Application to licence the release of a classical non-native invertebrate biological control agent (IBCA) on Ascension Island

This document is a modified version of the Department for the Environment, Food and Rural Affairs (Defra), UK, form for the application to licence the release of an invertebrate biological control agent (IBCA) in England. It was originally adapted from work undertaken by the EU-funded 'REBECA' (Regulation of Biological Control Agents) (http://www.rebeca-net.de/) project and by the European and Mediterranean Plant Protection Organisation (EPPO) to support the use of IBCAs.

The release of a non-native IBCA into Ascension is regulated under the Biosecurity Ordinance, 2020, and requires a licence issued by the Governor of St Helena on the recommendation of the Ascension Island Government (AIG) Chief Biosecurity Officer.

Using this form

This form should be used for IBCAs, which refer to arthropods as well as entomopathogenic nematodes, but not micro-organisms. Applications to release non-native micro-organisms from quarantine conditions should be made separately.

Guidance on the completion of this form is provided in the accompanying Guidance Document. This form is valid for an application relating to a single IBCA. An IBCA should be allocated to one of the following identifiable and recognisable taxonomic levels: species, sub-species, population, strain, or biotype.

The intention to submit a licence application should first be notified to the AIG Conservation Department. The completed application should be submitted to an appropriate expert for an independent risk assessment. Defra (Risk and Policy Team, non-nativebiocontrol.licensing@defra.gsi.gov.uk) may be able to provide advice on an appropriate expert. At all times and in all communications, the application will be regarded as confidential. The independent expert will provide advice to the AIG Government Conservation and Fisheries Directorate (AIGCFD) who will make the final recommendation on whether a licence to import and release is granted. A licence will be valid for a fixed period of time, after which a renewal maybe sought. If there are no changes to the application at renewal, this form does not need to be filled in again.

Submitting an application and further information:

Tiffany Simpson
Director of Conservation and Fisheries
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1. Applicant and product information

Section 1A: Information on the applicant	
Who will apply for the licence?	
Name of organisation	CABI
Name of applicant	Norbert Maczey
Affiliation of applicant	CABI
Address	Bakeham Lane, Egham, Surrey, TW20 9TY, United Kingdom
Phone	+44 (0) 1491 829029
E-mail	n.maczey@cabi.org
Who is the contact person?	
Name of contact person	Norbert Maczey
Affiliation of contact person	Details as above
Address	"
Phone	"
E-mail	"

Section 1B: Purpose of the application and use	
Information on the application	
Application type (new or renewal)	New
Renewal (application number and expiry date of current licence)	N.A.
Positive List organism (yes/no)	Yes
Relation to previous/other applications	N.A.
Application or registration elsewhere in Europe	N.A.

Existence of an earlier risk assessment	No
Purpose of use	
Target species	*Neltuma juliflora (Sw.) Raf.* (Mexican Thorn, Mesquite)
Receiving environment	All of Ascension Island
Area of release	Dense *Neltuma juliflora* stands at lower elevation of island.
Rearing/research facilities and procedures Describe how the risks, and the extent or probability of escape into the wild will be managed (if applicable)	
Address	Host range testing and culturing of the IBCA has been conducted at the CABI Facilities in Egham, UK. Import and release to Ascension Island are only planned after approval of this RA, where no measures to prevent escape into the wild will be required.
Labelling, packaging and storage	Packaged at CABI quarantine facilities according to international standards and hand-carried on a direct flight from the UK to Ascension Island. Transferred initially to net cages before release.
Facility	Temporary culture facility established on Donkey Plain, Ascension Island.
Quality control management system	N.A.
Accreditation/ PH quarantine license	CABI is entitled to import invertebrates, which meet GB's quarantine pest criteria under INV licence 212997-10. However, consultation with the Animal and Plant Health Agency (APHA) looking into specifically adding *Evippe* sp. #1 to be a named species on this licence revealed that this was not required: "*Evippe* sp. #1 does not meet GB provisional quarantine pest criteria. Further assessment may be required in the future for these pests that are not: GB quarantine pests, GB provisional quarantine pests or pest free area quarantine pests for Great Britain, if legislative requirements change or new evidence regarding the pest comes to light.

	A scientific licence is not required to import, move or keep these species; however, we advise that this pest should be held securely and not released or allowed to escape into the environment in accordance with Article 14 of the Wildlife and Countryside Act 1981 which prohibits the release of animals that are not ordinarily resident in, and are not a regular visitor, of Great Britain into the environment." A culture of *Evippe* sp. #1 is held within CABI's quarantine facilities at Egham (UK). A licence for release on Ascension Island is only to be issued after approval of this Risk Assessment (RA).
Safety information	The agent is safe to be released on Ascension Island. Transport will be in sealed containers, but even in case specimens would be able to escape there are no safety risks expected as both the UK and the Falkland Island (endpoint of the flight link between the UK and Ascension Island) both have a climate unsuitable for the survival of the IBCA.

2. Initiation

2.1. Identity

2.1.1. Name of the IBCA

Domain	Eukaryota
Kingdom	Metazoa
Phylum	Arthropoda
Subphylum	Uniramia
Order	Lepidoptera
Family	Gelechiidae
Genus	*Evippe*
Species	*Evippe* sp. #1
Authority	Chambers, 1873 (for genus only)
Synonymy:	*Agnippe* Chambers, 1872

Species previously listed under synonymized *Evippe* Chambers, 1873 have recently been synonymized with *Agnippe* Chambers, 1872 (Lee & Brown, 2008). The genus *Agnippe* comprises about 30 species occurring mainly in the Holarctic Region (Bidzilya & Li, 2010). There are also a few records for the Neotropical Region (Becker, 1984). Nearctic species are listed by Hodges (1983).

Wikipedia already lists *Evippe* as a synonym of *Agnippe* (https://en.wikipedia.org/wiki/Agnippe). Other synonyms for *Evippe* are:

- *Aganippe* Chambers, 1880 (misspelling)
- *Phaetusa* Chambers, 1875 (preocc. Wagler, 1832)
- *Tholerostola* Meyrick, 1917

So far, in all publications, reports and press releases this taxon has always been dealt with under the name *Evippe* sp. #1. Simply for practical reasons, we therefore suggest to continue using this placeholder name until a full description of this species has been published.

The applicants are not aware of any common names for this species. There is no information available on any micro-organisms associated with this species.

2.1.2 ID confirmation

Evippe sp. #1 refers to a taxon belonging to the genus *Evippe*, which is currently still undescribed. This taxon has been cultured and widely released in Australia and South Africa. Some specimens are now being processed to be sent off from the Agricultural Research Council (ARC) in South Africa to the Smithsonian for possible ID/ description (F. Heysteck, ARC, pers. comm., July 2022). Several specimens of the taxon have also been sequenced under the name *Evippe* sp. ANIC1. Details can be accessed under: https://v3.boldsystems.org/index.php/Public_SearchTerms?query=Evippe[tax].

Voucher specimens of adult *Evippe* sp. #1 are deposited in the collections of CABI, Bakeham Lane, Egham, TW20 9TY, UK.

2.1.3 Collection and culture

Evippe sp. #1 specimens cultured for release on Ascension Island were provided by the ARC in South Africa. This country has recently licensed the species for release to control invasive *Neltuma* species (Fabaceae). Because *Evippe* sp. #1 is not native to South Africa no export permit from this country was required. A batch of circa 300 caterpillars and pupae hidden in *Neltuma* leaf-ties were shipped to quarantine facilities at CABI Egham in August 2022 for host range testing and the development of a clean pest-free culture. The shipment was imported into the UK under the Defra plant health licence no 51073/212997-5, but no additional licensing to keep the species in culture in the UK was required. This has been confirmed through consultation with APHA (see above).

The population in South Africa itself originates from Australia where a control programme for *Neltuma* using *Evippe* sp. #1 was conducted some years ago. Australia was using a licensed export of the species from Argentina, which is part of its natural range. The resolution No. 410/19 from Argentina, which establishes the minimum standards for the application of Nagoya protocol, in its article 6 c) says "The use of biological resources that does not imply the use of their genetic and/or biochemical components (e.g. conducting host specificity tests; using natural enemies as biocontrol agents) are excluded from ABS requirements'. A specific license from Argentina for a third country provision of the control agent was therefore not required.

Culturing of *Evippe* sp. #1 currently takes place under controlled conditions at the CABI quarantine facilities at Egham, UK. The species is reared on caged *Neltuma juliflora* (Sw.) DC. (Mexican Thorn, Mesquite) plants grown from seeds of the naturalised population present on Ascension Island. Rearing of host plants and the IBCA itself follows the guidelines provided by Cowie (2022).

Evippe sp. #1 (Fig. 1) is easy to culture under controlled conditions and at the time of writing several generations have been reared already to a) conduct host range testing and b) to produce sufficient numbers for shipments for the release on Ascension Island.

A B

C D

Figure 1: A: *Evippe* sp. #1 caterpillar in leaf tie; B: caterpillar; C: pupa in leave-tie; D: adult female. © Chris Berman, CABI

2.2. Distribution of the IBCA

Gelechiid leaf-tiers on *Neltuma* are widespread in both Argentina and Paraguay (Cordo & DeLoach, 1987), but few have been reliably identified. The presence of *Evippe* sp. #1 has been confirmed in La Rioja and Santiago del Estero Provinces of Argentina (van Klinken et al., 2003). All *Evippe* sp. #1 in use for classical biological control (CBC) originate from a culture established from over 20 adults bred from *Neltuma* x *vinalillo* (Stuck) C. E. Hughes & G. P. Lewis which was collected 24 km west of Suncho Corral, Santiago del Estero Province, Argentina (30°22'S 63°27'W) (van Klinken & Heard, 2000).

