Assessment of natural enemies for *Carpobrotus edulis* biocontrol

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Foreword

Invasive alien plants have serious economic and ecological impacts, for example, by displacing native plants and invertebrates, and their management is often costly and ineffective in the long term. Classical biological control (biocontrol) is advocated as an alternative to conventional invasive species management that has the potential for long term, self-perpetuating and effective control, especially in more sensitive environments such as protected areas or riparian habitats.

CABI was commissioned by Natural England to conduct a biocontrol feasibility assessment for *Carpobrotus edulis*, which has been identified as a significant threat to a number of sensitive habitats in coastal regions. This current study improves our current knowledge on the natural enemies thought to have potential as classical biocontrol candidates for GB.

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Executive summary

A Natural England stakeholder workshop in 2022, which aimed to prioritise species for biological control, identified *Carpobrotus edulis* (Aizoaceae) as a significant threat to sensitive UK habitats primarily in coastal regions, and as such CABI was commissioned to conduct a biocontrol feasibility assessment for *C. edulis*. The study identified a number of natural enemies recorded as damaging to *C. edulis*, one of which – the scale insect *Pulvinariella mesembryanthemi* – was known to have had significant impacts on the plant in California following its introduction and had been recorded as present on *C. edulis* in the southwest of England. The current study included a survey to this region (Cornwall and the Isles of Scilly) to assess natural enemies of *C. edulis* in the field and to locate the scale and review its impacts. All natural enemies and other *C. edulis* associated invertebrates and fungi were identified and are reported herein but were broadly non-damaging and/or generalist.

The scale, *P. mesembryanthemi* was located at several sites, but in low densities, and found to be having minimal impact on *C. edulis* where present. The scale was cultured at CABI, its parasitoids cleared from the culture and identified, and its impact on *C. edulis* under warm controlled temperature conditions assessed. *Pulvinariella mesembryanthemi* was able to reach high densities over a series of months, causing significant die-back and ultimately death of *C. edulis* plants. A related non-native plant species (*Disphyma crassifolium*) from the same family as *C. edulis* was found to be growing in high density monospecific mats at Lizard Point, Cornwall and was brought into culture at CABI where it was exposed to *P. mesembryanthemi*, which is known to target multiple species within the Aizoaceae (a family with no native representatives in the UK). The scale was able to develop to maturity on the host and it's feeding again led to the plant's death.

It is apparent that under suitable climatic conditions and in the absence of predators and parasitoids, *P. mesembryanthemi* can have a significant impact on *C. edulis* (and certain related species). As such, a climatic assessment was made using CLIMEX, including known worldwide distribution records of *P. mesembryanthemi* to determine the climatic suitability of the UK for the scale. This assessment indicated that significant regions of the UK, particularly to the south and overlapping extensively with *C. edulis* distribution records would be suitable for the establishment of the scale. The scale may still be limited in its impact on *C. edulis* by the climate and natural enemies, but, subject to host range assessment, rearing and redistribution of the scale to more *C. edulis* locations could improve establishment and ultimately impact, particularly under warming conditions brought about by climate change.

An assessment of damaged plants on Saint Helena identified a number of potential *C. edulis* natural enemies, however, none of the identified invertebrates would be considered suitably specific and damaging to the plant for consideration as biocontrol agents.

A test plant list for assessment of candidate biocontrol agents for *C. edulis* in the UK (with consideration of European flora) is provided, developed in consultation with an expert botanist experienced in biocontrol research.

Finally, an assessment of natural enemies reported in the native range of South Africa was made. Of promise and worthy of detailed assessment is a novel pathogen recorded from dying *C. edulis* plants in the plant's native range (the potential future export of which has been coordinated), along with a number of armoured scale insects with one in particular reported to cause damage to the plant in spite of parasitoid pressure (which would be reduced on introduction to a new region such as the UK where co-evolved parasitoids would be lacking). In addition, the natural enemy complex of *C. edulis* in its native range has not been thoroughly assessed as part of a classical biological control research programme and further suitably specific and damaging natural enemies with potential as biocontrol candidates could be expected. As such, the logical progression of the biocontrol research into *C. edulis* for the UK would be to conduct native range surveys in South Africa for pathogen and invertebrate natural enemies. CABI has been in contact with key research institutions in the region and willing collaborators for both pathology and invertebrate surveys have been identified.

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Photo 1. *Carpobrotus edulis* Photo © CABI

Introduction

A Natural England stakeholder workshop in 2022, which aimed to prioritise species for biological control, identified *Carpobrotus edulis* as a priority species given its impacts upon sensitive coastal habitats. This mat-forming succulent, listed on Schedule 9 of the Wildlife and Countryside Act is a transformer species which is challenging to control across its coastal range. A biocontrol feasibility assessment commissioned by Natural England was carried out by CABI in 2021-22 and flagged several natural enemies of *C. edulis* with potential to impact upon the plant. Of those species, the scale insect *Pulvinariella mesembryanthemi* was recorded as having a significant impact on *C. edulis* in California following its accidental introduction in the 1970s from South Africa, the native range of the plant and the scale. In addition, the literature indicated that *P. mesembryanthemi* had been recorded outdoors in the UK on the Isles of Scilly, with an additional anecdotal report of the scale's presence on *C. edulis* on the mainland in Cornwall. The current study aimed to assess *C. edulis* populations across key regions of the plant's invasive range in the UK, identify any natural enemies present with a particular focus on *P. mesembryanthemi*, record impacts in the field and maintain cultures of the plant and scale to establish potential impacts and any apparent parasitoid complex. Additional components of the study included a CLIMEX assessment to better understand the current and potential distribution of the scale; assessment of natural enemies on St Helena found to be damaging invasive *C. edulis* there; a review of potential additional candidate agents including scale insects and a novel pathogen reported from South Africa; and production

of a test plant list for candidate biocontrol agents of *C. edulis* for the UK. This report presents the project findings.

Survey

Informed by a review of *C. edulis* records and accounting for the reported presence of *P. mesembryanthemi* on the Isles of Scilly and potentially Cornwall, a field survey was conducted from 5th-8th September 2022 in southwest Cornwall and the Isles of Scilly, with assessment of numerous *C. edulis* mats of varying size. The survey areas centred on the following locations (see Figure 1):

- Cape Cornwall, Cornwall
- Gwynver Beach, Cornwall
- Porthcurno Beach, Cornwall
- Town Beach, St Marys, Isles of Scilly
- West coast, Tresco, Isles of Scilly

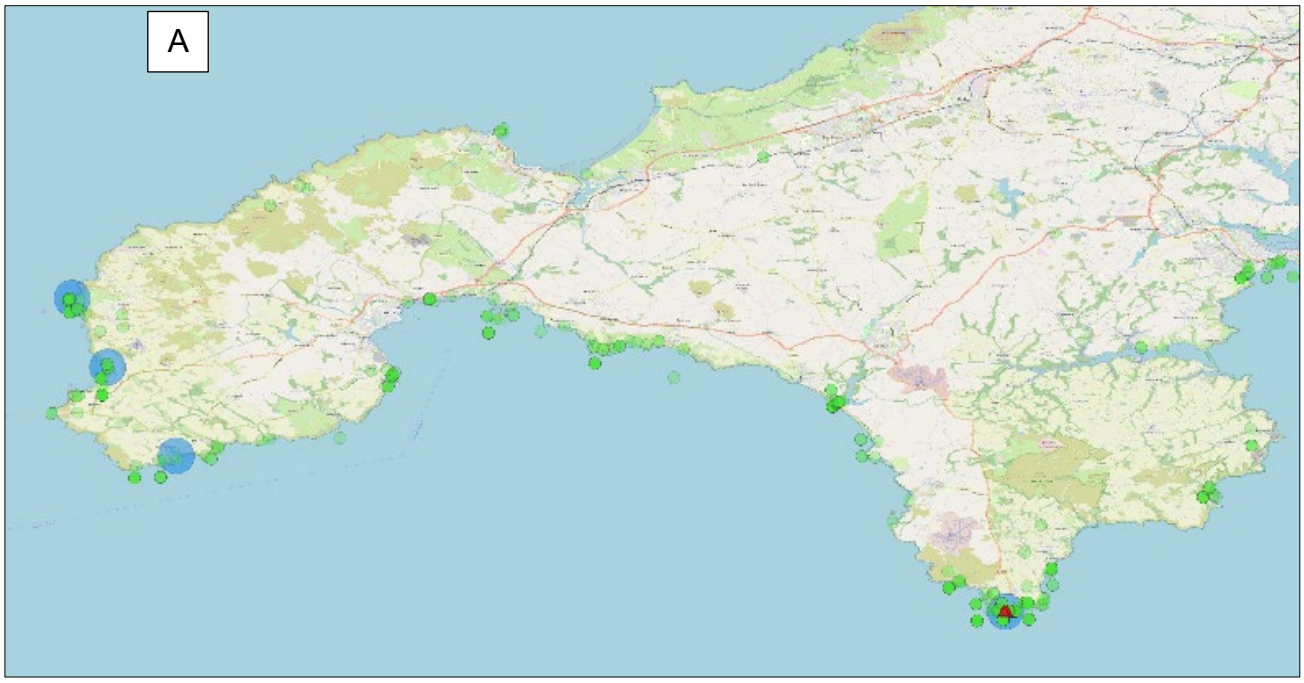

Figure 1A: Field survey areas for *Carpobrotus edulis* and associated natural enemies (blue circles) with *C. edulis* records (green circles) and *Pulvinariella mesembryanthemi* collection sites (red triangles) in Cornwall. (*C. edulis* records from BSBI Distribution Database, 2023). Contains, or is derived from, information supplied by Ordnance Survey. © Crown copyright and database rights 2023.

