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CBC

Centre for Biological Control

Annual Report 2021

Front cover photograph
Phillippa Muskett, Zezethu Mnqeta and Iain Paterson in front of a Queen of the Night cactus infected with the biocontrol agent *Hypogeococcus*.
Photo: David Taylor

Back cover photograph
Byron Soetland and Karin Goliath monitoring a release site for *Dactylopius opuntiae* 'stricta' on *Opunita stricta* in Salem, Eastern Cape.
Photo: David Taylor

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Centre for Biological Control

Annual Report 2021

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DIRECTOR'S REPORT

Professor Martin Hill with his family. **Photo:** Julie Coetzee

I write this report in the context of South Africa and the world having been through two very strange years that have no doubt affected us all in some way or another. In spite of this, when I read through this annual report, I am amazed at how much progress has been made. The year 2021 was a very productive one for the Centre for Biological Control in which several new biological control agents were released, establishment of others was confirmed, and the impacts of biological control on many weeds quantified. There were many highlights in 2021: Julie Coetzee was appointed to the rank of full professor; Sean Moore was made a visiting professor at Rhodes University, and Julie was appointed President of the Entomological Society of Southern Africa (ESSA). We graduated five Honours, two Masters of Commerce, ten Masters of Science and seven PhD graduates as they walked across the virtual stage in two graduation ceremonies at Rhodes University and numerous other graduates at consortium universities. We published over 40 peer-reviewed research papers and book chapters, had nearly 50 conference and symposium papers, and convened two conferences. Our students performed spectacularly at conferences and, at the National Symposium on Biological Invasions; we were awarded the Best Oral Paper by a student (Matthew Paper) and runner-up (Sage Wansell); the best poster by a student (Siya Mnciva); and at the ESSA conference, we again were awarded best student presentation (PhD) (Rosali Smith) and runner-up (Clarke van Steenderen), and best student presentation (MSc) (Garyn Townsend).

One of the highlights of the year was the production of the 4th set of decadal reviews on weed biological control in South Africa (*African Entomology* 29 (3): 685-1142). This is a very impressive collection of work done over the last ten years and I have no doubt that it will become a highly cited text, as the previous issues were. Congratulations to Iain Paterson and his team – Grant Martin, Alana Den Breeÿen and Terry Olckers – for producing such a high-quality publication.

The CBC faced some funding challenges during 2021 due to delays within the Department of Forestry, Fisheries and the Environment (DFFE) on the outcome of our tender application. In spite of this, the CBC had a highly productive year, and it has been very pleasing to see the resilience of people in stretching the limited funds further, and seeking additional sources of funding. We have been able to attract considerable funding from overseas research institutions, most notably in the USA, Australia, and New Zealand to work on indigenous plants which have become problematic elsewhere. This research has enhanced our international reputation, and we thank our international collaborators for supporting us. Our agricultural entomology projects have been well supported, mostly through the citrus industry who see direct benefits from our research into novel ways of controlling pests. I think that the current situation has shown us that we should not be too reliant on one funder and need to diversify our funding streams. The successful project on the biological control of water hyacinth on Hartbeespoort Dam and surrounding dams is a case in point. Much of the implementation work on these dams is now being funded directly by private landowners who have seen the benefits of biological control. We are developing a similar approach to the biological and integrated control of *Prosopis* in the Northern Cape. We do need to get better at communicating the benefits of biological control, and once we do, I believe that access to private industry funding will be available.

Unfortunately, with the passing of Stefan Neser in February 2021, biological control bade farewell to one of its most significant scientists. I do not have sufficient space here to do Stefan justice, but he had a very positive effect on all who were fortunate enough to have come in contact with him. I was privileged to have been mentored by Stefan during my tenure at the ARC and can think of no better education for a biologist than going into the field with him, or listening to him at the tea table. He is sorely missed.

I have no doubt that 2022 will present challenges to the CBC, but I believe that we have all grown a little more resilient over the last two years and, with the challenges will come opportunities. We need to make sure that we align ourselves to make use of these opportunities as they present themselves.

Liam Yell in the field collecting grass. **Photo:** David Taylor

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AQUATIC WEEDS

Research into the biological and integrated control of a number of invasive aquatic weed species in South Africa in 2021 included post-release evaluations and implementation of biological control of four of the five major floating aquatic weeds (water hyacinth, parrot's feather, salvinia, water lettuce), as well as new studies on the biological control of common salvinia (*Salvinia minima*). Biological control continues to be the most successful method of control for the majority of these floating aquatic weeds, with continued success in the control of water hyacinth, following a focused mass-rearing and release campaign of the water hyacinth hopper, *Megamelus scutellaris* at a number of invaded sites around the country, including Hartbeespoort, Roodeplaat and Bospoort dams.

The CBC has also been very active in biological control research of submerged and emerging aquatic weeds, which have been termed the 'low-hanging fruit' projects. These projects encompass a far more holistic approach to the biological and integrated control of invasive aquatic plants in South Africa and its neighbouring countries. Post-release evaluations of the recently released hydrellia fly on the submerged Brazilian waterweed (*Egeria densa*) have confirmed the fly's establishment at all release sites. The release of an agent against the emergent delta arrowhead (*Sagittaria platyphylla*) was halted due to the requirement of further testing on a rare native Alismataceae which, fortunately, was found in Mkhuze Game Reserve. Testing the chrysomelid, *Aphthona nonstiata* for release against yellow flag iris (*Iris pseudacorus*) is nearing completion.

The Aquatic Weeds Programme also keeps a number of potentially serious aquatic invaders, some of which already occur in South Africa, on a 'watch list', with the aim of conducting feasibility studies into their biological control. These weeds include pickerelweed (*Pontederia cordata*), watercress (*Nasturtium officinale*), yellow heart (*Nymphoides peltata*), water poppy (*Hydrocleys nymphoides*), Amazon frogbit (*Limnobium laevigatum*) and Canadian waterweed (*Elodea canadensis*). This programme will also be on the lookout for additional species such as alligator weed (*Alternanthera philoxeroides*), and fanwort (*Cabomba caroliniana*) which are serious invaders elsewhere, but not yet recorded as established in South Africa.

Despite the restrictions of the COVID pandemic in 2021, the Aquatic Weeds Programme included a number of activities: identification, screening, release and, most importantly, evaluation of biological control agents, as

PROGRAMME HIGHLIGHTS IN 2021

- Water hyacinth cover on Hartbeespoort Dam was reduced to less than 5% through biological control alone by the end of May 2021.
- Cumulatively, over 1 million *Megamelus scutellaris* have been released from the Waainek Mass-rearing Facility.
- Biological control of water hyacinth at Roodeplaat Dam also reduced cover to less than 5% through an inundative mass rearing and release campaign in 2020–2021.
- *M. scutellaris* rearing stations were set up at key sites around South Africa through stakeholder partnerships.
- Significant numbers of *Cyrtobagous salviniae* were released on giant salvinia, *Salvinia molesta*, compared to previous years, including releases in in Zimbabwe and Zambia.
- The Florida biotype of *C. salviniae* was imported into quarantine for testing against common salvinia, *Salvinia minima*, which has invaded Hartbeespoort Dam.

well as integration of biological control into management by transferring appropriate technology to water managers. This programme also made a significant contribution to capacity building and knowledge transfer in the field of weed biological control, and results were published in top international journals, placing this research into an international context.

Water hyacinth

Biological control of water hyacinth has been a highlight for the Aquatic Weeds Programme this year. Since 2019, we have mass-reared and released vast numbers of the water hyacinth hopper, *Megamelus scutellaris*, at targeted sites around the country. We increased our rearing efforts at the CBC's Waainek Mass-rearing Facility, and set up a number of smaller rearing facilities with the help of stakeholders at invaded sites, allowing us to release hundreds of thousands of hoppers throughout the country. This has resulted in unprecedented control at sites where we never thought biological control alone would be successful. Besides our mass rearing and release efforts, the research on the biological control of water hyacinth includes a range of studies, from post-release evaluations, to the effects of sub-lethal doses of herbicides on control agent efficacy, and the effects of elevated CO2 on water hyacinth biological control.

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Water hyacinth post-release evaluations

This year, the CBC took a more targeted approach in monitoring biological control in systems invaded by water hyacinth in Gauteng and the North-West by posting Dr Rosali Smith, a postdoctoral fellow, in the area. Sites were monitored bi-monthly to monthly to document the different stages of water hyacinth biological control in an effort to improve implementation. Water quality was also monitored as some of these sites are highly eutrophic, which can reduce biocontrol success. Targeted sites included the Vaalkop, Bospoort and Hartbeespoort dams in the North-West province, as well as Roodeplaat and Witfield dams, Bronkhorstspruit River, and the Blesbokspruit wetland in Gauteng. In addition to monitoring the infestations on these systems, we embarked on a community-focused programme

Figure B1: Inside of a community partner's rearing tunnel on the banks of Inanda Dam **Photo:** Ben Miller

Table B1: Community driven mass-rearing stations for the production of *Megamelus scutellaris*

System	Province	Number of stations
Nahoon River	Eastern Cape	3
Blesbokspruit Wetland	Gauteng	1
Bronkhorstspruit River	Gauteng	1
Roodeplaat Dam	Gauteng	3
Cato Ridge farm dams	KwaZulu-Natal	1
Inanda Dam	KwaZulu-Natal	1
Crocodile River	Mpumalanga	1
Letaba River	Mpumalanga	1
Hartbeespoort Dam	North-West	3
Vaalkop Dam/Hex River	North-West	1
	TOTAL	**16**

establishing rearing stations to produce *M. scutellaris* at sites managed by various stakeholders (Fig. B1).

Regular site visits have allowed for fine-tuned implementation of water hyacinth biological control, and frequent engagement with parties implementing other control methods in accordance with management plans. With this information, we were able to adjust the biological control programme to allow optimal agent establishment and population build-up.

CBC personnel were asked to consult on several other systems around the country; a list of the main systems where rearing stations have been initiated is given in Table B1.

Hartbeespoort Dam Inundative Biological Control programme

The CBC has been involved in biological control efforts at the Hartbeespoort Dam since 2018. Many hundreds of thousands of biological control agents have been released on water hyacinth at the dam as part of an inundative approach to augmentative biological control. *Megamelus scutellaris* has been the most intensively released agent on the dam in recent years because it is easy to rear in high numbers. The CBC has engaged with stakeholders around the dam, including landowners, water users, and the numerous local governing bodies and government departments to assist with the extensive invasion of water hyacinth on the dam. This approach has enabled successful biological control events over successive years, showing drastic reductions in the total biomass of the weed, despite the negative effects of the temperate climate and high levels of eutrophication.

Earlier this year, CBC researchers analysed the changes in water hyacinth coverage relative to increases in control agent populations by determining if the inundative biological control technique applied to *M. scutellaris* releases successfully reduced water hyacinth at the Hartbeespoort Dam in the absence of chemical control. The change in water hyacinth cover from January 2019 to May 2021 was estimated using Sentinel-2 MultiSpectral Instrument satellite imagery at a 10-metre resolution. An imaging frequency of <5 days, combined with the cloud computing resources of Google Earth Engine, enabled a detailed time series of its high spatiotemporal dynamics.

The first significant decline in water hyacinth cover occurred in summer, between October 2019 and February 2020, falling from 820 ha (45%) to 55 ha (3%) (Fig. B2). After recovering over the 2020 winter period (June–August) following seed germination, the cover declined again from a peak in summer, of 747 ha (41%) in December 2020 to 128 ha (7%) in March 2021. *Megamelus scutellaris* populations were very low in late spring (October 2020) but gradually increased over the growing season due to natural population build-up and constant releases from the Waainek Mass Rearing Facility, as well as from satellite rearing stations at the dam. As a result, *M. scutellaris* reached a peak population density by late summer (March 2021). The decline in water hyacinth cover decreased correspondingly with the increase in *M. scutellaris* density. By late summer, April 2021, the water hyacinth covered less than 91 ha (5%) of the dam, and where plants could be sampled, the *M. scutellaris* populations had declined.

The negative relationship between water hyacinth cover and *M. scutellaris* density indicates that delphacid herbivory reduced water hyacinth cover at Hartbeespoort Dam. While biological control has been responsible for the

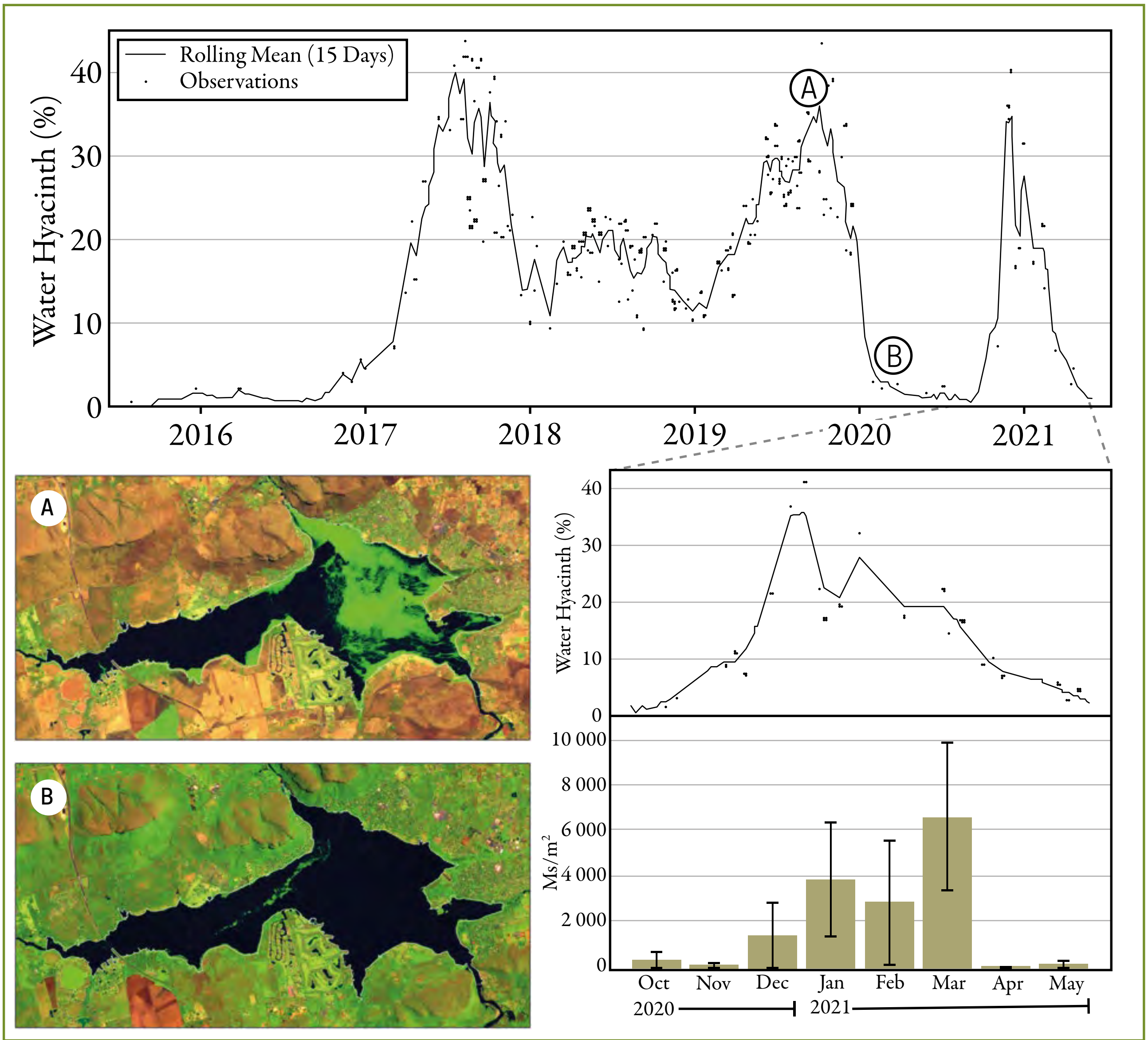

Figure B2: The first significant decline in water hyacinth occurred between October 2019 (A) and February 2020 (B), falling from 45% to 3%, respectively. Water hyacinth appears bright green against the black water in the satellite images. After recovering over the 2020 winter period, water hyacinth cover declined from a peak coverage of 41% in December 2020 down to 7% in March 2021 as a result of damage from high densities of *Megamelus scutellaris*. **Images:** Sentinel-2

decline in water hyacinth on tropical Lake Victoria in the late 1990s, large-scale declines in water hyacinth cover at temperate, hypertrophic sites due to biological control alone have not been recorded in the literature. *Megamelus scutellaris* has been released in Florida and California, USA, and at sites throughout South Africa. While it has established at many of these sites, the population increase has been relatively slow, and always negatively affected by cold winter temperatures. Our results show that adopting an inundative approach to water hyacinth biological control can indeed significantly reduce water hyacinth infestations over a season and that the strategic adaptive management of these programmes at key points of the invasion must be implemented to reduce the lag time between plant and control agent population build-up. To this end, we have established a citizen scientist programme with stakeholders around the dam to continue the mass rearing and releasing of *M. scutellaris*, especially in spring following large-scale germination of water hyacinth seeds.

Similar declines were observed at Roodeplaat Dam in early 2021 (see Fig. B5 inset), with significant numbers of biological control agents, in particular *M. scutellaris*, observed on the water hyacinth (Fig. B3). Even though other control methods were used at the dam, biological control was responsible for damaging most of the water hyacinth biomass, despite the significant eutrophication (Fig. B4). The impact of timeous inundative biological control releases contributed to the reduction in total cover of water hyacinth, below 5% by the end of March.

Figure B3: Winged *Megamelus scutellaris* on a water hyacinth leaf at the Roodeplaat Dam, January 2021 **Photo:** Ben Miller

Figure B4: Water hyacinth at the Roodeplaat Dam showing signs of damage from biological control, January 2021 **Photo:** Ben Miller

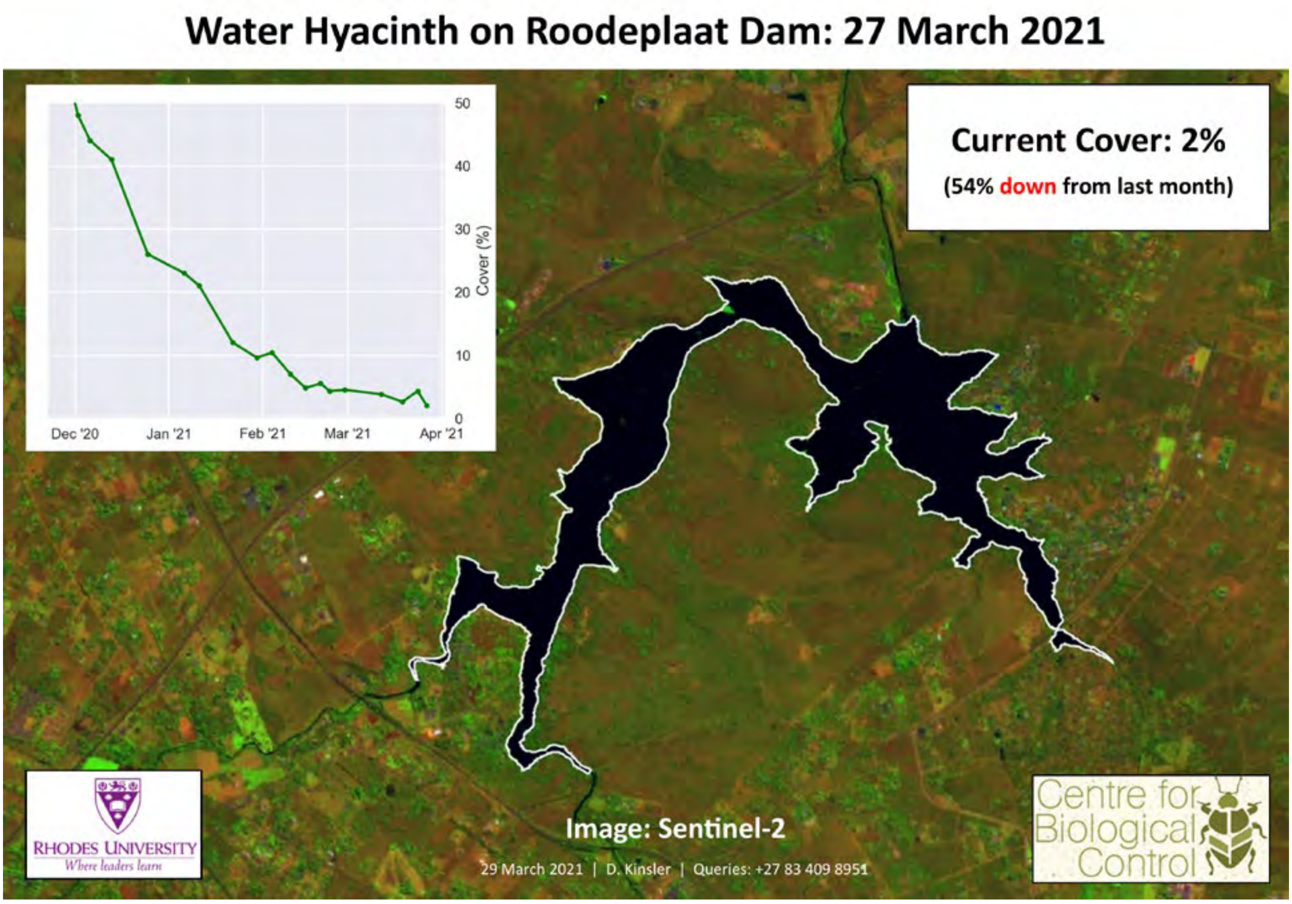

Figure B5: Satellite imagery showing the % cover in late March 2021.

Genetic identification of quarantined Acrididae specimens

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

Because establishment success could be improved through acclimation of individuals prior to release, or through the re-collection of *C. aquaticum* populations from colder regions within the native range, populations from five different sites were collected from Argentina in December 2019, and brought back to the CBC quarantine. The genetic relatedness of these populations, and their similarity to the original South African quarantine population was assessed prior to conducting further experiments.

There were significant genetic differences between the populations of *C. aquaticum*. Phylogenetic comparisons between the South African group and the Argentinian group indicated a difference of 11%, suggesting that the populations are genetically and, therefore, taxonomically distinct (Fig.B6). Morphological taxonomic analysis reported all specimens to be the same species, *C. aquaticum*, suggesting that the long period of time in quarantine might have resulted in these genetic differences.

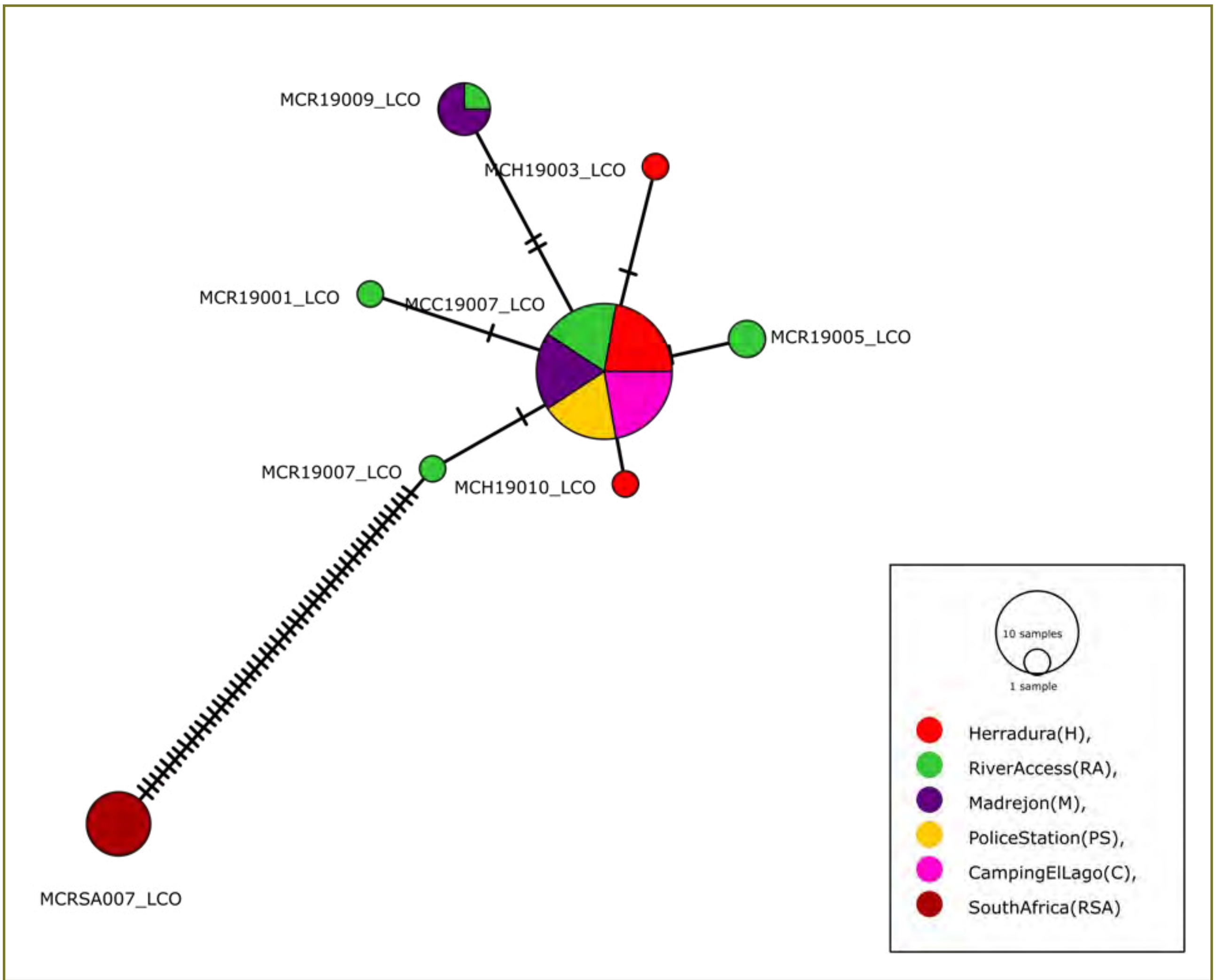

Figure B6: Haplotype network of South African *Cornops aquaticum* Bruner specimens collected from various sites along the Parana River, from Buenos Aires Province to Formosa Province, highlighting the genetic difference between the South African and South American populations.

Kariba Weed

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Salvinia molesta or giant salvinia remains under excellent biological control in South Africa. The programme against this weed involves the mass rearing of the agent, *Cyrtobagous salvinae*, for inundative releases onto sites where the weed might have been removed through a flooding event and has come back in the absence of the weevil, or at sites where it is necessary to reduce the time for classical biological control to take effect. This weed is also subject to ongoing post-release evaluation to measure the level of success, which is being measured as biodiversity recovery. Thanks to the success of *C. salviniae* in South Africa, the CBC has consulted on projects outside South Africa, sending *C. salviniae* to Zambia and Zimbabwe, and advising projects in Botswana.

Post-release evaluation

Salvinia molesta is considered under complete biological control in South Africa, by the weevil, *Cyrtobagous salviniae*. Nonetheless, infestations of the weed still occur at various sites, in particular, along the Garden Route of the southern Cape, through to the City of Cape Town. The CBC embarked upon a mass-rearing and release campaign at a number of these sites, followed by post-release evaluations earlier in 2021. In February 2021, 25 *S. molesta* sites were investigated; only three sites had persistent *S. molesta* cover, ranging from 5% to 10%. Only one site (Somerset West) did not have *C. salviniae* present. Therefore, over 80% of the sites surveyed were under complete or substantial control by the weevil. Changes in the rearing strategy for *C. salviniae* made 2021 one of the most successful years for producing the insect.

Systematics of *Helicosporidium* sp. in *Cyrtobagous salviniae*, a biological control agent of *Salvinia molesta*

In 2007, the parasitic alga, *Helicosporidium* sp., was detected in field collected *Cyrtobagous salviniae* adults in South Africa. The distribution and impacts of this disease on the weevil and its efficacy as a control agent were not known. Tshililo Mphephu determined the prevalence, infection load, and impact of *Helicosporidium* sp. on *Cyrtobagous salviniae* for his PhD to measure the impact the infection might have on the biological control programme.