Outside its natural range, *Evippe* sp. #1 has become established in Australia (van Klinken et al., 2003). Here it has been released on a large scale in NSW, Queensland, Northern Territory, and Western Australia (van Klinken, 2012). It is also in the process of becoming established in South Africa where a release programme is still ongoing. The leaf feeding moth *Evippe* sp. #1 was released into South Africa for the control of invasive *Neltuma* species (mesquite) for the first time on 24 February 2021 at the Meerkat Reserve at SKA, and a second release was made in the North-West Province, near Bloemhof on 3 March 2021 (press release by ARC Marketing and Communications).

2.3. Biology of the IBCA

2.3.1. Morphology

Evippe sp. #1 is a small leaf-tying moth (gelechiid) native to parts of Argentina. It is an as yet undescribed taxon and at the date of writing no detailed morphological assessment has been published.

Externally, it is very similar, or identical, to *E. omphalopa* Meyrick, which is known only from type material collected in Ecuador (Sattler, pers. comm., 1997). Both species share strongly asymmetrical valvae (the principal character that distinguishes them from other described *Evippe* species) but differ from each other in the shape of the uncus and gnathos (Sattler, pers. comm., 1997). Voucher specimens of *Evippe* sp. #1 have been lodged in the Australian National Insect Collection (Canberra: LPL 10041-3) and the Natural History Museum, London (van Klinken & Heard, 2000).

Males and females can easily be distinguished with males have two long palpi protruding the head capsule, which are missing in the female (Fig. 2)

Figure 2: Adult female *Evippe* sp. #1. © Norbert Maczey, CABI

2.3.2. Life cycle

Both the adults (moths) and larvae (caterpillars) feed on the leaves of *Neltuma*, although only larval feeding and leaf-tying is damaging. Overall, the biology of *Evippe* sp. #1 allows for the moth to be easily reared with a rapid development time on a single plant of ~35 days from egg to adult (Cowie, 2022).

Between 75 to 300 eggs are oviposited directly onto the plant where they are mostly placed into cracks and fissures in the bark (van Klinken et al., 2003). Oviposition begins within a day of emergence and continues through the female's life, although 77% of eggs are oviposited in the first week and 99.9% by day 15 (van Klinken & Heard, 2000). An average of 5.4 eggs per female were found in the foliage. This accounts for only about 7% of the total number of leaf mines found (75 leaf mines per female), implying that most contributing eggs are oviposited elsewhere (van Klinken & Heard, 2000).

Eggs hatch within a few days and the larvae begin leaf mining, subsequently forming leaf-ties within which they feed, develop, and pupate (Cowie, 2022). Van Klinken & Heard (2000) provide an overview of the larval development: '*Evippe* sp. #1 has four larval instars which may be distinguished by head capsule width. First instars mine mature pinnules leaving a webbed entry hole, which is also used by larvae to rid the mine of frass. Leaf mines appear externally as a pale blister. Most first instar larvae moult and continue to feed within the leaf mine before exiting and constructing a leaf tie. Leaf ties are constructed from opposing and adjacent pinnules tightly webbed together to form a capsule in which the second instar continues feeding and moults. New, independent leaf ties are made by the third and fourth instars, and pupation occurs in the third leaf tie. These later leaf ties are often larger (incorporating up to 20 pinnules) than those made by second instars (about 2± 10 pinnules), and use more extensive sheaths of webbing with which to bind pinnules together. Adults eclose (the term used for the emergence of adult insects from a pupal case) within the leaf tie and exit via a gap in the silk sheaths between the apex of the terminal pinnules. Complete development took between 34 and 48 days from oviposition. Leaf mining begins about 8 days after eggs were laid, leaf tying from day eleven, and pupation from day 26. The four larval instars each take a minimum of 3± 5 days.'

Under controlled conditions (27 °C day, 23 °C night; 60 % RH; L:D 16:8 photoperiod plus limited oblique natural lighting) egg to adult development takes between 34 and 48 days. Adults are short-lived, rarely surviving for more than 20 days with 70% still alive after day seven and 14% after day 15 (Klinken & Heard, 2000; van Klinken et al., 2003). *Evippe* sp. #1 diapause as fourth instar larvae. Diapause is thought to be initiated primarily by changes in daylength, as it is entered independently of temperature and moisture conditions, and can be prevented through the use of grow lamps. In Australia under field conditions, larvae enter diapause in late March or early April and exit it in July. The timing of diapause appears to be independent of latitude (van Klinken et al., 2003).

Host plants

In Argentina, *Evippe* sp. #1 was bred from *Neltuma vinalillo*. In Australia, cultures were maintained on whole plants of *Neltuma* species belonging to the Chilensis series (*N. velutina, N. juliflora, N. glandulosa*) (van Klinken & Heard, 2000).

Apart from *N. juliflora, Evippe* sp. #1 developed well on *N. velutina* and to a lesser degree on *N. pallida* (Pallidae series) (van Klinken & Heard, 2000; Heystek & Kistensamy, 2020). There was also full development on an unidentified *Neltuma* hybrid sourced from South Africa (*Neltuma* spp. x?*velutina* hybrid) (Heystek & Kistensamy, 2020).

There was also some initial larval development (first instar leave-mines) on closely related Mimosoideae (*Leucaena leucocephala, Senegalia mellifera, Vachellia erioloba, Vachellia karroo, Vachellia nilotica, Vachellia tortilis, Vachellia robusta, Dichrostachys cinerea, Xerocladia viridiramis, Burkea africana, Schotia brachypetala, Tamarindus indica*) (van Klinken & Heard, 2000; Heystek & Kistensamy, 2020). However, caterpillars never advanced to the next stage of building leaf-ties on those plants, and therefore could not sustain a population of *Evippe* sp. #1.

Prosopis species native to Africa (*P. africana, P. farcta, P. cineraria*) were not attacked by *Evippe* sp. #1 at all (Heystek & Kistensamy, 2020).

It is unknown whether *Evippe* sp. #1 attacks other *Neltuma* species within its natural range in South America.

3. Risk assessment

3.1. Establishment

3.1.1. Geographic range of hosts in Ascension Island

The target host for *Evippe* sp. is Mexican thorn (*Neltuma juliflora*), which was introduced to Ascension Island in the 1960s during the construction of Two Boats village to help consolidate the soil and prevent erosion. Since the 1980s, the tree has naturalised and spread rapidly into all the low altitude, dry lava regions in the entire western half of the island. Feral donkeys and sheep eat the seedpods and excrete intact seeds in their dung. Rodents also consume the pods, but seeds are destroyed in the process (Fowler, 1998:14). Mexican thorn thrives in difficult physical conditions and spreads aggressively (White, 2009).

3.1.2. Alternate hosts and other essential species

Evippe sp. #1 does not require alternative hosts or other essential species to complete its life cycle. Both the adults (moths) and larvae (caterpillars) feed on the leaves of Mexican thorn, although only larval feeding and leaf-tying is damaging. The biology of *Evippe* sp. #1 allows for the moth to be easily reared with a rapid development time on a single plant of approximately 35 days from egg to adult (Cowie, 2022).

3.1.3. Temperature tolerance

Evippe sp. #1 has been introduced throughout Australia, but performs best where conditions are warm to hot all year round (mean average monthly temperature 19.8 to 30.1 °C such as the Pilbara region of Western Australia (van Klinken et al., 2009)). Well-lit and warm to hot conditions are also key to the optimal growth and development of *Evippe* sp. #1 in cultures reflecting on the requirements for successful establishment. Temperatures within rearing rooms should ideally be maintained between 25 to 30 °C during the day and 20 to 25 °C at night (if possible). Avoid allowing temperatures to drop below 15 °C or exceed 32 °C as this can stress the developing larvae and plants in the rearing cages (Cowie, 2022).

We expect the climate on Ascension Island to fully support a successful establishment of *Evippe* sp. #1. Ascension lies in the path of the South-East Trade Winds, which stabilise the climate ensuring generally low rainfall and reduces the chance of thunderstorms. The climate is generally dry, tropical and oceanic, with relatively constant temperatures and little change to the weather during seasons. However, differences are seen across the island with a temperature difference normally of about 6 °C between Georgetown at sea level and the weather station on Green Mountain at 660m, and an average annual rainfall of 680 mm on Green Mountain, compared to approximately 340 mm in Two Boats village at the centre of the island and 130 mm on the western coast at Georgetown (White, 2009; Ashmole & Ashmole, 2000:190-191). Temperatures range between 20 to 38 °C. Showers occur throughout the year with slightly heavier rains in between the January to April period. Annual precipitation on the island indicates it is very arid, with a long-term average of only 165mm. Occasionally, very heavy monthly precipitation totals occur (between February and June), which bring severe damage to the island’s infrastructure and ecosystems.

3.1.4. Other abiotic factors

No detailed information has been found on the major abiotic factors required for the establishment of *Evippe* sp. #1 aside from temperature. Such factors may include humidity and rainfall, pH, salinity, topography and soil characteristics. *Evippe* sp. #1 does not inhabit the soil at any stage of its life cycle; therefore, soil characteristics and pH are not likely to directly affect its establishment. Ascension Island experiences occasionally strong wind and the impact of such winds on a small, weak flying insect is unknown, although the *Neltuma* stands themselves will most likely provide sufficient shelter.

There are two climatic factors, which could potentially impact negatively on a successful establishment:

The first one is how well *Evippe* sp. #1 is able to cope with a low variation of day length throughout the year. The length of the day on Ascension Island does not vary substantially over the course of the year, staying within 35 minutes of 12 hours throughout. The shortest day is 21 June, with 11 hours, 40 minutes of daylight; the longest day is 22 December, with 12 hours, 35 minutes of daylight (https://weatherspark.com/y/147647/Average-Weather-at-RAF-Ascension-Island-St.-Helena-Year-Round). In climates with a pronounced summer/winter change of day length *Evippe* sp. #1 tends to go into diapause during winter months. However, it is not known, what exact minimum day length is required for a continued development. This is also difficult to test under laboratory conditions as light spectrum and intensity can be expected to be significant factors to be considered as well and which are difficult to replicate to resemble natural outdoor conditions.