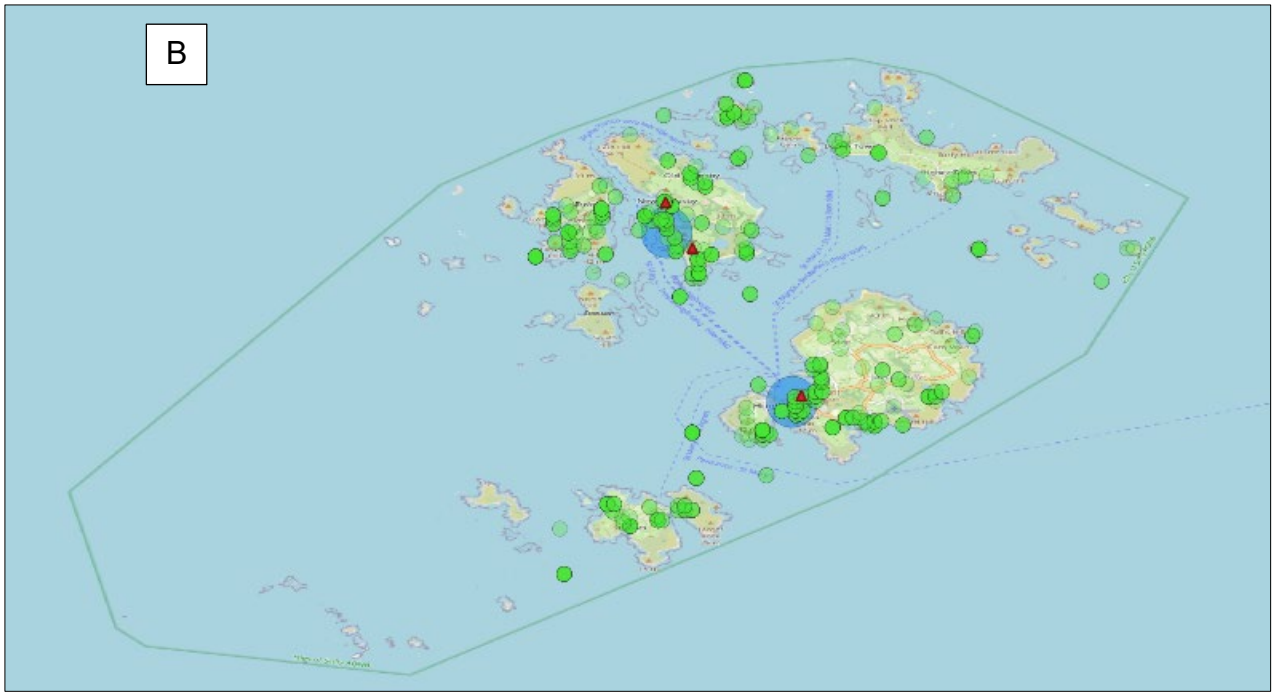

Figure 1B: Field survey areas for *Carpobrotus edulis* and associated natural enemies (blue circles) with *C. edulis* records (green circles) and *Pulvinariella mesembryanthemi* collection sites (red triangles) in the Isles of Scilly. (*C. edulis* records from BSBI Distribution Database, 2023). Contains, or is derived from, information supplied by Ordnance Survey. © Crown copyright and database rights 2023.

The survey conditions ranged from cool with heavy rain, to warm and sunny. The late summer timing was to target invertebrates, including the scale *P. mesembryanthemi* (in its late reproductive period) along with its potential parasitoids. Across all sites there were very limited signs of damage to the *C. edulis* mats which were broadly post-flowering, and where potential herbivores were present, they were in low density (see Figures 2 and 3).

Figure 2: A) Aphid colony on *Carpobrotus edulis*; B) Snails on *C. edulis*; C) Potential surface herbivory on *C. edulis* leaves; D) Leaf damage to *C. edulis* by unknown agent. Photos © CABI

Figure 3: A) Late flower of *Carpobrotus edulis*; B) dense hanging bankside mat of *C. edulis* in Cornwall; C) Ovisacs of scale insect *Pulvinariella mesembryanthemi* visible on *C. edulis* plants, Isles of Scilly; D) Norbert Maczey assesses *P. mesembryanthemi* ovisac with hand lens. Photos © CABI

Various invertebrate species found in association with *C. edulis* were collected during the survey for lab identification (see Identifications sections). In addition, *C. edulis* plant material was collected to propagate for establishment of scale cultures. The targeted scale, *P. mesembryanthemi*, was located and collected from sites in St Mary's and Tresco (at two locations) on the Scilly Isles, along with Lizard Point (at two locations) in Cornwall. The scale was only collected on *C. edulis*. When observed, scales were typically clustered, but in low numbers and with most plants and mats assessed appearing to be scale free. Where *P. mesembryanthemi* was present, no visible plant damage or field impact was discernible.

Identifications

In addition to *P. mesembryanthemi*, various herbivorous species found in association with the *C. edulis* assessed during the survey were identified and their life histories reviewed to ascertain their likely relationship with the plant (and potential suitability as biocontrol candidates):

Herbivores

Leafhoppers/Planthoppers

Polyphagous, possible *C. edulis* mesophyll feeding:

- *Philaenus spumarius*
- *Empoasca* sp.

Vagrant:

- *Megophthalmus* sp.
- *Aphrodes makarovi*
- *Stenocranus minutus*

Unknown:

- Unknown sp. of *Cixiidae*

Heteroptera

Somewhat polyphagous on flowers/fruit:

- *Phytocoris varipes*

Aphids

- cf *Aphis fabae* [and associated hoverfly]

Orthoptera

Possible Carpobrotus seedpod feeder:

- *Platycleis albopunctata*

Grass feeder:

- *Chorthippus brunneus*

Molluscs

Polyphagous, potential for some limited damage to Carpobrotus:

- *Cornu aspersum*

Likely vagrants:

- *Theba pisana*
- *Cepea nemoralis*
- *Cochlicella acuta*
- *Lauria cylindracea*

Fungi

A fungal isolate from a potentially diseased leaf was identified as *Penicillium* sp., thus the leaf damage observed was due to secondary infection.

Predators and parasitoids

In addition to the various herbivorous species reported, several predators and parasitoids were also found in association with *C. edulis* and its natural enemies. Of particular importance in relation to *P. mesembryanthemi* and its potential to impact upon *C. edulis* in the field was the identification of two parasitoid species emerging from the scales held in culture. One individual *Trichomasthus* sp. emerged from the scale population ex. Lizard Point and numerous male and female *Microterys nietneri* (naturalised in the UK) emerged from scales collected on the Isles of Scilly (see Figure 4). None of the South African parasitoid species associated with *P. mesembryanthemi* in its native range or released in California to control the scale into the 1980s when *C. edulis* was considered a desirable species were observed, but it appears that UK naturalised or native species have adopted the scale as a host. The identified parasitoid species may contribute to limiting the widespread establishment and population growth that would be necessary for the scale to exert significant impacts on *C. edulis* populations. The scale culture held at CABI was cleared of parasitoids to allow ongoing culturing and impact assessment, with the parasitoid *M. nietneri* moved onto an alternative host, the brown soft scale *Coccus hesperidum* reared on Malabar spinach. The full list of associated predators and parasitoids identified from the field collection and subsequent rearing, with life history comments is as follows:

Coccinelids

Aphid and scale feeder, may impact *P. mesembryanthemi*:

- *Chilocorus bipustulatus*

Ants

Potentially protecting aphids and scales from parasitoids and predators:

- *Lasius niger*

Parasitoids

Ex. *P. mesembryanthemi*, Lizard. One individual emergent. May limit scale impact and population growth:

- *Trichomasthus* sp. (poss. *T. albimanus*)

Ex. *P. mesembryanthemi*, Isles of Scilly. Numerous emergent males and females. May limit scale impact and population growth:

- *Microterys nietneri*

[culture now established on brown soft scale, *Coccus hesperidum*]

