Centre for Biological Control

Annual Report 2019

Front cover photograph
Benjamin Miller sampling water hyacinth in the Nahoon River, East London.
Photo credit: Esther Mostert

Back cover photograph
Waainek mass-rearing facility in Makhanda (Grahamstown)
Photo credit: Matt Powell

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Contents

DIRECTOR'S REPORT

CBC Director, Prof. Martin Hill. **Photo credit:** Julie Coetzee

Biological control at Rhodes University dates back to the 1970s, initiated through highly successful biological control programmes against red scale on citrus, and the suppression of invasive cacti. From 2002, those involved in the teaching, research and implementation of biological control combined as an informal research team called the Biological Control Research Group (BCRG), based in the Department of Zoology and Entomology. The BCRG grew over a period of 15 years and by 2017, it was clear that a new structure was needed, and the Centre for Biological Control (CBC) was formed. The CBC provides an easily identifiable and coordinated entity that builds skills and capacity through undergraduate teaching and postgraduate supervision, and provides a holistic approach to biological control as an environmentally safe and sustainable supplement to other control methods aimed at the suppression of invasive organisms and pests.

Initially the CBC represented a consortium of Rhodes University, the University of Cape Town, the University of the Witwatersrand, the University of KwaZulu-Natal, Citrus Research International, and the Agricultural Research Council – Plant Health and Protection. During 2019, memoranda of agreement were signed with the University of the Free State, the University of Pretoria – Forestry and Agricultural Biotechnology Institute (FABI), and the University of Mpumalanga. Further afield, the CBC has agreements with the Foundation for the Study of Invasive Species (FuEDEI) in Hurlingham, Argentina; the National University of Hurlingham, Argentina; the Queensland Department of Agriculture, Australia; New Mexico State University, USA; the Department of Primary Industries, New South Wales, Australia; the Commonwealth Scientific and Industrial Research Organisation (CSIRO), Australia, and Universidade Regional de Blumenau (FURB), Curitiba, Brazil, and collaborates with many more institutions globally. During the course of 2019, the CBC also played host to many international visitors and our researchers, and students were able to visit labs across the globe and attend international conferences, ensuring that the CBC fulfils its mandate as a leading, internationally recognised research centre in biological control.

The CBC has adopted a hub-and-spoke approach with researchers, technical staff and students spread across several campuses, including Rhodes University main campus, the Waainek research facility, the Uitenhage research facility, the University of Witswatersrand, the University of Cape Town, the University of KwaZulu-Natal, The University of the Free State (QwaQwa and Bloemfontein), the University of Mpumalanga (Nelspruit) and the University of Pretoria. The CBC consists of some 42 staff and more than 50 postgraduates, including postdoctoral fellows, PhD and Masters students. Facilities include insect quarantine facilities, mass-rearing facilities and analytical laboratories. One key member of the team worth mentioning is Jeanne van der Merwe, the centre's administrator who plays a key role in ensuring the smooth operations of the various and diverse aspects of the CBC, including the management of funds. Funding for the CBC comes mainly from the Department of Environment, Forestry and Fisheries: Natural Resources Management Programmes, but also industry (Citrus Research International, and other commodity groups), several international contracts, the Water Research Commission of South Africa, and the National Research Foundation of South Africa through the South African Research Chairs Initiative: Insects in sustainable agricultural ecosystems. The contributions by the universities that constitute the consortium are also significant.

In this annual report we present a synopsis of the research conducted within the centre during 2019 on some 54 invasive alien plant species and ten crop pests. The research is divided into six key programmes: the aquatic weeds, cacti weeds, northern temperate weeds, invasive trees, grasses, and agricultural pests. Additionally, there are a

number of other smaller research programmes which focus on specific species. There are also several cross-cutting activities that include human capital development, community engagement and outreach, socio-economic studies, and mapping and GIS. Individual research projects within the programmes are multi-disciplinary by nature and, on the Rhodes campus alone, the CBC conducts research across ten academic departments. The research covers the full spectrum of the biological control discipline from pre-release studies such as host-specificity trials, post-release evaluation, and implementation through mass-rearing and release efforts.

Highlights for 2019 include:

- The initiation of a project of insects associated with honeybush tea in collaboration with the South African Honeybush Tea Association (SAHTA)
- Collaboration with Forestry and Agricultural Biotechnology Institute (FABI) on the impact and control of the polyphagous shot hole borer
- The start of the 'mass development of aquatic macrophytes – causes and consequences of macrophyte removal for ecosystem structure, function, and services' (MADMACs project) on Hartbeespoort Dam, in the North West Province
- The introduction of several new species of potential biological control agents into the CBC quarantine
- New project on the biological control of prosopis (mesquite) – a plant invasive predominantly in the Northern Cape
- The release of two new biological control agents for two cactus species in Namibia

When the CBC was launched in November 2017, the vision for the centre was to conduct research to sustainably control environmental and agricultural pests 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.

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AQUATIC WEEDS

Historically, biological control efforts against aquatic macrophytes in South Africa have focused on the floating weeds, water hyacinth (*Pontederia crassipes*)[1], salvinia (*Salvinia molesta*), water lettuce (*Pistia stratiotes*), parrot's feather (*Myriophyllum aquaticum*) and red water fern (*Azolla filiculoides*). Post-release evaluations over the last twenty years have shown that, with the exception of water hyacinth, all of these problematic aquatic plants have been effectively suppressed using classical biological control. However, in eutrophic water bodies at high elevations that experience cold winters, an integrated approach that includes herbicide application and augmentative biological control is required against water hyacinth.

In contrast, there is a dearth of research into the invasion and control of submerged and emergent aquatic macrophytes, with numerous invasive aquatic species already established in South Africa. Horticultural and aquarium industries, nutrient-rich water systems, and a limited knowledge of the drivers of invasions of submerged and emergent macrophytes means that South Africa is highly vulnerable to a second phase of aquatic plant invasions. The Aquatic Weeds Programme therefore also has a focus on submerged and other emergent invasive species – Brazilian waterweed (*Egeria densa*), yellow flag iris (*Iris pseudacorus*), Mexican water lily (*Nymphaea mexicana*) and delta arrow head (*Sagittaria platyphylla*).

The main aim of the Aquatic Weeds Programme is to determine pathways of new invasions, and the invasion ecology and management of emerging aquatic weeds. Competitive interactions between introduced and native species are crucial to understanding the invasion process, including modes of spread and phenology. Initiating and implementing biological control programmes on these species is the ultimate goal of the Aquatic Weeds Programme.

Prof. Julie Coetzee is the Aquatic Weeds Programme Manager and there is a range of students in Entomology, Botany and Economics who are doing their research on different aspects of waterweed invasion ecology in this programme.

PROGRAMME HIGHLIGHTS IN 2019

- The ephydrid fly, *Hydrellia egeriae*, has established on Brazilian waterweed, *Egeria densa* at two sites
- A new culture of the water hyacinth grasshopper, *Cornops aquaticum*, was received from Argentina
- A culture of the 'minima' strain of weevil, *Cyrtobagous salviniae* for *Salvinia minima*, was received

[1] Water hyacinth was formally known as *Eichhornia crassipes* but was recently changed to *Pontederia crassipes*.

Water hyacinth

Water hyacinth (*Pontederia crassipes*) is one of the world's worst floating aquatic weeds. It originates in the Amazon Basin, but has been distributed globally and was first recorded in South Africa in 1910. Biological control was initiated in 1973 and to date, nine different biological control agents have been released to control it.

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New agent for water hyacinth released on the Nahoon River

The Nahoon River is a heavily invaded fresh water system in the Eastern Cape, with four of the five major floating aquatic weeds present along its lower reaches. In December 2018, the CBC received some 200 adult water hyacinth grasshoppers (*Cornops aquaticum*) from South African Sugarcane Research Institute (SASRI) for the control of water hyacinth. *Cornops aquaticum* is a semi-aquatic grasshopper from the Amazonian Basin, and was maintained in quarantine at Agricultural Research Council Plant Protection Research Institute (ARC-PPRI) Rietondale for 10 years, until permission for its release was granted in 2011. None of the releases of this grasshopper made at three locations in South Africa in 2011 established. Since then, populations have been maintained at SASRI's mass-rearing station. The CBC requested that it be allowed to mass rear and release the insect intensively, to see if it could achieve establishment. Experiments are currently underway at the Nahoon River in East London to determine the best methods to gain establishment. The positive news is that the grasshopper overwintered in quarantine, and in the field. Efforts to increase the population in quarantine are underway so that more field releases may be made. A new culture of the insect was imported from Corrientes Province in Argentina into the CBC quarantine in December 2019. This new population will be kept separate and subjected to host-specificity testing before release.

Release of the water hyacinth grasshopper (insert), *Cornops aquaticum,* on the Nahoon river in East London in March 2019. **Photo credit:** Esther Mostert, **insert credit:** Lynette Rudman

Population dynamics of four biological control agents on water hyacinth on the Kubusi River

A PhD study investigated the seasonal population dynamics of water hyacinth and four of the associated biological control agents: the weevils (*Neochetina eichhorniae* and *N. bruchi)*; the plant hopper (*Megamelus scutellaris),* and the mirid (*Eccritotarsus catarinensis)*. The study was conducted on the Kubusi River, Eastern Cape, which is the coldest continuous monitoring site in South Africa. The study aimed to investigate the effect of the prevailing climate on the biological control agents over three years, from May 2016 until April 2019. The results revealed several patterns of the population dynamics of both water hyacinth and its biological control agents.

Climate patterns have a significant influence on both host plant abundance and quality, and consequently on the agent abundances. In this study, resource availability, which is subject to seasonal change and associated temperatures, affected the population dynamics of all the biological control agents on the Kubusi River. This study highlighted the challenge of lag phases between the target plant and the biological control agents, showing that augmentation at the beginning of the growing seasons is necessary in order to curb plant growth at the onset.

Parasitoid on water hyacinth biological control agent

From January to August 2019 the CBC hosted American postdoc, Emily Kraus. Emily completed her PhD at Louisiana State University. Her dissertation involved plant-insect interactions in agriculture, specifically induced resistance in rice, *Oryza sativa*, and its utility as part of an integrated pest management program. She joined to CBC to and apply her knowledge of induced resistance to aquatic weeds. During her time at the CBC she focused on understanding the relationship between aquatic weeds and their biological control agents, with particular reference to induced resistance.

Early on during her stay, while Emily was learning how to identify male and female insects of the water hyacinth planthopper, *Megamelus scutellaris*, she noticed something strange attached to one of the insects. She wasn't sure what it was and took it up to the lab to be identified. After some discsuiions, it was deteremined that it was a parasitoid wasp form the Dryinidae family. The insect, *Megamelus scutellaris*, was brought from abroad to control water hyacinth and should not have had any natural enemies other than generalist predators in the new environment so this was very exciting and surprising. Emily was able to rear out dozens of the parasitoid wasps from the insects and send them to Dr. Simon van Noort, based at the Iziko Museum in Cape Town, and his collaborator Dr. Massimo Olmi for identification. They determined the insect was *Ecthrodelphax migratorious*, a wasp native to South Africa. This was very interesting as it represented a new relationship between two species which would never have come into contact if not for human intervention. It was also very interesting as there is no record of the native organism that the parasitoid would normally use to complete its' lifecycle. Emily wrote up this observation in a Short Communications paper in Biocontrol Science and Technology, which was published in August 2019.

Thermal physiology, digestion and metabolism of biocontrol agents

Dr Candice Owen has been delving deeper into the thermal physiology of the widely-established water hyacinth biocontrol agent, the weevil, *Neochetina eichhorniae*. Assessment of insect thermal physiology is a tool that has recently been gaining popularity in weed biological control, but the classical methods used look

Megamelus scutellaris nymph parasitised by two *Echthrodelphax migratorius* larvae (top) and a male parisitoid wasp *Echthrodelphax migratorius* (bottom). **Photo credit:** Simon van Noort, Iziko Museum

only at the effects of temperature on the behaviour and development of the insects. The present work aims to delve deeper into the root causes of these observed patterns by assessing the effect of temperature on the metabolism itself. Once the metabolic patterns and budgets under different thermal regimes can be outlined and understood, it is hoped that these can be linked back to observed behaviours and control success, thereby allowing more predictive power for future releases.

To understand the metabolic dynamics in *N. eichhorniae*, Candice has collaborated with Prof. John Terblanche of Stellenbosch University, as well as one of his PhD students, Henriek Bosua. They have used an open system respirometer maintained at different temperatures to track the metabolic rates of field-collected weevils through the digestive period following a meal. Results are still preliminary, and only a small sample size has been assessed thus far, but digestion appears to require less energy at hotter temperatures. This suggests that more energy is available for other important life history traits like dispersal and reproduction at hotter temperatures, whereas insects in cold conditions may have less energy available for these. These results may provide a good explanation for why many agents fail to establish in cold conditions.

To further the work on metabolism, the researchers plan to track water loss during the respirometry trials to gain a better understanding of the results, and to run additional trials to obtain a dataset with a larger sample size. These trials were started in July 2019, but the respirometer developed a leak and required several months for repairs. While waiting for the machine to be repaired further experiments on the digestive characteristics of *N. eichhorniae* are planned, in particular, gut passage times, meal sizes, digestion efficiency, and the effect of time on feeding efficiency at different temperatures. A range of methods, including bomb calorimetry, will be used to record these data. Candice Owen has secured funding for one Masters-level student for 2020 to work on the project.

The group has also started looking at aspects of thermal plasticity in control agents released against aquatic weeds in South Africa. A study by Jordan Porter showed that the mirid, *Eccritotarsus catarinensis,* was able to increase or decrease its thermal limits, indicating that it exhibits thermal plasticity. It was therefore decided to test the thermal physiology and plasticity of field and laboratory populations of the water hyacinth planthopper, *Megamelus scutellaris* to see if similar patterns exist.

Populations of the planthopper were collected from the Kubusi River in the Eastern Cape, and from cultures maintained under mass-rearing conditions. The mean CTMin (Critical Thermal Minimum) was higher for the Kubusi population than for the mass-rearing populations. This result was unexpected, as the climate at Kubusi is much colder than in Makhanda (Grahamstown), but it does indicate plasticity. Although *M. scutellaris* did not display the same thermal trend has been described for *E. catarinensis* (the Kubusi population had significantly lower CTMin than the mass-reared population), the species did display a similar trend to that described for *N. eichhorniae*, whose Kubusi population had a significantly higher average CTMin than the mass-reared populations. Interestingly, the mean CTMax of the Kubusi population was significantly lower than the mass-reared populations. A similar trend was seen in *E. catarinensis*, whose mass-reared populations had a significantly higher mean CTMax than field populations. Although the Kubusi population of *M. scutellaris* does not appear to have adapted to the local conditions, this does not mean that the species is not thermally plastic since, during the thermal acclimation experiment, results showed that the mean CTMin of *M. scutellaris* can be lowered by nearly 2°C by keeping insects at low temperatures for only five days. This is a promising result as it indicates that pre-acclimating *M. scutellaris* is not only possible, but does not require extended periods of time.

Salvinia molesta and *Salvinia minima*

Salvinia molesta, referred to as giant salvinia, is a free-floating fern from South America that inhabits still and slow-moving freshwater bodies. The fern is sterile and reproduces by vegetative growth of the rhizomes.

Salvinia molesta has been present in South Africa since the early 1900s and is most troublesome in the tropical and subtropical regions of the country. Its distribution includes both inland areas, generally below 500m, and coastal regions. The weevil, *Cyrtobagous salviniae* is an effective biological control agent for *Salvinia molesta*, and was first released in South Africa in 1985.

Salvinia minina is referred to as common salvinia, and is also is a species of aquatic, floating fern that grows on the surface of still waterways. It is native to South America, Mesoamerica, and the West Indies, and was first recorded in South Africa from Hartbeespoort Dam in early 2012. The species, *Salvinia minima*, can be distinguised form *Salviania molesta* by the structure of the tiny water-repellent hairs (trichomes) on the leaf surface. Trichomes of *Salvinia minima* have an open structure, in comparison to the closed cage-like 'egg beater' structure of *Salvinia molesta* trichomes.

Cyrtobagous salviniae is a semi-aquatic weevil that has been extremely successful in controlling invasive *Salvinia minima* in the southern states of the United States. At present, *S. minima* has been reported at a single location in South Africa, within the North-West Province, on Hartbeespoort Dam. The Florida ecotype of *Cyrtobagous salviniae* was imported into quarantine at Rhodes University on 31 November 2018 for host-specificity testing. Unfortunately, the culture of weevils died out due to fungal infection, but a new population was collected in Louisiana and imported to quarantine in early October 2019.

A pair of newly imported *Cyrtobagous salviniae* on *Salvinia minima* in the CBC quarantine. **Photo credit:** Matthew Paper

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Because the Brazilian ecotype of *C. salviniae* is host specific and already widely distributed throughout South Africa, host-specificity testing of the Florida ecotype will only include no choice and paired choice tests on *Salvinia minima* and *Salvinia hastata,* which is the only indigenous African species. Host-specificity testing is scheduled to start in early 2020.

The role of *Helicosporidium* sp. in the efficacy of the weevil, *Cyrtobagous salviniae* biological control of *Salvinia molesta*

At the CBC mass-rearing station, numbers of the weevil, *Cyrtobagous salviniae,* for released as a biological control against *Salvinia molesta* have decreased due to a parasitic algal infection in the culture. The alga, *Helicosporidium* sp., was first detected in 2007 from field-collected adults of *C. salviniae* in South Africa and consequently, the effects of *Helicosporidium* sp. infection in *C. salviniae* populations established on *S. molesta* across the country were assessed.

