory feeding response while the phloem-feeding M. scutellaris experienced a significant increase in population density rather than feeding damage as a result of eCO_2. Although both agents experienced an eCO_2 stimulated response, the population-level response of *M. scutellaris* is of significant importance to the biological control programme and future control strategies. The population-level response of *M. scutellaris* suggests the potential of the agent for improved efficacy at future eCO_2 conditions in which the eCO_2 stimulated increases in population density may lead to increased agent loads on water hyacinth infestations over a shorter interval, and effectively mitigate the eCO_2 fertilisation of water hyacinth.

The Open Topped CO_2 facility at Rhodes University, Makhanda where experiments were conducted. **Photo:** Brad Ripley

Insect thermal physiology plasticity, and links to metabolism and life history

Because insects are ectothermic, the thermal physiology of insects is an important component of biological control initiatives. Insects introduced into invaded areas with thermal regimes that frequently extend beyond their thermal tolerance limits are unlikely to establish in the introduced site. If they do manage to establish, their performance could be severely impacted thereafter. Lack of establishment or poor performance is a financial and effort loss for a biological control programme.

Nonetheless, there are examples of unanticipated agent establishment and success in areas of introduction outside of the insects' predetermined thermal ranges. Research in this area aims to understand better the thermal tolerance of the insect in order to improve predictability of agent establishment and control success, both for new releases and for the future under altered climates.

There are two primary focus areas: the first is a better understanding of thermal tolerance plasticity. Many studies have indicated that the thermal physiology of insects is adaptable over both short and long time spans. Such plasticity and adaptability could explain unexpected establishments. Understanding which agents display tolerance plasticity, the duration over which such adaptability operates, and how greatly tolerances can change would help explain past, or predict future establishment patterns. Secondly, the programme investigates the link between thermal tolerance and metabolism in insects, which could be used to explain shifts in life history characteristics under differing thermal regimes. Once again, understanding these dynamics could be used to interpret unexpected results or predict possible future changes in control.

Understanding past patterns in agent performance is important for future releases or the development of new agents. Predicting possible future agent performance is equally important, as it could help pre-empt whether alternative species need to be sourced for invaded regions where the insects will no longer be able to survive or perform well. Alternatively, this research may indicate that range expansions could be possible for certain agents currently restricted to limited areas, thereby providing hope for improved future control.

In South Africa, the weevil, *Neochetina eichhorniae,* is one of the longest-established biological control agents on water hyacinth. The species has established at various sites around South Africa and is exposed to a range of thermal conditions, making the weevil a subject of interest for investigation into whether thermal tolerance acclimatises to local conditions over time.

In 2020, as part of his BSc Honours project, Khalipha Dambuza conducted a desktop study on data collected by Candice Owen in 2016 and 2017. Candice determined the critical thermal and lethal limits of male and female *N. eichhorniae* specimens collected from the hottest-on-average and coldest-on-average sites where the weevil has established in South Africa. Critical thermal limits are the upper and lower temperatures at which insects lose the ability to control their musculature and thus normal behaviour. Lethal limits indicate the temperatures beyond which individuals will die. Khalipha's analysis of the results indicated that the critical thermal limits of the insects collected from the two sites did not differ significantly. However, the *N. eichhorniae* individuals from the colder site survived significantly colder conditions before death (lower lethal limits) than the specimens collected from the warmer site. There were no differences between the sexes in either of the thermal limits. These results indicate that thermal limit acclimatisation has enabled this species to better tolerate local conditions at the two sites. This adaptation is possibly only observable in the more extreme thermal limits because the insect is capable of behavioural thermoregulation. Such a behavioural adaptation could relieve some selective pressure for adaptation in the physiological processes behind critical thermal limits.

Impacts of water hyacinth on freshwater fish

Macrophyte invasions are extremely complex, and the precise impacts are difficult to quantify. One recent discovery is the potential impact of invasive macrophytes on predator-prey interactions of freshwater fish. Increased structural complexity, such as that generated by aquatic plants, is known to influence the mechanisms and outcomes of predator-prey interactions. For example, habitat structure can reduce predation by providing refugia for prey, or conversely, increase predation by providing ambush predators with cover. Predicting these impacts is challenging, as different predators employ various prey-capture strategies, and different prey species have different vulnerabilities. As South Africa does not boast any native floating plants, our native fish species are not adapted to them. Invasive floating water hyacinth could provide invasive predators with an advantage, further aggravating the impacts of invasive floating plants.

Benjamin Coetzer's research focuses on two of the most prevalent freshwater invaders in South Africa, the largemouth bass (*Micropterus salmoides*) and water hyacinth. The prey is a native freshwater fish, banded tilapia (*Tilapia sparrmanii*). Juvenile largemouth bass and banded tilapia were placed into microcosms with varying levels of water hyacinth cover. Their behaviour was observed, and survival rate determined under the various percentages of cover to establish whether increased cover was beneficial to either species.

The aim of this study is to gain further insight into the influence of water hyacinth on predator-prey interactions; an aim that was achieved by focusing on the effect of water hyacinth on the predation efficiency of select invasive predators, and by assessing the potential for certain native species to use it as a refuge. This is likely to provide a better understanding of the effects of invasive aquatic plants on native species and food webs and to inform future management decisions.

Experimental setup in the Department of Zoology and Entomology. Microcosms with varying levels of habitat cover generated by water hyacinth. **Photo:** Benjamin Coetzee

Kariba weed

The weevil, *Cyrtobagous salviniae*, was released as a biological control agent against *Salvinia molesta,* also known as Kariba weed or giant salvinia, in South Africa in 1985. This agent has been highly successful against *Salvinia molesta* and has significantly reduced the weed's populations around the country. However, in 2007, the parasitic algae, *Helicosporidium* sp. (an undescribed species), was detected in field-collected *Cyrtobagous salviniae* adults in South Africa. The distribution and impacts of this disease on the weevil and its efficacy as a control agent were not known.

Tshililo Mphephu conducted a study for his PhD which determined the prevalence, infection load, and impact of *Helicosporidium* sp. on *Cyrtobagous salviniae*. In 2019, adult weevils were collected from 10 sites across the Eastern Cape, KwaZulu-Natal, Limpopo, and Western Cape provinces and screened to determine the occurrence, infection load, and geographic distribution of *Helicosporidium* sp. Transmission mechanisms of this disease in *C. salviniae* were then evaluated. The possible impact of *Helicosporidium* sp. was assessed by comparing the feeding rates and the reproductive output of diseased and healthy adults of *C. salviniae*. An attempt was then made to eliminate the disease in *C. salviniae* by applying the antibiotic, ketoconazole. The role of temperature on infection load in *C. salviniae* was also assessed. Finally, recommendations were made for the long-term biological control programme against *S. molesta* in South Africa.

The disease covers the entire distribution range of *C. salviniae* in South Africa, with the occurrence rate ranging from 92.15% to 100% of insects infected per site. *Helicosporidium* sp. was found to transmit vertically within the populations of *C. salviniae*, reducing the reproductive output of *C. salviniae* as well as its impact on biomass reduction of *S. molesta*. Multiple applications of ketoconazole concentrations under *in vitro* trials reduced 98.44 to 98.55% of *Helicosporidium* sp. loads. *In vivo* treatments resulted in 70% control of *Helicosporidium* sp. in the adults of *C. salviniae* that were fed ketoconazole three times over a 21-day period. Adult *C. salviniae* feeding and survival performances were similar when fed fronds of *S. molesta* inoculated with ketoconazole and water. The lowest and highest disease loads of *Helicosporidium* sp. were recorded when the weevils were reared at 30°C and 14°C, respectively. As expected, the highest impact of *Helicosporidium* on reproductive output of *C. salviniae* were at 30°C.

RESEARCH AND TECHNICAL STAFF
Prof. Julie Coetzee, Prof. Martin Hill, Matthew Paper, Maretha Boshoff

STUDENTS
Samuel Motitsoe (PhD), Tshililo Mphephu (PhD), Getrude Tshithukhe (MSc)

This study highlights the role of diseases on biological control agents, and the gaps in both the pre-release and post-release monitoring that should integrate screening of diseases in these studies. Although the combined application of the antibiotic and temperature will reduce *Helicosporidium* sp. loads and impact, this technology is most likely only applicable where the weevils are reared in small numbers in a rearing facility and is not really applicable to the field situation. It is important to release healthy agents that will efficiently control the target weed species; therefore, when introducing new biological control agents, the health status of such agents needs to be understood. Long-term field monitoring and assessment of the impact of *C. salviniae* on *S. molesta* should be conducted to track all the changes that may result owing to the presence of *Helicosporidium* sp. Such long-term monitoring and assessment will provide information on the role of *Helicosporidium* sp. in field populations of *C. salviniae*.

Damage by the weevil *Cyrtobagous salviniae* to Kariba weed.
Photo: Lynette Rudmann

Parrot's feather

Parrot's feather (*Myriophyllum aquaticum*) is an invasive aquatic weed that is currently considered under control in South Africa by the biological control agent, the beetle *Lysathia* sp. This insect has successfully established at many sites with highly variable thermal regimes, indicating that it can tolerate a wide range of thermal conditions. It is also relatively easy to rear in the laboratory, and hardy to handling. These characteristics make it an attractive model organism for research.

RESEARCH TEAM
Prof. Julie Coetzee, Prof. Martin Hill, Dr Candice Owen

STUDENTS
Perryn Richardson (Hons), Batandwa Masiko (MSc)

COLLABORATORS
Stellenbosch University

In 2020, Candice Owen and Perryn Richardson, a BSc Honours student, conducted an experiment where *Lysathia* sp. individuals were maintained in a controlled environment chamber for an extended period. The temperature and light conditions within the chamber mimicked normal daily cycles. Access to the plant was restricted to set periods for specific groups of insects, such that only one group had constant access to food. A second group were only allowed to feed during the hottest day-time period and the last group only received plant material during the coldest night-time period. The results indicated the impact of temperature at the time of feeding on body composition, body mass, mortality and fecundity. The experiment needs to be repeated under a hotter thermal regime to confirm the results, but the COVID-19 pandemic disrupted these plans. They are likely to go ahead in 2021.

Batandwa Masiko began an MSc in 2020 that also focusses on the digestive energetics of *Lysathia* sp. In particular, this project uses bomb calorimetry and quantum dots to track digestion efficiency and gut passage times when feeding under variable temperature and carbon dioxide conditions. This work attempts to predict how the metabolism, digestion and thus, performance of *Lysathia* sp. in controlling *M. aquaticum* might change under an altered climate. At the time of writing this report, Batandwa's experiments were still underway.

In early 2020, Perryn Richardson and Henrika Bosua from Stellenbosch University, collected respirometry data in Prof. Terblanche's laboratory at Stellenbosch University from *Lysathia* sp. individuals exposed to two different constant temperatures. This experiment was designed based on prior pilot studies conducted in 2019 by Candice Owen and Henrika, using a different agent. The data indicated that the respiratory rate of the insect differs, depending on the thermal environment, and showed that digestion requires less energetic expenditure under hot conditions than colder ones. Digestion efficiency thus improves under hotter conditions, explaining why the insects perform better. Specific respiratory rates calculated for each temperature from these experiments can now be factored into other experiments to explain the observed results.

Perryn Richardson working on *Lysathia sp.* body macronutrient assays in the Applied Physiological Ecology laboratory at Stellenbosch University. **Photo:** Perryn Richardson

Lysathia adult on Parrots feather. **Photo:** Lynette Ruddman

Brazilian waterweed

Over the last seven years, the CBC targeted for biological control one of the worst submerged aquatic weeds in South Africa, *Egeria densa*, commonly known as Brazilian waterweed, or Brazilian elodea. It is a rooted, submerged macrophyte that grows vigorously in slow-moving water. The control agent, the Egeria fly (*Hydrellia egeriae)* was imported into South Africa in 2014 for quarantine host-specificity testing. Results showed that the leaf-mining fly is specific and damaging, and it was first released in South Africa in October 2018. The biology of the fly is similar to *Hydrellia pakistanae* and *H. balciunasi,* which were released as biological control agents for the submerged weed, *Hydrilla verticillata* in the USA. Female *H. egeriae* oviposit eggs on protruding leaves, and after eclosion, the fully aquatic larvae mine the photosynthetic tissue of the leaves. Adults emerge and float on the water surface in an air bubble. They feed on dead or trapped insects on the water surface, or pollen and sugary substances. *Hydrellia egeriae* is the first biological control agent released against a submerged aquatic weed in South Africa, and the first invertebrate released against *Egeria densa* in the world.

Since the release and establishment of the fly in South Africa, a number of post-release studies are underway. As part of her PhD research, Rosali Smith, conducted investigations into the thermal physiology of *H. egeriae*. Her results showed that both adults and larvae have a wide thermal range, being able to survive freezing temperatures and warm temperatures of > 40°C. In addition to these favourable attributes for agent establishment, climatic modelling has shown that the biological control agent is able to complete multiple generations at *E. densa* invaded sites in South Africa.

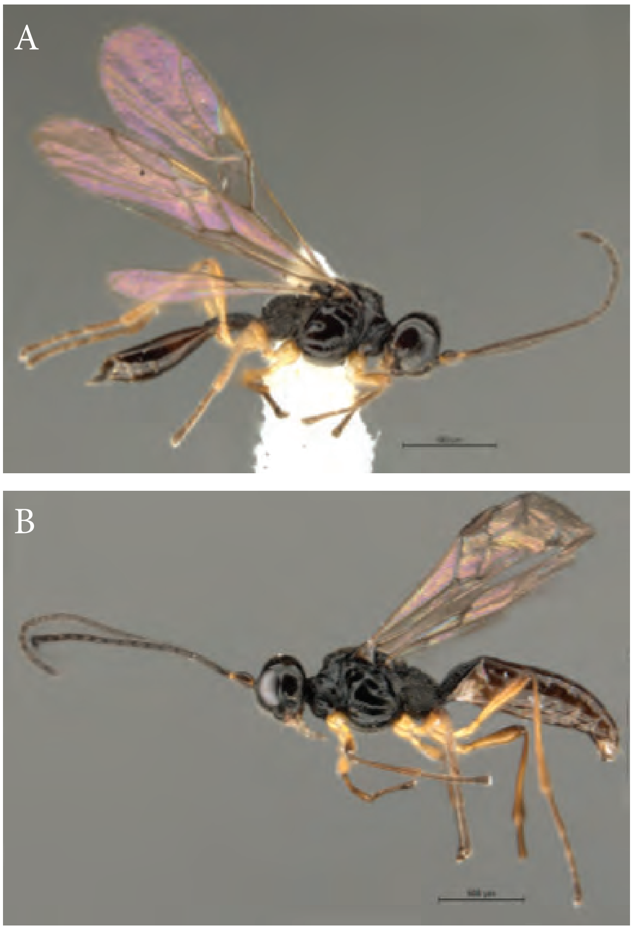
Ground-truthing these predictions through post-release surveys showed that *H. egeriae* established at both sites where releases where made: the Nahoon River, East London and the Midmar Dam, KwaZulu-Natal. Only one release was done in the Nahoon River (October 2018), whereas multiple releases were done in Midmar Dam. Augmentative releases significantly affected the population size of *H. egeriae* between the two sites.

Although the number of release events contributed to agent field populations, post-release surveys also showed that the biological control agent acquired novel parasitoids. These parasitoids were collected in the field and identified as wasps, *Ademon lagarosiphonae* and *Chaenusa seminervata* (Figure x). They are both native parasitoids to the fly *Hydrellia lagarosiphon*, a specific herbivore to *Lagarosiphon major*, an indigenous South African submerged plant.

RESEARCH AND TECHNICAL STAFF
Prof. Julie Coetzee, Prof. Martin Hill

STUDENTS
Rosali Smith (PhD)

COLLABORATORS
FuEDEI



The parasitoid wasps *Chaenusa seminervata* (a) and *Ademon lagarosiphonae* (b). **Photo:** Simon van Noort (Iziko Museum)

Dense monoculture stand of flowering Brazilian waterweed in the Nahoon River, East London. **Photo:** Rosali Smith

Delta arrowhead

Another new macrophyte target for biological control in South Africa is *Sagittaria platyphylla,* also known as delta arrowhead. *Sagittaria platyphylla* is an emergent freshwater aquatic macrophyte that has become an invasive weed in freshwater systems in South Africa. The plant was first discovered in the Kranzskloof Nature Reserve, KwaZulu-Natal, in 2008, followed by the Makana Botanical Gardens, Makhanda, Eastern Cape, in 2009, from where it has spread. *Sagittaria platyphylla* is already regarded as one of the fastest spreading invasive species in the country; the plant's ability to reproduce sexually and asexually contributes to its ability to spread.

The weevil, *Listronotus appendiculatus,* has been identified as a promising candidate for *S. platyphylla* control, and has been imported into quarantine at the Centre for Biological Control (CBC). Pre-release studies (biology, host-specificity and impact trials) have been completed, and results indicate that the fruit- and flower-feeding weevil is a suitable biological control agent for *S. platyphylla* in South Africa. The weevil damages the fruits and flowers of the plant, reducing the chances of spread between catchments, and may significantly reduce the spread of the weed within catchments, thereby greatly contributing to management operations.

RESEARCH AND TECHNICAL STAFF
Prof. Julie Coetzee, Prof. Martin Hill, Dr Grant Martin

STUDENTS
Mpilonhle Ndlovu (MSc), Daniel Rogers (MSc)

COLLABORATORS
DPI, USACE, SANBI

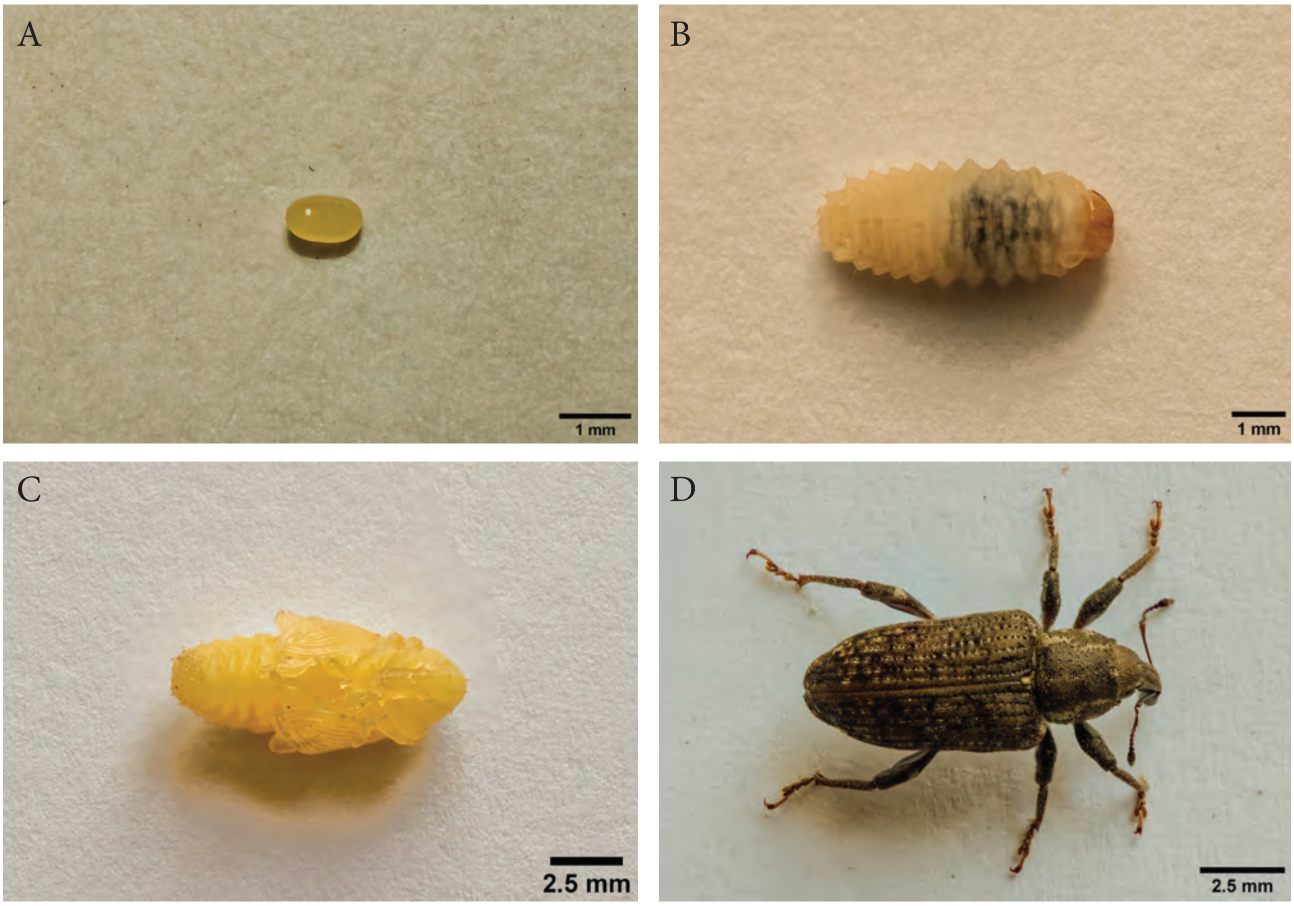
Host-specificity testing conducted in South Africa on closely related native species as well as alien ornamental species found in South Africa show that the weevil is specific to its host plant, *S. platyphylla*. From the host-specificity studies concluded, the CBC suggested that *L. appendiculatus* is safe for release and will assist in the management of *S. platyphylla* in South Africa. As such, a release application was submitted to the Department of Agriculture, Land Reform and Rural Development, in October 2020, and their decision is still pending.

Makana Botanical Gardens infestation of delta arrowhead. **Photo:** Daniel Rogers

The flowers and fruit of delta arrowhead, are a primary source of food for the weevil *Listronotus appendiculatus*. **Photo:** Daniel Rogers

During 2020, the life history characteristics of *L. frontalis* were determined. The weevil completes four larval stages, and the entire life cycle takes approximately 42 days to be completed, from egg to ovipositing adults. On average, adult weevils live for 67 days. Impact studies also indicated that adult herbivory significantly affected plant health.