In order to identify this disease, morphological features of *Helicosporidium* sp. isolated from *C. salviniae* in South Africa were recorded using Scanning Electron Microscopy (SEM) and the molecular identification of *Helicosporidium* sp. was determined, using genomic DNA. The prepared nucleotide sequences of *Helicosporidium* sp. were aligned with other related sequences of *Chlorophyta* and *Prototheca* spp. obtained from GenBank.

The SEM micrographs showed additional morphological features of the vegetative, mature cyst and filamentous cells (Fig. B7). The mature cyst and vegetative cells measured 3.2–6.2 µm and 3.6–5.4 µm (n= 60, each), respectively. Coiled filamentous cells with barbs were observed enclosing or forming the mature cyst (Fig. B7). Uncoiled filamentous cells also showed barbs (Fig. B8). The ruptured pellicle cells (Fig. B8), which could have contained either the mature cyst or vegetative cells, were also observed. These morphological features of *Helicosporidium* sp. have not been previously detected under light microscopy.

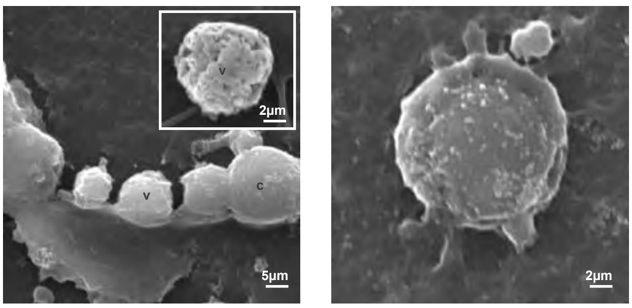

Figure B7: Scanning electron micrographs of *Helicosporidium* sp. cells. Abbreviations: (A) Vegetative and cyst cells (B) Mature cysts. Abbreviations: V (vegetative cell), C (Cyst).

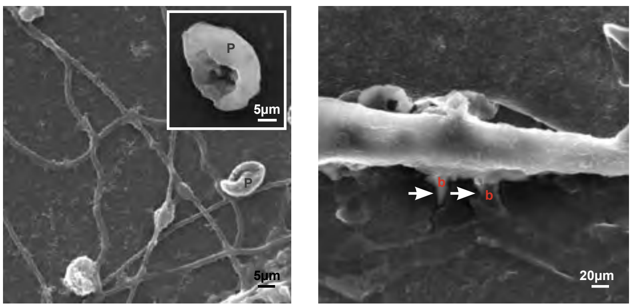

Figure B8: Scanning electron micrographs of filamentous cells. Abbreviations: F (filament), P (pellicle) and B (barb).

The extracted genome of *Helicosporidium* sp. is illustrated in Fig. B9. The sequenced PCR (polymerase chain reaction) products (size: 500 bp) resulted from the specific amplification of *Helicosporidium* 18S, while rDNA fragments were recovered. The 18S rDNA nucleotide sequences of *Helicosporidium* sp. 18S rDNA had formed a polyphyly relationship with other *Helicosporidium* spp., showing a paraphyletic relationship with both *Helicosporidium* spp. and *Prototheca* sp. isolates (Fig. B9). These sequences had strong support (high bootstrap value) with the ancestral root of the phylogenetic tree, Chlorophyceae. Both SEM morphological features and molecular results support the identification of this isolate of *C. salviniae* as a parasitic alga, *Helicosporidium* sp. The identity sequence of *Helicosporidium* sp. ex *C. salviniae* will be deposited into GenBank as reference for future studies.

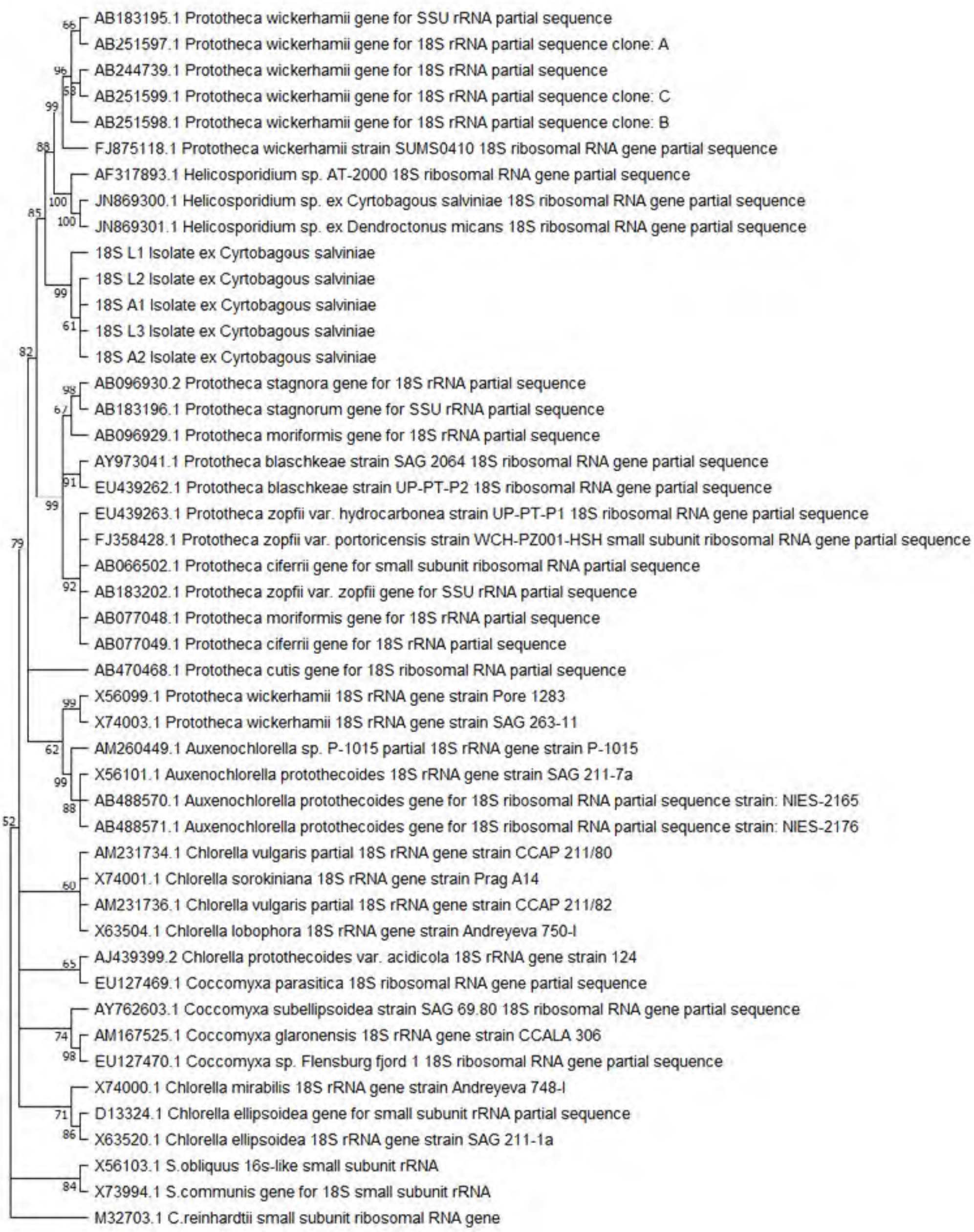

Figure B9: Maximum Likelihood (ML) phylogram constructed from 18S rDNA gene sequences from Trebouxiophyceae and incorporating isolates made from *C. salviniae* sequences. Comparative sequences were downloaded from GenBank (accession or reference numbers are presented). The tree shows that all isolates from *C. salviniae* correspond to *Helicosporidium*, and provide support for the polyphyly of the genus *Helicosporidium*, and paraphyletic of both the genera *Helicosporidium* and *Prototheca*. This genus appears as polyphyly taxa to a group of many *Prototheca* spp. The tree is rooted in Chlorophyceae. ML bootstrap values >50 (1000 replicates) are indicated by numbers at the nodes, whereas nodes that were not supported by bootstrap analysis (value <50) are excluded from the tree nodes.

Common salvinia

A previously unrecorded alien species of salvinia was discovered at Hartbeespoort Dam in the North-West Province. The discovery was made in December 2011 by retired ARC-PPRI entomologist and biocontrol scientist, Dr Carina Cilliers, while undertaking zooplankton studies of water samples from the dam. Fern taxonomist, Dr Ronell Klopper of SANBI in Pretoria, confirmed that the Hartbeespoort Dam plants were neither *Salvinia molesta*, which has long been invasive in southern Africa, nor *S. hastata*, which is indigenous to east Africa. The new plants were identified as *Salvinia minima* Baker (Salviniaceae) (common salvinia), whose native range extends from Mexico through Meso-America to northern Argentina.

Salvinia minima is native to Central and South America, and has been introduced to and is invasive in the southern United States of America, Bermuda, Puerto Rico, Spain and now South Africa. This species' mode of introduction is most likely through the nursery and aquarium trade.

The first record of common salvinia in South Africa is from 2011, from Hartbeespoort Dam. How or when *S. minima* was introduced into South Africa, or how it got into Hartbeespoort Dam is unknown, but given its mode of introduction elsewhere, it was probably through the aquarium trade. Until 2021, records of *S. minima* were limited to Hartbeespoort Dam, and ponds on housing estates around the dam. However, in May 2021, its presence was confirmed downstream of the Crocodile River on Roodekoppies Dam, as well as on two unconnected systems, Bon Accord and Roodeplaat dams. Anglers, recreational boaters, as well as birds are likely to spread it more systems, given its small size.

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There are ten species of salvinia worldwide, but there is only one African species, *S. hastata*, which occurs naturally on the Shire and lower Zambezi rivers in tropical east Africa. The newly recorded common salvinia at Hartbeespoort Dam could easily be mistaken for the more widespread *S. molesta*. However, even under the extremely high nutrient conditions of the dam, its smallest leaves are only ~8 mm wide, with the largest leaves only reaching ~15 mm wide. *Salvinia molesta*, under the same conditions,

Figure B10: A canoeist's view of *Salvinia minima* on Hartbeespoort Dam. **Photo:** Julie Coetzee

Figure B11: An aerial view of Hartbeespoort Dam, highlighting the extent of the *Salvinia minima* invasion. **Photo:** Julie Coetzee

would be expected to have leaves 20—40 mm wide or more. *Salvinia minima* on Hartbeespoort Dam has the potential to become as widespread and problematic as its congener, *S. molesta*, against which a successful biocontrol programme has been implemented in South Africa. Research conducted in Florida, USA by the USDA shows that there is a damaging 'Florida' biotype of the weevil *C. salviniae* which could be used in South Africa.

In South Africa, *C. salviniae*, first released in 1985, has been developed as an extremely successful biocontrol agent against *S. molesta*, resulting in 100% control at a number of sites throughout South Africa. Unfortunately, the biotype of the weevil present in South Africa, originally collected from Brazil, does not control *S. minima* to the same degree as it does *S. molesta* due to plant's size – it cannot complete development on the smaller *S. minima*. Research in the USA has shown that a smaller 'Florida' biotype of *C. salviniae* is able to complete development on both the smaller *S. minima*, and *S. molesta*. Narrower rhizomes in *S. minima* may restrict usage of this species by the larger 'Brazil' weevils, whereas the smaller larvae of the 'Florida' weevils are better able to burrow in a wider range of plant sizes.

Figure B12: Adult *Cyrtobagous salviniae* moving across a *Salvinia minima* frond. **Photo:** David Taylor

Host specificity testing of Cyrtobagous salviniae

The Florida biotype of *Cyrtobagous salviniae* was imported into the CBC's quarantine in July 2021, and is currently in the end phases of its host specificity testing. We have focused the testing on *S. minima* and *S. hastata* to ensure that the weevil will not pose a threat to an indigenous African species. The results to date suggest that *S. hastata* will not be able to host the weevil. A release application will be submitted to DFFE as soon as the host specificity testing is complete.

Brazilian Waterweed

Biological control has been successful in controlling several of the floating aquatic weeds, which has allowed South Africa's aquatic ecosystems to be invaded by submerged weeds. In 2014, the CBC targeted one of the worst submerged aquatic weeds in South Africa, *Egeria densa*, commonly known as Brazilian waterweed, or Brazilian elodea for biological control. It is a rooted, submerged macrophyte that grows vigorously in slow-moving water. The control agent, the Egeria fly (*Hydrellia egeriae*) was released in 2018, and is the first control agent released against a submerged weed in South Africa and the first agent released against *E. densa* in the world.

Rosali Smith mass-reared and released the agent, and monitored its establishment and impact at a number of sites in South Africa.

Mass-rearing and releases

Rearing of the biological control agent, *Hydrellia egeriae*, is conducted at the Waainek Mass-Rearing Facility, in a polytunnel and a laboratory. The population reached a mean of 28 064 immatures/kg *E. densa* in March (Fig. B14). During this time, approximately 103 600 *H. egeriae* were released in the Groot River, Baviaanskloof, Eastern Cape (Fig. B15), which reduced the Waainek population to 1 348 immatures/kg *E. densa* (April), which slowly increased to 5 146 immatures/kg *E. densa*. In October, approximately 8 059 *H. egeriae* were released in the Groot River, Baviaanskloof.

RESEARCH TEAM
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COLLABORATOR
FuEDEI

Figure B13: *Hydrellia egeriae* rearing in a laboratory room at the Waainek Mass-rearing Facility **Photo:** Rosali Smith

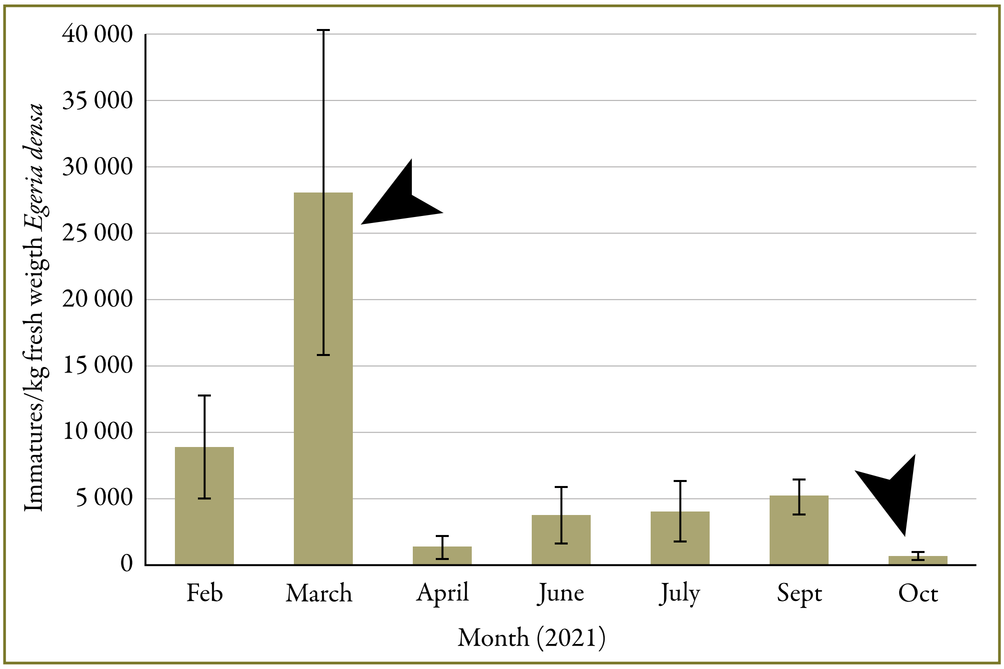

Figure B14: Monthly mean (±SE) *Hydrellia egeriae* population size (immatures/kg fresh weight *Egeria densa*) for 2021. Immatures represent eggs, larvae, and pupae. Arrows indicate when insects were collected for field releases.

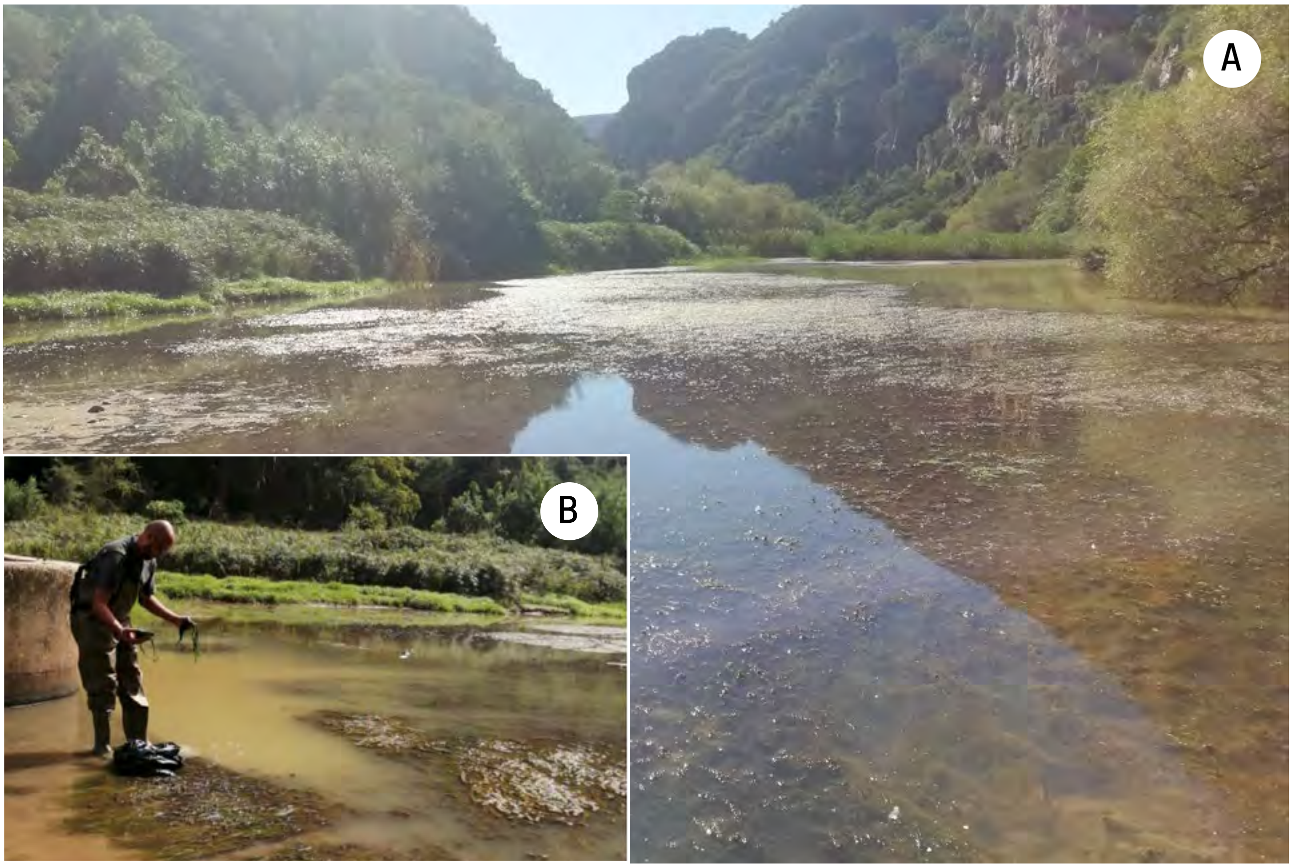

Figure B15: A) *Egeria densa* monoculture stand in the Groot River, Baviaanskloof in May 2021. B) Lyle Titus releasing *H. egeriae* in March 2021. **Photos:** Rosali Smith, Samella Ngxande-Koza

Brazilian waterweed post-release monitoring

Since permission for release has been granted, *H. egeriae* has been released at various sites in KwaZulu-Natal and the Eastern Cape. However, travel restrictions and budget cuts in 2021 restricted post-release monitoring for *H. egeriae* to the Eastern Cape. The Nahoon River was the first site for the release of *H. egeriae*, which occurred in 2018. Post-release monitoring of this site in August this year showed that *E. densa* is no longer present in the Nahoon River.

Hydrellia egeriae has established in the Groot River and reached a maximum density of 4 761 insects/kg *E. densa* only three months after its release (Fig. B16). A post-release survey in July showed that the population had decreased to 312 insects/kg *E. densa*. However, previous work has shown that *H. egeriae* is affected by native parasitoids, mostly in the winter months. Since its release, the *E. densa* infestation has decreased from 82% to 75% in cover (Fig. B16).

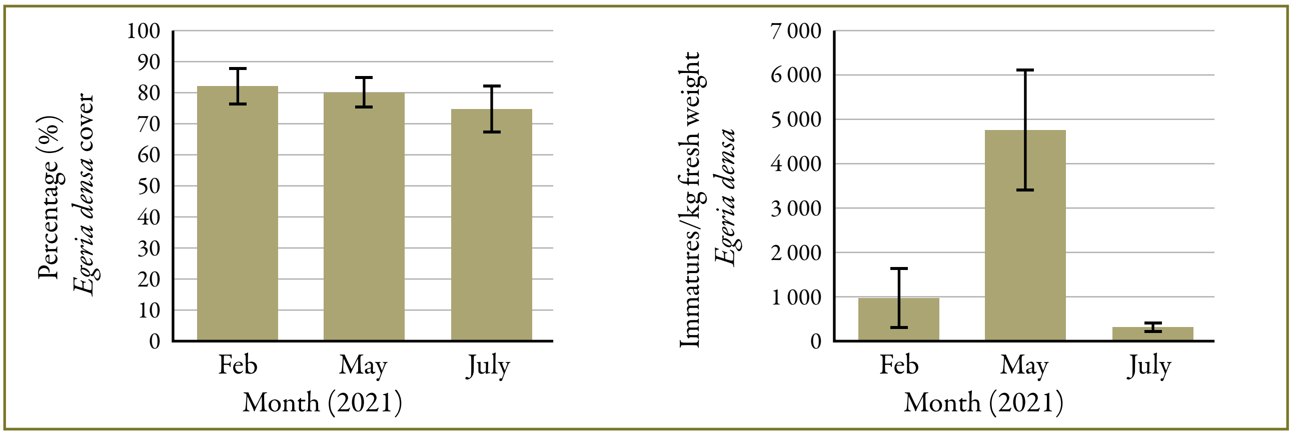

Figure B16: Extent of the *Egeria densa* invasion, and the population size of its biological control agent in the Groot River, Baviaanskloof in 2021.

Delta Arrowhead

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

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Biological control options for delta arrowhead

Listronotus appendiculatus has been identified as a promising candidate for *S. platyphylla* control, and a release application was submitted to the Department of Agriculture, Land Reform and Rural Development, in October 2020. Unfortunately, the Department did not grant permission for release. The independent reviewers felt that the insect shows great promise as a biological control agent; however, they suggested that in order to confirm that the weevil is completely safe for release in South Africa, paired choice or multiple generation tests should be conducted on the closely related aquatic macrophyte, *Limnophyton obtusifolium*. The initial no-choice test results on this species were inconclusive, as finding enough test plants proved challenging.

Limnophyton obtusifolium is known from only five locations in South Africa in seasonal pans in the extreme northern regions of the country where the occurrence of the plant is occasional. Since the start of the project, the plant has only been found at one site in Mkhuze Game Reserve, KwaZulu-Natal, despite numerous exploratory trips to find it. A trip was therefore conducted to Mkuze in May to search for the species; only three immature plants were located from one of the pans in Mkuze. These plants were carefully dug up and returned to the CBC's quarantine facility at Rhodes University for paired choice testing with the target species. Although we managed to conduct two paired choice replicates before the plants died, the

Figure B17: Dr Grant Martin in search of *Limnophyton obtusifolium* in Mkhuze Game Reserve, Kwa Zulu-Natal Province.
Photo: Julie Coetzee

results are still inconclusive and additional trips to search for the plant species will need to be conducted in 2022.

A second weevil, *Listronotus frontalis*, is also being considered as a biological control agent of *S.platyohylla platyphylla*. *Listronotus frontalis* larvae damage the subterranean tubers of the plant, while the adults damage the flowers and young leaves. In 2021, Daniel Rogers completed his MSc investigating the impact, as well as the biology of the weevil. He was able to show that the weevil develops from egg to ovipositing adult within just over 40 days. Females were recorded to lay up to 48 eggs within a period of one week. Impact studies showed that adult feeding led to a reduction in all but one of the 11 measured plant growth and developmental parameters, including a reduction in the mean mass of the above-ground plant material, as well as a reduction in size and abundance of tubers and stolons. These results are encouraging and suggest that if the insect is released in South Africa, it may prove to be a valuable biocontrol agent.

Since completing his MSc, Daniel and Lab technician, Getrude Tshithukhe, have further investigated the host specificity of the weevil. This has previously proved difficult to test as it has been unknown how the insect reaches the buried tubers under natural conditions. However, by manually inserting eggs into the substrate it was possible to obtain positive controls for the first time. In addition, no-choice tests were conducted on the crowns of test species and the target. The trials have shown that *L. frontalis* was not able to complete its life cycle on any of the tested whole plants or crowns other than *S. platyphylla*, suggesting that *L. frontalis* may be suitably host specific to consider as a biological control agent in South Africa. Similar results have been recorded by researchers in Australia who are also investigating the host specificity of the insect.

Sagittaria platyphylla remains a damaging, rapidly spreading, invasive macrophyte in South Africa. The delay in the release of *L. appendiculatus* has been a major setback; however, the additional testing will be completed and we hope that both *L. appendiculatus* and *L. frontalis* will be approved for release.

Yellow Flag Iris

Yellow flag iris, *Iris pseudacorus*, was targeted for early detection and eradication, because its distribution in South Africa was underestimated, leading to its listing as a Category 1a invasive species (NEM:BA). It is native to Eurasia and north Africa, but has been declared invasive in the USA, Canada, New Zealand, Argentina, and South Africa, where it was introduced as an ornamental and then escaped cultivation.

Management of *I. pseudacorus* is difficult due to its rapid dispersal. It is referred to as an ecosystem engineer with the ability to drastically reduce native plant diversity in invaded water. Wetlands are particularly vulnerable to invasion, where the plant alters the hydrology by reducing water flow and trapping sediment in its rhizomes. In time, this species can form monocultures that modify the structure and functioning of aquatic ecosystems.

Emma Sandenbergh worked on a variety of aspects of the *I. pseudacorus* invasion in South Africa for her MSc. Results of field surveys conducted by Emma in 2019 and 2020 show that *I. pseudacorus* infestations are greater in number in South Africa's major cities, but more severe (in terms of abundance) in the Limpopo and Mpumalanga provinces. While the severity of invasion appears to be linked to temperature and precipitation, the data were insufficient, probably due to South African *I. pseudacorus* invasions being in the 'lag' phase.