In 2019 a total of 1005 adult weevils were collected from 10 of 15 surveyed sites across the Eastern Cape, KwaZulu-Natal, Limpopo, and Western Cape provinces and screened to determine the occurrence, infection rate, and distribution of *Helicosporidium* sp. The current transmission mechanism that is building-up the infection rate of *Helicosporidium* sp. in the populations of *C. salviniae* was investigated. Further, experimental trials to determine the effect of *Helicosporidium* sp. on the effectiveness of *C. salviniae* against *Salvinia molesta* were also investigated. This was investigated through the determination of the feeding and reproductive performance of the infected and healthy populations of *C. salviniae* in a collaboration between South Africa (Rhodes University) and the United States of America

Samuel Motitsoe and Zolile Maseko on the Kogmanskloof river tributary of the Breede river, after successfully biological control of *Salvina molesta*. **Photo credit:** Daniel Rogers

(Louisiana State University). The infection covers the entire distribution range of *C. salviniae* in South Africa, with the infection occurrence rate ranging from 92.15% to 100% per site. *Helicosporidium* sp. was found to transmit vertically within the populations of *C. salviniae*. The female adults of *C. salviniae* were found as the carriers of *Helicosporidium* sp. infection. *Helicosporidium* sp. infection reduces the reproductive output of *C. salviniae* as well as the impact on the biomass of *S. molesta*. These findings attested the necessity of subjecting the infected *C. salviniae* to a trial antibiotics treatment, which is currently underway at the CBC, Rhodes University. A total of five antibiotics were selected for this treatment trial. As part of this collaboration, early detection surveys, collections, and screening of *C. salviniae* for *Helicosporidium* sp. are also being conducted in Louisiana.

Ecological recovery following management of *Salvinia molesta* in South Africa.

In an attempt to understand ecosystem recovery following the biological control of free-floating invasive alien aquatic plant species, a mesocosm experiment was set up. The experiment consisted of twelve pools arranged in three blocks of four, making up 12 sampling units. The experiment followed an extension of the Before-After (period), Control-Impacted (treatment) design, by incorporating a 'during invasion' period and a 'restored' treatment. Epilithic algae and aquatic macroinvertebrate recruitment were assessed as a measure of ecosystem recovery. For phase 1, all treatments were free of *Salvinia molesta* (open water) and left for a period of six weeks for complete epilithic algae and aquatic macroinvertebrate colonisation to occur before baseline (before invasion) data were collected. After six weeks, epilithic algae and aquatic macroinvertebrate recruitment was assessed.

A similar study was conducted on the Swartkops River in the Eastern Cape, where Getrude Tshithukhe's Masters research project investigated how the composition of aquatic flora and fauna changed in response to urban land use, and determined which biological indicators/ indices best reflected the disturbance. It also investigated the use of aquatic weeds (*Pontederia crassipes* and *Salvinia molesta*) as bio-accumulators of metals. Her research provides specific biological indicators for different pollution sources in the urban environment and adds to our knowledge on the use of traits and stable isotope analysis as an alternative early warning detection technique for freshwater eutrophication.

Brazilian water weed

Egeria densa, commonly known as Brazilian waterweed or Brazilian elodea, is a submerged aquatic weed.

A rooted macrophyte found in both lentic and lotic systems, it grows vigorously in slow-moving water. The plant was first recorded in South Africa in the 1963, and is actively traded through the aquarium trade.

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Release of the fly, *Hydrellia egeriae* on Brazillian waterweed

A highlight for the CBC in October 2018 was the release of the biological control agent, the Egeria fly (*Hydrellia egeriae),* against Brazilian water weed on the Nahoon River, East London. Since then, monthly post-release evaluations have been conducted to monitor agent establishment, population size and weed abundance.

Mass rearing of the fly was initially conducted in a temperature-controlled room at the Waainek research facility from August 2018. In April 2019, mass rearing was also initiated in 600L tubs situated in a polytunnel, providing *Hydrellia egeriae* with more natural conditions. The fly population has gradually increased with a population size of 48 681 immatures per kilogram of Brazilian waterwwed fresh weight for December 2019, compared to 7707 immatures per kilogram for March 2019.

Hydrellia egeriae was released at the southern foot of Midmar Dam in April 2019. A post-release survey three months later showed that the fly was present at the release site, and had spread out along the release site. During this trip, the fly, together with the necessary equipment, was delivered to Mr. Ernie Steenkamp, a teacher at Hilton College, to establish a mass-rearing station.

In October 2019, around 20 000 *Hydrellia egeriae* immatures were released in the Groot River in the Baviaanskloof Reserve. The Groot River runs into the Kouga River at Andrieskraal, where 80 000 immatures were released. *Egeria densa* populations appeared patchy at release sites, and it is anticipated that the fly will spread and establish on *E. densa* downstream, where citrus farming is contributing to nutrient inflow into the Kouga river.

The potential parasitism of *Hydrellia egeriae* by native parasitoids was investigated. *Egeria densa* was collected from five quadrats within an established *H. egeriae* site, placed in black bags, and transported back to the lab for further dissection. Parasitoids reared from these collections were sent to Dr Simon van Noort at the Iziko Museum of South Africa, Cape Town, for identification. Recent results show that these may be new parasitoid species not yet described in South Africa. At this stage, it is unclear what impact the parasitoid will have on populations of the fly in the field, but this is being monitored.

Matthew Paper releasing immatures of the Egeria leaf-mining fly into the Groot River, in the Baviaanskloof Nature Reserve. (top)
A fly pupariaim developing on an *Egeria densa* strand – mined leaves of its three instar stages. (bottom)
Photo credits: Rosali Smith

Yellow flag iris

Yellow flag iris (*Iris pseudacorus*) is an herbaceous perennial wetland plant, native to Europe, western Asia and northwest Africa, which was introduced into South Africa as an ornamental plant.

In 2017, a biological control programme within the CBC was initiated on this wetland weed, and significant progress has been made. Yellow flag iris 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 the National Environmental Management: Biodiversity Act (NEMBA), and is an eradication target of the South African National Biodiversity Institute (SANBI) Invasive Species Programme, but because of its rapid regenerative growth, its ability to spread via seeds, and the fact that its range is now well beyond what it was five years ago, biological control is being considered as an option in South Africa.

Introduction of two agents for the biological control of yellow flag iris

In April 2018, a potential biological control agent, the flea beetle, *Aphthona nonstriata*, was imported into the CBC quarantine from collaborators in Belgium. A good population of this potential control agent is currently in quarantine at Rhodes University and host-specificity testing of *A. nonstriata* commenced in January 2019. Fourteen plant species are being maintained at Waainek for host-specificity testing, and the CBC will continue to obtain additional species for required testing. The host-specificity testing is still in progress as we test more non-target species in the Iridaceae.

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The CBC received a consignment of yellow flag iris seed capsules containing pupae of the weevil, *Mononychus punctumalbum*, from Belgium in mid-September. Twenty- one adult weevils eclosed and have been placed on flowering yellow flag iris. In Europe, weevils emerge from the soil in late May, following winter diapause, and congregate on yellow flag iris flowers to feed and mate. Eggs are laid in developing seeds, and then larvae complete their development to pupation by the end of the summer in late August, early September. Adults eclose and move into the soil to overwinter.

In South Africa, yellow flag iris starts to flower towards the end of September, early October. In order to try to prevent the weevils from Europe entering diapause they were placed directly onto flowering plants in the hope that they would feed, mate and lay eggs in quarantine. It is hoped that larval development can be confirmed in the next few months.

Emma Sandenbergh surveying yellow flag iris in Hogsback, Eastern Cape. **Photo credit:** Sara Astudillo

Mexican water lily

In 2016 the CBC embarked on a programme investigating the biological control of the Mexican water lily, *Nymphaea mexicana*, which has the potential to replace water hyacinth in South Africa as the most serious invasive alien. As its name suggests, *Nymphaea mexicana* is native to Mexico and southern parts of the United States of America. The species was first introduced into South Africa through the ornamental plant trade, and has since established numerous populations in dams, ponds and rivers around the country. Because it grows rapidly the water lily can become weedy at an alarming rate, and this in turn, negatively affects aquatic biodiversity and ecosystem functioning.

Surveys for potential control agents in the United States of America in 2018 identified two species with high potential for biological control, namely a weevil, *Bagous americanus*, and a plant hopper, *Megamelus toddi*. Based on pilot studies and literature, *Bagous americanus* appears to have a narrow host range, restricted at least to the family Nymphaeaceae, and 120 individuals were imported into quarantine at Rhodes University at the end of October 2018. Attempts were made to start a culture of these insects, but while multiple generations successfully completed development, populations eventually died out in March 2019. In 2020, another culture of these weevils will be imported into quarantine, and host-specificity testing will take place.

RESEARCH TEAM
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The invasive Mexican water lily (left) has bright yellow flowers with pointed petals. The leaves have smooth edges, are sometimes red on the underside, and may have purple or red blotches. Hybrids of the invasive Mexican water lily look similar (middle), but the flowers are often paler with more rounded petals. The flowers of the indigenous South African water lily (right), are normally blue, purple, or sometimes pink. The leaves have serrated edges and the leaves are smoother. Young leaves may have spots, but the larger more mature leaves are not spotted. **Photo credit:** Megan Reid

Pickerelweed

Pickerelweed (*Pontederia cordata*) is an invasive emergent macrophyte from North America that forms dense vegetative colonies in shallow waterbodies causing the loss of native species and posing a serious threat to South Africa's biodiversity and water security.

In 2017, pickerelweed became one of the focus weeds of the CBC for feasibility studies. In the past, the weed was a Category 3 species, but the new NEMBA regulations list it as a Category 1b invader. Past annual surveys by the CBC showed that the number and density of the populations had rapidly increased; for this reason, it was prioritised for implementation of a biological control programme in South Africa. Collation of information from the Southern African Plant Invaders Atlas (SAPIA) database, SANBI database and field surveys has made it possible to record and map invasion sites of pickerelweed throughout South Africa to determine the extent of invasion.

Pickerelweed invasions have been recorded in urban areas, possibly dispersed via the horticultural trade as the showy purple flowers make it a popular ornamental pond plant. Control and removal of pickerelweed is crucial, as its rapid spread in South Africa may already have had deleterious effects on the native ecosystems. DNA analyses are currently underway to determine what variety is present in South Africa. Pickerelweed may be a sterile horticultural variety with little genetic diversity and may be spreading clonally in South Africa. These DNA outcomes will help to determine a biological control programme for pickerelweed.

Other aspects of pickerelweed invasion in South Africa are also being investigated, such as identifying its pollinators. It may be possible that invasive populations are not producing seeds because of a lack of appropriate pollinators. Identifying pollinators of pickerelweed in South Africa by looking for insects pollinating flowers in the field will exclude this possibility. In addition, artificial self-pollination and cross-pollination greenhouse experiments are well underway to determine whether the invasive plants in South Africa can produce seed and thus reproduce sexually. These findings will be crucial in determining how pickerelweed is spread in South Africa: if plants cannot reproduce sexually because only short-morphed plants are present, and cannot produce seeds without cross-pollination from medium- and long-morphed plants, then preventing the import and trade of medium- and long-morphed pickerelweed plants into South Africa will be crucial. If more than one morph of pickerel weed establishes in SA and plants start producing seed, then it is possible that propagation and spread will become even more aggressive and detrimental to the native ecosystem and water security.

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These studies will aid the development of a biological control programme. Surveying and identifying potential biological control agents should be based on the agent's ability to decrease asexual spread (e.g. by damaging the plant's rhizomes) to ensure the greatest impact and control of pickerelweed invasion.

A pickerelweed infestation in Hogsback.
Photo credit: Emma Sandenbergh

Delta arrowhead

Daniel Rogers in the thick of sampling delta arrowhead at Maden Dam, an invaded site outside of King Williams Town in the Eastern Cape. **Photo credit:** Matthew Paper

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Since 2014, the CBC has been investigating potential biological control for delta arrowhead, *Sagittaria platyphylla*, an emergent aquatic macrophyte native to southern United States of America.

To work on potential control agents against delta arrowhead, the CBC established excellent collaboration with the Australian Victorian Department of Primary Industries (DPI) and the United States Army Corps of Engineers (USACE), Mississippi, which resulted in the collection of four *Listronotus* species from the Mississippi basin in 2014.

Two weevil species have been shelved, as they are currently unsuitable for release in South Africa. Basic biology, host-specificity and impact studies continue on two remaining potential agents – the weevils, *Listronotus frontalis* and *Listronotus appendiculatis*. Additionally, in collaboration with SANBI, pre-release and impact surveys are being conducted across the populations in South Africa.

Red water fern

Azolla filiculoides, or red water fern, was introduced to South Africa from South America as a pond ornamental in 1948 and, by 2000, had infested slow and still water bodies at over 400 localities throughout the country.

The frond-feeding weevil, *Stenopelmus rufinasus*, was released as a biological control agent for *Azolla filiculoides* in South Africa in 1997. Annual field post-release surveys have been undertaken to most of the *Azolla filiculoides* infested sites since the early 2000s, and show that the weed has been controlled to extinction at most sites. At the few sites where the weed persists, it is in the form of a mono layer of plants that have no negative impacts. This study shows the value of long-term post-release evaluation over a wide geographical area. On

RESEARCH STAFF
Prof. Martin Hill

6 March 2018, an application was made to the South African Department of Environment, Forestry and Fisheries to have *Azolla filiculoides* delisted as a restricted species on the basis that it no longer poses a threat to the environment. In 2016, a new invader, *Azolla cristata*, was confirmed to have established in the eastern part of South Africa. Fortunately, *Stenopelmus rufinasus* also feeds on this species, although not as well as on *Azolla filiculoides*. The post-release evaluation on this species shows that the weevil will also keep it in check.

Climate change research: The effects of elevated CO_2 on the biological control of four invasive aquatic weeds in South Africa

Climate change and invasive species are two of the most prevalent features of the Anthropocene, with a predicted doubling of atmospheric CO_2 concentration by the end of the 21st century. Numerous studies have shown that increased CO_2 levels in the atmosphere have a significant impact on photosynthesis of both native and invasive species, and this may lead to disturbed plant-insect interactions. However, due to their competitive nature, invasive species might benefit more from such changes. In South Africa, most of the aquatic ecosystems are eutrophic, and therefore are particularly prone to invasion by a number of alien aquatic weeds. Although most of the floating aquatic weeds are under effective biological control, this situation may be under threat due to changes in atmospheric CO_2.

The aim of this study is to investigate the effects of elevated CO_2 and nutrient status on the growth and defence of four invasive aquatic weeds red water fern (*Azolla filiculoides*), parrot's feather (*Myriophyllum aquaticum*), Kariba weed (*Salvinia molesta*) and water lettuce (*Pistia stratiotes*) that are a threat to natural resources but are currently being successfully controlled by their respective biological control agents (*Stenopelmus rufinasus*, *Lysathia sp.*, *Cyrtobagous salviniae* and *Neohydronomus affinis*, respectively).

The study species were grown at ambient (400 ppm) and elevated (800 ppm) CO_2 concentrations at the Rhodes University Elevated CO_2 Facility, with and without their respective biological control agents. The results of this study will be instrumental in determining whether additional agents will be needed for continued control of these invasive macrophytes.

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A bird's eye view of the open topped CO_2 chamber facility at Rhodes University. **Photo credit:** Mike Cramer

Economic evaluation of weed biological control: evaluation of chemical and biological control of four aquatic weeds

The Department of Environment, Forestry and Fisheries (DEFF) in South Africa should be in a position to use a cost-effective approach to decide how to control exotic aquatic weeds; the continued use of cost-ineffective methods will result in continued waste of scarce financial resources. *Azolla filiculiodes*, *Salvinia molesta*, *Pistia stratoites*, and *Myriophyllum aquaticum* are under complete biological control in South Africa. In the absence of biological agents, these weeds would have been controlled using herbicides.

Applying the Cost Benefit Analysis (CBA) framework as the main technique of evaluation, and using site coordinates from the Southern African Plant Invaders Atlas (SAPIA), the study first retrospectively estimated the total number of hectares cleared as at July 2019. The study also estimated the cost of clearing using both biological and chemical control methods. To estimate the cost of chemical control, water hyacinth costs were used as surrogate costs and were adjusted accordingly to accommodate biological differences of the four selected weeds. These chemical control costs were expressed in terms of three possible spray approaches that the Department of Environmental Affairs (DEA) would have taken: boat, vehicle, and knapsack approach. Data on costs were obtained from the CBC and the Natural Resource Management (NRM) programme. Mary Maluleke's results showed that, over the last 20 years, biological control has saved the country many millions of Rands in herbicide costs.

RESEARCH TEAM
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STUDENT
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COLLABORATORS
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International collaboration

In addition to rolling out biological control of aquatic weeds in South Africa, the CBC is committed to neighbours further north in Africa who rely on the technology developed in South Africa. Collaborative projects with Kenya, Cameroon, Botswana, Namibia, Morocco, Uganda, Malawi and Madagascar are ongoing through consultation work with Martin Hill.