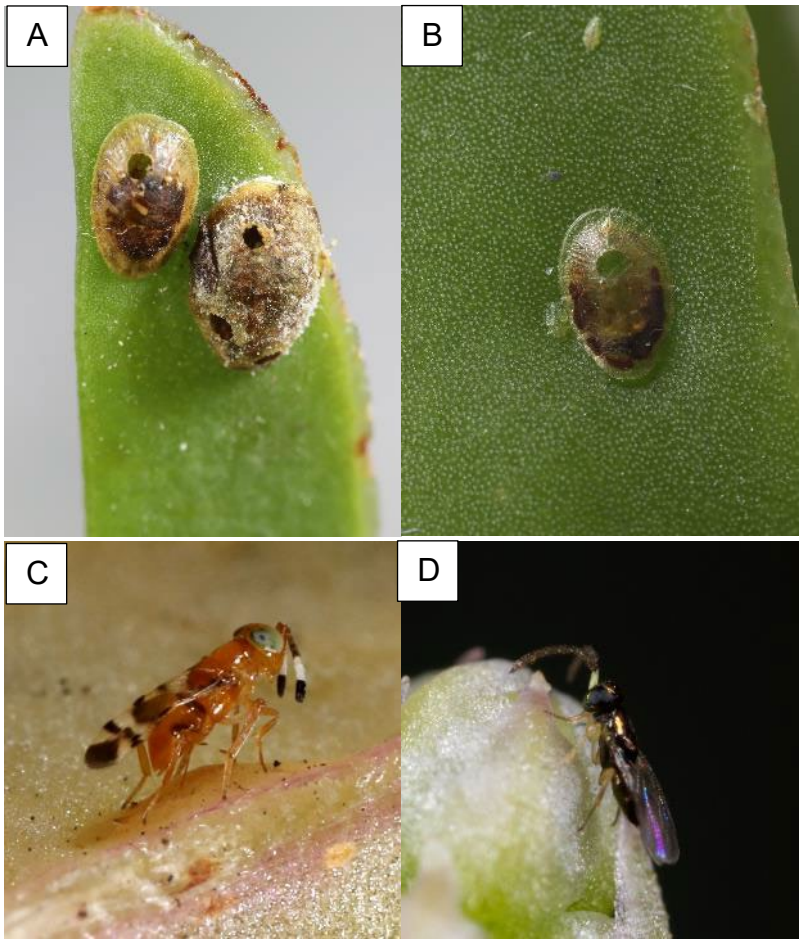

Figure 4: A) and B) Parasitised *Pulvinariella mesembryanthemi* scales in culture with parasitoid exit holes; C) *Microterys nietneri* female; D*) Microterys nietneri* male. Photos © CABI

Scale culturing and impact assessment

In the lab, plant parts hosting various life stages of *P. mesembryanthemi* from the field were placed amongst planted *C. edulis* plants to allow the movement of mobile stages to viable plant material (see Figure 5). After around one month of culturing with multiple ovisacs apparent at the time of collection, small crawlers (~1mm) were observed moving across *C. edulis* leaves (see Figure 6), beginning to settle over time. Over the subsequent month, plant quality started to decline, however, the cultures had been maintained in a naturally lit laboratory and abiotic factors may have been influencing plant performance as autumn set in. The plant and scale cultures were therefore moved to a controlled temperature room with artificial lighting (14:10 light:dark) at 22±2˚C to allow the ongoing development of the scales and to provide the plants with ideal growth conditions. Additional growing plant material was added to the cultures to ensure the scale culture could persist and to assess the potential impact of *P. mesembryanthemi* on *C. edulis* in relatively warm conditions. By December 2022, there was extensive settlement of crawlers and significant growth and development of scales through early to intermediate stages (See Figure 6).

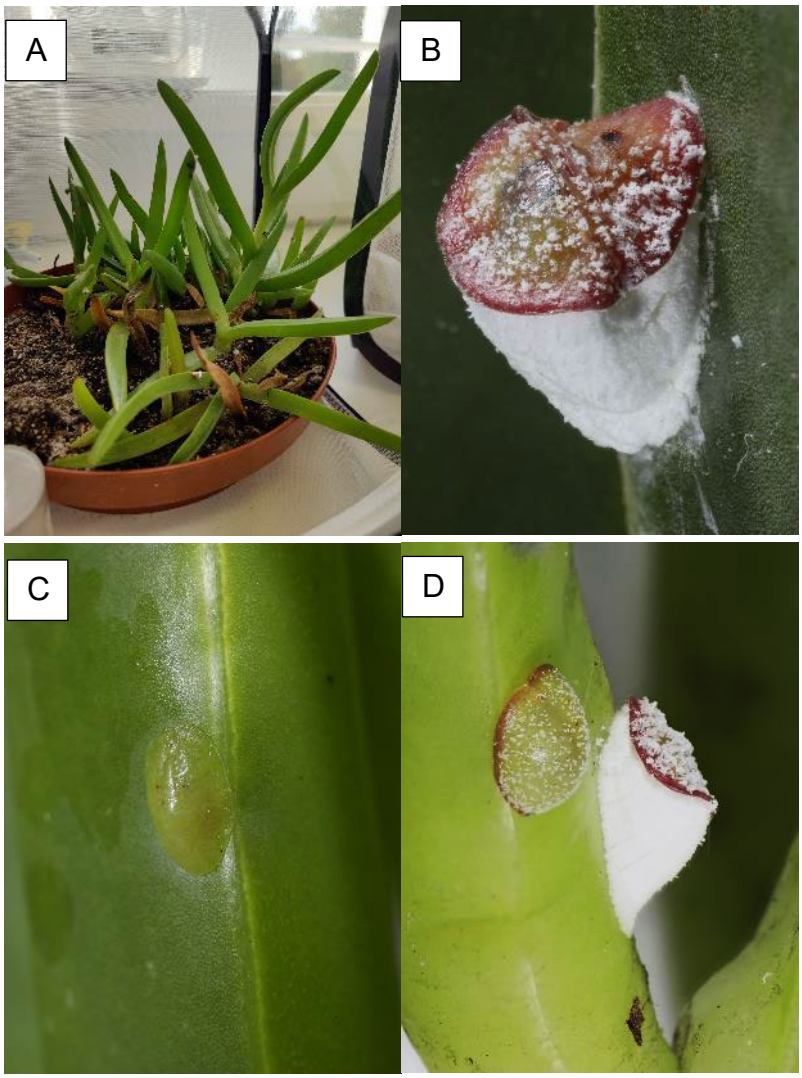

Figure 5: A) Propagated *Carpobrotus edulis* with visible *Pulvinariella mesembryanthemi* ovisacs visible; B) *P. mesembryanthemi* ovisac developing under female scale; C) Intermediate stage *P. mesembryanthemi*; D) Mature *P. mesembryanthemi* and female with ovisac. Photos © CABI

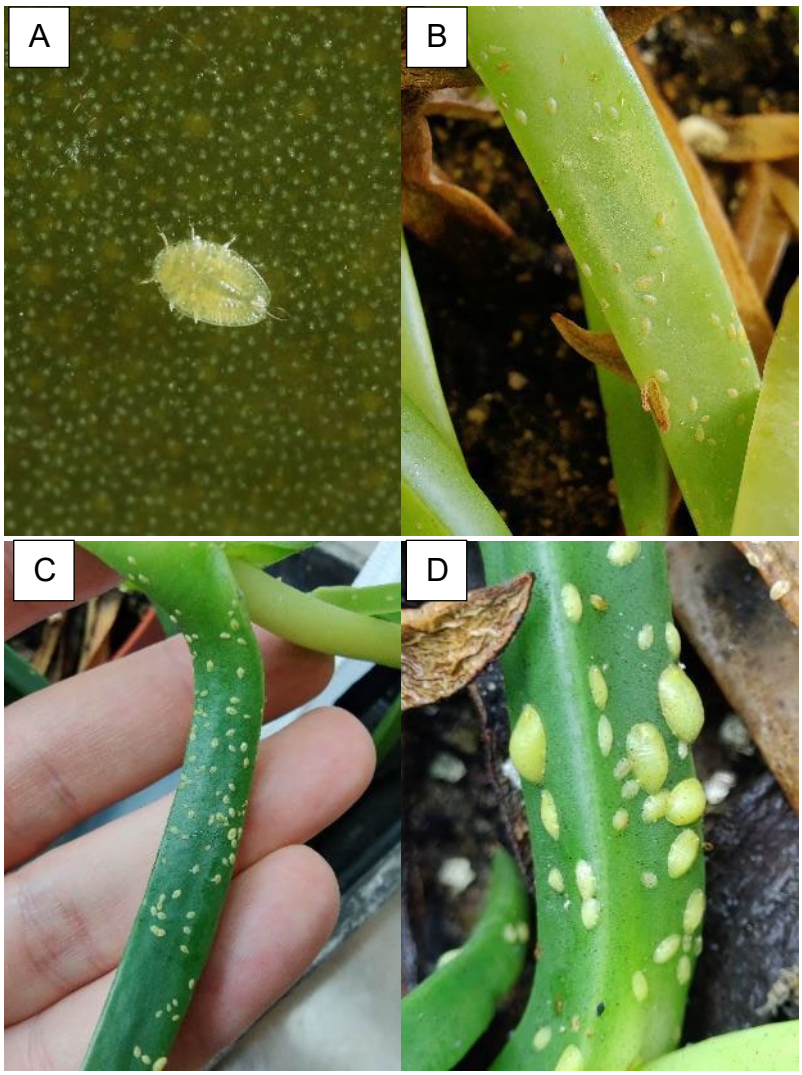

Figure 6: A) *Pulvinariella mesembryanthemi* crawler on C. edulis; B) Juvenile *P. mesembryanthemi* in November 2022; C) Increasing settlement of early stages of P. *mesembryanthemi in December 2022; D) High density of various stages* of *P. mesembryanthemi* in December 2022. Photos © CABI

Following extensive scale settlement and maturation, the *C. edulis* plants declined quite swiftly resulting in extensive dieback of leaves and stems and ultimately plant death (see Figures 7 and 8). Fresh plant material was provided, depleting plant stocks which had reduced vigour over winter and early spring despite being held in a warm propagation chamber. It is clear from this assessment, that given consistent, warm conditions, good quality plant material and in the absence of predators and particularly parasitoids, the scales can quickly reach high densities and cause significant dieback and plant death in *C. edulis*. It seems likely that factors including climate, predation/parasitism and year-round plant quality may be limiting the ability of *P. mesembryanthemi* to build large, high-density populations that would ultimately lead to *C. edulis* impacts in the field, the type observed in California in the 1970s before the introduction of natural enemies to control the scale.