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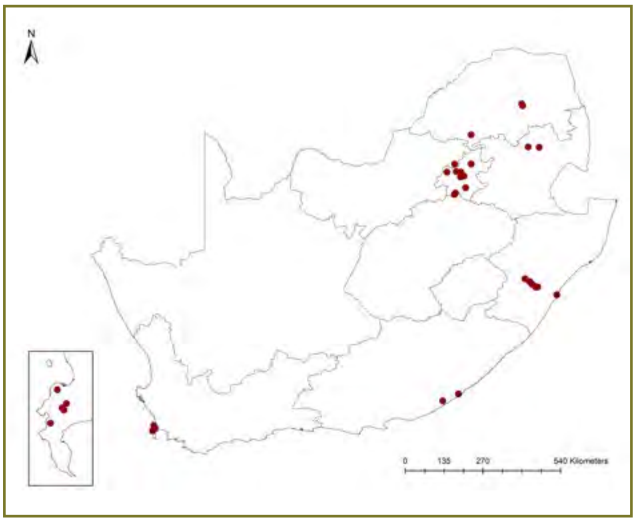

Figure B18: Distribution of confirmed *Iris pseudacorus* populations in South Africa, sampled between October–November 2019 and February–March 2020

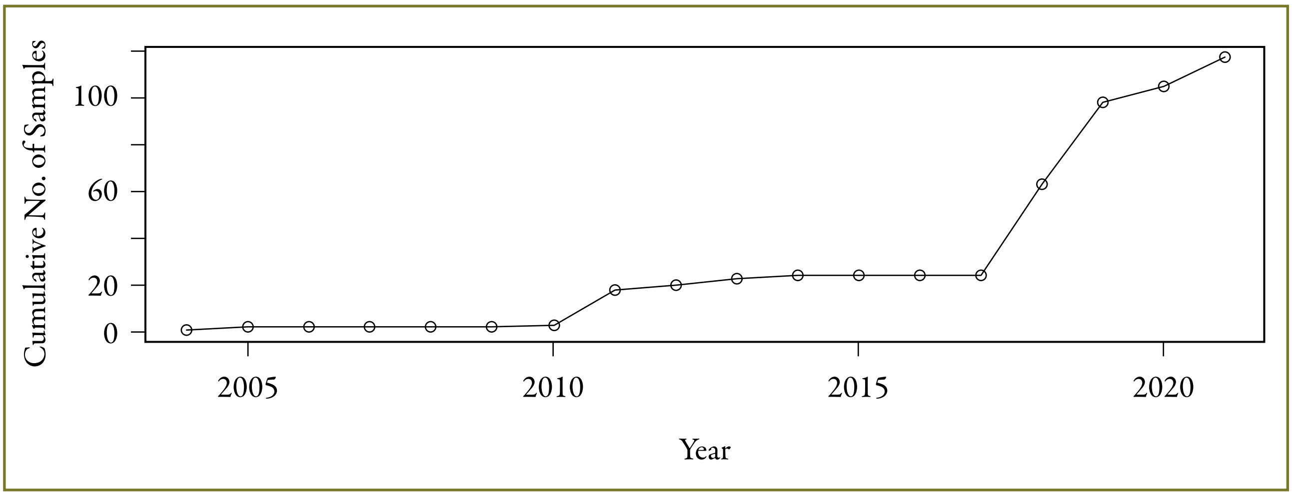

Figure B19: Cumulative records of South African *I. pseudacorus* populations recorded from 2004 to 2021

Genetic analysis

Iris pseudacorus is capable of reproducing both sexually and asexually, spreading via both seed dispersal and vegetative propagation, and it was initially thought that the primary mode of invasion was through clonal vegetative reproduction by rhizomes. However, recent studies in the USA have shown that its spread is the result of prolific seed production. The seeds are buoyant to allow for dispersal by water, and high levels of both seed viability and germinative power have been reported in *I. pseudacorus* populations in the northern hemisphere, Argentina, and South Africa. As a result, the genetic diversity between populations should be high.

The analyses of the genetic diversity indicated a high degree of genetic diversity between and within South African *I. pseudacorus* populations, suggesting the occurrence of gene-flow through sexual reproduction (Fig. B20). While aquatic invaders usually reproduce asexually, similar results were found for *I. pseudacorus* populations in the United States. Unlike the results obtained in the United States, a negative correlation was observed between geographic distance and genetic similarity, suggesting fewer founding events in South Africa than was the case in the US. However, the correlation was weak, and most likely indicates a largely artificial spread of *I. pseudacorus* populations in South Africa.

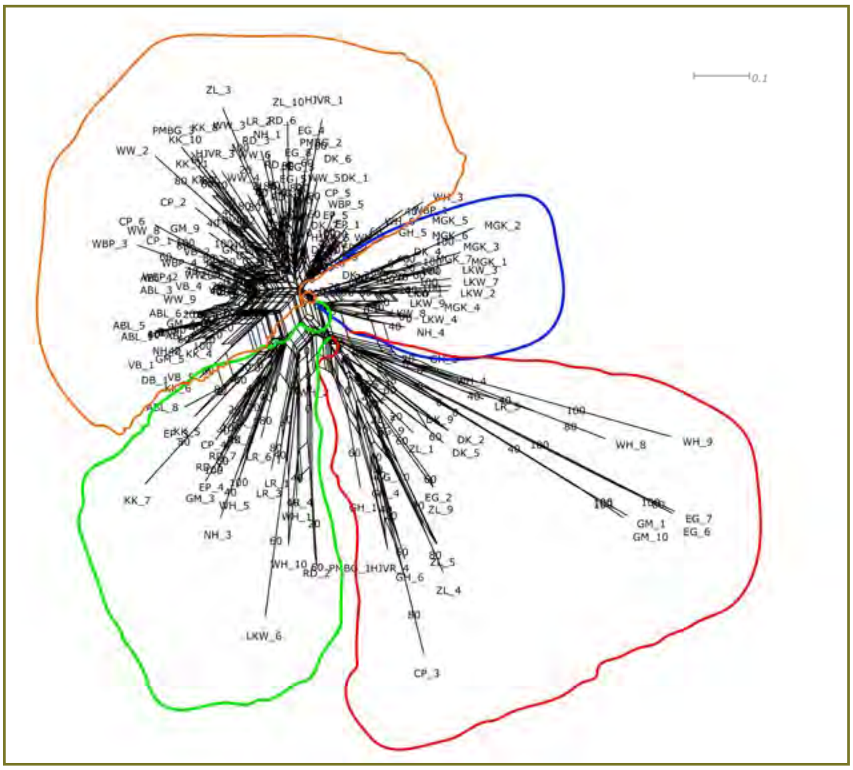

Figure B20: NeighbourNet tree produced in SplitsTree using Jaccard's distance. Different coloured outlines represent potential clusters of *Iris pseudacorus* populations according to genetic relatedness of individuals.

Figure B21: Paula Gervazoni sampling insects on Iris in Argentina. **Photo:** Paula Gervazoni

Figure B22: Insects observed in *Iris pseudacorus* in the field **Photo:** Paula Gervazoni

Competition with native species

Emma also concluded a competition experiment assessing the competitive interactions occurring between *I. pseudacorus* and the co-occurring native, *Typha capensis*. With a greater biomass accumulation and RGR, *T. capensis* outperformed *I. pseudacorus*, increasing its growth in the presence of *I. pseudacorus*. A high RSR was observed for *I. pseudacorus*, and the species appeared unaffected by the presence of *T. capensis*. Because *I. pseudacorus* can form dense rhizomatic mats, it is possible that a different result would have been observed had the experiment run for an extended time period.

Iris pseudacorus: Associated fauna in the introduced range

PhD student, Paula Gervazoni, sampled insects associated with *I. pseudacorus* in 2018, 2019 and 2020 in Argentina to compare these communities with the ones present in South Africa and Europe (Fig. B21). In 2021, the collected material was analysed in collaboration with specialists, and a total of 343 individual insects were collected, the most abundant orders being Hemiptera, Diptera and Coleoptera. A coleopteran was prioritised (Fig. B22), as it could contribute to controlling the invasive plant in the invaded range. More field work has been done this year,

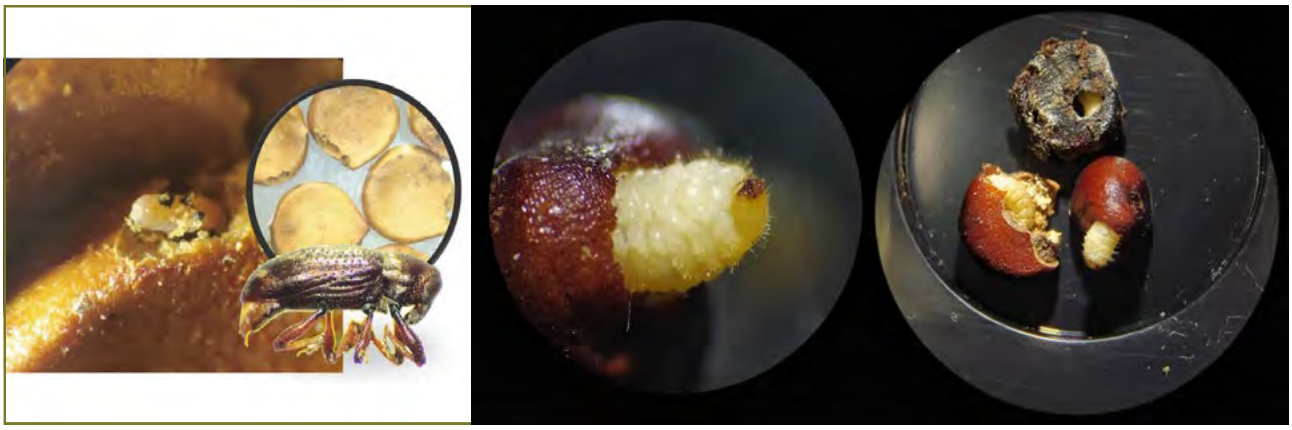

Figure B23: Coleoptera developing within seeds of *I. pseudacorus* collected in Argentina collecting and dissecting mature capsules of the plant; different insects were found developing inside *I. pseudacorus* seeds. Taxonomists have assisted in identifying these specimens. Figure B23 shows another insect that is being prioritised as it damages the plant, too.

Biological control: *Aphthona nonstriata*

In South Africa, *I. pseudacorus* is currently listed as an eradication target under NEM:BA (i.e., Category 1a invasive species), but the only control options are manual/ mechanical and herbicides. Its growth characteristics, abundance at sites, and widespread distribution make it a difficult target for these methods, which seldom, if ever, result in eradication. Chemical control using glyphosate has been used in some countries, but large-scale herbicide application is environmentally unsustainable, especially in proximity to aquatic ecosystems. For these reasons, a biocontrol programme was initiated with natural enemy surveys in Europe in 2018, followed by the importation of a potential control agent, the chrysomelid, *Aphthona nonstriata* Goeze (Coleoptera: Chrysomelidae), from Belgium.

Host specificity

A list of test species including *Iris* species and genera in the Iridaceae was compiled for host specificity, across various invaded ranges. Each taxon was assessed based on phylogenetic relatedness, geographic overlap and ecological similarity with respect to *I. pseudacorus*. Native Iridaceae species' seeds are also being collected in invaded countries for further testing.

A no-choice full development experiment was conducted in greenhouse conditions in Belgium by PhD student, Gianmarco Minuti. He tested four *Iris* spp., two ornamental Iridaceae (*Crocosmia* sp. and *Gladiolus* sp.) and a *Carex* sp. (Cyperaceae) which commonly co-occurs with *I. pseudacorus* in the native range. *Aphthona nonstriata* only completed development on *I. pseudacorus* and *I. foetidissima*, suggesting it is an *Iris sp*. specialist.

In South Africa, the host specificity development trials of *A. nonstriata* are ongoing and nearing the end with 26 species tested (Table B2). None of the indigenous test species supported the development of this potential agent, while some *Iris* species available in the ornamental industry in South Africa did support development, adding to the results from Belgium that *A. nonstriata* is an iris specialist. This finding has been supported in both the no-choice and choice tests conducted on *Iris domestica*, *I. confusa*, *I. laevigata* (June x Self) and (Regal x Open) and *I. spuria* (Archie Owen) and (Destination). There are a few remaining species to be tested: *I. orientalis*, *Moraea serpentine*, *M. ochroleuca*, *Gladiolus crassifolius*, *G. elliotii*, two species from New Zealand, *Libertia ixioides* and *L. grandiflora*, and a few from Argentina.

Table B2: List of species used in testing the host specificity of *Aphthona nonstriata*

Species	n	*x* adults
Iris pseudacorus	61	44.3
I. domestica	5	19.6
I. confusa	5	6.4
I. laevigata (June x Lake)	5	14.2
I. laevigata (Regal x Open)	5	41.8
I. spuria (Destination)	5	8
I. spuria (Archie Owen)	3	20
Dietes grandiflora	5	0
D. bicolor	5	0
D. iridiodes	5	0
Moraea huttonii	4	0
M. reticulata	4	0
Watsonia angusta	5	0
W. borbonica	5	0
W. pillansii	3	0
Gladiolus grandiflora	3	0
Neomerica gracilis	5	
Aristea ecklonii	5	0
Dierama igneum	3	0
Hesperantha coccinea	3	0
Iris coffee	3	0
Gold Kist	3	0
Louisiana iris	5	0
Annie's song	3	0
Louisiana for Dad	3	0
Garden blue iris	5	0

Species distribution modelling

The potential bioclimatic niche of *I. pseudacorus* and *A. nonstriata* was modelled from presence-only data, using the software MaxEnt. The results indicate that *I. pseudacorus* has the potential to expand its invasive range in the southern hemisphere, but that *A. nonstriata* is likely to establish across humid temperate climates invaded by the weed (Figures B24 and B25). These results were presented at the 18th Symposium on Insect-Plant Interactions (https://www.universiteitleiden.nl/sip2021) and have been accepted by the journal, *Biological Control*, for publication.

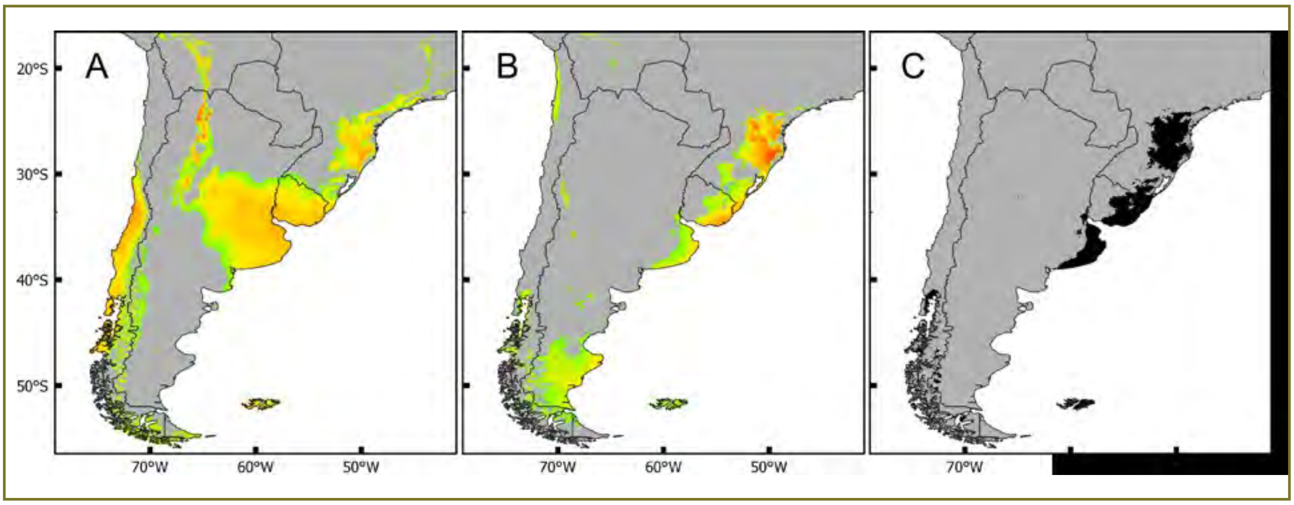

Figure B24. Predicted climatic suitability for *Iris pseudacorus* (A) and *Aphthona nonstriata* (B) in South America. Warmer colours indicate higher suitability. The overlap between the potential distribution of the two organisms is indicated in black (C).

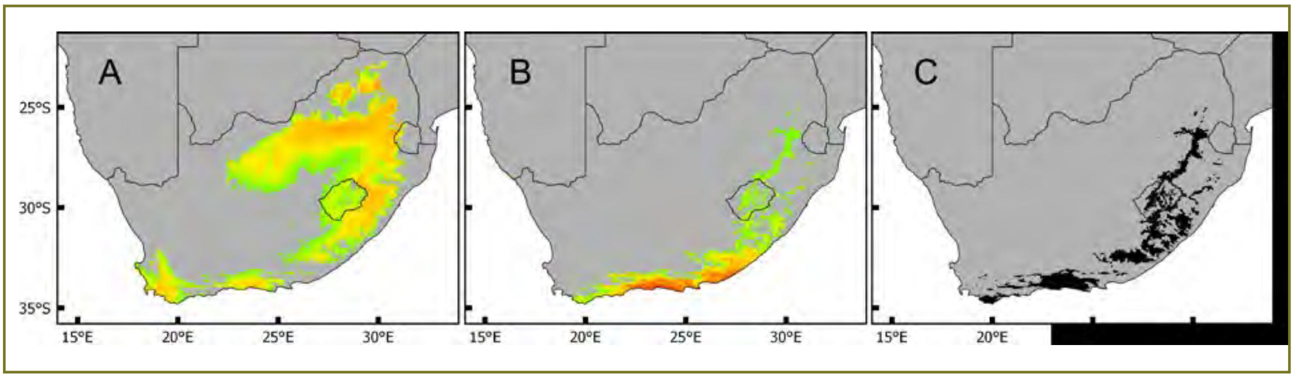

Figure B25. Predicted climatic suitability for *Iris pseudacorus* (A) and *Aphthona nonstriata* (B) in South Africa. Warmer colours indicate higher suitability. The overlap between the potential distribution of the two organisms is indicated in black (C).

Using *Iris pseudacorus* distribution data from different sources, Paula also conducted ecological niche modelling of I. pseudacorus in Argentina. This was done to compare current distribution with potential distribution, detecting vulnerable areas, and to compare model performance from different datasets (distribution reports obtained from researchers as opposed to researchers plus citizen science). This highlights the importance of citizen science as a tool in invasion distribution. Results revealed an important increase in suitable area when including citizen science, which demonstrates the relevance of combining different data sources in invasive alien species research.

Mexican Water Lily

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Mexican waterlily (*Nymphaea mexicana* Zuccarini (Nymphaeaceae)) has become a problematic invasive species in South Africa after being introduced from southern USA and Mexico, most likely through the horticultural trade. Biological control research for this species was initiated by Megan Reid, then an MSc student, in 2016. Since then, two potential biological control agents have been identified during surveys in the native range, and it was determined that hybrids of *N. mexicana* also exist at invaded sites in South Africa. In 2020, as part of her PhD work, Megan found a native weevil, *Bagous longulus* Gyllenhal (Coleoptera: Curculionidae), feeding and reproducing on *N. mexicana* at an invaded site in Port Elizabeth/Gqeberha during pre-introductory surveys. In 2021, continuation of these surveys identified a second site in Knysna, Western Cape, where *B. longulus* populations are being sustained on *N. mexicana*.

To further investigate the potential of *B. longulus* as a biological control agent, surveys were conducted on native waterlilies in South Africa to determine the distribution and field host range of *B. longulus*, as well as the levels of damage exerted at different sites. The surveys finished in November 2021, and preliminary data suggest that *B. longulus* was specific at least to the genus *Nymphaea*, was widely distributed across South Africa, and could exert high levels of damage on the host plants. Data obtained from these surveys also suggest that *B. longulus* does not significantly damage *N. mexicana* hybrids. Preparations are underway to begin host specificity experiments to compare feeding damage on native *Nymphaea* with *N. mexicana* collected from two separate populations in South Africa, and on a hybrid (*Nymphaea marliacea chromatella*). Rearing of *B. longulus* cultures at Waainek Research Facility is ongoing and is being optimised.

Using biological control to manage hybrids can be challenging, but the chances of success may improve if the parent species of the hybrids are surveyed to identify further potential biological control agents. Hence, genetic studies are also underway to attempt to identify the putative parents of the *Nymphaea* hybrids in South Africa. Finally, *N. mexicana* plants in quarantine are being prepared for the importation of *Megamelus toddi* (Hemiptera: Delphacidae) from Florida for investigation as a potential biological control agent.

Figure B26: Side view of *Bagous longulus*. **Photo:** David Taylor.

Figure B27: Megan looking for *Bagous longulus* weevils on native *Nymphaea* in the Eastern Cape. **Photo:** David Taylor

Ecological Restoration

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

In 2021, the CBC continued to expand its research agenda into the role of ecosystem restoration after aquatic weed control to ensure ecosystem recovery. The restoration team has set up an ongoing whole-pond manipulation experiment at the Waainek Mass-rearing Facility. The aim is to mimic a situation of a highly invasive floating plant (*Pistia stratiotes*) invaded system at first, followed by

Figure B28: *Pistia stratiotes* (water lettuce) growing in the experimental ponds at Waainek Research Facility, Rhodes University. **Photo:** Antonella Petruzzella successful invasive plant control by biological control agent *Neohydronomus affinis* (Coleoptera: Curculionidae)). After achieving complete control, native vegetation will be actively re-established and then a secondary invader, the South American rooted submerged plant *Egeria densa*, will invade the system. The native species will be established following three native plant species priority effects: native plants will be planted at the (1) same time, (2) earlier and (3) later, relative to the secondary invader arrival, the rooted submerged plant *E. densa*. In addition, other biotic communities will also be monitored, including phytoplankton, periphyton, zooplankton and macroinvertebrates, together with water quality parameters. This will allow us to assess how the biota and water quality change and/or recover during invasive plant control management and native vegetation re-establishment. We hope to guide future restoration efforts in a way to maximise the likelihood of desired species establishment by strengthening native species' priority effects to curb future re-invasions and/or secondary invasions.

Insect Thermal Physiology Plasticity, and Links to Metabolism and Life History

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

Nonetheless, there are examples of unanticipated agent establishment and success in areas of introduction outside of the pre-determined insects' thermal ranges. This programme thus aims to better understand insect thermal tolerance to improve predictability of agent establishment

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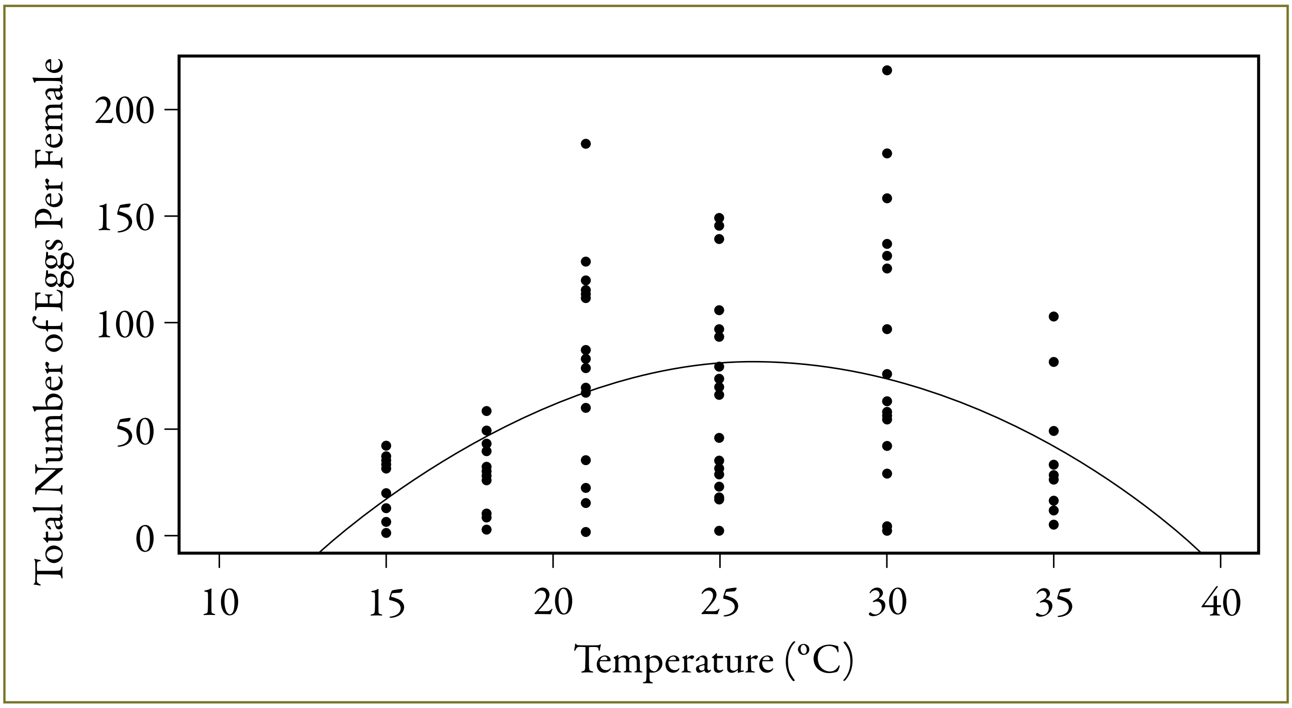

Figure B29: The total number of eggs per female at each temperature treatment with a plot of the quadratic regression model (solid line).

and control success, both for new releases and for the future under altered climates.

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

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

Lysathia sp.

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

Batandwa Masiko began an MSc in 2020 that focusses on the development and digestion of *Lysathia* sp. under constant and fluctuating thermal regimes. This project determines development time, adult size, digestive rates, and body composition of the species when feeding and developing under either constant or variable temperature conditions. This work aims to elucidate the impacts that experimentation using constant conditions, which traditional experiments have always used, could have on results when compared to those gathered under fluctuating conditions, which are more ecologically relevant. Mr Masiko's experiments are still underway at present but will be complete early in 2022.

Tristan Pitcher and Sipho Manqele both investigated the fecundity of *Lysathia* sp. under different conditions. Tristan developed a fecundity performance model for the species, indicating that it has a wide range of temperatures in which oviposition can occur, while Sipho's experiments indicated that fecundity is not adversely impacted by cold exposure before oviposition (Fig. B29). These results contribute to the growing body of knowledge on the thermal physiology of *Lysathia* sp. that indicates that its high tolerance of adverse temperatures contributes to its success as a biocontrol agent. These data indicate the possible suitability of the species as a biocontrol agent in colder European countries that are also invaded by parrot's feather.

CACTUS WEEDS

Some of the most successful weed biological control programmes globally have been against cactus weeds. Probably the most well-known weed biological control programme ever was the control of the Australian Pest Pear *Opuntia stricta*, a cactus weed that covered over 24 million hectares in Queensland and New South Wales in Australia. It was estimated that the biomass of cactus in these infested areas was about 250 000kg/ha and 16 000 plants/ha. Like all cactus weeds, this plant resulted in large tracts of land being unsuitable for agriculture, with many farms being abandoned because the land became completely unusable. Two biocontrol agents were released in Australia for the control of the pest pear: the cactus moth, *Cactoblastis cactorum*, and the cochineal insect, *Dactylopius opuntiae*. In just a few years, the massive and dense infestations were reduced to the point that the weed was no longer a problem. This was largely due to the impact from the cochineal insect, and it is various cochineal insects that are the focus of the South African biological control programmes against many of our cactus weeds.

Invasive alien cacti are also a serious problem in South Africa, and they are equally susceptible to biocontrol. The spiny thickets that are formed by cactus invasions restrict access to grazing, reducing the carrying capacity of farms, restricting movement of livestock and wildlife and therefore reducing access to water and shade; the spines are directly harmful to animals and a pollutant of wool. In South Africa, we rely primarily on cochineal insects and the galling mealybug, *Hypogeococcus*, as biocontrol agents for invasive Cactaceae, and the results have been almost as impressive as the famous control of *O. stricta* in Australia.

Cochineal insects only feed on cacti in the tribe Opuntieae, so they are all suitably host-specific for release in South Africa. The family Cactaceae is endemic to the New World, with only a single species of cactus, a mistletoe cactus called *Rhipsalis baccifera*, being indigenous outside of the Americas; so there are no indigenous Opuntieae in Africa. This means that all cacti that are problematic in the arid parts of South Africa are alien, and many of them are susceptible to cochineal insects that can be used as biological control agents. It is, however, important that the correct cochineal species is used for the control of the correct target weed. There are ten species or lineages of cochineal that are used to control 12 target weeds in South Africa, but there are always new cactus species arriving in the country and these species need new biological control agents if they start to become invasive.

There are also several invasive alien cactus species in South Africa that are not Opuntieae which are controlled by another biological control agent, the galling mealybug, *Hypogeococcus*. *Hypogeococcus* feeds on several cactus species

PROGRAMME HIGHLIGHTS IN 2021

- Post-release evaluations of cactus biocontrol agents released from the CBC mass-rearing facility have indicated that the reproductive output and biomass of cactus weed populations has been dramatically reduced by biological control.
- New promising potential agents for *Opuntia elata* and *Opuntia megapotamica* are under evaluation in quarantine.
- Australian biological control experts are considering two biocontrol agents used for the control of *Pereskia aculeata* in South Africa for release in Australia. Host specificity testing conducted by the CBC has indicated that they are both safe for release.

all within the subtribe Cereanae, which includes some of the worst cactus weeds in South Africa. This agent cannot survive on plants outside the subtribe and is therefore suitably host-specific to be safe for use in South Africa without any threat to indigenous plants.