The CBC also collaborates with scientists from the United States Army Research and Development Center - Environmental Laboratory, in Vicksburg, Mississippi, and the Victoria DPI in Australia, on the control of *Sagittaria platyphylla*. Nathan Harms visited South Africa in March 2019 to strengthen this collaboration. Scientists from FuEDEI, Argentina, and the USDA laboratory in Albany, California, are collaborating with us on the control of *Egeria densa*. Another collaboration between Landcare Research, New Zealand, and the CBC is testing the natural enemies' hypothesis, using *Lagarosiphon major* and *Egeria densa* as models. We have a collaboration with Dr Iris Stiers from the Vrije Universiteit Brussels, Belgium, on the *Iris pseudacorus* project which includes the FuEDEI team in Argentina. In May this year, Julie Coetzee visited Iris Stiers, and Gianmarco Minuti, a PhD student working on the yellow flag iris control programme.

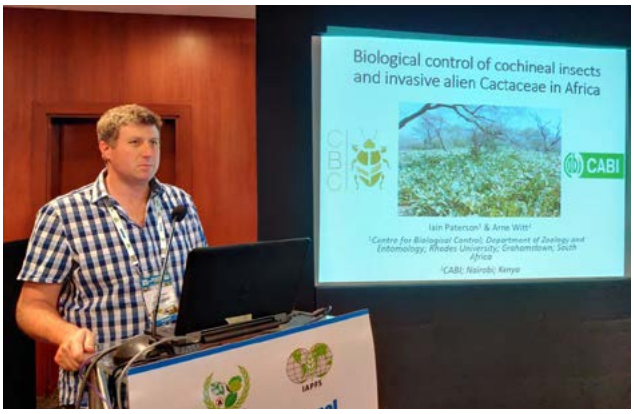
The partnership with FuEDEI in Hurlingham, Argentina has strengthened over the last few years, with a bilateral agreement bringing funding for opportunities for students and staff to come on exchanges. The project aims to investigate aspects of enemy release on the agents widely used for the biological control of water hyacinth. Tómas Righetti visited the CBC from February to May 2019 to work on aspects of *Megamelus scutellaris* biological control.

Julie Coetzee is collaborating on an international project coordinated by the Water JPI, with scientists from Norway, Germany, France, and Brazil, investigating 'Macrophyte development and mass removal' (MadMacs) using the water hyacinth invasion on Hartbeespoort Dam as a case study. PhD student Keneilwe Sebola joined Julie in Rennes, France for a workshop on the programme in June 2019.

Prof. Julie Coetzee with CBC PhD Student, Keneilwe Sebola, and German postdoctoral student, Dr Sarah-Faye Harpenslager from the Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, at Grand-Lieu Lake, Nantes, France. Photo credit: MadMacs

CACTACEAE WEEDS

The Cactaceae Weeds Research Programme aims to reduce the negative impacts of invasive alien cactus weeds in South Africa through biological control research and implementation. Biological control is the most effective, affordable and environmentally friendly manner in which to control invasive alien cactus species in South Africa; this project aims to better utilise biological control, develop new biological control agents, and evaluate the efficacy of biological control in the country. Dr Iain Paterson leads this research programme.



Dr Iain Paterson presenting research on cochineal and cactus biological control in Africa at the XIX International Plant Protection Congress in India (November 2019)

The programme has two components: pre-release and post-release. The pre-release component includes the development of biological control agents for new target weeds. Round-leaved prickly-pear (*Opuntia engelmannii*) and torch cactus (*Trichocereus spachianus*) are widespread, destructive cactus weeds that require new biological control agents. *Opuntia elata* and *Cylindropuntia pallida* are considered pre-emptive targets that are not widespread and destructive, but are very likely to increase in future if no control is implemented. Surveys for new potential agents for *Trichocereus spachianus* and *Opuntia elata* are being conducted in South America; promising potential agents for *Opuntia engelmannii* are under evaluation in quarantine, and an application for the release of a new agent for *Cylindropuntia pallida* has been submitted to the relevant authorities.

The post-release component includes mass rearing and releases of cactus biological control agents; quantification of biological control success through long-term monitoring and public opinion surveys, and maximising the impact of biological control agents through the development of new techniques and strategies. This approach ensures that the maximum possible benefit can be obtained from the cactus biological control agents that have been released in South Africa in the past. Six different cactus biological control agents for twelve cactus weed species are mass reared, released and monitored by the CBC cactus team.

The mass-rearing facility in Uitenhage, outside Port Elizabeth, is responsible for rearing and releasing agents on infestations, in consultation with landowners.

PROGRAMME HIGHLIGHTS IN 2019

- Long-term monitoring and public opinion surveys have confirmed that releases of cactus biological control agents conducted by the CBC have reduced the density of invasive cactus weeds
- New potential agents for the control of some of the varieties of round-leaved prickly-pear have been identified
- Host-specificity testing of the Pereskia stem-wilter (*Catorhintha schaffneri*) has confirmed that it is suitably specific for release in Australia
- A release application for the release of a new biological control agent for thistle cholla (*Cylindropuntia pallida*) in South Africa has been submitted

Quantifying the benefits of cactus biological control

Releases of biological control agents from the mass-rearing facility have undoubtedly contributed towards the control of invasive alien cactus weeds in South Africa, but it is essential that this contribution is quantified.

Releases conducted by the mass-rearing team are followed by long-term monitoring, most of which is conducted within the Eastern Cape because it is more feasible for the team to visit these sites regularly. Monitoring consists of either setting up permanent transects along which cactus and agent densities are quantified over time, or tagging individual plants at release sites and assessing the size and damage by cochineal on each of the tagged plants. By recording these basic measurements, it is possible to assess the amount of cactus and the agent at a site after releases and therefore assess the impact of the agent. Long-term monitoring on nine different cactus species is currently being conducted at 44 sites.

Biological control users, including land-users and conservationists, have also been interviewed, using a questionnaire. The aim of the questionnaire is to assess the opinion of people who have had biological control agents released on their land by the CBC. The questionnaires are another way of assessing the benefits of the mass-rearing and release effort of this project. While it is not possible for staff of the CBC to monitor every release site, especially those a long distance from the Eastern Cape, it is possible for every person who receives biological control agents from the CBC mass-rearing facility to complete a questionnaire. Thus far, over 80 land-users who have received agents from the CBC mass-rearing facility have completed questionnaires.

Post-release monitoring has shown that the releases of cactus biological control agents have reduced the density of all *Opuntia* and *Cylindropuntia* species. For example, the release of the cochineal insect, *Dactylopius austrinus*, on jointed cactus, *Opuntia aurantiaca*, has resulted in a steady decline in the density of the weed over time, from an average of over 30 joints/m^2 to less than 10 joints/m^2. Jointed cactus is sterile in South Africa, so fruits were not included in this analysis. Fruits are, however, important for the reproduction of many other species of cactus, including the Australian pest-pear, *Opuntia stricta*. Releases of the cochineal insect, *Dactylopius opuntiae*, on Australian pest-pear has reduced the weed biomass by over 70%, and the average number of fruits produced by each plant has decreased from about 140 fruits per plant to about 10. These changes occurred within six months of releasing the biological control agent, and weed biomass and fruit production has remained at the same low level ever since. For other species of cactus weeds, including three *Harrisia* species and the Queen of the night cactus, *Cereus jamacaru*, biological control is much slower. For these species, the spread and density of the agents have increased over time, but densities of the weed start to reduce only five to ten years after the establishment of the agent.

RESEARCH TEAM
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STUDENT
Zezethu Mnqeta (PhD)

The data from long-term monitoring are supported by data from surveys of the perceptions of land-users who are using biological control agents from the CBC mass-rearing facility. Over 70% of the land-users reported that the agents had reduced cactus infestations, and over 90% said that they would recommend the use of cactus biological control agents to other land-users. This is particularly impressive, given that biological control is a long-term solution and many land-users have not had the agent on their land for long enough for the full impact of the agent to be realised.

CBC members and staff rangers after the release of 640 *Hypogeococcus festerianus* galls on the Queen of the Night Cactaceae in Thomas Baines Nature Reserve in April 2019. **Photo credit:** Sithembiso Mhlongo

This programme has established a relationship with nature reserves around the country to assist in controlling their cactus invasions. Releases have been made in the private Kwandwe Game Reserve, Thomas Baines Nature Reserve (Eastern Cape Parks) and many other conservancies in the Eastern Cape Province. In collaboration with SANParks, the biological control agent for Devil's rope cactus, *Dactylopius tomentosus* 'imbricata' was released in the buffer zone around Camdeboo National Park and is proving to be a successful management tool for keeping the invasions under control. Collaboration with Cape Nature has seen the release of biocontrol agents to control creeping prickly-pear (*Opuntia humifusa*) in the Western Cape where the weed was a direct threat to three critically endangered indigenous succulents.

The Uitenhage research facility team with PhD candidate, Zezethu Mnqeta, joined the Mountain Zebra Camdeboo Protected Environment Ecologist at Camdeboo National Park to monitor released biological control agents in the buffer zone of the national park.

CBC Staff and students conducting long-term monitoring on the Devil's rope cactus (top) outside of Graaf-Reinet in the Eastern Cape, and the same site a few months later (bottom). **Photo credit:** Maryke Stern

Developing a new agent for the four varieties of round-leaved prickly-pear

Round-leaved prickly-pear (*Opuntia engelmannii*) is considered one of the most problematic invasive alien cactus species in South Africa.

It is also problematic in Kenya and is one of the few very abundant cacti without an effective biological control agent. It is therefore spreading and increasing in density at an alarming rate. *Opuntia engelmannii* is divided into at least 13 intraspecific taxa that are referred to as varieties. There are three morphologically distinct varieties in South Africa, and a fourth variety in Kenya. At present, there are eleven lineages of cochineal (*Dactylopius* sp.) housed under quarantine conditions at ARC-PHP and the University of the Witwatersrand. This project aims to identify the most effective cochineal lineage for each of the problematic varieties of cacti in Africa and to test whether 'new' or 'old' associations between cochineal agents and their host plant varieties are more effective. Hybridisation between different cochineal lineages is also an issue that could change the efficacy of cochineal agents that may be released in future, or have already been released for the control of other cactus weeds. Hybridisation between any new cochineal agents and cochineals that are already used for biological control must therefore be assessed.

Of the eleven lineages of cochineal currently under investigation, three lineages are damaging to the Eastern Cape variety of round-leaved prickly-pear, and two are damaging to the Kenyan variety. Hybridisation trials are currently underway and, if no adverse implications from hybridisation are evident, the most damaging lineages for each of these two cactus varieties will be released in Kenya and the Eastern Cape. None of the lineages are effective against the Northern Cape variety of the weed, which is a particularly problematic variety in South Africa. Fortunately, there has been recent success in controlling the Northern Cape variety at a site in Douglas, Northern Cape Province, using the biological control agent for the Australian pest-pear, another cochineal insect, *Dactylopius opuntiae* 'stricta'. The Australian pest-pear agent has been released at other infestations of the Northern Cape variety of round-leaved prickly-pear around the country to test if it is an effective agent for this variety of the weed.

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STUDENT
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The Northern Cape variety of *Opuntia engelmannii* near Douglas, Northern Cape, in 2016. Although *Dactylopius opuntiae* 'stricta' was released in 2014, the release site was about 3 km from this photo and the cochineal had not reached the area where this photo was taken. (top)
(bottom) The identical location in 2019 after the cochineal had naturally dispersed to the area some time since 2016. Many plants have died and all living cladodes are covered with cochineal. In parts of the site where the cochineal has been present since 2014, all the *Opuntia engelmannii* is dead.
Photo credits: Iain Paterson

DNA barcoding of biological control agents

Six cochineal species/lineages are already present in South Africa, with a further twelve species/lineages in quarantine facilities around the country.

Plans are underway to release at least one more *Dactylopius opuntiae* lineage for the control of *Opuntia engelmannii* and another *Dactylopius tomentosus* lineage for the control of *Cylindropuntia pallida*. All of these cochineal species are very similar in appearance to non-specialists and the various lineages within species are morphologically identical. Not being able to identify the cochineal insects makes cactus biological control difficult because if the wrong cochineal is used it will not effectively control the weed. .

As part of his MSc, Clarke van Steenderen has identified non-coding DNA regions of the genomes of cochineal that can be used to identify the various lineages and species. The technique has been used to confirm whether the correct lineage of cochineal is present at various field sites, and that the culture of 'stricta' cochineal at the CBC mass-rearing facility and the Skukuza mass-rearing facility in Kruger National Park is the correct lineage.

RESEARCH TEAM
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Included in the analyses are all the cochineal lineages that have been released, or are likely to be released in the near future, for the biological control of Cactaceae in Australia. This work has been conducted in collaboration with Australian colleagues and will be beneficial both for the control of cacti in Australia and for our understanding of DNA barcoding of cochineal generally.

Another new development is a webpage interface that can be used to identify lineages of cochineal from sequencing data. A cochineal can be collected and sequenced following the protocols used in this study and the data can be easily uploaded through the webpage in order to quickly identify what the cochineal is. The webpage is a tool that makes the process of identifying cochineal insects much quicker and simpler. It can be found at: https://clarkevansteenderen.shinyapps.io/Dactylopius_ID_version_1/

Clarke van Steenderen at work in the Zoology and Entomology Molecular Lab (ZEML) at Rhodes University.
Photo credit: Iain Paterson

Orange-tuna cactus

The orange-tuna cactus (*Opuntia elata*) is an emerging weed in South Africa. It is widespread and abundant in the arid parts of the country and has become increasingly problematic over the past few years.
This species is morphologically similar and closely related to some of the worst cactus weeds in South Africa, so the chances are high that it will become more problematic in future. No biological control agents have been released against this species and none of the cochineal lineages already released in South Africa damage it. In the last ten years, the number of localities reported for this plant has increased from zero to 22, and a pre-release survey conducted by the CBC revealed many new sites, bringing the new total to 52 sites. At some of these sites, the weed forms extensive infestations.

Opuntia elata is indigenous in northern Argentina, Paraguay, Uruguay and the southern Brazilian state of Rio Grande Do Sul. A cochineal insect, identified as *Dactylopius ceylonicus,* has been recorded on *Opuntia elata* in the indigenous range and is likely to be an effective agent. Permits have been obtained to export *Dactylopius* from Uruguay and Brazil, but permits are difficult to obtain from Argentina. However, cultures of potential agents can be housed at the FuEDEI laboratories, with whom the CBC has a strong collaboration, until such time that permits are secured.

RESEARCH TEAM
Phillippa Muskett, Dr Iain Paterson

COLLABORATORS
FUEDEI

The CBC intends to import a variety of cochineal lineages for *Opuntia elata* in February 2020 and test which are the most effective at damaging South African *Opuntia elata* plants. The CBC will then conduct host-specificity tests and hybridisation trials using the most damaging lineage. A genetics project will also determine the origin of the South African *Opuntia elata,* which could be important in answering the question whether 'new' or 'old' associations are more effective biological control agents. This project is likely to result in an effective agent to combat a serious emerging invasive cactus.

Opuntia elata, the orange-tuna cactus. **Photo credit:** Iain Paterson

Leaf cactus

Two agents that have been released on the leaf cactus (*Pereskia aculeata*) in South Africa: the leaf-feeding beetle, *Phenrica guerini*, and the stem-wilting bug, *Catorhintha schaffneri*. Both agents are established at sites in the Eastern Cape and KwaZulu-Natal and there is evidence that the leaf feeding beetle is damaging at some sites where high densities of the beetle are present. The stem-wilting bug was released relatively recently and it is too soon to quantify its efficacy. Leaf-cactus has recently been targeted for biological control in Australia, and the CBC is collaborating with the Department of Primary Industry of New South Wales to release both agents in Australia.

RESEARCH TEAM
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The host-specificity testing that was required by the Australian authorities for the stem-wilting bug has now been completed. A release application is being prepared and the agent will probably be released in Australia in 2020. The CBC will be conducting host-specificity testing for the leaf-feeding beetle during 2020 with the intention of releasing that agent in Australia during 2021.

Namibia joins the biological control community

Three of the cactus weeds that are under effective biological control in South Africa have become serious environmental pests in Namibia where no biological control agents are available. In collaboration with the Namibian Chamber for Environment (NCE) and the Botanical Society of Namibia, the CBC obtained permission from all the relevant Namibian Ministries to release three biological control agents against the three cactus weeds. The releases were conducted near Windhoek on the 22 August 2019. This is a significant development for the control of invasive alien plant species in Namibia because it is the first time since Namibian independence that a biocontrol release has been conducted and it provides opportunities for developing a process for the release of other agents in the future. The released agents are known to be very effective, and will result in a significant reduction in cactus infestations in the country to the benefit of indigenous biodiversity and agricultural productivity.

A crowd of interested members of the public, as well as BotSoc, NUST and NCE representatives at the first release of cactus biocontrol agents in Namibia in August 2019. **Photo credit:** Rob Thompson

(right) Iain Paterson releasing the first cochineal for Devil's rope cactus in Namibia. **Photo credit:** Rob Thompson.

Torch cactus

The torch cactus (*Trichocereus spachianus*) has recently been identified as one of the top ten new invaders in South Africa, based on the rapid increase of its distribution and densities over the last ten years.

Based on the past success with cactus weeds and records of potential agents in the native distribution of Argentina, biological control is likely to be an effective solution to this increasing problem. The galling mealybug, *Hypogeococcus* sp., has been recorded on the plant in Argentina, and is likely to be an effective agent. The cochineal species, *Dactylopius confertus*, has also been recorded in association with close relatives of this species and may, therefore, also be an effective option for biological control.