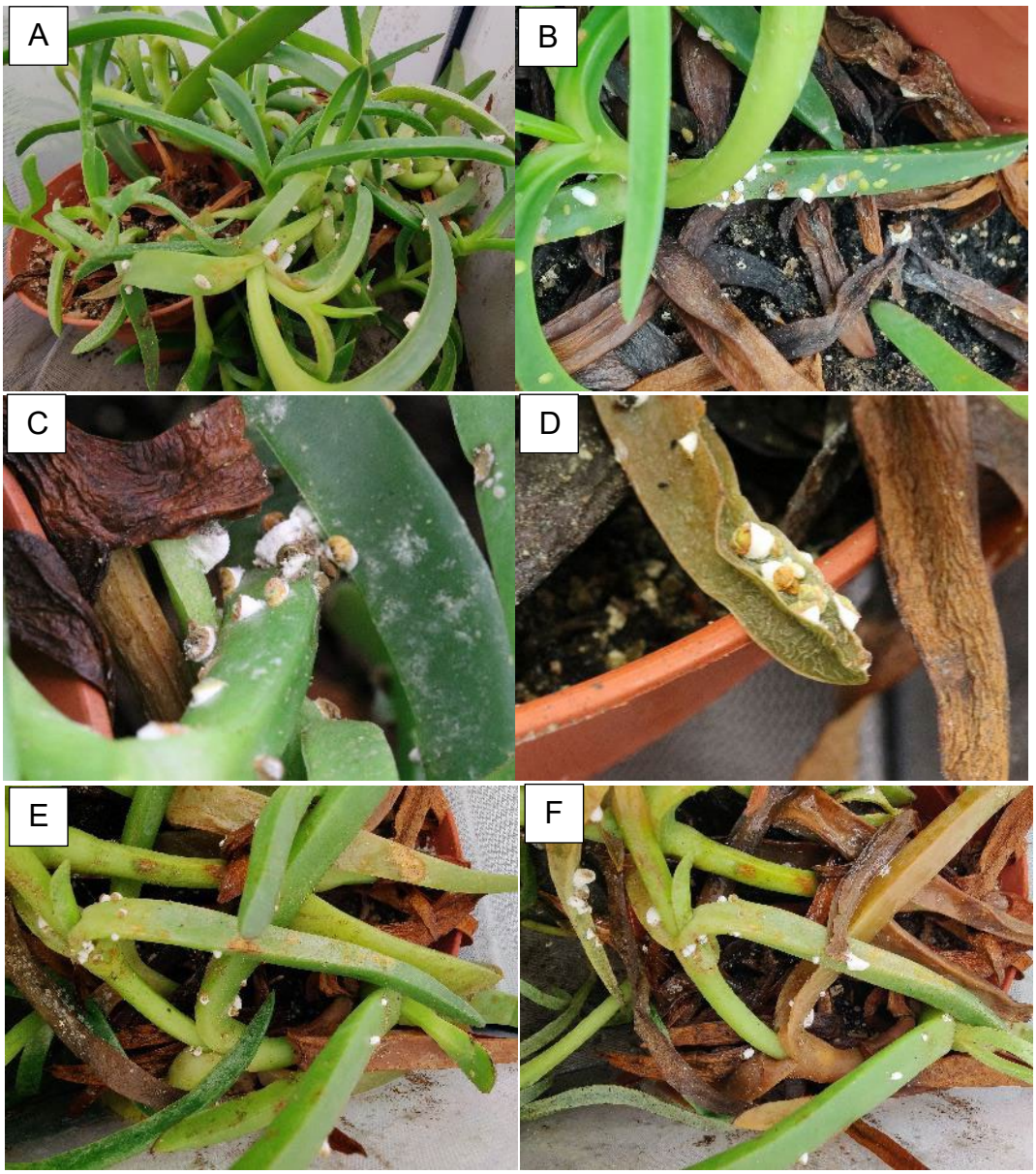

Figure 7: A) to C) High densities of various developmental stages of *Pulvinariella mesembryanthemi* on *Carpobrotus edulis*; D) Female *P. mesembryanthemi* with ovisacs on dying *C. edulis* leaf; E) and F) The same section of *C. edulis* hosting *P. mesembryanthemi* and beginning to die off from 6th to 22nd March 2023. Photos © CABI

Figure 8: Two plants kept under the same conditions from 7th February to 24th April 2023. Plant on the left exposed to *Pulvineriella mesembryanthemi* scales on 7th February, with the plant on the right free from scales. Photo © CABI

It is known that *P. mesembryanthemi* can develop on numerous Aizoaceae species, with differing levels of host suitability (Washburn & Frankie, 1985). There are no UK native Aizoaceae, however, in addition to those species actively propagated as ornamentals a number of species have become naturalised, and some invasive. *Disphyma crassifolium* was one of several Aizoaceae observed during the survey in Cornwall and Scilly and itself appeared to be displaying invasive tendencies, growing in high density mats and to the exclusion of other species, particularly at Lizard Point. Samples of this species were collected and propagated, then exposed to *P. mesembryanthemi* in the CT chamber. The plant was an acceptable host for the scale, although the scales - most notably the females with ovisacs - did trend smaller than those developing on *C. edulis*. Scales developed on the plant and over a series of weeks caused extensive die off and ultimately plant death (see Figure 9). We did not locate the scale on *D. crassifolium* in the field, although the plant was a secondary focus of the survey and did not attract as much scrutiny as *C. edulis*. It may be that although a viable host, it is not a preferred host for *P. mesembryanthemi*. Were population levels of the scale to increase at Lizard it would be interesting to see which plant species would be impacted, with extensive *C. edulis* and *D. crassifolium* growing in close proximity. Given the invasive tendencies of both species, *P. mesembryanthemi* impacts to either could be considered a positive.

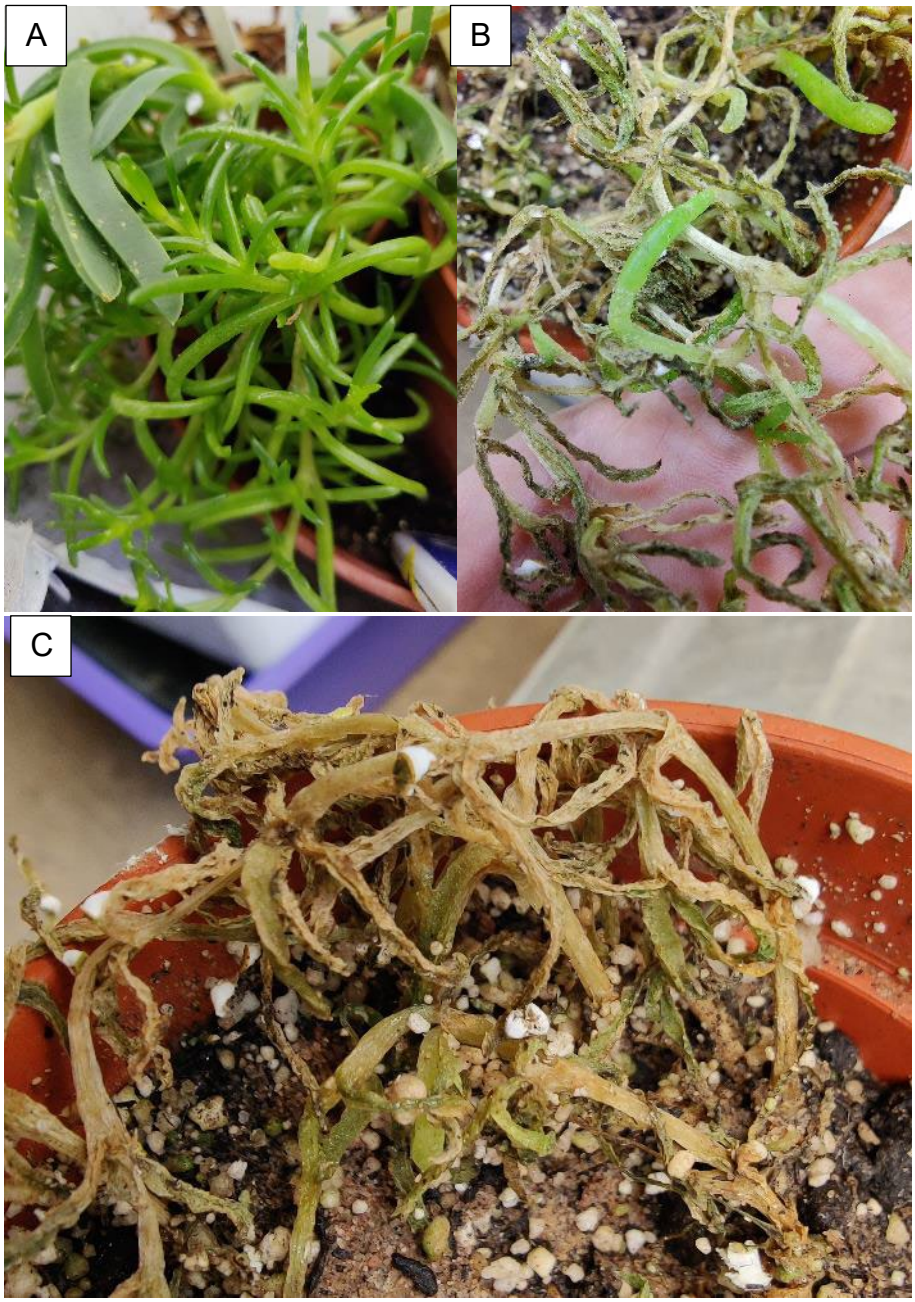

Figure 9: *Disphyma crassifolium* exposed to *Pulvinariella mesembryanthemi* in A) mid-January 2023 and in B) and C) suffering significant die off by early March. Photo © CABI