Field surveys of delta arrowhead populations showed that populations were healthier and more prolific in high nutrient sites, as well as sites that had more shelter from direct sunlight. Analysis of factors affecting *S. platyphylla* parameters showed significant relationships between tuber numbers and the environmental factors, water depth and seasonality, but no other factors. A separate experiment showed that a higher percentage of tubers grew in coarse sediment than in finer sediment.



Some developmental stages of *Listronotus frontalis*: a) a newly laid egg, b) a third instar larva, c) a pupa, d) an adult weevil. **Photos:** Daniel Rogers

Yellow flag iris

A biological control programme was initiated on the wetland weed, *Iris pseudacorus* (yellow flag Iris) in 2017, and since then, significant progress has been made through collaborative research with the Vreije Universiteit, Brussels, and Conicet, Argentina. *Iris pseudacorus* is an herbaceous perennial wetland plant, native to Europe, western Asia and northwest Africa, introduced into South Africa as an ornamental plant. It has been recorded from a number of localities in the country, most notably from Gauteng, KwaZulu-Natal and Mpumalanga. It is a Category 1a invader under NEMBA, and is an eradication target of the SANBI Invasive Species Programme. Owing to its rapid regenerative growth and ability to spread via seeds, and the fact that its range is now well beyond what it was ten years ago, biological control is being considered as an option in South Africa.

The attractive yellow flowers of *Iris pseudacorus*, lead to its introduction as an ornamental plant in South Africa. **Photo:** Julie Coetzee

Seed germination

In 2020, Emma Sandenbergh completed the fieldwork for her MSc project, collecting data on distribution, density and reproduction for yellow flag iris across South Africa. She found the plant in all provinces except the Northern Cape, with most occurrences in urban areas. During these trips, Emma collected seeds and assessed germination rates in the laboratory. The mean proportion of germinated seeds was 83%, but this is likely to be higher as a large number of seeds germinated prior to the experiment and were therefore left out of the analysis. The germination rates of *I. pseudacorus* seeds in South African populations appears to be higher than that reported in its native range, but possibly lower than that reported in the United States, where it is also invasive.

RESEARCH AND TECHNICAL TEAM
Prof. Julie Coetzee, Samella Ngxande-Koza

STUDENTS
Emma Sandenbergh (MSc), Gianmarco Minuti (PhD), Paula Gervazoni (PhD)

COLLABORATORS
Vreije Universiteit Brussels (VUB), FUEDEi, Conicet

Surveys for natural enemies of *Iris pseudacorus* in the native and invaded range

The first native range surveys for natural enemies of *Iris pseudacorus* led to the identification of three potential candidate biological control agents, namely the sawfly, *Rhadinoceraea micans*, the seed weevil, *Mononychus punctumalbum*, and the flea beetle, *Aphthona nonstriata*. The potential of these species as candidate biological control agents was evaluated based on their habitat adaptability, damage (type and extent), phenology of attack, and inferred host range. The flea beetle, *A. nonstriata,* was given higher priority, and a population of this species was imported to quarantine at the CBC, where it is currently undergoing host-specificity testing. Importation and establishment of the two remaining candidates is expected shortly.

Additional surveys are ongoing, in both the native and invaded ranges (South Africa, Argentina) in an attempt to find evidence of enemy release, data regarding plant architecture, vegetative growth, sexual reproduction potential; associated fauna and flora will also be compared. Within the invaded range, control sites are visited in order to assess the ecological impact of the weed on native vegetation and invertebrate communities. In order to assess the feeding preferences and short-term survival of *A. nonstriata*, a preliminary no-choice experiment was conducted in the CBC quarantine facilities. A total of 18 plant species, chosen from native South African and South American Iridaceae, as well as important economic irises, were tested.

Full development trials of *A. nonstriata* have been carried out on the following test plants: *Dietes grandiflora, D. bicolor, D. iridiodes,* Louisiana iris, *Neomarica gracilis, Aristea ecklonii, Watsonia angusta, Watsonia borbonica* and garden blue iris. The results showed no emergence of the first generation following no-choice tests. However, *Belamcanda chinensis*, now named *Iris domestica*, a close relative or *I. pseudacorus*, did support development to adult under no-choice conditions. Choice tests are now underway to determine further specificity. No-choice trials with a number of other Iridaceae are currently underway.

Paula Gervazoni sampling yellow flag iris in Gauteng Province, South Africa, for invertebrates and signs of damage, to observe if there are native herbivores attacking the plant in South Africa and compare them with the community found in Europe. **Photo:** Gianmarco Minuti

A range of species were used in the host specificity testing of Aphthona nonstriata in the CBC quarantine facility. Eighteen Iridaceae species from the introduced ranges in South Africa and Argentina were tested, being selected by relatedness to *Iris pseudacorus* and/or economic importance. **Photo:** Paula Gervazoni

Gianmarco is also exploring the potential eco-climatic niche of the yellow flag iris in South Africa and other invaded countries, using the results to assess its invasion potential as well as to refine the search for climatically matched areas within the native range of the plant to prioritise future surveys for natural enemies. In addition to that, the potential climatic distribution of the currently prioritised agents is being examined.

Thermal requirements of *A. nonstriata* were assessed by conducting a short experiment that determined the critical thermal minima and maxima, as well as the lower and upper lethal temperatures. This information will be associated with thermal characteristics of the invaded range and used to evaluate the possible future establishment of the agent in selected areas, complementing niche modelling.

Iris pseudacorus distribution in the invaded range

Paula Gervazini, a PhD student based in Argentina, has developed an online questionnaire to gain more insight into the current worldwide distribution of *Iris pseudacorus*. The questionnaire was distributed on social media in 2020, allowing her to obtain hundreds of new reports of the iris in the invaded range South Africa – Argentina – New Zealand, as well as citizen opinions on different topics, including biological control and invasive alien species.

Mexican water lily

The attractive flowers of the Mexican water lily (*Nymphaea mexicana*) contributed to its introduction to South Africa through the ornamental plant trade. Native to Mexico and the southern parts of the United States of America, this plant has spread rapidly in dams, ponds and rivers around South Africa. In 2016, the CBC embarked on a programme to investigate a biological control option for this floating weed. Pre-release surveys on Mexican water lily began in early March 2020 to determine the state of a number of sites invaded by the lily in Gauteng, Eastern Cape, and Western Cape. The plan was to survey each site twice in the year, but travel plans were altered as a result of the COVID-19 restrictions. The Gauteng sites were surveyed once in March 2020 and again in December 2020. Eastern Cape sites were surveyed in September 2020 and again in January 2021. Western Cape sites were surveyed in September 2020 and will be surveyed again in February 2021. These surveys yielded information on current coverage at the sites, estimates of water parameters, and the presence of insect assemblages on the invasive lily populations.

An important note in 2020 was the discovery of the native weevil, *Bagous longulus* (Coleoptera: Curculionidae), sustaining populations on and causing damage to *N. mexicana* at a site in Port Elizabeth in the Eastern Cape. The native water lily, *Nymphaea nouchali*, is known to host *Bagous longulus* but may show promise as a biological control agent of *N. mexicana* through the development of a new association between the species. Attempts are currently being made to establish a culture of *B. longulus* at Waainek mass-rearing facility, and host specificity and impact studies will be conducted to establish its suitability as a potential biological control agent.

In August 2020, attempts were made to import another potential biological control agent, the weevil, *Bagous americanus*, from its native range of southern USA. Unfortunately, delays in couriering these insects, and difficulty in rearing the few adults that survived transportation in quarantine led to failure to establish a culture. Further attempts to establish cultures will have to be delayed until springtime in the USA.

RESEARCH AND TECHNICAL TEAM
Prof. Julie Coetzee, Prof. Martin Hill

STUDENTS
Megan Reid (MSc)

COLLABORATORS
USDA-ARS, Florida, Louisiana State University

Intaka Island, Century City, Western Cape (top) where no weevils (*Bagous longulus*) were present, and growth of Mexican water weed was extensive; as compared to the site at King's Beach skate park, Port Elizabeth, Eastern Cape, where hundreds of weevils were present, and growth was considerably less extensive. **Photo:** Megan Reid

Pickerelweed

RESEARCH AND TECHNICAL TEAM
Prof. Julie Coetzee, Prof. Sjirk Geerts

STUDENTS
Sage Wansell (MSc)

COLLABORATORS
Cape Peninsula University of Technology (CPUT)

Pontederia cordata, commonly known as pickerelweed, is an invasive emergent waterweed originating from North and South America that has had detrimental environmental, agricultural and socio-economic impacts in South Africa. Sage Wansell investigated the invasive ecology of pickerelweed in South Africa as part of her MSc by determining the population genetics, pollination ecology and floral traits of invasive populations throughout the country. The results of genetic studies using phylogenetic analysis of over 130 leaf samples collected throughout the Western Cape, Eastern Cape, Gauteng and KwaZulu-Natal indicated that genetic diversity of pickerelweed populations is low within and amongst invasive populations in South Africa compared to native populations in the USA. This finding suggests that sexual reproduction is not present in invasive populations, and that only a single or very few introductory events have occurred in South Africa. Invasive pickerelweed populations shared the highest genetic similarity with native samples from Belle Haven, Virginia, USA; further sampling and future genetic studies should be conducted in this area to identify source populations to survey for potential biological control agents. DNA analyses are currently underway to determine what variety of pickerelweed is present in South Africa.

Pickerelweed has three flower forms; preliminary field surveys suggest that only one of the three tristylous flower forms of pickerelweed, namely the short, morphed plant, is invading South Africa, but no seeds have been observed in any invasive populations. Pollination studies tested the speculation that the absence of native insect pollinators (such as the specialist bumblebee from the USA) may be responsible for the absence of sexual reproduction and seed production in the invasive range. However, generalist insect pollinators are present. Artificial, hand-pollination experiments on 8865 flowers were conducted to determine whether an incompatibility system was present which prevented seed production. No seeds were produced, and it was concluded that illegitimate pollination of the short-morphed plants prevented seed production due to self-incompatibility. Rhizomes are therefore responsible for the invasion of *P. cordata* throughout South Africa.

This research considered possible management strategies such as biological control and recommended control programmes should target the plant rhizomes to prevent and reduce spread. Preventing the introduction of medium- and long-morphed plants into South Africa is crucial to prevent *P. cordata* from producing seeds and intensifying invasion further through both asexual and sexual spread. Surveys in the native range should also target potential biological control agents that cause damage to the rhizomes of pickerelweed plants because rhizomes are the reproductive organ responsible for the rapid spread of pickerelweed in South Africa.

Future studies should be considered, such as determining the extent to which pickerelweed rhizomes can be fragmented and continue to grow (this may help us understand how pickerelweed spreads so efficiently via rhizomes), and rhizome desiccation experiments that may provide insight into how tolerant rhizomes are of climatic changes (such as waterbodies drying up). It is assumed that rhizomes are spread through humans planting them and from flooding events.

Lagarosiphon major

In 2018, Manaaki Landcare Research, New Zealand, approached the CBC to initiate research into the biological control of *Lagarosiphon major*, a submerged aquatic macrophyte indigenous to South Africa, but highly invasive in New Zealand's lakes. Previous surveys with collaborators from University College Dublin, Ireland, identified two potential control agents, the fly, *Hydrellia lagarosiphon* (Ephydridae), and the midge, *Polypedilum tuburcinatum* (Chironimidae) occurring with *L. major* in South Africa. Subsequent host-specificity testing has shown both species to be suitably host specific to *L. major* for use in Ireland (although biological control is not currently practised by the European Union). The collaboration with New Zealand aims to determine host specificity further, including species indigenous to New Zealand. Nompumelelo Baso is conducting this research towards to her PhD.Nompumelelo's study aims to investigate the role of the Enemy Release Hypothesis (ERH) on the invasiveness of *L. major* in New Zealand and will be achieved by measuring and comparing plant parameters (biomass and cover) between South Africa (native range) and New Zealand (invaded area).

The study will also quantify the rate of parasitism of the candidate biological control agent, a fly, *Hydrellia lagarosiphon,* by a braconid parasitoid in South Africa in order to predict its performance in the introduced range, which will be the enemy-free space. Ultimately, the study will determine whether *Polypedilum tuburcinatum* and *Hydrellia lagarosiphon* are safe and effective enough to be used as biological control agents of *L. major* in New Zealand.

RESEARCH AND TECHNICAL TEAM
Prof. Julie Coetzee

STUDENTS
Nompumelelo Baso (PhD)

COLLABORATORS
Manaaki Landcare Research, New Zealand

Nompumelelo Baso sampling *Lagarosiphon major* at Rosetta Village Dam in KwaZulu-Natal. The submerged indigenous *Lagarosiphon major* looks similar to the invasive *Egeria densa*, but can be distinguished by the curly leaf structure.
Photo: Nompumelelo Baso (top) and Wandisile Mdiza (bottom)

CACTACEAE WEEDS

Biological control of cactus weeds has a long and well-documented history of success in South Africa. The very first biological control agent released against any weed in the country was against the cactus weed, *Opuntia monacantha*, commonly referred to as drooping prickly-pear. Like many cactus plants that become overabundant outside of their indigenous distribution, drooping prickly-pear restricted the movement of livestock and wildlife through areas that it infested, reduced grazing capacity, reduced indigenous plant diversity and caused direct harm to wildlife and livestock because of its sharp spines. The agent, a cochineal insect, *Dactylopius ceylonicus*, was released in 1913 and very quickly reduced the density and distribution of the weed to a fraction of what it had been, opening up land for agriculture and protecting the indigenous diversity of the area. This control has been permanent, and although *O. monacantha* is still present in the Eastern Cape today, it is kept at such low levels that it is not considered problematic.

Many other successes have been recorded over the past 107 years, but new problem cacti are constantly arriving in the country, mostly due to the global trade in cacti as ornamental plants. Biological control agents are highly specific to their target weeds, so new agents are required for these new weeds, or they will become increasingly problematic in time. The CBC Cactus Biological Control Programme aims to ensure that biological control agents for cactus weeds that are already available in the country are utilised to their full potential, and that new biological control agents are developed for cactus weeds that do not have effective agents. The programme emphasises post-release evaluation with the aim of implementing biological control effectively and monitoring its success.

The overall aim of the project is to use biological control to control cactus weeds safely and effectively for the benefit of land-users, such as farmers, and to protect South Africa's indigenous biodiversity, employing a combination of scientific research, implementation and community engagement. The team is split across four locations and includes a large team at the Kariega mass-rearing facility, Rhodes University main campus, Wits University, and ARC-PHP.

PROGRAMME HIGHLIGHTS IN 2020

- Post-release evaluations of cactus agents released by the CBC cactus mass-rearing team have effectively reduced the target weed populations. Through a questionnaire study that forms part of Zezethu Mnqeta's PhD, land-users confirmed that the agents have been beneficial.
- New potential biological control agents for three of the worst cactus weeds in South Africa have been sourced in Argentina and imported into quarantine at Rhodes University for host-specificity and impact studies.
- Permission for the release of a new biological control agent, the cochineal, *Dactylopius tomentosus* '*californica* var. *parkeri*', for the control of the thistle cactus, *Cylindropuntia pallida*, has been granted in South Africa.
- An application to release the leaf cactus, *Pereskia aculeate,* biological control agent, and the stem-wilting bug *Catorhintha schaffneri*, has been submitted for possible release in Australia.
- Funding received from Red Meat Research and Development South Africa for research into biological control on *Opuntia elata*.

Quantifying the benefits of cactus biological control

RESEARCH TEAM
Prof. Iain Paterson, Ruth Scholtz

STUDENT
Zezethu Mnqeta (PhD)

Biological control of cactus weeds is generally very successful, and control is achieved much more quickly than for most other target weed species. The limiting factor for cactus biological control is dispersal of the agents. Biological control agents for invasive alien cactus weeds are either cochineal insects of the genus *Dactylopius* or galling mealy bugs in the genus *Hypogeococcus*. Both make excellent biological control agents if they are released on the correct target weed; but if they are released on the wrong weed species, they are ineffective; they are all very poor dispersers and are unlikely to get to new sites without assistance. Mass-rearing and releasing the correct agent at the correct site is therefore essential for the success of cactus biological control in South Africa.

Ruth Scholtz manages a team of eight people who are responsible for mass-rearing and redistributing cactus weed agents across South Africa. The mass-rearing facility is located at the old ARC-PPRI building near the Spring Resort outside Kariega (Uitenhage), where the agents are reared in several greenhouse and shade-houses (see page 64 for further details). Six biological control agents for the control of nine target cactus weeds are mass-reared at the facility. Land-users, such as farmers and conservationists, send requests for agents to the CBC and the team at Kariega will either travel to the site to conduct a release, or send a smaller consignment of the agent via courier if the site is far from the facility and travel is not possible.

While getting the agents out into the field is the main objective of the cactus mass-rearing facility, it is also essential that the benefits of these releases are quantified. Zezethu Mnqeta, who is registered as a PhD student with the CBC, has been quantifying the benefits of these biological control releases with the help of Ms Scholtz and her team. As a first step, every person who receives agents from the cactus facility is asked to complete a questionnaire about the status of the cactus weed on their land and what they expect from biological control. Follow-ups are then made with all land-users, who provide an assessment of how well they perceive the biological control to have worked in reducing their cactus problem. The results of these questionnaires overwhelmingly support the fact that biological control has been effective in reducing cactus invasions in almost all cases, and that land-users have benefited from the releases.

Zezethu, Ruth and the team also quantify the changes in agent and cactus density at selected sites that are close enough to the mass-rearing facility to allow for bi-annual follow-up assessments. Transects or plants are marked at the sites and the abundance of both the plant and the agent are monitored over time. While for some cactus species the results vary, depending on climate, in the vast majority of cases there is an increase in abundance of the agent followed by a decrease in cactus density. These data therefore corroborate the perception of the land-users who have completed the questionnaire surveys, and confirm that the releases of the biological control agents have successfully controlled the cactus weeds.

Fifty-seven sites have been monitored for ten species of target weed over the past four years, and most sites are visited every six months. Monitoring will continue at all these sites, and target weed species with only a few sites being monitored will be prioritised for further releases and monitoring. The overall objective is to quantify the long-term reduction in cactus weeds associated with the releases of biological control agents conducted by the CBC. The results so far have clearly indicated that the releases have been beneficial and that biological control effectively reduces cactus infestations.

Zezethu Mnqeta with Byron Scoetland, counting recording parameters (number of cladodes and cochineal-infested cladodes) on *Cylindropuntia imbricata* for long-term monitoring data at Awies's Farm in Graaff-Reinet. **Photo:** Chumakwande Makehle

Developing new agents for round-leaved prickly pears in South Africa

RESEARCH TEAM
Prof. Iain Paterson, Prof. Marcus Byrne, Hildegard Klein, Nic Venter, Dr Samalesu Mayonde (Postdoc), Phillippa Muskett

STUDENT
Zanele Machimane (MSc,Wits), Kedibone Mofokeng (MSc)

COLLABORATORS
FUEDEI, CABI

Opuntia engelmannii, commonly known as round-leaved prickly pear, is considered one of the most problematic invasive alien cacti in South Africa. Many other cacti were more problematic in the past, but most of these are now under effective biological control. *Opuntia engelmannii,* however, does not have an effective biological control agent and is becoming increasingly problematic; it is also one of the least understood species of *Opuntia*, a genus itself that many botanists regard as the least understood of all plant genera. The result is taxonomic confusion in the indigenous distribution as well as in South Africa, where the plant is invasive. At least three varieties of *O. engelmannii* were recognised in South Arica until very recently when Samalesu Mayonde of Wits University initiated a DNA study to identify the origin of each of these three varieties. This study led to the startling discovery that one of the *O. engelmannii* varieties, called the Eastern Cape *O. engelmannii* variety because it is so abundant in that province, is in fact a South American species of cactus, *Opuntia megapotamica*. *Opuntia engelmannii* is indigenous to North America, so biological control practitioners have been trying to source agents for this weed in the south-western USA for many years. Samalesu's DNA analysis indicates that South America is a better place to look. Two lineages of cochineal were collected off very close relatives of *O. megapotamica* in Argentina in early 2020. With the help of our collaborators at FUEDEI, the cochineal have been successfully imported into quarantine at Rhodes University and are under evaluation for their efficacy against *O. megapotamica*. If either of these cochineal genotypes is suitably damaging, host-specificity testing will be conducted, and the agent will be considered for release.


Opuntia engelmannii, also know as round leaf cactus. **Photo:** Iain Paterson

Further DNA analysis confirmed another variety of *O. engelmannii,* the Northern Cape/Free State variety, as a North American species, but whether it should be called *O. engelmannii* is still debatable. This variety was successfully controlled using the same cochineal insect that is used for another North American weed, *Opuntia stricta*. The 'stricta' lineage of the cochineal insect *Dactylopius opuntiae* which was originally collected in the USA and now effectively controls *Opuntia stricta* in South Africa, was also very effective at controlling the Northern Cape/Free State variety of *O. engelmannii* at a huge infestation near Douglas in the Northern Cape. Experiments to repeat this success in other parts of the country, such as the Eastern Cape, are underway. If these releases prove to be effective, the 'stricta' lineage may be an effective agent for the Northern Cape/Free State variety of *O. engelmannii*, which is one of the most problematic of all the round-leaved prickly-pears.

Understanding the taxonomy of target weeds is essential for successful biological control, and with difficult taxa, such as the round-leaved prickly-pear species in South Africa, genetics has proved to be a useful tool to resolve these issues. In this case, genetic techniques have helped resolve the identification of two of the worst cactus weeds in South Africa, and will, we hope, result in effective agents being sourced for both of them.