South African *Trichocereus spachianus* is morphologically distinct from plants that are found in the indigenous distribution, probably due to manipulation of the plant in the horticultural trade. In order to overcome this problem, South African *Trichocereus spachianus* will be exported to Argentina, and potential agents will be tested on the South African form of the plant.

RESEARCH TEAM
Dr Iain Paterson

STUDENT
Tamzin Griffith (PhD)

COLLABORATORS
FuEDEI

A PhD student from the CBC, Tamzin Griffith, has been conducting surveys for new potential agents at the FuEDEI laboratories in Argentina since August 2019. Several promising candidate agents have been found and she is conducting impact studies, as well as preliminary host-specificity studies, at the FuEDEI laboratories, with the help of our Argentine collaborators.

The torch cactus forms dense thickets that are impenetrable to livestock and wildlife. **Photo credit:** Iain Paterson

Thistle cactus

Thistle cactus, *Cylindropuntia pallida*, is an invasive alien plant in South Africa that is indigenous in southern USA and Mexico.

In the arid parts of South Africa, it forms dense infestations, to the detriment of indigenous biodiversity and agricultural productivity. The plant is extremely spiny, and these spines are harmful to wildlife and livestock. It is considered an emerging weed because it is at an early stage of the invasion process, but is likely to increase in distribution and density if left unmanaged.

Cylindropuntia pallida has recently been targeted for biological control in Australia, where the cochineal insect, *Dactylopius tomentosus* 'californica var. parkeri' has been released and has been extremely damaging to infestations of *Cylindropuntia pallida*. *Cylindropuntia pallida* is therefore an excellent target for biological control in South Africa with a very high likelihood of success. An application for the release of this promising new agent has been submitted to the South African authorities and the CBC hopes to release this agent in 2020.

RESEARCH TEAM
Dr Iain Paterson

STUDENT
Ekhona Zozo (MSc)

COLLABORATORS
Department of Primary Industries, Australia

Thistle cactus is an extremely spiny plant that is harmful to wildlife and livestock. **Photo credit:** Iain Paterson

International collaboration

A strong collaboration between Fundação Universidade Regional de Blumenau (FURB) in Brazil and FuEDEI in Argentina is essential for this programme. New agents for two of the main target weeds of the project, *T. spachianus* and *O. elata*, are indigenous to Argentina and Brazil, respectively. Tamzin Griffith, a PhD student from CBC, has been based at the FuEDEI laboratories in Argentina since August 2019 and will be based there during 2020.

Work in Namibia was conducted in collaboration with the Namibian Chamber of Environment (NCE) and the Botanical Society of Namibia.

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

The International Organization for Biological Control (IOBC) Global Cactus Working Group (GCWG) aims to improve the control of invasive Cactaceae internationally by creating a platform where the stake holders from different countries can collaborate and engage. The CBC coordinates the GCWG while Dr Iain Paterson is the Chair of the group and a meeting for all stakeholders is scheduled for 2020.

CBC researchers, Tamzin Griffith, Phillippa Muskett, Iain Paterson with Guillermo Logarzo from FuEDEI doing fieldwork on *Opuntia elata* in Argentina. **Photo credit:** Phillippa Muskett

NORTHERN TEMPERATE WEEDS

Initiated in March 2017, the Northern Temperate Weeds Programme, led by Dr Grant Martin, is a new and ambitious programme within the CBC. This programme aims to bring the benefits of biological control to the high-elevation grasslands of South Africa. Grasslands are central to the livelihoods and economies for both small-scale and commercial farmers as well as a number of rural communities. Furthermore, the high-altitude mountain catchments of South Africa are the systems most important to water security as they provide nearly half of all water run-off in South Africa. Unfortunately, these high-elevation grasslands are being over-run by northern temperate woody invasive species. Surprisingly, prior to the initiation of this programme in 2017, none of the invasive species had been considered for biological control, even though a number of species would make excellent targets.

The Northern Temperate Weeds Programme is multifaceted and has involved conducting feasibility studies on a number of the worst invasive species currently invading the high-elevation regions of South Africa. This initial feasibility study was carried out to determine which species should be prioritised for biological control. The programme also includes pre- and post-release studies.

This programme has also aimed to initiate biological control programmes against two invasive tree species: black locust (*Robinia pseudoacacia*) and honey locust (*Gleditsia triacanthos*). These species are both native to the United States of America and are becoming highly invasive in the high-elevation regions of South Africa. Researchers based in the plants' native range in Blacksburg, Virginia USA, have identified a number of potential biological control agents for black locust. To ensure this programme covers all aspects of biological control, pre-release studies have been conducted so that, if these agents are released, the benefits derived from biological control can be accurately measured and reported.

This programme has benefited from support and collaboration of both local and international entities. In South Africa, close collaboration has been established with the University of the free State Bloemfontein campus and the Afromontane Research Unit based at the University of the Free State QwaQwa campus.

Invasive species prioritised under the Northern Temperate Weeds Programme

Species	**Feasibility**	**Development**	**Evaluation**
Cotoneaster spp.	x		
Rubus spp.	x		
Gleditsia triacanthos (Honey locust)		x	x
Populus alba (Silver poplar, silverleaf poplar)	x		
Populus canescens (Grey poplar)	x		
Pyracantha angustifolia (Firethorn)	x		
Robinia pseudoacacia (Black locust)		x	
Rosa rubiginosa (Rosehips)	x		
Salix fragilis (Crack willow, brittle willow)	x		
Salix babylonica (Weeping willow)	x		

International collaboration has been developed between the CBC and the Chinese Academy of Sciences; the Sustainable Management of Agricultural Ecosystems Laboratory (UTAGRI-ECO) in Rome Italy; the Department of Entomology, Virginia Tech Blacksburg, and the Centre of the Region Haná for Biotechnological and Agricultural Research, Crop Research Institute, Czech Republic.

The seed weevil, *Amblycerus robiniae,* cultured on Honey locust pods in the CBC quarantine. **Photo credit:** Esther Mostert

A highlight has been the first introduction of a potential biological control agent for the honey locust, into the Rhodes University quarantine facility. The seed weevil, *Amblycerus robiniae*, identified as a potential agent for the biological control of honey locust, was sent to the CBC from Virginia, USA, in November 2019 and is now in the CBC quarantine facility. CBC MSc Student, Sara Astudillo will be testing this species as part of her MSc research. A number of promising agents for the control of black locust have been identified and will be imported into the CBC quarantine in 2020. This will from part of the research of PhD candidate, Abigail Wolmarans.

A further highlight, looking at invasive species from an economic point of view, has been studied to determine if biological control of rosehips (*Rosa rubiginosa*) and berry species *Rubus* sp. would have any negative social or economic impacts.

PROGRAMME HIGHLIGHTS IN 2019

- Importation of the brucid seed weevil, *Amblycerus robinae*, on honey locust into the CBC quarantine
- Confirmation of birds as main contributed to spread of *Pyracntha angustifolia* in grassland biome
- Prioritisation of potential agents associated with *Robinia pseudoacacia* in the native range
- Extensive pre-release surveys conducted on *Robinia pseudoacacia* in South Africa

What is fuelling the firethorn invasion in the grasslands?

The invasive shrub, *Pyracantha angustifolia*, commonly known as firethorn, is a Category 1b invader in South Africa. It is the focus of Lehlohonolo Adams' Master's research (based at the University of the Free State, QwaQwa campus). Members of the plant genus *Pyracantha* (commonly known as firethorns, family Rosaceae), brought into South Africa as ornamental plants, have become some of the worst invaders in the grassland biome.

Native fauna often act as substitute pollinators and seed dispersers for alien species, so knowledge of how native species may be contributing to the spread of these invasive species is thus valuable for any invasive species control programme. This study set out to determine the seed dispersal system of invasive *Pyracantha* species in afromontane areas of the eastern Free State and northern KwaZulu-Natal.

The small orange- to red-coloured, apple-like fruits of Pyracantha species are typically eaten and dispersed by frugivorous birds. The presence of these fruit-bearing bushes in the Afromontane grasslands of eastern South Africa present an unusual situation in that the grassland flora is largely depauperate of brightly coloured, fleshy, fruit-bearing plant species. The brightly coloured fruits of fruiting firethorn bushes in an otherwise drab-coloured winter landscape are visible from a considerable distance.

Lehlohonolo Adams investigated the reproductive ecology in the eastern Free State where firethorn is widely distributed. This study shows that more than one million seeds are produced per square metre of invasion in all three of the invaded areas within the eastern Free State Province, with a probability of up to 10 million seeds being produced per square metre of *Pyracantha agustifolia* invasions per annum. Camera traps and direct observations revealed that seeds are dispersed by native frugivorous birds and fallen fruits are consumed by rodents. The spread of *Pyracantha angustifolia* is facilitated by the dispersal by frugivores, but hindered by low persistence of propagules in the soil.

RESEARCH TEAM
Dr Grant Martin (CBC), Dr Sandy Steenheisen (UFS)

STUDENT
Lehlohonolo Adams (MSc, University of Free State)

COLLABORATORS
Afromontane Research Unit, University of the Free State, QwaQwa Campus, Phuthaditjhaba; Department of Plant Sciences, University of the Free State, QwaQwa Campus; Rhodes University Physics and Electronics Department, The Commonwealth Scientific and Industrial Research Organisation (CSIRO) ·Division of Ecosystem Sciences

Lehlohonolo Adams collecting *Pyracantha* berries to conduct feeding trials. **Photo credit:** Sandy Steenhuisen

Black locust tree

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

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

COLLABORATORS
SANParks; University of the Free State, University of Kentucky, Virginia Tech

Owing to the adverse impact of the invasive black locust tree (*Robinia pseudoacacia*) on indigenous biodiversity, its disruption of ecosystem services and its prolific ability to propagate and disperse in terrains where mechanical and chemical control is not feasible, it has been earmarked for biological control using insects.

Although it has established in all South African provinces, several questions remain surrounding the seedbank and population dynamics of black locust, its interaction with indigenous insect and plant species, and nutrient cycling among South African grasslands. Having these baseline data will ensure that once biological control agents are released against *Robinia pseudoacacia,* the benefits derived from control can be accurately determined. This is the PhD work of student Gerald Chikowore, based at the University of the Free State. For her PhD, Abigail Wolmarans will be identifying and developing biological control agents for the invasive black locust tree.

Black locust tree invasion outside of Clarens in the Free State Province. **Photo credit:** Grant Martin

Honey locust tree

RESEARCH TEAM
Dr Grant Martin (CBC)

STUDENT
Sara Salgado (MSc, CBC)

COLLABORATORS
University of Kentucky, Virginia Tech, Blacksburg

The invasive honey locust tree (*Gleditsia triacanthos*) 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 that 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 and the impact of the weevil on the seeds of the tree may influence future management options. If the seed-feeding brucid is not impacting the plant seeds sufficiently, additional seed-feeding biological control agents will be considered, such as the damaging honey locust seed beetle, *Amblycerus robiniae*. Sara Astudillo, MSc student, will focus on this as part of her research.

Rosehips

Timothy Westwood undertook this study as part of his Master's degree in Economics. The study aimed to determine if there is a conflict of interest between the management of rosehips (*Rosa rubiginosa*) and the market surrounding its fruits, also known as hips.

RESEARCH TEAM
Dr Grant Martin, Prof. Gavin Fraser

STUDENT
Timothy Westwood (MComm)

Rosehips is a compact, deciduous shrub native to Europe and Asia. In South Africa, the plants are distributed mainly in the higher elevations of the grassland biome. The plant is classified as a Category 1b invasive species under the NEMBA regulations, meaning it is illegal to propagate the species. However, the plant has significant economic value. Rosehips (fruits) are the fifth largest organic wild-collected product globally. In South Africa and Lesotho, the rosehips are harvested in the wild for the global market; however, the size of the market has not yet been accurately determined, nor have any potential conflicts of interest that may arise from the management of the species in South Africa been considered. It was found that most landowners are not particularly concerned with the species as there are other invasive species that have more significant impacts.

The rosehip market turned out to be far bigger than expected, with an estimated 5000 tonnes of rosehip going through production annually; it provides an informal income for approximately 30 000 people in two countries. The plant could potentially be grown from cuttings in regions where the climate is not suitable for seed germination and would therefore not be invasive. In these regions, the plant could be grown commercially to supply the current global demand for the species.

Rubus

Brett Mason is a MComm student in the Economics Department at Rhodes University. The focus of his research is to evaluate the economic costs and benefits of a selected geographic distribution of invasive *Rubus* genus in South Africa. The *Rubus* genus contains edible berries, such as blackberries and raspberries The plants are native to Europe and America, but were imported into South Africa to be grown for berry production. The shrubs have spindly rambling branches with thorns, and in invaded ranges stands of Rubus can cause impenetrable areas, leading to loss of arable land.

RESEARCH TEAM
Dr Grant Martin, Prof. Gavin Fraser , Dr. Costas Zachariades

STUDENT
Brett Mason (MComm)

COLLABORATORS
University of Cape Town; ARC-PPRI, 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

Species that present both benefits and costs are often conflict-generating species. This study aims to determine the positive and negative socio-economic impacts of invasive *Rubus* species in South Africa. The ultimate objective of the study is to guide policy regarding the management of these species. The species in this genus present a unique management conundrum, given the complex phylogeny of indigenous, alien, and hybrid species. The Rand value of the costs and benefits associated with the species have been determined through structured interviews with relevant stakeholders. Results show that costs and benefits differ between regions and depend largely on the land usage and authorities involved. In some regions, such as the Free State, vintage brews are produced by local cottage industries that use the berries of invasive *Rubus* species. In other regions, such as KwaZulu-Natal, invasive *Rubus* species are regarded as a menace and, by and large, controlled wherever possible. Management of the species in the *Rubus* genus requires a nuanced management approach, potentially predicated on a regional basis.

Another important component of this study is to determine if biological control is a viable management option for the invasive *Rubus* sp. in South Africa. To ascertain this the insects associated with the indigenous, invasive, and hybrid species in South Africa are being investigated. If the indigenous species have a suite of phytophagous insects associated with them that do not spill over onto the invasive species, it might be possible to find potential biological control agents in the invasive species' native ranges that are specific enough and will not pose a threat to our indigenous species.

INVASIVE TREES

Australian acacias

Many Australian acacias are considered invasive in South Africa and have negative impacts on biodiversity, agriculture and water. Historically, biocontrol efforts have focused on seed-reducing agents to avoid potential conflicts of interest, which could arise as a result of commercial utilisation of several of the species. While there are more than ten invasive Australian acacias present in South Africa, research focusses on *Acacia baileyana*, *Acacia cyclops* (rooikrans), *Acacia dealbata* (silver wattle), *Acacia decurrens* (green wattle), *Acacia longifolia* (long-leaved wattle), *Acacia mearnsii* (black wattle), *Acacia podalyriifolia* (pearl wattle) and *Acacia saligna* (Port Jackson willow). Currently, the biological control agents used include gall-forming wasps, flower-galling midges and seed-feeding weevils. An additional gall-forming wasp is being investigated. The research on this group of weeds includes all aspects of a biocontrol programme, from development and screening of new agents, to post-release evaluations, many of which have been ongoing for almost 20 years.

John Hoffmann and Daleen Strydom emptying seed traps. **Photo credit:** Fiona Impson

In addition to looking into seed banks and seed longevity, much of the research on Australian acacias focuses on gaining a better understanding of the seed dynamics of this group of weeds by carrying out long-term studies to investigate annual seed rain of several species in different climatic regions of the country. Running parallel to this, studies investigating insect damage levels, including mortality studies of agents, will facilitate insight into the impacts of biological control agents that reduce seed production of their host plants.

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

The gall-forming wasp, *Perilampella hecataeus*, on two acacias

This gall-forming wasp, *Perilampella hecataeus*, has been investigated since 2013 when initial surveys and collecting took place in Australia, followed by preliminary host-specificity testing in quarantine in South Africa, and field testing in Australia. Further extensive surveys and collecting in Australia in 2016 confirmed the specificity of the agent to *Acacia baileyana* and *Acacia dealbata* with occasional galling on *Acacia decurrens* and limited 'spillover' onto *Acacia mearnsii*. However, subsequent ongoing tests with a small culture of wasps maintained in quarantine have been unable to confirm any oviposition or development on *Acacia mearnsii*. A new import of gall material in December 2019 did not result in any female wasps for final tests against *Acacia mearnsii*.

The midge, *Dasineura pilifera*, on *Acacia baileyana*, *Acacia dealbata* and *Acacia decurrens*

Permission to import and release this flower-galling midge, *Dasineura pilifera*, was granted in 2015, and the first releases took place in 2016 in Stellenbosch. The midge established, but a fire in early 2018 destroyed the only

release site, and adults emerging from a consignment shipped from Australia in May 2018 failed to establish at any of the release sites. Finally, in 2019, a shipment of galls resulted in successful establishment of midges at two sites in the Stellenbosch area, and an additional import and further releases are planned for 2020.

Seed dynamics – seed banks, seed rain, and seed burial

A project initiated in 2004 to investigate seed banks of *A. cyclops* now includes a number of the more important and abundant invasive *Acacia* species. In addition, annual seed production (seed rain) of several species is monitored across the country in order to establish seed rain figures both pre- and post- introduction of biocontrol measures. In the case of *Acacia mearnsii*, the long-term data set has shown dramatic and consistent declines in seed rain following the introduction of a gall-forming midge. Overall, such data promise to contribute towards a better understanding and improved management of these weeds.