The CBC Cactus Team mass rears, releases, and monitors the success of cactus biological control agents that have already been released in South Africa. It also aims to develop new biological control agents for cactus weeds that do not have effective agents already. There are also always new cactus species being introduced which will become problematic in the future if biological control is not implemented now. The best way to avoid the negative impacts of an invasive weed is to stop it from invading an area in the first place, so the biological control of weeds that are in the early phase of invasion process is very beneficial.

Cactus biological control has resulted in the control of several of the worst invasive alien plants in South Africa, with significant benefits to land-users, agriculture, natural ecosystems, and society in general. The research of the CBC Cactus Team has contributed to this success through developing improved implementation techniques and new biological control agents for cactus species that are not under suitable levels of control.

Quantifying the benefits of cactus biological control

RESEARCH TEAM
Prof. Iain Paterson, Ruth Scholtz

STUDENT
Zezethu Mqeta (PhD)

One very important, but often neglected, step in a biological control programme is post-release evaluation. Without post-release evaluations it is impossible to tell whether an agent has been successful or not, and it is therefore impossible to quantify the benefits and success of biological control. Quantifying success is important in leveraging further support for biological control, and it is also important for determining whether further control interventions are required.

Zezethu Mnqeta has been working on post-release evaluations of the cactus biological control agents that are released by the CBC for her PhD, which was submitted for examination at the end of 2021. Her work included an assessment of where the agents have been released in the country compared to the distribution of the weed, measuring the biomass and reproductive output of cactus infestations before and after release, and an assessment of the benefits of the biocontrol agents to land-users, including farmers and conservationists.

Figure C1: Zezethu Mnqeta monitoring *Hypogeococcus* sp. on Queen of the Night cactus in the Eastern Cape. **Photo:** David Taylor

The CBC has released agents at 173 sites in the country, with six agents on ten target weed species since the Kariega Facility reopened in 2015. Releases have been made in every province in the country, but only 26% of the quarter degree squares (an area of about 27 km^2) where the target weeds are present have had releases in them. This indicates that a much larger release effort is required to ensure that the agents that we have available are released throughout the country. A part of the country where very few releases have been conducted and many cactus species are widely distributed and abundant, is Limpopo Province, and this suggests that Limpopo would benefit from a new cactus biocontrol mass-rearing facility.

Post-release evaluations at sites where the agents have been released indicated that, for most species, there was a significant increase in the density of the agent after release, followed by a decline in plant biomass and reproductive output. For example, the number of jointed cactus cladodes (that is, stem segments of a cactus) per/m^2 decreased from 31 (S.E. ± 0.78) to 18 (S.E. ± 1.5) over the time that the study was conducted, and for drooping prickly pear the number of fruits declined from 135 (S.E. ± 15.6) to 56 (S.E. ± 15.1) and cladodes declined from 89 (S.E. ± 12.7) to 32 (S.E. 5.6). Some species of cactus take longer to control than others, and for the devil's rope cactus, no change in the number of cladodes was measured, although a significant increase in the number of cladodes infested with cochineal was recorded, and this was associated with a small decline in diameter of the cactus trees. This decline in diameter is due to the plants becoming unhealthy and wilting because of the cochineal feeding on them. Unlike the other cactus species that are controlled by biological control, the devil's rope cactus has a large woody stem, which the cochineal cannot feed on. To get good control of this species of cactus, an integrated approach using biocontrol and mechanical control is required. When the plants become wilted and covered with cochineal, the main stem of the cactus should be cut, and this will effectively kill the plant.

Interviews with 85 landowners indicated that 69% of the people who received biological control agents from the CBC believe that the agents reduced the density of the cactus weeds on their land, and 49% of these landowners said that the negative impacts caused by cactus infestations had declined. This is a very positive result because most land-users are only seeing the first benefits of biological control, having had the agents released on their land just a year or two before the interview. The benefits of the biological control agents are expected to increase as time passes.

Zezethu's PhD has confirmed that biological control is an effective method of dealing with invasive alien cactus species, and has highlighted that further investment in implementation, including the development of new mass-rearing facilities, is warranted.

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

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

STUDENT
Kedibone Mofokeng (MSc)

COLLABORATORS
FuEDEI, CABI

Opuntia engelmannii and *O. megapotamica* were considered to be the same species until a genetic analysis conducted by the CBC team at Wits University confirmed that they are two species, one from North America (*O. engelmannii*) and one from South America (*O. megapotamica*). Potential agents for the two species were collected in USA and housed at Agricultural Research Council – Plant Health and Protection (ARC-PHP) and Wits University in quarantine. Another potential agent for *O. megapotamica* was imported from Argentina to the Rhodes University quarantine facility. All the potential agents are cochineal insects, with both *Dactylopius opuntiae* and *D. confusus* imported from USA, and *D. ceylonicus* imported from Argentina.

There are at least two varieties of *O. engelmannii* present in South Africa, with the most damaging and widespread of them being the Northern Cape/Free State variety, which is a low growing, extremely spiny variety, with dense glochids (the small spines of the cactus that are damaging to animals that feed on them). None of the new cochineals were suitably damaging for either of the two varieties that are problematic in South Africa, but one cochineal from the USA was damaging to another variety of the plant that is a problem in Kenya. The Wits University team conducted impact assessments and host specificity testing on the cochineal that was damaging to the Kenyan variety of *O. engelmannii*, and this cochineal has now been released in Kenya. There is still some hope for the control of the Northern Cape/Free State variety because at least one large infestation has been controlled using the biocontrol agent for *Opuntia stricta*, *Dactylopius opuntiae* 'stricta'. Whether it will be suitably effective at controlling the weed in other parts of the country is not yet known, but experimental releases have been conducted.

Two of the cochineals, a lineage of *D. ceylonicus* from Argentina, and a lineage of *D. opuntiae* from the USA, are both damaging to *O. megapotamica*. Further studies to compare the efficacy of the two cochineals are required before one can be selected as the most damaging potential agent for *O. megapotamica*. The cochineal from USA has no evolutionary history with the target weed because the plant and its close relatives are not present in North America, while the cochineal from Argentina was collected from a very close relative of the target weed in an area where the target weed is present. This is a good opportunity to test whether new or old associations are more damaging to cactus weeds. The most damaging of the two cochineals will be released for the control of *O. megapotamica*.

Orange-tuna cactus

Opuntia elata is an emerging weed in South Africa. In 2000, before the biological control programme against the weed was started, there were no locations where the plant was known to have naturalised in the country, but by 2016 there were 22 localities and, in 2019, 41 localities were confirmed. This species is also very similar to other invasive Opuntia, and is therefore likely to become a more problematic weed in the future if nothing is done to curb its spread. It is found primarily in the more arid regions of the country, where few of the other *Opuntia* species can survive. We would like to acknowledge Red Meat Research and Development South Africa for assisting us with funding in this project.

RESEARCH TEAM
Phillippa Muskett, Prof. Iain Paterson

COLLABORATORS
FuEDEI

Opuntia elata is indigenous to Argentina and Uruguay, and with the help of our collaborators at FuEDEI in Argentina, a lineage of *Dactylopius ceylonicus* was imported into quarantine and is currently under evaluation as a potential biocontrol agent. As the lineage is extremely unlikely to feed on any plants outside the genus *Opuntia* and is therefore likely to be suitably host-specific for release, the CBC has started by assessing the efficacy of the potential agent, with the intention of conducting host specificity testing if it proves to be suitably damaging. To assess the efficacy of the agent, Pippa Muskett has been comparing the fitness and level of damage of this cochineal lineage on the proposed target plant, *O. elata*, with the damage that other cochineal already present in South Africa do to *O. elata* and their respective host plants. We know how damaging each of the cochineals are to their own host plants in the field, so we can compare the relative damage done to *O. elata* by the new cochineal in our quarantine experiments and extrapolate how damaging it is likely to be in the field. The preliminary results indicate that the cochineal from Argentina is likely to be suitably damaging to consider as an agent for *O. elata*. Interestingly, another cochineal insect from South America, *D. austrinus*, which is used as an agent against jointed cactus in South Africa, is also damaging to *O. elata*. Further studies will determine which of the two cochineals is the most damaging, and that cochineal will be promoted for the control of this emerging weed.

Figure C2: Flowering *Opuntia elata*. **Photo:** Phillippa Muskett

Leaf cactus

Leaf cactus, or *Pereskia aculeata*, is a very different sort of cactus. It grows as a vine or scrambling shrub and has well-developed leaves and a woody stem. Leaf cactus is indigenous to the Caribbean, Venezuela, southern Brazil and northern Argentina. It is a serious invasive in South Africa, where it grows over and kills indigenous vegetation in coastal forest habitats, resulting in dramatic declines in biodiversity. It is extremely difficult to control mechanically or with herbicides because it grows intertwined with indigenous vegetation and it can reproduce from fragments of leaves and stems. Two biological control agents have been released in South Africa, the flea beetle, *Phenrica guerini*, and the stem-wilting bug, *Catorhintha schaffneri*. The flea beetle is widely established and at some sites is very damaging to the target weed, while *C. schaffneri* was released much more recently and has established at only a few sites in the country.

Leaf cactus has recently become increasingly problematic in Australia, and colleagues at the Department of Primary Industry in New South Wales, Australia, are working together with the CBC to get both agents released there. The necessary host specificity testing for the release of the stem-wilting bug has been completed and an application for its release has been submitted. Elizabeth van der Merwe has conducted the host specificity testing and an efficacy assessment for the flea-beetle as part of her MSc degree. Her work has shown that the beetle is suitably host specific for release in Australia, and is suitably damaging to warrant release. An application for the release of the flea-beetle will therefore be compiled with Elizabeth's data soon. Hopefully, releasing biocontrol agents for leaf cactus in Australia at this early stage of the invasion will stop it from becoming as problematic as it has become in South Africa.

RESEARCH TEAM
Phillippa Muskett, Prof. Iain Paterson

STUDENT
Elizabeth van der Merwe (MSc)

COLLABORATORS
Department of Primary Industries, Australia

Figure C3: Adult *Phenrica guerini*. **Photo:** David Taylor

Figure C4: Damage on *Pereskia* from the flea beetle. **Photo:** Elizabeth van der Merwe

Torch cactus

RESEARCH TEAM
Prof. Iain Paterson

STUDENT
Tamzin Griffith (PhD)

COLLABORATORS
FuEDEI

Although cacti are usually very good targets for biological control, taxonomic confusion can result in some of them being very difficult targets. Taxonomic confusion is rife in the family Cactaceae because many cacti are highly variable within a single species, while others lack morphological differences between species. The problem is further complicated by the fact that creating herbarium voucher specimens for cacti is very difficult and the specimens are usually almost unrecognisable from the living plant that they were taken from. Torch cactus, *Trichocereus spachianus*, is a good example of how taxonomic confusion regarding the target weed can hamper biological control efforts.

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

Figure C5: A torch cactus invasion in flower. **Photo:** Tamzin Griffith

Torch cactus is widely naturalised in Europe, but its indigenous range is believed to be the arid parts of northern Argentina. The indigenous distribution is where any potential biological control agents would be found, so PhD student, Tamzin Griffith, spent most of 2019 and part of 2020 in Argentina working in collaboration with our colleagues at FuEDEI. Torch cactus is a columnar cactus, and many similar cacti have been successfully controlled using the galling mealybug, *Hypogeococcus*. The *Hypogeococcus* that has already been released in South Africa is not sufficiently damaging to torch cactus, but new lineages or species of *Hypogeococcus* were considered the most promising new potential agents, based on their success on other similar and closely related cactus weeds. Tamzin's main objective in Argentina was therefore to find a *Hypogeococcus* associated with torch cactus or its close relatives.

This search proved to be challenging as none of the surveys conducted in Argentina resulted in finding wild populations of torch cactus. After checking the known records of torch cactus, it is now thought that the species either never existed in Argentina, or it is very rare, or extinct. The species was described from potted plants at a botanical garden and there are no natural populations in South America that could be verified. Several *Hypogeococcus* species/lineages were collected from close relatives of torch cactus, and all of these have now been preliminarily screened for their efficacy against torch cactus in South Africa. Unfortunately, none of the *Hypogeococcus* are suitably damaging to warrant release. One more attempt will be made to find a suitable biocontrol agent for torch cactus, and surprisingly, this will be a cochineal insect rather than *Hypogeococcus*. The cochineal insect, *Dactylopius confertus*, differs from most other cochineal species in that it feeds on *Trichocereus* and *Harrisia* species, not the usual Opuntieae hosts. *Dactylopius confertus* was introduced into Namibia accidentally by an unknown pathway, and is now present on *Harrisia* species that are invasive around the capital city of Windhoek. Since this cochineal is known to be damaging to other *Trichocereus* species, it is worth testing its efficacy on torch cactus.

Figure C6: An experiment being run in quarantine with *Hypogeococcus*. **Photo:** Tamzin Griffith

NORTHERN TEMPERATE WEEDS

In March 2017, the CBC started a new programme, Northern Temperate Weeds (NTW). The programme aims to bring the benefits of biological control to the mountain grasslands of South Africa. The high elevation grasslands of South Africa are important systems for water security, as they provide nearly half of all the country's water run-off and are a key biodiversity hotspot with high plant endemism. The biome is also an invaluable resource, supporting livestock central to the livelihoods and economies of commercial, small-scale, communal farming/agriculture, but is under severe threat from invasive alien plants, particularly species deriving from the cooler northern temperate regions of the globe.

Thus far, research has focused on biological control feasibility and ecological and socio-economic impact studies on several northern temperate weeds, including *Berberis julianae*, *Pyracantha angustifolia*, *Rosa rubiginosa*, *Salix* spp., *Cotoneaster pannosus*, *Robinia pseudoacacia*, *Gleditsia triacanthos* and *Rubus* spp. In addition, research conducted in the USA and Europe on the pests associated with two of these species, *R. pseudoacacia* and *G. triacanthos* (both native to the USA), has provided South African researchers with the necessary foundation to initiate programmes against these weeds. Research in South Africa is currently focused on pre-release studies on the biological control of *R. pseudoacacia*, using the leaf miner, *Odontota dorsalis* Thunberg, and the locust midge, *Dasineura pseudoacacia* Fitch, and on *G. triacanthos*, using a seed bruchid, *Amblycerus robiniae* Fabricius.

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

In addition, the programme has recently also combined with Range X, an ambitious project aimed at investigating the ecological drivers of range-expanding plant species at high altitudes. The overall aim of the Range X programme is to contribute to efforts to mitigate the effect of climate change on the environment and the communities reliant on those environments. The NTW programme aims to inves-

PROGRAMME HIGHLIGHTS IN 2021

- The seed-feeding bruchid, *Megabruchidius tonkineus*, was determined to be a suitable, safe biological control agent for *Gleditsia triacanthos*.
- Collaboration with researchers at Virginia Tech University, Blacksburg, Virginia and USDA Forest Service, USA, as well as with CABI, Delémont, Switzerland have helped prioritise potential biological control agents for *Robinia pseudoacacia*.
- The drivers of *Cotoneaster pannosus* invasion in South Africa grasslands were determined.
- A new collaboration was developed with Range X project, which aims to investigate ecological drivers of range-expanding plant species at high altitudes.

tigate how future climate scenarios may affect the growth parameters of a number of the species being considered for biological control.

The programme has also championed the development of the Southern African Mountains Invasive Alien Plants Working Group. This working group seeks to increase collaboration among researchers and conservation managers to facilitate the best management and research practice for invasive alien plants (IAP) Southern African with the ultimate goal of reducing the impact of plant invasions.

Rosa rubiginosa

Rosa rubiginosa L. is a Category 1b invasive and highly abundant in the mountain regions of the eastern Free State Province; however, *R. rubiginosa* has the potential to provide an economic benefit in South Africa due to the expanding market for the shrub's fruit, rosehips. Legislation prohibits cultivation of the shrub in South Africa, limiting the economic potential as the rosehips can only be collected from wild *R. rubiginosa* shrubs. Previously the programme showed *R. rubiginosa* to have great economic potential and market growth, with very little negative economic impact, making it a conflict-of-interest species. However, the species remains an important invasive species in the South African grassland biome and the project continues investigating the abiotic and biotic drivers which facilitate or prohibit the spread of the species so that management options can be recommended.

One of the primary drivers of invasive Rosacea in South Africa is the copious amount of seed produced by the species. The importance of seeds to the reproductive success of the species is currently being determined by MSc student, Patricia Masole. Through several field measurements and laboratory experiments, Patricia is investigating fruit production, fruit availability, the number of seeds per fruit, soil seed banks, seed survival in the soil, seed viability and seed dispersers. Finally, as the plant seems to be limited to the colder regions of South Africa, she is also considering the importance of cold stratification to seed germination potentially confirming why the species is more dominant on colder north-west-facing slopes.

In South Africa, the shrub has acquired an invasive seed-feeding wasp, *Megastigmus aculeatus*, commonly known as the rose seed chalcid. Collections of seeds have revealed a high percentage of seeds are damaged by the wasp which could potentially be limiting, or at least reducing, the spread of the species. Further studies into the alternative host of the wasp as well as its biology in South Africa are underway.

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

STUDENT
Patricia Masole (UFS-MSc)

Rosa rubiginosa is part of a suite of invasive Rosaceae invading the grasslands of South Africa including the native Leucosidea sericea, commonly known as "ou hout". Interactions between seedlings and nurse plants are known to play an important role in the dynamics of plant communities, including the enhancement of invasions by exotics. Growth under exotic and native nurse species is said to encourage the growth of shade-tolerant species. Patricia is testing the nurse-plant hypothesis with *Rosa rubiginosa* by comparing the influence of the invasive thorny *R. rubiginosa* to the thornless native *L. sericea* to the recruitment of native and exotic woody species in the grazed grasslands of the eastern Free State.

Figure D1: MSc Students Patricia Masole, Karabo Moloi and Dr Sandy Steenhuisen investigating thorny Rosacea species acting as 'nurse plants' for woody invasive species. **Photo:** Grant Martin

Orange firethorn

Lehlohonolo Adams conducted a MSc within the CBC, finishing in 2020, where he showed that, where it is found invading the grassland biome, more than one million seeds are produced per square metre of *P. angustifoia* (orange firethorn) invasion, with a probability of up to 10 million seeds being produced per square metre. 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 a low persistence of propagules in the soil. This research has prioritised orange firethorn as one of the fastest spreading and most damaging invasive species currently invading the grassland biome. Lehlohonolo is still affiliated to the CBC but has since started a PhD with Prof. Colleen Downs at the School of Life Sciences, University of KwaZulu-Natal, where he aims to investigate what is driving fleshy fruit invasions globally and aims to conduct several investigations into fleshy fruited plants in South Africa.

RESEARCH TEAM
Dr Grant Martin (UFS-CBC), Sandy Steenhuisen (UFS-ARU), Colleen Downs (UKZN)

STUDENT
Lehlohonolo Adams

COLLABORATOR
Yunnan University, China

Figure D2: PhD candidate Lehlohonolo Adams deploying a camera trap to determine the effect of altitude on seed dispersers. **Photo:** Grant Martin

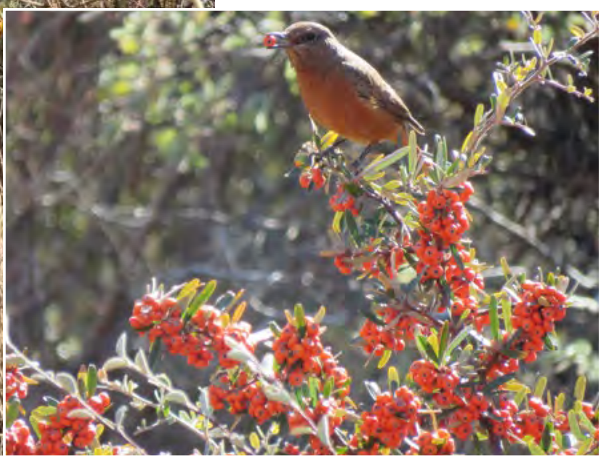

Figure D3: Cape Rock Thrush eating the berries of the *Pyracantha angustifolia*. **Photo:** Grant Martin

Silverleaf cotoneaster

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

STUDENT
Karabo Moloi (MSc)

Cotoneaster pannosus (silverleaf cotoneaster), a native species of the Himalayan mountain range, is another red-fruited, fleshy rosacea species becoming increasing problematic in the grassland biome of South Africa. The species forms dense, impenetrable thickets, particularly along river courses, excluding other species and making access to water difficult. It was probably introduced as a horticultural species as it has impressive bouquets of berries which are clearly visible in winter. These berries are assumed to be the mechanism driving the invasions.

Karabo Moloi is currently completing her MSc on the species, exploring its seed biology by investigating fruit production, fruit availability, soil seed banks and seed dispersers. Results show that *C. pannosus* has a long fruiting duration that persists from April until October and produces an average of 4 million seeds per m^3 with a significantly positive relationship between shrub volume and seed production. Seeds were found to survive extended periods in the soil bank and demonstrated over 50% seed viability after being in the soil for over a year. In addition, consumption of fruits by frugivorous birds confirmed that long-distance seed dispersal depends on birds, and remote camera observations revealed that rodents seem to be seed predators. The long seed dormancy of *C. pannosus* means that seeds dispersed in hostile environments remain viable until conditions are ideal for successful germination. A number of the identified traits suggest *C. pannosus* will be a formidable invader, with the ability to spread rapidly within and to new ecosystems. Karabo aims to start a PhD in 2022 further investigating a number of ecological drivers of fresh fruit invasions into South African grasslands.

Figure D4: MSc student Karabo Moloi standing in a dense *Cotoneaster* invasion near the town of Clarens in the Free State Province. **Photo:** Grant Martin

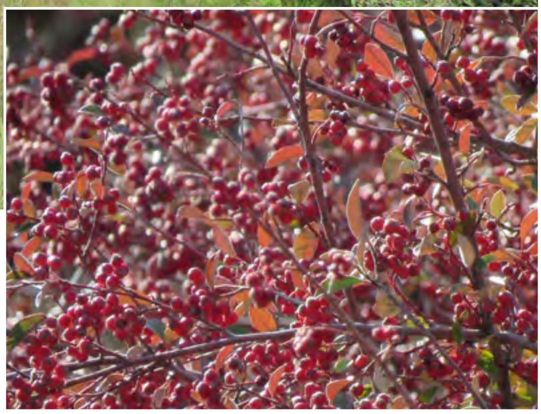

Figure D5: Dense red berries typical of *Cotoneaster* species often seen in the winter months in the grassland biome. **Photo:** Grant Martin

Black locust tree

Gerald Chikowore (PhD student) was tasked with determining how our native grassland ecological systems are being impacted by a prolific invader from North America, *Robinia pseudoacacia* (Fabaceae), commonly called black locust. Gerald showed that black locust alters the biophysical components of grassland ecosystems and subsequently native plant and arthropod community assembly. He found that mechanisms, such as shading, significantly reduce temperatures and light availability in the understory. These alterations drive changes in vegetation communities with differences of approximately 96% in grasses, and also drive out native grassland arthropods, particularly grasshoppers (Acrididae). Age and density of black locust invasions also influence the composition of understory vegetation with a noticeable successional trend from the absence of understory vegetation in dense younger stands (Canopy cover index = 60%), the dominance of ruderal herbaceous species in intermediate aged stands (Canopy cover index = 27%), and dominance of alien nitrophilous grasses in older, sparse stands. As a result, ecosystem services such as grazing are significantly reduced. The tree also competes with commercial fruit trees for pollination services, potentially compromising fruit quality and yield. Furthermore, surrounding communities do not significantly utilise black locust trees, and prefer their removal instead.

Figure D6: PhD student Gerald Chikowore setting up a time lapse camera to monitor *Robinia pseudoacacia* phenology. **Photo:** Gerald Chikowore

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

STUDENTS
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COLLABORATORS
Virginia Tech, USA and CABI (Switzerland)

These impacts justify the need to sustainably manage black locust invasions and other invasive alien species to preserve the integrity and function of montane grassland ecosystems. Gerald's work has not only highlighted the impact of invasive trees in the grassland biome, but has also derived important baseline data with which management operations such as biological control can be compared. Gerald is starting a postdoc within the CBC in 2022.

Options for biological control agents for *Robinia pseudoacacia*

Research into the invasion, ecology and management options of *Robinia pseudoacacia* continued into 2021. PhD candidate, Abigail Wolmarans, once again conducted annual field surveys as part of a pre-release study to confirm that no natural enemies of *R. pseudoacacia* have been inadvertently introduced into South Africa as has happened in many other countries. However, some trees were found to have damaged leaves. Further investigation showed isolated stands in the montane grassland biome were in exceptionally good condition with very little damage to their leaves, while trees that formed mixed stands and were usually found close to habitation had a higher number of damaged leaves. Generalist herbivores such as *Anoplognathus* sp. were found to be doing most of this damage.

Seed ecology studies with the addition of sites in Switzerland were also continued. These studies continue to show very low seed numbers in the soil bank in South Africa compared to high numbers which were recorded in Switzerland. In order to determine the destiny of fallen seeds in South Africa, camera traps were set around bait stations under undisturbed stands. Interestingly these did not provide much evidence about what is occurring to the seeds in South Africa, while bait stations in Switzerland showed rodents to be prevalent seed predators of *R. pseudoacacia* seed. Owing to the COVID 19 pandemic, desk-top prioritisation studies have become even more valuable. By using available resources such as pre-release survey data from South Africa, the native range in the USA as well as Europe (where both *R. pseudoacacia* and some of its associated insects have become naturalised), phylogenetics, ecological niche modelling, citizen science initiatives and available literature, we were able to identify close to 64 candidate biological control agents and have prioritised three foliage feeding agents namely, *Odontota dorsalis* (locust leaf miner), *Phyllonorycter robiniella* and *Obolodiplosis robiniae* (locust gall midge). Unfortunately, *Obolodiplosis robiniae* has not survived in quarantine facilities in South Africa, making conventional host specificity testing impossible, but we are currently considering if there is enough evidence available to support the interpretation that *O. robiniae* is sufficiently host specific for release in South Africa. No congeners occur in South Africa, but representatives of the Robinioid clade of species are present. Two of these, *Sesbania sesban* and *Sesbania punicea* (itself an invasive species) are morphologically similar to *R. pseudoacacia*. Evidence from surveys on closely related species conducted in in the USA (native range) and Europe (where *O. robiniae* is established) support the interpretation that the host range of *O. robiniae* is restricted to *R. pseudoacaacia*. This finding was not unexpected as *Obolodiplosis robiniae* belongs to a group of closely related gall midge species which are all associated with the Leguminosae and which all have restricted host ranges. This study should provide sufficient evidence to seek approval for the release of *O. robiniae* as the first biological control agent for the black locust in South Africa.

Figure D7: PhD candidate Abigail Wolmarans and Grant Martin setting up seed fall traps . **Photo:** Grant Martin

Honey locust tree

Gleditsia triacanthos L. (Fabaceae) (honey locust) is a fast-growing, deciduous tree indigenous to the United States of America. Introduced around the world as an ornamental tree, it has become invasive in a number of countries. Where it is invasive, *G. triacanthos* competes and replaces indigenous species; it creates dense stands along watercourses, posing a significant environmental threat. In South Africa, *G. triacanthos* is regarded as one of the country's fastest-spreading weeds. *Gleditsia triacanthos* produces numerous seeds contained in large hanging pods. Once dislodged from the pods, the seeds are dispersed by birds and mammals, including livestock, which eat the pods. It has been suggested that the seeds should be the target for a biological control programme. Sara Salgado Astudillo completed her MSc in 2021, with distinction for her study. In the study, Sara used two different modelling programmes, CLIMEX and MaxEnt, to predict areas where *G. triacanthos* could find favourable growing conditions in South Africa; both species distribution models showed that most of the country is suitable for *G. triacanthos* and that it will probably continue to spread, if left unmanaged, into new bioregions, such as the Karoo.