The second factor of uncertainty is the on average high relative humidity on Ascension Island (with 67% average humidity being lowest in July hovering around 80% for most of the year). In confined rearing cages relative humidity of between 40 to 70% is well suited to the development of *Evippe* sp. #1, but humidity should not be too high (> 75%) given the increased likelihood of fungal pathogens becoming more enabled to attack the IBCA (Cowie, 2022). However, this does not reflect outdoor conditions with a much stronger and constant air flow. In Argentina, *Evippe* sp. #1 develops throughout the humid subtropical summer months with the highest relative humidity being reached in March (73%) in Cordoba, the nearest town to the place where the species had been collected from (https://en.climate-data.org/south-america/argentina/cordoba/cordoba-878817/).

3.1.5. Competition and natural enemies

Competition

Biological control agents of Mexican thorn were experimentally introduced to Ascension in 1997. This involved the release of two species of bruchid beetles, *Algarobius prosopis* (LeConte) and *Neltumius arizonensis* (Schaeffer), around Travellers Hill, Two Boats Road, the golf course and near Butt Crater (White, 2009). During the release program, it was noted that many trees were showing signs of substantial insect herbivore attack in the form of dieback. Sap-sucker insects causing most of the damage were found to be a *Rhinocloa* species of the Miridae (mirid) family as well as *Heteropsylla reducta* (Caldwell & Martorell) of the family Psyllidae (psyllid) also causing damage. These accidentally introduced biocontrol agents, were found to be reasonably host specific to Mexican thorn (Fowler, 1997a:2, 1998:13, 25-26). On Ascension Island all three biocontrol agent groups turned out to be host specific to *Neltuma* (White, 2009).

The introduction of the bruchids has not prevented the spread of *Neltuma* across Ascension. However, effects of dieback from the sap-suckers, may be reducing the pod production of the plant. This, along with bruchid control and mammal damage of pods, was predicted to reduce future spread (White, 2009).

In addition, there is herbivory on *N. juliflora* through introduced mammals, namely rats and donkeys.

Natural enemies

There is no information on natural enemies attacking *Evippe* sp. #1 in its natural range. In Australia, the primary parasitoid fauna consisted of at least 14 hymenopteran parasitoids attacking either larvae or pupae and at least one tachinid fly attacking the larvae (van Klinken & Burwell, 2005).

Evippe sp. #1 was expected to be vulnerable to parasitism as it has a semi-concealed feeding habit, is a member of a well-represented family, and is an herbivore on a well-represented family of trees and shrubs. Surveys were conducted in four regions across Australia to determine whether parasitoids were responsible for differential performance of the moth in rangeland Australia, and what the consequences might be for the release of further semi-concealed Lepidoptera (van Klinken & Burwell, 2005).

The parasitoid fauna was found to be diverse, but the composition was similar across the four regions surveyed. It included primary and hyperparasitoids of both larvae and pupae. Parasitism rates were generally low, rarely above a few percentage points in any one survey, and therefore, unlikely to threaten moth populations. There was also no relationship between parasitism rates and leaf-tie abundance. These results suggest that any new semi-concealed lepidopteran biological control agents in rangeland Australia might also be parasitized by a diverse fauna. However, observed parasitism rates were actually very low, rarely above 2%. Predator-prey relationships may also be affected by the presence of other prey species (van Klinken & Burwell, 2005).

Parasitoids found in association with *Evippe* sp. #1 (source: van Klinken & Burwell, 2005)

Order: **Hymenoptera**

Family/subfamily: **Braconidae: Microgastrinae**

- *Apanteles* spp.
- *Dolichogenidea*
- Microgastrine (unidentifiable)

Family/subfamily: **Braconidae: Cheloninae**

- *Chelonus* sp.

Family/subfamily: **Ichneumonidae**

- Ichneumonid sp.

Family/subfamily: **Chalcididae**

- *Brachymeria* sp.
- *Proconura* spp.
- Chalcidid (unidentifiable)

Family/subfamily: **Eulophidae: Elasminae**

- *Elasmus funereus* Riek
- *Elasmus* spp. (not *funereus*)
- Family/subfamily: **Eulophidae: Entedoninae**
- *Pediobius bruchicida*
- Family/subfamily: **Eupelmidae**
- *Eupelmus* (*Eupelmus*) sp.
- Eupelmine (unidentifiable)

Family/subfamily: **Bethylidae**

- *Sierola* sp.

Order: **Diptera**

Family/subfamily: **Tachinidae**

Introduced species on Ascension are likely to predate *Evippe* sp. #1 if it is released the island. The non-native ant species, *Monomorium subopacum* (Smith), is abundant on Mexican thorn across Ascension and will predate *Evippe* sp. #1. Black rats are also widespread on Ascension and capable of climbing trees to reach and predate *Evippe* sp. #1. Ant and rat control measures will be used around the culture and control sites to reduce losses of *Evippe* sp. #1.

3.1.6. Other factors that may influence establishment

Parthenogenesis

Cultures of *Evippe* sp #1 in South Africa are heavily skewed towards female dominance (f/m >20). This has become even more pronounced in the culture maintained in the UK for host range testing and release on Ascension Island. Here males have become very rare. Tests using only freshly hatched females without the opportunity for mating still produced fertile eggs and this process can be maintained over several generations. It is therefore likely that the culture kept for release is mainly parthenogenetic, even though there is an occasional reversal and the production of a low number of males.

Parthenogenesis, a natural form of asexual reproduction produced from unfertilized eggs, occurs very rarely in Lepidoptera (Liu et al., 2018). Only recently has this been demonstrated for the Potato Tuber Moth (*Phthorimaea operculella* (Zeller)) (Liu et al., 2018), which belongs to the same family (Gelechiidae) as *Evippe* sp. #1.

In theory, a population able to sexually reproduce should be able to adapt to specific or changing environmental conditions rapidly. This should be more difficult or impossible altogether for a parthenogenetic population. Therefore, there is a small risk that parthenogenetic *Evippe* sp. #1 released on Ascension Island may initially not establish as quickly as desired. However, the observations of low numbers of males indicated that sexual reproduction can still take place and mid-to long term there should be a selection towards a more even sex ratio in case sexual reproduction will lead to the evolutions of a population better adapted to the conditions on Ascension Island compared to the specimens initially released.

It is notable, that in island ecosystems parthenogenetic colonisers often do very well. Only recently, a parthenogenetic leafhopper population of the genus *Empoasca* has been discovered on St Helena, which has become rapidly widespread. Another example is the successful colonisation of the islands of the Tristan da Cunha group by the parthenogenetic brown scale *Coccus hesperidum*.

Diapause (see also under 3.1.4)

Van Klinken et al. (2003) state that *Evippe* sp.#1 diapauses as fourth instar larvae. Diapause is thought to be initiated primarily by changes in daylength, as it is entered independently of temperature and moisture conditions, and can be prevented through the use of grow lamps. In the field in Australia, larvae enter diapause in late March or early April and exit it in July. The timing of diapause appears to be independent of latitude (van Klinken et al., 2003). During culturing in Australia, a photoperiod of L:D 16:8 was used to prevent caterpillars entering diapause. During culturing in the UK initially a photoperiod of L:D 12:12 was used similar to the light regime on Ascension Island throughout most part of the year. However, under these conditions caterpillars in two cages started to enter diapause and this was only reversed after switching to a regime of L:D 16:8. It may be that the initiation of diapause at 12:12 was caused by low light intensity compared to outdoor conditions. Therefore a 12:12 light regime on Ascension Island is highly likely to be sufficient to prevent establishment by inducing diapause too frequently and/or over long periods.

3.2. Spread

3.2.1. Natural spread

Dispersal rate on Ascension Island will probably be rapid. Even a small flying insect will be able to reach all *Neltuma* stands on the island in a short amount of time, particularly if the insect can also be carried by wind.

Evippe sp. #1 established easily in most regions across Australia. Rates of increase were greatest at sites with warm winters and hot summers, possibly allowing a greater number of generations per year (van Klinken et al., 2009). The species is also an excellent disperser. It spread 1.3 to 3.6 km/year following release (almost certainly an underestimate), and in one case spread ca. 115 km from a release site within three years (van Klinken et al., 2003), and over 1,300 km between isolated mesquite populations, presumably by wind (van Klinken et al., 2009). The excellent dispersal ability of the moth and its rapid rate of increase, means that it will quickly re-establish on surviving or regenerating plants following a control program.

Based on the published rates of spread form Australia, it is predicted that *Evippe* sp. #1 would reach all parts of Ascension within two years of its release. The natural spread of *Evippe* sp. #1 is likely to be restricted to areas where its target host *Neltuma* occurs. It is not likely to spread or at least establish in areas where its host is absent.

3.2.2. Artificial spread

Based on the experience gained in Australia and South Africa it is recommended to directly release any imported *Evippe* sp. #1 into a small number of suitable naturalised *Neltuma* stands and not to delay progress through artificial on-site mass rearing. The control of non-native ants and rats at the release sites would aid establishment. On a small island like Ascension, artificial spread to numerous release sites should not be necessary. It may, however, be desirable to maintain a small culture in controlled conditions to allow for any unexpected events around the primary release and to allow for introductions to be made in other areas at a later date if seen as beneficial. Under guidance from South African releases, cut branches of *N. juliflora* hosting *Evippe* sp. #1 leaf ties would be transported in contained boxes directly from the UK quarantine facility to Ascension. These branches would then be placed into polystyrene boxes with holes cut for moth emergence and at the base of the box to allow water egress (see Fig. 3). This box (or boxes) would be placed directly into a healthy *N. juliflora* tree, ideally with sufficient shade to stop the box from overheating and with exclusionary measures against rats. Additional small releases could be made inside mesh sleeves along *N. juliflora* branches which would allow for monitoring of moth emergence, which may otherwise be hard to detect. Sticky traps could also be placed in surrounding trees to assess emergence and dispersal, but could risk excess moth mortality and may be surplus to monitoring requirements. Given good establishment and spread, the leaf mining and tying by *Evippe* sp. #1 should be suitable for assessing the moth's progress on Ascension.