PROGRAMME HIGHLIGHTS IN 2019

- Release and establishment of a flower-galling midge, *Dasineura pilifera*, for *Acacia baileyana*, *Acacia dealbata* and *Acacia decurrens*, following destruction of the original release site
- Collaboration with the University of Coimbra, Portugal, to initiate a biological control programme against *Acacia dealbata* and *Acacia melanoxylon*, using Melanterius seed weevils, in addition to a combined post-release evaluation study on the bud-galling wasp, *Trichilogaster acaciaelongifoliae*, on *Acacia longifolia* in Portugal and South Africa
- Collaboration with University of Tel Aviv to initiate a biological control programme against *Acacia saligna* in Israel, using a Melanterius seed weevil
- Completion of testing of *Coelocephalapion gandolfoi* for deployment against prosopis; permission to release has been secured

Prosopis

The negative impacts of prosopis (also known as mesquite) invasions globally are widely recognised, but a conflict of interest stemming from the useful attributes of these trees has historically hampered biocontrol interventions. To date, only mature seed-feeding beetles have been released in South Africa with one of these species both widespread and capable of inflicting high levels of damage. However, interference from livestock reduces its effectiveness.

At a stakeholder workshop organised by the CBC in conjunction with Agri SA in February 2019, there was a clear call for more concerted management efforts targeting prosopis, and a call to deploy more damaging biocontrol agents. Several potentially damaging candidates warrant investigation.

In 2015, CBC consortium partners based at the University of Cape Town took over responsibility for overseeing the development and deployment of an additional seed-feeding weevil targeting prosopis. The seed-feeding weevil, *Coelocephalapion gandolfoi* attacks the immature, developing seed of prosopis and was earmarked as particularly promising in earlier surveys and testing. It was approved for release in 2015, subject to the testing of an additional southern African legume (*Xerocladia viridirmis*) considered at potential risk.

After substantial delays resulting from permit issues, permission was obtained for an export from Argentina in 2019 and a collecting trip undertaken by Catharina Kleinjan (UCT) and Dr Fernando McKay (FuEDEI). Although collecting conditions were poor, a small consignment was successfully imported into quarantine and the requisite testing completed. These tests showed that *Xerocladia viridiramis* is unequivocally not a suitable host for *Coelocephalapion gandolfoi*, and the Department of Agriculture, Land Reform and Rural Development (DALRRD) granted permission to release in January 2020.

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

COLLABORATORS
Dr Fernando McKay, FuEDEI

Prosopis trees produce prolific flowers (top) and seeds which are dispersed by browsers or washed away during flood events and deposited on floodplains to form dense impenetrable thickets (bottom). **Photo credit:** Graham Harding

BUGWEED

Bugweed, *Solanum mauritianum*, indigenous to South America, is one of South Africa's most widespread environmental weeds. Considerable research efforts in South Africa have, however, culminated in the release and establishment of only two insect agents, the lace bug, *Gargaphia decoris* and flower feeding weevil, *Anthonomus santacruzi* in 1999 and 2008, respectively. Despite both agents establishing in the field, damage to bugweed is regionally local and has been largely insignificant, possibly due to agent climatic incompatibility. Much of the bugweed invasion in South Africa occurs at high altitude (> 1000 m) regions that experience cold winters. These issues have stimulated renewed testing of new agents, and re-collecting existing agents, such as *Anthonomus santacruzi,* that may potentially be better adapted to cold.

In March 2019, Nic Venter and Blair Cowie from Wits University accompanied Hugh Gourlay of New Zealand Land Care on a joint expedition in Uruguay to survey and collect potential biological control agents for bugweed. Sampling occurred in the south-eastern region of Uruguay, predominantly between Aigau and Rocha over a seven-day period. Trees were initially mapped for the region and then actively sampled, with flower, bud, and stem material collected from trees prior to beating. *Anthonomus* spp. were brought back to quarantine at Wits University for ongoing testing.

RESEARCH STAFF
Prof. Marcus Byrne, Nic Venter, Blair Cowie

COLLABORATORS
UKZN; LandCare Research New Zealand

Blair Cowie, a PhD candidate from Wits University surveying bugweed in its native Uruguay for biocontrol agents; The flower feeding weevil, *Anthonomous morticinus*, is currently being considered as an additional biocontrol agent for bugweed. **Photo credit:** Nic Venter

TAMARIX

Invasive *Tamarix* are terrestrial weeds infesting riparian ecosystems and dams. The weeds are believed to have been introduced in South Africa in the early 1900s for unknown reasons and have since escaped their point of introduction and are problematic in the Eastern and Western Cape Provinces, with the native species occurring more in the Northern Cape Province. Remote sensing data have shown that invasive *Tamarix* have almost doubled their infestations in a period of 11 years between 2007 and 2018. Negative impacts of *Tamarix* are evident as they are known to reduce ground and underground water levels, increase soil salinity through salt deposits, and reduce native biodiversity.

Two Eurasian species, *Tamarix* chinensis and *Tamarix* ramosissima, together with their hybrids, have since been declared invasive, and efforts to suppress their infestations are underway. *Tamarix usneoides*, the only species native to the southern Africa region including South Africa, poses the challenge for 'non-target effect' to the biocontrol programme against the invasive genotypes. However, phylogenetic analysis has strongly separated the indigenous *T. usneoides* from the invading genotypes, placing them in separate clades, and thus suggesting that there is a chance of finding host-specific biocontrol agents with no non-target effects on the native species. In contrast, the leaf-feeding beetle, *Diorhabda carinulata,* and the scale insect, *Trabutina mannipara,* have been discontinued as potential biocontrol agents because of non-target effects on the native *T. usneoides*. As a result, differences in secondary metabolites in host plants are being investigated to elucidate chances of finding host-specific biocontrol agents since studies show that insect parasitism depends on the secondary metabolites of the host plant. In addition to our previous phylogenetic inference, we are analysing the second metabolites of *Tamarix* and associate that with herbivory to infer host specificity.

RESEARCH TEAM
Prof. Marcus Byrne, Dr Samalesu Mayonde (Postdoc)

STUDENTS
Sivenathi Hatile (MSc, Wits)

COLLABORATORS
Dr Sivu Situngu – Wits University; USDA-ARS-European Biological Control Lab

Host-range testing of *Tamarix* Eriophyoid mites.

Gall-forming mites have been discovered on two distinct *Tamarix usneoides* populations in Noorspoort, Eastern Cape, and Dwyka in the Western Cape. The fact that the gall-forming mites have not been seen on any of the invasive species and occur only on specific *T. usneoides* populations suggests that the usually highly host-specific mites would present an opportunity to find suitable biocontrol agents against invasive *Tamarix* with no non-target effect on the native species. There are about 11 eriophyoid species from the family Eriophyidae that are associated with *Tamarix*. Eriophyoid mites collected in Noorspoort and Dwyka are currently being reared in quarantine at Wits University prior to host-range testing. Once the mites are confirmed as highly host specific, exploration of biocontrol agents in the native range of the invasive Eurasian species will focus on collecting mites.

Secondary metabolites analysis and exploration for new biocontrol agents

A field trip to Eurasia to collect new biocontrol agents is scheduled for April/May 2020 and will be conducted with the help of our European collaborators, Dr Massimo Cristofaro and/or Dr Rene Sforza. The insects will be reared in the quarantine at Wits University by MSc student, Sivenathi Hatile, who will also be testing for any differences in secondary metabolites of *Tamarix* species present in South Africa. The outcome will be compared against feeding of the collected insects.

AFRICAN BOXTHORN

African boxthorn (*Lycium ferocissimum*) is a plant indigenous to the Western and Eastern Cape provinces of South Africa and has become invasive in a number of countries, including Australia, where attempted chemical and mechanical control methods are costly and unsustainable. In Australia, biological control is being considered as a management option; however, the herbivorous insects associated with the plant in South Africa are not well known. This placed the CBC in an ideal position to assist Australian colleagues when they started to investigate potential biological control options for the management of the species.

At the initiation of the programme, a number of taxonomic uncertainties with regard to the plant in both South Africa and Australia posed significant challenges to any potential biological control programme. In collaboration with Australia, it was possible to clear up these uncertainties, ensuring surveys for potential biological control agents took place on the most appropriate boxthorn populations. Surveys for phytophagous insects on African boxthorn were carried out repeatedly over a two-year period in both regions. The number of insect species found in the Eastern Cape Province (55) was higher than that found in the Western Cape Province (41). The tortoise beetle, *Cassida distinguenda* , ladybird beetle, *Cleta eckloni* and the weevil, *Neoplatygaster serietuberculata* (Curculionidae) were prioritised as potential biological control agents

In addition to providing potential solutions for the management of the species in Australia, the programme has resulted in excellent collaboration between the two research entities. Since the start of the programme, a researcher from CSIRO has travelled to South Africa to collect a rust fungus *Puccinia rapipes* from field sites, and PhD candidate, Evans Mauda, was able to transport the prioritised insect biological control agent, the ladybird beetle (*Cleta eckloni*) to Australia in February 2019.

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

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

COLLABORATORS
CSIRO, Australia

The ladybird beetle (*Cleta eckloni*) on African boxthorn. **Photo credit:** Evans Mauda

PROGRAMME HIGHLIGHTS IN 2019

2019 was an excellent year for the programme as much of the initial groundwork conducted at the start of the programme was analysed and completed. The Western Cape populations of *Lycium ferocissimum* were confirmed as the origin of the populations currently invading Australia, suitable agents for Australia could be prioritised and be the CBC could export the insects to Australia for further host-specificity testing. Two papers were submitted for review. Evans Mauda was able to hand-carry the first insect biological control agent, *Cleta eckloni,* to Australia. Evans used this opportunity to conduct morphology studies in the plants' invaded range.

GRASSES

The biological control of invasive grass species is receiving more interest globally, and the CBC has started a number of novel and innovative projects aimed at contributing towards this growing body of knowledge, as well as providing biological control options for the management of invasive grass species.

Historically, grasses have been avoided as targets for biological control, despite being some of the most abundant and damaging invasive plants worldwide. This reluctance has stemmed from the perception that grasses lack sufficiently host-specific and damaging natural enemies to use as biological control agents. There are also concerns that the risk posed to economically important crop/pasture species and closely-related native species is too great to consider implementing biological control for invasive grasses. 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, given that practitioners follow appropriately rigorous methods when assessing the specificity of candidate biological control agents.

Biological control could play an important role in the management of invasive grasses, but greater ecological and evolutionary understanding of grass-consumer interactions is required to harness the full potential of this weed control strategy. The CBC encourages biological control practitioners to consider grasses as suitable targets for biological control.

PROGRAMME HIGHLIGHTS IN 2019

- A review of grass biological control was published in the International Organisation for Biological Control (IOBC) journal, *Biocontrol*. The paper was authored by members of the CBC and collaborators, and calls for invasive grasses to be considered as targets for classical biological control
- Promising potential agents were sourced for invasive *Sporobolus* sp. in Australia

Common reed, *Phragmites australis*

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

STUDENT
Guy Sutton (PhD)

COLLABORATORS
Department of Agriculture and Fisheries, Australia

The common reed (*Phragmites australis*) found in most wetland ecosystems, is a native reed in South Africa and is one of the most abundant plants in the country. In recent years, a pattern of reed expansion has been observed that shows similar trends to cryptic invasions in North and South America where the introduction of non-native lineages outcompetes local lineages of *Phragmites*.

The CBC undertook a genetic study to investigate whether or not this was occurring in South Africa. The study confirmed the native status of *Phragmites australis* and found no evidence of the introduction of non-native lineages. This work has helped guide management strategies on expansive reed populations and determined that biological control cannot be considered as the reed is indigenous. Instead, other means of control, such as mechanical control, and the reduction of the number of impoundments on our river systems, need to be considered.

Expansive *Phragmites* reed stands in the Western Cape, South Africa. **Photo credit:** Kim Canavan

Giant reed, *Arundo donax*

RESEARCH TEAM
Dr Kim Canavan (Postdoc), Guy Sutton

Arundo donax invasion along the Fish River in Cradock, Eastern Cape. **Photo credit:** Kim Canavan

The giant reed, *Arundo donax,* is one of South Africa's worst invasive alien species, present in all provinces and seriously impacting riparian ecosystems. It is indigenous in Europe and Asia, but has become a damaging invasive alien species in riverine areas worldwide. A biological control programme is being considered for *Arundo* in South Africa and the CBC initiated pre-introductory surveys to provide information to guide this programme. Surveys were carried out across the reed's distribution to determine the herbivore assemblages that are present in South Africa. This work was able to identify a biological control agent, the wasp, *Tetramesa romana,* that is already established from an unknown introduction. A number of other herbivores were also identified and recommendations could be made on potential new agents that could be brought in. In addition, a genetics study explored the origin and genetic diversity of *Arundo* populations in South Africa which revealed that reed populations are clonal in South Africa (no genetic diversity) and share the same lineage as other invasive *Arundo* populations worldwide, which have an ancient origin in the Indus Valley, Asia.

Tussock grasses *Sporobolus* spp.

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

STUDENT
Guy Sutton (PhD)

COLLABORATORS
Department of Agriculture and Fisheries, Australia

The invasive tussock grasses, *Sporobolus pyramidalis* and *Sporobolus natalensis* (giant rat's tail grass), 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, in collaboration with the Queensland Department of Agriculture and Fisheries (Michael Day) and funded by AgriFutures Australia. Surveys across South Africa have identified three stem-boring wasps, namely two *Tetramesa* spp. and a *Bruchophagus* sp. (Hymenoptera: Eurytomidae) that appear to attack only *S. pyramidalis* and *S. natalensis*, despite surveying 47 other non-target grass species. While all three of these wasps reduce seed production and plant survival, *Tetramesa* sp. 1 accounts for the vast majority of this damage (approximately 84%), reducing seed production by up to 95%, and so making it the top-priority candidate agent for this project. *Tetramesa* sp. 1 will be imported into quarantine in Brisbane, Australia in 2020 for laboratory-based host-range testing. Prospects for the biological control of *S. pyramidalis* and *S. natalensis* in Australia are good.

Gamba grass *Andropogon gayanus*

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

COLLABORATORS
CSIRO Australia

Gamba grass (*Andropogon gayanus*), a noxious weed in Australia, is native to tropical and subtropical savannahs of Africa. Although the grass is highly invasive in northern Australia, it remains an economically important foraging grass for livestock. The negative impacts of the grass in Australia include reducing indigenous biodiversity and disrupting fire regimes and therefore ecosystem functioning. Since January 2018, the CBC has been conducting preliminary surveys for phytophagous insects associated with *A. gayanus*. The aim of these surveys was to determine the feasibility of biological control as a strategy for managing the grass. These surveys highlighted the damaging stem-mining insects associated with the grass: two species of flies, five wasps, and two moths. The damage inflicted by these stem-mining species is encouraging because weakening the stem of gamba grass may reduce the environmental risk from fires in Australia but still allow the grass to be used for grazing. Going forward, the project aims to include a larger area of the grass' native range, including Nigeria, Benin and Kenya.

Collaboration with the Alien Grass Working Group

The CBC has worked with the Alien Grass Working Group since its initiation in 2013. The group aims to bring relevant experts together with a shared interest in alien invasive grasses in South Africa. The CBC has helped to plan and coordinate the next annual meeting to be held with the South African Association of Botanists (SAAB) at the University of the Free State, QwaQwa campus from 6 –10 January 2020. The meeting will include a grass identification course and a special session within SAAB on alien invasive grasses.

AFRICAN TULIP TREE

The African tulip tree, *Spathodea campanulata*, is indigenous to West Africa, but has been introduced in many regions outside its native distribution. It has become a serious environmental and agricultural pest in the Pacific Islands. African tulip tree has been targeted for biological control in Rarotonga, the largest of the Cook Islands in the southern Pacific. Potential biological control agents were sourced by CBC staff from Ghana and imported into quarantine in South Africa in 2016. In 2017, members of the CBC, together with LandCare Research New Zealand and members of the Ministry of Agriculture in the Cook Islands, released a leaf-galling eriophyid mite (*Colomerus spathodeae)*, the first biological control agent released against African tulip tree, on Rarotonga. The mite is now established across the whole island and has infected most African tulip trees.

RESEARCH TEAM
Dr Iain Paterson, Phillippa Muskett, Guy Sutton

COLLABORATORS
LandCare Research New Zealand, CSIRO Ghana, Ministry of Agriculture (Cook Islands)

During 2019, host-specificity testing of an additional biological control agent, the flea beetle *Paradibolia coerulea*, was completed, and the Cook Islands authorities granted permission to release the agent in Rarotonga. The population of *P. coerulea* will be exported from the CBC quarantine to Rarotonga in early 2020. It is hoped that a combination of these two agents will reduce the density of African tulip trees on Rarotonga with benefits to indigenous biodiversity, including many rare endemic species found only on the island.