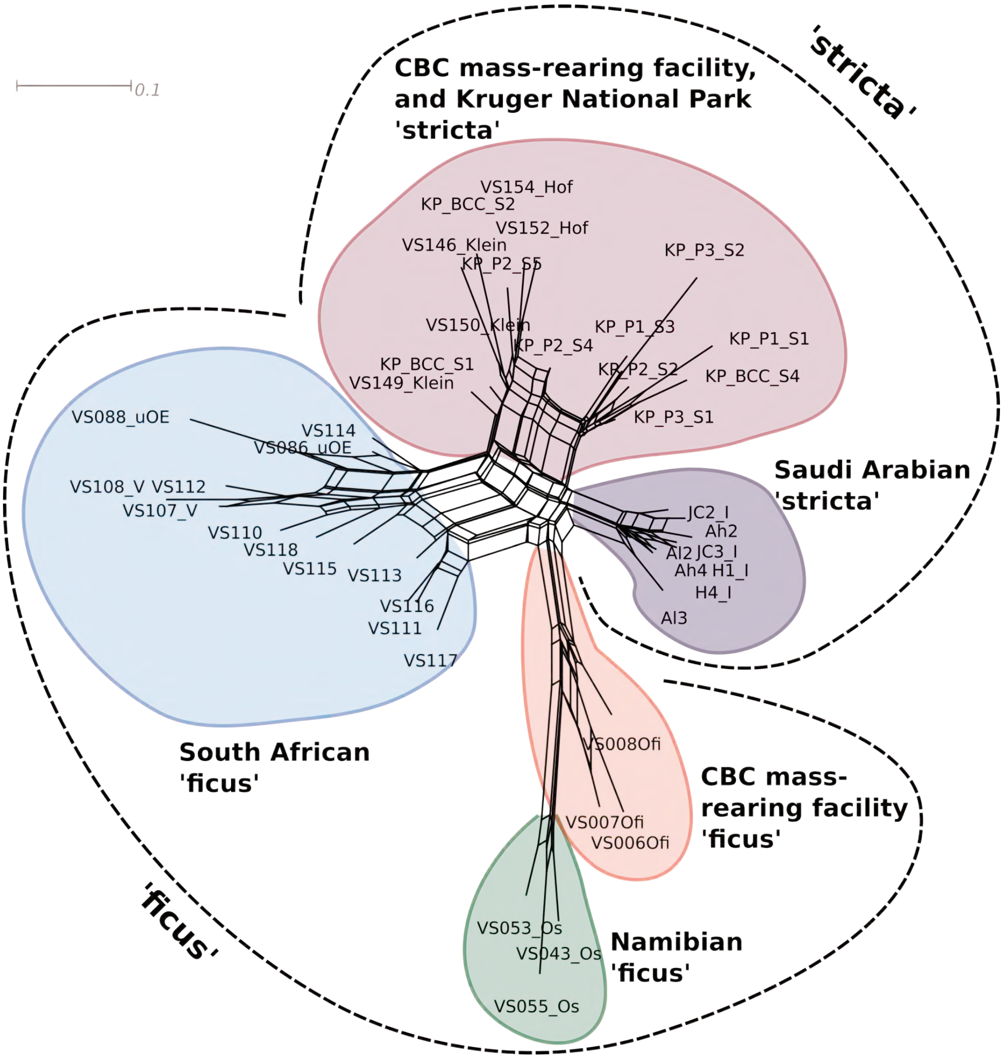
DNA barcoding of biological control agents

RESEARCH TEAM
Prof. Iain Paterson

STUDENT
Clarke van Steenderen (MSc)

COLLABORATORS
Queensland Department of Agriculture, Australia

The different species of cochineal used for biological control are morphologically very similar in appearance to anyone not trained in *Dactylopius* taxonomy. Even expert taxonomists struggle to identify the different species. Within these cochineal species, there are distinct lineages that are particularly damaging to certain target weeds and not to others. These lineages are morphologically indistinguishable from each other. South Africa and Australia both utilise multiple species and lineages of cochineal for cactus biological control, and being able to identify the lineage and species quickly and accurately is essential for research and implementation purposes. Clarke Van Steenderen, who graduated with his MSc at Rhodes University through the CBC in 2020, solved this problem by developing DNA barcoding techniques to identify all the lineages and species of cochineal used for cactus weed control in South Africa and Australia. This work was done in collaboration with the Queensland Department of Agriculture and has resulted in a publication in the journal *Biological Control*. The methods developed in this study have already proved useful, highlighting cochineal cultures that have been contaminated with other lineages of cochineal, and identifying cochineals that are either effective or ineffective at controlling cactus species in the field.



A relationship tree showing that two cochineal lineages within the same species are genetically different (Dactylopius opuntiae “ficus” and “stricta”) based on a DNA fingerprinting method (ISSR). **Credit:** Clarke van Steenderen

Orange-tuna cactus

Opuntia elata, orange-tuna cactus, was selected as a target for biological control because it is closely related to other very problematic invasive Cactaceae in South Africa, and the invaded distribution in South Africa appeared to be increasing rapidly, suggesting it is likely to become more and more problematic in future if it is not controlled. Phillippa Muskett, a PhD candidate with the CBC, started working on the biological control of *O. elata* in 2019. The first step in the biological control programme, surveys of the target weed in South Africa, revealed 30 previously unrecorded sites, bringing the total number of recorded sites in South Africa to 52. These surveys also confirmed that none of the cochineal lineages currently released in South Africa were damaging *O. elata* in the field. Surveys of the native region were therefore planned so that new biological control agents could be sourced to control *O. elata.Opuntia elata* is native to Argentina, Uruguay and southern Brazil. In February 2020, a native range survey was undertaken in Argentina and Uruguay. In some cases, insects found on closely related Cactaceae have been more effective biological agents than those found on the actual target weed, so the close relative of *O. elata*, *Opuntia rioplatense*, was included in the surveys. In Argentina, genetic samples of *O. elata* and *O. rioplatense* were collected at 15 sites and cochineal was collected off *O. elata* at one site and *O. rioplatense* at two sites. In Uruguay, genetic samples were collected at 14 sites but no cochineal was found.

The permits to transport the cochineal populations to South Africa were approved, and in early December 2020 the cltures of cochineal, which had been housed at the FuEDEI laboratories in Argentina since the field trip, arrived in South Africa where they are currently in quarantine at Rhodes University. Genetic testing will help confirm the identification of the cochineals collected in Argentina, and trials to determine which of these are most promising as biological control agents are underway. If one of these promising potential agents proves to be a suitable biological control agent, it will be released and, we hope, will bring populations of *O. elata* in South Africa under control before they become more problematic. The Red Meat Research and Development fund agreed to support this research with additional sponsorship.

RESEARCH TEAM
Phillippa Muskett, Prof. Iain Paterson

COLLABORATORS
FUEDEI

Cochineal (*Dactylopius ceylonicus*) in Entre Rios, Argentina, where CBC researchers, Iain Paterson, Tamzin Griffiths and Phillippa Muskett, undertook fieldwork in early 2020 to search for a potential biological control agent for Orange tuna cactus (*Opuntia elata*). This cochineal insect is considered a promising candidate agent and was imported into the CBC quarantine where it is under evaluation. **Photo:** Phillippa Muskett

Leaf cactus

Pereskia aculeata is a creeping cactus unlike typical cacti in that it has well-developed leaves and a woody, rather than succulent, stem. It is also known as leaf cactus. It is indigenous to South America and is a serious problem in both South Africa and Australia. Outside of the indigenous distribution, this plant forms dense and impenetrable spiny thickets that displace indigenous plants, resulting in changes to the functional composition of ecosystems and a reduction in biodiversity. Two biological control agents have been released in South Africa to control *P. aculeata:* the flea-beetle, *Phenrica guerini*, which was released in 1991, and the stem-wilting bug, *Catorhintha schaffneri*, which was released in 2014. *Phenrica guerini* has effectively reduced the invasiveness of *P. aculeata*, and at some sites has reduced densities of the plant significantly, although a sustained impact is required over many years to achieve this level of control. *Catorhintha schaffneri* has established at very few sites in the country, but we anticipate that establishment success will improve now that the drought in KwaZulu-Natal, where the climate is most suited for this agent, has broken, and the CBC has made new releases of the agent. Both agents are of interest to Australian biological control research scientists from the Department of Primary Industry (DPI) in New South Wales. *Pereskia aculeata* is a problem in Australia, but it is less problematic than in South Africa, suggesting that the negative impacts in Australia may increase in time. Releasing a biological control agent before the weed becomes a more serious problem in Australia would be very beneficial in protecting Australia's indigenous ecosystems.

The CBC, in collaboration with DPI Australia, conducted host-specificity testing of *C. schaffneri* for release in Australia, and has submitted an application for release of this agent to relevant authorities. As soon as international travel to Australia is possible, the agent will be transported to quarantine in Australia and released if and when the Australian authorities accept the application for release.

Host specificity testing of *P. guerini* is currently underway and is being led by CBC MSc student, Elizabeth van der Merwe. When this testing is completed, we will submit an application for release in Australia for *P. guerini*.

RESEARCH TEAM
Phillippa Muskett, Prof. Iain Paterson

STUDENT
Elizabeth van der Merwe (MSc)

COLLABORATORS
Department of Primary Industries, Australia

Phillippa Muskett using an innovative method to spot the stem wilter, *Catorhintha schaffneri* on leaf cactus in East London. **Photo:** Iain Paterson

Catorhintha schaffneri adult on *Pereskia aculeata.* **Photo:** Phillippa Muskett

Torch cactus

The torch cactus (*Trichocereus spachianus*) is fast becoming a worrying invasive species, particularly in the dry savannah and Karoo biomes in South Africa. *Trichocereus spachianus* is believed to originate in north-west Argentina and southern Bolivia, but its origins are uncertain. Extensive field surveys in Argentina failed to locate this plant in natural populations, suggesting that it is either extremely rare or does not exist in the country. There are also no confirmed records of *T. spachianus* existing in natural populations in its native distribution and the species was described from plants grown in the horticultural trade. This makes biological control of torch cactus particularly challenging because there are no populations of the target weed in the indigenous distribution from which to collect agents. Close relatives of the target weed must therefore be used as a source for potential agents.

There are currently no effective biological control agents for *T. spachianus* in South Africa. A PhD student from the CBC, Tamzin Griffith, conducted surveys for potential agents in Argentina in collaboration with the research team at FuEDEI in 2019 and 2020. Owing to its success in other cactus control programmes, we believe a galling mealybug in the genus *Hypogeococcus* to be potentially the most successful candidate for such a programme. Collection efforts were thus focused on insects in the genus *Hypogeococcus* that are associated with close relatives of *T. spachianus*. Five lineages of the undescribed species *Hypogeococcus* sp. were collected, as well as a single lineage of *Hypogeococcus spinosis* Ferris and *H. festerianus* Lizer & Trelles. Cultures of these populations were maintained at the FuEDEI laboratories in Argentina, and once permits were acquired were exported to South Africa where Tamzin has been conducting host-specificity tests and other important trials to establish their suitability for release as biological control agents for *T. spachianus* in South Africa.

RESEARCH TEAM
Prof. Iain Paterson

STUDENT
Tamzin Griffith (PhD)

COLLABORATORS
FuEDEI

The CBC team is looking for a potential biological control agent in the native range of this invasive cactus, *Trichocereus spachianus*, seen in Kariega (Uitenhage), Eastern Cape. **Photo:** Iain Paterson

Thistle cactus

The thistle cactus, *Cylindropuntia pallida*, is an emerging weed in South Africa. It is not problematic over a wide area, but is increasing in distribution and density very quickly. Biological control of thistle cactus using the cochineal insect, *Dactylopius tomentosus* '*californica* var. *parkeri*', has been very successful in Australia, so this agent is likely to be successful in South Africa, too.

The CBC submitted an application to release *Dactylopius tomentosus* '*californica* var. *parkeri*' in 2019; this application was accepted by the relevant authorities in South Africa in 2020. Unfortunately, the culture of *D. tomentosus* '*californica* var. *parkeri*' was contaminated by the widespread cochineal insect used for the control of another cactus weed, *Cylindropuntia imbricata*. The agent for *C. imbricata* is a different lineage of *D. tomentosus* and will likely hybridise with the new '*californica* var. *parkeri*' lineage, reducing the efficacy of the agent for *C. pallida*.

RESEARCH TEAM
Prof. Iain Paterson

STUDENT
Ekhona Zozo (MSc)

COLLABORATORS
Department of Primary Industries, Australia

A new consignment of *D. tomentosus* '*californica* var. *parkeri*' must be sourced from Australia to ensure that the agent that is released is effective. Once international travel is possible, a new consignment of the agent will be imported and released. The release of this agent is likely to be highly effective at controlling *C. pallida,* based on its success in Australia, but we must ensure that a pure culture of the correct cochineal is released.

Cylindropuntia pallida in Hotazel, Northern Cape. **Photo:** Iain Paterson

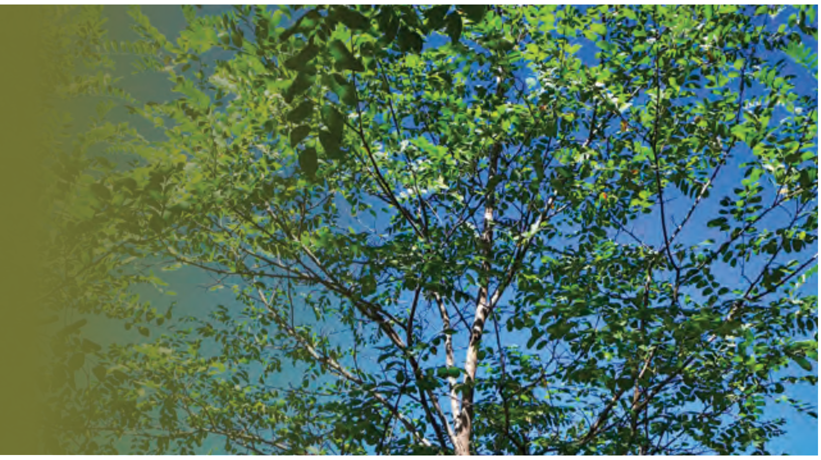
Collaborators

A strong collaboration with Fundação Universidade Regional de Blumenau (FURB) in Brazil and FuEDEI in Argentina is essential for this programme. Three of the main target weeds of the programme, *T. spachianus*, *O. elata* and *O. megapotamica*, are indigenous to Argentina and Brazil, and FuEDEI has facilitated and conducted field trips on which agents for these species were sourced. FuEDEI has housed these agents at its laboratories, and facilitated the importation of these potential agents into quarantine in South Africa.

Mike Day from the Queensland Department of Agriculture has collaborated to include Australian biological control agents in the DNA barcoding of the cactus biological control agents' project.

Andrew McConnachie from the New South Wales Department of Primary Industry is working in collaboration with the CBC to release leaf-cactus agents in Australia and has facilitated the importation of agents for thistle cactus.

NORTHERN TEMPERATE WEEDS



Historically, biological control efforts against invasive terrestrial trees and shrubs in South Africa have focused on species from the southern hemisphere, and there has been a dearth of research into invasions by, and control of, a number of tree and shrub species from the temperate regions of the northern hemisphere (northern temperate weeds). Northern temperate weeds are common, widespread and problematic in South Africa, particularly in high elevation grasslands. In addition, several new species, which may pose additional threats in these grassland regions, have been identified. The high elevation grasslands of South Africa are important systems for water security, as they provide nearly half of all the country's water run-off and are a key biodiversity hotspot with high plant endemism. Invasive alien plants are increasingly threatening both water security and biodiversity. The biome is also an invaluable resource, supporting livestock which are central to the livelihoods and economies of commercial, small-scale and communal farming/agriculture. These invasive plants are driving changes in native flora, thereby reducing the grazing capacity of these rangelands.

The Northern Temperate Weeds Programme is a relatively new programme, which aims to provide management options to try and reduce the negative impacts of these weeds. Thus far, research has focused on biological control feasibility and ecological and socio-economic impact studies on several northern temperate weed species, including *Pyracantha angustifolia, Rosa rubiginosa, Salix* spp., *Cotoneaster* spp. and *Rubus* spp. In addition, research conducted in the USA and Europe on the pests associated with two of these species, *Robinia pseudoacacia* and *Gleditsia triacanthos* (both native to the USA), have provided South African researchers with the necessary foundation to initiate programmes against these weeds. Research in South Africa is currently focused on pre-release studies on the biological control of *R. pseudoacacia*, using the leaf miner, *Odontota dorsalis,* and the locust midge, *Dasineura pseudoacacia*, and on *G. triacanthos*, using a seed bruchid, *Amblycerus robiniae*.

This programme has benefited from support of and collaboration with both local and international entities. In South Africa, close collaboration exists with the University of the Free State, Bloemfontein campus, and the Afromontane Research Unit based at the University of the Free State, QwaQwa campus, as well the ARC-PPRI. International collaboration has been developed between the CBC and Yunnan University China; the Department of Entomology, Virginia Tech Blacksburg; the Centre of the Region Haná for Biotechnological and Agricultural Research, Crop Research Institute, Czech Republic, and CABI Switzerland.

Invaded farmlands by Popular, Eucalyptus and wattle species in the eastern Free State. **Photo:** Grant Martin

PROGRAMME HIGHLIGHTS 2020

- Completion of feasibility studies on *Rubus* species as well as rosehip, *Rosa rubiginosa*.
- Prioritisation of insects associated with the black locust tree, *Robinia pseudoacacia,* in Europe.
- A review of firethorn, *Pyracantha angustifolia,* was published as the first article of *Invasive Plant Science and Management*

Black locust tree in Afromontane grasslands of South Africa

The growing invasion of Afromontane grassland ecosystems of South Africa by *Robinia pseudoacacia*, a tree from North America commonly known as black locust tree, is threatening this fragile and fast disappearing biome. Gerald Chikowore, a PhD student at the University of the Free State, undertook multi-scaled surveys to determine and quantify the impacts of this IAP in the eastern Free State, South Africa. With the species targeted for biological control, the biggest question was how well we understand impacts of this tree in a local context. Thus, Gerald assessed interactions between the invasive alien tree, between biotic and abiotic components, as well as the implications for human well-being. *Robinia pseudoacacia* functional traits such as phenology, canopy cover, tree diameter, and population demographics like population density were determined, together with their influence on abiotic components of the ecosystem, such as temperature and light availability (microclimatic conditions). Gerald evaluated the cascading effects of these environmental modifications on native vegetation and grassland arthropods as well as the interruption of key ecosystem services such as rangeland condition and pollination in agro-ecological ecosystems. Gerald has shown that *R. pseudoacacia* significantly lowers maximum temperatures and illumination in understory habitats. As a result, grass communities between invaded and uninvaded habitats differed by 96%, with understory communities dominated by invasive alien grasses. Invasion of grasslands also resulted in the elimination of Acridids (Orthoptera) in favour of detritivorous coleopterans. Veld condition was significantly lower in invaded habitats (180 ± 24.3) than uninvaded veld (401 ± 24.3) at one of the sites, compromising livestock production. The flowering phenology of *R. pseudoacacia* coincided with that of apples, a fruit of economic importance. This resulted in the sharing of pollinators, including honeybees, *Apis mellifera*. Thus, fruit production may be affected owing to inadequate pollination. All these impacts form a baseline for evaluation of future management interventions against *R. pseudoacacia* of which biological control will be significant as current attempts seem to be ineffective.

RESEARCH TEAM
Dr Grant Martin, Dr Frank Chidawanyika (UFS)

STUDENT
Abigail Wolmarans (PhD), Gerald Chikowore (PhD)

COLLABORATORS
Virginia Tech USA & CABI (Switzerland)

Selecting and prioritising agents for black locust tree

Numerous insects that attack an invasive weed are usually found in the insect's natural range; however, selecting host-specific and damaging insects out of these insects can be difficult and time consuming. PhD candidate Abigail Wolmarans identified high priority potential agents prior to collection, using all available literature, established prioritisation techniques, as well as rapidly developing citizen science.

She used three criteria in an attempt to determine the most promising candidates for further investigation: whether the insect was monophagous or not according literature; its feeding damage and guild (if it was a leaf-feeding insect, sucking insect or gall insect), and whether it was abundant and host-specific in areas outside of its native range. After applying these criteria, Abigail identified the five most promising candidates, including *Obolodiplosis robiniae,* the locust gall midge.

Having identified the most promising candidate agents, we undertook targeted field surveys in the native range as well as in areas where the species had been introduced outside their native range. Surveys showed *O. robiniae to* be specific and widespread in Europe, however, the midge was susceptible to parasitoids.

Shipments of *O. robiniae* were made to South Africa from Switzerland. Unfortunately, they were not received and the insects became dormant in the Northern Hemisphere. One shipment of other prioritised agents reached South Africa, but due to delays, most of the insects did not survive. Rearing the surviving insects was also unsuccessful. Insects will be collected when they become active again in the new season so that quarantine trials can begin to confirm our desktop prioritisations, as well as the native range survey results.

Honey locust tree

Gleditsia triacanthos, commonly known as honey locust, is a fast-growing deciduous tree, indigenous to North America. The tree has been introduced around the world, including South Africa, where it has become invasive. Wherever the tree establishes, it competes with and replaces indigenous species. Dense stands along watercourses significantly reduce stream flow. In South Africa, it is regarded as Category 1b under the NEMBA regulations. It has been listed as one of the nine fastest-spreading weeds in South Africa and thus a species that poses a significant environmental threat.

The tree is assumed to be spread primarily by seed, and within waterbodies by stream flow, suggesting the seeds should be a target for biological control. In South Africa the seed-feeding bruchid, *Megabruchidius tonkineus*, has been recorded in the plant's seed pods. Although the insect was not released as part of a formal biological control programme, it is an unintentional introduction, and is still considered a biological control agent. However, neither host-specificity nor impact studies were conducted on the species prior to its introduction into South Africa. The impact of the weevil on the seeds of the tree may influence future management options. Sara Salgado Astudillo's initial host-specificity testing as part of her MSc suggests the insect is specific to honey locust; however, extensive surveys of the tree suggest that, even though the insect is widely distributed, its impact on seed is limited. This research is still ongoing. Sara aims to complete her MSc in 2021 and start a PHD investigating the positive and negative impacts of honey locust in South Africa while prioritising and developing additional biological control agents, including *Dasineura gleditchiae,* a gall midge.