Figure 3: A polystyrene box used for *Evippe* sp. #1 releases in South Africa and resulting in field establishment. Base with emergence/water egress holes displayed. © Corin Pratt, CABI

3.3. Host range

3.3.1. Target hosts

The target host is Mexican thorn (*Neltuma juliflora*), also known as mesquite or algarrobo in its native neotropical region. It is a tough and resilient tree growing to about 13 m (-20 m) in height, and producing robust, hard and strong timber suitable for construction purposes and furniture production. It can grow on any type of soil, including highly alkaline and saline soils. It requires very little rainfall and can survive on 70 mm per year. The pods provide fodder and human food crop. The foliage, however, is not palatable, even to camels and goats, except the tips of very tender twigs; thus, the species is suitable for use in hedges and live fences. It has been used in reclamation reforestation of mining spoil, wastelands and sand dunes (Hocking, 1993). However, due to its tenacity and drought resistance, it has the potential to aggressively invade and colonise areas quickly, becoming a weed (https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.43942).

Neltuma juliflora is a shrub or small tree native to Mexico, Central and northern South America. It has shown itself to be a very aggressive invader, especially in frost-free arid and semi-arid natural grasslands, both in its native range and in particular, where introduced. *Neltuma* as a genus is treated as one of the world's worst invasive plant species, and *N. juliflora* is by far the most invasive species. This has led to the declaration of *N. juliflora* as an invasive and/or noxious weed in many African countries notably Kenya, Ethiopia and Sudan, Pakistan and other Asian countries, and also in Australia and South Africa. *N. juliflora* was widely introduced and planted as a fuel and fodder species, particular during fuelwood programmes in the 1980s, and the seed are spread widely by grazing animals. It is a nitrogen-fixing and very drought and salt-tolerant species, which can rapidly out-compete other vegetation. The thorniness and bushy habit of *N. juliflora* enable it quickly to block paths and make whole areas impenetrable (https://www.cabidigitallibrary.org/doi/10.1079/cabicompendium.43942).

Neltuma juliflora was intentionally introduced to Ascension Island in the late-1960s (Belton, 2008), and now forms impenetrable stands over large parts of the island (Fig. 4). Its range is continuing to expand and current control efforts are labour intensive and therefore limited to protecting the most sensitive sites and important infrastructure (Belton, 2008; AIG pers. comm., 2020).

Mexican thorn on Ascension Island is a significant threat to the nesting beaches of green turtles (*Chelonia mydas*), breeding seabirds including sooty terns (*Oncychoprion fuscatus*) and the critically endangered Ascension Island spurge (*Euphorbia origanoides*) (Belton, 2008; Pickup, 1999). This can be through direct habitat encroachment or the harbouring of non-native pests, in particular introduced rodents. No endemic invertebrates and few native invertebrates are able to persist in areas where Mexican thorn is present, probably as a result of the non-native predatory invertebrates the plant harbours. The spread of Mexican thorn into coastal areas could threaten populations of endemic crickets (*Discophallus* spp.) and pseudoscorpions.

The rapid spread of Mexican thorn is altering the landscape character of large swathes of the island and having profound effects on hydrology, soil formation and microclimate. It also threatens to obscure the volcanic nature of the island that was identified by the local community as capturing the 'essence' of Ascension (Canales et al. 2019). It also hampers maintenance of infrastructure with significant costs attached to its removal.

Control of Mexican thorn is a high priority in the Ascension Biodiversity Strategy and Action Plan and the Ascension Biosecurity Strategy. Established mechanical and chemical methods of controlling Mexican thorn are currently used at localised sites on Ascension. However, these methods are resource-intensive and limited to a small number of very high priority sites. This puts native species and habitats at risk and even the current level of control is not sustainable within existing AIG capacity.

Past releases of biocontrol agents have likely slowed the spread of Mexican thorn on Ascension Island, but not reduced cover.

Figure 4: *Neltuma juliflora* infestation on Ascension Island. © Norbert Maczey, CABI

3.3.2. Host testing

An extensive range of plants have been tested for susceptibility to *Evippe* sp. #1 during biological control programmes conducted in Australia and South Africa (van Klinken & Heard, 2000; Heystek & Kistensamy, unpublished). In addition, plant species endemic or native to Ascension Island plus a selection of species cultivated horticulturally or for ornamental purposes on Ascension Island have been tested as part of the ongoing project DPLUS134 during 2022 and 2023. Tables 1 and 2 provide overviews over the plant species assessed.

The non-target plants most at risk are *Prosopis* species native to tropical Africa and Asia (Pasiecznik & Felker, 2006) and other genera within the Tribe Mimoseae (van Klinken et al., 2009). Four *Prosopis* species are native to tropical Africa and Asia. They belong to sections *Prosopis* (*P. cineraria, P. farcta*, *P. koelziana, P. africana*) (Burkart, 1976). Both *P. africana* and *P. cineraria* are economically important resources and under some threat from overexploitation and land-use changes in parts of Africa (Pasiecznik & Felker, 2006). Genetic analyses suggest that these native Afro-Asian species are distinct and are only distantly related to the exotic invasive species in section *Algarobia* (Pasiecznik et al., 2006; van Klinken et al., 2009).

During the extensive host range tests conducted for releases in Australia and South Africa, *Evippe* sp. #1 has turned out to be very host specific to the genus *Neltuma* where it is able to develop fully on a number of species belonging to this genus. Outside of the genus *Neltuma,* the control agent could only be observed to start development on several species very closely related to *Neltuma* belonging to the same tribe (Mimoseae) and of which *Leucaena leucocephala* is the only species also occurring on Ascension Island. However, *Evippe* sp. #1 caterpillars can only survive and develop on *L. leucocephala* and some other closely related species for a limited period, causing initial leaf-mines and to a very limited degree leaf-ties. The species can however never fully develop to adulthood on this host and therefore cannot sustain a population (van Klinken & Heard, 2000). *Leucaena leucocephala* is a widespread non-native plant on Ascension Island. Although some initial feeding may be observed on this species after a release of *Evippe* sp. #1 this will not contribute to the establishment of the control agent, because any caterpillars hatched from eggs laid on this plant will perish before turning into an adult moth. The persistence of the *Evippe* sp. #1 on Ascension Island will solely depend on the availability of Mexican Thorn.

Table 1: Plants tested in Australia and South Africa

Family Leguminosae
Subfamily Mimosoideae
Tribe Mimoseae
Neltuma pallida (Humb. & Bonpl. ex Willd.) Kunth
Neltuma velutina Wooton
Neltuma juliflora (Sw.) DC.
Neltuma spp. (?*velutina* hybrid) (ex. Bloemhof (P1))
Prosopis africana (Guill. & Perr.) Taub.
Prosopis farcta (Banks & Sol) J.F. Macbr.
Prosopis cineraria (L.) Druce
Elephantorrhiza burkei Benth.
Adenanthera pavonina L.
Desmanthus virgatus (L.) Willd.
Dichrostachys cinerea (L.) Wight & Arn.
Dichrostachys spicata (F. Muell.) Domin
Leucaena leucocephala (Lam.) de Wit
Mimosa pudica L.
Neptunia gracilis Benth.
Neptunia major (Benth.) Windler
Neptunia monosperma F. Muell.
Neptunia dimorphantha Domin
Entada phaseoloides (L.) Merr.
Entada rheedi Spreng.
Tribe Ingeae
Albizia lebbeck (L.) Benth.
Archidendron lucyi F. Muell.
Cathormion umbellatum (Vahl) Kosterm.
Pararchidendron pruinosum (Benth.)
Paraserianthes lophantha (Willd.) I.C.Nielsen
Samanea saman (Jacq.) Merr.
Tribe Acacieae
Acacia farnesiana (L.) Willd.
Acacia baileyana F. Muell.

Acacia deanei (R.Baker)Welch, Coombs & McGlynn
Acacia irrorata Sprengel
Acacia oshanesii F. Muell. & Maiden
Acacia spectabilis Benth.
Acacia aulacocarpa Benth.
Acacia leiocalyx (Domin) Pedley
Acacia mangium Willd.
Acacia falcata Willd.
Acacia fimbriata G. Don
Acacia macradenia Benth.
Acacia pulchella R.Br.
Acacia complanata Benth.
Acacia simsii Benth. N
Acacia stenophylla Benth. N
Faidherbia albida (Delile) A.Chev.
Senegalia galpinii (Burtt Davy) Seigler & Ebinger
Senegalia caffra (Thunb.) P.J.H.Hurter & Mabb.
Senegalia mellifera (M. Vahl) Seigler & Ebinger
Senegalia nigrescens (Oliv.) P. J. H. Hurter
Vachellia farnesiana (L.) Wight & Arn.
Vachellia erioloba (E.Mey.) P.J.H.Hurter
Vachellia karroo (Hayne) Banfi & Galasso
Vachellia nilotica (L.) P.J.H.Hurter & Mabb
Vachellia tortilis (Forssk.) Galasso & Banfi
Vachellia robusta subsp. *robusta* (Burch.) Kyal. & Boatwr.
Vachellia sieberiana (DC.)
Vachellia xanthophloea (Benth.) P.J.H.Hurter
Subfamily Caesalpinioideae
Tribe Amherstieae
Schotia brachypetala Sond.
Tamarindus indica L.
Afzelia quanzensis Welw.