UK climatic suitability for *Pulvinariella mesembryanthemi*

The fact that a significant period of reproduction was apparent (numerous females with ovisacs, followed by crawler emergence in culture) moving into the autumn months in the UK may reflect the situation reported in California where *P. mesembryanthemi* was found to be bivoltine displaying prolonged reproductive episodes in spring and autumn, with delayed development in cooler coastal regions. The cool autumns (and relatively cold winters) in the UK may, however, limit the survival of the scale over winter, with significant crawler emergence potentially coinciding with the onset of lower temperatures and reduced plant quality. Washburn and Frankie (1985) found that survivorship for *P. mesembryanthemi* was lower at cooler temperatures (14.5˚C and 17˚C) than in warmer conditions (>20˚C), as was growth rate. As such, it is possible that *P. mesembryanthemi* is somewhat climate-limited in the cool temperate conditions of the UK, preventing it from reaching the densities necessary to exert significant impacts on *C. edulis* populations. In addition, with its primary host plant, *C. edulis*, intolerant to frost, it may be that the scale populations are knocked back significantly in areas where harsh winters lead to *C. edulis* mortality, even if the scale itself were able to tolerate these conditions. In order to further examine the potential range of *P. mesembryanthemi* in the UK, records of its worldwide distribution both native (South Africa) and introduced (North America, South America and Australasia) were downloaded from the Global Biodiversity Information Facility (GBIF) (GBIF.org, 2023) (see Figure 10) for use in a CLIMEX analysis. Although the scale has been reported in the literature as present in other regions including parts of Europe and now the UK, coordinates were not available on GBIF for use in the analysis.

Figure 10: GBIF records of *Pulvinariella mesembryanthemi* in A) North America; B) South America; C) Australasia; and D) Africa. Contains, or is derived from, information supplied by Ordnance Survey. © Crown copyright and database rights 2023.

The CLIMEX Match Regions (CMR) function was used to compare climatic values between the recorded range of *P. mesembryanthemi* both native and introduced with the UK in order to assess the likelihood and extent of field establishment that may be expected based on climatic considerations alone. Insufficient biological data, such as specific thermal requirements for development, were known for the scale to develop a full CLIMEX model, however, the CMR algorithm allows simultaneous specification of multiple home locations (climate stations throughout the known distribution of the organism in question) to be matched against a selection of away locations (stations throughout the UK) using a simple set of climate match indices against long-term datasets (Robertson *et al*., 2008). Composite Match Index (CMI) values are generated for away locations giving an indication of climatic comparability to the home locations. The four default climatic variables were used for CMR (minimum and maximum temperature; annual rainfall total and rainfall pattern – equally weighted with a value of 1). The output of CMI values for the away locations can be mapped to give a visual indication of climatic similarity of this region to the established range of the organism and thus an idea of the likelihood of its persistence across the away region. CMI values range from 0 (poorly matched) to 1 (perfectly matched) with a value of 0.7 or greater typically indicating moderate to high biological relevance (Kriticos, 2012).

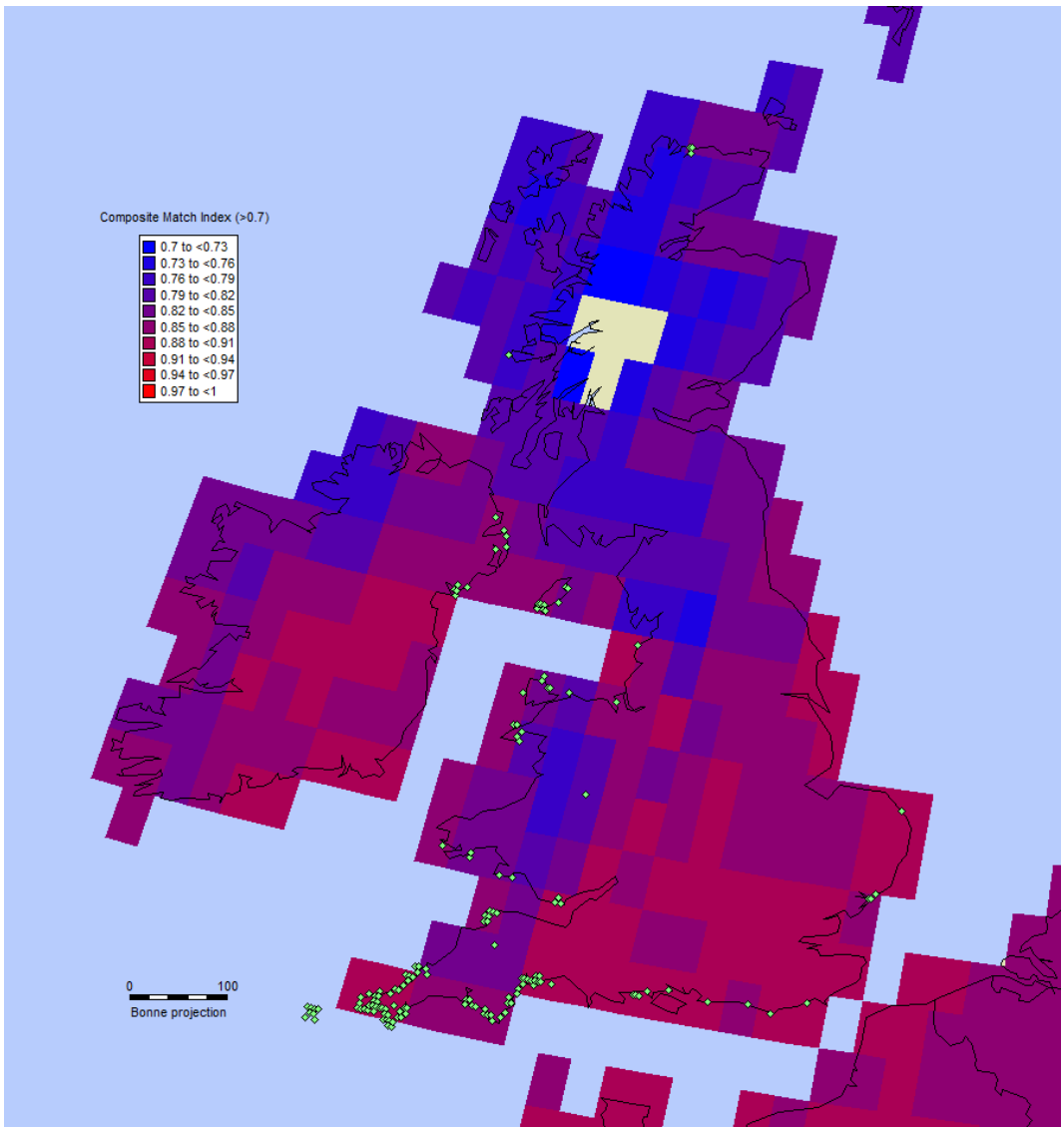

Figure 11: CLIMEX Match Regions output of climatic similarity of recorded international distribution of *Pulvinariella mesembryanthemi* to the UK. Composite Match Index values >0.7 displayed. Green circles indicate *Carpobrotus edulis* records for the UK (NBN Atlas, 2023).

From the CLIMEX Match Regions analysis it is apparent that significant regions on the UK should be suitable for establishment by the scale *P. mesembryanthemi*, where appropriate host plants are present. All areas with *C. edulis* records are somewhat suitable for the scale, particularly southern regions. This is understandable given that the scale is already established at some level in the UK as evidenced by the survey described above, and by records of the scale in regions with somewhat comparable climatic conditions. For example, a comparison of average seasonal temperature ranges is given below (Figure 12) for Newquay (UK), Christchurch (New Zealand) and Osorno (Chile), all of which have warm summers and cold winters and have proximate records of *P. mesembryanthemi* presence (see Figure 10).