In South Africa, the Asian seed-feeding bruchid, *Megabruchidius tonkineus* (Pic, 1914) (Coleoptera: Chrysomelidae: Bruchinae) has been recorded in the plant's seed pods and has been considered as a biological control agent. The insect was not released as part of a formal biological control programme and neither host specificity nor impact studies were conducted on the species prior to its discovery. In 2017, a decision was made to reconsider its status as a biological control agent until further details of its biology, host specificity, and impact on the seeds of *G. triacanthos* in South Africa were available. Sara was able to show that *Megabruchidius tonkineus* has established across the entire *G. triacanthos* population in South Africa, damaging approximately 9% of seeds. Laboratory studies show that *M. tonkineus* completes its larval development in the seeds of *G. triacanthos* in about 66.80 ± 0.6880 SE days before eclosing. In addition, the adult females oviposit on the following Fabaceae species: *Arachis hypogaea*, *Albizia julibrissin*, *Cicer arietinum*, *Pisum sativum*, *Dipogon lignosus*, *Peltophorum africanum*, *Podalyria buxifolia*, *Senegalia burkei*, *Umtiza listerina* and *Vachellia sieberiana*. However, larval development was limited to *G. triacanthos*. It was concluded that the seed-feeding beetle is not a threat to native Fabaceae species in South Africa and is once again considered as a biological control agent in South Africa.

RESEARCH TEAM
Dr Grant Martin, Prof. Martin Hill

STUDENT
Sara Salgado Astudillo (MSc)

COLLABORATORS
Virginia tech, USA & CABI (Switzerland)

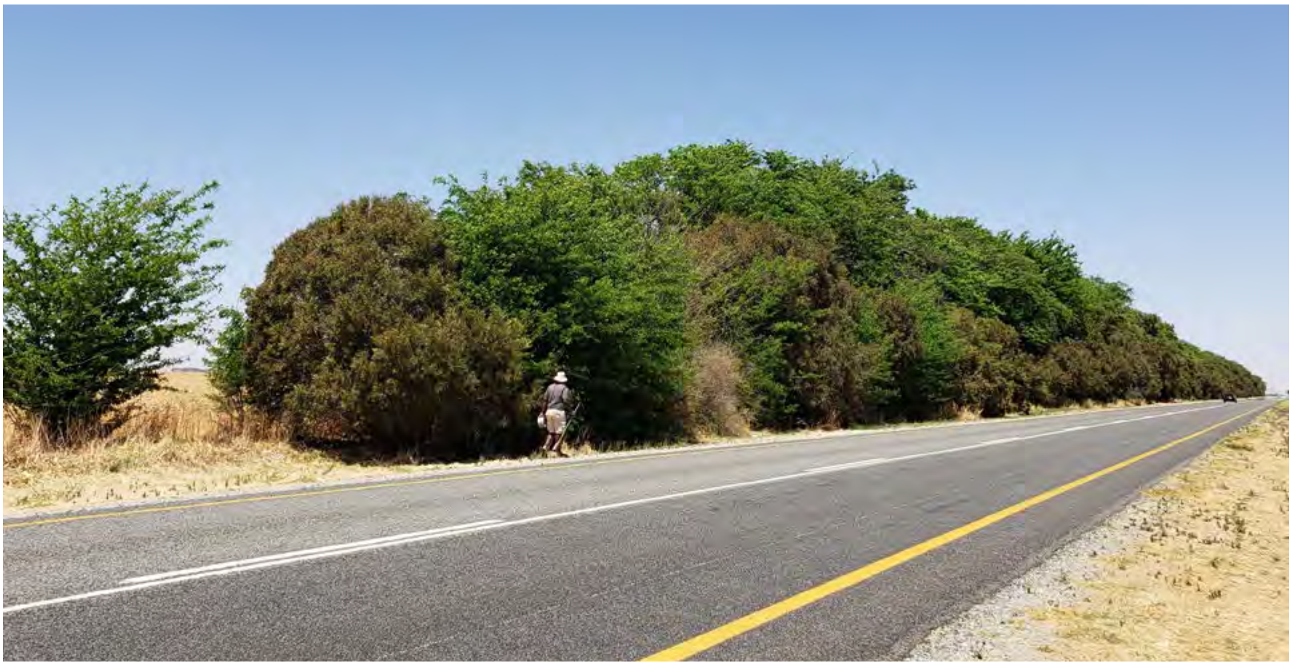

Figure D8: Dr. Evans Mauda assisting MSc student Sara Salgado Astudillo surveying *Gleditsia triacanthos* growing roadside on the Road- R700 in Free State Province. **Photo:** Grant Martin

Figure D9: Grant Martin and Sandy Steenhuisen in Golden Gate National Park. **Photo:** Patricia Masole

Wintergreen barberry

Berberis julianae, commonly known as wintergreen barberry, is a shrub species native to China that has been introduced into South Africa and is found growing along the riverbanks of a few highveld locations. Keet *et al.* (2016) identified a need to put control measures in place to manage an increasing population in Golden Gate Highlands National Park (GGHNP) in the Free State Province. After assessing the plant demographics within the park, cut stump and seedling removal control measures were implemented. The authors recommended that follow-up research should be done to check if their control measures were successful or not. Patricia Masole has conducted a very successful Honours project aimed at mapping and comparing the current distribution of *B. julianae* in GGHNP and to investigate the change in population demographics to that recorded by Keet *et al.* (2016). She was able to show that the number of seedlings had multiplied in number from 6 in 2014 to 588 in total in 2020, while the number of mature shrubs had decreased. In conclusion, the control measures that were put in place by Keet *et al.* (2016) were successful in reducing the adult population; however, follow-up control of seedlings is still required. The population will be monitored over the next couple of years to determine if further management options should be considered.

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

STUDENT
Patricia Masole (UFS-MSc)

COLLABORATORS
Virginia Tech, USA and CABI (Switzerland)

The Range X project

The high elevation regions of South Africa are some of the most severe environments for plant species resulting in unique, specialised, and endemic species surviving there. However, with climate warming, it can be expected to change, opening up this unique niche to both native and invasive 'lowland' plants. This will have a major impact on ecology, livelihoods, endemic alpine species, and water production in these high areas. It is therefore increasingly important to understand the process of species migrating upwards as a result of climate warming to inform and guide policy as well as management within these regions. The Afromontane Research Unit, who are pioneering mountain research in Africa, have partnered with the European Union's Horizon 2020 initiative called 'The Range X project' which aims to investigate mechanisms underlying the success and impacts on biodiversity and ecosystem functioning of range-expanding species under climate change, across the globe. The ARU component of Range X is funded by the Department of Science and Innovation (DSI) through BiodivERsA. The CBC's Northern Temperate Weeds programme is collaborating with the ARU in this initiative and are involved in a number of dynamic and interesting investigations within the project which will have direct implications for the management of invasive species in mountain regions in a changing climate.

RESEARCH TEAM
Dr Grant Martin, Dr Ralph Clark (UFS-ARU), Dr Sandy Steenhuisen (UFS-ARU), Dr Ona Gwate (UFS-ARU), Muxe Dlomu (UFS-ARU)

Specifically, the CBC is involved in investigating how invasive *Rosacea* species might perform at different elevations under climate change conditions. This is being done by using a full-factorial warming experiment using Open top chambers (OTCs) placed on the top of Sentinel Peak (3,100 m) and comparing them to OTCs positioned at lower altitudes down below near the Witsieshoek (2,180 m). Open top warming chambers are widely used to mimic global warming as they are very effective in elevating ambient temperatures by 1-2 °C. Secondly, the CBC aims to determine how the invasive species and the native biodiversity responds to standard management practices at different altitudes by using matched plot designs and clearing large areas of woody invasives over an increasing altitude gradient. Being part of this global initiative should contribute to the understanding of the impact of climate change of invasive species in our mountain systems and provide insights on how it may affect management.

Figure D10: Open top chambers set up for the Range X project. **Photo:** Grant Martin

Figure D11: Muxe Dlomu, Ona Gwate and Grant Martin setting up temperature buttons in the open top chambers. **Photo:** Sandy Steenhuisen

A proposed national strategic framework for the management of invasive alien plants for southern African mountains

The mountains of southern Africa support critically important ecosystem services – notably water production – and are exceptionally rich in floral and faunal biodiversity and endemics. However, these mountains are marginalised regions and are under threat from detrimental land-uses, unsustainable use of natural resources, climate change, poor governance, and IAP. Invasive alien plants in particular pose a substantial and continuously increasing problem in driving ecosystem changes, often with dire results. The different climate, altitude and relief found in these mountains support a unique suite of IAPs compared to their surrounding landscapes, and because of the steep slopes and dangerous terrain, conventional methods of research and management are inappropriate. This working group seeks to increase collaboration among researchers and conservation managers to facilitate best management and research practices for IAPs on southern African mountains, with the ultimate goal of reducing their spread and impact. Thus far, the group has taken some initial steps such as presenting the concept and goals of the working group at the Annual Invasive Species Symposium. The presentation received significant support. In addition, the group has also started developing lists of invasive species within each mountain range which can be used as a benchmark for the group to start management plans (See Canavan et al., 2021a; Canavan et al., 2021b)

INVASIVE TREES PROGRAMME

The Invasive Trees Programme team is made up of Emeritus Associate Professor John Hoffmann, Fiona Impson, Catharina Kleinjan and Cliff Moran all of whom are based at the University of Cape Town within the Plant Conservation Unit (PCU) in the Department of Biological Sciences and have been part of the CBC Consortium since 2017. This team has many years of experience in the field of biological control of invasive trees and has developed strong collaborations with several international institutions.

The Invasive Trees Programme continues its focus on the biological control of several Australian *Acacia* (wattle) species, and also *Prosopis* (mesquite) originating from both South and North America. Australian acacias and *Prosopis* remain particularly important and destructive invaders across large parts of South Africa. Both groups of trees share common uses in being beneficial agroforestry trees, providing timber, fuel, fodder, and shade, but where they have become invasive, their negative impacts on biodiversity and water resources outweigh these benefits and management and control of further spread of these species is necessary.

The commercial utilisation of several *Acacia* species has largely restricted biological control to the use of agents that have no impact on the vegetative growth of these trees (i.e., agents that attack the buds, flowers or seeds). Despite this constraint, several agents are now well established and curbing the invasiveness of the acacias, and efforts are underway to introduce additional, different species of agents.

While there has been some success with biological control of *Prosopis* to date using two seed-feeding beetles, levels of control have not been enough to alleviate the problem. Engagement with stakeholders in the farming community created an incentive to expand the biological control programme to include agents that are destructive on other parts of the trees; permission for release of two additional agents has been granted and releases of both commenced in the spring of 2021.

Despite the continued challenges during 2021, both with lockdown measures and funding, good progress has been made. Reviews on the biological control of *Acacia* and *Prosopis* in South Africa, which are updated every 10 years, have been completed and have been published. Furthermore, analyses of the results of some of the ongoing long-term Acacia studies have been consolidated and published. Collaboration with the Instituto Politécnico de Coimbra in Portugal, Tel Aviv University in Israel, and Fundación para el Estudio de Especies Invasivas in Argentina has continued.

PROGRAMME HIGHLIGHTS IN 2021

- Release of the flower-galling midge, *Dasineura pilifera* on *Acacia baileyana* and *Acadia decurrens*.
- Release of *Coelocephalapion gandolfoi* for the control of *Prosopis* spp.

Australian acacias

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

COLLABORATORS
University of Tel Aviv, Israel; Instituto Politecnico de Coimbra, and Centre for Functional Ecology, University of Coimbra, Portugal; FuEDEI

Thanks to the lifting of some international travel restrictions earlier in the year, colleagues from Tel Aviv University, Israel managed to travel to South Africa in late March to collect the seed-feeding weevil, *Melanterius castaneus*, to further their biological control programme against *Acacia saligna* in Israel. Approximately 800 adult weevils were collected, which enabled the establishment of five release sites in Israel.

In early April, an opportunity arose to import material of the flower-galling midge, *Dasineura pilifera*, from Australia. During mid-late August, 833 individuals (of which 515 were female) emerged from quarantine in Stellenbosch and were released into five sites in and around Stellenbosch and at one site at Grabouw. Galls were also shipped to Makhanda, where a further 431 adults (211 females) were released at three sites. By late October, the presence of galls was confirmed at three of the five release sites in the Stellenbosch area and one of three sites in the Eastern Cape.

During September 2021, an application requesting permission to release the gall-forming wasp, *Perilampella hecataeus*, was submitted to the Directorate of Plant Health in the Department of Agriculture Land Reform and Rural Development. It is hoped that further progress will be made on this biocontrol agent in 2022.

Small collections of both *Melanterius acaciae* (441 adults) and *Melanterius maculatus* (267 adults) were made during November. These were shipped to Portugal to allow further host specificity testing to be carried out.

Figure E1: *Dasineura pilifera* galls to show signs of initial establishment. **Photo:** Fiona Impson.

Prosopis

Permission was granted in 2019 for release of the *Prosopis* podlet weevil (*Coelocephalapion gandolfoi*). This weevil feeds and oviposits on immature *Prosopis* pods and the adults emerge before the pods ripen. It is anticipated that the life cycle of this species will allow it to circumvent the impacts of vertebrate pod consumers. Consumption of the pods by livestock and wildlife has proved to be an impediment to the success of the seed-feeding beetles already present in South Africa as biological control agents on *Prosopis*.

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

STUDENT
Gretha van Staden

COLLABORATOR
Dr Fernando McKay, FuEDEI.

The lifting of travel restrictions enabled the import of a consignment of adult weevils in November 2021. These were collected in Argentina by Dr Fernando McKay (FuEDEI) and shipped to Dr Blair Cowie at the quarantine facility at the University of the Witwatersrand (a CBC consortium partner). After processing in the quarantine facility, five releases, consisting of just over 125 weevils per site, were undertaken in the central Karoo.

An additional notable development for 2021 is that releases of the leaf-tying moth *Evippe* sp. #1 were initiated by Fritz Heystek and Yogi Kistensamy (Agricultural Research Centre, Roodeplaat). The CBC consortium partners will be involved in further development of this agent and monitoring of its impacts.

Figure E2: The journey of the *Prosopis* podlet weevil. **Photos:** Fernando McKay, Blair Cowie and John Hoffmann

BUGWEED

Figure F1: *Anthonomus morticinus* feeding on a bugweed flower in quarantine at Wits University. **Photo:** Nic Venter.

The well-known invasive tree, bugweed (*Solanum mauritianum* Scoop.), indigenous to subtropical South America, is one of our most long-standing and widespread environmental weeds. Research efforts in South Africa have culminated in the release and establishment of two insect biological control agents, namely the lace bug, *Gargaphia decoris*, and the flower-feeding weevil, *Anthonomus santacruzi*, in 1999 and 2008, respectively. Both agents have established in the field at low-altitude sites, but with damage being localised and largely limited, possibly owing to climatic incompatibility. Prominent bugweed invasions in South Africa also occur in high-altitude regions (> 1000 m) that experience cold winters. Owing to this, an additional agent, the flower-feeding weevil, *Anthonomus morticinus*, was collected from temperate regions of Uruguay during 2020. These regions are climatically more similar to the cooler high-altitude regions of South Africa where bugweed remains a problem. Laboratory thermal assessments have since indicated that *A. morticinus* appears better adapted to lower temperatures than its congener, *A. santacruzi*. Allied experiments on the humidity requirements of *A. morticinus* are also underway, with encouraging indications that the species is more tolerant of lower humidity than *A. santacruzi*.

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

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

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

Host range testing of *A. morticinus* is ongoing with results being promising thus far. Compared to *A. santacruzi*, *A. morticinus* appears to exhibit an even narrower host range on commercially grown Solanaceae. As plants from the Solanum family are important agricultural plants, mainly grown for human consumption, it is imperative that any potential bugweed biocontrol agents do not favour these species. Work is still underway to test *A. morticinus*' host range on Solanaceae species that are native to South Africa.

In addition, Manaaki Whenua LandCare Research in New Zealand is collaborating with the University of the Witwatersrand to conduct host range testing of *A. morticinus* on Solanaceae species indigenous to New Zealand. Seeds have been imported from New Zealand and work is underway to grow and conduct the host range testing at the Wits Quarantine Facility.

Figure F2: *Solanum* species being grown in the Wits' Quarantine Facility for host testing of Solanum weevils as potential biocontrol agents of bugweed. Although *A. santacruzi* has already been cleared for release in South Africa, it is being retested in these trials to benchmark the feeding behaviour of the new species, *A. morticinus*. **Photo:** Marcus Byrne

TAMARIX

Invasive *Tamarix* species (Tamaricaceae) are trees that become dominant weeds in riparian ecosystems found mainly in the central to western half of South Africa. The National Environmental Management Biodiversity Act 2014 classifies two Eurasian species, *T. chinensis* Lour. and *T. ramosissima* Ledeb., as Category 1b invaders. In 2014, a research programme was initiated at Wits University to explore the feasibility of using biological control against these species. However, *Tamarix usneoides* E. Mey. ex Bunge is an additional species that is native to South Africa and therefore poses a challenge to avoid non-target effects from any biological control agents used against the invasive species. The indigenous *T. usneoides* grows in semi-arid regions with low annual rainfall and occurs widely in the Northern Cape and some parts of the Western Cape Province, close to the border with Namibia. The invasive *Tamarix* are largely distributed in the cooler, wetter interior of the Eastern and Western Cape Provinces. However, the two invasive species and the indigenous *T. usneoides* readily hybridise to form three additional hybrid genotypes. Interestingly, the Swart River in Prince Albert, Western Cape and the Groot River, Eastern Cape are the only localities known where all six *Tamarix* genotypes co-occur (the three parental species and their respective hybrids). Measurements of *Tamarix* land cover in the Eastern and Western Cape Provinces show that the weed continues to spread. Hence, management interventions are required, of which biological control offers the best long-term prospects.

The Wits' research has to date rejected the leaf-feeding beetle, *Diorhabda carinulata*, and the scale insect, *Trabutina mannipara*, as potential biological control agents of alien *Tamarix* due to non-target feeding on native *T. usneoides*. However, a phylogenetic disjuncture between the indigenous *T. usneoides* and the invasive genotypes, which places them in separate clades (groups), provides hope of finding host-specific biological control agents for the invasive species that will not recognise the native species as a potential host. Ongoing research is currently testing the *Tamarix* weevil *Coniatus tamarisci* in quarantine, for host specificity on the full range of *Tamarix* genotypes that occur in South Africa. In addition, differences in secondary metabolites between the *Tamarix* genotypes are being investigated to explore the signals that herbivorous insects use to select between potential host plants. Studies on other weeds have shown that insect host plant selection can be influenced by the secondary metabolites of the potential host plant. Being from different taxonomic clades suggests that the indigenous and alien species should differ in their secondary metabolite profile and help guide the selection of potential biocontrol agents.

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

STUDENTS:
Sivenathi Hatile (MSc, Wits)

COLLABORATORS:
Alekzandra Szewczuk (PhD, Wits, and Centre for Invasion Biology, Stellenbosch University)
Dr Massimo Cristofaro (Biotechnology and Biological Control Agency (BBCA), Rome, Italy)

A culture of *C. tamarisci* was imported into the Wits Quarantine Facility in October 2020. This has recently been supplemented by additional importation of weevils in December 2021. The weevils have been tested in small-scale, petri-dish trials against the range of *Tamarix* genotypes they will encounter in the field in South Africa. The results so far are encouraging, suggesting some level of feeding discrimination against the indigenous *T. usneoides*. This is now being further explored in full-scale host specificity experiments, using whole trees in quarantine, that are tracking the weevils' behaviour through from oviposition to complete larval development. In addition, qualitative exploration of secondary metabolites has revealed some differences in polyphenols and terpenoids between the different Tamarix taxa. This is now being quantitatively investigated further, using high-performance liquid chromatography (HPLC).

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

Figure G1: Alekzandra Szewczuk collecting GPS points of the invasive *Tamarix chinensis* in the field near Prince Albert, for ground truthing satellite images. The GPS coordinates are collected as reference points for either training or validation points in the classification models. **Photo:** Tyler Goodburn

INTERNATIONAL WEED PROJECTS

The CBC has been contracted by several international biological control research institutions, most notably in the USA, Australia and New Zealand, to undertake surveys on indigenous plants that have become invasive elsewhere for potential agents.

African boxthorn

African boxthorn (*Lycium ferocissimum*) is a noxious weed in Australia that is native to southern Africa. The CBC is assisting CSIRO, Australia in developing a biological control programme against the weed with the ultimate goal of reducing the negative impacts associated with the plant. Since 2017, the CBC staff and students at Rhodes University have conducted native-range surveys for potential biological control agents in South Africa. Over this period, a number of herbivorous arthropods has been collected and identified. Four insect species and one pathogen species have been prioritised as potential biological control agents owing to their impact on the plants and their host specificity. Host specificity testing on the fungus, *Puccinia rapipes*, the ladybird beetle, *Cleta eckloni*, and the flea weevil, *Neoplatygaster serietuberculata* has already taken place at CSIRO, Australia. Over the last year, there has been a greater emphasis on plant host specificity testing for *Neoplatygaster serietuberculata*. Preliminary results of 'no-choice' tests have revealed that the weevil can consume other *Lycium* species of economic and biodiversity importance in the absence of *L. ferocissimum*. Consequently, 'multi-choice' and multi-generational tests are now being carried out at Rhodes University to determine if this non-target feeding on other *Lycium* species is incidental, exploratory, or forced, or if the weevil can successfully live for multiple generations on the non-target *Lycium* species. Most of these tests will be conducted at Rhodes University as quarantine space is limited at CSIRO, Australia. Native range surveys continue to be carried out in South Africa to identify stem- and root-mining/feeding insects with biological control potential and that were missed in the initial surveys that focused on foliage feeders.

RESEARCH TEAM
Dr Grant Martin, Dr Lenin Chari (Postdoc), Mr Wandisile Mdiza (research technician)

COLLABORATOR
CSIRO, Australia

PROGRAMME HIGHLIGHTS IN 2021

- Three shipments of *Neoplatygaster serietuberculata* were exported to Australia.
- An Australian indigenous *Lycium* species, *Lycium australe*, was imported into South Africa and 25 plants are currently being reared in quarantine for multi-choice host specificity testing.
- 25 Goji berries (*Lycium barbarum*; an economically important crop in Australia) are currently being reared at the Waainek Facility (Rhodes University) to be used for multi-choice host specificity testing to access any damage that could be done by the weevil.
- Two surveys for stem-mining insects were conducted in the Western Cape Province.

Figure H1 (A&B): Stem-boring *Curculionidae* larvae collected from dissected stems of *Lycium ferocissimum* showing tunnels created by the larvae and a still unidentified insect larva. **Photos:** Lenin Chari

Adult weevil

Leaf mining larva

Weevil egg

Figure H2: Developmental stages of leaf feeding weevil, *Neoplatygaster serietuberculata*; a potential biological control agent of *Lycium ferocissimum* that is undergoing host specificity testing in Australia and South Africa. **Photos:** David Taylor and Lenin Chari

Ice plant

Cryophytum crystallinum commonly called crystalline ice plant, gets its name from the massively enlarged bladder-cells that make it appear to have droplets of dew or icicles on the leaves and stems. The cells are filled with hypersaline solution that the plant accumulates from its surroundings. In South Africa, it is indigenous and found along the coast of the Western Cape and Northern Cape provinces. In California (USA) and Mexico, it has become a serious environmental pest along the coastal region and on offshore islands. The plant becomes so abundant that it excludes all other plants, resulting in a dramatic decline in indigenous plant diversity and associated faunal diversity. The offshore islands of California have some very rare and endemic plant and animal species that are directly threatened by crystalline ice-plant infestations. The weed is an ecosystem engineer because it accumulates salt in the bladder cells, and this is then leached back into the topsoil when the plant dies (it is usually a short-lived annual). This results in an increase in salt in the topsoil, making it unsuitable for the growth of almost all other plants species except the specialist halophyte (salt-loving plant), crystalline ice-plant.

Hopefully, a solution for this problem will be found in the indigenous distribution in South Africa. Caitlin Webb, an MSc student at the CBC, has conducted surveys for natural enemies and two very promising potential agents have been discovered. These are the stem-boring weevil, *Lixus carinerostris*, and root-girdling weevil, *Calodemas prolixus*. The only known host plants for both species are *C. crystallinum* and one other very closely related, and very similar, congeneric species. This is promising, because there are very few plants in USA or Mexico that are closely related to ice-plant, so there is a good chance that one or both of these potential agents may be suitably host-specific for release. Caitlin's MSc will determine where in the indigenous distribution the ice-plant in USA and Mexico came from, and she is conducting preliminary host specificity tests to determine if one of the potential agents (the stem-boring weevil) should be imported into quarantine in USA for further testing.

RESEARCH TEAM (WITS):
Prof. Iain Paterson

STUDENT
Caitlin Webb (MSc)

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

Figure I1: Ice plant flowers **Photo:** Iain Paterson

Figure I2: The potential agent, stem-boring weevil, *Lixus carinerostris* that is being investigated. **Photo:** Iain Paterson

GRASSES

Invasive alien grasses have largely not featured in biological control efforts globally, yet they are often associated with major environmental and socio-economic impacts. Many of the most problematic invasive grasses are native to South Africa, so the CBC is in an excellent position to develop biocontrol programmes against these species. The CBC have established an alien grass team and their research has included native range surveys and host specificity testing of potential biocontrol agents. Evidence from these projects is increasingly showing that prospects for grass biocontrol are good, whereby target grasses have been found to support damaging and host-specific natural enemies. Continued assessments are anticipated to lead to the rollout of biocontrol on a number of new alien grass targets.

PROGRAMME HIGHLIGHTS IN 2021

- Two stem-boring wasps of Giant rat's tail grass, both *Tetramesa* spp., have been tested against >65 non-target grass species, and are suitably host-specific to export to Australia for further testing as potential biocontrol agents of *Sporobolus* spp.
- Two additional stem-boring wasps, *Tetramesa* spp., have been recorded on African lovegrass. They are currently undergoing field and laboratory-based host specificity testing in South Africa to assess their suitability as potential biocontrol agents.
- A DNA barcoding database of > 120 sequences has identified at least four potentially new *Tetramesa* species from South Africa that need to be formally described. DNA barcoding has allowed us to test the host specificity of *Tetramesa* specimens collected from an array of non-target grasses.
- Dr Guy Sutton, Dr Canavan and Prof Paterson contributed to a paper that highlighting important advances made towards improving methods of conducting grass biocontrol programmes in the field within the native distribution, which was published in the Journal of Applied Ecology.

Giant rat's tail grass

The giant rat's tail grasses, *Sporobolus pyramidalis* and *Sporobolus natalensis*, are two African grasses that invade rangelands and pastures in eastern Australia, costing the livestock industry approximately AUS$ 60 million per annum in grazing losses. The CBC has been evaluating prospects for biological control of these species since 2017. Surveys across South Africa have identified three stem-boring *Tetramesa* wasps (Hymenoptera: Eurytomidae) that appear to attack only *S. pyramidalis* and *S. natalensis*, despite surveying more than 65 other non-target grass species, and reduce seed production and plant survival. The two most damaging species, *Tetramesa* sp. 1 and *Tetramesa* sp. 2, have been tested against >20 non-target grasses in

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no-choice host specificity tests, which confirmed that the wasps are host specific. The Covid-19 pandemic has prevented the importation of both species into quarantine in Australia. Once travel restrictions are lifted, both wasps will be sent to Australia where they will be tested against a number of native Australian grasses before being considered for release. The work performed during this project has recently been published in the *Journal of Applied Ecology*, providing a framework and case study of how increasing the focus and effort during native-range surveys may improve and streamline the detection and prioritisation of potential biological control agents.

African lovegrass

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STUDENT
Liam Yell (M.Sc.)

COLLABORATOR
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African lovegrass, *Eragrostis curvula*, is another African grass that has becomes highly problematic in Australia, particularly in New South Wales (NSW). Dense infestations can reduce grazing capacity by over 50% and farm profitability by 25%. In 2019, the CBC initiated preliminary surveys across southern Africa in search of potential biocontrol agents, in a collaboration with the NSW Department of Primary Industries. Surveys conducted in 2021 on *E. curvula* and other closely related grasses (particularly other *Eragrostis* species) identified two promising candidate control agents: two undescribed *Tetramesa* spp., whose larvae feed within the grass culm. Subsequent surveys, however, have recorded both *Tetramesa* spp. from other native South African Eragrostis species growing sympatrically with *E. curvula*. Six and 13 non-target grass species have been offered to the two *Tetramesa* spp., respectively, under greenhouse conditions in South Africa. No non-target damage or larval development has been recorded on any grass species other than *E. curvula*, to date. Additional no-choice and choice host range testing is underway to determine the suitability of the two *Tetramesa* spp. as potential control agents for *E. curvula* in Australia, including the testing of a number of native Australian *Eragrostis* species under quarantine conditions in South Africa.