RESEARCH TEAM
Dr Grant Martin

STUDENT
Sara Salgado Astudillo (MSc)

COLLABORATORS
Virginia Tech USA & CABI (Switzerland)

Sara Salgado sampling honey locust. **Photo:** Sara Salgado

Firethorn

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

STUDENT
Lehlohonolo Adams (PhD, UFS)

COLLABORATORS
Yunnan University, China

In November 2020, the journal, *Invasive Plant Science and Management (IPSM),* announced the launch of a new series focused on the biology and ecology of invasive plants. *Invasive Plant Science and Management* is a journal of the Weed Science Society of America, a non-profit scientific society focused on weeds and their impact on the environment. The inaugural article was a review of *Pyracantha angustifolia,* or firethorn, written by members of the Northern Temperate Weed Programme on the research that we have conducted on the species over the last three years. The article highlighted a year of success for this programme, including Lehlohonolo Adams completing his MSc on "Reproductive ecology of *Pyracantha angustifolia* in Afromontane grasslands of the Eastern Free State" through the Afromontane Research Unit at the University of the Free-State. Lehlohonolo has since been awarded a Doctoral bursary through South African National Biodiversity Institute – Biological Invasions to continue looking at Northern Temperate invasive shrubs invading the highlands of South Africa. *Pyracantha angustifolia,* a medium-sized, evergreen shrub native to north-eastern China, 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 shrub's berries are eaten and distributed by birds and mammals. In its introduced range, the shrub primarily invades grass- and heathland, rocky ridges, and riparian habitats where it forms dense thickets that shade out native plants and impede the growth and regeneration of shrubs and trees. The species is regarded as an invasive species in several countries where it is recorded as having negative environmental and potentially negative economic effects. The species has been identified as one of the most damaging and fastest-spreading species in the grasslands of South Africa and therefore a candidate for biological control. In order to start the process of identifying potential biological control agents in China, we are collaborating with Yunnan University in China to identify the full suite of potential biological control agents for *P. angustifolia* in China. Starting in 2021, initial host-specificity testing will be done on closely related species in China; however, given the number of indigenous Rosaceae species present in South Africa, additional testing will also have to be conducted on prioritised agents here.

A common fiscal shrike perching on *Pyracantha angustifolia* – the abundant red berries contribute to the name firethorn.
Photo: Lehlohonolo Adams

Cotoneaster species

Cotoneaster species are northern temperate shrubs characterised by numerous small red fruits; a number of species are invading the highland grasslands of South Africa. One of the most abundant in the highland grasslands is *Cotoneaster pannosus,* known by its common name: silver leaf cotoneaster. This woody shrub is native to south central China and was probably introduced as an ornamental. The fruits are red-orange pomes which are very attractive to birds, and are assumed to be the main agent of seed dispersal. It is assumed that their reproductive ecology would be similar to *Pyracantha angustifolia* in South Africa; however, this has not been confirmed. Karabo Moloi, based at the University of Free State QwaQwa campus, is currently conducting her MSc, investigating the reproductive ecology and impacts of *Cotoneaster pannosus* on the highland areas of South Africa.

Karabo Moloi and Sandy Steenhuisen (below), surveying the landscape on a field trip which involved investigating the seed viability of *Cotoneaster pannosus* (right), **Photos:** Grant Martin

RESEARCH TEAM
Dr Grant Martin, Dr Sandy Steenhuisen (UFS)

STUDENT
Karabo Moloi (MSc, UFS)

Rubus species

Rubus species (brambles, blackberries, raspberries, or dewberries) are a globally recognised genus on account of the edible fruit as well as the many negative impacts they can have as invasive species. The at least 23 species of the *Rubus* subgenus *Rubus* in South Africa include native, alien, naturalised alien, and invasive alien species. The invasive *Rubus* species are becoming increasingly problematic in South Africa, but the taxonomy of indigenous, alien, and alien invasive species is poorly understood and therefore efforts to understand the genus, the impacts and solutions to those impacts have been avoided thus far. There has also been little research conducted into the economic or ecological impacts on the taxon in South Africa, and yet, the regulations list species in the genus in categories that require the landowner to manage the species on their land. The potential value of certain species, as well as the cost associated with their impacts, could create a scenario where a complex conflict of interest may arise; this requires investigation.

MComm candidate Brett Mason has investigated the impacts of the *Rubus* species and tried to place a value on both the positive and negative attributes of the species. He has shown that all economic impacts of invasive *Rubus* species in South Africa are externalities. Invasive *Rubus* species in South Africa can (i) harbour vermin, (ii) impede human and animal livestock, (iii) reduce crop yields, (iv) encroach on grazing land, (v) present fire hazards, and (vi) potentially impact fire regimes.

The edible blackberry is part of the *Rubus* genus and is invasive in South Africa. **Photo:** Brett Mason

RESEARCH TEAM
Dr Grant Martin, Prof. Gavin Fraser, Dr Costas Zachariades (ARC-PPRI)

STUDENT
Brett Mason (MComm)

COLLABORATORS
ARC-PPRI Cedara, Centre of the Region Haná for Biotechnological and Agricultural Research, Department of Genetic Resources for Vegetables, Medicinal and Special Plants, Crop Research Institute, Czech Republic

These species also:

1. provide berries
   a. that have been for sale on a commercial level;
   b. that are used for personal consumption;
   c. that provide an ingredient for alcohol-brewing processes;
4. could potentially facilitate forest regeneration;
5. could provide infrequent grazing for certain livestock;
6. could potentially provide ingredients for anti-inflammatory drugs or modern cosmetics in South Africa, as they do in other regions of the globe.

Brett found that the costs associated with the invasive species can be presented dichotomously; the private cost-benefit ratio, for costs and benefits incurred or enjoyed by private economic agents, stands between 1:1.15 and 1:2.9. A private-public cost-benefit ratio, that incorporates both private and public costs and benefits, stands at 1:0.07. The private-public cost-benefit includes public expenditure, or government control measures, directed at invasive *Rubus* and shifts the cost-benefit analysis in favour of costs. Brett's study revealed that the benefits per hectare are R13.14/ha, the private costs, R5.12/ha and the holistic cost R176.22/ha, which includes both private and public expenditure. He found that that the monetary values, when expressed per hectare, are misleading owing to a large standard deviation in the spread of benefits received as only a small sample of the respondent population derive benefits. The distribution of costs and benefits was not uniform across the regions assessed. All benefits in this study accrue to economic agents in the Free State Province, while most of the costs accrue to economic agents in KwaZulu-Natal. The benefits appear to be primarily derived from an alien species, *Rubus* sect. *Arguti*. It appears few benefits are derived from the invasive species of *Rubus* while many of the costs are attributable to these species.

INVASIVE TREES

The Invasive Trees Programme focuses on Australian acacias and *Prosopis*. The core Invasive Trees Programme team comprises Emeritus Associate Professor John Hoffmann, Fiona Impson, Catharina Kleinjan and Cliff Moran, based at the University of Cape Town within the Plant Conservation Unit (PCU) in the Department of Biological Sciences, all of whom have been part of the CBC Consortium since 2017. This team has many years of experience in the field of biological control of invasive trees and have developed strong collaborations with several international institutions. Initially, officials brought Australian acacias into South Africa in the early 1800s to stabilize dunes, but several species have since become highly invasive. Despite being problematic, the various species are also useful as sources of tannin, wood pulp, firewood and timber. The commercial utilisation of most of the species has largely restricted biological control to the use of agents that have no impact on the vegetative growth of these trees (e.g., agents that attack the buds, flowers or seeds). Despite this constraint, several agents are now well established and curbing the invasiveness of the acacias.

Prosopis comprises a group of leguminous thorn tree species and their hybrids, which originate in South and North America. Officials introduced *Prosopis* to South Africa in the 1960s as useful agroforestry trees to provide shade, timber, fuel and fodder in arid areas. Farming communities planted *Prosopis* widely; the trees have subsequently become invasive, particularly along watercourses where they outcompete native vegetation and deplete available water reserves. There has been some success with biological control using two seed-feeding beetles but not enough to alleviate the problem. Recent engagement with stakeholders in the farming community has created an incentive to extend the biological control programme to include agents that are destructive on other parts of the trees, as it is clear that, on a national scale, the costs of *Prosopis* far outweigh the benefits.

Despite the challenges of 2020, the team made good progress. We have consolidated the analysis of the results of ongoing long-term *Acacia* studies and prepared these for publication. Collaboration with the Instituto Politécnico de Coimbra in Portugal and with Tel Aviv University has continued.

PROGRAMME HIGHLIGHTS IN 2020

- As part of an ongoing collaboration with Tel Aviv University, the team collected 2200 adult *Melanterius castaneus* weevils in South Africa in early November and sent these to Israel. This consignment will facilitate efforts towards achieving biological control of *Acacia saligna* in Israel.
- Approximately 1000 *Melanterius acaciae* adults, 500 *Melanterius maculatus* adults and 2 kgs of *Trichilogaster acaciaelongifoliae* galls were shipped to colleagues at the University of Coimbra in Portugal in December.
- Despite COVID-19 travel restrictions, the team completed local fieldwork to gather seed bank and seed rain data, ensuring the continuity of long-term data sets.

Australian *Acacia* species

Biological control efforts against invasive Australian *Acacia* species have been ongoing since the 1970s in South Africa, when surveys for potential agents began. Since then, researchers have introduced two bud-galling wasps, five seed-feeding weevils, three flower-galling flies, and a rust fungus against 10 of the most invasive *Acacias* in South Africa. The research by this team includes all aspects of a biological control programme (i.e., developing and screening new agents; release and monitoring, and post-release evaluation). The local and international travel restrictions during 2020 heavily compromised some of the research plans for the year although progress was made through our collaboration with the University of Tel Aviv in Israel. Continued research on a bud-galling wasp, *Perilampella hecataeus* (a potential new agent for *Acacia dealbata* and *Acacia baileyana*) had to be put on hold as collection and importation from Australia was prevented, and likewise, additional importation of a flower-galling fly, *Dasineura pilifera*, (also for *Acacia dealbata* and *Acacia baileyana*) was not possible.

Post-release evaluation studies investigating acacia seed banks and seed rain in various parts of the country have been ongoing since 2004. This aspect of the research programme was carried out during 2020, and the results contribute to a long-term data set providing information on the status of seed dynamics, both pre- and post-biological control being implemented.

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

(right) Nir Bonda (MSc student, Tel Aviv University) removing the sleeved pods to confirm larval development of *Melanterius castanaeus* weevils, on *Acacia saligna*. The weevils were sent to Israel from South Africa in early 2020. **Photo:** Netta Dorchin

Prosopis

The University of Cape Town's component of the *Prosopis* biological control programme is currently restricted to the development and deployment of an apionid beetle, *Coelocephalapion gandolfoi,* which the Department of Agriculture approved for release in January 2020. While this beetle is not directly damaging to the adult *Prosopis* plants, it could cause a substantial reduction in seed production. The larvae develop within maturing canopy-held pods so that they complete their development before livestock initiate feeding on the pods. Due to travel restrictions, we had to postpone plans to import *C. gandolfoi* in 2020, in collaboration with Dr Fernando McKay (FuEDEI, Buenos Aires, Argentina). South Africa has recently expanded biological control initiatives to include evaluation of potential agents that are directly damaging to *Prosopis.* There is a wealth of largely unexplored potential in this regard, particularly in South America. In collaboration with Dr Fernando McKay, we have planned further surveys, focussing specifically on damaging agents. The initial intended focus is three gall forming *Eschatocerus* wasps (Cynipidae), but travel restrictions precluded progress in 2020.

Colleagues and partners at the Agricultural Research Council's directorate of Plant Health and Protection successfully applied for permission to release the biological control agent, *Evippe* species #1. Fritz Heystek and fellow researchers will release this agent during the early part of 2021. *Evippe* species #1 is a leaf-tying moth that will defoliate *Prosopis* under suitable environmental conditions. The CBC looks forward to supporting and working alongside Fritz and his colleagues to make sure that this agent has the best possible chance of successful establishment in South Africa. The CBC plans to maintain overwintering populations of the insect in facilities that we are developing with Oasis Skills Development and the Association of and for People with Disabilities in Upington.

As reported in the Community Engagement section of the report (page 66), Philip Ivey from Rhodes University continues to work with partners in the agricultural sector to facilitate implementation of *Prosopis* control. This work is essential after a workshop held in 2019 showed that there is a great need for stakeholder engagement around the control of *Prosopis* in South Africa.

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

COLLABORATORS
Dr Fernando McKay, FuEDEI, ARC-PHP

Prosopis trees produce a prolific number of seed pods. **Photo:** John Hoffmann

BUGWEED

The widely known Bugweed, *Solanum mauritianum*, indigenous to subtropical South America, is one of our most long-standing and widespread environmental weeds. Research efforts in South Africa have, however, culminated in the release and establishment of only two insect biological control agents, namely the lace bug, *Gargaphia decoris* and the flower-feeding weevil, *Anthonomus santacruzi,* in 1999 and 2008, respectively. Both agents have established in the field at low altitude but damage is local and has largely been insignificant, probably owing to agent climatic incompatibility. Bugweed invasion in South Africa also occurs in high altitude regions (greater than 1000 m) that experience cold winters. These issues have renewed interest in testing new agents that may be better adapted to low temperatures. Climate matching of potential collection sites in Uruguay with colder regions of South Africa suggested that Uruguay would be a viable region in which to search for more damaging candidate biological control agents.The flower-feeding weevil, *Anthonomous morticinus*, was collected in Uruguay in 2019, but a culture failed to establish in quarantine. Nic Venter and Vusimuzi Mkhomazi, an MSc candidate from the University of the Witwatersrand, undertook a second trip in March 2020. Vusimuzi resolved the rearing issues and a viable culture has been established in quarantine. Preliminary results from host-range testing on non-target plants show promise; however, this remains ongoing.

Although host specificity of a candidate agent is imperative, the new candidate agent also needs to be climatically better suited than *A. santacruzi* to survive the temperatures experienced throughout much of bugweed's invaded range. Results from thermal tolerance trials thus far indicate that *A. morticinus* appears to be better adapted to lower temperatures than its congener, *A. santacruzi*. Despite climate playing a large role in agent establishment, a further impediment could be a genetic mismatch of the target weed in its invaded range to the host plant in its native range from which the agent was collected. To understand if such a mismatch is affecting *A. santacruzi's* establishment and that of the potential new agent *A. morticinus*, population genetics are being conducted on bugweed from South Africa and Uruguay. Preliminary results indicate that there are no significant genetic differences between plants from South Africa and Uruguay.

RESEARCH TEAM (WITS)
Prof. Marcus Byrne, Nic Venter, Blair Cowie, Giuseppe Venturi, Samalesu Mayonde.

STUDENT
Vusimuzi Mkhomazi (MSc, Wits), Yaron Keizan (MSc, Wits)

COLLABORATORS
UKZN; LandCare Research New Zealand

Manaaki Whenua LandCare Research in New Zealand have collaborated with the University of the Witwatersrand to conduct full host-range testing of *A. morticinus* on *Solanaceae* plants indigenous to New Zealand. These tests will be conducted on two of their *Solanum* species at the Wits Quarantine Facility.

Vusumuzi Mkhomazi conducting thermal tolerance trials on the weevil *Anthonomus morticinus*, a candidate biological control agent for bugweed. **Photo:** Nic Venter

Anthonomus morticinus weevils collected in Uruguay are currently being tested as candidate biocontrol agents for *Solanum mauritianum*. **Photo:** Nic Venter

TAMARIX

Alien invasive *Tamarix* species are terrestrial weeds that invade riparian ecosystems in South Africa. *Tamarix* is a genus in the Tamaricaceae, comprising 54 tall shrubs and trees. In South Africa, the National Environmental Management Biodiversity Act 2014 classifies two Eurasian species, *T. chinensis* Lour. and *T. ramosissima,* as Category 1b invaders; investigations of biological control agents to suppress their infestations are underway. However, *Tamarix usneoides*, is a species native to South Africa and therefore poses a challenge in avoiding "non-target effects" from any biological control agents used against the invasive genotypes. The indigenous *T. usneoides* grows successfully in semi-arid regions with low annual rainfall, and is widely distributed in the Northern Cape and some parts of the Western Cape Province, close to the border with Namibia. The invasive *Tamarix* in South Africa is more widely distributed in the cooler, wetter interior of the Eastern and Western Cape Provinces. The Swart River in Prince Albert/Western Cape and the Groot River/Eastern Cape are the only localities known to contain all six *Tamarix* genotypes present in South Africa (the three parental species and their respective hybrids). Remote sensing data shows that invasive *Tamarix* land cover in the Eastern and Western

Galls caused by eriophyid mites on the indigenous *Tamarix usneoides* in Noorspoort, Eastern Cape. **Photo:** Samalesu Mayonde

RESEARCH TEAM)WITS)
Prof. Marcus Byrne, Dr Samalesu Mayonde (Postdoc), Lyriche Drude, Nic Venter

STUDENTS
Sivenathi Hatile (MSc, Wits)

COLLABORATORS
Dr Sivu Situngu (Wits), Dr Massimo Cristofaro (BBCA, Rome, Italy) & Dr Rene Sforza – USDA-ARS-European Biological Control Lab

Cape Provinces doubled from 2007 to 2018. Hence, immediate management interventions are required. The leaf-feeding beetle, *Diorhabda carinulata,* and the scale insect, *Trabutina mannipara,* have been rejected as potential biological control agents due to non-target feeding on the native *T. usneoides*. However, there is a phylogenetic disjuncture between the indigenous *T. usneoides* and the invasive genotypes, which places them in separate clades (groups). This disjuncture provides hope of finding host-specific biological control agents for the invasive species which will not see the native species as a potential host. In addition, differences in secondary metabolites between the *Tamarix* species are being investigated. Studies have shown that insect host-plant selection can be influenced by the secondary metabolites of the potential host plant. The search for additional biological control agents against invasive *Tamarix* continues, looking at Eriophyid mites, and the weevils, *Corimalia tamarisci* and *Coniatus tamarisci*.

Host-range testing of *Tamarix* Eriophyid mites

Eriophyid mites are phytophagous, and rely on above-ground plant material for development. Approximately one-third of species from the Phytoptidae and Eriophyidae are gall-formers. This lifestyle places a physiological strain on the host plant because the mites inject toxic saliva into the host's tissue. In 2018, gall-forming mites were discovered on two distinct *Tamarix usneoides* populations in Noorspoort, Eastern Cape and Dwyka, Western Cape. However, these mites have not been seen on any of the invasive species and, at the moment, are only known to occur on specific *T. usneoides* populations. This suggests that the mites are highly host-specific and therefore present an opportunity to look for similar host-specific biological control agents against the invasive *Tamarix*. About 11 eriophyid species are associated with *Tamarix*; if the local mites are found to be host specific, our aim will be to include other mite species in our exploration for biological control agents in Eurasia, the native range of the invasive species.

Eriophyid mites collected in Noorspoort and Dwyka are currently the subject of rearing trials in quarantine at Wits University prior to host-range testing. In November 2019, *T. usneoides* plants were inoculated with eriophyid mites, which did not establish galls. However, a second attempt in early 2020 showed some signs of gall development, raising hopes of successful rearing. In November 2020, specimens of *T. usneoides*, *T. ramosissima*, *T. chinensis* and *T. chinensis* × *T. ramosissima* hybrids were planted under the galled trees at Noorspoort farm near Steytlerville in the Eastern Cape. These will provide a field test of host range and hopefully establish mites which can be transported to the Wits Insectary.

Coniatus sp. (left) and Corimalia sp (right) feeding on *Tamarix ramosissima*. Insects were collected in Italy and imported into quarantine at Wits University for host range testing. **Photo:** Nic Venter

AFRICAN BOXTHORN

Evans Mauda searching for stem mining insects and any damage signs to the stem of boxthorn at Elands Bay in the Western Cape. **Photo:** Lenin Chari

RESEARCH TEAM
Dr Grant Martin, Dr Lenin Chari (Postdoc)

STUDENTS
Evans Mauda (PhD), Emma Stirk (BSc Honours)

COLLABORATORS
CSIRO, Australia

African boxthorn (*Lycium ferocissimum*) is a noxious weed in Australia that is native to southern Africa. The ultimate goal of this CBC programme is to reduce the negative consequences of this weed in Australia. Since 2017, the CBC staff and students at Rhodes University have conducted native-range surveys for potential biological control agents in South Africa. Over this period, a number of herbivorous arthropods has been collected and identified. Four insect species and one pathogen species have been prioritised as potential biological control agents owing to their impact on the plants and their host specificity. Host-specificity testing on the fungus, *Puccinia rapipes,* and the ladybird beetle, *Cleta eckloni,* is currently taking place at CSIRO, Australia. Two other insects, another ladybird beetle, *Cleta* sp1, and the weevil, *Neoplatygaster serietuberculata* are currently being kept in cultures at Rhodes University, awaiting shipment to Australia.

PROGRAMME HIGHLIGHTS IN 2020

- During 2020, surveys were limited owing to the COVID-19 national lockdown. However, the easing of restrictions allowed surveys to be resumed, and insect cultures to be re-established.
- Post-lockdown, two shipments of *Cleta* sp1 and *Neoplatygaster serietuberculata* were exported to Australia. Additionally, surveys for stem-mining insects that have been generally missed in past surveys, have begun.
- Two students working on this project; Evans Mauda (PhD) and Emma Stirk (BSc Honours), graduated with a PhD and BSc Honours, respectively, and two papers were published.