Tribe Caesalpinieae
Erythrophleum chlorostachys (F. Muell.) Baillon
Gleditsia triacanthos L.
Caesalpinia decapetala (Roth) Alston
Caesalpinia pulcherrima (L.) Sw.
Peltophorum africanum Sond.
Peltophorum pterocarpum (DC.) K. Heyne N
Tribe Cassieae
Chamaecrista mimosoides (L.) Greene
Petalostylis labicheoides R.Br.
Burkea africana Hook.
Ceratonia siliqua L.
Cassia abbreviata Oliv.
Senna artemisioides (DC.) Randell
Senna petersiana (Bolle) Lock
Tribe Cercideae
Bauhinia galpinii N.E.Br.
Lysiphyllum hookeri (F. Muell.) Pedley
Subfamily Papilionoideae
Tribe Aeschynomene
Arachis hypogaea L.
Stylosanthes hamata (L.) Taub cv verano
Tribe Bossiaeeae
Hovea acutifolia G. Don
Tribe Desmodieae
Desmodium tortuosum (Sw.) DC.
Tribe Galegeae
Swainsona maccullochiana F. Muell.
Tribe Mirbelieae
Pultenaea villosa Willd.
Tribe Phaseoleae
Cajanus cajan (L.) Millsp.
Centrosema pubescens Benth.
Glycine max (L.) Merr.
Lablab purpureus (L.) Sweet
Macroptilium atropurpureum (DC.) Urb.
Vigna mungo (L.) Hepper
Tribe Tephrosieae
Pongamia pinnata (L.) Pierre
Tribe Vicieae
Pisum sativum L.

Family Rosaceae
Eriobotrya japonica (Thunb.) Lindley
Fragaria X *ananassa*

Table 2: Plants tested in Egham UK for susceptibility to *Evippe* sp. #1 in current project DPLUS134

Scientific names	Common names	Source
Ascension Island endemics		
Pteris adscensionis Sw.	Feather fern	~5000 spores imported from Ascension Island
Nephrolepis sp. Schott	Sword fern	~1000 spores imported from Ascension Island
Euphorbia origanoides L.	Ascension Spurge	50 seeds imported from Ascension Island
Sporobolus caespitosus Kunth	Hedgehog grass	100 seeds imported from Ascension Island
Ascension Island natives		
Portulaca oleracea L.	Common Purslane	Seeds obtained from commercial supplier in UK
*Ipomoea pes-caprae (*L.) R. Br.	Beach Morning Glory	Seeds obtained from commercial supplier in UK
Ascension Island horticulturally cultivated/introduced ornamental		
*Delonix regia (*Bojer ex Hook.) Raf.	Flame Tree	40 seeds imported from Ascension Island
Tamarindus indica L.	Tamarind	Seeds obtained from commercial supplier in UK
Tecoma stans (L.) Juss ex Kunth	Yellowboy	~600 seeds imported from Ascension Island
Ficus benjamina L.	Rubberplant	Obtained from garden centre in the UK
Ficus robusta Corner	Rubberplant	Obtained from garden centre in the UK
Psidium guajava L.	Guava	Seeds obtained from commercial supplier in UK
Rubus idaeus L.	Raspberry	Obtained from garden centre in the UK
Lactuca sativa	Lettuce	Seeds obtained from garden centre in the UK including 'Romaine', 'Red Salad Bowl' (a mixture of unspecified red-leaved lettuce varieties), 'Butterhead' (Justice) and 'Romana'.
Ascension Island invasives		
Neltuma juliflora (Sw.) Raf.	Mexican Thorn	~300 seeds imported from Ascension Island
Leucaena leucocephala (syn. *L. glauca*) (Lam.) de Wit	Horse Tamarind	~400 seeds imported from Ascension Island

Plants were cultivated at CABI's propagation facilities at Egham, UK. All species could be grown to a suitable size for testing with the exception of guava (*Psidium guajava*), where repeated trials to germinate seeds failed. It was however decided, that this plant could be taken off the test plant list as it is on the one hand extremely unlikely that *Evippe* sp. #1 would develop on this species and on the other hand because guava is itself becoming increasingly invasive on Ascension Island. Any development of *Evippe* sp. #1 on guava could therefore be seen as a positive non-target effect.

Seeds from seven endemic or valued plant species and of *N. juliflora* from Ascension Island were sent to CABI. The plants are currently being cultivated in the CABI propagation facilities at Egham, UK. Some difficulty was experienced while trying to grow the endemic Ascension spurge (*Euphorbia origanoides)*, but after repeated trials, seeds could be germinated successfully and plants were grown to the flowering stage when they reached a size sufficient for host range testing.

Most of the test plant species could readily be propagated and grew well under the controlled temperature conditions indoors in Egham. It was only possible to germinate a small number of *Euphorbia origanoides*, but these plants grew well into the flowering stage and provided sufficient material to conduct the host range testing. The endemic grass *Sporobolus caespitosus* grew prolifically and equally started to set seeds (Fig. 5). Both ferns on the test plant list (*Nephrolepis* sp. and *Pteris adscensionis*) propagated well (Fig. 6 & 7), although it took several months until spores germinated and eventually turned into plants of a size sufficient for testing.

Neltuma plants raised from the seeds obtained from Ascension Island propagated very well and enough mature plants were grown to maintain a culture of *Evippe* sp. #1 from August 2022 onwards, to conduct the host range testing experiments, and to eventually produce enough *Evippe* sp. #1 specimens for any planned release later in 2023 (Fig. 8).

Figure 5: *Sporobolus caespitosus* in the propagation facilities at Egham, March 2023. © Norbert Maczey, CABI

Figure 6: *Nephrolepis* sp. in the propagation facilities at Egham, March 2023. © Norbert Maczey, CABI

Figure 7: *Pteris adscensionis* in the propagation facilities at Egham, March 2023. © Norbert Maczey, CABI

Figure 8: *Neltuma juliflora* in the propagation facilities at Egham, March 2023. © Norbert Maczey,

3.3.3 Non-target hosts

Through the host range testing regime conducted in Australia, South Africa, and now for this RA, *Evippe* sp. #1 has only been found to be able to develop on a limited range of *Neltuma* species. None of these, apart from the target species *N. juliflora*, occurs on Ascension Island.

One of the main aims of this RA was to conduct tests to establish host specificity of *Evippe* sp. #1 to the genus *Neltuma* and in this case specifically to *N. juliflora* with regard to the flora of Ascension Island. Host range tests conducted already in South Africa confirmed that *Evippe* sp. #1 is only attacking *Neltuma* species of the clade of species originating from Central and South America but not the species native to Africa, which are only distantly related to American *Neltuma* species (Catalano et al., 2008; Zachariades et al., 2011). This in combination with the fact that *Evippe* sp. #1 has now been released and become established in continental Africa restricts any safety testing to the flora of Ascension Island because of possible side effects in case of an accidental introduction of the IBCA to mainland Africa are not relevant under these circumstances.

A selection of a wide range of species for host range testing in Australia and South Africa including many closely related species from the subfamily Mimosoideae and here within the tribe, Mimoseae has already established that *Evippe* sp. #1 is very host-specific.

The results from previous testing programmes could be confirmed through the test regime in this project (DPLUS134). None of the test plants showed any susceptibility to any life stage of the control agent. This includes *Leucaena leucocephala*, which did show in rare cases the development of initial leaf mines caused by the first instar caterpillars during testing in South Africa and Australia. This species is also introduced to Ascension Island and is regarded here to be invasive.

After a late request from stakeholders, lettuce (*Lactuca sativa*, Asteraceae) - due to its remote relationship with Mexican thorn a very unlikely host plant of *Evippe* sp. #1 - was only included into the test plant list in the second half of 2023 and test results for this species became only available in November 2023.

In all tests the controls using *Neltuma* plants grown from seeds collected on Ascension Island were always heavily damaged by the caterpillars of the agent. We suspect that in the few cases where the control has failed some abiotic factors played a role that no successful egg laying or subsequent development took place, such as variation in humidity or light composition. In some cases, when two *Neltuma* plants were exposed to adult *Evippe* sp. #1 moths egg laying and development was initially confined to only one of the plants. We suspect that this may be due to variable levels of volatile metabolites exuded by individual plants resulting in one 'smelling' stronger than the other and attracting all adults of the cage to lay their eggs on this plant only. When subjected to emergent generations of moths these plants were found to be fully susceptible to oviposition and subsequent feeding and development of larvae through to adulthood.

Table 3: Results of the host range testing conducted within the ongoing DPLUS134 project

Test plants	HR tests completed	Mean No. leaf ties per plant (±SE)	Mean No. emergent adults per plant (±SE)
Neltuma juliflora	27	86.0 (±14.7)	37.7 (±10.3)
Delonix regia	1	0	0
Leucaena leucocephala	2	0	0
Tecoma stans	1	0	0
Ipomoea pes-caprae	1	0	0
Nephrolepis sp.	3	0	0
Euphorbia origanoides	3	0	0
Tamarindus indica	2	0	0
Sporobolus caespitosus	4	0	0
Pteris adscensionis	4	0	0
Portulaca oleracea	2	0	0
Ficus robusta	1	0	0
Ficus benjamina/ elastica	1	0	0
Rubus idaeus	2	0	0
Lactuca sativa	4	0	0

3.4. Direct negative impacts of IBCA

3.4.1. Non-target host impacts

Based on the results of all host range testing programmes conducted for *Evippe* sp. #1 so far, which includes the testing provided in the ongoing project DPLUS134, it is extremely unlikely that there will be any non-target host impacts on Ascension Island.

3.4.2. Hybridisation

No other *Evippe* moths or closely related species are found on Ascension, so hybridisation would not be possible.

There are no reports indicating evidence of hybridisation of *Evippe* sp. #1 with other closely related moth species either in the field or in the laboratory though there is no indication that this has been actively investigated in either setting.

3.4.3. Trophic effects

No known direct trophic effects of *Evippe* sp. #1 impacting on native species communities have been reported from Australia or South Africa where the species has been deliberately released for the control of several *Neltuma* species. *Evippe* sp. #1 is not a predator and only feeds on Mexican thorn. This means it is highly unlikely to interact with any endemic or non-native invertebrate species on Ascension.