Figure J1: *Eragrostis curvula* found in grasslands, Free State. **Photo:** Kim Canavan.

Guinea grass

Megathyrsus maximus is a perennial African grass that has been introduced in many regions outside of its native distribution for pasture development. It has become a serious environmental pest in Texas, USA, where it reduces grazing capacity, alters natural fire regimes, and negatively effects native wildlife populations. Previous biocontrol surveys had been performed in Kenya. However, DNA samples provided by the CBC indicated that Guinea grass populations in Texas originated from southern Africa. This resulted in the CBC initiating a collaboration with the USDA, BBCA and the University of Texas to identify potential biocontrol agents for Guinea grass from southern Africa. Preliminary surveys in KwaZulu-Natal identified at least four potential agents, including a seed-attacking weevil (Curculionidae), a stem-boring beetle (Scolytidae), an eriophyoid mite (Eriophyidae) and a stem-boring wasp (*Tetramesa* sp.). Additional surveys in 2021 identified an additional two potential biocontrol agents, a stem-galling cecidomyid fly (Cecidomyiidae) and a root-and-crown feeding scale insect (Coccidae). Preliminary field host range surveys have not recorded either insect on any grass species other than Guinea grass. Efforts are underway to obtain formal identifications for all the above insects. Laboratory cultures and controlled no-choice host range testing will be established to determine their potential as biocontrol agents.

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Figure J2: Unidentified galling cecidomyid found on *Megathyrsus maximus* in KwaZulu-Natal. **Photo:** Kim Canavan

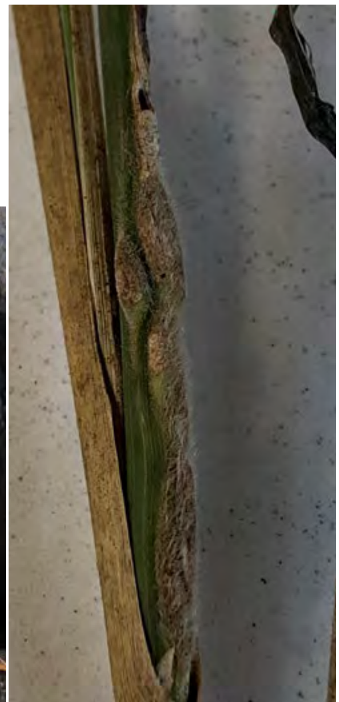

Figure J3: Unidentified root-and-crown feeding scale insect found on *Megathyrsus maximus* in KwaZulu-Natal. **Photo:** Iain Paterson

Gamba grass

Gamba grass (*Andropogon gayanus*), a noxious weed in Australia, is native to the tropical and subtropical savannas of Africa. Although the grass is highly invasive in northern Australia, it remains an economically important foraging grass for livestock. The negative impacts of the grass in Australia include reducing indigenous biodiversity and disrupting fire regimes and therefore ecosystem functioning. Since January 2018, the CBC has been conducting preliminary surveys for phytophagous insects associated with gamba grass. The aim of these surveys has been to determine the feasibility of biocontrol as a strategy for managing the grass. It is anticipated that the damage inflicted by stem-mining insect species may weaken the stem of gamba grass and reduce the environmental risk from fires in Australia but still allow the grass to be used for grazing.

Between 2018 and 2020, roadside surveys in unprotected sites (disturbed) in the Limpopo and KwaZulu-Natal Provinces, highlighted the possibility of the presence of

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Figure J4: Dr Lenin Chari doing field surveys on Gamba grass at the Phinda Private Game Reserve, KwaZulu-Natal. **Photo:** Kim Canavan.

Figure J5: Field surveys at Phinda Private Game Reserve looking for potential biological control agents in the native range (from left: Liam Yell, Dr Guy Sutton, Dr Lenin Chari and Prof. Iain Paterson) **Photo:** Kim Canavan.

stem-damaging insects associated with the grass. During these surveys, more than 40 insect species associated with the grass were identified to at least family level, but none of them showed any biocontrol potential. More recently (in 2021), new sites have been surveyed inside four game reserves. It is assumed that natural enemies of the grass would be more abundant inside these protected areas, where disturbance forces are significantly lower and grass populations are less fragmented, hence increasing the chances of finding potential biocontrol agents of the grass. Preliminary results indicate that indeed a higher diversity and abundance of insects (and their parasitoids) associated with the grass occur on Gamba grass collected from the four game reserves than all previous unprotected roadside sites. However, the majority of these insects appeared to be parasitoids or generalist herbivores with no biocontrol relevance. Full taxonomic identifications of these insects are pending and will be reported on in the following year. Genetic sequencing was done to investigate the relatedness of some of the collected wasps to known species of herbivorous wasps such as the *Tetramesa* genus, but no matches were found.

A long-term monitoring site has been established inside one of the game reserves, Phinda Private Game Reserve, and we have already begun conducting surveys for Gamba grass, together with several other grass species in the vicinity. During the most recent survey, whitefly (Aleyrodidae) pupae were observed on Gamba grass, upon which a field host range survey revealed that the whitefly pupae were only found on Gamba grass and not on any of the other grass species nearby. Attempts have been made to rear the whitefly to investigate its biology and potential impact on the growth of the grass, but currently no emergence of the adults has taken place in captivity. Samples have been sent for taxonomic and genetic analyses. Going forward, the project aims to collect infested grass from more areas within the grass's native range, including Benin, Kenya, Nigeria and Zimbabwe. Currently, an *ad hoc*/opportunistic survey is being conducted in Zimbabwe by an associate (Dr Gerald Chikowore) and promising insect damage signs have been observed on the grass stems.

Figure J6: Field surveys at the Phinda Private Game Reserve. **Photo:** Kim Canavan.

Nassella spp.

The CBC, in collaboration with the Afromontane Research Unit and the Centre for Invasion Biology, are assisting in the supervision of PhD student, Anthony Mapaura. Anthony has investigated three invasive alien *Nassella* species in South Africa: *Nassella trichotoma* and *N. tenuissima* are declared weeds under Category 1b of the National Environmental Management: Biodiversity Act (NEM:BA) and occur mainly in the montane grasslands of the Western and Eastern Cape Provinces; *Nassella neesiana* is not listed in NEM:BA but is naturalised in the Eastern Cape, Western Cape, and Free State provinces. Anthony's work has involved looking at the *Nassella* distributions, potential spread under predicted climatic changes and impacts on biodiversity. The encroachment of *Nassella* presents a serious threat to South African grasslands and this work will play an important role in guiding future management strategies, including biocontrol.

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STUDENT
Anthony Mapaura

Tetramesa as grass biocontrol agents

The *Tetramesa* genus (Hymenoptera: Eurytomidae) comprises at least 200 species that feed exclusively on grasses. The highly host-specific behaviour of these wasps, and the damage that they can cause to their host plants, makes them ideal biological control agent candidates for invasive grasses. Little is known about the Afrotropical Hymenoptera in general, and to date, *Tetramesa* surveys and taxonomy have an almost complete northern hemisphere bias. Only four African species have been described, none of which are from South Africa. The CBC has been investigating biological control options for several African grasses that have become invasive in Australia and the Americas and has been collecting *Tetramesa* specimens across South Africa since 2017. The insect communities associated with more than 55 different native grasses have been surveyed over this period. The uniform morphology of adult and larval *Tetramesa* has, however, made it impossible to determine whether these wasps are a single polyphagous species, or multiple oligophagous and/or monophagous species.

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We are currently using genetic barcoding tools (mitochondrial *cytochrome c oxidase* I and nuclear *internal transcribed spacer* regions), species delimitation methods, and image recognition tools to solve this problem. We currently have over 120 DNA sequences per gene, and our preliminary results have identified at least four putative species. These were collected from single host plants, confirming their host specificity and potential as biological control agents. It is likely that we will uncover many more undescribed species in the region as our sampling effort escalates.

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. This year Dr Guy Sutton and Dr Kim Canavan worked with the group to review an alien grass risk assessment and also helped raise awareness of the growing threat of pampas grass (*Cortaderia selloana* and *C. jubata*) in South Africa. This included communication with government officials working on alien plant legislation enforcement and has led to a SANBI bursary for a MSc student to commence work on pampas at the University of the Free State, Qwaqwa campus in 2022.

For more information on the group please see the following link:

https://invasives.org.za/working-group/alien-grass-working-group/

AGRICULTURAL ENTOMOLOGY

From the molecular quantification of virus occlusion bodies, used for the control of the false codling moth, *Thaumatotibia leucotreta*, in citrus, to the identification of alternative hosts of the oriental fruit fly, *Bactrocera dorsalis*, the Agricultural Entomology Research Programme at the CBC has continued its work on understanding and managing important insect pests which pose a risk to the South African agricultural industry. Key to the success of these projects, is the collaboration between the CBC and Citrus Research International (CRI), who provide vital technical and financial support. Through this collaboration, projects have emerged that focus on understanding and overcoming a variety of challenges encountered within the existing biological control options that are used in Integrated Pest Management (IPM) programmes. This includes the continued development and evaluation of techniques which have recently been employed to mitigate the negative effects of ultraviolet radiation on virus-based biopesticides. Similarly, research has been conducted to better understand the impact of hyperparasites on parasitoid-based biocontrol programmes. This research has led to the identification of three hyperparasites affecting two primary parasitoids. These findings provide fundamental information in implementing and managing parasitoid-based strategies. The increase in netted orchards has also created a variety of research opportunities. Projects evaluating differences between open and enclosed orchards have been established to better understand changes in both insect pest complexes and the impact of nets on control strategies used in these orchards. The results and progress of these and other projects in the Agricultural Entomology Research Programme are listed below, providing an overview of the interesting and important work being conducted here at the CBC.

PROGRAMME HIGHLIGHTS IN 2021

- Presentation of several papers at the 2021 International Congress on Invertebrate Pathology and Microbial Control and 53rd Annual Meeting of the Society for Invertebrate Pathology.
- Publication of multiple papers in peer-reviewed journals.

Development and optimisation of a qPCR assay for the enumeration of betabaculoviruses used for commercial applications

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

STUDENTS
Thuthula Mela (MSc)

Thaumatotibia leucotreta (Meyrick) (Lepidoptera: Tortricidae) is an insect pest that poses a threat to the South African citrus industry. The pest is highly significant as it has caused an annual loss well in excess of R100 million to the industry. To control *T. leucotreta* in South Africa, an IPM programme has been used. One of the components of this programme is Cryptophlebia leucotreta granulovirus (CrleGV) which has been formulated into commercial biopesticides, such as Cryptogran which has been successfully applied in the field for over 15 years. Using these viruses as biopesticides requires accurate quantification of the viral particles to determine appropriate concentrations when conducting bioassays, field trials and for commercial formulation among other applications. Dark-field microscopy is the traditional method used to quantify baculoviruses, including CrleGV; however this method is subjective, tedious, labour intensive and time consuming.

This project saw the development of an accurate quantification method using quantitative polymerase chain reaction (qPCR). First, several genomic DNA extraction methods were evaluated to determine which yielded the greatest quantity of gDNA from CrleGV-SA OBs. The results obtained indicated that pre-treatment of viral OBs with sodium carbonate and neutralisation with Tris-HCl prior to DNA extraction with a commercial extraction kit, yielded the most DNA of the greatest purity. The development of this extraction process provides an improved method for the extraction of baculovirus DNA, as compared with the commonly used CTAB process. The next objective saw the development of recombinant plasmids for use as standards to which viral DNA extracts could be compared and enumerated. Three recombinant plasmids were successfully constructed as standards, with each targeting a unique region of the viral genome, enabling multiple and independent evaluation of unknown samples during qPCR assays, and resulting in highly accurate quantification of CrleGV OBs. The results obtained from the qPCR assays were compared with results obtained using dark-field microscopy, which showed that the qPCR technique could accurately quantify unknown CrleGV samples in relation to the predefined standards. This technique could see application in research and commercial settings where the accurate quantification of CrleGV samples is routinely performed.

Figure K1: Thuthula Mela analysing and imaging an agarose gel for detection of baculovirus DNA. **Photo:** David Taylor

Selection for a UV-resistant isolate of a nucleopolyhedrovirus, for improved field persistence and efficacy against false codling moth (FCM)

Thaumatotibia leucotreta, commonly known as the false codling moth, is a phytophagous insect endemic to southern Africa. It is highly significant to the South African citrus industry due to its classification as a phytosanitary pest. The most effective approach for controlling T. *leucotreta* has been using an IPM programme. Specific treatments are strategically combined to suppress *T. leucotreta* early in the season, preventing the build-up of subsequent potentially harmful population numbers later on.

Entomopathogenic viruses are one of the major biological control agents used in IPM programmes. Baculoviruses are primarily used as they have a narrow host range and do not harm non-target pests or humans. One of the greatest shortcomings of insect viruses as biopesticides is their ultraviolet (UV) sensitivity and hence rapid breakdown in the field. Baculoviruses are susceptible to UV radiation and lose their activity within hours to a few days after exposure. Several substances have been tested as UV protectants to improve the persistence of baculovirus biopesticides in the laboratory, such as optical brighteners, UV absorbers and antioxidants. However, they have not been as successful in the field. Recently a study was completed at Nelson Mandela University, demonstrating that consecutive exposure of Cryptophlebia leucotreta granulovirus (CrleGV-SA) to UV irradiation cycles, with parameters set to mimic those experienced within the field,

RESEARCH TEAM

Dr Marcel van der Merwe, Dr Michael Jukes, Prof. Caroline Knox, Prof. Sean Moore, Prof. Martin Hill selected for a UV-resistant isolate. This resulted in about a 1000-fold improvement in virulence after exposure to UV, relative to the wild-type isolate used in Cryptogran™. Recently a novel alphabaculovirus (NPV) was isolated from a laboratory culture of *Cryptophlebia peltastica*. The novel NPV isolate was identified as Cryptophlebia peltastica nucleopolyhedrovirus (CrpeNPV). The biological activity of this alphabaculovirus was tested against *T. leucotreta*, *Cryptophlebia peltastica* and *C. pomonella*. Cryptophlebia peltastica nucleopolyhedrovirus was found to be highly virulent against all three species. In this study, we propose developing a UV-resistant CrpeNPV isolate that will persist in the field for longer and be an effective alternative to CrleGV for the control of *T. leucotreta*. Once a UV-resistant isolate has been selected, various UV-protectants will be tested alongside it. Finally, semi-field trials are to be conducted to examine whether the UV resistance observed in the laboratory is carried over into the field.

Biology, chemical ecology, and management of Fruit-piercing moth, *Serrodes partita*, in the Kat River Valley

The Catapult moth, *Serrodes partita* (Fabricus) (Lepidoptera: Erebidae), is a polyphagous, multivoltine pest of tropical and subtropical fruit, including citrus. The adult moth pierces the skin of ripening or ripe fruit and sucks the juices. Wounds promote the entry of secondary fungal pathogens and fruit fall is typical in citrus. In the Kat River valley, the population dynamics of *S. partita* have evolved over the years, with recent observations showing recurring occurrences as opposed to usual seasonal outbreaks approximately five to ten years apart. As a result, little research has been undertaken for this pest. Owing to the feeding behaviour and elusive nature of the moth, current pest management practices are limited and only adaptable for small orchards, with huge disadvantages on large-scale production. Furthermore, such pest management practices lack specificity and could negatively impact the environment and non-target pests. Innovative, sustainable, and eco-friendly strategies that control the problem while preventing the erosion of biodiversity are needed. The use of attractive baits (banana trap) has shown to be promising but impractical in the long term. The biology and chemical

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STUDENT
Tapiwa Mushore (PhD)

Figures K2 and K3: Adult fruit piercing moth, *Serrodes partita*, feeding on soft citrus; the damage it causes. **Photos:** David Taylor.

Figure K4: Tapiwa Mushore examining fruit damaged by the fruit piercing moth. **Photo:** David Taylor ecology of *S. partita* has not been well enough investigated to allow for the development of synthetic lures. Therefore, this study aims to develop monitoring and management tactics for *S. partita* through behavioural and electrophysiological approaches. Pest populations and damage assessments are being conducted to establish intervention thresholds for control as well as to establish population peaks in citrus orchards. Efforts are being made towards artificial rearing to facilitate future studies, and bioprospecting for pathogens aimed at controlling larval stages.

Investigating the biological and genetic stability of UV-tolerant baculoviruses following *in vivo* and *in vitro* passaging for improved control of *Thaumatotibia leucotreta*

RESEARCH TEAM
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STUDENT
Petrus Iita (PhD)

Ultraviolet (UV) radiation of sunlight is a major factor that disrupts the integrity of a biopesticide in the field. Baculoviruses are effective biocontrol agents, but they are sensitive to UV irradiation which gradually decreases their biological activity following exposure to direct sunlight. Two baculoviruses are known to infect *Thaumatotibia leucotreta*, the FCM, namely Cryptophlebia leucotreta granulovirus (CrleGV) and Cryptophlebia peltastica nucleopolyhedrovirus (CrpeNPV). The CrleGV-SA isolate is currently applied as a biopesticide for *T. leucotreta* control, whereas CrpeNPV is undergoing field trials. Recently, a variant of CrleGV-SA (referred to as CrleGV-C5) was selected for by successive exposure of occlusion bodies to UVA and UVB irradiation and shown to have increased tolerance to UV irradiation. A project is currently underway to utilise this process to select for UV tolerance in CrpeNPV (referred to as CrpeNPV-UVT).

To enable mass production of the CrleGV-C5 or CrpeNPV-UVT isolates for downstream usage, including laboratory experimentation, field trials and commercial application, the passage of the virus through *T. leucotreta* late instar larvae is required. Alternatively, mass production could also be performed in a recently developed *T. leucotreta* cell-line, which is permissive for CrpeNPV and potentially permissive for CrleGV-SA. The latter option is preferable for mass production of the virus because it does not require insect cultures and it also minimises the potential risk of contamination arising from covert-overt infections by baculoviruses in host insects.

The continued passage of baculoviruses in host insects has been shown to result in changes to specific traits of the virus (i.e., virulence and genetic composition). Whether the biological activity and genetic composition of CrleGV-C5 or CrpeNPV-UVT isolates can be maintained as the virus is continuously passaged through insect larvae or cells is unknown. This project aims to a) determine whether the virulence of CrleGV-C5 or CrpeNPV-UVT has changed following continued passage in *T. leucotreta* larvae or cells, b) determine whether the genetic composition of CrleGV-C5 or CrpeNPV-UVT has changed following passage, c) determine whether CrleGV can infect a *T. leucotreta* cell-line, enabling the development of a CrleGV cell-based infection and production system.

A new method of infection based on the diet-plug bioassay technique was developed, enabling CrleGV-C5 to be passaged five times through *T. leucotreta* late instar larvae. The purity of the virus recovered between each passage was assessed by a multiplex PCR assay to ensure that no contamination with CrpeNPV in the subsequent passage and downstream experiments occurred. Initial bioassays of CrleGV-C5 against neonate *T. leucotreta* larvae were conducted along with the wild type, CrleGV-SA as control, with results showing similarity in the LC_{50} and LC_{90}. Subsequent work will evaluate the virulence of CrleGV-C5 recovered from larvae after five passages and this will be performed in biological assays against neonate *T. leucotreta* larvae. The genome of CrleGV-C5 recovered after five passages will be fully sequenced to determine potential genetic changes. An infection assay in a *T. leucotreta* cell-line will be developed and optimised to determine its potential as an alternative for the mass production of the CrleGV-C5 isolate.

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

RESEARCH TEAM
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STUDENT
Tahnee Bennett (MSc)

The baculovirus Cryptophlebia leucotreta granulovirus (CrleGV-SA), is one of the components in the IPM programme used for the control of *Thaumatotibia leucotreta*, a serious citrus pest. Baculoviruses have been formulated into biopesticides which are greatly impacted by ultraviolet radiation (UVA and UVB) from the sun. The viral DNA is damaged by exposure to UV light which decreases the efficiency of the biopesticide.

In a previous study conducted by Mwanza (2019), a UV-tolerant Cryptophlebia leucotreta granulovirus isolate (CrleGV-C5) was developed following successive cycles of UV exposure. This UV-tolerant strain has potential as a biocontrol agent for improving the control of *T. leucotreta* in the field. The aim of the study was to determine the biological and genetic stability of the UV-tolerant strain of CrleGV-SA, which can potentially improve *T. leucotreta* control. The genome of the UV-tolerant isolate was reassembled by de novo assembly followed by a sequence comparison with the CrleGV-SA genome. Seven single-nucleotide polymorphisms (SNPs) were detected after sequence comparison. Four of the SNPs detected were the same as those reported by Mwanza (2022). Two SNPs detected were unique to the current study and the seventh SNP detected was previously reported by van der Merwe et al. (2017). Three target regions encompassing five of the identified SNPs were amplified by PCR using specifically designed oligonucleotides. Polymerase chain reaction amplicons were ligated into the pJET1.2/blunt cloning vector for sequencing to determine if the SNPs remained detectable in the genome. Four of the five SNPs were detected in the CrleGV-C5 genome and could potentially be used as genetic markers for discerning the wild-type isolate, CrleGV-SA from the UV-tolerant isolate CrleGV-C5.

Lethal concentrations (LC) of the UV-tolerant strain were determined after conducting surface-dose biological assays. A LC$_{90}$ value of $8.75\times10_9$ OBs/ml was determined, with this concentration used in subsequent experiments for producing additional CrleGV-C5 OBs by sequential passage through 3rd/4th instar *T. leucotreta* larvae in laboratory assays. Sufficient OBs were generated after two rounds of sequential passage through the host, with DNA successfully extracted from these OBs, enabling the PCR amplification of the three target regions mentioned above. These were again sequenced to determine the genetic integrity of the UV-tolerant CrleGV-SA isolate after sequential passage. All five of the target SNPs remained detectable in the genome after this process, suggesting successful maintenance of the CrleGV-C5 isolate in infected host larvae. These OBs were subsequently used in detached fruit bioassays, to evaluate whether the isolate still exhibited increased UV-tolerance as compared to the wild type, CrleGV. Bioassays comprised fruit treated with either CrleGV-C5 (UV-tolerant), CrleGV-SA (wild type) or non-treated (Control), which were evaluated in triplicate over two weeks with half exposed to natural UV radiation, and the remainder kept under cover. Statistical analysis of the collected data is still in progress.

Figure K5: Tahnee Bennett examining biological assay plates to determine the effect of a novel UV tolerant isolate of CrleGV against *T. leucotreta*. **Photo:** David Taylor

An assessment of the reasons for lower False Codling Moth (FCM) infestation in organic versus conventional citrus orchards

Packhouse assessments in the Eastern Cape recorded significantly lower levels of FCM infestation in fruit from organic orchards than fruit from conventional citrus orchards, in. Conventional wisdom might conclude that naturally occurring biological control would be greater on an organic farm. However, this cannot simply be assumed. This study evaluated the possible factors that could be contributing to this difference, namely above- and below-ground biocontrol, fruit biochemistry and farming practices.

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Luke Cousins (MSc)

Analysis of FCM ecology began in October 2019 and ended in October 2021. Total moth catches on conventional farms were higher than organic farms. Average weekly moth catches in conventional Palmer Navel and Newhall Navel orchards were significantly higher than organic orchards ($p<0.05$). The total number of infested fruits collected from conventional orchards was higher than on conventional farms, but overall numbers were too low to lead to any statistical significance. Nutrient analysis revealed significantly higher magnesium, calcium, and boron content ($p<0.05$) in conventional fruit. Mean rind thickness of organic fruit was higher than conventional fruit. No significant difference has been revealed between the susceptibility of organic and conventional fruit to infestation by FCM larvae. Oviposition preference trials have shown a slight preference of females to lay eggs on conventional fruit; however, differences were not significant to a 95% confidence level ($p=0.065$).

Fruit powder bioassays are currently under way to determine the effect of different nutrient compositions on the survival, growth rate, weight and fecundity of FCM. So far, a higher number of survivors have been recorded in artificial diet supplemented with conventional fruit powder than with organic fruit powder, with fecundity still to be determined. Eight soil sample sets (over 450 individual soils samples) have been collected and baited. Differences in abundance between entomopathogenic fungi (EPFs) and entomopathogenic nematodes (EPNs) in organic and conventional soil remain unclear; however, the prevalent genera of EPF appear to differ between farming practices, with Metarhizium EPF more commonly found in the organic farms (55% of isolates), while Beauveria has been more commonly found on conventional farms (67% of isolates). Numbers of rove beetles and ants, known predators of FCM pupae, were higher on organic farms, based on preliminary examination of pitfall traps. The ultimate objective is to identify factors leading to reduced incidence of FCM on organic farms and to determine how any of these influential factors can be transferred to conventional farming.

Integrated Pest Management (IPM) under nets in Mpumalanga Province

With the growing consumer demand for fresh produce worldwide, South African citrus and litchi growers are forced to review current production practices to ensure optimal marketable yields every growing season. However, an increase in marketable yield brings about a concomitant increase in production costs per hectare. Current factors reducing fruit quality include wind damage, sunburn, fruit splitting, insect pests, diseases, and physiological disorders. Wind damage in citrus is predominantly reduced by means of windbreaks; however, the windbreak most often used currently, *Casuarina cunninghamiana* (beefwood), affects the production potential of row-end trees and does not eliminate wind scars on all fruit in the orchard. Hence,

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Karlien Grobler (MSc)

alternative solutions to mitigate wind damage should be considered. Fruit cracking in litchi, due to high temperatures and low humidity, is yet another challenge. Netting of crops has shown to be an effective method in reducing wind damage, sunburn, and litchi fruit cracking. However, the effect of netting on yield, pests and diseases for litchi and grapefruit in Mpumalanga Province is unknown.

In this study, insect pest complexes were assessed over a period of three years in an enclosed netting structure (20% shade) over Star Ruby grapefruit and Mauritius litchi and compared to open areas. Under the net and in a comparable orchard outside the net, three replicates of ten data trees each were marked for regular monitoring of citrus thrips, bollworm, mealybug, red scale, mites, FCM and fruit fly species throughout the growing season. Seychelles scale, mango scale, pink wax scale, fruit fly species, FCM and litchi moth were monitored in the litchi orchard. Twenty fruit per tree in both citrus orchards were inspected at harvest and graded according to the CRI guidelines for export standards for pest damage and wind blemishes. Three samples per orchard were sent to Hortec Analytical Services for residue analysis.

The incidence and severity of citrus thrips infestation throughout the citrus growing season were found to be less under shade net than in the open orchard; however, the netted orchard had more severe infestations of mealybug. False codling moth total trap catches were higher under citrus shade net than in the open orchard, while fruit fly species were caught in higher numbers in the open orchard for both litchi and citrus. Little fruit infestation by either FCM or fruit flies was recorded during citrus and litchi harvesting. Citrus fruit sampled for residue tests revealed a higher number of active ingredients and higher concentrations (especially dithiocarbamates and pyraclostrobin) under the enclosed netting than in the open orchard. However, these never exceeded permissible residue levels. By harvest, fruit damage was lower and export pack outs higher from the netted orchard. Seychelles scale and pink wax scale were found to be more frequent under the litchi net than in the control orchard.

The practice of erecting shade netting over citrus orchards is growing throughout the country. The findings in the study will help to better understand and manage pest, natural enemy, and pesticide trends under nets, and to meet market demands for clean, high quality, pest-free fruit. Thus far, netting of citrus and litchi does appear to improve external fruit quality and thus Class 1 export volumes.

Figure K6 and K7: (top) netted vs open orchards (bottom) Litchi under nets.
Photos: Karlien Grobler

Host relationships of hyperparasites in citrus orchards

Classical biological programmes usually introduce natural enemies from the native range of the target organism. Results from these introductions are often less than desirable and can be seen as a failure of the biological control agent to control the target organism. There is clear evidence from some biological control programmes that hyperparasitism can impact the parasitoid biological control agent, but it has not been clear whether it can result in control failure. The main explanations of failure include climatic conditions, predation or parasitism of the primary biological control agent and lack of alternative hosts or food, or it can be explained as a result of intrinsic and extrinsic factors. Hyperparasitism is a highly evolved behaviour in the Hymenoptera and in a few species of Diptera and Coleoptera, in which an adult hyperparasite (or secondary parasitoid) oviposits on or in a primary parasitoid host that has attacked another insect species.