GRASSES

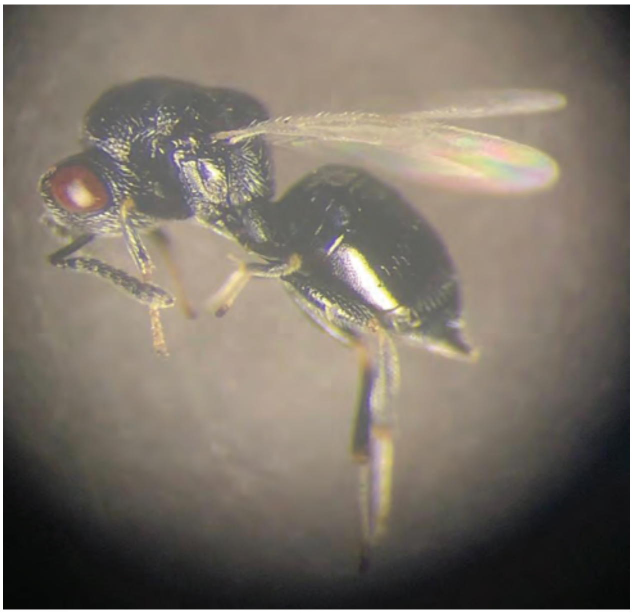
Biological control of invasive alien grasses has had very limited implementation globally, despite their severe environmental and socio-economic impacts. This neglect has largely been due to an irresolute perception that grasses are poor targets for biological control because they are thought to lack host-specific herbivores, tolerate herbivory, and are too closely related to valuable economic cereals and pasture species. A review paper published by three members of the CBC and their collaborators evaluated whether grasses really are unsuitable biological control targets. Contrary to expectations, grasses often support host-specific and damaging natural enemies that could be developed as potential biological control agents. Host-specificity testing provides a scientifically rigorous and reliable assessment of the risk posed to important economic crops, ornamentals, and native plant species. As such, the risks associated with biological control of grass are no greater than for other weedy taxa, and grasses should therefore be considered suitable targets for biological control. The CBC has started a number of novel and innovative projects that will improve our knowledge in the field of grass biological control and help pave the way in extending this approach to more invasive grasses.

PROGRAMME HIGHLIGHTS IN 2020

- After extensive field and laboratory host-specificity trials were conducted in South Africa, an undescribed species of stem-boring wasp called *Tetramesa* sp. 1 has been shown to be host specific to *Sporobolus* sp. that are problematic in Australia. *Tetramesa* sp. 1 will be imported into quarantine in Brisbane, Australia in 2021, where it will be tested against a number of native Australian grasses before being considered for release.
- Native range field surveys in South Africa on *Eragrostis curvula* (commonly known as weeping lovegrass) have resulted in the identification of three potential biological control agents for Australia where this species is a problematic invasive.
- A review of the invasive grass genus, *Nassella,* in South Africa was published in the *South African Journal of Botany*. The paper, authored by Anthony Mapaura, co-supervised by Kim Canavan from the CBC, and other collaborators, has helped determine the current status and identified knowledge gaps that will assist in managing these grasses in South Africa.

African lovegrass, *Eragrostis curvula*

African lovegrass, *Eragrostis curvula,* is another African grass that has become highly problematic in Australia, particularly in New South Wales (NSW). Dense infestations can reduce grazing capacity by over 50% and farm profitability by 25%. In 2019, the CBC initiated preliminary surveys across southern Africa in search of potential biological control agents, in collaboration with the NSW Department of Primary Industries. Surveys were performed on several closely related grasses (particularly other *Eragrostis* species), in addition to *E. curvula*, to identify potential agents. Three candidate agents have been identified so far, including: two undescribed *Tetramesa* spp., whose larvae feed within the culm, and a presently unidentified gall-midge (Diptera: Cecidomyiidae), whose larvae feed within developing caryopses and ovules. A small, greenhouse culture has been established and preliminary no-choice testing initiated for one of the *Tetramesa* sp. Going forward, an MSc. student (Liam Yell) has been appointed to continue surveys across southern Africa in search of additional candidate agents and to further investigate the field host-range of the three candidate agents already identified. Greenhouse cultures and no-choice testing are currently being initiated for the second *Tetramesa* and the gall-midge. Prospects for the biological control of *E. curvula* in Australia are good.



A microscope photo of the wasp, *Tetramesa* sp. 5, which was found on the grass, *Eragrostis curvula* and is being investigated as a potential biological control agent for release in Australia where the grass is highly invasive **Photo:** Kim Canavan

RESEARCH TEAM
Dr Kim Canavan (Postdoc), Guy Sutton

Kim Canavan sampling the grass, *Eragrostis curvula* to look for potential biological control agents to release on invasive populations in Australia. **Photo:** Kim Canavan

Guineagrass, *Urochloa maxima* (= *Panicum maximum*)

RESEARCH TEAM
Prof. Iain Paterson, Guy Sutton

COLLABORATORS
USDA, BBCA (Massimo Cristofaro, Francesca Marini)

Urochloa maxima (Guinea grass) is a perennial African grass that has been introduced for pasture development in many regions outside its native distribution. It has become a serious environmental pest in Texas, USA, where it reduces grazing capacity, alters natural fire regimes and negatively effects native wildlife populations. Previous biological control surveys had been performed in West Africa and Kenya. However, DNA samples provided by the CBC indicated that Guinea grass populations in Texas probably originated from southern Africa. As a result, the CBC initiated a collaboration with the United States Department of Agriculture (USDA) and the Biotechnology and Biocontrol Agency (BBCA) in 2019 to identify potential biological control agents for Guinea grass from southern Africa. Preliminary surveys in KwaZulu-Natal identified at least four potential agents, including a seed-attacking weevil (Curculionidae), a stem-boring beetle (Scolytidae), an eriophyoid mite (Eriophyidae), and a stem-boring wasp (*Tetramesa* sp.). The seed-attacking weevil was sent to the USDA laboratory in Texas where host-range trials are underway. An expert taxonomist in Europe is currently identifying the eriophyid mite. In 2021, additional surveys will be performed across southern Africa to refine the host-range of the four candidate agents and identify any additional candidates. A consignment of the *Tetramesa* sp. attacking Guinea grass will soon be sent to the USDA lab in Texas for host-range testing.

Gamba grass *Andropogon gayanus*

RESEARCH TEAM
Dr Grant Martin, Dr Iain Paterson, Dr Lenin Chari (Postdoc)

COLLABORATORS
CSIRO Australia

Gamba grass, a noxious weed in Australia, is native to tropical and subtropical savannas in Africa. Although the grass is highly invasive in northern Australia, it remains an economically important foraging grass for livestock. The negative impacts of the grass in Australia include reducing indigenous biodiversity and disrupting fire regimes and, therefore, ecosystem functioning. Since January 2018, the CBC has been conducting preliminary surveys for phytophagous insects associated with gamba grass. The aim of these surveys has been to determine the feasibility of biological control as a strategy for managing the grass. We anticipate that the damage inflicted by stem-mining insect species may weaken the stem of the grass and reduce the environmental risk from fires in Australia while still allowing the grass to be used for grazing. Surveys between 2018 and 2019 highlighted the possibility of the presence of stem-damaging insects associated with the grass. A total of 24 insect species associated with the grass were identified to at least family level, but none of them showed any potential as biological control agents. More recently, large tussocks of potentially infested gamba grass have been collected from the field and regrown inside insect cages at Rhodes University, where emergence of stem-dwelling insects is being monitored. So far, three hymenopteran species (*Allodape* sp., *Braunsapis* sp. and *Sphecidae* sp.) have emerged from the grass, and more insects are expected to emerge. Going forward, the project aims to collect infested grass from more areas within the grass's native range, including Benin, Kenya, Nigeria and Zimbabwe. Sites have also been identified inside protected areas where a higher diversity of natural enemies is anticipated, thereby improving the chances of finding potential biological control agents for the grass.

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

RESEARCH TEAM
Dr Iain Paterson, Dr Kim Canavan (Postdoc)

STUDENT
Guy Sutton (PhD)

COLLABORATORS
Department of Agriculture and Fisheries, Australia

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 of 47 other grass species across South Africa identified three stem-boring *Tetramesa* wasps (Hymenoptera: Eurytomidae) that appear to attack only *S. pyramidalis* and *S. natalensis*. These wasps have been shown to reduce seed production and plant survival. The most damaging wasp, *Tetramesa* sp. 1, has been tested against 20 non-target grasses in laboratory based no-choice testing, confirming that the wasp is host specific. *Tetramesa* sp. 1 will be imported into quarantine in Brisbane, Australia, in 2021 where it will be tested against a number of native Australian grasses before being considered for release.

Nassella spp.

RESEARCH TEAM
Dr Kim Canavan (Postdoc), Dr Sandy-Lynn Steenhuisen (UFS, ARU), Dr Vincent R. Clark (UFS, ARU), Prof David Richardson (CIB, US)

STUDENT
Anthony Mapaura (PhD, UFS)

The CBC in collaboration with the Afromontane Research Unit (ARU), the Department of Plant Sciences at the University of the Free State (UFS) and the Centre for Invasion Biology (CIB) are assisting in the supervision of PhD student, Anthony Mapaura. Anthony will be investigating three invasive alien *Nassella* species in South Africa. *Nassella trichotoma* and *N. tenuissima* are declared weeds under category 1b of the National Environmental Management: Biodiversity Act (NEM:BA) and occur mainly in the montane grasslands of the Western and Eastern Cape provinces. *Nassella neesiana* is not listed in NEM:BA but is naturalised in the Eastern Cape, Western Cape and Free State provinces. Anthony's work will include determining *Nassella* distributions, potential spread under predicted climatic changes, impacts on biodiversity, and competition with native grasses. The encroachment of *Nassella* presents a serious threat to South African grasslands and this work will play an important role in guiding future management strategies, including biological control.

Tetramesa as biological control agents for grass

RESEARCH TEAM
Prof. Iain Paterson, Guy Sutton, Dr Kim Canavan (Postdoc), Dr Lenin Chari (Postdoc), Clarke van Steenderen (PhD candidate)

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

The stem-boring wasps, *Tetramesa* (Hymenoptera: Eurytomidae), have been identified as high-priority candidates for biological control of invasive grasses, as they usually only attack a single grass species and can be extremely damaging (stopping seed production and causing tillers to die-off). They play a key role in our grass biological control programmes (barring gamba grass, but we hope to find one soon!). Unfortunately, little is known of the African *Tetramesa* fauna, with only four described species, none of which occur in southern Africa, and adult wasps are exceptionally difficult to identify because they all look the same. Not knowing if the wasps collected from the different grasses represent different wasp species made assessing the suitability of *Tetramesa* spp. as biological control agents impossible.

To overcome this, the CBC has undertaken an exciting project to catalogue, identify and describe the *Tetramesa* fauna from southern Africa. To date, we have developed a reference library of more than 60 DNA sequences of *Tetramesa* spp. recorded from different host grasses. Preliminary results from the DNA library indicate that each grass species plays host to its own host-specific *Tetramesa* species. Representative specimens of at least four putative *Tetramesa* spp. identified using our DNA library have been sent to an expert taxonomist in Iran to be formally described for the first time. Building this reference library of DNA sequences has been invaluable to our assessment of *Tetramesa* spp. as biological control agents; it facilitates the development of new grass biological control programmes against invasive grasses in the future and contributes towards our knowledge of the amazing biodiversity of Africa.

Biological control prospects for invasive alien grasses in South Africa

RESEARCH TEAM
Guy Sutton, Dr Kim Canavan (Postdoc),

COLLABORATORS
Dr Angela Bownes (ARC and UKZN), Dr Vernon Visser (UCT), Anthony Mapaura (UFS)

To date, there have been no biological control agents released on invasive alien grasses in South Africa. However, evidence shows that many of these grasses have major impacts in the country, and in most cases, chemical and manual control are not sustainable options for their management. The CBC, together with collaborators, evaluated the prospects for implementing novel grass biological control projects in the country over the next ten years. Using the CBC's recently developed Biological Control Target Selection system, the highest risk grasses were ranked according to biological control priority. From this, five grasses were identified as most suitable for biological control: *Arundo donax*, *Glyceria maxima*., *Nassella trichotoma*, *Cortaderia selloana* and *Cortaderia jubata*.

Arundo donax is an invasive giant reed grass which can form dense stands. **Photo.** Kim Canavan

Collaboration with the Alien Grass Working Group

Field excursion to the Golden Gate National Park as part of the grass identification course (left: Dr Ian Rushworth – Ezemvelo KZN Wildlife, middle: Dr Maria S. Vorontsova – Kew Gardens, right: Aluoneswi Caroline Mashau – SANBI). **Photo:** Kim Canavan

The CBC has worked with the Alien Grass Working Group since its inception in 2013. The group aims to bring relevant experts together with a shared interest in alien invasive grasses in South Africa. The CBC helped to plan and coordinate the group's annual meeting held with the South African Association of Botanists (SAAB) at the University of the Free State, QwaQwa campus, on the 6–10 January 2020.

The meeting included a grass identification course and a special session within SAAB on alien invasive grasses. The meeting was a great success and was attended by representatives from fourteen research institutes. Going forward, the working group will assist in reviewing alien grass risk assessments that will then feed into their legislation.

The Alien Grass Working Group members at the annual meeting held at in January 2020 at the University of Free State's QwaQwa campus. **Photo:** Kim Canavan

AGRICULTURAL ENTOMOLOGY

The Agricultural Entomology Research Programme at the CBC focuses on developing effective biological control agents that can be incorporated into integrated pest management (IPM) programmes primarily for the control of citrus pests. The programme aims to identify, develop, and apply natural insect parasitoids, predators, and entomopathogens (viruses, nematodes, and fungi) in an environmentally friendly manner, while maintaining high levels of pest control. The programme is run in collaboration with Citrus Research International (CRI), enabling greater technical and financial support, ensuring continued protection of this economically important sector in South Africa. The 2020 programme included important projects undertaken by postgraduate students and postdoctoral fellows, investigating topics ranging from synergistic effects between viruses and yeast to whether orchard shade nets influence pest dynamics and IPM programmes. While each of these projects focuses on individual aspects of biological control in IPM programmes, their joint contribution has significant implications for the control of agricultural pests in the field.

PROGRAMME HIGHLIGHTS 2020

- The publication of several peer reviewed articles.
- The identification of several yeast isolates with potential synergistic characteristics.
- Completion of two PhD and four MSc projects.

False coding moth (FCM), *Thaumatotibia leucotreta*, is a major insect pest of citrus in South Africa. **Photo:** David Taylor

An investigation into yeast-baculovirus synergism for the improved control of false codling moth

RESEARCH TEAM
Prof. Caroline Knox, Prof. Sean Moore, Prof. Martin Hill

STUDENT
Marcel van der Merwe (PhD)

Studies conducted between yeasts and insects have highlighted their importance, not only in food acquisition, but also in the development and behaviour of insects. The diversity of yeasts within the environment and their persistence in the gastrointestinal system of many insects indicate that they possess great potential for use in biological control. Only a handful of studies have focused on the associations between yeast and insects of the order Lepidoptera; these insects are of great importance as they are well-known pests of economically important crops, such as citrus and cotton.A mutualistic association between *Cydia pomonella*, also known as codling moth, and epiphytic yeasts, has been reported. This mutualistic relationship was manipulated for the purposes of biological control, by combining the yeast with the baculovirus Cydia pomonella granulovirus (CpGV) to significantly increase larval mortality. We proposed to determine which species of yeast are associated with *Thaumatotibia leucotreta* larvae and to examine whether any of these yeasts, when combined with the Cryptophlebia leucotreta granulovirus (CrleGV), increase larval mortality.

The bioprospecting process led to the isolation and identification of six yeast isolates, namely *Meyerozyma guilliermondii*, *Pichia kluyveri*, *Pichia kudriavzevii*, *Hanseniaspora uvarum*, *Candida lusitaniae* and *Kluyveromyces marxianus*. The isolated yeasts were investigated to a) see if they enhance the oviposition preference of female *T. leucotreta* moths, b) aid in neonate *T. leucotreta* larval attraction and development, and c) examine whether any of these yeasts, when combined with CrleGV, have a synergistic effect in increasing the mortality of neonate *T. leucotreta* larvae. *Meyerozyma guilliermondii*, *P. kluyveri*, *P. kudriavzevii* and *H. uvarum* yielded promising results, with *P. kudriavzevii* ultimately being selected for field trials.

Future work for this project will involve identifying the yeast volatiles responsible for causing attraction and conducting additional field trials using *P. kudriavzevii* to show its efficacy in increasing larval mortality when combined with CrleGV.

Marcel spraying a yeast-baculovirus mixture on citrus trees to evaluate potential synergistic interactions which could lead to improved control of false codling moth in the field. **Photo:** David Taylor

Investigating synergistic interactions between CrleGV-SA and CrpeNPV to improve control of false codling moth

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

STUDENT
David Taylor (MSc)

The project continued the investigation into the synergistic interaction between CrleGV-SA and CrpeNPV in mixed infections of FCM. It was previously shown that the lethal concentrations (LC) were improved by using mixed infections. This project first looked at the lethal concentrations of each virus alone and then in a 1:1 combination of the two, using surface-dose biological assays. Following this, the lethal times (LT) of each virus alone and in the 1:1 mixture of the two were investigated. The LC_{50} and LC_{90} of the 1:1 mixture were approximately five and ten times lower, respectively, than CrleGV-SA by itself and approximately five times lower than both the LC_{50} and LC_{90} of CrpeNPV. This confirmed results that had previously been reported by Jukes (2018) and warrants further investigation by field trials. In terms of the speed of kill, the LT for CrleGV-SA and CrpeNPV were determined to be very similar, while the 1:1 mixture was significantly slower. The LT_{50} and LT_{90} of the 1:1 mixture were 139 hours and 193 hours respectively. In terms of pest management, the differences in LT_{90} of different treatments are more important than the LT_{50}. The LT_{90} of CrleGV-SA and CrpeNPV were 40 and 34 hours faster, respectively, than the 1:1 mixture. When considering that FCM larvae typically take about four days (96 hours) to penetrate the skin of citrus, a difference of 34 hours and 40 hours potentially allows the pests to cause damage before the infection kills them or changes their behaviour. These results therefore have implications for future management of FCM on citrus.

Improving baculovirus virulence against the false codling moth by serial passage

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

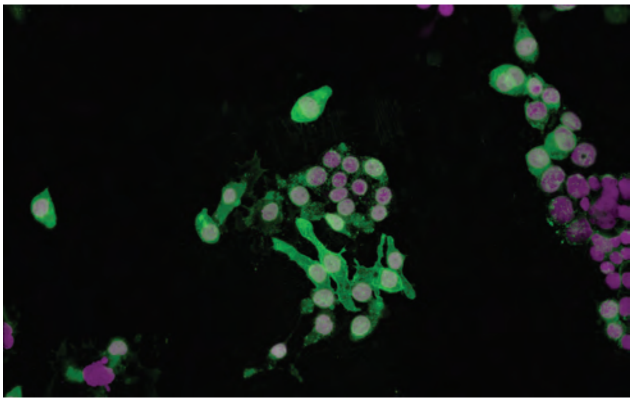
STUDENT
Petrus Iita (MSc)

The discovery of Cryptophlebia peltastica nucleopolyhedrovirus (CrpeNPV) offered a unique opportunity for continued investigation into this baculovirus as a potential biological control agent. Of interest to this study is the application of CrpeNPV against the heterologous host, FCM. There is evidence that the virulence of a baculovirus in a heterologous host can be improved via serial passage. The aim of this study was therefore to determine if the virulence of CrpeNPV can be improved by sequential passage through FCM larvae in laboratory assays. A total of 12 serial passage assays of CrpeNPV through FCM have been conducted to determine the potential for improved virulence against it. Laboratory bioassay results against neonate larvae using similar concentrations of the virus have shown that both the wildtype virus and the virus recovered following serial passage resulted in lethal times (LT) for 50% larval mortality (LT_{50}) of 3 days 12 hours, and 3 days 16 hours post treatment, respectively. Unfortunately, there was no significant improvement in virus efficacy against FCM following serial passage. Although an improved LT for the virus was the desired outcome, CrpeNPV has still demonstrated its potential as a biological control agent for the control of FCM and hence could be used alongside the current entomopathogenic virus Cryptophlebia leucotreta granulovirus (CrleGV) in IPM programmes, or as an alternative, should signs of resistance to CrleGV be observed in the future.

The development of a novel cell line derived from false codling moth eggs for the manipulation and production of baculoviruses

To further develop and characterise the baculoviruses used to control *Thaumatotibia leucotreta*, the false codling moth (FCM), an insect cell line has been established that can be utilised for *ex vivo* (*in vitro*) experimentation. The cell line has been shown to be susceptible to the baculovirus, Cryptophlebia peltastica nucleopolyhedrovirus (CrpeNPV), with infection successfully achieved using budded virus extracts from diseased larvae. Initially, successful infections were screened via microscopic examination of the cells and through the detection of viral mRNA. The detection of viral mRNA was expanded from a single gene to multiple genes, with each expressed during different periods of infection, either immediate, early, or late. A surprising result was the detection of mRNA for all three genes within the first 24 hours of infection, long before morphological changes were observed at around 120 hours. CrpeNPV occlusion bodies were collected from the cells 144 hours post infection and evaluated against neonate FCM larvae. This was done to determine whether the virus produced in the cell line is still able to infect the larvae *per os*. All larvae in the virus treatment died after seven days, while no mortality was recorded in the control. Experimentation with the cell line has progressed with efforts now focused on testing the stability of budded virus samples generated, ensuring these can be reliably maintained and utilised in downstream applications. This will ensure that upcoming experiments that generate viral samples, such as those relating to the isolation of genotypes or the passage, bulk-up and maintenance of UV-resistant CrleGV strains, can be conducted in the long term in a reliable and consistent manner.