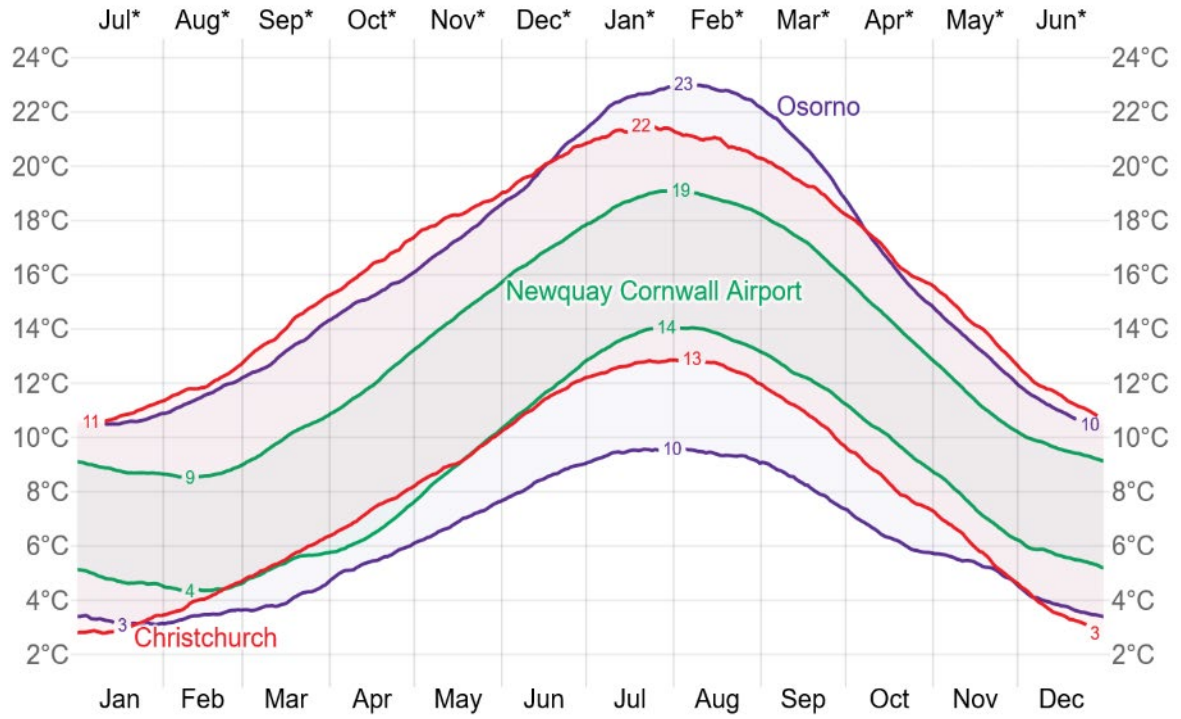

Figure 12: Average high and low temperatures in Newquay (UK) [GREEN], Christchurch (New Zealand) [RED] and Osorno (Chile) [PURPLE], all of which have nearby records of *Pulvinariella mesembryanthemi*. Southern hemisphere vs northern hemisphere locations offset by 6 months to synchronise seasons. (www.WeatherSpark.com)

While establishment and persistence may be possible in suitable regions of the UK, climate may still be limiting scale population density and spread, with scale survivorship known to be lower in cool conditions and the likelihood of reduced host plant quality over cooler months, particularly in areas experiencing frost and associated dieback of *C. edulis* (and potentially related host plants such as *C. acinaciformis*). Degree day accumulation may also be lower in parts of the UK, compared even with somewhat climatically comparable regions where the scale persists (see Figure 13).

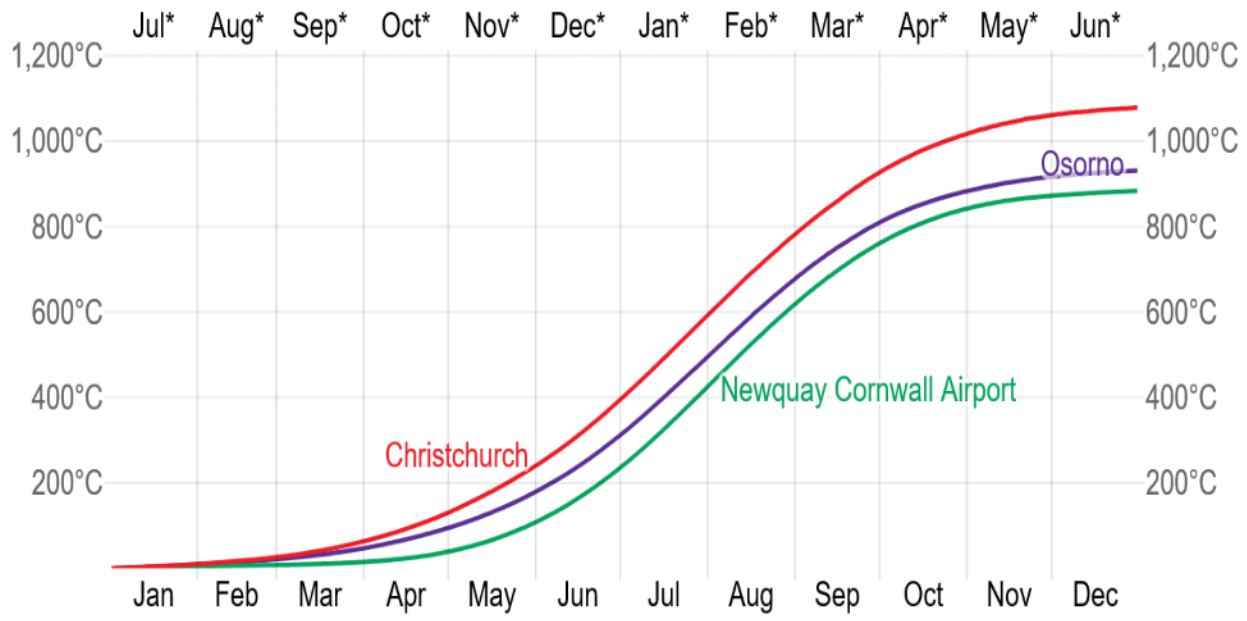

Figure 13: Growing degree days above 10˚C for Newquay (UK) [GREEN], Christchurch (New Zealand) [RED] and Osorno (Chile) [PURPLE]. Southern hemisphere vs northern hemisphere locations offset by 6 months to synchronise seasons. (www.WeatherSpark.com)

With climate change, it could be expected that UK conditions will become increasingly suitable for *P. mesembryanthemi*, which spread rapidly in warm regions of California following its introduction, thought to be dispersed primarily by wind (Washburn & Frankie, 1985). It is understandable that what appears to be a long established and persistent (though low density) population of *P. mesembryanthemi* exists on the Isles of Scilly, since the climate is influenced by the warm Gulf Stream and frost and snow are rare. Significant impacts on *C. edulis* are unlikely to be seen in the field until persistent, high-density populations of the scale can establish. An additional limitation to *P. mesembryanthemi* population growth is likely to come from predation and particularly parasitism, as evidenced by the significant rates of parasitism observed in field collected scales. In the US, *C. edulis* was considered desirable in the 1970s when it came under attack by *P. mesembryanthemi* (and *Pulvinaria delottoi*) and a suite of parasitoids from the native range of South Africa were released, bringing the scale under effective control (and allowing a resurgence of *C. edulis*). It is not clear that the current parasitoid complex attacking the scale in the UK would be as efficient as those co-evolved species from its native range but given their relative abundance from a relatively small number of scales collected, they could well be limiting population growth.

Carpobrotus edulis natural enemies on Saint Helena

Observations made by Norbert Maczey in recent field trips to Saint Helena, the British Overseas Territory in the South Atlantic Ocean, indicated extensive visible feeding damage to the invasive *C. edulis* present on the island and during a survey in October 2022 Norbert was able to assess likely candidates for the herbivory as follows:

- Banded fruit weevil *Phlyctinus callosus* – likely key damaging agent; polyphagous (Figure 14)
- African armyworm, *Spodoptera littoralis* – polyphagous
- Millipedes – present, but likely polyphagous

Figure 14: Mating banded fruit weevil adults, *Phlyctinus callosus*, on *C. edulis* with feeding damage apparent. Photo © CABI

Additional *C. edulis* herbivory candidates proposed by Liza Fowler, Saint Helena National Trust Invertebrate Specialist, but not observed by Norbert Maczey were:

- Crambid moth *Spoladea recurvalis* – polyphagous
- Fuller's rose beetle *Naupactus godmanni* – polyphagous

Unfortunately, none of the observed or proposed species detailed for Saint Helena would likely make suitable biocontrol candidates due to their polyphagous nature. Any future observations of more specific species will be recorded.

Test plant list for *Carpobrotus edulis* biocontrol agents

The determination of a potential biocontrol agent's host range is critical in order to assess its safety. In weed biological control, the centrifugal phylogenetic method, proposed by Wapshere (1974), has been used successfully for several decades and continues to serve as the basis of current host-range testing protocols as recognized by the IPPC Code of Conduct for the Import and Release of Exotic Biological Control Agents (ISPM No.3). Plants are selected based on their phylogenetic relatedness to the target weed, with those that are the most closely related being the species at higher risk and those that are more distantly related at lower risk. Biogeographic overlap and ecological similarity are also considered when selecting a test plant list (Briese, 2002).