Damage by the flea beetle on the African tulip tree. **Photo credit:** Phillippa Muskett

African tulip tree infestation in Fiji. **Photo credit:** Iain Paterson

PROGRAMME HIGHLIGHTS IN 2019

- A leaf-galling mite, *Colomerus spathodeae,* has successfully established and spread throughout Rarotonga
- Host-specificity testing of the flea beetle, *Paradibolia coerulea,* was completed and the agent is suitably host specific for release in the Cook Islands for control of the African tulip tree, *Spathodea campanulata*
- An application for release was submitted and accepted by the Cook Island authorities

AGRICULTURAL ENTOMOLOGY

All the plants that we use as crops are also attacked by insect pests, with an estimated loss of about 50% of crops to pests worldwide. In an attempt to reduce the amount of chemical pesticides sprayed in the environment, the Agricultural Entomology Research Programme, undertaken in the Entomology Department at Rhodes University, aims to identify and exploit predatory and parasitic insects, such as beetles and wasps, and entomopathogens, such as fungi and viruses, to control insect pests. This research focusses mainly on the citrus industry, but projects on pests of pome fruit (apples and pears), cabbage, potato, litchi, macadamia and pecans are also underway. These biological control techniques are self-perpetuating and thus sustainable; they do not pollute or disrupt the environment, nor do they leave chemical residues on the food.

Dr Sean Moore manages the Agricultural Entomology Research Programme and a number of students, from third-year level to post-doctorate, are carrying out research within this programme. The aim of this programme is to research and develop novel biological control solutions to pest problems in agriculture, and to present these in a practical and usable form to the relevant agricultural industries and the scientific community. Various research projects are being conducted by the CBC here at Rhodes University to improve, expand and develop novel biological control options in South Africa.

False codling moth cultures at Rhodes University

A large proportion of CBC agricultural students conduct research on the key citrus pest, false codling moth. As such, this insect is maintained at Rhodes University for research purposes. These insects are reared on an artificial maize-based diet from eggs to pupae and subsequently maintained as adults in specialised oviposition cages under controlled conditions. Currently five genetically distinct populations are maintained, aptly named after the area from which they were originally collected: Citrusdal, Nelspruit, Marble Hall, Addo and an Old colony of mixed origin. In nature, FCM populations are often localised resulting in genetically differentiated populations on local and regional scales. These differences may affect the efficacy of certain control options currently investigated or used. Thus, testing hypotheses across a range of different populations may help foresee challenges in these pest management strategies that may then be addressed.

PROGRAMME HIGHLIGHTS IN 2019

- Multiple presentations were given at local and international conferences
- Several peer reviewed papers and a book chapter were published
- A PhD student attended the postgraduate course in Analytical Methods in Chemical Ecology held at the Swedish University of Agriculture in Alnarp, Sweden

Is pest freedom under nets possible?

Netting citrus orchards is thought to provide protection from various abiotic factors, but the effect on biotic factors, particularly insect pests, requires further study. This study focused on the effect nets have on key pests, residue levels, and various horticultural aspects. False codling moth (FCM), *Thaumatotibia leucotreta*, is a major insect pest of citrus and other commercial fruit in South Africa and causes a substantial financial loss every year.

Orchards of similar cultivars were monitored inside and outside nets in several orchards at three sites throughout the Sundays River Valley, Malelane, and Burgersfort. Pest management programmes inside and outside of nets were similar, including the release of sterile moths in both environments at two of the sites. Data trees inside and outside the nets were monitored weekly for fruit infestation during the 2018 and 2019 seasons. During the 2018 season, false codling moth (FCM) infestation was higher under nets, possibly because nets had been erected over orchards with previously high FCM infestation. During the 2019 season, no FCM was found inside or outside of nets owing to an overall low number of FCM present in the Sundays River Valley. Pheromone traps were also monitored for sterile and wild moth catches. Higher numbers of wild and sterile moths were caught under nets, and a higher ratio of sterile moths was recaptured under nets in both seasons. These same orchards were used to scout for key citrus pests: red scale, mealybug, thrips, brown and green leafhopper, bud mite, and fruit fly. Red scale and mealybug were significantly higher under nets for both seasons. Thrips damage was significantly higher outside of nets. Spiders and predatory mites were significantly higher under nets, which may explain the lower thrips damage. There was no significant difference between bud mite and leafhopper damage between trees inside and outside nets. Further research, particularly on the increased number of mealybug and red scale infestations, as well as further monitoring of netted orchards would help in determining if similar trends are recorded in the following seasons.

RESEARCH TEAM
Dr Tamryn Marsberg, Prof. Martin Hill, Dr Sean Moore, Mellissa Peyper and Wayne Kirkman

STUDENTS
Karlien Grobler (PhD), Wayne Mommsen (MSc)

Aerial view of a netted citrus orchard. **Photo credit:** Inge Cilliers - Rotorworx

Improving quality control testing of sterile moths for false codling moth sterile insect technique

The sterile insect technique (SIT) for false codling moth (FCM) has been commercially implemented in citrus in South Africa since 2007, with generally good success. However, a few possible problems have been identified and there is a continual pursuit to improve the quality and performance of the sterile moths. Thus, this study focused on various aspects: first, the lack of activity of sterile moths compared to wild moths at cooler temperatures.

False codling moth larvae (top), pupae (middle) and adult moth (below). **Photo credits:** David Taylor

RESEARCH TEAM
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STUDENTS
Karlien Grobler (PhD), Wayne Mommsen (MSc)

Previous work identified trehalose as an effective cryoprotectant for sterile moths if added to the larval diet. Consequently, a field trial was conducted during autumn and winter of 2018 and 2019, to compare recaptures of moths reared on a trehalose-augmented diet with those reared on the normal diet. Recaptures were higher for trehalose moths for both years. Trap catches also indicated that trehalose-fed moths survived longer in the field than control moths.

A reliable quality control test to measure the mating competitiveness of sterile male moths was required and was investigated in laboratory trials by determining the spermatophore transfer between sterile males vs. wild females, and wild males vs. wild females. Simultaneously, choice-trials were conducted in a field net to compare the mating incidents between sterile and wild moths. Wild males were found to have a significantly higher spermatophore transfer than sterile males, with sterile males showing no preference for sterile females. A statistically significant correlation was also recorded between mating incidents in cages involving sterile males and spermatophore transfer in laboratory trials. Therefore, laboratory-based spermatophore transfer trials can be used as a reliable quality control measure for sterile males.

Finally, amplified fragment length polymorphism (AFLP) tests are being developed as a means to differentiate between wild larvae and commercially-released sterile (Xsit) larvae, in order to determine whether any larvae infesting fruit in the field are F1 steriles.

Sexual attraction and mating compatibility between false coding moth populations

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

STUDENT
Jennifer Upfold (MSc)

Semiochemical control technologies using the sex pheromone have become an important strategy in controlling FCM population numbers in orchards. However, the compounds of the sex pheromone vary in FCM populations in different geographical locations. Research using AFLP analysis shows that, in the genetically differentiated populations of FCM in South Africa, males have a significantly greater attraction to females from their own population. Furthermore, regional attractiveness assessed through choice/ no-choice mating compatibility trials found significant sexual isolation occurring between genetically distinct FCM populations. However, no significant sexual isolation occurred between commercially-released sterile (Xsit) moths of mixed origin and regionally distinct FCM populations. This indicates that the sterility process does not significantly affect fitness when the population is of mixed origin. Further research involves measuring the mixing ratio of the FCM sex pheromone for the different populations in South Africa.

Yeast-baculovirus synergism

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

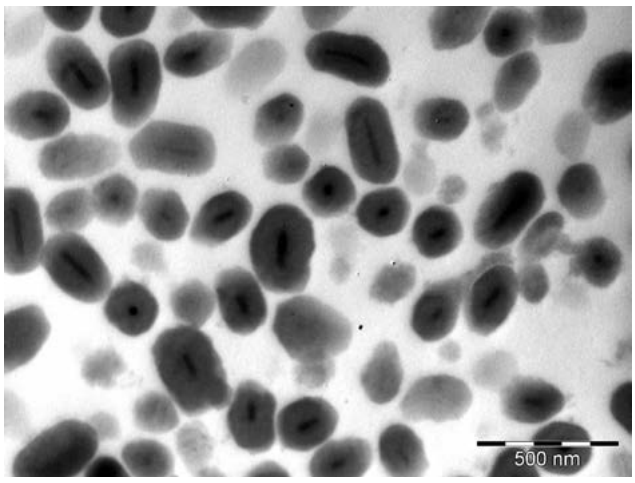
STUDENT
Marcel van der Merwe (PhD)

A mutualistic association has previously been reported between *Cydia pomonella,* also known as codling moth, and epiphytic yeasts. Laboratory assays and field trials show that combining yeast with *Cydia pomonella* granulovirus significantly increased larval mortality. We propose to determine which species of yeast occurs naturally in *Thaumatotibia leucotreta* larvae and to examine whether any of these yeasts, when combined with the *Cryptophlebia leucotreta* granulovirus, increase larval mortality.

Navel oranges infested with *T. leucotreta* larvae were collected from geographically distinct citrus-producing regions in South Africa. This bioprospecting process led to the isolation and identification of several yeast isolates, namely *Meyerozyma caribbica*, *Pichia kluyveri*, *Pichia kudriavzevii*, *Hanseniaspora opuntiae*, *Pichia fermentans*, *Kluyveromyces marxianus* and *Candida lusitaniae*.

Over the course of the last year, these isolated yeasts have been subjected to oviposition and attraction assays to investigate the effect that they have on mated female *T. leucotreta*. Some promising results have been obtained. Additionally, detached fruit bioassays to determine the optimal yeast/virus concentration and to identify the best performing yeast candidate have been successfully completed.

Future work for this project will involve analysing the volatiles produced by the isolated yeast species via gas chromatography-mass spectrometry (GCMS) analysis, optimising the yeast/virus mixture with the addition of adjuvants, and then finally conducting field trials.



Transmission electron micro-graph of purified Cryptophlebia leucotreta granulovirus (CrleGV) occlusion bodies embedded in resin and sectioned. **Photo credit:** Michael Jukes

Investigating improved lethal time through synergistic interactions between a granulovirus and nucleopolyhedrovirus

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

STUDENT
David Taylor (MSc)

The project investigates potential synergistic effects between an alpha- and beta-baculovirus for enhanced control of false codling moth (FCM). To date, seven-day dose-response bioassays have been performed on FCM neonates for three different treatments: CrleGV-SA, Cryptophlebia peltastica nucleopolyhedrovirus (CrpeNPV), and a 50:50 mixture of CrleGV-SA and CrpeNPV. The LC_{90} values for CrleGV-SA and CrpeNPV support results previously described in the literature, with CrpeNPV having a dose approximately 5-fold lower than that of CrleGV-SA, indicating that the experimental procedures used are valid and repeatable. The LC_{90} of the 50:50 mixture was approximately 2.5-fold lower than that achieved for CrpeNPV alone and approximately 13-fold lower than that of CrleGV-SA. While many of these results confirm past measurements, they form the foundation for the upcoming experiments which will evaluate the effect of combining these viruses on lethal time. A series of time-response bioassays for each of these treatments is ongoing, with the hope that improved speed of kill will be measured for these viruses when applied in combination.

Selection for improved virulence of CrpeNPV in false codling moth

RESEARCH TEAM
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STUDENT
Petrus Iita (MSc)

Cryptophlebia leucotreta granulovirus (CrleGV-SA) has been used as a biocontrol agent for *Thaumatotibia leucotreta* in citrus orchards in South Africa as part of an integrated pest management (IPM) system. Although CrleGV-SA has been very successful in IPM, there is growing concern about the possible risks of resistance development in *T. leucotreta*. Restrictive host range and slow speed of kill are other challenges that limit CrleGV-SA's competitive edge in the biocontrol industry. Although this is the only baculovirus registered for *T. leucotreta* control in South Africa, a newly described insect virus, Cryptophlebia peltastica nucleopolyhedrovirus (CrpeNPV) also infects and kills *T. leucotreta*. This provided the opportunity to test for improved efficacy of CrpeNPV to *T. leucotreta* for improved control in the field. Improved efficacy, coupled with a wider host range of CrpeNPV, provides the opportunity for future biopesticide formulations in citrus. CrpeNPV can also be a potential alternative to CrleGV-SA, should any sign of resistance be observed in the future. Serial passaging of a virus through a heterologous insect host has been shown to be an effective method for improving virulence or speed of kill against that host.

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

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

The betabaculovirus Cryptophlebia leucotreta granulovirus (CrleGV) forms part of the biological control options used against False codling moth (FCM), *Thaumatotibia leucotreta,* and has been applied successfully in the field for more than 15 years. The alphabaculovirus Cryptophlebia peltastica nucleopolyhedrovirus (CrpeNPV) was recently identified in the litchi moth, *Cryptophlebia peltastica*, and is being developed into a biological pesticide. This virus was found to have a broad host range, which includes FCM, making it a promising candidate as a biopesticide for use against this pest. Although the use of these viruses provides a strong foundation for the sustained control of FCM, continued development is necessary to achieve long-term management. Recently, a novel insect cell line derived from FCM eggs was established and has been maintained for more than 50 passages. Inoculation with CrpeNPV-budded virus resulted in the formation of occlusion bodies 144 h post infection. The susceptibility of the cell line to other beta-baculoviruses such as Cryptophlebia leucotreta granulovirus, Cydia pomonella granulovirus, Phthorimaea operculella granulovirus and Plutella xylostella granulovirus, is currently being evaluated. The ability to manipulate these viruses *in vitro* may make it possible to isolate distinct genotypes or to genetically modify viral DNA, resulting in baculovirus-based biopesticides with improved activity.

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

RESEARCH TEAM
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STUDENT
Samantha Prinsloo (MSc)

False codling moth, *Thaumatotibia leucotreta* (FCM), 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 which may be used in FCM management plans are *Steinernema yirgalemense*, *S. jeffreyense* and *Heterorhabditis noenieputensis*. In addition, previous research has shown that a synergistic relationship might occur between EPN and EPF when they are combined for the management of late instar lepidopteran larvae. The synergistic relationship between EPF and EPN on FCM is not yet known. Thus, this study is investigating the control potential of *M. anisopliae* FCM Ar 23 B3 and several EPN species alone and in combination with one another on late fifth instar FCM, using standardised laboratory bioassays. The individual bioassay results were as follows: *M. anisopliae* FCM Ar 23 B3 (84% mortality), *Steinernema yirgalemense* (96% mortality), *S. jeffreyense* (75% mortality) and *Heterorhabditis noenieputensis* (91% mortality). The trials also assessed potential synergism between the EPF and three EPN species. However, this research is still ongoing, and synergism bioassays are currently being conducted.

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

RESEARCH TEAM
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STUDENT
Wayne Kirkman (PhD)

False codling moth (FCM), *Thaumatotibia leucotreta*, is one of the most important pests on citrus. The European Union, by far the largest market for South African citrus, has regulated FCM as a phytosanitary pest. Several effective pre-harvest control measures exist for FCM, but there is no 'silver bullet' to ensure that no infested fruit reaches the packhouse. Such control measures are inadequate for a pest for which there is zero tolerance and there is an urgent necessity to investigate methods for post-harvest detection of FCM.

Previous studies showed that a Solid Phase Micro-extraction (SPME) probe effectively trapped and concentrated headspace volatile compounds surrounding intact citrus fruit. Volatile compound detection was then achieved by using a GCMS system. GCMS analysis was conducted on five major volatile compounds of citrus: 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 infested Witkrans navel oranges, D-limonene levels decreased with time after infestation, while levels of naphthalene 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 Navel oranges and Midknight and Delta Valencia oranges, these same trends were not observed. This difference was mainly caused by variability in D-limonene levels in all cultivars: the result of extremely unusual climatic conditions in the Eastern Cape which caused excessive splitting and fruit drop, and uncharacteristic berg winds which scorched Valencia orchards.

In the trials conducted in 2018 on infested Washington and Witkrans Navel oranges, as well as Midknight and Delta Valencia oranges, D-limonene levels decreased significantly and naphthalene levels increased with time after infestation; the ratio between the two compounds (D-limonene/naphthalene) was significantly lower than with healthy fruit. In Clementine Mandarins, there was a significant increase in beta-Ocimene levels with time after infestation, where levels were undetectable in the control fruit. Infested Midknight Valencia oranges also showed significantly lower levels of caryophyllene with time after infestation. The ability of an electronic nose to detect FCM infested fruit was investigated. In the trial conducted on Washington Navel oranges, 80%, 90% and 90% were correctly detected for two, six and ten days after infestation. Twenty percent of the control fruit were incorrectly classed as infested. A Selected Ion Flow Tube Mass Spectrometry (Sift-MS) unit at the University of Leuven in Belgium could differentiate between healthy fruit and fruit injured 24 hours earlier after a few seconds of real-time volatile detection and analysis.

Gas chromatography-mass spectrometry (GCMS) analysis of citrus fruit volatiles. **Photo credit:** Wayne Kirkman

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)

This new research project within the CBC was initiated at the beginning of 2019 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. The honeybush tea industry has grown substantially in recent years as demand by foreign markets has increased and *Cyclopia* spp. are now being grown organically in moderate to large monoculture fields to increase product output. The South African Honeybush Tea Association (SAHTA) highlighted several insect species as potentially damaging and for which control options may be required. The key species of concern is *Leto venus*, more commonly referred to as the silver-spotted ghost moth or Keurboom's moth, although this project may be extended to include other insect species. Dr Candice Coombes is the manager of the project.