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



False codling moth cells stained with two fluorescent dyes, one targeting cellular membranes and another DNA in the nucleus. **Photo:** Michael Jukes

Genetic analysis and field application of a UV-resistant strain of CrleGV for improved control of *Thaumatotibia leucotreta*

Thaumatotibia leucotreta is a serious citrus pest which has been controlled through several control strategies within an integrated pest management (IPM) programme. One of the components in the IPM programme is the use of Cryptophlebia leucotreta granulovirus (CrleGV-SA). Formulated as Cryptogran™ (River Bioscience (Pty) Ltd, South Africa), it has been used in the field successfully for many years to control *T. leucotreta*. One of the main factors influencing baculovirus insecticides such as Cryptogran™ and their application in the field is UV irradiation. The DNA of the virus is damaged by exposure to UV light, and this decreases the efficiency of the biopesticide. Thus, the resistance of the occlusion bodies (OBs) to UV needs to be improved. In a recent study, a UV-resistant virus strain of CrleGV-SA that differed genetically and biologically from the wildtype virus was selected after repeated

RESEARCH TEAM
Prof. Caroline Knox, Prof. Sean Moore, Prof. Martin Hill, Dr Michael Jukes, Prof. Gill Dealtry (NMU)

STUDENTS
Tahnee Bennett (MSc)

exposure of viral OBs to UV-irradiation. This UV-resistant strain has potential as a biological control agent for improving the control of *T. leucotreta* in the field. Whether the genetic and biological stability of this strain is maintained when passaged repeatedly through the host for commercial purposes is not known. A literature review has been submitted and the virus genome has been evaluated to identify the genetic mutations that have taken place. Fourteen single-nucleotide polymorphisms (SNPs) have been identified and the SNPs have been mapped to the published CrleGV-SA genome sequence. Three sets of primers targeting the regions where the SNPs occur have been designed and tested. Subsequent work includes evaluating these primers on the UV-resistant CrleGV genomic DNA as well as determining whether the genetic integrity of the UV-tolerant CrleGV strain is retained when bulked up i*n vivo*. A real-time PCR assay will be developed to screen samples for these genetic mutations. This study then aims to conclude with evaluating the efficacy of the UV-resistant CrleGV in semi-field trials.

Integrated Pest Management under nets in Mpumalanga Province

The use of protective shade netting over citrus and litchi orchards has become an increasingly popular practice in South Africa of late. However, relatively little on the impact of this practice on pest dynamics has been studied. This project aims to fill some of these knowledge gaps.

RESEARCH TEAM
Prof. Sean Moore, Prof. Martin Hill

STUDENT
Karlien Grobler (MSc)

In 2019/20, the incidence and severity of citrus thrips throughout the citrus growing season was found to be less under 20% enclosed shade net than in an open citrus orchard. Mealybug populations were higher and more concentrated under the citrus netting than in the open orchard, contradicting the recordings in the previous growing season. The parasitoids, *Coccidoxenoides perminutus* and *Anagyrus* sp. were released simultaneously in equal quantities under citrus netting and in the open orchard. Mealybug infestation declined, while percentage mealybug parasitised increased six weeks after release in both the enclosed and open orchard. Bollworm and orange dog populations did not seem to be any different in the two citrus orchards. Total trap catches of FCM were higher under citrus shade nets than in the open orchard, while fruit fly species were caught in higher quantities in the open orchard. However, these quantities have not yet been compared statistically. Minimal fruit infestation by both FCM and fruit flies was recorded during harvesting. Mummified mealybugs were collected in citrus sites and placed in emergence boxes. Only *Coccidoxenoides perminutus* emerged. Red scale was collected and inspected for parasitism by *Aphytis* spp. None have been detected to date. Citrus fruit sampled for residue tests resulted in higher concentrations of dithiocarbamates and pyraclostrobin under the enclosed netting than in the open orchard. Seychelles scale and pink wax scale infestation was higher in litchi orchards under shade net than in the control. Mango scale infestation seems to be similar in litchi orchards in both scenarios. No litchi moth adults were trapped and no larval infestation was detected. False codling moth trap counts and larval infestation were higher under the netted litchi orchard. Fruit fly trap catches and larval infestation were found to be higher in the open orchard than under shade net. *Cryptolaemus montrouzieri* was released in equal quantities simultaneously in the netted litchi orchard and open orchard for the control of Seychelles scale and pink wax scale. Both pest populations were reduced to acceptable IPM levels before harvest. Predators (*Rodolia cardinalis* and *Chilocorus* spp.) were found to occur naturally under litchi shade nets, but none in the open orchard.

Netted and open citrus orchards near Kirkwood in the Sundays River Valley. **Photo:** Rotorworx Aviation

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

RESEARCH TEAM
Prof. Sean Moore, Prof. Martin Hill

STUDENT
Wayne Mommsen (MSc)

Augmentation of the parasitoid, *Anagyrus vladimiri*, for control of mealybugs on citrus is now fairly widely practised. However, there is still a dearth of knowledge about the ideal or most effective protocols for parasitoid releases pertaining to both timing of releases and release numbers. Furthermore, the integration and management of biological control with a strict chemical treatment regime for thrips and citrus blackspot remains a challenge and needs to be refined. The objective of this study is to answer these questions. Non-target effects on *A. vladimiri* by agricultural chemicals, used for control of red scale, mealybug and thrips will also be evaluated. Augmentation trials with *A. vladimiri* were given priority and commenced in the 2019/20 season on three different cultivars in two different growing regions, Burgersfort and Hoedspruit. Mealybug infestation of fruit in the *A. vladimiri*-treated orchards peaked at an average of 14.0% (Dec/Jan), 14.3% (Feb/Mar) and 10.0% (Dec/Jan) in Burgersfort open orchards, Burgersfort under net, and Hoedspruit open orchards, respectively. Infestation declined to averages of 1.5%, 5.3% and 0.8% in the weeks prior to harvest. Maximum-minimum trends for the control blocks were 15%-5%, 2%-0% and 6%-0%. The T1 orchards, where the earliest releases of *A. vladimiri* were made, shortly after petal drop, showed the lowest incidence of mealybug-infested fruit over time compared to an untreated control, and resulted in the lowest percentage of mealybug-infested fruit before harvest in two of the three experiments.

Close up of a citrus mealybug, *Planococcus citri,* which damages citrus by sucking sap causing wilting and premature fruit drop while also secreting honeydew which promotes the growth of fungal mold on leaves and fruit. **Photo:** Peter Stephen, Citrus Research International

Identifying volatile emissions associated with false codling moth infestation of citrus fruit

RESEARCH TEAM
Prof. Sean Moore, Prof. Martin Hill, Prof. Rui Krause, Prof. Roman Tandlich

STUDENT
Wayne Kirkman (PhD)

Due to the phytosanitary status of false codling moth (FCM), *Thaumatotibia leucotreta,* for several of South Africa's citrus export markets, this project aimed to develop a post-harvest technology for detecting infested fruit. A Solid Phase Microextraction (SPME) probe was shown to effectively trap and concentrate headspace volatile compounds surrounding intact fruit. Volatile compound detection is achieved by inserting the probe into a Gas Chromatography-Mass Spectrometry (GCMS) system. The GCMS analysis was conducted on five major volatile compounds of interest previously shown to be released by FCM-infested oranges. These major volatile compounds are D-limonene, 3,7-dimethyl-1,3,6-octatriene, (E)-4,8-dimethyl-1,3,7-nonatriene, caryophyllene and naphthalene. In trials conducted in 2016 on Witkrans Navel oranges infested 14 and 21 days previously, as well as naturally infested fruit from the same orchard, D-limonene levels decreased with time after infestation, while levels of a naphthalene derivative increased. The ratio of these compounds was significantly different between healthy and infested fruit for all time periods. In similar trials conducted in 2017 on Mor Mandarins, Washington and Witkrans Navels, and Midknight and Delta Valencias, these same trends were not observed. This finding was mainly due to variability in D-limonene levels in all cultivars, a finding which can probably be ascribed to the extremely unusual climatic conditions in the Eastern Cape, which resulted in excessive splitting and fruit drop, as well as uncharacteristic berg winds scorching the Valencia orchards. However, in the trials conducted on Witkrans Navel oranges, as well as Midknight and Delta oranges in 2018 and 2019, the trend of decreased levels of D-Limonene and increased levels of naphthalene, with time after infestation, and the ratio between the two compounds (D-Limonene/naphthalene) was similar to 2016. Trials on Washington Navel oranges in 2019 revealed the same trend. In trials conducted on Clementine Mandarins in 2018 and 2019, there was an increase in beta-Ocimene levels with time after infestation, where levels were undetectable in the control fruit. A Select Ion Flow Tube Mass Spectrometry (SIFT-MS) unit was evaluated at the University of Leuven in Belgium. The unit could clearly differentiate between healthy fruit and fruit injured 24 hours prior to evaluation. The presence of D-Limonene could be identified after one second of SIFT analysis following 20 seconds of headspace collection. In 2021, research on this technology will continue in collaboration with our Belgian partners.

An assessment of the reasons for lower false codling moth infestation in organic versus conventional citrus orchards

RESEARCH TEAM
Prof. Sean Moore, Prof. Martin Hill

STUDENT
Luke Cousins (MSc)

Packhouse assessments in the Eastern Cape record notably lower levels of FCM infestation in fruit from organic than fruit from conventional orchards. Conventional wisdom might conclude that naturally occurring biological control would be greater on an organic farm. However, this cannot simply be assumed. Consequently, this study evaluates the possible factors that could contribute to this difference, namely above- and below-ground biological control, fruit biochemistry and farming practices. The progress of the project is on schedule. Analysis of FCM ecology has been underway since October 2019. Numbers of wild FCM caught within study sites have been very low in all but one conventional orchard, resulting in an observed difference between organic and conventional orchards, but with a high level of variation. Due to the low FCM numbers, egg counts have also been very low, with no clear differences. Four soil samples have been taken, and the final sample analysis in progress. So far, there have been no clear differences in abundance between entomopathogenic fungi (EPF) and entomopathogenic nematodes in organic and conventional soil. There does seem to be a difference in EPF species between the organic and conventional farms in Kirkwood, which will be confirmed by further samples and final genetic identification. Fruit oviposition preference studies have almost been completed for the season and results show no obvious trend. There was an observed oviposition preference for organic fruit in the choice experiments; however, later in the season, there seemed to be slightly more eggs laid on conventional fruit in no-choice trials. Fruit susceptibility trials indicate a possible higher susceptibility of conventional Navels towards the end of the season. Late-season trials with Delta Valencias are currently underway. Wet analysis

Larval damage of an orange infested with false codling moth.
Photo: Peter Stephen, Citrus Research International

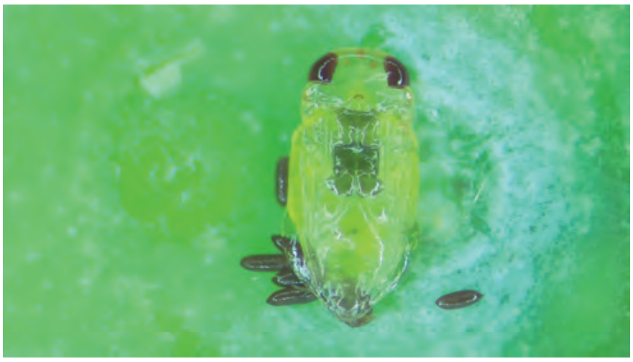
of early Palmers and Deltas, both early and late in the season, has been completed by Bemlab laboratories, and all analysis reports have been received. A common trend throughout the season for both cultivars is a higher magnesium content for conventional fruit. CAL laboratories have conducted a separate dry analysis of the ripe fruit for both cultivars, indicating a clearly higher crude fat content for conventional fruit. Physical measurements and juice analysis of fruit has been completed for immature and ripe Navels and Valencias. Physical parameter measurements show that organic fruit is larger, with a corresponding higher mass and a thicker peel. Juice analysis indicates a similar sugar/acid ratio for fruit of both farming types, but a higher overall sugar and acid content for conventional fruit. Pitfall trap specimen collection has been added to the project, and two collections have been completed so far. Analysis of these samples is still taking place.

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

Current and future market trends require fruit with lower or no chemical residues and originating from a source where sound IPM protocols are applied. The active ingredients of remedies used to control red scale are the most common active ingredients to be found amongst citrus fruit residue analyses done in the last 10 years. To be able to reduce or remove such active ingredient(s), a better understanding of the role of *Aphytis* spp. as a part of integrated red scale control is necessary. During the first season, *A. melinus* was augmented into seven orchards versus seven control orchards in the Eastern and Western Cape. Results so far have not shown any differences between treated and untreated orchards, and although *Aphytis* spp. were effective in most cases in reducing red scale infestation, *A. melinus* did not contribute significantly towards this. However, the naturally occurring indigenous *A. africanus* showed a strong natural presence in most orchards, probably outcompeting the augmented *A. melinus*. Ongoing trials will be aimed at optimising augmentation to possibly increase overall parasitism levels through self-established populations.

RESEARCH TEAM
Prof. Sean Moore, Prof. Martin Hill

STUDENT
Ernst de Beer (PhD)



The parasitic wasp, *Aphytis melinus*, about 1mm in size, is an augmented parasitoid of red scale. The wasp lays eggs under the scale covering, and after hatching the larva feeds on the scale. **Photo:** Ernst de Beer

Synergism and formulation of entomopathogenic fungi for foliar control of various citrus pests

RESEARCH TEAM
Prof. Sean Moore, Prof. Martin Hill

STUDENT
Samantha Prinsloo (MSc)

False codling moth (FCM), *Thaumatotibia leucotreta*, is a major insect pest of citrus in South Africa. Owing to restrictions on the use of insecticides, non-chemical control options, including the use of entomopathogenic fungi (EPF) have been investigated. Laboratory and field trials have highlighted several isolates, including *Metarhizium anisopliae* FCM Ar 23 B3, capable of inducing mortality in FCM soil-dwelling life stages. Other potential control agents of such life stages include entomopathogenic nematodes (EPN). Three species of nematodes that may be used in FCM management plans are *Steinernema yirgalemense*, *S. jeffreyense* and *Heterorhabditis noenieputensis*. Previous research has shown that a synergistic relationship might occur between EPN and

EPF when they are combined to manage late instar lepidopteran larvae. Alone, all four microbial agents induced more than 75% FCM mortality. Preliminary results from the EPN-EPF interaction trials suggest interactions to be mostly additive or antagonistic. Only one synergistic interaction was reported between the EPN species *S. yirgalemense* and the EPF isolate *M. anisopliae* FCM Ar 23 B3 when applied simultaneously. EPF interactions with the EPN species *S. jeffreyense* were considered antagonistic, irrespective of the timing of EPN application (0, 24, 48, 72 and 96h post-EPF application). Further trials are currently in progress to confirm these findings.

(right) False codling moth cadaver infected with the entomopathogenic fungus *Metarhizium anisopliae* FCM Ar 23 B3. **Photo:** Samantha Prinsloo

Honeybush tea

RESEARCH TEAM
Dr Candice Coombes, Prof. Martin Hill

STUDENT
Tapiwa Mushore (MSc)

COLLABORATORS
South African Honeybush Tea Association (SAHTA), Gillian McGregor (Department of Geography, Rhodes University), Matt Sephton (Living Lands)

The honeybush research project, initiated in 2019, aims to investigate insect pests associated with the cultivation of honeybush (*Cyclopia* spp.), a plant indigenous to the fynbos region of South Africa and grown for the production of honeybush tea. This unique herbal tea, with a pleasant honey-like taste and flavour, has gained economic importance owing to increased demand both globally and locally. The increase in demand has led to a recent shift from wild harvesting to commercial cultivation of honeybush. Successful large-scale cultivation of honeybush faces several challenges, including managing insect pests, and if the shift from wild harvesting to commercial cultivation is to be achieved, insect pests associated with honeybush need to be better understood and managed.

Tapiwa Mushore, a MSc student, has been hard at work conducting research on two important insect pests of honeybush, the Keurboom moth, *Leto venus,* and the blue-winged leafhopper, *Molopopterus* sp. Both these insect species are endemic to South Africa and have the potential to negatively impact the cultivation of this indigenous crop. The larval life stage of the Keurboom moth is the most damaging as it bores into plant stems and branches, feeding on the plant tissues and creating large tunnels in the process. Left uncontrolled, this may lead to entire plant dieback owing to extensive damage to structural integrity and damage to vascular plant tissues. Both the adults and all nymphal life stages of the blue-winged leafhopper may cause damage to the leaves of the honeybush plant due to their feeding mode as a sapsucking species. This feeding causes phytotoxic symptoms termed "hopperburn", which can result in complete desiccation, ultimately leading to loss in potential product. Leafhoppers also secrete honeydew, which may serve as a nutritional food source for other insect species and may encourage the growth of sooty mould under high humidity conditions. Although not known for this species, leafhoppers have also been reported as vectors of plant pathogens and thus are important pests for which control is needed.

Research conducted over the 2019–2020 period was to establish factors responsible for field infestation of the Keurboom moth; results showed the infestation was a product of four fixed effects: stem diameter, plant species, farm location and plant age. These results suggested that only older plants of the species *Cyclopia subternata*, with a stem thickness of greater than 10 cm diameter, need be monitored for infestation. As larval entry into the plants was always recorded at ground level or just above, it may be possible to prevent infestation by treating the base of the plants. Soil samples were collected from various honeybush fields and surrounding refugia and baited, using mealworms to isolate and identify entomopathogenic fungi as a natural control option. Twenty isolates of entomopathogenic fungi from two species, *Metarhizium anisopliae* and *Fusarium oxysporum,* were identified. At the time of this research, Keurboom moth abundance within sampled fields was low and new infestations rare. As a result, the control potential of these microbial agents could not be evaluated against the pest, and it was concluded that control practices already employed may be sufficient to control the pest at current population levels.

Pest bionomics, host preference and factors influencing the distribution and abundance of *Molopopterus* sp. were explored. Under laboratory conditions, leafhopper development from egg to adult was approximately 50 days with five nymphal instars recorded. A strong preference towards *Cyclopia longifolia* over other commonly cultivated species was found, both in the laboratory and field, with *Molopopterus* sp. abundance and distribution recorded as being influenced by age of the plant, location of the farm, harvesting regimes and plant species. The density of leafhoppers was observed to decrease with an increase in age; however, a surge in density was observed in recently harvested stands. Differential densities were also observed at different farms, with Kurland having the highest pest densities. Given the problematic nature of this pest, the collected entomopathogenic fungal isolates were evaluated as a potential management tool. Although experiments are still in progress against the nymphal life stages, the results of bioassay against the adults identified seven isolates capable of inducing 60% mortality or above. These isolates have the potential to reduce leafhopper populations upon successful formulation for field application.

Alongside this research, field monitoring of insect presence within honeybush fields of several ages was undertaken, using both active and passive sampling techniques at Kurland Estate in the Western Cape, from January 2019 to January 2020. Numerous insect species were recorded, including several species of thrips, leafhoppers, gall midges, aphids, and an abundance of natural enemies such as lacewing larvae, ladybug larvae, parasitoid wasps, and spiders. Compared to earlier sampling events, the number of potential insect pests recorded declined, suggesting a natural level of pest control and good management practice. However, thrips have been reported as increasingly problematic elsewhere, and as such, this may need to be investigated further. Positively, the entomopathogenic fungal isolates identified by Tapiwa may offer a control solution against these notorious insect pests.

Tapiwa conducting leafhopper host preference trials at the CBC tunnels. **Photo:** Daniel Rogers

The adult leafhopper, *Molopopterus* sp., as observed under a dissecting microscope. **Photo:** Tapiwa Mushore

POLYPHAGOUS SHOT HOLE BORER

The polyphagous shot hole borer (PSHB) is an ambrosia beetle native to Southeast Asia that has become a highly invasive tree pest in the United States and Israel. In 2017, it was discovered in a botanical garden in Pietermaritzburg, KwaZulu-Natal and has now become well established in eight of the nine provinces of South Africa.

PSHB bores into trees and releases a symbiotic fungus, *Fusarium euwallaceaea*, which grows in the vascular system of the tree, inhibiting water and nutrient uptake which, in many cases, kills the tree. It has been found attacking various ornamental trees and more importantly, several native tree species. It poses a major threat not only to agricultural crops and urban trees, but to native forests throughout South Africa. Currently, research projects focusing on the impact of the PSHB on susceptible agricultural and urban trees, as well as its impact in indigenous forests throughout South Africa, are being conducted by Forestry and Agricultural Biotechnology Institute (FABI) researchers.

Current results show that the beetle has not had any major impacts on agricultural crops. It has, however, been recorded on 44 indigenous tree species, 11 of which are reproductive hosts of the beetle. Preliminary data from permanent monitoring plots in indigenous Afrotemperate forests has shown that the spread of the beetle in these natural areas is slow, but consistent increases in the number of attacked trees have been evident in the last year. Forest patches near urban areas with lower tree-species richness appear to be more susceptible to attack, and larger trees in poor health or already under stress are at greater risk. The continued attacks by PSHB on trees in indigenous forests may have serious consequences, and continued long-term monitoring is required to determine potential ecological impacts. Monitoring the spread and impacts of the beetle continue, with eight universities throughout South Africa, as well as overseas collaborators, forming a research network that is constantly sharing new information and ideas. This monitoring forms part of Garyn Townsend's MSc; he is registered at the University of Pretoria, with the Forestry and Agricultural Biotechnology Institute (FABI), but based at Rhodes University, and is co-supervised by professors from both institutions.

RESEARCH TEAM
Prof. Martin Hill, Prof. Wilhelm de Beer (FABI, UP)

STUDENT
Garyn Townsend (MSc, UP)

COLLABORATORS
FABI

A polyphagous shot hole borer beetle damages trees because of the impact of a symbiotic fungus which grows in the tunnels that the borer makes, and inhibits water and nutrient uptake **Photo:** Samantha Bush, FABI

Garyn Townsend is working in indigenous Afrotemperate forests along the Garden Route where his research is focusing on assessing the impacts of the invasive polyphagous shot hole borer (PSHB) on native tree species such as this yellowwood (*Afrocarpus falcatus*). **Photo:** Garyn Townsend

MASS-REARING

Once an insect is approved for release to control a specific invasive plant, the next stage is to mass-rear the insect so that it can be released in large numbers at the sites around South Africa where the invasive plant occurs. The CBC has two facilities where mass rearing of insects takes place – one in Makhanda, focused on waterweed agents, and a second facility in Kariega (formerly Uitenhage), where the focus is on cactus agents. The process by which insects are mass-reared is, firstly, by cultivating invasive alien plants in controlled greenhouse environments; these plants are then used to culture the biological control agent specifically approved for control of a particular plant. Once a large enough population exists, these insects are collected, carefully packaged and distributed throughout South Africa. The sites where they are released are recorded, and the invasive plant populations are monitored at these sites. Getting a large number of healthy insects in the field helps to increase the speed of control and improve the efficacy of the control agent in situations where the biological control agents might spread slowly owing to environmental conditions, such as cooler winters.