Trials conducted in Burgersfort, Mpumalanga, South Africa, where different timing of *Anagyrus vladimiri* releases was compared, effectively showed reduction in mealybug infestation on citrus fruit before harvest in April of 2020. However, in the follow-up season, augmentations of *A. vladimiri* were unable to control mealybug and a major outbreak of citrus mealybug occurred. One of the main objectives of the study was to investigate the impact

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of external factors, other than effects of insecticides, on *A. vladimiri* augmentations such as hyperparasitism.

Field surveys for parasitic wasps were conducted in 31 citrus orchards over a two-year period between October 2019 and April 2021. Orchards hosting citrus mealybug as the predominant species were selected, based on historical scouting records. Eleven orchards were covered by net-house structures and the remaining orchards were open-air orchards. Scouting of the designated orchards for mealybug commenced in October, with a total area of approximately 60 hectares of commercial citrus orchards under survey. Sampling was subject to the availability of mealybug colonies, with ten citrus fruits sampled

Figures K8 and K9: (Left) *Leptomastix dactylopii* (primary parasitoid) (Right) Wayne Mommsen examining parasitism in the field. **Photos:** Wayne Mommsen selectively within an orchard at two-week intervals to observe parasitism of *Plannococcus citri* as well as the presence of hyperparasites in the samples. No more than ten third-instar mealybug were inspected on fruit infested with mealybug because numbers of mealybug on fruit ranged from one to over 100 for the duration of the trial. The fruit from each orchard were placed together in 4-litre plastic emergence containers with ventilation windows made from voile material. Containers were kept at room temperature, and the containers inspected daily for the emergence of parasitoids. Containers were kept for approximately 21 days and then emptied, and each fruit carefully inspected under a microscope for the presence of emerging parasitoids. All the parasitoids were collected by aspirating them into Eppendorf tubes and preserved with 70% ethanol. Representative samples were sent to the ARC biosystematics division for identification, after which the proportion of hyperparasites to primary parasitoids was determined.

Two primary parasitoids (*Anagyrus vladimiri* and *Coccidoxenoides perminutus*) and three hyperparasites (*Coccophagous rusti* Compere, *Chartocerus* sp. and *Pachyneuron* sp.) emerged. The level of hyperparasitism over two seasons was

Figures K10 and K11: (Left) - *Pachyneuron* spp. (hyperparasite) (Right) *Coccophagous rusti* Compere (hyperparasite). **Photos:** Wayne Mommsen

thought to be high, with the sample ratio of hyperparasites totalling 51% in 2020 and increasing to 79% in 2021. This is believed to be the cause of poor mealybug biocontrol in the field. The impact of hyperparasites on the efficacy of commercial augmentations of *Anagyrus vladimiri* has not been published previously; thus, observations in this study have provided us with valuable insights and adequate motivation for future research to continue. Understanding the impact of hyperparasites on augmentations will help optimise control strategies reliant on effective biocontrol.

Alternate hosts of the Oriental Fruit Fly, *Bactrocera dorsalis*, in the Sundays River Valley

RESEARCH TEAM
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Bactrocera dorsalis detection has occurred in the Sundays River Valley (SRV) citrus production area since 2015. Trap monitoring is present throughout the region, and in 2020 a total of 26 *B. dorsalis* males were detected. Of these 26 males, one was detected in a trap that was 5 km away from any cultivated hosts, indicating that a low-level establishment has occurred on alternate host plant species within the Sundays River area. This project aims to determine the range of hosts for *B. dorsalis* in the natural thicket vegetation surrounding citrus growing regions in the SRV.

Twenty-four Methyl Eugenol and 24 Bio-lure traps were set up in citrus orchards and surrounding natural vegetation across three sites along the length of the SRV, with those in the natural vegetation a minimum of 500 m from the nearest cultivated hosts. The traps are monitored monthly. To date, a total of three individuals of *B. dorsalis* have been recorded in the traps, with one individual detected in a natural vegetation trap. With the help of Gareth Coombs (PhD), a comprehensive list of species occurring in the natural vegetation of the SRV was produced. *Capparis sepiaria* and *Opuntia ficus-indica* are confirmed hosts of *B. dorsalis* and are commonly found in the thicket surrounding the citrus production regions, potentially acting as a population reservoir. Fruits of any species in the natural vegetation are currently being collected as they come into season and placed into emergence boxes in controlled environment rooms, to determine the degree of infestation.

Future work for this project involves the continuation of fruit collection and insect emergence to determine if *B. dorsalis* can reproduce and complete its life cycle on any natural vegetation in the SRV. Trap collections and monitoring of fruit fly populations will continue.

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

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False codling moth *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) is a key phytosanitary pest in citrus orchards across South Africa. The mere presence of a single moth in consignments destined to overseas markets may result in the rejection of the entire shipment. For this reason, multiple control strategies have been implemented to reduce FCM populations in South Africa. Such strategies include the use of semiochemicals as insect pest repellents. There has been an extensive research on the use of semiochemicals in IPM programmes, much of which has been focused on pest species of veterinary and medical importance. Very little research has been done on the potential use of insect repellents in agricultural IPM programmes. More importantly, to the best of our knowledge, there are no past studies that have been conducted on potential repellents to FCM. This gap provides an opportunity for a study to improve the control of FCM using repellents.

This study assessed FCM oviposition repellence in plants, essential oils, and common commercial insecticides. Repelling oviposition could improve FCM control since the larval stage is considered the only damaging stage of its life cycle. Repellence trials were conducted in a CE room under nocturnal conditions. Oranges treated with solutions of potential repellents were placed one by one into a cage of gravid FCM females for four hours, with oviposition being recorded every hour. So far, seven potential repellents have been identified: two essential oils (Lavender and Peppermint), two plant crude extracts (Garlic and Marigold), and three chemicals (Delegate, Coragen, and Warlock). The next step is to identify the chemical compounds responsible for eliciting the repellent effect in these potential repellents using GC-MS, and to determine if any of them have a dual action (both repellent and ovicidal properties). If a compound can be identified that repels FCM from ovipositing, it may be valuable for dispensing in an orchard or for spraying onto trees. This means that the efficacy of these repellents can be further tested in field trials.

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

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Around the world, commodity markets are demanding that citrus be produced with less chemical intervention to increase the health benefits of consuming the fruit. Insecticide usage has had a long history in the citrus industry and, prior to 1948, insecticides were mainly safe for humans as their residual effects were short and they were also 'soft' on beneficial insects. However, after 1948, new chemicals, such as the organophosphates, were developed which were effective against a wide range of pests and which led to a global boom in citrus production. However, these insecticides were non-selective; natural enemies of pests were killed and high levels of chemical residue were left on fruit. Naturally this level of spraying also increased the production costs.

The aim of this study was to determine if it was possible to obtain the same yield and pack out percentage in citrus orchards where an integrated management programme heavily based on biological control was applied, in comparison to orchards that relied solely on chemical pest control. In particular, the interactions between citrus mealybug, *Planococcus citri*, citrus thrips, *Scirtothrips aurantii*, red

Table K1: A comparison of the cost, pack out and defect percentages, and citrus yield under either IPM or chemical pest control programmes.

	ZC1	**ZC2-IPM**		**ZC2-Bio**		**ZC3**	**ZC3-Bio**	
Input	Chemical	Chemical	Insects	Chemical	Insects	Chemical	Chemical	Insects
Cost/Ha	R 58 212	R 59 236	R 27 182	R 47 080	R 29 312	R 37 786	R 32 359	R 18 677
Total	**R 58 212**	**R 86 419**		**R 76 392**		**R 37 786**	**R 51 036**	
Pack out %	83.8	92.4		88.4		92.3	93.8	
Defects %	14	6		9		6	4	
Yield (t/ha)	71.6	40.6		50.8		47	47	

scale, *Aonidiella aurantii*, and citrus red mite, *Panonychus citri*, and their natural insect enemies were investigated.

The project started in September 2020 and has been running for 14 months. Several sites were selected, as shown in the map below. ZC1 is a commercial orchard of approximately 16 ha under a net and is farmed on a chemical programme. ZC2-IPM, is an orchard of about 10 ha under net and is managed under an IPM programme where 'soft' chemicals will be used together with beneficial releases. Additionally, a separate section of ZC2-Bio, approximately 4 ha in size, is farmed under nets, and is the orchard where increased amounts of beneficial insects and biological products will be used. Lastly, ZC3 is a commercial orchard of about 20.5 ha with no net, which follows a chemical programme. A small section of ZC3, of approximately 2 ha, has no nets and is following a biological programme.

During the first season, different control strategies were applied. Scouting still led the spraying and releasing actions. However, in December we had an outbreak of citrus mealybug and different interventions had to be applied to protect the crop. Within two orchards, ZC1 & ZC2-IPM, more chemicals were sprayed. Within

Figure K12: Map of the different trial sites and orchards on Zandvliet Estate, Ashton, Western Cape.

ZC2-Bio, more beneficial insects, including predators such as *Cryptolaemus* and *Nephus*, were released. Before the harvest, thrips, scale, red mite, and even citrus mealybug were controlled and most sooty mould had washed off. Certain fruit did show signs of mealybug rind damage. These interventions increased the cost/ha considerably as can be seen in the table below, alongside pack house data from the 2020–2021 season.

The lessons learnt in the season of 2020–2021 led to certain changes in the control strategy within ZC2. Certain chemicals got switched for others and increased numbers of beneficial insects were added early in the season . The aim is to get the IPM programme under nets effective, sustainable, and economical. One season only gives one set of data and to make correlations, deductions, or predictions on only one set of data can be dangerous. What can be said is that lessons have been learnt and improved alterations have been made from the control strategies of the previous season. In theory this should give us higher pack outs, fewer defects and increased yield.

After receiving the new Plant Protection Products List and list of requirements for special markets for next season, it is becoming more and more evident that we, as a citrus industry, need to find the balance between chemical and beneficial insects sooner rather than later. Through this study we hope to do so, and to find a commercially viable option to farm sustainably and economically with an IPM programme.

POLYPHAGOUS SHOT HOLE BORER

The polyphagous shot hole borer or PSHB (Euwallacea fornicatus) is an invasive ambrosia beetle native to South-east Asia. It has become a highly invasive tree pest in the United States, Israel and, more recently, in Australia. In 2017, the beetle was discovered in a botanical garden in Pietermaritzburg and is now well established in eight of the nine provinces of the country. The PSHB bores into trees and releases a symbiotic fungus, Fusarium euwallaceaea, which grows in the vascular system of the tree, inhibiting water and nutrient uptake which, in many cases, can kill 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 also to native forests throughout South Africa. Various projects investigating the impacts of PSHB on agricultural crops such as pecan nut, grapevine and deciduous fruits are ongoing, but the beetle has not yet had a major impact on crop trees.

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STUDENT
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COLLABORATOR
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Figure L1: Mesfin Gossa, a postdoctoral fellow at FABI, and Garyn Townsend looking at an infested Coral tree (*Erythrina caffra*) in KZN. **Photo:** Brett Hurley

The primary impact of PSHB is seen in urban forests where it usually attacks non-indigenous trees. Research into the spread and impact of PSHB in indigenous forests is ongoing and, to date, the beetle has been recorded on 61 indigenous tree species, 19 of which are reproductive hosts of the beetle. Preliminary data from permanent monitoring plots in indigenous Afrotemperate forests has shown that the spread of the beetle in these natural areas is slow, but consistent. Beetle populations and tree attacks are highest near urban areas, where source populations are present. Forest patches with high tree species richness appear to be less at risk of attack. Certain tree species are more susceptible to attack by PSHB and six attacked tree species were found to be reproductive hosts of the beetle. The PSHB beetle is spreading at a rapid rate and poses a major risk to natural forest health, not only in Afrotemperate forests, but also to other native forests throughout Africa.

As part of Garyn Townsend's PhD, based at the Forestry and Agricultural Biotechnology Institute (FABI), investigations into the potential for biological control of the beetle and/or its fungus are now underway. In addition to the ongoing monitoring of the impact of PSHB in indigenous forests, this project aims to better understand the ecology of PSHB in its native range, including characterising the diversity and prevalence of natural enemies and identifying their host specificity. It also aims to characterise the diversity and prevalence of potential natural enemies in its invaded range of South Africa, as well exploring the impacts of PSHB on the abundance and diversity of indigenous ambrosia beetle populations. This work is being done in collaboration with eight universities throughout South Africa, as well as overseas, forming a research network that is taking a holistic approach to understanding the biology of PSHB and its ecological, urban and agricultural impacts. The research will contribute to protocols and policies aimed at controlling the spread and impacts of PSHB both in South Africa and throughout its entire invaded range.

MASS-REARING

Weed biological control practitioners usually rely on classical biological control, that is, a small amount of the agent is released and once a population is established, no further releases are conducted. The population of the agent is expected to increase naturally and reach an equilibrium with the target weed population, hopefully at a level where there is significantly less of the weed. An alternative to this approach is augmentative biological control, which aims to increase the number of agents in the field to gain quicker and better control.

An augmentative approach can improve the level of control for both water weeds and cactus weeds. The CBC aims to produce large numbers of the relevant agents for several target weed species and release them in large numbers, at the correct time of year, to make the maximum impact in the field. The agents are therefore used more as green herbicides than as classical biological control agents. Producing large numbers of agents is a labour-intensive process, so the CBC has two teams working to produce these agents at two different facilities, one for water weed biocontrol agents and the other for cactus agents.

Figure M1: The Kariega team at the mass-rearing facility. Byron Soetland (top left), Karin Goliath, Ruth Scholtz, Ronel Roman, Carmen Peters, Arthur Scholtz (bottom left), Daniel Scholtz, Lubabalo Malinga, Gugulethu Mkile. **Photo:** David Taylor

Waainek mass-rearing facility

From 21 June 2021, the management of Waainek Mass-rearing Facility was changed. With the substantial support from the CBC team, especially the six members of CBC's Sisonke Team, the change went well. New rearing practices were successfully implemented to increase the populations of the biocontrol agents, in particular *M. scutellaris* and *Cyrtobagous salviniae*, and to make sure that when the agents are sent where they are needed, they arrive timeously and in good health. Table M1 shows the release numbers of each species. We have begun to develop a data management tool to monitor the value chain of the implementation of biological control in collaboration with Information Systems Department at Rhodes University.

Every week, biological control agents are sent out to be released at targeted sites in South Africa, while *C. salviniae* has been released in Zambia and Zimbabwe as part of new collaborations beyond our borders. A new tunnel was built before the funding delay but is not yet in use owing to low funds as the contract with DFFE has not yet been renewed.

Funding delays led to the retrenchment of members of the rearing staff at the end of October 2021. An effort to raise money through a crowd funding platform enabled two of the employees to be brought back temporarily to assist during the summer. In memory of our colleague, Lulama Poni, who passed away at the end of 2020, a tree was planted at the Waainek Mass-rearing Facility.

Table M1: Releases of waterweed biological control agents from the CBC mass-rearing facility

WEED	AGENT	Number of releases made	Total number of insects or immature larvae released
Pontederia crassipes	*Megamelus scutellaris*	39	179 850
Myriophyllum aquaticum	*Lysathia sp.*	11	11 475
Egeria densa	*Hydrellia ergeriae*	2	111 659
Pistia stratiotes	*Neohydronomous affinis*	6	3 569
Salvinia molesta	*Cyrtobagous salviniae*	5	4560
TOTAL		**54**	**311 113**

Figure M2: Vuyani Ntyinkala, Landile Booi and Lyle Titus collecting biological control agents for release. **Photo:** David Taylor

Kariega mass-rearing facility

The Kariega team is made up of eight mass-rearing technicians led by the facility manager, Ruth Scholtz, as well as PhD student, Zezethu Mnqeta. The facility is a former Agriculture Research Council biocontrol laboratory where much of the early work on cactus biocontrol was conducted. Biocontrol came back to the facility in 2015 when the CBC moved in to mass-rear cactus agents. There are several large greenhouses with open sides that provide the perfect hot and dry conditions to mass-rear cactus agents. Six agents are mass-reared at the facility, and these are used to control ten target weeds, and are made freely available to land-users anywhere in South Africa. The agents are either cochineal insects or the galling mealybug, *Hypogeococcus*, so the releases are done by releasing either the galls of the mealybug or cactus cladodes (stem segments) infected with the correct cochineal species.

Figure M3: Karin Goliath, Arthur Scholtz and Carmen Peters working with *Harrisia martinii.* **Photo:** David Taylor

This year, 111 releases were conducted, with a total of 15 508 galls/infected cladodes being released (Table M2). We have released in all nine provinces in South Africa, on ten target weed species using six agents. This brings the total number of releases of biocontrol agents by the cactus team since the project was started in 2015 to 454 releases, totalling 390 521 galls/infected cladodes, covering every province of the country.

Zezethu's PhD has assessed the impacts of these releases, and her data show that the efforts of the CBC mass-rearing team have resulted in significant reductions in invasive alien cactus, with associated benefits to land-users such as farmers and conservationists, as well as indigenous biodiversity, on a national scale. Her results also indicated that much more could be done and that increasing capacity in mass-rearing of cactus weed agents would be beneficial for the country.

Table M2: Releases of cactus biological control agents from the CBC mass-rearing facility

		Total (2021)	
WEED	**AGENT**	**# releases**	**# cladodes/galls**
Opuntia aurantiaca	*Dactylopius austrinus*	22	9 463
O. stricta	*D. opuntiae* 'stricta'	16	1 541
O. humifusa	*D. opuntiae* 'stricta'	8	540
O. monacantha	*D. ceylonicus*	5	336
O. ficus-indica	*D. opuntiae* 'ficus-indica'	12	216
O. engelmannii	*D. opuntiae* 'stricta'	1	215
Cylindropuntia imbricata	*D. tomentosus* 'imbricata'	15	1 674
Cereus jamacaru	*Hypogeococcus* sp.	18	942
Harrisia martinii	*Hypogeococcus* sp.	11	448
Harrisia pomanensis	*Hypogeococcus* sp.	3	133
TOTAL		**111**	**15 508**

COMMUNITY ENGAGEMENT

The CBC prides itself on its engagement with local communities around Makhanda, as well as invaded sites across South Africa and even abroad. Staff and students engage and interact with people on different topics from insects in general to invasive alien species and biological control. The aim of these engagement activities is to interact with the public so that they can learn about invasive species and the impacts they have on our environment, and that there is a way of controlling some invasive species biologically. The global pandemic has led to the postponement and cancellation of many of our face-to-face activities, but we continue to try to engage with communities online and in person where possible and safe. Below we highlight our activities over the course of 2021.

Agricultural partnership

The CBC continued its partnership with AgriSA during 2021. This partnership has linked us to important people in helping manage invasive species. In partnership with Agri Noord-Kaap, the CBC has continued to work on the development of a strategy for management of *Prosopis*. In 2021, Gretha van Staden joined the CBC family to pursue her MSc and, together with Philip Ivey, started a WhatsApp group and an Instagram page which shares information and news around *Prosopis* management allowing farmers, researchers and stakeholders to interact. Gretha's work focuses on the development of farm-scale management plans that promote the use of biological control.

Prosopis engagement

The management of *Prosopis* is vital to protect lives and livelihoods in the arid regions of South Africa. Ross Shackleton's research clearly demonstrated that integrated management which includes mechanical harvesting and increased biological control is required to successfully manage this species. The CBC has worked with Agri Noord-Kaap and partners in the Northern Cape to prepare funding applications to assist in implementing an integrated plan. The National Research Foundation has funded the engagement activities with stakeholders. Discussions and re-working of the draft strategy for management of *Prosopis* is underway; the CBC hopes for sufficient progress to be made this year as there will be more opportunities to meet in person.

The CBC plans to maintain overwintering populations of biological control agents released on *Prosopis*. We will house these agents in facilities managed by Oasis Skills Development and the Association for People with Disabilities in Upington.

Cactus outreach work

The Cactaceae team have continued their work with protected areas and have strong relations with both Camdeboo and Addo Elephant National Parks. These parks both have cactus management programmes, which we continue to support.

The South African Cactus Working Group normally has two meetings annually, co-hosted by the CBC and SANBI, but owing to the COVID-19 pandemic, these were cancelled; instead, we hosted a well-attended virtual meeting in 2021 to reconnect and share new information. The CBC recently took over the coordination of the International Cactus Working Group (ICWG). We had planned to have the first meeting in 2020 in Windhoek, Namibia. Unfortunately, pandemic restrictions meant this had to be postponed and we now have a proposed date for the second half of 2022.

Weed Biological Control Short Course

The CBC hosts an annual Weed Biological Control Short Course which is accredited through Rhodes University. Owing to the global pandemic we had to cancel both the 2020 and 2021 courses. We will revisit dates for 2022 and look at alternate ways of delivering the course.

School engagement

In the last few years, the CBC has been actively engaged with schools in the Hartbeespoort Dam and Nahoon River areas as part of the school mass-rearing projects. COVID-19 and the constraints and restrictions at the schools has meant that some schools have put this activity on the back burner for now. The CBC has been able to support and assist where possible with keeping these projects going; it has been easier with the two schools in East London as they are within driving distance from Makhanda. These two stations are going well, and releases have been happening throughout the year. Engagement with schools in Gauteng and North-West have been slow, but we will continue to engage and support teachers who want to set up facilities.

Community and school mass-rearing projects

In 2019, the CBC approached community groups and concerned citizens to participate in mass-rearing projects after the success of biological control on the Hartbeespoort Dam over the summer of 2019/2020. The aim was to increase the number of insects released onto the dam. Throughout 2020 and 2021, despite the national lockdown and the COVID-19 pandemic, the CBC team set up 13 rearing facilities and provided scientific consultation on three others that were funded by the stakeholders themselves. To date, there have been many new releases of the biological control agent *Megamelus scutellaris* on water hyacinth invasions around the country, and this has contributed immeasurably to the biological control effort in the country while also helping to improve support for the biological control national programme by increasing the awareness of the benefits of biological control. Having Rosali Smith based in Gauteng has been great for community members to have the presence and support more often than in the past.

In addition to the water hyacinth rearing projects, there was scope to start a rearing project for Cyrtobagous salviniae in areas such as the Garden Route Dam in George, where *Salvinia molesta* was prevalent. Local community members and government have acted together to assist in controlling the weed. The CBC implemented a satellite mass-rearing station at the Garden Route Botanical Gardens in partnership with a local schoolin March. As of September 2021, complete control of the system was achieved. The CBC was asked to assist the local school's programme (which has a substantial environmental focus) with biological control-based projects, and we are happy to announce that over 60% of students made use of these projects for their final year hand-ins.

The CBC has also been assisting a school in Zimbabwe with the mass-rearing and release of Cyrtobagous salviniae for the control of *Salvinia molesta* on a dam on the school's property. This partnership has become important for getting biological control in Zimbabwe and getting learners to engage with the practice.

Figure N1: Charmaine Stockdale and Rosali Smith at Witfield Rehabilitation. **Photo:** Grant Stockdale

Partners in the CBC's community engagement activities

AgriSA, 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, Peterhouse Boys School, Graeme College, Merrifield College, Stirling High School and Study House Private School.

Figure N2: Susa from Peterhouse Boys School releasing *Cyrtobagous salviniae* on Kariba weed in their mass-rearing facility. **Photo:** Ben Miller

Funders

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

National Funders

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

Citrus Research International (CRI)

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

National Research Foundation (NRF).

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

Red Meat Research and Development South Africa

River BioScience

Drakenstein Trust

South African Water Research Commission (WRC)

International Funders

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

Shire of Ravensthorpe, Western Australia

Queensland Department of Agriculture, Australia

New South Wales Department of Primary Industry, Australia

LandCare Research, New Zealand

Research Outputs

Graduates

In 2021, the CBC had a number of students graduate in the different virtual graduation ceremonies. Five Honours, two Masters of Commerce, ten Masters of Science and seven PhD graduates walked across the virtual stage in the course of the year

DEGREE OF BACHELOR OF SCIENCE WITH HONOURS

1. Khalipha Dambuza, in Entomology
2. Perryn Richardson, in Entomology, with distinction
3. Caitlin Webb, in Zoology
4. Liam Yell, in Zoology
5. Patricia Masole, in Botany at UFS

DEGREE OF MASTER OF COMMERCE

1. Brett Mason, MComm in Economics, in the Department of Economics and Economic History. Thesis: An evaluation of the socio-economic costs and benefits of the invasive *Rubus* (Blackberry/Bramble) genus species at selected sites in South Africa. Supervisor: Professor GCG Fraser. Co-supervisors: Dr GD Martin and Dr C Zachariades (ARC).
2. Timothy Westwood, MComm in Economics, in the Department of Economics and Economic History. Thesis: The potential conflict of interest associated with the management of *Rosa rubiginosa* L. (Rosehip) in South Africa. Supervisor: Professor GCG Fraser. Co-supervisor: Dr GD Martin.

DEGREE OF MASTER OF SCIENCE

1. Lehlohonolo Adams, MSc in Botany at UFS. Title: Reproductive Ecology of *Pyracantha* species in the Afromontane Grasslands of the Eastern Free State. Supervisor: Dr S Steenhuisen. Co-supervisors: Dr VR Clark and Dr GD Martin (RU).
2. Petrus Iita, MSc in Microbiology, **with distinction**, in the Department of Biochemistry & Microbiology. Thesis: Selection for improved virulence of Cryptophlebia peltastica nucleopolyhedrovirus (CrpeNPV) to False Codling Moth, *Thaumatotibia leucotreta*, by serial passage through a heterologous host. Supervisor: Professor CM Knox. Co-supervisors: Distinguished Professor MP Hill and Dr S Moore (CRI).
3. Siyasanga Mnciva, MSc in Entomology, with **distinction**, in the Department of Zoology and EntomologyThesis: Enhancement of *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) by naturally occurring phytopathogens for biological control of Pontederia crassipes (C. Mart) Solms (Pontederiaceae) in South Africa. Supervisor: Professor JA Coetzee. Co-supervisor: Dr C Coombes.
4. Tapiwa Mushore, MSc in Entomology, in the Department of Zoology and Entomology. Thesis: Biology, ecology, and management of the keurboom moth, *L. venus* Cramer and the leafhopper *Molopopterus* sp. Jacobi in cultivated Honeybush (*Cyclopia spp.*). Supervisor: Distinguished Professor MP Hill. Co-supervisor: Dr C Coombes.
5. Samantha Prinsloo, MSc in Entomology, **with distinction**, in the Department of Zoology & Entomology. Thesis: Potential synergism between entomopathogenic fungi and entomopathogenic nematodes for the control of false codling moth (*Thaumatotibia leucotreta*). Supervisor: Distinguished Professor MP Hill.
6. Daniel Rogers, MSc in Entomology, **with distinction**, in the Department of Zoology & Entomology. Thesis: The tuber-feeding weevil *Listronotus frontalis* as a candidate biological control agent for the invasive semi-aquatic plant *Sagittaria platyphylla* within South Africa. Supervisor: Dr GD Martin. Co-supervisor: Professor JA Coetzee.
7. Sara Salgado Astudillo, MSc in Entomology, **with distinction**, in the Department of Zoology & Entomology. Thesis: Evaluation of *Megabruchidius tonkineus* (Coleoptera: Chrysomelidae: Bruchinae), a candidate biological control agent for *Gleditsia triacanthos* L. (Fabaceae) in South Africa. Supervisor: Dr GD Martin. Co-supervisor: Distinguished Professor MP Hill.