The only invertebrate endemic to Ascension that is found on Mexican thorn is the psocid, *Indiopsocus mendeli*. This species evolved on the island long before Mexican thorn was introduced and, though it may lay its eggs in the foliage of Mexican thorn, it is most likely to feed on fungus or decaying matter rather than the foliage of Mexican thorn. There should be no competition between *Evippe* sp.#1 and endemic invertebrates on Ascension.

In Australia, a number of native parasitoids belonging to Hymenoptera and Diptera have been able to use this IBCA for development. However, parasitism rates by these not co-evolved parasitoids remain low (van Klinken & Burwell, 2005), and no significant impact on the performance of *Evippe* sp. #1 is expected or vice versa by the IBCA on the parasitoid communities themselves.

On Ascension Island, there is a small risk that predators, in particular non-native ants and rats, may feed on caterpillars and pupae, and potentially hamper establishment and impact exerted by the IBCA. The presence of the IBCA is unlikely to significantly increase populations of either of these generalist predators because they have multiple alternative food sources and overall ant and rat populations are expected to decrease with an overall reduction of the *Neltuma*-habitat.

We do not expect that any of the natural enemies of *N. juliflora* already present on Ascension Island will compete with *Evippe* sp. #1 in a negative way. On the contrary, it is hoped that the release of *Evippe* sp. #1 will lead to the suite of natural enemies synergistically becoming effective enough to exert significant control on *N. juliflora* reducing its impact below a threshold where it is still posing a significant problem both environmentally as well as economically. For possible interactions with already established IBCAs on *N. juliflora* on Ascension Island see also chapter 3.1.5.

3.4.4. Human and animal health effects

Evippe sp. #1 is a small so-called micromoth, which in the natural environment will only rarely come into contact with humans and other vertebrates. It will not actively fly into houses and is not reported to be attracted to light. It does not bite or sting and does not have any known adverse impacts on humans or other vertebrates. However, as with all Lepidoptera, scales from the wings of adults can lead to irritation, and also to sensitisation and allergies in a very small number of individuals. This is, however, only something to consider when working closely with a culture of the species in enclosed environments such as inside a laboratory.

Although caterpillars of many species are known to ingest and accumulate toxic metabolites from their host plants as a defence mechanism against herbivores, this has not been recorded for *Evippe* sp. #1 or any closely related species. We therefore do not anticipate any impact caused by caterpillars or pupae being accidentally ingested by herbivores such as donkeys or rats, which goes beyond any impact the feeding on *Neltuma* leaves has on livestock or other herbivores.

3.4.5. Vector capacity

There are no reports that indicate *Evippe* sp. #1 is capable of vectoring any plant pathogens.

3.4.6. Other potential negative impacts

Contamination of *Evippe* sp. #1 culture

Despite strict phytosanitary measures in place to keep *Evippe* sp. #1 in clean cultures it may be possible that other arthropods, in particular plant-feeding mites, could be accidentally introduced to Ascension along with the IBCA itself. This is mainly due to the fact that *Evippe* sp. #1 can only be transported as caterpillars/pupae inside leaf ties, requiring the transport of a substantial amount of *Neltuma* plant material along with the control agents. The culture obtained from South Africa was initially contaminated with red spider mites. Currently, mites are successively removed from the cultures by transferring adults only to new clean *Neltuma* plants between individual generations. It needs to be pointed out that red spider mite is already present on Ascension Island and reaching high densities, particularly on aubergine grown inside polytunnels (Morris, 2019).

3.5. Indirect negative impacts

It is anticipated that long term the density of *N. juliflora* stands will decrease due to the control effect of *Evippe* sp. #1 and that overall cover by this invasive tree will diminish. This will have indirect impacts on Ascension Island.

3.5.1. Soil erosion

The successful release of the IBCA would lead to the defoliation of *Neltuma* trees and the increased exposure of open ground. This increased exposure to wind may increase dust levels and soil erosion. However, the expected biological control exerted by *Evippe* sp. #1 would be a prolonged process, with stands of *Neltuma* only gradually reducing in size over a relatively high number of years. The roots of the trees would still be present to bind soils and stabilise slopes.

Periods of heavy rainfall on Ascension have resulted in erosion and damage to roads and other infrastructure. This has occurred in the presence of Mexican thorn. To protect infrastructure, engineering solutions that can be targeted at priority sites would provide a more effective solution without the negative impacts associated with Mexican thorn.

3.5.2. Habitat modification

Evippe sp. #1 is currently highly effective in suppressing the populations of *Neltuma* in the Pilbara region of Australia and is also expected to do so in South Africa. Successful control by *Evippe* sp. #1 is also expected to result in a retreat of *Neltuma* and consequently significant habitat modification on Ascension Island. Experience from Australia indicates that the presence of the moth will not result in the death of all *Neltuma* trees, but the frequent defoliation is expected to make the canopies less dense. The defoliation will look similar to that seen during recent drought periods on Ascension. Other non-native plants are expected to move into areas where Mexican thorn dies back, but this may take many years. It is unlikely the lowlands of Ascension will return to their natural barren state, but they will become less green than they are currently, at least in the short term.

N. juliflora is a pioneer species moving into barren landscapes where many other plants are unable to grow. The leaf litter it creates can lead to the development of soil in previously rocky habitats. Signs of this early soil development are evident on Ascension. Reducing the cover of *Neltuma* would slow or halt the development of soil, making it more difficult for other invasive species to colonise these areas.

Neltuma has a long and extensive root system that draws water from a large area, drying the soil and reducing the amount of water available for other plants. A reduction in the cover of *Neltuma* will lower this water extraction and possibly lead to higher soil moisture at depth, but Ascension does not have a water table that would be affected.

3.5.3. Competitive release of other non-native plants

No observations on a replacement of *Neltuma* with other invasive plant species have so far been published from other areas with successful control of members of this genus. On Ascension Island, other acacia species may flourish once Mexican thorn is removed and find it easier to outcompete *N. juliflora* and gradually replace this species. This may in particular be true for *Leucaena leucocephala*. To a lesser degree, this may also be expected for other invasives such as *Tecoma stans* (Yellow boy). The degree of such replacements is difficult to predict but overall, we expect a more beneficial outcome both environmentally and economically even if there is a significant replacement of *N. juliflora* with other woody invasive plants. Many of the potential candidates, which could replace *N. juliflora* are known to be invasive to a lesser degree, which can also be managed (removed) in an easier and more cost-effective way. Some of these invasives will also be less severe in their negative impacts such as providing a habitat for rats or even might exert some benefits as providing habitat and/or shelter for some of the native or endemic invertebrates and contributing to dust suppression and erosion control.

3.5.4. Climate impacts

Reduction in the cover of Mexican thorn would cause a reduction in transpiration rates from the Mexican thorn trees, but this is unlikely to have an impact on Ascension's weather overall since the effect will be dwarfed by evaporation coming from the ocean surrounding Ascension.

3.5.5. Fire risk

Large stands of *Neltuma* increase the risk of wildfires, which can be dangerous due to smoke inhalation and as a threat to installations and settlements. We expect that initially - over the course of a number of years - the mortality rate of *N. juliflora* on Ascension Island due to repeated defoliation by *Evippe* sp. #1 will lead to a comparably high number of dead trees, which have an elevated risk of catching fire relative to live trees. However, long-term this risk will reduce as the extent of *N. juliflora* decreases and the deliberate cutting of fire breaks becomes sustainable due to the reduced spread and regeneration of *Neltuma* in the presence of the IBCA. Eventually, the risk will fall below the current level of fire hazard caused by the spread of *Neltuma*.

3.5.6. Impact on native and endemic species

Ascension's endemic species, including the land crabs and endemic invertebrates, became established on the island and evolved in the absence of *Neltuma*. They, therefore, do not require it for their survival.

Land crabs may suffer some negative effects through the loss of shade on their spawning migration routes, but this will be balanced by the reduction in cover for non-native predators/competitors such as rats. There are other sources of shade for land crabs to use and if climate change or other factors result in high mortality of land crabs due to desiccation, then artificial shading could be considered on key migration routes.

The only endemic invertebrate that may be affected by the loss of *Neltuma* cover is the psocid (*Indiopsocus mendeli)*, which has been found in areas of *Neltuma*, though it has also been recorded from other plant species and so *Neltuma* is unlikely to be significant for its survival. Of the native invertebrate fauna, only very generalist species such as two species of barkfly, have a loose association with *Neltuma*. They will all have established and survived on Ascension in the absence of *Neltuma* and so will not be reliant on it. The only invertebrate species that show a strong association with *Neltuma* are abundant non-native ants, webspinners, and sac spiders.

3.5.7. Impact on feral mammals

Feral donkeys and sheep present on Ascension are known to eat the seed pods of *Neltuma*, which are nutritious and used as livestock feed in some areas. They have other sources of food and it is usually only the seed pods of Mexican thorn that they eat during the periods when these are available. The donkey population is feral and unmanaged meaning it will expand up to the limit imposed by food resources. An ephemeral food resource such as Mexican thorn seed pods will not result in fewer hungry donkeys or support a larger population size in this long-lived species. The Mexican thorn trees do provide shade for donkeys, but there are other areas of shade available to them.

3.6. Potential Benefits of the IBCA

In summary, the long-term benefits of licensing the IBCA are expected to be positive (see Sections 3.6.1-3.6.5) and are anticipated to reduce impacts on infrastructure and lower the risk of extinction for some of the native invertebrates and support conservation efforts for endemic plants, turtles and seabird colonies. Long-term, we expect also a lowering of standing operational costs for ongoing control efforts in the form of mechanical removal and chemical control, which are expected to result in significant economic benefits.