The CBC does not charge any fees for the distribution of these agents to farmers, researchers, implementation officers and reserve managers; the costs are carried by government funding as the CBC is largely funded by the DFFE. All biological control agents mass-reared by the CBC are safe and approved for release in South Africa by the appropriate regulatory bodies.

Waainek mass-rearing facility

The Waainek mass-rearing facility focuses on mass rearing and releasing insects for biological control of waterweeds around South Africa. The facility was established in 2008, and is funded by the DEFF. Maretha Boshoff, manager at this facility, manages a team of eight staff (the Sisonke team) who ensure healthy plant material is available for the insects to thrive, and who assist with the collection and packaging of these insects in response to any requests.

The Waainek mass-rearing facility overcame several challenges in 2020. The water problems were addressed in February when the Rhodes University borehole system was linked up to the Waaninek mass-rearing facility system, ensuring a constant water supply. A generator was installed in July, which meant an end to the challenges of trying to manage the tunnel temperatures during power cuts. COVID-19 restrictions also impacted the workforce, but three staff members were allowed to return to work in July, when the capacity of managing mass-rearing of waterweed agents was increased. In October 2020 all staff were back at work.

In 2020 the Waainek mass-rearing facility made a total of 85 releases of a range of biological control agents at various sites in South Africa, and over 400 000 insects were released.

The Waainek mass-rearing facility with tubs of waterweeeds. **Photo:** Samella Ngxande-Koza

Aquatic weed biological control agents released by the CBC Waainek mass-rearing facility in 2020

Target Weed	Biological control agent	Number of releases made	Total number of insects or immature larvae released
Pontederia crassipes	*Megamelus scutellaris*	70	367 500
Myriophyllum aquaticum	*Lysathia sp.*	7	6280
Egeria densa	*Hydrellia ergeriae*	3	24400 + 2kg of plant material
Pistia stratiotes	*Neohydronomus affinis*	5	5250
TOTAL		85	403430

Sisonke team

An important programme with a long-standing history within the CBC (which started out as a research group) is the Sisonke team. This group started in 2009 when three people living with disabilities were employed to assist with mass-rearing and implementing the aquatic weed biological control programme. Since then, the Sisonke team has grown and is currently made up of eight dedicated people (some of whom live with disabilities, some of whom are able-bodied) who all work together in the important implementation part of the CBC's mandate. The team is based at the Waainek mass-rearing facility where they are given opportunities to upskill wherever possible. Members of the team have also been involved in training and setting up the City of Cape Town's Invasive Species Programme's mass-rearing facility.

As mentioned in the Director's Report, at the end of 2020, the Sisonke team was saddened to lose one of its longest serving members, Lulama Poni. Lulama started out with this group in 2009 and was one of the first people with a disability to join the group; he opened up the door for many more. He passed away on 26 December 2020 after a short illness. He will be sorely missed for his sense of humour, and his knowledge of biological control and current affairs.

Lulama Poni talks with Prof Marcus Byrne from Wits and Prof Alejandro Sosa from Argentina, at the opening of the CBC in November 2017. **Photo:** Kay Montgomery

Summer is always busy with the Waainek team collecting biological control agents to send out. Landile Booi, Vuyani Ntyinkala and Wandisile Mdiza are pictured here with automatic pooters in hand. **Photo:** Julie Coetzee

Kariega mass-rearing facility

The team based at the Kariega mass-rearing facility comprises eight staff who are responsible for mass-rearing, releasing, and monitoring cactus biological control agents throughout the country. The facility manager, Ruth Scholtz, and CBC PhD student, Zezethu Mnqeta, work together to ensure that the team releases as many agents at as many sites as possible. They also ensure that a database of releases is kept, that land-users are informed and educated about biological control of cactus weeds, and that the impact and success of the agents are monitored and recorded.

Six different cactus biological control agents are mass-reared by the team, and these are used for the control of eleven different cactus weeds. The cochineal insects, which all fall into the genus *Dactylopius*, each control a single weed species, while the galling mealybug, *Hypogeococcus* sp., is used for the control of several closely related cactus weeds. The cochineal insects are reared on the cactus pads (called cladodes), and the cochineal-infested cladodes are then released by placing them in the canopy of the target weed in places where the weed is a problem. For *Hypogeococcus*, the agent is reared on live plants, and the galls that are formed by the agent on these plants are then attached to the growing tip of the target weed in the field. When possible, the team will travel to the sites where cactus infestations occur and conduct large mass-releases of the correct biological control agent, but if travel is not possible, as was the case for much of 2020, smaller releases are made by couriering the agent to the land-user with the cactus problem and instructing them how to conduct the release themselves.

In 2020, 117 releases were made with a total of over 9000 cochineal-infested cactus cladodes or galls. This is a significant achievement, given the restrictive circumstances of 2020; it would not have been possible without the help of the network of land-users and the DFFE Biodiversity Officers who requested and released agents. Long-term monitoring to evaluate the success of these biological control agent releases was continued through 2020 at 33 sites, and the data from this monitoring indicate that the vast majority of releases have resulted in a significant reduction in the biomass and density of the targeted cactus weeds. This success is corroborated by the land-users themselves who are all interviewed by the team as part of Zezethu's research. The vast majority of land-users believe that biological control has reduced the cactus problems on their land in a safe and effective manner.

The Kariega mass-rearing facility has a number of green houses in which cactus species are grown in order to rear the biological control agent – *Hypogeococcus* on *Harrisia martinii* shown here. **Photo:** Tamzin Griffiths

Releases of cactus biological control agents from the CBC mass-rearing facility

Target weed	Biological control agent	Total (2020)	
		Number of releases made	Total number of cladodes or galls released
Opuntia aurantiaca	*Dactylopius austrinus*	8	2383
Opuntia stricta	*Dactylopius opuntiae* 'stricta'	27	1383
Opuntia humifusa	*Dactylopius opuntiae* 'stricta'	13	959
Opuntia monacantha	*Dactylopius ceylonicus*	13	1298
Opuntia ficus-indica	*Dactylopius opuntiae* 'ficus-indica'	8	143
Opuntia robusta	*Dactylopius opuntiae* 'ficus-indica'	1	29
Cylindropuntia imbricata	*Dactylopius tomentosus* 'imbricata'	17	1901
Cereus jamacaru	*Hypogeococcus* sp.	24	815
Harrisia martini	*Hypogeococcus* sp.	6	195
TOTAL		117	9106

Kariega mass-rearing manager, Ruth Scholtz, inspecting cochineal on cactus cladode of *Opuntia monocantha*. **Photo:** Tamzin Griffiths

COMMUNITY ENGAGEMENT

A key aspect of the work the CBC does is engaging with their community in Makhanda as well as the broader South African community about the topics of invasive alien species and biological control. The aim of these engaged activities is to interact with the public so that they can learn about invasive species and the impacts they have on our environment, and that there is a way of controlling some invasive species biologically. The CBC team does a great job at getting together to engage with people on these topics. Owing to the COVID 19 global pandemic, many of our face-to-face activities have had to be postponed or cancelled. We have continued to try to engage with communities online and in person where possible and safe. Below we highlight our activities over the course of 2020.

Agricultural partnership

The CBC continued its partnership with Agri SA during 2020, although the COVID-19 pandemic prevented any in-person meetings. The CBC published the first of a series of three articles on management of waterweeds in the Agri SA online newsletter; the subsequent articles need to be completed. These articles called for a clear strategy to manage waterweeds through integrated management, with an emphasis on effective use of biological control. In partnership with Agri Noord-Kaap, the CBC has continued to work on developing a strategy to manage *Prosopis*.

Restrictions on international travel during 2020 prevented our CBC team, as well as our partners in the Agricultural Research Council, from travelling to Argentina to investigate possible biological control agents. Through collaboration with partners in Argentina, the CBC will test potential agents during the next few years on *Opuntia elata*. Professor Iain Paterson secured funding for three years from Red Meat Research and Development for research into biological control of *Opuntia elata;* the funder understands the delay and supports the retention of funds until the resumption of international travel and research. The ARC team are working on the inkberry species, *Cestrum parqui* and *Cestrum laevigatum* and hope to travel soon to investigate possible biological control options.

Cactus outreach work

The Cactaceae programme has strengthened their relationships with protected areas over the last few years. Camdeboo National Park and Addo Elephant National Park both have programmes where they are managing various cactus species which we have supported from a safe distance this year. The CBC has a long history of collaboration with Enseleni Nature Reserve near Richards Bay in KwaZulu-Natal, conducting research into and implementation of the control of water hyacinth on the river. Recently the cactus group has been assisting, as many areas around Enseleni are negatively impacted by the invasive alien cactus, *Pereskia aculeata*. Dr Roy Jones, a CBC PhD graduate and employee of Ezemvelo Wildlife, has motivated for the development of a small mass-rearing facility at Enseleni to rear the biological control agent for *P. aculeata*, the *Pereskia* stem-wilter, *Catorhintha schaffneri*.

Iain Paterson visited Enseleni to train Enseleni staff in how to mass-rear the agent and provide some basic background about biological control, and especially what can be expected for biological control of *Pereskia* at their small facility.

The South African Cactus Working Group normally has two annual meetings co-hosted by the CBC and SANBI, but owing to the COVID-19 pandemic, these could not take place. This long-standing working group has diverse membership and meetings are productive. The CBC has recently taken over the coordination of the International Cactus Working Group (ICWG) and had planned to have the first meeting in 2020 in Windhoek, Namibia. Unfortunately, again, owing to the pandemic, this meeting had to be postponed and we have a proposed date for the second half of 2021.

Iain Paterson providing some background and training about how to rear the Pereskia stem-wilter, *Catorhintha schaffneri*, to staff at the Enseleni Nature Reserve near Richards Bay. **Photo:** Guy Sutton

Prosopis engagement

Research has clearly demonstrated the need for a well-supported strategy and an integrated approach for the management of *Prosopis,* using both a more effective biological control and physical removal of plants. The CBC has worked with Agri Noord-Kaap and partners in the Northern Cape to prepare funding applications for projects that utilise *Prosopis* biomass. With the correct nutritional additives, the leaf and small twig biomass from *Prosopis* can possibly be used in supplementary fodder. Unfortunately, funding applications have not been successful, but further bids for funds are underway. The CBC has prepared a draft strategy for management of *Prosopis* and circulated this for discussion, but owing to the lack of opportunities to meet, this outreach has not made sufficient progress.

Weed Biological Control Short Course

The CBC hosts an annual Weed Biological Control Short Course which is accredited through Rhodes University. COVID-19 meant we had to postpone and then subsequently cancel the course for the financial 2020/2021 year. We will revisit dates for 2021 and also look at alternative ways of delivering the course.

Undergraduate vacation apprenticeship and experience for UKZN students

The apprenticeship opportunity was advertised to second-year students in the School of Life Sciences, UKZN, Pietermaritzburg campus in 2020. Ten second-year students were chosen for the apprenticeship and were covered by UKZN insurance while at their respective research centres. These ten students then took part in the January–February 2020 vacation apprenticeship where they were allocated to research partners for the apprenticeship period. Some students were exposed to the various aspects of alien invasive problems, research, and potential biological control. However, as documented in their thank-you letters to the respective mentors, they gained far more: they learned about the particular problems researchers are involved in, experimental design, data collection, replication of experiments, and computer information searches and data input. Students were familiarised with the institution's facilities; they visited study sites and developed a rapport with their particular researchers. Some students undertook small research projects for the researchers they were assigned to.

As a consequence of the COVID-19 restrictions, undergraduate students were unable to participate in the July vacation biological control apprenticeship. We therefore gave five postgraduates between degrees the opportunity to participate and assist with research involving alien invasives from July–December 2020. In November 2020 we selected ten second-year students for the apprenticeship in January–February 2021 at the respective research centres.

The CBC would like to acknowledge Prof. Colleen Downs (School of Life Sciences, UKZN) for coordinating this project, and the following for hosting students: Dr Des Conlong, and Thobeka Khumalo (SASRI), Dr Terry Olckers (School of Life Sciences, UKZN), Dr Costas Zachariades (Cedara Weeds laboratory, ARC-PPRI); students also worked with Dr Caswell Munyai, Dr Ziv Tsvuura, Dr Michelle Tedder, and Prof. Kevin Kirkman, (School of Life Sciences, UKZN).

Implementation training

For a number of years, small teams of people have been employed in the Western Cape to implement biological control through the Department of Environment, Forestry and Fisheries (Natural Resource Management Programme) funding. Historically, the two teams have been based in George and Grabouw, and have consisted of between 6–12 team members each. Responsibilities include collecting, redistributing and monitoring specific biological control agents of problematic invasive species, namely Australian acacias, hakea, *Leptospermum* and sesbania. The respective biological control agents cannot be mass-reared, but must be collected in the field and redistributed by trained individuals. Together with staff from ARC/PHP, Stellenbosch, researchers at UCT have played an active role in training these teams: providing a basic background to biological control, outlining the steps in biological control programmes, providing focus on certain invasive plants and their biological control agents in the Western Cape, and then more specifically, how to go about collecting and releasing such agents.

During 2020, Cape Nature became the implementing agent for overseeing these teams, and three teams are in the process of being appointed in George (Outeniqua Nature Reserve), Robertson (Vrolijkheid Nature Reserve) and Stellenbosch (Jonkershoek Nature Reserve). On 1 December 2020, training was provided to a new team of incumbents (see photo), and will be rolled out to the additional two teams during early 2021.

Fiona Impson undertakes some field training in the Western Cape. **Photo:** Fiona Impson

School engagement

The CBC is involved in a number of school engagement activities. The longest-standing one is the Science Internship Programme, which is a great way of exposing learners to what being a scientist is all about. School learners apply through their educators and are then selected for the programme and placed at different Science Faculty Departments. The learners spend three weeks of their holiday with the different research groups. During this time, the learners are exposed to what it is to be an applied entomologist in the field of biological control at the CBC. Unfortunately, we have not been in the position to welcome learners onto campus during the national lockdown period, but the centre looks forward to welcoming them back again when it is safe.

In the last few years, the CBC has been actively engaged with schools in the Hartbeespoort Dam and Nahoon River areas as part of the school mass-rearing projects. For obvious reasons, some schools have put this activity on the back burner for now. The CBC has been able to support and assist where possible with keeping these projects going; it has been easier with the two schools in East London as they are within driving distance from Makhanda. Before the national lockdown, the CBC team took the two schools' Eco Clubs to the Nahoon River to show them how to assess the water hyacinth and biological control.

The schools' mass-rearing project was growing organically with schools showing the initial interest, which is a good sign, as schools need to take ownership of the projects for them to be effective. We will continue to expand and reach out once regulations allow, and it is safe to do so.

The CBC's focus with this programme is not so much on the numbers of agents reared and released into the water systems, as on the learning and eagerness generated through the activities, and the involvement of the learners. The CBC hopes to grow young environmental stewards and possibly future entomologists!

Teachers from Stirling High School releasing the water hyacinth planthopper, *Megamelus scutellaris*, on the pool set up for mass-rearing; **Photo:** Benjamin Miller

Learners from two East London schools get an insight into the fieldwork sampling process in the field of aquatic biological control, at the Nahoon River. **Photo:** Esther Mostert

Community mass-rearing projects

After the success of biological control on the Hartbeespoort Dam over the summer of 2019/2020, community groups and concerned citizens identified by the CBC in 2019 were encouraged to participate in mass-rearing projects to increase the effective number of insects released onto the dam. Throughout 2020, despite the national lockdown and the COVID-19 pandemic, the CBC team (Prof. Julie Coetzee, Benjamin Miller and Kim Weaver) set up 13 rearing facilities and provided scientific consultation on two others that were self-funded. Most of the facilities are in Gauteng, the

North-West province and KwaZulu-Natal and include private landowners, estates, non-profit organisations and who want to make a difference to their local systems. These mass-rearing facilities have enabled many new releases of the biological control agent, *Megamelus scutellaris,* on water hyacinth invasions around the country, and fostered invaluable support for the biological control national programme, which may have otherwise been unknown to the layperson. This effort has been supported and coordinated through releases from the Waainek mass-rearing facility at Rhodes University.

The project, which will expand in 2021, is crucial to producing healthy insects in high numbers close to the infested waterbody where they are required, thus reducing the time between insect collection and release, and so increasing insect health and effectiveness. The projects also aim to reduce the time between plant recovery after winter and biological control insect population recovery.

Partners in the CBC's community engagement activities

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

Schools: Ntsika Secondary School, Nombulelo Secondary School, Victoria Girls High School, Graeme College, Merrifield College, Stirling High School and Mountain Cambridge School.

Patrick Ganda, community partner from the Blesbokspruit Wetland Reserve and Benjamin Miller releasing biological control agents on the Blesbokspruit River, Springs. **Photo:** Matthew Paper

Ezekiel Khosa, Llewellyn Foxcroft from SANParks scientific services, together with and Benjamin Miller checking for biological control agents on water hyacinth on the Letaba River, Kruger National Park. **Photo:** Julie Coetzee

Phillip van Tonder from the Canoe Academy at Roodeplaat inoculating the mass-rearing facility's plants with *Megamelus scutellaris*. **Photo:** Benjamin Miller

Funders

The CBC would like to acknowledge the various funders for 2020:

National Funders

Citrus Research International (CRI)

Department of Forestry, Fisheries and the Environment (DFFE): NRM: Working for Water (WfW) programme

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

HORTGRO (Alternative Crop Fund)

National Research Foundation (NRF)

Red Meat Research and Development South Africa

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

Rhodes University

River BioScience

South African Honeybush Tea Association (SAHTA)

Water Research Commission (WRC)

International Funders

AgriFutures Australia (formerly the Rural Industries Research and Development Corporation (RIRDC)).

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

Biosecurity South Australia (PrimaIndustries and Regions South Australia)

LandCare Research New Zealand

New South Wales Department of Primary Industry; Australia

Queensland Department of Agriculture, Australia

Shire of Ravensthorpe, Western Australia

Research Outputs

Graduates

PhDs

1. Mavis Acheampong. 2020. Suitability of entomopathogenic fungal isolates for microbial control of citrus pests: biological traits. PhD. Supervisors: Martin Hill, Candice Coombes and Sean Moore. Rhodes University.
2. Blair Cowie. 2020. *Parthenium hysterophorus*: understanding the invasion and potential controls. PhD. Supervisors: Marcus Byrne and Ed Witkowski. University of the Witwatersrand.
3. Zolile Maseko. 2020. Post-release evaluation of the distribution and efficacy of *Eccritotarsus catarinensis* and *Eccritotarsus eichhorniae* on water hyacinth in South Africa. PhD. Supervisors: Julie Coetzee and Martin Hill. Rhodes University.
4. Evans Mauda. 2020. Investigations into biological control options for *Lycium ferocissimum* Miers, African Boxthorn (Solanaceae) for Australia. PhD. Supervisors: Grant Martin and Martin Hill. Rhodes University.
5. Samuel Motitsoe. 2020. Quantifying ecological benefits of the biological control of invasive alien macrophytes in Southern Africa. PhD. Supervisors: Martin Hill, Julie Coetzee and Jaclyn Hill. Rhodes University.
6. Jeanne Mukarugwiro. 2020. The abundance and extent of water hyacinth in Rwanda and selection of appropriate biological control. PhD. Supervisor: Marcus Byrne. University of the Witwatersrand.
7. Giuseppe Venturi. 2020. Thermal physiology and mechanistic model construction of the grasshopper *Cornops aquaticum* (Brüner, 1906) (Orthoptera: Acrididae). PhD. Supervisors: Marcus Byrne and Frances Duncan. University of the Witwatersrand.

MScs

1. Lehlohonolo Adams. 2020. Reproductive ecology of the invasive alien plant *Pyracantha angustifolia* in Afromontane grasslands of the eastern Free State. MSc. Supervisors: Sandy Steenhuisen, Grant Martin and Ralph Clarke. University of Free State.
2. Nompumelelo Baso. 2020. The effects of elevated atmospheric CO2 on the biological control of invasive aquatic weeds in South Africa. MSc. **With Distinction.** Supervisors: Julie Coetzee and Angela Bownes. Rhodes University.
3. Nwabisa Magengelele. 2020. Genetic characterisation and climatically suitability of the invasive alien plant, Brazilian pepper tree (*Schinus terebinthifolia*). Supervisors: Grant Martin, Iain Paterson and Unathi Heshula (UFH). Rhodes University.
4. Mary Maluleke. 2020. Economic evaluation of chemical and biological control methods on four selected aquatic weeds in South Africa. MComm. Supervisors: Gavin Fraser and Martin Hill. Rhodes University.
5. Mpilo Ndlovu. 2020. Managing the invasive aquatic plant *Sagittaria platyphylla* (Engelm.) J.G. Sm (Alismataceae): problems and prospects. MSc. Supervisors: Grant Martin and Julie Coetzee. Rhodes University.