The target invasive plant, Carpobrotus edulis (L.) N.E.Br., belongs to the Aizoaceae within the order Caryophyllales. The family is widely distributed throughout the drier and warmer parts of the globe. It is particularly genus and species rich in Southern Africa where the majority of the (approximately) 124 genera and 1,722 species are found (https://www.mobot.org/MOBOT/research/APweb/). The genus Carpobrotus is one of a small number of genera that are found naturally outside this area. The genus is usually accepted as being native to South Africa, Mozambique, Chile, Argentina and Australia. C. edulis originates from South Africa where two subspecies are recognised, the largely coastal subsp. edulis and the montane subsp. parviflorus Wisura and Glen. On a global scale, there is a need for a taxonomic revision of the genus and the number of species is unclear (Campoy et al., 2018) but the currently accepted number is 13, the majority of which are from Southern Africa (https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:328405-2). The status of one species, C. chilensis (Molina) N.E.Br., which is often considered native to Chile and Argentina, is questionable and it may be that the taxon is a hybrid derived from species originating (https://floranorthamerica.org/Carpobrotus_chilensis, Campoy and al. 2018) from Southern Africa.

Of the 13 species, 3 (Carpobrotus acinaciformis (L.) L.Bolus, Carpobrotus edulis and Carpobrotus glaucescens (Haw.) Schwantes) are accepted as occurring in the wild as non-natives in GB; additionally, another two species (Carpobrotus aequilaterus (Haw.) N.E.Br. and Carpobrotus chilensis) may also occur (Stace 2019). Hybridization between C. acinaciformis and C. edulis is also known to occur (Campoy et al., 2018) but is undocumented in GB. Within GB, C. edulis subsp. edulis appears to be the most widespread taxon (subsp. parviflorus is not recorded here) with C. acinaciformis and C. glaucescens being much more restricted in their distribution (Isles of Scilly, West Cornwall, Isle of Wight, Suffolk, Norfolk and Wigtownshire; BSBI Maps). According to Stace (2019), there are three forms of C. edulis in GB, the most frequent being yellow-flowered (var. edulis), however some plants have pinkish purple petals (var. rubescens Druce) or pink petals with yellow petal bases (var. chrysophthalamus C.D. Preston and P.D. Sell). It is probable that the latter two variants are hybrid in origin, and it is notable that in South

Africa, C. edulis plants are always yellow-flowered (http://pza.sanbi.org/carpobrotus-edulis).

The genus Carpobrotus is closely related to Disphyma N.E.Br. which, like Carpobrotus, is also known to occur as a native genus outside South Africa, in Australia and New Zealand. Hybridization has been recorded between the two genera (C. edulis x D. australe (Aiton) J.M.Black and C. aequilaterus x D. australe) in New Zealand (Chinnock 1972; Preston 1989). In the UK, one species of Disphyma (D. crassifolium (L.) L.Bolus) is naturalised in similar habitats to C. edulis and it is possible that some 'Carpobrotus' plants in the GB may be intergeneric hybrids (Preston, 1989).

Systematic overview of Carpobrotus edulis

Clade:	Angiosperms
Clade:	Eudicots
Order:	Caryophyllales Juss. ex Bercht. and J.Pres
Family:	Aizoaceae Martinov
Genus:	*Carpobrotus* N.E.Br.
Species:	*Carpobrotus edulis* (L.) N.E.Br.

Test species selection within the Caryophyllales

The order contains approximately 37 families, 749 genera, and 11,620 species (https://www.mobot.org/MOBOT/research/APweb/). It is a rather unusual order, there is a widespread tendency towards succulency and other adaptations to living in saline or dryer environments. The placement of the order on phylogenetic trees is often not well supported but overall, it appears that the order is probably sister to, alongside the Cornales, the Asterid clade (Figure 15). Similarly, within the order relationships between families are sometimes unclear but overall, there appear to be two main clades (Figure 16). The first clade is the smaller and is itself divided into two groups, one consisting of largely carnivorous families and the other dominated by the Polygonaceae. Of the carnivorous group (Droseraceae, Nepenthaceae, Droserophyllaceae, Ancistrocladaceae and Dioncophyllaceae) only the Droseraceae is represented in the wild and in commercial horticulture in the UK, therefore the remaining largely tropical families will not be considered further. The second group (Frankeniaceae, Tamaricaceae, Plumbaginaceae and Polygonaceae) are widespread in temperate regions and representatives of all four families are found in the wild and in commercial horticulture in the UK.

The second clade is much larger and more diverse. A large number of families (Rhabdodendraceae, Simmondsiaceae, Astropeiaceae, Physenaceae, Microteaceae, Achatocarpaceae, Stegnospermataceae, Limeaceae, Lophiocarpaceae, Barbeuiceae, Gisekiaceae, Sarcobataceae, Molluginaceae, Halophytaceae, Didieraceae, Basellaceae, Talinaceae and Anacampserotaceae), are characteristic of warm temperate and tropical regions and are not, with the exception of specialist collections and botanic gardens, present in the wild or in commercial horticulture in the UK; these families will not be considered further (with the exception of Basella alba and Mollugo cerviana that are on the

list for the continental European perspective). The remaining families, Caryophyllaceae, Amaranthaceae, Phytolaccaceae, Nyctaginaceae, Montiaceae, Portulacaceae and Cactaceae are either represented by widespread wild plants or have varying degrees of importance in agriculture and horticulture. A summary of the caryophyllalean families relevant to this test list is given here:

The small family Droseraceae has three carnivorous representatives growing in the wild in the UK (Drosera anglica, D. intermedia and D. rotundifolia). However, their distribution and ecologies do not overlap with Carpobrotus (BSBI Maps), therefore they are excluded from the test list.

The Frankeniaceae is represented by one native species, the Sea Heath (Frankenia laevis), which is a nationally scarce and restricted to coastal habitats that are likely to be impacted by Carpobrotus. It is therefore included in the test list.

The non-native Tamaricaceae is widely planted in coastal areas and frequently grows on habitat impacted by invasive Aizoaceae (particularly Carpobrotus and Disphyma). Tamarisk is often considered important as a windbreak/dune stabiliser and is valued for its aesthetic appeal. Therefore, the most frequently planted species, Tamarisk (Tamarix gallica), is included on the test list.

The family Plumbaginaceae is represented by two genera, Armeria and Limonium, in the wild in the UK. One other, Plumbago auriculata (and is closely related to the Southern European P. europaea), is popular in horticulture and is included on the list. There is significant ecological and distributional overlap between the native genera and Carpobrotus in the UK (BSBI Maps), therefore two species Thrift (Armeria maritima) and Rock Sea-lavender (Limonium binervosum agg.) are included on the list.

The Polygonaceae is quite a large and important family, both in the wild (Stace, 2019) and in horticulture. The generic boundaries within this family have undergone major changes over the last decade and the nomenclature can be confusing. Two wild representatives, Sea Knotgrass (Polygonum maritimum) and Shore Dock (Rumex rupestris) have been selected to reflect this diversity and because both species are rare and endangered; furthermore, their habitat and range strongly overlaps with that of Carpobrotus in SW England. It is recognised that both of these species will be very challenging to source, therefore the following closely related and ecologically overlapping more widespread taxa may be substituted – Ray's Knotgrass (P. oxyspermum subsp. raii) and Curled Dock (Rumex crispus), the latter species is known to hybridise with Shore Dock (Stace, 2019).

The Caryophyllaceae is a large and ecologically significant wild plant family in the UK. It is also of importance to horticulture (particularly Dianthus and Silene spp. and their hybrids). Several groups from the family also have species that occupy similar niches to Carpobrotus. There is a tendency towards succulency in these plants. Therefore, three species (Sea Sandwort, Honckenya peploides; Lesser Sea-spurrey Spergularia marina and Sea Campion, Silene uniflora) have been selected for testing. Some salt-tolerant or requiring plants may be challenging to cultivate and testing may have to be limited to

harvested samples and not cultivated material; this challenge may impact other proposed test species, particularly halophyte (salt-adapted) members of the Amaranthaceae.

Like the Caryophyllaceae, the Amaranthaceae is an important plant family in wild in the UK. Due to their small flowers, they are less popular in horticulture but some species, particularly Root Beet (Beta vulgaris subsp. vulgaris), Spinach (Spinacia oleracea) and Quinoa (Chenopodium quinoa) are economically important, particularly Root Beet of which the very important agricultural crop Sugar Beet is a form. Quinoa is closely related to the widespread Fathen (C. album) which has selected for the test list. Similarly, the UK native Sea Beet (B. vulgaris subsp. maritima) is selected due to its importance and a Crop Wild Relative (see also Wild Cabbage below) of Root Beet and because of the significant ecological and distributional (BSBI Maps) overlap between this plant and Carpobrotus in the UK. The family is characterised by various adaptations to saline environments, consequently several genera are of importance ecologically in coastal environments and are likely to grow in close proximity to Carpobrotus. Additionally, like Carpobrotus, several of these genera are (semi-)succulent and should therefore be considered for testing. In addition to the above species, the following taxa: Red Goosefoot, Oxybasis (syn. Chenopodium) rubra; Babington's Orache, Atriplex glabruiscula; Pedunculate Sea-purslane, Atriplex pedunculata; Sea-purslane, Atriplex portulacoides; Perennial Glasswort, Sarcocornia perennis; Common Glasswort, Salicornia europaea; Shrubby Sea-blite, Suaeda vera and Prickly Saltwort, Salsola kali, are recommended for testing. As with Rock Sea-spurrey, it is recognised that cultivation may be challenging (particularly for Sarcocornia perennis, Salicornia europaea, Suaeda vera and Salsola kali) and that testing may have to be limited to harvested samples and not cultivated material (NOTE: Using harvested plant material will work with insect herbivores but is more problematic when working with plant pathogens. It has been done in the past, but sometimes results are not reliable). Three species of Atriplex s.l. are proposed for testing; some species such as A. glabruiscula are widespread and ecologically important in coastal habitats. The other two species are only distantly related and are sometimes separated into another genus, Halimione (Kaderit et al., 2010). A. pedunculata is extremely rare (or extinct) in the UK (one very vulnerable population in Essex) whereas A. portulacoides is widespread; if material of A. pedunculata is unavailable (which is highly likely it may be possible to obtain plants from continental Europe but import restriction may apply as it is a rare and protected plant in most countries where it occurs) it is probable that A. portulacoides will be a suitable proxy.