3.6.1. Efficacy against target hosts

Although no data on its efficacy in South Africa is yet available, *Evippe* sp. #1 was established easily in most regions across Australia and quickly started to impact heavily on the target hosts. Rates of population increase were greatest at sites with warm winters and hot summers, possibly allowing a greater number of generations per year (van Klinken et al., 2009). Although the IBCA has been introduced throughout Australia, it performs best where conditions are warm to hot all year round (mean average monthly temperature 19.8-30.1 °C) such as in the Pilbara region of Western Australia (van Klinken et al., 2009). It maintains exceptionally high densities in this region, resulting in greatly reduced growth rates and seed production (van Klinken et al., 2009).

In Australia, *Evippe* sp. #1 caused over 90% defoliation within 12 months of release and has since maintained similarly high rates of defoliation (van Klinken et al., 2003; Anderson et al., 2006; van Klinken & White, 2009). As a result, *Neltuma* plants in Australia where *Evippe* sp. #1 has become established rarely produce much foliage. Repeated defoliation has contributed to the very low seed production and growth rates observed since 2000, and tree death is becoming apparent (van Klinken et al., 2009).

The excellent dispersal ability of the moth and its rapid rate of increase means that it will quickly re-establish on surviving or regenerating plants following a control program (van Klinken & Burwell, 2005). Impact on mesquite was contingent on the leaf-tying moth *Evippe* sp.#1 maintaining very high densities for long periods. In Australia, this was assisted by low parasitism rates in the field despite immature stages attracting a diverse range of generalist parasitoids (van Klinken & Burwell, 2005).

If this can be repeated on Ascension Island, a long-term removal of *N. juliflora* will be beneficial for any endemic plants and invertebrates as the habitat will be restored to its original composition. Although *Neltuma* will not immediately die even after a high degree of defoliation and is even expected to survive consecutive attacks by the IBCA, it is expected that overall the vigour of trees will decrease and, long-term, increased mortality rates will be high enough that the overall cover of *Neltuma* will diminish and the species will become confined to a significantly smaller niche.

The degree to which *Evippe* sp. #1 can defoliate *Neltuma juliflora* is also demonstrated by the pictures below showing the density of leaf ties under laboratory conditions (Figs. 9 & 10).

Figure 9: Leaf ties on *Neltuma julifora* caused by *Evippe sp. #1*. © Norbert Maczey, CABI

Figure 10: Individual leaf tie and leaf mines caused by *Evippe sp. #1*. © Norbert Maczey, CABI

3.6.2. Reduced impact on infrastructure

Neltuma has spread rapidly across Ascension since the 1980s and its range and density are still increasing on the island. Whilst much of the area it has invaded was bare ground with no current land use, *Neltuma* is encroaching on roads, buildings, pipelines, and cables. The roots of *Neltuma* can damage foundations and surfaces and the above-ground parts of the plant obscure structures and block access. A reduction in *Neltuma* coverage and vigour caused by the release of the IBCA would reduce this damage and remove the threat from infrastructure on the edge of the current range of *Neltuma*.

Figure 11: *Neltuma juliflora* encroaching on infrastructure © Chrisna Visser, AIG

3.6.3. Reduced cost of treatment

Neltuma species are considered to be causing substantial negative economic, environmental, and social impacts over large parts of the world (van Klinken & Campbell, 2001; Mauremootoo, 2006; Ogutu & Mauremootoo, 2006; Zimmermann et al., 2006).

Control efforts based on chemical and mechanical treatments are labour-intensive and costly. On Ascension Island estimated annual costs for the treatment and removal of *Neltuma* by AIG are:

Table 4: Estimated cost to AIGCFD to control Mexican thorn

Costs to AIGCFD	
Staff costs	£ 20 000.00
Costs of interns / volunteers (£120 per day)	£ 680.00
Herbicide	£ 5 000.00
Equipment and PPE	£ 3 550.00
TOTAL COST	**£ 24 930.00**

Other organisations on the island also carry out *Neltuma* control to protect their infrastructure, but it was not possible to obtain cost estimates for this work.

It is expected that mid to long-term the self-sustaining impact of the IBCAs will significantly reduce the amount required to control *N. juliflora* in future years. Chemical and mechanical control will still be used for targeted removal from sensitive areas as part of an Integrated Control Plan, but the reduced plant vigour caused by the IBCA will mean clearance work will have longer-lasting results as regeneration is suppressed and the rate of spread of *Neltuma* diminished. This will not only cut down on direct costs for material and consumables but more importantly on required staff time.

In the presence of the IBCA, other non-native plant species such as tree tobacco (*Nicotiana glauca*) and yellow boy (*Tecoma stans*) may replace *Neltuma* in at least part of its range. These species are also invasive and may require management, but they have much smaller root systems and are significantly easier to control by chemical and mechanical means than *Neltuma* so overall there should still be a substantial reduction in the resources required for treatment.

3.6.4. Environmental benefits

The main benefit will be to stop its encroachment towards sensitive areas for biodiversity. Any *Neltuma* cover in these areas would be damaging to the globally important nesting populations of green turtle (*Chelonia mydas*) and seabirds and the endemic invertebrates found on Ascension. This is a result of direct impacts caused by the loss of bare ground and loose sand required by these species as well as indirect impacts caused by the presence of non-native predators such as black rats, ants, and spiders within *Neltuma* stands.

The release of the IBCA will slow the spread of *Neltuma*, preventing it from reaching areas of coastline that currently support green turtle, seabird and endemic cricket and pseudoscorpion populations. This will prevent habitat degradation and the establishment of non-native predators in these areas. Mechanical and chemical control efforts to remove *Neltuma* from protected areas will be more successful and long-lasting in the presence of the IBCA.

3.6.5. Human and animal health benefits

Potentially significant health benefits can be expected following a reduction of *N. juliflora* on Ascension Island caused by a reduction of allergy risks. There is recent evidence that pollen of *N. juliflora* is highly allergenic and can in particular induce cross allergies to a range of food products (Killian & McMichael, 2004; Al-Frayh et al. 1999).

In the USA, Mexico, Saudi Arabia, Kuwait, United Arab Emirates, India and South Africa the pollen has been identified as a major allergen (e.g. Killian & McMichael, 2004), and Dhyani et al. (2008) described *N. juliflora* as an 'important source of respiratory allergens in tropical countries'. Killian and McMichael (2004) identified at least 13 human allergens in the pollen.

Apart from allergic reactions such as asthma caused by pollen, thorns of Mexican thorn are poisonous and/or promotive to secondary infections on prickling. When replaced by other invasive woody plants it is expected that these would not form such impenetrable thickets, reducing significantly the risk of injury when work has to take place close to or inside tree stands to the same degree as with *Neltuma* thickets.

3.7. Uncertainty

3.7.1. Establishment of *Evippe* sp.#1

Parasitism

*Evippe s*p. #1 has attracted a diverse range of larval and pupal parasitoids in Australia. However, parasitism rates remain low, averaging only 1.8% (van Klinken & Burwell, 2005, van Klinken et al., 2009). We therefore do not expect that any parasitoids of moths already present on Ascension Island will have a significant impact on its establishment and efficacy, but this risk cannot be excluded entirely at this stage.

Climate

Conditions on Ascension Island are generally very favourable for the establishment of *Evippe* sp. #1 but there remains a small risk of them entering diapause during the largely L:D 12:12 regime and not continuing development. In the area, where *Evippe* sp. #1 has originally been obtained from, as well as at release sites in Australia and South Africa local climates show a more pronounced seasonality and on average lower levels of humidity. We do not anticipate that higher humidity levels on Ascension Island will become a problem by impacting negatively on the establishment, spread, and subsequently impact, but it cannot be excluded as a risk completely at this stage.

3.7.2. Impact of *Evippe* sp.#1 release

Impacts caused by *Evippe* sp.#1 in Australia appeared only a few months after its release in Australia. However, as climatic conditions differ somewhat on Ascension Island there remains some uncertainty about the time span until the control agents become established or until first defoliations or the dieback of entire trees can be observed.

On the basis of the information presented in this risk assessment, there is minimal uncertainty surrounding the direct or indirect negative impacts of the IBCA. There are unlikely to be any potential non-target effects (attacking alternative hosts or hybridization). If the IBCA is established, there is also minimal uncertainty regarding the direct or indirect positive impacts.

3.8. Conclusion

3.8.1. Establishment

We expect the climate on Ascension Island to fully support the successful establishment of *Evippe* sp. #1. The climate on Ascension is generally dry, tropical, and oceanic, with relatively constant temperatures and little change to the weather during seasons. There are, however, two climatic factors, which could potentially impact negatively on a successful establishment. The first one is how well *Evippe* sp.#1 is able to cope with a low variation of day length throughout the year as the species needs to have comparably long hours of daylight to develop and not enter or remain in a diapause. The second is the on average high relative humidity on Ascension Island, which is on average higher compared with the natural range of the control agent.

We do not expect any parasitoids able to reduce the full potential of *Evippe* sp. #1 to be present on Ascension

Island. Even in biodiversity-rich Australia, rates of parasitism by non-co-evolved parasitoids remain very low.

Despite a certain amount of uncertainty regarding some of the climatic conditions, it is expected that *Evippe* sp. #1 should be able to establish anywhere on Ascension Island where the target host *N. juliflora* exists. We also do not expect that the likely prevalence of parthenogenesis in our *Evippe* sp. #1 culture and potential competition with other natural enemies of *Neltuma* already established on Ascension Island will impact the establishment of our control agent.

Predation by rats and ants could be a significant threat to the establishment. Rat and ant control methods would be put in place at culture and release sites to mitigate this threat.

3.8.2. Spread

Evippe sp. #1 has been observed to spread rapidly over comparably long distances in Australia. We therefore expect the dispersal rate on Ascension Island also to be high. The excellent dispersal ability of the moth and its rapid rate of increase at some of its release sites means that it will quickly re-establish on surviving or regenerating plants following a control program. The natural spread of *Evippe* sp. #1 is likely to be restricted to areas where its target host *Neltuma* occurs. It is not likely to spread or at least establish in areas where its host is absent.