CBC students celebrating their 2020 virtual graduations – Getrude Tshithukhe (MSc), Jennifer Upfold (MSc) and Nompumelelo Baso (MSc). **Photo:** Getrude Tshithukhe, Jennifer Upfold, Nompumelelo Baso

6. Megan Reid. 2020. Initiating biological control for *Nymphaea mexicana* Zuccarini (Nymphaeaceae) in South Africa. MSc. **With Distinction.** Supervisors: Julie Coetzee and Martin Hill. Rhodes University.
7. Getrude Tshithukhe. 2020. Assessing invasive alien aquatic plant species' phytoremediation success using biological indicators. MSc. Supervisors: Samuel Motitsoe and Martin Hill. Rhodes University.
8. Jennifer Upfold. 2020. Sexual attraction and mating compatibility between *Thaumatotibia leucotreta* populations and implications for semiochemical dependent technologies. MSc. Supervisors: Martin Hill, Candice Coombes and Sean Moore. Rhodes University.
9. Clarke van Steenderen. 2020. The phylogeny of *Dactylopius* with special reference to the potential for DNA-barcoding of biological control agent lineages. MSc. Supervisors: Iain Paterson and Shelley Edwards. Rhodes University.
10. Timothy Westwood. 2020. The potential conflict of interest associated with the management of *Rosa rubiginosa* L. (rosehip) in South Africa. MComm. Supervisors: Gavin Fraser and Grant Martin. Rhodes University.

Peer reviewed articles

Rhodes University

1. Acheampong, M.A., Coombes, C.A., Moore, S.D. and Hill, M.P. 2020. Temperature tolerance and humidity requirements of select entomopathogenic fungal isolates for future use in citrus IPM programmes. *Journal of Invertebrate Pathology* 174: 107436.
2. Acheampong, M.A., Hill, M.P., Moore, S.D. and Coombes, C.A. 2020. UV sensitivity of *Beauveria bassiana* and *Metarhizium anisopliae* isolates under investigation as potential biological control agents in South African citrus orchards. *Fungal Biology* 134: 304–310.
3. Aigbedion-Atalor, P.O., Abuelgasim Mohamed, S., Hill, M.P., Zalucki, M.P., Azrag, A.G.A. Srinivasan, R. and Ekesi, S. 2020. *Dolichogenidea gelechiidivoris* (Hymenoptera: Braconidae), a candidate for classical biological control of *Tuta absoluta* in Africa. *Biological Control* 144: 104215.
4. Albertyn, S, Moore, S.D., Marsberg, T., Coombes, C.A. and Hill, M.P. 2020. The influence of citrus orchard age on the ecology of entomopathogenic fungi and nematodes. *Biocontrol Science and Technology*: 1–17.
5. Baso, N.C., Delport, G.R. and Coetzee, J.A. 2020. Nutrient-mediated silica uptake from agricultural runoff in invasive floating macrophytes: implications for biological control. *Hydrobiologia* 847: 3397–3407.
6. Baso, N. C., Coetzee, J. A., Ripley, B. S. and Hill, M. P. 2020. The effects of elevated atmospheric CO2 concentration on the biological control of invasive aquatic weeds. *Aquatic Botany*: 103348.
7. Blanckenberg, M., Mlambo, M.C., Parker, D., Motitsoe, S.N. and Reed, C. 2020. Protected and un-protected urban wetlands have similar aquatic macroinvertebrate communities: A case study from the Cape Flats Sand Fynbos region of southern Africa. *PLoS ONE* 15 (5): e0233889.
8. Canavan, K., Canavan, S., Harms, N.E., Lambertini, C., Paterson, I.D. and Thum, R. 2020. The potential for biological control on cryptic plant invasions. *Biological Control*, 104243.
9. Chari, L., Mauda, E., Martin, G.D. and S Raghu. 2020. Insect herbivores associated with *Lycium ferocissimum* (Solanaceae) in South Africa with reference to potential biological control agents in Australia. *African Entomology* 28:2.
10. Chari, L., Martin G.D., Steenhuisen, S., Adams L.D. and Clark. V.R 2020. The Biology of Invasive Plants. 1. Pyracantha angustifolia (Franch.) C.K. Schneid. *Invasive Plant Science and Management*. Ms. No. IPSM-D-20-00034.
11. Coetzee, J.A. and Hill, M.P. 2020. *Salvinia molesta* D.Mitch. (Salviniaceae): impact and control. *CAB Reviews* 15, No. 033. 1–11.
12. Coetzee, J.A., Langa, S.D.F., Motitsoe, S.N. and Hill, M.P. 2020. Biological control of water lettuce, *Pistia stratiotes* L., facilitates macroinvertebrate biodiversity recovery: a mesocosm study. *Hydrobiologia* 847:3917–3929.
13. Cruaud, A., Delvare, G., Nidelet, S., Sauné, L., Ratnasingham, S., Chartois, M., Blaimer, B.B., Gates, M., Brady, S.G., Faure, S. and van Noort, S. 2020. Ultra-Conserved Elements and morphology reciprocally illuminate conflicting phylogenetic hypotheses in Chalcididae (Hymenoptera, Chalcidoidea). *Cladistics* 37: 1–35.
14. Egbon, I., Paterson, I.D., Compton, S. and Hill, M.P. 2020. Evolution of growth traits in invasive *Pereskia aculeata* (Cactaceae): testing the EICA hypothesis using its specialist herbivore, *Catorhintha schaffneri* (Coreidae). *Pest Management Science*. 76: 4046–4056.
15. Gervazoni, P., Sosa, A., Franceschini, C., Coetzee, J.A., Faltlhauser, A., Fuentes-Rodriguez, D., Martínez, A. and Hill, M.P. 2020. The alien invasive yellow flag (*Iris pseudacorus* L.) in Argentinian wetlands: assessing geographical distribution through different data sources. *Biological Invasions* 22:3183–3193.
16. Hattingh, V., Moore, S.D., Kirkman, W., Goddard, M., Thackeray, S., Peyper, M., Sharp, G., Cronje, P. and Pringle, K. 2020. An improved systems approach as a phytosanitary measure for *Thaumatotibia leucotreta*

(Lepidoptera: Tortricidae) in export citrus fruit from South Africa. *Journal of Economic Entomology* 113(2): 700–711.

17. Hoffmann J.H., Moran V.C., Zimmermann H.G. and Impson F.A.C. 2020. Biocontrol of prickly pear cactus in South Africa: Reinterpreting the analogous renowned case in Australia. *Journal of Applied Ecology,* 57: 2475–2484.
18. Hofstra, D., Schoelynck, J., Ferrell, J., Coetzee, J., de Winton, M., Bickel, T.O., Champion, P., Madsen, J., Bakker, E.S., Hilt, S. and Matheson, F. 2020. On the move: New insights on the ecology and management of native and alien macrophytes. *Aquatic Botany* 162: 103190.
19. Langa, S.D.F., Hill, M.P. and Compton, S.G. 2020. Agents sans frontiers: cross-border aquatic weed biological control in the rivers of southern Mozambique. *African Journal of Aquatic Science* 45: 329–335.
20. Mapaura, A., Canavan, K., Richardson, D., Clark, V.R. and Steenhuisen, S. 2020. Invasive *Nassella* species in South Africa: a review. *South African Journal of Botany,* 135, 336–348.
21. Martin, G.D., Paterson, I.D., Sutton, G.F. and Magengelele, N.L. 2020. Climate modelling suggests a review of the legal status of Brazilian pepper, *Schinus terebinthifolia*, in South Africa is required. *South African Journal of Botany.* 132: 95–102.
22. McCulloch, G.A., Mauda, E.V., Chari, L.D., Martin, G.D., Gurdasani, K., Morin, L., Walter, G.H. and Raghu, S. 2020. Genetic diversity and morphological variation in African boxthorn (*Lycium ferocissimum*)–Characterising the target weed for biological control. *Biological Control*, 143: 104206.
23. Miller, B.E., Coetzee, J.A. and Hill, M.P. 2020. Mind the gap: the delayed recovery of a population of the biological control agent *Megamelus scutellaris* Berg. (Hemiptera: Delphacidae) on water hyacinth after winter. *Bulletin of Entomological Research*, 111(1), 120–128.
24. Minuti, G., Coetzee, J. A., Ngxande-Koza, S., Hill, M. P. and Stiers, I. 2020. Prospects for the biological control of *Iris pseudacorus* L. (Iridaceae). *Biocontrol Science and Technology*, 1–22.
25. Motitsoe, S.A., Hill, M.P., Avery, T.S. and Hill, J.M. 2020. A new approach to the biological monitoring of freshwater systems: mapping nutrient loading in two South African rivers, a case study. *Water Research* 171: Article 115391.
26. Motitsoe, S.N., Coetzee, J.A., Hill, J.M. and Hill, M.P. 2020. Biological control of *Salvinia molesta* (D.S. Mitchell) drives aquatic ecosystem recovery. *Diversity* 12: 204.
27. Musedeli, J.N., Simelane, D.O., Hill, M.P. and Marais, M. 2020. Additive interaction between a root-knot nematode *Meloidogyne javanica* and a root-feeding flea beetle *Longitarsus bethae* on their host *Lantana camara. Pest Management Science* 76: 189–204.
28. Muskett, P.C., Paterson, I.D. and Coetzee, J.A. 2020. Ground-truthing climate-matching predictions in a post-release evaluation. *Biological Control* 144: 104217. (IF:2.60).
29. Ndlovu, M.S., Coetzee, J.A., Nxumalo, M.M., Lalla, R., Shabalala, N. and Martin, G.D. 2020. The establishment and rapid spread of *Sagittaria Platyphylla* in South Africa. *Water* 12(5):1472.
30. Netshiongolwe, N.R., Cuthbert, R.N., Maenetje, M.M., Chari, L.D., Motitsoe, S.N., Wasserman, R.J., Munyai, L.F. and Dalu, T. 2020. Quantifying metal contamination and potential uptake by *Phragmites australis* (Poaceae) along a subtropical river system. *Plants* 9: 846.
31. Paynter, Q., Paterson, I.D. and Kwong, R.M. 2020 Predicting non-target impacts. *Current Opinion in Insect Science* 38: 79–83.
32. Reid, M.K., Coetzee, J.A., Hill, M.P., Diaz, R., Gettys, L.A., Cuda, J.P. and Reid, C.S. 2020. Insect herbivores associated with *Nymphaea mexicana* in southern United States: Potential biological control agents for South Africa. *Florida Entomologist* 103: 54–63.
33. Schaffner, U., Hill, M.P., Dudley, T. and D'Antonio, C. 2020. Post-release monitoring in weed biological control for assessing impact and increasing predictability. *Current Opinions in Insect Science* 38: 99–106.
34. Silvestri, L., Sosa, A., Mc Kay, F., Diniz Vitorino, M., Hill, M.P., Zachariades, C., Hight, S., Weyl, P., Smith, D., Djeddour, D. and Mason, P.G. 2020. Implementation of access and benefit-sharing measures has consequences for classical biological control of weeds. *BioControl* 65: 125–141.
35. van Steenderen, C.J.M., Paterson, I. D., Edwards, S. and Day, M.D. 2020. Addressing the red flags in cochineal identification: the use of molecular techniques to identify cochineal insects that are used as biological control agents for invasive alien cacti. *Biological Control* 152: 104426.
36. Rogers, D., Terblanche, J. S. and Owen, C. A. 2020. Low temperature physiology of climatically-distinct South African populations of the biological control agent *Neochetina eichhorniae*. *Ecological Entomology*, 46(1): 138–141.

University of Witwatersrand

1. Drude, L., Marlin, D., Venter, N. and Byrne, M.J. 2020. Increased soil salinity has no effect on the host preference and suitability of the Tamarix biological control agent, *Diorhabda carinulata*. *Biocontrol Science and Technology*, 30 (12): 1340–1350.

2. Abutaleb, K., Newete, S.W., Mangwanya, S., Adam, E. and Byrne, M.J. 2020. Mapping eucalypts trees using high resolution multispectral images: A study comparing WorldView 2 vs. SPOT 7. *The Egyptian Journal of Remote Sensing and Space Science*.
3. Mayonde, S., Cron, G.V., Glennon, K.L. and Byrne, M.J. 2020. Effects of cadmium toxicity on the physiology and growth of a halophytic plant, *Tamarix usneoides* (E. Mey. ex Bunge). *International Journal of Phytoremediation:* 1–9.
4. Mukarugwiro, J.A., Newete, S.W., Adam, E., Nsanganwimana, F., Abutaleb, K. and Byrne, M.J. 2020. Mapping spatio-temporal variations in water hyacinth (*Eichhornia crassipes*) coverage on Rwandan water bodies using multispectral imageries. *International Journal of Environmental Science and Technology*, 114: 1–12.
5. Reynolds, C., Venter, N., Cowie, B.W., Marlin, D., Mayonde, S., Tocco, C. and Byrne, M.J. 2020. Mapping the socio-ecological impacts of invasive plants in South Africa: Are poorer households with high ecosystem service use most at risk? *Ecosystem Services*, 42: 101075.
6. Cowie, B.W., Byrne, M.J., Witkowski, E.T., Strathie, L.W., Goodall, J.M. and Venter, N. 2020. Parthenium avoids drought: Understanding the morphological and physiological responses of the invasive herb *Parthenium hysterophorus* to progressive water stress. *Environmental and Experimental Botany*, 171: 103945.
7. Cowie, B.W., Venter, N., Witkowski, E.T. and Byrne, M.J., 2020. Implications of elevated carbon dioxide on the susceptibility of the globally invasive weed, *Parthenium hysterophorus*, to glyphosate herbicide. *Pest Management Science*. 76: 2324–2332.
8. Katembo, N., Witkowski, E.T., Simelane, D.O., Urban, A.J. and Byrne, M.J. 2020. Impact of biocontrol agents on *Lantana camara* in an inland area of South Africa. *BioControl*, 65: 143–154.
9. Jombo, S., Adam, E., Byrne, M.J. and Newete, S.W. 2020. Evaluating the capability of Worldview-2 imagery for mapping alien tree species in a heterogeneous urban environment. *Cogent Social Sciences*, 6 (1): 1754146.
10. Sasa, A., Gosik, R., Witkowski, E.T.F., Byrne, M.J. and Mazur, M.A. 2020. Sexual dimorphism in *Anthonomus santacruzi* (Coleoptera: Curculionidae): a biological control agent of *Solanum mauritianum* Scopoli (Solanaceae). *Neotropical Entomology* 49 (6): 840–850.
11. Singh, G., Reynolds, C., Byrne, M. and Rosman, B. 2020. A Remote Sensing Method to Monitor Water, Aquatic Vegetation, and Invasive Water Hyacinth at National Extents. *Remote Sensing* 12 (24): 4021.

Book chapters

1. Byrne, M.J., du Plessis, D., Ivey, P.J., Measey, J. Robertson, M.P., Robinson, T.B. and Weaver, K.N. 2020. Chapter 25: Education, Training and Capacity-Building in the Field of Biological Invasions in South Africa. In van Wilgen B.W. et al. (eds.), *Biological Invasions in South Africa*, *Invading Nature - Springer Series in Invasion Ecology'* vol 14. Pp. 731-758.
2. Faulkner, K.T., Burness, A., Byrne, M.J., Kumschick, S., Peters, K., Robertson, M.P., Saccaggi, D.L., Weyl, O.L. and Williams, V.L. 2020. Chapter 12: South Africa's pathways of introduction and dispersal and how they have changed over time. In: van Wilgen B., Measey J., Richardson D., Wilson J., Zengeya T. (eds.) *Biological Invasions in South Africa. Invading Nature - Springer Series in Invasion Ecology*, vol 14. pp. 313–354.
3. Hill, M.P., Moran, V.C., Hoffmann, J.H., Neser, S., Zimmermann, H.G., Simelane, D.O., Klein, H., Zachariades, C., Wood, A.R., Byrne, M.J., Paterson, I.D., Martin, G.D. and Coetzee, J.A. 2020. Chapter 19: More than a century of biological control against invasive alien plants in South Africa: A synoptic view of what has been accomplished. In: (Eds) van Wilgen, B.W., Measey, J., Richardson, D.M., Wilson, J.R. and Zengeya, T.A. *Invading Nature - Springer Series in Invasion Ecology*: *Biological Invasions in South Africa*. pp. 553–572.
4. Hill, M.P., Coetzee, J.A., Martin, G.D., Smith, R. and Strange, E.F. 2020. Chapter 4: Invasive alien aquatic plants in freshwater ecosystems. In: (Eds) van Wilgen, B.W., Measey, J., Richardson, D.M., Wilson, J.R. and Zengeya, T.A. *Invading Nature - Springer Series in Invasion Ecology*: *Biological Invasions in South Africa*. pp. 97-114.
5. Hill, M.P. and Coetzee, J.A. 2020. Chapter 20. How can progress in the understanding of antagonistic interactions be applied to improve biological control of plant invasions? In: Eds) Traveset, A. and Richardson, D.M. *Plant Invasions: The Role of Biotic Interactions* CABI, Wallingford, UK. pp. 361–374.

Conference proceedings

1. Adams, L.D., Martin, G.D., Clark, V.R., Downes, C.T., Thabethe, A., Raji, A., Steenhuisen, S. 2020. The effects of birds on seed dispersal and germination of invasive firethorn. 46th annual conference South African Association of Botanists. 7-10 January 2020. Qwaqwa South Africa.
2. Canavan, K., Paterson, I.D., Lambertini, C. and Hill M.P. 2020. *Phragmites australis* – alien invasion or disturbed wetlands? *South African Association of Botanists*, Qwaqwa, 7–10 January.

3. Martin, G.D., Fraser, G., Westwood, T. 2020. Guns and Rosehips. *46th* annual conference South African Association of Botanists. 7–10 January 2020. Qwaqwa South Africa.
4. Mason, B.A., Martin, G.D., Frazer, G., Zacharaides, C. 2020. Invasive alien plants in an economic crucible: conducting an economic evaluation of the invasive *Rubus* genus in South Africa. *46th* annual conference South African Association of Botanists. 7–10 January 2020. Qwaqwa South Africa.
5. Moloi, K., Martin, G.D. Steenhuisen, S. 2020. A review of the invasive genus Cotoneaster (Rosaceae) in southern Africa. *55th Annual Congress & Webinar Series. Virtual Congress & Webinars.* 30 June–2 July 2020.Virtual conference.
6. Moore, S. 2020. Specialist Panel Member: Adoption of biological control in the citrus industry. *Biosolutions Africa*. 14–16 July 2020. Virtual conference.
7. Sutton, G.F., Canavan, K., Visser, V., and Paterson, I.D. 2020. Invasive grasses as suitable biological control targets: a South African perspective. *44th South African Association of Botanists Annual Conference.* 7–10 January 2020, Qwa-Qwa, South Africa.
8. Van Steenderen, C. 2020. A tale of two weevils: hybridisation in the biological control agents of water hyacinth. *Rhodes University's Centre for Postgraduate Studies Virtual Conference*, October 2020.
9. Mnciva, S. 2020. Tackling water scarcity issues caused by the destruction of water weeds in South Africa. *Rhodes University's Centre for Postgraduate Studies Virtual Conference*, October 2020.

CBC Team

Samella Ngxande-Koza, Brett Mason Samalesu Mayonde, Kim Canavan, Clarke van Steenderen, Colleen Downes, Samuel Motitsoe, Daniel Rogers, David Taylor, Benjamin Miller, Marcus Byrne, Tapiwa Mushor

Ruth Scholtz, Hildegard Klein, Phillippa Muskett, Byron Soetland, Tahnee Bennett, Grant Martin, David Kinsler, Evans Mauda, Rosali Smith, Nic Venter, Lyle Titus , Mpilo Ndlovu, Karin Goliath

Gerald Chikowore, Kim Weaver, Iain Paterson, Lulama Poni, Blair Cowie, Esther Mostert, Petrus Iita , Carmen Peters, Abigail Wolmarans, Lubabalo Malinga, Sage Wansell, John Hoffmann, Keneilwe Sebola

Megan Reid, Thuthula Mela, Sariana Faure, Candice Owen, Sara Salgado Astudillo**,** Vuyani Ntyinkala, Martin Hill, Getrude Tshithukhe, Luke Cousins, Pascal Osa Aigbedion-Atalor, Tshililo Mphephu , Matthew Paper, Marcel van der Merwe

Bongiswa Ngxanga, Vusumzi Sukula, Benjamin Coetzer, Philip Ivey, Landile Booi, Candice Coombes, Zezethu Mnqeta, Emma Sandenbergh, Jeanne van der Merwe, Guy Sutton, Siyasanga Mnciva, Catharina Kleinjan, Zanele Machimane

Tamzin Griffith, Julie Coetzee, Lehlohonolo Adams, Gugu Mkile, Sean Moore, Mlamli Mbangi, Michael Jukes, Lenin Chari, Antonella Petruzzella, Garyn Townsend , Fiona Impson

Lunga Ngeju, Ronel Roman, Ernst de Beer, Maretha Boshoff, Frank Chidawanyika, Samantha Prinsloo, Arthur Scholtz, Caroline Knox, Slovo Dyira, Wayne Kirkman, Daniel Scholtz, Nompumelelo Baso

Acronyms

ARC-PPRI	Agricultural Research Council – Plant Protection Research Institute
ARU	Afromontane Research Unit
BBCA	Biotechnology and Biocontrol Agency
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 (Australia)
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
NEMBA	National Environmental Management: Biodiversity Act
NRF	National Research Foundation
NRM	Natural Resource Management
OBs	Occlusion bodies
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
SARChI	South African Research Chairs Initiative
SASRI	South African Sugarcane Research Institute
U3A	University of the Third Age
UCT	University of Cape Town
UFS	University of the Free State
UKZN	University of KwaZulu-Natal
USACE	United States Army Corps of Engineers
USDA	United States Department of Agriculture
WfW	Working for Water
Wits	University of Witwatersrand

THE CENTRE FOR BIOLOGICAL CONTROL (CBC)
Department of Zoology and Entomology
Life Science Building
Barratt Complex
Rhodes University
African Street
Makhanda

https://www.ru.ac.za/centreforbiologicalcontrol/

Phone: +27 46 603 8763
Email: cbcinfo@ru.ac.za

Twitter: https://twitter.com/RhodesUniCBC
Facebook: https://www.facebook.com/RhodesUniCBC/

CBC Vision

The CBC seeks to:

(i) Sustainably control environmental and agricultural pests for the protection of ecosystems and the societies that depend on them, and

(ii) Ensure that the maximum benefits of biological control are realised through excellence in research, implementation and community engagement.

CBC Mission

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