The Keurboom moth is endemic in the Southern Cape region of South Africa, particularly where its natural hosts, the Keurboom tree (*Virgilia* spp.), are apparent. Although often not seen, the adults are visibly striking on account of their size and patterning. Before the female dies, eggs are deposited around the base of host plants, where upon hatching, early instars exhibit a litter-phase prior to boring into the stems of host plants. Here they remain feeding on plant tissue as they develop through several more instars until pupation occurs, with adult emergence apparent in February to March. Larval tunnelling, as evident by 'sawdust' at the base of infested plants, has been shown to damage the structural integrity and vascular tissues of plants, resulting in stem dieback, which may lead to plant death if not controlled. Given the enigmatic nature of this moth, very limited information on its biology and life history, especially on *Cyclopia*, exists; such information is fundamental for the development of successful pest management programmes. Current control measures used by honeybush farmers involve physical control methods that may become too labour intensive as field size increases. Finding alternate, organic, and sustainable management options for the Keurboom moth is thus important.

'Sawdust' - tell-tale damage in a Honeybush plant from the Keurboom's moth larvae. **Photo credit:** Candice Coombes

In 2019, research took two approaches: firstly, monitoring insect populations in fields of different ages on a large farm using active (scouting) and passive sampling (yellow sticky traps). This monitoring was important given the lack of knowledge surrounding insects associated with cultivated honeybush, with respect to the insects present, their abundance, and their seasonality. Three monitoring events have occurred to date. Insects with pest potential and in highest abundance during each sampling event include thrips and leafhoppers. The latter has subsequently been identified as a species in the genus *Molopopterus*. Aphids were also commonly recorded, but positively, many of these in the second and third sampling event exhibited signs of parasitism. Other natural enemies that were recorded, either on the plants themselves or yellow sticky traps, include predatory ladybird beetles and larvae, green lacewings, spiders, and various parasitic wasps. A final sampling event will occur in early 2020, one-year post initiation of monitoring to determine whether any further action is necessary.

Secondly, surveys for the Keurboom moth were initiated on several farms visited in early 2019. Based on these surveys, the way forward with this research was planned. As no laboratory culture of this insect exists, owing to its typical non-pest status, and the challenges faced in mass rearing of Hepialid moths in general, much of this research is field-based. Research to be carried out in early 2020 includes establishing the host preference of the moth, its developmental life cycle and the extent

of damage with which it is associated in cultivated honeybush fields. This will involve extensive sampling of cultivated honeybush fields, natural honeybush stands and surrounding refugia, especially where Keurboom trees are present. Complementing this research, several soil samples have been collected from the previously surveyed farms and used to isolate entomopathogenic fungi. These fungi may offer a potential natural management tool for this pest species, and other pest species associated with honeybush. Attempts to rear this insect using artificial diet will be investigated.

Traditionally wild harvested, Honeybush tea is now grown commercially. **Photo credit:** Tapiwa Mushore

The adult Keurboom's moth is striking but seldom seen. **Photo credit:** Colin Ralston

POLYPHAGOUS SHOT HOLE BORER

The polyphagous shot hole borer (PSHB) beetle is an ambrosia beetle native to Southeast Asia. Over the past decade it has become a highly invasive pest of trees in the United States and Israel, and in 2017 it was discovered in a botanical garden in Pietermaritzburg, Kwa-Zulu Natal. It is thought that the beetle was introduced through lumber shipments or dunnage from Asia. Since its discovery, it has been recorded in eight of the nine provinces in South Africa.

The beetle bores into trees and releases a symbiotic fungus, *Fusarium euwallaceaea*, that 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. Preliminary results show that the beetle has not had any major impacts on agricultural crops. It has been recorded on 35 indigenous tree species, mostly in urban areas. In indigenous forests, the spread of the beetle appears to be slow, with a few individual trees being attacked. The biggest threat it poses currently is to urban and ornamental trees such as English oaks (*Quercus robur*), box elders (*Acer negundo*), and London planes (*Plantus × acerfolia*). Monitoring efforts of the spread and impacts of the beetle are ongoing, 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 co-supervised by professors from both institutions.

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

STUDENT
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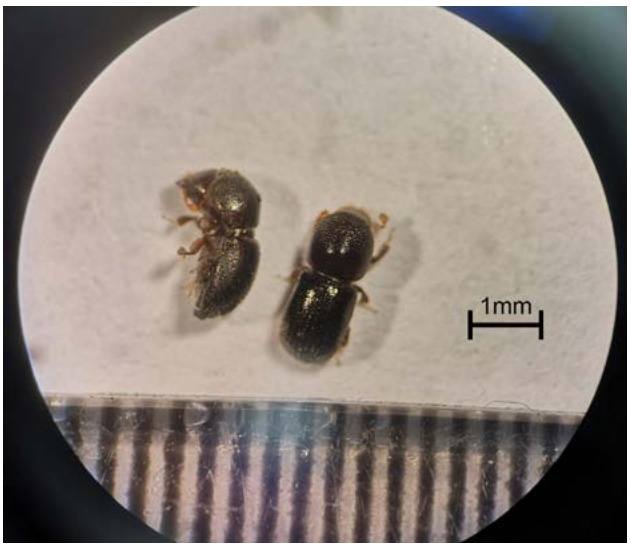
COLLABORATORS
FABI



The polyphagous shot hole borer beetle – adults are on average 2.6mm in length. **Photo credit:** Garyn Townsend

The entry hole from the polyphagous shot hole borer beetle on an Oteniqua yellowwood in the Western Cape. **Photo credit:** Garyn Townsend

MASS-REARING

In a move away from classical biological control, the CBC mass-rears insects for augmentative releases against most of the waterweeds and cacti. It is vital to get high numbers of healthy insects in the field to increase the speed of control, and improve the efficacy of the control agent in situations where the biological control agents might be slowed by cooler winters. The CBC cultivates invasive alien plants in controlled greenhouse environments. These plants are then used to culture the biological control agent specifically approved for control of that particular plant, to be eventually released at recognised and monitored invasive plant populations across South Africa. These biological control agents are available for free to researchers, implementation officers, reserve and water quality managers, farmers and concerned members of the public who want to get involved in preserving biodiversity and controlling invasive species in their local natural environments.

All biological control agents mass reared by the CBC are approved for safe release in South Africa by the regulatory body, the Department of Environment, Forestry and Fisheries and conform to current legislation. The identification and control of invasive species is a continuous and ever evolving problem. Every year more species are identified as problematic, and more effort is invested into finding new biological control agents. Thus, the CBC also house two quarantine facilities, to expedite research into host specificity and the fecundity of potential biological control agents.

Two research facilities at the CBC serve to mass rear biological control agents. The focus of the Waainek facility in Makhanda (Grahamstown) is on mass-rearing waterweed biological control agents – particularly for water hyacinth, water lettuce, parrots feather and salvinia. The facility in Uitenhage, outside Port Elizabeth, rears biological control agents which can be released on various cacti species.

Waainek mass-rearing facility

In order to maximise the potential of weed biological control, high numbers of healthy insects are required, and these agents can be mass reared. This waterweed mass-rearing facility was established in 2008 at Waainek in Makhanda (Grahamstown) during the first phase of the Working for Water-funded aquatic weed project. Since then, the mass-rearing programme has been upgraded and expanded. In 2009, the first people were employed including three people living with disabilities. There are currently six people with disabilities employed at the facility. The mass-rearing of biological control agents at this facility has enabled millions of agents to be released in invaded areas all over the country to reduce alien invasive plant populations. Maretha Boshoff, the Waainek mass-rearing facility manager, manages a team of eight staff. A brand-new quarantine facility has been built at Waainek to maximise space for research into new biological control agents.

The new quarantine facility at the Waainek mass-rearing facility. **Photo credit:** Kim Weaver

One of the tunnels at the Waainek mass-rearing facility with pools of water hyacinth. **Photo credit:** Esther Mostert

In 2019, the Waainek mass-rearing facility made a total of 55 releases of various biological control agents at sites around South Africa. More than 440 000 insects or immature larvae were released in seven provinces – no releases were made in the North West or Northern Cape. A large portion of releases (47%) were made in Gauteng.

Aquatic weed biological control agents released by the CBC Waainek mass-rearing facility in 2019:

Target Weed	Biological Control Agent	Number of releases made	Total number of insects or immature larvae released
Pontederia crassipes	*Cornops aquaticum*	3	4 876
Pontederia crassipes	*Eccritotarsus catarinensis*	12	83 000
Pontederia crassipes	*Megamelus scutellaris*	24	228 000
Salvinia molesta	*Cyrtobagous salviniae*	1	300
Myriophyllum aquaticum	*Lysathia sp.*	9	15 500
Egeria densa	*Hydrellia ergeriae*	3	110 000
Pistia stratiotes	*Neohydronomus affinis*	3	1 800
TOTAL		55	443 476

Uitenhage mass-rearing facility

The CBC took over the facility outside Uitenhage in April 2015, after the success of the Waainek mass-rearing facility in Makhanda (Grahamstown). The Uitenhage facility focuses on rearing agents for alien invasive cacti, which are serious environmental and agricultural weeds throughout South Africa. Biological control of cacti has been very successful in both South Africa and Australia.

At present, a team of eight people are mass rearing six species of agents on six cactus weeds at the Uitenhage facility. Farmers, conservationists and landowners make requests and the agents are provided free of charge. The team is also involved in monitoring how effective the releases are in controlling the weeds.

A total of 62 releases of cactus biological control agents were made at sites around South Africa, in 2019, from the Uitenhage Mass Rearing Facility. Ten of the releases were at sites where the CBC had conducted releases in the past and the remainder were releases at new sites.

Trays of jointed cactus cladodes are used to mass rear cochineal (*Dactylopius austrinus*) in one of the tunnels at the Uitenhage mass-rearing facility. **Photo credit:** Kim Weaver

The total number of infected cladodes or galls released in 2019 was nearly 30 000. Most of the releases were made in the Eastern Cape (62%), but releases were conducted througout South Africa.

Cactus biological control agents released by the CBC Uitenhage mass-rearing facility in 2019:

Target Weed	Biological Control Agent	Number of releases made	Total number of cladodes or galls released
Opuntia aurantiaca	*Dactylopius austrinus*	12	8 690
Opuntia monacantha	*Dactylopius ceylonicus*	6	926
Opuntia ficus-indica	*Dactylopius opuntiae "ficus-indica"*	5	74
Opuntia engelmannii	*Dactylopius opuntiae "stricta"*	2	3 142
Opuntia humifusa	*Dactylopius opuntiae "stricta"*	5	241
Opuntia stricta	*Dactylopius opuntiae "stricta"*	12	1 622
Cylindropuntia imbricata	*Dactylopius tomentosus*	13	14 185
Cereus jamacaru	*Hypogeococcus festerianus*	3	740
Harrisia martinii	*Hypogeococcus festerianus*	3	152
Hylocereus undata	*Hypogeococcus festerianus*	1	20
TOTAL		62	29 792

COMMUNITY ENGAGEMENT

The CBC prides itself on engaging with their community in Makhanda (Grahamstown) as well as the broader South African community about the topics of invasive alien species and biological control. The aim of the activities within the Community Engagement Programme 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. Kim Weaver leads the Community Engagement Programme with the rest of the CBC team and liaises with a wide range of stakeholders and partners.

SciFest Africa and Bathurst Agricultural Show

Locally, the CBC is involved in SciFest Africa and the Bathurst Agricultural Show. The exhibitions the CBC displays at these events have different audiences, but are equally important.

The CBC students and staff have an opportunity to share their knowledge on invasive species and biological control with the many people that come to these events.

CBC PhD Student, Zezethu Mnqeta engaging with learners at the CBC's SciFest Africa exhibit. **Photo credit:** Kim Weaver

Business School miniSASS outing

The CBC takes the Master of Business Administration (MBA) students from the Rhodes Business School on an excursion each year to measure a local river's health using the stream assessment scoring system. Samuel Motitsoe facilitates the outing where aquatic invertebrates are used to assess the river health. The environment plays an important role in the business model that the Business School uses as a foundation of their course.

MBA students getting stuck in with identifying insects from their river sample. **Photo credit:** Kim Weaver

Agricultural partnership

The CBC has formed a partnership with Agri SA and its affiliated members. As a result of this partnership, the CBC is able to communicate with the agriculture sector through their media and at their meetings. We have been able to grow interest in better management of invasive species and raise awareness of the value, safety, and effect of biological control. As a corporate member, the CBC's engagement with Agri SA enables them to develop a strong and sustainable partnership in efforts use biological control more effectively. Stakeholders in the agricultural sector have expressed interest in work on the following invasive plants: Prosopis species, ink

berries (*Cestrum parqui* and *Cestrum laevigatum*), satansbos (*Solanum elaeagnifolium*), spear thistle *(Cirsium vulgare*), as well as all the invasive cactus species, particularly jointed cactus (*Opuntia aurantiaca*).

The CBC and Agri Noord Kaap coordinate a representative working group on prosopis which aims to better manage the invasion and impacts of this species through using existing biological control agents more effectively, developing new agents, and integrating biological control with other control methods. The working group shares information on biological control and coordinates the development of a Provincial Management Programme for Management of Prosopis, and a communication plan to promote the work of biological control.

Through an introduction to the Red Meat Producers' Organisation, the CBC was invited to submit research proposals to Red Meat Research and Development. Funding was received for a literature review on *Opuntia elata* and the likelihood of finding a suitable biological control for this species which is toxic to cattle is of concern to red meat producers. In partnership with the Agricultural Research Council, Plant Health and Protection, a project on ink berries has been funded by the Red Meat Research and Development fund.

Cactus outreach work

The cactaceae programme has strengthened their relationships with protected areas this year. One especially significant and very successful interaction is that between the Uitenhage research facility team and Camdeboo National Park's management team in managing various cactus species in the buffer zone of the park.

The South African Cactus Working Group has two annual meetings co-hosted by the CBC and SANBI. This is a long-standing working group with a diverse membership. The CBC has taken over the coordination of the International Cactus Working Group (ICWG) and will be hosting the first meeting in early 2020 in Windhoek, Namibia. The CBC hopes to start other working groups going forward to ensure that they are collaborating broadly with both research and implementation.

Prosopis engagement

The CBC have recently taken over the prosopis management programme and with this have engaged with concerned stakeholders, including government officials, chemical companies, farmers and landowners, around coordinating a prosopis management plan. In February 2019, the CBC hosted a meeting in Kimberly where this issue was discussed and a way forward is in progress. A committee has been selected to lead this planned development. However, the current drought has meant farmers have had other concerns which have taken priority over the prosopis management plan.

Weed Buster Week

Weed Buster Week is an initiative that was started by the Department of Environment, Forestry and Fisheries (DEFF) to create awareness around weeds. For Weed Buster Week this year, CBC had an invasive awareness event in our local botanical gardens in collaboration with the Makhanda/Grahamstown Park Run. The attendance was great and the community members enjoyed removing weeds and hearing about biological control from our staff and students. We look forward to making this an annual event.

Weed buster week in the Botanical Gardens, Grahamstown. **Photo credit:** Kim Weaver

Weed Biological Control short course

The CBC hosts an annual Weed Biological Control Short course which is accredited through Rhodes University. In 2019 the course was held from the 25–30 August and saw 23 delegates qualify with a certificate. Implementation officers, government officials, protected area managers and students made up the course delegates this year.

Weed biological short course participants in the field. **Photo credit:** Julie Coetzee

Undergraduate vacation apprenticeship and experience for UKZN students

A total of 25 students applied for the apprenticeship in May 2019 after the opportunity was advertised to second-year students in the School of Life Sciences, at the University of KwaZulu-Natal (UKZN), Pietermartizburg campus. 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 June–July 2019 vacation apprenticeship. Students 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 were 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.

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

School engagement

The CBC is involved with a number of school engagement activities. The longest standing one is the Science Internship Programme. In this programme, school learners who apply and are selected for the programme, spend three weeks of their holiday periods with us in the laboratory. During this time, they are exposed to what it entails to be an applied entomologist in the field of biological control. Fifteen learners from four local schools are part of this programme annually.

In the last two years the CBC has been actively engaged with schools in the Hartbeespoort area as part of the school mass-rearing projects. Two schools rear water hyacinth biological control agents to release on the dam as often as they can. A school in East London approached the CBC in April 2019 with an interest in becoming involved with rearing insects for the control of invasive species on the Nahoon River system. The CBC has supported them in setting us a small-scale rearing station and the first number of insects reared at the school, water hyacinth leaf hoppers, *Megamelus scutellaris*, were released on the river on 4 December 2019. Midmar Dam in KwaZulu-Natal has also had some invasive species management attention this year. A school nearby the dam reached out to the CBC with an interest to becoming involved with rearing insects to control the submerged invasive Brazilian water weed in the dam.

The schools' mass-rearing programme seems to be growing organically with schools showing the initial interest, which is a good sign, as schools need to take ownership of the programme for it to be effective. The CBC's attention with this programme is not so much on the numbers of agents reared and released on the water systems, as on the learning and eagerness generated through the activities involved for the learners. The CBC hopes to grow young environmental stewards and possibly future entomologists!

Kim Weaver assisting Merrifield College learners in East London placing their first set of water hyacinth plant hopper insects in their mass rearing tubs in October 2019. **Photo credit:** Alison Randall

PARTNERS IN THE CBC'S COMMUNITY ENGAGEMENT ACTIVITIES

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

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

Funders

The CBC would like to acknowledge the various funders for their ongoing support without which the research could not take place.

National

Citrus Research International (CRI)

Department of Environment, Forestry and Fisheries (DEFF) - Natural Resource Management (NRM): Working for Water (WfW) programme

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

Drakenstein Trust

HORTGRO (Alternative Crop Fund)

National Research Foundation (NRF).