8. David Taylor, MSc in Entomology, **with distinction**, in the Department of Zoology and Entomology. Thesis: Improvement of baculovirus–based management options for Thaumatotibia leucotreta Meyr. (Lepidoptera: Tortricidae) by exploiting synergistic relationships among different baculoviruses. Supervisor: Distinguished Professor MP Hill. Co-supervisors: Professor CM Knox and Dr S Moore (CRI).
9. Garyn Townsend, MSc in Microbiology, in the Department of Biochemistry, Genetics and Microbiology. Thesis: Initial assessment of the polyphagous shot hole borer (PSHB) in indigenous Afrotemperate forests. Supervisor: Professor ZW de Beer. Co-supervisors: Distinguished Professor MP Hill, Professor F Roets.
10. Sage Wansell, MSc in Botany, in the Department of Botany. Thesis: The invasion ecology of *Pontederia cordata* L. (Pontederiaceae) in South Africa. Supervisor: Professor JA Coetzee. Co-supervisor: Professor S Geerts.

DEGREE OF DOCTOR OF PHILOSOPHY

1. Pascal Aigbedion-Atalor, Doctorate in Entomology, in the Department of Zoology and Entomology. Thesis: Unravelling and ameliorating the impacts of *Tuta 19 absoluta* (Meyrick) invasion in eastern Africa: spread, socio-ecological impacts, and potential of a newly imported parasitoid for classical biological control. Supervisor: Distinguished Professor MP Hill. Co-supervisor: Dr M Samira.
2. Gerald Chikore, Doctorate of Entomology, in the Entomology Department, University of Free State, Bloemfontein. Thesis: Impact of *Robinia pseudoacacia* in the grassland biome. Supervisor: Dr F Chidawanyika. Co- Supervisor: Dr GD Martin.
3. Sariana Faure, Doctorate in Entomology, in the Department of Zoology & Entomology. Thesis: Systematics of the Afrotropical *Chalcididae* (Hymenoptera: Chalcidoidea). Supervisor: Dr S van Noort. Co-supervisor: Professor S Crompton (Leeds).
4. Adolphe Lehavana, Doctorate in Entomology, in the Department of Zoology and Entomology. Thesis: Distribution, and ecological and economic impact, of the invasive alien aquatic weeds *Pontederia crassipes*, *Salvinia molesta*, *Azolla filiculoides* and *Pistia stratiotes* in Madagascar. Supervisor: Distinguished Professor MP Hill. Co-supervisor: Dr C Zachariades (ARC) and Dr C Birkinshaw

Figure 01: The CBC's Masters and Doctorate graduates: Brett Mason, Tim Westwood, Lehlohonolo Adams, Petrus Iita, Siya Mnciva, Tapiwa Mushore, Samantha Prinsloo, Daniel Rogers, Sara Salgado Astudillo, David Taylor, Garyn Townsend, Sage Wansell, Pascal Aigbedion-Atalor, Sariana Faure, Adolphe Lehavana, Rosali Smith, Guy Sutton, Marcel van der Merwe.

5. Rosali Smith, Doctorate in Entomology, in the Department of Zoology & Entomology. Thesis: The biological control of *Egeria densa Planchon* (Hydrocharitaceae) in South Africa. Supervisor: Distinguished Professor MP Hill. Co-supervisor: Professor JA Coetzee.
6. Guy Sutton, Doctorate in Entomology, in the Department of Zoology and Entomology. Thesis: Prioritisation of biological control agents for Giant Rat's Tail Grass. Supervisor: Professor I Paterson. Co-supervisor: Dr K Canavan.
7. Marcel van der Merwe, Doctorate in Microbiology, in the Department of Biochemistry & Microbiology. Thesis: An investigation into yeast-baculovirus synergism for the improved control of *Thaumatotibia leucotreta*, an economically important pest of citrus. Supervisor: Professor C Knox. Co-supervisors: Distinguished Professor MP Hill and Dr S Moore (CRI).

Peer-reviewed articles

Rhodes University

1. Aigbedion-Atalor, P. O., Hill, M. P., Ayelo, P. M., Ndlela, S., Zalucki, M. P. & Mohamed, S. A. 2021. Can the Combined Use of the Mirid Predator *Nesidiocoris tenuis* and a Braconid Larval Endoparasitoid *Dolichogenidea gelechiidivoris* Improve the Biological Control of *Tuta absoluta*? *Insects*, **12**(11): 1004.
2. Albertyn, S., Moore, S.D., Marsberg, T., Coombes, C.A. & Hill, M.P. 2021. The influence of citrus orchard age on the ecology of entomopathogenic fungi and nematodes. *Biocontrol Science & Technology* **31**(1): 80–96.
3. Baso, N.C., Coetzee, J.A., Ripley, B.S. & Hill, M.P. 2021. The effects of elevated atmospheric CO2 concentration on the biological control of invasive aquatic weeds. *Aquatic Botany* **170**: 103348.
4. Buitenhuis, R., Cock, M.J., Colmenarez, Y.C., De Clercq, P., Edgington, S., Gadaleta, P., Gwynn, R.L., Heimpel, G.E., Hill, M., Hinz, H.L., Hoddle, M.S., Jäkel, T., Klapwijk, J.N., Leung, K., Mc Kay, F., Messelink, G.J., Silvestri, L., Smith, D., Sosa, A. & Wäckers, F.L. 2021. Draft study on sustainable use and conservation of microbial and invertebrate biological control agents, and biostimulants. FAO. http://www. fao. org/3/ng961en/ng961en. Pdf.
5. Byrne, M.J., Mayonde, S., Venter, N., Chidawanyika, F., Zachariades, C. & Martin, G.D. 2021. Three new biological control programmes for South Africa: Brazilian Pepper, Tamarix and Tradescantia. *African Entomology*, **29**(3): 965–982.
6. Canavan, K., Paterson, I.D., Ivey, P., Sutton, G.F. & Hill, M.P. 2021. Prioritisation of targets for weed biological control III: a tool to identify the next targets for biological control in South Africa and set priorities for resource allocation. *Biocontrol Science and Technology* **31**(6): 584–601.
7. Canavan, K., Magengelele, N. L., Paterson, I. D., Williams, D. A. & Martin, G. D. 2021. Uncovering the phylogeography of *Schinus terebinthifolia* in South Africa to guide biological control. *AoB Plants* **14**(1).
8. Canavan, K., Canavan, S., Clark, V. R., Gwate, O., Richardson, D. M., Sutton, G. F. & Martin, G. D. 2021. The alien plants that threaten South Africa's mountain ecosystems. *Land*, **10**(12): 1393.
9. Chikowore, G., Mutamiswa, R., Sutton, G. F., Chidawanyika, F. & Martin, G. D. 2021. Reduction of Grazing Capacity in High-Elevation Rangelands After Black Locust Invasion in South Africa. *Rangeland Ecology & Management*, **76**: 109–117.
10. Chikowore, G., Martin, G.D. & Chidawanyika, F. 2021. An assessment of the invasive alien tree, *Robinia pseudoacacia*, canopy traits and its effect on grassland microclimates and subsequent arthropod assemblages. *Journal of Insect Conservation* **25**(3): 429–439.
11. Chikowore, G., Mutamiswa, R., Steenhuisen, S., Martin, G.D., & Chidawanyika F. 2021. Integration of invasive tree, Black locust, into agro-ecological flower visitor networks induces competition for pollination services. *Arthropod-Plant Interactions* **15** (5): 787–796.
12. Chikowore, G., Chidawanyika, F. & Martin, G. D. 2021. Contributions of black locust (*Robinia pseudoacacia* L.) to livelihoods of peri-urban dwellers in the Free State Province of South Africa. *GeoJournal*, 1–14.
13. Coetzee, J.A., Bownes, A., Martin, G.D., Miller, B.E., Smith, R., Weyl, P.S.R. & Hill, M.P. 2021. A review of the biocontrol programmes against aquatic weeds in South Africa. *African Entomology*, **29**(3): 935–964.
14. Downey, P.O., Paterson, I.D., Canavan, K. & Hill, M.P. 2021. Prioritisation of targets for weed biological control I: a review of existing prioritisation schemes and development of a system for South Africa. *Biocontrol Science and Technology* **31** (6): 546–565.

15. Harms, N.E., Knight, I.A., Pratt, P.D., Reddy, A.M., Mukherjee, A., Gong, P., ... & Diaz, R. 2021. Climate Mismatch Between Introduced Biological Control Agents and Their Invasive Host Plants: Improving Biological Control of Tropical Weeds in Temperate Regions. *Insects* **12** (6): 549.
16. Hill, M.P., Conlong, D., Zachariades, C., Coetzee, J.A., Paterson, I.D., Miller, B.E., Foxcroft, L. & Van Der Westhuizen, L. 2021. The role of mass-rearing in weed biological control projects in South Africa. *African Entomology* **29**(3): 1030–1044.
17. Hinsberger, A., Blanchère-Lopez, C., Knox, C., Moore, S.D., Marsberg, T. & Lopez-Ferber, M. 2021. CpGV-m replication in type I resistant insects: Helper virus and order of ingestion are important. *Viruses* **13**(9): 1695
18. Hussner, A., Heidbüchel, P., Coetzee, J. & Gross, E. M. 2021. From introduction to nuisance growth: a review of traits of alien aquatic plants which contribute to their invasiveness. *Hydrobiologia*, 1–33.
19. Ivey, P.J., Hill, M.P. & Zachariades, C. 2021. Advances in the regulation of weed biological control in South Africa. *African Entomology*, **29**(3): 1060–1076
20. Jukes, M. D. 2021. Reads in a haystack: extracting complete mitogenome sequences hidden in baculovirus datasets. *Insect Molecular Biology*, **30**(6): 541–551.
21. King, A.M, Paterson, I.D, Simelane D.O., Van Der Westhuizen L., Mawela, K.V. & Mnqeta, Z. 2021. Biological control of invasive climbing plants in South Africa. *African Entomology*, **29**(3): 905–934.
22. Kleinjan, C.A., Hoffmann, J.H., Heystek, F., Ivey, P. & Kistensamy, Y. 2021. Developments and prospects for biological control of *Prosopis* (Leguminosae) in South Africa. *African Entomology*, **29**(3): 859–874.
23. Maluleke, M., Fraser, G.C.G. & Hill M.P. 2021. Economic evaluation of chemical and biological control of four aquatic weeds in South Africa. *Biocontrol Science and Technology*: 1–16.
24. Martin, G.D. 2021. Prospects for the biological control of Northern Temperate Weeds in South Africa. *African Entomology*, **29**(3): 791–808.
25. Maseko, Z., Coetzee, J.A. & Hill, M.P. 2021. Population dynamics of the biological control agent, *Eccritotarsus catarinensis* Carvalho (Miridae) on *Pontederia crassipes* Pellegrini and Horn (Pontederiaceae) at a seasonally cold site. *African Entomology* **29**(1): 307–310.
26. Miller, B., Coetzee, J.A. & Hill, M.P. 2021. Mind the gap: Evidence for delayed recovery of a biological control agent population on water hyacinth in the Eastern Cape Province (South Africa) after winter. *Bulletin of Entomological Research* **111**(1): 120–128.
27. Minuti, G., Coetzee, J.A., Ngxande-Koza, S., Hill, M.P. & Stiers, I. 2021 Prospects for the biological control of *Iris pseudacorus* L. (Iridaceae). *Biocontrol Science and Technology* **31**(3): 314-335.
28. Moore, S.D. 2021. Biological Control of a Phytosanitary Pest (*Thaumatotibia leucotreta*): A Case Study. *International Journal of Environmental Research and Public Health* **18**(3): 1198.
29. Olckers, T., Coetzee J.A., Egli, D., Martin, G.D., Paterson, I.D., Sutton, G.F. &Wood A.R. 2021. Biological control of South African plants that are invasive elsewhere in the world: a review of earlier and current programmes. *African Entomology* **29**(3): 1005–1029.
30. Paterson, I.D., Hill, M.P., Canavan, K. & Downey, P.O. 2021. Prioritisation of targets for weed biological control II: the South African Biological Control Target Selection system. *Biocontrol Science and Technology* **31**(6): 566–583.
31. Paterson, I.D, Klein, H, Muskett, P.C, Griffith, T.C, Mayonde, S., Mofokeng, K., Mnqeta, Z. & Venter, N.C. 2021. Biological control of Cactaceae in South Africa. *African Entomology*, **29**(3): 713–734.
32. Paterson, I.D., Den Breeÿen, A., Martin, G.D. & Olckers, T. 2021. An introduction to the fourth decadal review of biological control of invasive alien plants in South Africa (2011–2020). *African Entomology*, **29**(3): 685–692.
33. Reid, M. K., Naidu, P., Paterson, I. D., Mangan, R. & Coetzee, J. A. 2021. Population genetics of invasive and native *Nymphaea mexicana* Zuccarini: Taking the first steps to initiate a biological control programme in South Africa. *Aquatic Botany* **171**: 103372.
34. Sutton, G.F., Canavan, K., Day, M.D. & Paterson, I.D. 2021. Field-based ecological studies to assess prospective biological control agents for invasive alien plants: an example from giant rat's tail grass. *Journal of Applied Ecology* **58**: 1043–1054.
35. Sutton, G.F., Bownes, A., Visser, V., Mapaura, A. & Canavan, K. 2021. Progress and prospects for the biological control of invasive alien grasses (Poaceae) in South Africa. *African Entomology*, **29**(3): 837–858.

36. Strathie, L., Cowie, B., Mcconnachie, A., Chidawanyika, F., Musedeli, J., Sambo, S.M.C., Magaso E.X. & Gareeb, M. 2021. A decade of biological control of *Parthenium hysterophorus*. L. (Asteraceae) in South Africa reviewed: introduction of insect agents and their status. *African Entomology*, **29**(3): 809–836;

37. Tshithukhe, G., Motitsoe, S.N. & Hill, M.P. 2021. Heavy Metals Assimilation by Native and Non-Native Aquatic Macrophyte Species: A Case Study of a River in the Eastern Cape Province of South Africa. *Plants* **10** (12): 2676.

38. van Noort, S., Smith, R. & Coetzee, J. A. 2021. Identity of parasitoid wasps (Hymenoptera, Braconidae and Eulophidae) reared from aquatic leaf-mining flies (Diptera, Ephydridae) on invasive Brazilian waterweed *Egeria densa* in South Africa. *African Invertebrates* **62**: 287.

39. van Rooyen, E., Paap, T., De Beer, W., Townsend, G., Fell, S., Nel, W., Morgan, S., Hill, M. & Roets, F. 2021. The Polyphagous Shot Hole Borer (PSHB) beetle: current status of a perfect invader in South Africa: Current status of the Polyphagous Shot Hole Borer Beetle in South Africa. *South African Journal of Science* 2021;**117**(11/12), Art. #9736.

40. van Steenderen, C., Paterson, I.D., Edwards, S. & Day, M.D. 2021. Addressing the red flags in cochineal identification: the use of molecular techniques to identify cochineal insects that are used as biological control agents for invasive alien cacti. *Biological control* **152**: 104426.

41. Weaver, K.N., Hill, M.P., Byrne, M.J. & Ivey, P. 2021. Efforts towards engaging communities to promote the benefits of biological control research and implementation in South Africa. *African Entomology*, **29**(3): 1045–1059.

Wits

1. Abutaleb, K., Newete, S.W., Mangwanya, S., Adam, E. & Byrne, M.J. 2021. Mapping eucalypts trees using high resolution multispectral images: A study comparing WorldView 2 vs. SPOT 7. *The Egyptian Journal of Remote Sensing and Space Science* **24**(3): 333-342.

2. Buccellato, L., Fisher, J.T., Witkowski, E.T. & Byrne, M.J., 2021. The effects of a stem gall fly and a leaf pathogen on the reproductive output of Crofton weed, *Ageratina adenophora* (Asteraceae), in greenhouse and field trials. *Biological Control* **152**: 104453.

3. Byrne, M.J., Mayonde, S., Venter, N., Chidawanyika, F., Zachariades, C. & Martin, G. 2021. Three new biological control programmes for South Africa: Brazilian pepper, Tamarix and Tradescantia. *African Entomology* **29**(3): 965-982. (Duplicate)

4. Cowie, B.W., Byrne, M.J. & Witkowski, E.T. 2021. Feasible or foolish: Attempting restoration of a Parthenium hysterophorus invaded savanna using perennial grass seed. Journal of Environmental Management 280: 111686.

5. Cowie, B.W., Byrne, M.J. & Witkowski, E.T. 2022. Small-scale insights into the above-and below-ground invasion dynamics of *Parthenium hysterophorus* in a South African savanna: The potential role of stocking rate. *South African Journal of Botany* **144**: 229-237.

6. Gitonga, L.N., Cron, G.V., Glennon, K.L., McConnachie, A.J. & Byrne, M.J. 2021. Two ploidy levels present in the invasive *Campuloclinium macrocephalum* (pompom weed) in South Africa–Implications for biocontrol. *Weed Research* **62**(1): 59-67.

7. Hatile, S.L., Mayonde, S., Venter, N. & Byrne, M.J. 2021. The host specificity of *Trabutina mannipara* (Hemprich & Ehrenberg, 1829) (Homoptera: Coccoidea: Pseudococcoidae): a potential biocontrol agent of invasive *Tamarix chinensis* (Lour) and *T. ramosissima* (Ledeb) in South Africa. *Biocontrol Science and Technology*: 1-10.

8. Mayonde, S., Cron, G.V., Glennon, K.L. & Byrne, M.J. 2021. Effects of cadmium toxicity on the physiology and growth of a halophytic plant, *Tamarix usneoides* (E. Mey. ex Bunge). *International Journal of Phytoremediation* **23**(2): 130-138.

9. Mukarugwiro, J.A., Newete, S.W., Adam, E., Nsanganwimana, F., Abutaleb, K. & Byrne, M.J. 2021. Mapping spatio-temporal variations in water hyacinth (*Eichhornia crassipes*) coverage on Rwandan water bodies using multispectral imageries. *International Journal of Environmental Science and Technology* **18**(2): 275-286.

10. Musengi, K., Mbonani, S. & Byrne, M.J. 2021. Host suitability of three Opuntia taxa for the *Dactylopius opuntiae* (Hemiptera: Dactylopiidae) 'stricta' lineage. *Biocontrol Science and Technology*: 1-13.

11. Reynolds, C., Byrne, M.J., Chamberlain, D.E., Howes, C.G., Seymour, C.L., Sumasgutner, P. & Taylor, P.J. 2021. Urban animal diversity in the Global South. In: *Urban ecology in the Global South*. Springer, Cham, pp.169-202.

12. Tesfamichael, S.G., Newete, S.W., Adam, E. & Byrne, M.J. 2021. Discriminating pure Tamarix species and their putative hybrids using field spectrometer. *Geocarto International*: 1-20.

13. Venter, N., Cowie, B.W., Olckers, T. & Byrne, M.J. 2021. Current and future biological control efforts against *Solanum mauritianum* (Solanaceae) in South Africa. *African Entomology*, **29**(3): 875-888.

14. Venter, N., Cowie, B.W., Paterson, I.D., Witkowski, E.T. & Byrne, M.J. 2021. The Interactive Effects of CO2 and Water on the Growth and Physiology of the Invasive Alien Vine *Pereskia aculeata* (Cactaceae): Implications for its Future Invasion and Management. *Environmental and Experimental Botany* **194**: 104737.

15. Weaver, K.N., Hill, M.P., Byrne, M.J. & Ivey, P. 2021. Efforts towards engaging communities to promote the benefits of biological control research and implementation in South Africa. *African Entomology* **29**(3): 1045-1059. (Duplicate)

16. Williams, V.L., Burness, A., Wojtasik, E.M. & Byrne, M.J. 2021. Dataset, including a photo-guide, of alien plants sold in traditional medicine markets and healthcare outlets in three South African cities, specifically by traders of Indian, West African, East African, and Chinese origin. *Data in brief* **38**: 107395.

17. Williams, V.L., Wojtasik, E.M. & Byrne, M.J. 2021. A chronicle of alien medicinal plants used as traditional medicine in South Africa, and their status as invasive species. *South African Journal of Botany* **142**: 63-72.

Book chapters

1. Witt, A.B.R., Cock, M.J.W., Day, M.D., Zachariades, C., Strathie, L.W., Conlong, D.E., Hill M.P. & Roy, S. 2021. Biological control successes and failures: African region. In: Mason, P.G. (Ed) *Biological Control: global impacts, challenges and future directions of pest management*. CSIRO Publishing, Melbourne.

2. Barratt, B.I., Colmenarez, Y.C., Day, M.D., Ivey, P., Klapwijk, J.N., Loomans, A.J., Mason, P.G., Palmer, W.A., Sankaran, K.V. and Zhang, F., 2021. Regulatory challenges for biological control. In: Mason, P.G. (Ed) *Biological control: global impacts, challenges and future directions of pest management*. CSIRO Publishing, Melbourne.

Conference proceedings

1. Coetzee, J.A. 2021. Where's the water hyacinth? *National Symposium on Biological Invasions – virtual symposium*. 5–7 May 2021.

2. Coetzee, J.A. 2021. Managing aquatic weeds in southern Africa: the past, the present and the future. *The West African Symposium on Biological Invasions*, 14–16 December 2021, Saint Louis, Senegal.

3. Cousins, L., Moore., S., Peyper, M., Marsberg, T. & Hill, M.P. 2021. A comparison of *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae) infestation in organic versus conventional citrus orchards. *22nd Hybrid Congress of the Entomological Society of Southern Africa*. 28 June–1 July 2021. Tshipise, Forever Resort, Limpopo.

4. Cowie, B., Paterson, I., Kleinjan, C., Heystek, F., McKay, F., Thompson, D. & Ivey, P. 2021. Managing Prosopis: renewed promise for biocontrol. *National Symposium on Biological Invasions – virtual symposium*. 5–7 May 2021.

5. Griffith, T. & Paterson, I. 2021. The potential of *Hypogeococcus* (Pseudococcidae) as a biological control agent against torch cactus, *Trichocereus spachianus* (Cactaceae), in South Africa. *National Symposium on Biological Invasions – virtual symposium*. 5–7 May 2021. Poster presentation.

6. Hinsberger, A., Blachère-Lopez, C., Knox, C., Moore, S., Marsberg, T. & Lopez-Ferber, M. 2021. Multiple baculovirus infections in codling moth: CpGV-R5 help to CpGV-M cannot be substituted by CrpeNPV. 2021 *International Congress on Invertebrate Pathology and Microbial Control & 53rd Annual Meeting of the Society for Invertebrate Pathology*. 28 June–2 July 2021. Virtual Meeting.

7. Ivey, P., Koopman, R., Paterson, I.D. & Mnqeta, Z. 2021. Civil society, plant surveys and biological control researchers, working together! *National Symposium on Biological Invasions – virtual symposium*. 5–7 May 2021. Poster presentation.

8. Kinsler, D., Coetzee, J.C., Hill, M.P. & McGregor, G. 2021. Remote Sensing as a monitoring tool for Water Hyacinth (*Pontederia crassipes*) in the context of the biological control release programme at Hartbeespoort Dam. *National Symposium on Biological Invasions – virtual symposium*. 5–7 May 2021.

9. Kinsler, D.L., McGregor, G.K., Coetzee, J.A. & Hill, M.P. 2021. Remote Sensing as a monitoring tool for Water Hyacinth (*Pontederia crassipes*) in the context of the biological control release programme at Hartbeespoort Dam, South Africa. *Association for the Sciences of Limnology and Oceanography (ASLO), Aquatic Sciences Meeting. Virtual Meeting*. 22–27 July 2021.
10. Kirkman, W., Moore, S., Hill, M., Tandlich, R., Hertog, M. & Nicolaï, B. 2021. Post-harvest detection of infested fruit: The solution is hanging in the air. *36th Annual Meeting of the International Society of Chemical Ecology (virtual)*, 5–10 September 2021, Stellenbosch, South Africa.
11. Marsberg, T. & Moore, S.D. 2021. Synergism between a baculovirus and an insect growth regulator? *2021 International Congress on Invertebrate Pathology and Microbial Control & 53rd Annual Meeting of the Society for Invertebrate Pathology*. 28 June–2 July 2021. Virtual Meeting.
12. Martin, G.D. 2021. Invasive Alien Plants Working Group for southern African mountains. *National Symposium on Biological Invasions – virtual symposium*. 5–7 May 2021.
13. Miller, B.E., Coetzee, J.A., Kinsler, D. & Hill, M.P. Inundative release techniques: how mass releasing a biocontrol agent led to the reduction of water hyacinth on Hartbeespoort Dam. *22nd Hybrid Congress of the Entomological Society of Southern Africa*. 28 June–1 July 2021. Tshipise, Forever Resort, Limpopo.
14. Minuti, G., Coetzee, J.A. & Stiers, I. 2021. Climatic suitability of the invasive aquatic *Iris pseudacorus* L. and its candidate biocontrol agent *Aphthona nonstriata* (Goeze) in the Southern Hemisphere. *17th Symposium on Insect-Plant Interactions – virtual symposium*. 25–30 July 2021.
15. Mnciva, S., Coetzee, J.A. & Coombes, C. 2021. Enhancement of *Megamelus scutellaris* Berg (Hemiptera: Delphacidae) by naturally occurring phytopathogens for biological control of *Pontederia crassipes* (C. Mart) Solms (Pontederiaceae) in South Africa. *National Symposium on Biological Invasions – virtual symposium*. 5–7 May 2021. Poster presentation.
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46. Zozo, E. & Paterson, I.D. 2021. Biological control of *Cylindropuntia pallida*. *National Symposium on Biological Invasions – virtual symposium*. 5–7 May 2021. Poster presentation.

Student prizes

National Symposium on Biological Invasions

Best Oral Paper by a student:

Matt Paper

Sage Wansell

Best Poster:

Siya Mnciva

ESSA

Best student presentation (PhD)

Rosali Smith

Clarke van Steenderen

Best student presentation (MSc)

Garyn Townsend

Figure 02: Samella Ngxande-Koza, Petrus Iita, Samalesu Mayonde, Kim Canavan, Clarke van Steenderen, Colleen Downes, Samuel Motitsoe, Wayne Mommsen, Benjamin Miller, Marcus Byrne, Tapiwa Mushor

Ruth Scholtz, Hildegard Klein, Phillippa Muskett, Byron Soetland, Tahnee Bennett, Grant Martin, David Kinsler, Rosali Smith, Nic Venter, Lyle Titus, Gretha van Staden, Karin Goliath

Gerald Chikowore, Kim Weaver, Iain Paterson, Blair Cowie, Esther Mostert, Siphokazi Msengana, Carmen Peters, Abigail Wolmarans, Lubabalo Malinga, Daniel Scholtz, John Hoffmann, Keneilwe Sebola

Megan Reid, Thuthula Mela, Candice Owen, Vuyani Ntyinkala, Getrude Tshithukhe, Martin Hill, Anthony Mapaura, Luke Cousins, Pascal Osa Aigbedion-Atalor, Tshililo Mphephu, Matthew Paper, Marcel van der Merwe

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

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

Lunga Ngeju, Ronel Roman, Ernst de Beer, Maretha Boshoff, Frank Chidawanyika, Samantha Prinsloo, Arthur Scholtz, Caroline Knox, Wandisile Mdiza, Wayne Kirkman, Nompumelelo Baso, Emile van Son

Acronyms and Abbreviations

AFLP	Amplified Fragment Length Polymorphism
AgriSA	Agricultural Research Council of South Africa
ARC-PHP	Agricultural Research Council – Plant Health and Protection
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
CTAB	Cetyltrimethylammonium bromide
DEA	Department of Environmental Affairs
DFFE	Department of Forestry, Fisheries and the Environment
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
ESSA	Entomological Society of Southern Africa
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
KZN	KwaZulu-Natal
NCE	Namibian Chamber for Environment
NEMBA	National Environmental Management: Biodiversity Act
NPV	novel alphabaculovirus
NRF	National Research Foundation
NRM	Natural Resource Management
OBs	Occlusion bodies

PCR	polymerase chain reaction
PSHB	Polyphagous Shot Hole Borer
qPCR	quantitative polymerase chain reaction
SAAB	South African Association of Botanists
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
UFH	University of Fort Hare
UFS	University of the Free State
UKZN	University of KwaZulu-Natal
UMP	University of Mpumalanga
USACE	United States Army Corps of Engineers
USDA	United States Department of Agriculture
UV	Ultraviolet
WfW	Working for Water
Wits	University of Witwatersrand

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Instagram: https://www.instagram.com/rhodesunicbc/

CBC Vision

The CBC seeks to:

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

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

CBC Mission

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

CBC