3.8.3. Host range

Through host range testing regimes conducted in Australia, South Africa, and now for this RA as part of DPLUS134, *Evippe* sp. #1 has only been found to be able to develop on a limited range of *Neltuma* species and therefore is very host-specific. *Neltuma juliflora* is the only plant species on Ascension Island capable of supporting the full development of the control agent and none of the other plants occurring on Ascension Island is therefore at risk of being attacked by this IBCA. There had been limited initial development observed on *Leucaena leucocephala* in previous testing regimes. This could, however, not be confirmed during tests on plants originating from the invasive population present on Ascension Island. The fact that *Evippe* sp. #1 has now been released and become established in continental Africa also means that a release on Ascension Island does not pose any risks to native or endemic plants in mainland Africa.

3.8.4. Direct and/or indirect negative impacts

In summary, no significant direct impacts are anticipated on native, endemic, or introduced plant and animal species as a result of the successful establishment of *Evippe* sp. #1 on Ascension Island. There are, however, significant indirect habitat modifications expected to occur as a result of its release. Overall, negative impacts are expected to be low. However, there remains some uncertainty as to what degree other invasive woody plants may fill the gap caused by a retreat of *Neltuma* due to biological control. In all cases, the species that could potentially replace *Neltuma* are less invasive and more easily controlled.

There is also a temporary increased risk for wildfires if herbivory by *Evippe* sp. #1 increases the mortality rate of *Neltuma* leading to an increased amount of dry deadwood for some time. The overall fire risk would decrease over time as the extent of *Neltuma* is reduced and fire breaks could be maintained through chemical and mechanical control methods.

Despite strict phytosanitary measures in place to keep *Evippe* sp. #1 in clean cultures, it can't be fully guaranteed that other arthropods, in particular plant-feeding mites, could be accidentally introduced to Ascension Island along with the control agent itself. One particular species of concern is the red spider mite. However, this risk is insofar already mitigated as the mite species is already present on Ascension Island.

3.8.5. Benefits of the IBCA

The main benefit will be the reduction in the encroachment of *N. juliflora* towards sensitive areas such as turtle nesting sites, seabird colonies, and coastal strongholds of endemic invertebrates. Of particular importance is the anticipated reduction of the impact *Neltuma* has on native fauna and flora through its role as a habitat for rats and non-native predatory ants and spiders.

The main economic benefits will be a reduction in damage caused to infrastructure such as roads and pipelines and lower costs for the sustainable removal of trees through chemical and mechanical control methods

3.8.6. Overall conclusion

Based on the information available, and provided in this risk assessment, the risks of releasing *Evippe* sp.#1 into Ascension Island appear to be low. There are some uncertainties about the likelihood of establishment linked to daylength on Ascension and potential predation. However, there is little uncertainty regarding direct non-target effects (attacking alternative hosts or hybridisation) as there are no other plants present on Ascension Island, which can be attacked by *Evippe* sp. #1.

The benefits of releasing *Evippe* sp. #1 to manage *N. juliflora* will by far outweigh the risks or any anticipated negative indirect effects. As with all classical biological control it is not expected or even possible that *Evippe* p. #1 can eradicate its host completely. However, in combination with the natural enemies of *N. juliflora* already established on the island, it is expected that the density, biomass, and overall spread of *Neltuma* will decrease to a significantly more manageable level by being forced to occupy a largely reduced ecological niche.

4. Post-release monitoring and control measures

Is post-release monitoring intended to be carried out? **Yes**

Is a contingency plan intended to be prepared? **No**

There are no practical measures available for the eradication or containment of *Evippe* sp. #1 once it has been released and has established.

4.1. Monitoring methodology

Monitoring to confirm establishment of *Evippe* sp. #1 should be conducted at least once a month after the release and once a year thereafter. Most of the beneficial impacts such as a dieback of Mexican thorn may not be detectable for several years.

Monitoring establishment and efficacy

Monitoring should focus on the documentation of larval activity, namely defoliation and the presence of leaf-ties. Their presence and density can be evidenced by simply taking a series of photographs. It is then easy to complement these with photographs of caterpillars and pupae after opening a number of leaf-ties. The presence of adult moths will be more difficult to demonstrate but this is not necessary.

The extent of establishment and spread can easily be documented through a selection of monitoring points covering the majority of *N. juliflora* stands before the start of the monitoring. At each monitoring point, photographs should not only be taken of individual branches but also of whole trees or stands and should repeatedly be taken from the same place throughout the monitoring programme so that changes in tree health and cover can be documented.

Environmental impact

To assess the environmental impact, it is proposed to use earth observation satellite imagery and drone photography, to establish baselines prior to the release of the IBCA and measure cover by *N. juliflora* over subsequent years. This should be complemented by an assessment of potentially increasing cover by other non-native woody plant species.

4.2. Control measures

There are no practical measures available for the eradication or containment of *Evippe* sp. #1 on Ascension Island once it has been released and has been established.

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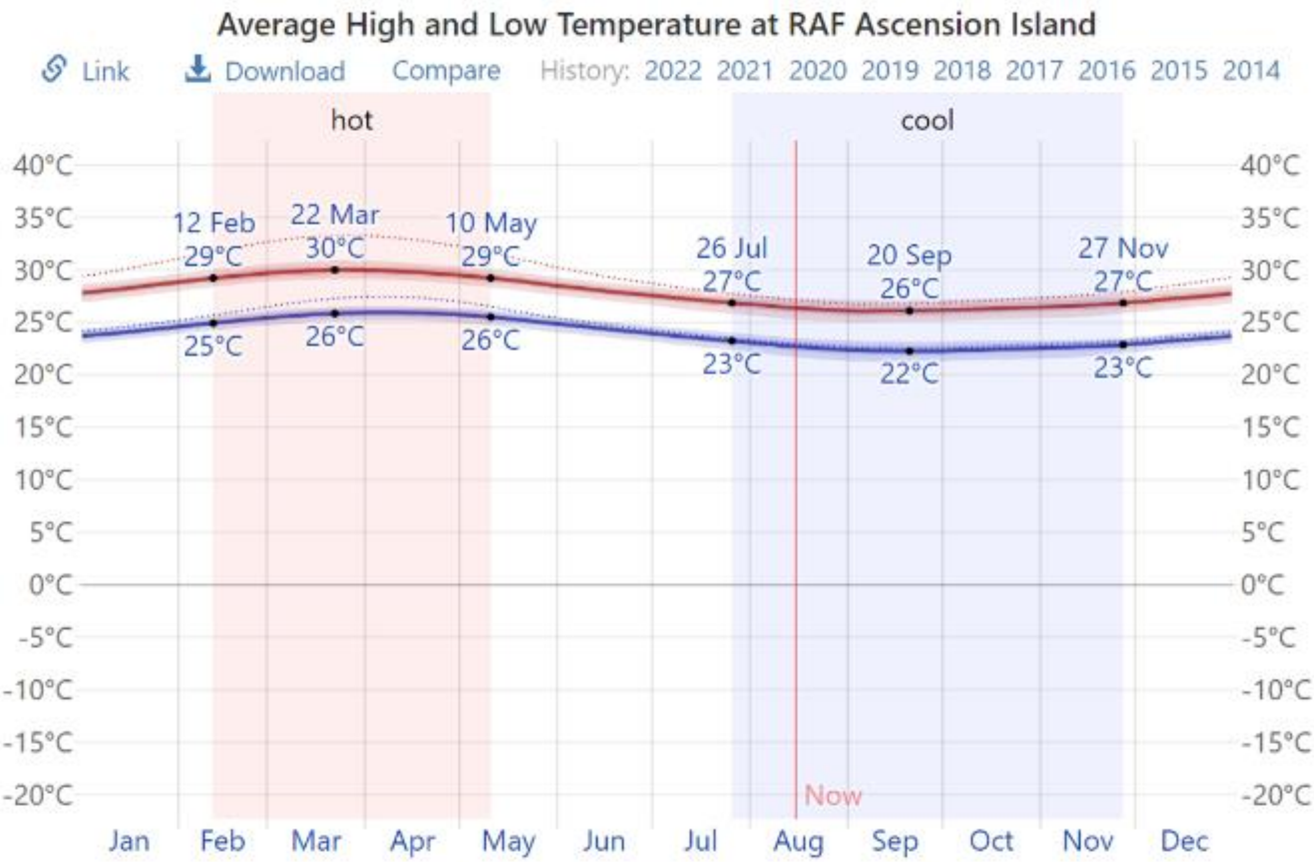
Appendices

Appendix 1. Climatic data for Ascension Island

	July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	June	Annual
Temperatures °C													
Average Maximum	14	14	14	15	17	19	20	21	20	18	17	15	17
Average Mean	12.5	12	12	13	15	17	18	19	18	16.5	15	13	15.1
Average Minimum	11	10	10	11	13	15	16	17	16	15	13	11	13.2
Sea Temperature	14	13	13	14	15	16	17	18	18	17	16	15	15.5
UV Index	2	3	5	7	10	11	11	10	8	5	3	2	6.4
Precipitation mm	160	175	169	151	128	127	93	113	121	129	155	160	1681
Number of wet days	25	26	24	22	18	19	18	16	17	20	23	23	251
Probability of rain per day %	81	84	80	71	61	61	58	57	55	67	74	77	69
Daylight Hours													
Hours of Daylight	10	11	12	13	14	14.5	14	13	12	11	10	9.5	12
% sun	34	32	33	33	32	29	31	35	38	38	35	34	34
% cloud	66	68	67	67	68	71	69	65	62	62	65	66	66
Relative Humidity %	79	79	78	79	79	80	79	77	75	78	78	79	78.3



The daily average high (red line) and low (blue line) temperature, with 25th to 75th and 10th to 90th percentile bands. The thin dotted lines are the corresponding average perceived temperatures.

Appendix 2. Temperature data for Ascension Island compared with Malvinas Argentinas, Buenos Aires, Mendoza, and Lago Argentino Aerodrome, Argentina