Red Meat Research and Development South Africa (RMRD SA)

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

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 - Primary Industries and Regions South Australia (PIRSA)

LandCare Research, New Zealand

New South Wales Department of Primary Industry, Australia

Queensland Department of Agriculture, Australia

Shire of Ravensthorpe, Western Australia

Research Outputs

PEER REVIEWED ARTICLES

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3. Clint Mac Aleer. 2019. An Integrated Management System to reduce False Codling Moth, *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) infested citrus fruit from being packed for export. MSc. Supervisors: Martin Hill and Sean Moore.

4. Slindile Nkqayi. 2019. Climatic suitability of *Dichrorampha odorata* Brown and Zachariades (Lepidoptera: Tortricidae), a shoot-boring moth for the biological control of *Chromolaena odorata* (L.) R.M. King and H. Robinson (Asteraceae) in South Africa. MSc. Supervisors: Julie Coetzee, Martin Hill and Costas Zachariades.

5. Jufter Musedeli. 2019. Interaction between the root-feeding beetle, *Longitarsus bethae* (Coleoptera: Chrysomelidae) and the root-knot nematode, *Meloidogyne javanica* (Nematoda: Heteroderidae): Implications for the biological control of *Lantana camara* L. (Verbenaceae) in South Africa. MSc. Supervisors: Martin Hill, David Simelane and Mariette Marais.

6. Benjamin Miller. 2019. Post-release evaluation of *Megamelus scutellaris* on water hyacinth in South Africa. MSc. Supervisors: Martin Hill and Julie Coetzee.

7. Prinavin Naidu. 2019. Invasion ecology of *Nymphaea mexicana*: assessing its suitability for biological control. MSc. Supervisors: Julie Coetzee and Iain Paterson.

8. Sandiso Mnguni. 2019. Reproductive isolation mechanisms of two cryptic species of *Eccritotarsus* (Hemiptera: Miridae), biological control agents of water hyacinth, *Eichhornia crassipes* (Martius) Solms-Laubach (Pontederiaceae). MSc. Supervisors: Julie Coetzee and Iain Paterson.

9. Thifhelimbilu Mulateli. 2019. Augmentative releases of *Dactylopius austrinus* De Lotto (Dactylopiidae; Hemiptera) for biological control of *Opuntia aurantiaca* Lindley (Cactaceae), in South Africa. MSc. Supervisors: Iain Paterson and Martin Hill.

CONFERENCE PROCEEDINGS

International conference proceedings

1. Jukes, M.D., Knox, C.M., Hill, M.P., Moore, S.D. 2019. The susceptibility of a novel cell line derived from *Thaumatotibia leucotreta* eggs to various alpha and beta baculoviruses. Oral presentation at the 52nd Annual Meeting of the Society for Invertebrate Pathology & 17th Meeting of the IOBC-WPRS Working Group "Microbial and Nematode Control of Invertebrate Pests" held at Valencia, Spain. 28 July-1 August 2019.
2. Albertyn, S., Mwanza, P., Marsberg, T., Hill, M.P., Dealtry, G.B., Lee, M.E., Moore, S.D. 2019. Influence of orchard age on the efficacy of a granulovirus: architecture trumps biochemistry. Oral presentation at the 52nd Annual Meeting of the Society for Invertebrate Pathology & 17th Meeting of the IOBC-WPRS Working Group "Microbial and Nematode Control of Invertebrate Pests" held at Valencia, Spain. 28 July-1 August 2019.
3. Knox, C.M., Jukes, M.D., Moore, S.D., Hill, M.P. 2019. Characterisation of novel baculovirus isolates for potential development and application as biopesticides against agricultural pests in South Africa. Oral presentation at the 52nd Annual Meeting of the Society for Invertebrate Pathology & 17th Meeting of the IOBC-WPRS Working Group "Microbial and Nematode Control of Invertebrate Pests" held at Valencia, Spain. 28 July-1 August 2019.
4. Baso, N.C., Coetzee, J., Hill, M., Ripley, B. The future of biological control in South Africa: Effects of elevated CO_2. *15th Conference on Ecology and Management of Plant Invasions* (EMAPI). 9-13 September 2019. Czech University of Life Sciences, Prague, Czech Republic.
5. Paterson, I.D., Witt, A. 2019. The implications of biological control of cochineal insects for the control of invasive alien Cactaceae. *XIX International Plant Protection congress*. 10-14 November 2019, Hyderabad, Telangana, India.
6. Paterson, I.D., Hill, M.P., Wannenburgh, A. 2019. The Working for Water programme: invasive plant control and poverty alleviation. *III Innovations in Invasive Species Management Conference*. Coeur d'Alene Resort, Idaho, U.S.A. 10th – 13th December 2019. (Keynote address).

National conference proceedings

1. Adams, D.L., Martin, G.D., Clark, R. and Steenhuisen, S. 2019. Reproductive ecology of the invasive firethorn species, Pyracantha angustifolia (Rosaceae) in the eastern Free State Province. 45th Annual SAAB, AMA and SASSB Joint Congress. 8 – 11 January 2019. Johannesburg, South Africa.
2. Adams, L.D., Martin, G., Clark, V.R. and Steenhuisen, S. 2019. Following the fate of seeds to investigate the spread of invasive *Pyracantha angustifolia* (Firethorn) in the eastern Free State, South Africa. *46th Annual Research Symposium on the Management of Biological Invasions*. 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape.
3. Baso, N.C, Coetzee, J., Hill, M. and Ripley, B. The impact of CO_2 on biological control of invasive macrophytes. *46th Annual Research Symposium on the Management of Biological Invasions*. 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape.
4. Canavan, K., Canavan, S., Lambertini, C., Thum, R. and Paterson, I.D. 2019. Cryptic invasions and the impact on weed biological control. 46th Annual Research Symposium on the Management of Biological Invasions. 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape.
5. Fraser, G., Martin, G. and Westwood, T. 2019. The conflict of interest associated with the management of *Rosa rubiginosa* in South Africa. 46th Annual Research Symposium on the Management of Biological Invasions. 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape.
6. Ivey, P. and Hill, M. 2019. From words into actions, the science of implementation. *46th Annual Research Symposium on the Management of Biological Invasions*. 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape.
7. Maluleke, M. Evaluating the cost-effectiveness of chemical and biological control methods on selected aquatic weeds in South Africa. *46th Annual Research Symposium on the Management of Biological Invasions*. 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape.
8. Martin, G. Investigations into high elevation weeds in South Africa. 46th Annual Research Symposium on the Management of Biological Invasions. 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape.

9. Maseko, Z., Coetzee, J.A., Hill, M. and Paterson, I.D. 2019. Seasonal population dynamics of *Eccritotarsus eichhorniae* on *Eichhornia crassipes* on the Kubusi River, Eastern Cape, South Africa's coldest site. 46th Annual Research Symposium on the Management of Biological Invasions. 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape.

10. Mason, B., Fraser, G. and Martin, G.D. Evaluating the socio-economic impacts of the invasive Rubus genus in South Africa. 46th Annual Research Symposium on the Management of Biological Invasions. 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape.

11. Mayonde, S., Paterson, I.D. and Byrne, M. 2019. Molecular genetics investigation of *Opuntia engelmannii* lineages in South Africa: implications for biocontrol. 46th Annual Research Symposium on the Management of Biological Invasions. 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape.

12. Mnqeta, Z., Paterson, I.D. 2019. Mass-rearing and monitoring of biological control agents on invasive alien cactus plants. 46th Annual Research Symposium on the Management of Biological Invasions. 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape

13. Mofokeng, K. and Paterson, I.D. 2019. The effect of hybridization of the 'ficus' and 'engelmannii' lineages of *Dactylopius opuntiae* on the host range of the offspring. 46th Annual Research Symposium on the Management of Biological Invasions. 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape

14. Mostert, E., Weaver, K., Coetzee, J and Hill, M. Strengthening the bridge – research and the public sphere. *46th Annual Research Symposium on the Management of Biological Invasions.* 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape.

15. Muskett, P.C., Paterson, I.D. 2019. Biological control of *Opuntia elata* in South Africa. 46th Annual Research Symposium on the Management of Biological Invasions. 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape.

16. Ndlovu, M. and Martin, G.D. Impact study of *Listronotus apendiculatus* on *Sagittaria platyphylla's* reproductive outputs. *46th Annual Research Symposium on the Management of Biological Invasions.* 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape.

17. Paterson, I.D. 2019. Biological control of invasive alien Cactaceae in South Africa. 46th Annual Research Symposium on the Management of Biological Invasions. 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape.

18. Sandenbergh, E., Coetzee, J. 2019. The future of yellow-flag iris invasions in South Africa. *46th Annual Research Symposium on the Management of Biological Invasions.* 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape.

19. Sutton, G.F., Canavan, K., Paterson, I.D. 2019. Are grasses suitable targets for biological control? A case study of two African grasses (*Sporobolus* spp.) invading Australia. 46th Annual Research Symposium on the Management of Biological Invasions. 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape.

20. Van Steenderen, C.J.M., Paterson, I.D., Edwards, S. 2019. The genetic barcoding of species and lineages of *Dactylopius* costa (Hemiptera: Dactylopiidae). 46th Annual Research Symposium on the Management of Biological Invasions. 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape.

21. Wolmarans, A., Martin, G., Paterson, I.D. Considering biological control options for *Robinia pseudoacacia*. 46th Annual Research Symposium on the Management of Biological Invasions. 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape.

22. Zozo, E., Paterson, I.D. 2019. Biological control of *Cylindropuntia pallida,* pink-flowering cholla. 46th Annual Research Symposium on the Management of Biological Invasions. 15-17 May 2019. Waterval Country Lodge, Tulbagh, Western Cape.

23. Marsberg, T., Peyper, M., Kirkman, W. and Moore, S. 2019. Integrated pest management under nets. *CRI IPM and disease management workshop,* Addo, Eastern Cape.

24. Motitsoe, S.N, Hill, M.P., Coetzee, J. and Hill, J.M. 2019. Trophic dynamics in alien invasive macrophytes dominated aquatic ecosystem with special emphasize to ecosystem structure and functioning. *Southern African Society of Aquatic Scientists (SASAqS*). Bela Bela, 30 June-4 July.

25. Tshithukhe, G., Motitsoe, S.N. and Hill, M.P. 2019. Invasive Alien Aquatic Plants for Phytoremediation: Case study of a South African urban river system. *South African Society of Aquatic Scientists (SASAqS)*. Bela Bela. 30 June-4 July.
26. Paterson, I.D. 2019. Biological control for the protection of water resources and rangelands (Plenary). *54th* Annual Grassland Society of South Africa Congress. Desert Palace Hotel and Casino. Upington. Northern Cape. 30 June-4 July 2019
27. Acheampong, M., Hill, M.P., Moore, S. and Coombes, C. 2019. Suitability of entomopathogenic fungal isolates for microbial control of citrus pests: biological traits and importance of formulation. *21st Congress of the Entomological Society of Southern Africa*. Umhlanga, Durban, 8-11 July.
28. Chari, L., Coetzee, J. Hill. M.P. 2019. A potential biological control agent for the control of *Iris pseudacorus* L. (Iridaceae) in South Africa. *21st Congress of the Entomological Society of Southern Africa*. Umhlanga, Durban, 8-11 July.
29. Kraus, E. 2019. Induced resistance in water hyacinth, *Eichhornia crassipes*, to the biological control agent *Megamelus scutellaris*. *21st Congress of the Entomological Society of Southern Africa*. Umhlanga, Durban, 8-11 July.
30. Marsberg, T., Peyper, M., Kirkman. W. and Moore, S. 2019. Netted orchards, do they have an effect on the management of key citrus pests? *21st Congress of the Entomological Society of Southern Africa*. Umhlanga, Durban, 8-11 July.
31. Mauda, E.V., Chari, L.D., Martin, G.D. and Raghu, S. Herbivorous insects associated with *Lycium ferocissimum* (Solanaceae) with reference to potential biological control of the plant in Australia. *21st Congress of the Entomological Society of Southern Africa*. Umhlanga, Durban, 8-11 July.
32. Mostert, E.A., Morgan, L.W., Martin, G.D., Coetzee, J.A. and Hill, M.P. 2019. Creating a more accessible database platform: introducing the new CBC Data Portal. *21st Congress of the Entomological Society of Southern Africa*. Umhlanga, Durban, 8-11 July.
33. Mphephu, T., Coetzee, J. and Hill, M.P. 2019. Parasitic alga, *Helicosporidium* sp. (Chlorophyta; Trebouxiophyceae) threatens effective biological control of the aquatic invasive, *Salvinia molesta* D.S. Mitchell (Salviniaceae) in South Africa. *21st Congress of the Entomological Society of Southern Africa*. Umhlanga, Durban, 8-11 July.
34. Mushore, T., Coombes, C. and Hill., M.P. 2019. Life history ecology and control of the Keurboom moth, *Leto venus* in cultivated Honeybush. *21st Congress of the Entomological Society of Southern Africa*. Umhlanga, Durban, 8-11 July.
35. Owen, C. A. and Terblanche, J. S. 2019. Insights into the metabolic costs of digestion in biocontrol insects. *21st Entomological Society of Southern Africa Congress*. Durban, 8-11 July 2019.
36. Prinsloo, S., Hill, M.P., Coombes, C., Malan, A. and Moore,S.2019.Synergisticeffectof entomopathogenic nematodes and entomopathogenic fungi for false codling moth management. *21st Congress of the Entomological Society of Southern Africa*. Umhlanga, Durban, 8-11 July.
37. Reid, M., Coetzee, J., Hill, M.P., Gettys, L., Diaz, R., Cuda, J. and Reid, C. 2019. Surveys for potential biological control agents for *Nymphaea mexicana* Zuccarini in the United States. *21st Congress of the Entomological Society of Southern Africa*. Umhlanga, Durban, 8-11 July.
38. Rogers, D., Terblanche, J. S. and Owen, C. A. 2019. Determining the freeze-tolerance strategy of *Neochetina eichhorniae* between two South African populations. *21st Entomological Society of Southern Africa Congress. Durban*, 8-11 July 2019.
39. Upfold, J., Joubert, F., Hill, M.P., Coombes, C. and Moore, S. 2019. A comparison of sex pheromones, sexual attraction and mating compatibility between false codling moth populations. *21st Congress of the Entomological Society of Southern Africa*. Umhlanga, Durban, 8-11 July.
40. Upfold, J., Hill, M.P., Marsberg, T., and Moore, S.D. 2019. Regional population structure of laboratory-reared FCM using fluorescent labelled amplified fragment length polymorphism (FL-AFLP). *21st Congress of the Entomological Society of Southern Africa*. Umhlanga, Durban, 8-11 July.
41. Mason, B. and Martin, G.D. 2019. An examination of the conflict of interest surrounding economically utilized Rubus genus plants with potential for biocontrol in South Africa. *Rethinking Economics for Africa REFA Festival*, University of Witwatersrand, 7-8 September, Johannesburg, South Africa.
42. Motitsoe, S.N, Hill, M.P., Coetzee, J. and Hill, J.M. 2019. Invasive alien aquatic plants species management: Ecosystem recovery and restoration. *8th World Ecological Restoration Conference*. Cape Town, South Africa, 24-28 September.

Acronyms

AFLP	Amplified Fragment Length Polymorphism
AgriSA	Agricultural Research Council of South Africa
ARC-PPRI	Agricultural Research Council – Plant Protection Research Institute
ARU	Afromontane Research Unit
BBCA	Biotechnology and Biocontrol Agency
CBA	Cost Benefit Analysis
CBC	Centre for Biological Control
CPUT	Cape Peninsula University of Technology
CRI	Citrus Research International
CrleGV	Cryptophlebia leucotreta granulovirus
CrpeNPV	Cryptophlebia peltastica nucleopolyhedrovirus
CSIRO	Commonwealth Scientific and Industrial Research Organisation
DEA	Department of Environmental Affairs
DEFF	Department of Environment, Forestry and Fisheries
DALRRD	Department of Agriculture, Land Reform and Rural Development
DPI	Department of Primary Industries
DST	Department of Science and Technology
EPF	Entomopathogenic fungi
EPN	Entomopathogenic nematodes
FABI	Forestry and Agricultural Biotechnology Institute
FCM	False Codling Moth
FuEDEI	Fundación para el Estudio de Especies Invasivas
FURB	Fundação Universidade Regional de Blumenau
ICWG	International Cactus Working Group
IPM	Integrated Pest Management
NCE	Namibian Chamber for Environment
NEMBA	National Environmental Management: Biodiversity Act
NRF	National Research Foundation
NRM	Natural Resource Management
PSHB	Polyphagous Shot Hole Borer
SAAB	South African Association of Botanists
SAHTA	South African Honeybush Tea Association
SANBI	South African National Biodiversity Institute
SANParks	South African National Parks
SAPIA	Southern African Plant Invaders Atlas
SARChI	South African Research Chairs Initiative
SASRI	South African Sugarcane Research Institute
SIT	Sterile Insect Technique
U3A	University of the Third Age
UCT	University of Cape Town
UFS	University of the Free State
UKZN	University of KwaZulu-Natal
UMP	University of Mpumalanga
USACE	United States Army Corps of Engineers
USDA	United States Department of Agriculture
WfW	Working for Water
Wits	University of Witwatersrand

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CBC Vision

The CBC seeks to:

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

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

CBC Mission

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