Members of the Phytolaccaceae are of very limited value to UK horticulture and are normally viewed as a weedy. There are several non-native members of the family growing in the wild in the UK. However, their distribution (BSBI Maps) and ecologies do not overlap, therefore they are excluded from the test list.

The fairly small family Nyctaginaceae is largely restricted to warm temperate and tropical regions; however, one species, Marvel-of-Peru (Mirabilis jalapa) is fairly widespread in outdoor cultivation in the UK and is recommended for testing.

There is one member of the Montiaceae growing wild in the UK, the diminutive and well-named Blinks (Montia fontana); this small semi-succulent plant is widespread in damp habitats, such as dune slacks and is recommended for testing.

Only one non-native species of Portulacaceae, Common Purslane (Portulaca oleracea) is present in the wild (mainly on the isles of Scilly) in the UK; however, it and several other species are cultivated. Therefore, this species in included on the test list.

While many genera of Cactaceae are popular as indoor plants, very few (e.g. Opuntia spp.) are grown outside in the mildest parts of the UK. The Portulacaceae are very closely related, and there are grounds for merging the two families; therefore, it is considered appropriate that P. oleracea represent these families on the test list.

While some members of the Aizoaceae are native to the Mediterranean basin, none are native to the UK and all the naturalised taxa originated from South Africa (with the exception of C. glaucescens). Due to their popularity in horticulture, and in some cases, their occurrence in the wild as invasives, twelves members of this family are proposed for testing. Several of the genera (Carpobrotus, Delosperma, Drosanthemum, Disphyma, Lampranthus, Oscularia and Ruschia) all belong to the same subfamily (Ruschioideae) and it may not be necessary to test all genera, in which case the following genera, Drosanthemum, Oscularia and Ruschia may be excluded from testing (or testing may be limited to harvested samples and not cultivated material). The remaining genera, Cleretum (syn. Dorotheanthus)

Mesembryanthemum (syn. Aptenia) and Tetragonia are from different subfamilies (the first two, Mesembryanthemoideae and the latter Aizooideae) and it is recommended that these genera are tested fully.

European members of the Aizoaceae may also be included to safeguard against any negative side-effects in cases where biocontrol agents released in the UK disperse onto continental Europe (or would be considered there for the control of Carpobrotus). It is recommended that the following taxa be included: Mesembryanthemum nodiflorum, Mesembryanthemum crystallinum and Aizoanthemum hispanicum.

Selection of additional test species

Selection of additional test species outside of the Caryophyllales has been driven by ecological and distributional (BSBI Maps) overlap with Carpobrotus in the UK as well as a tendency towards succulence. Within the exception of Brassica oleracea subsp. oleracea and Sedum anglicum test species selection has also focussed upon taxa within the Asterid clade (Figure 15).

Asterid

Ericales (**Primulaceae**): Sea Milkwort (*Lysimachia* (syn. *Glaux*) *maritima*)

Asterid I

Solanales (**Convolvulaceae**): Sea Bindweed (*Calystegia soldanella*).

Asterid II

Asterales (**Asteraceae**): Sea Aster (*Tripolium pannonicum*, syn. *Aster tripolium*)

Apiales (**Araliaceae**): Rock Samphire (*Crithmum maritimum*)

The final two species, English Stonecrop (*Sedum album*, Crassulaceae, Saxifragales) and Wild Cabbage (*Brassica oleracea* var. *oleracea*, Brassicaceae, Brassicales) have been selected because of their fleshy/succulent habit and because the UK has a significant proportion of their global populations and in the case of Wild Cabbage, because it is an important Crop Wild Relative.

The proposed UK test plant list is composed of 50 species (Table 1). It is not envisaged that all 50 species are tested as part of the current biocontrol agent test strategy; some species may only be testable using collected material, not cultivated plants. Plants can mostly be sourced from nurseries or field collected. Kew's Millennium Seed Bank, the Royal Horticultural Society and collaborators around the UK can also supply seeds and plant material of test species that are difficult to source. Any testing will be divided into two separate stages. Phase one includes species where any development by the control agents is most likely due to their close phylogenetic relationship to *Carpobrotus* or species of particular importance for the biodiversity in the target area. **Proposed Priority One species are typed in bold in the list below.** Priority 2 and 3 include a wider range of (ecologically, economically or horticulturally) important species from the Lamiales and the wider Asterid I and Asterid II clades.

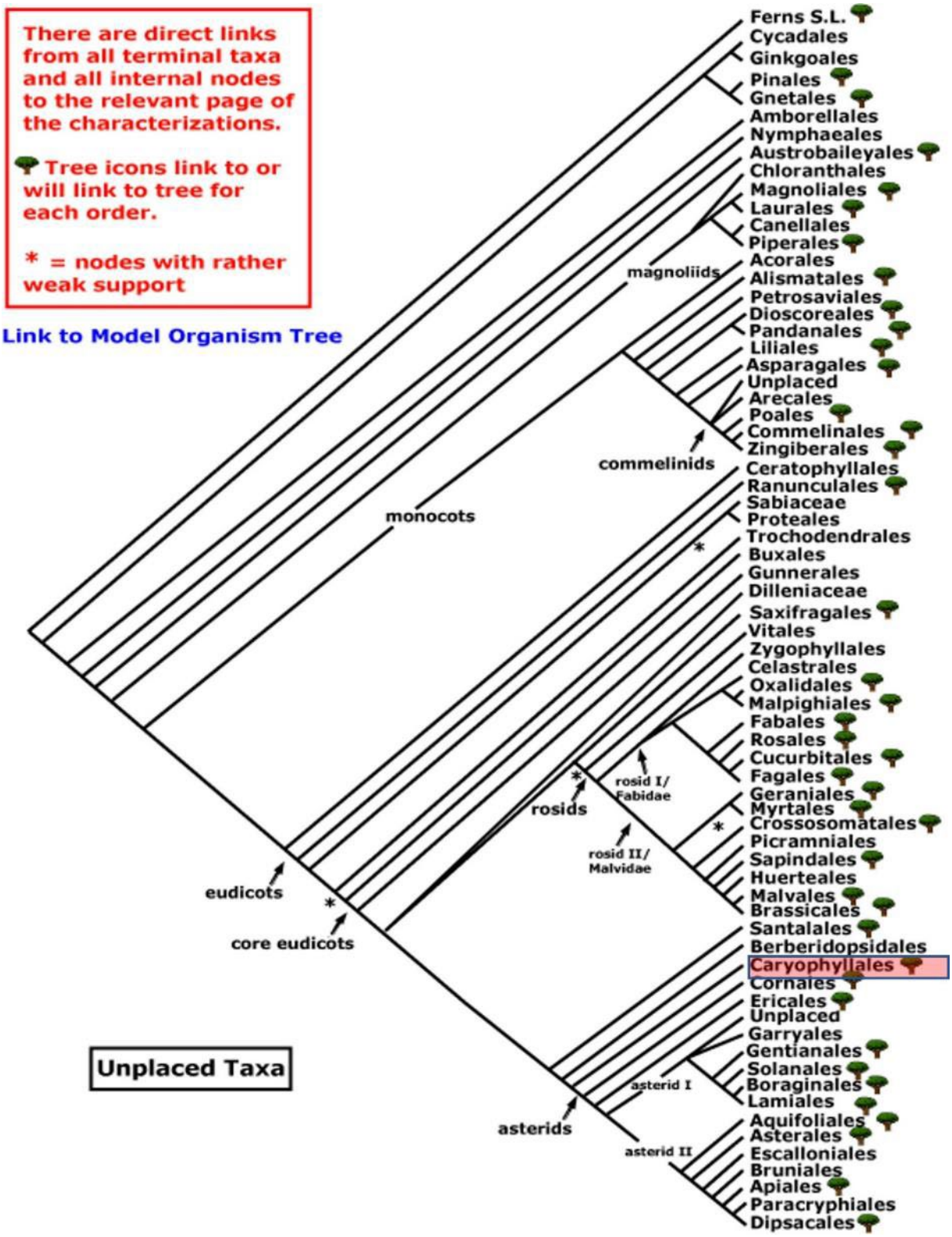

Figure 15: Phylogenetic tree of vascular plants showing the position of the Caryophyllales rooted within the core eudicots and sister to the Cornales and asterid clades (from the Angiosperm Phylogeny Website, see: https://www.mobot.org/MOBOT/research/APweb/).

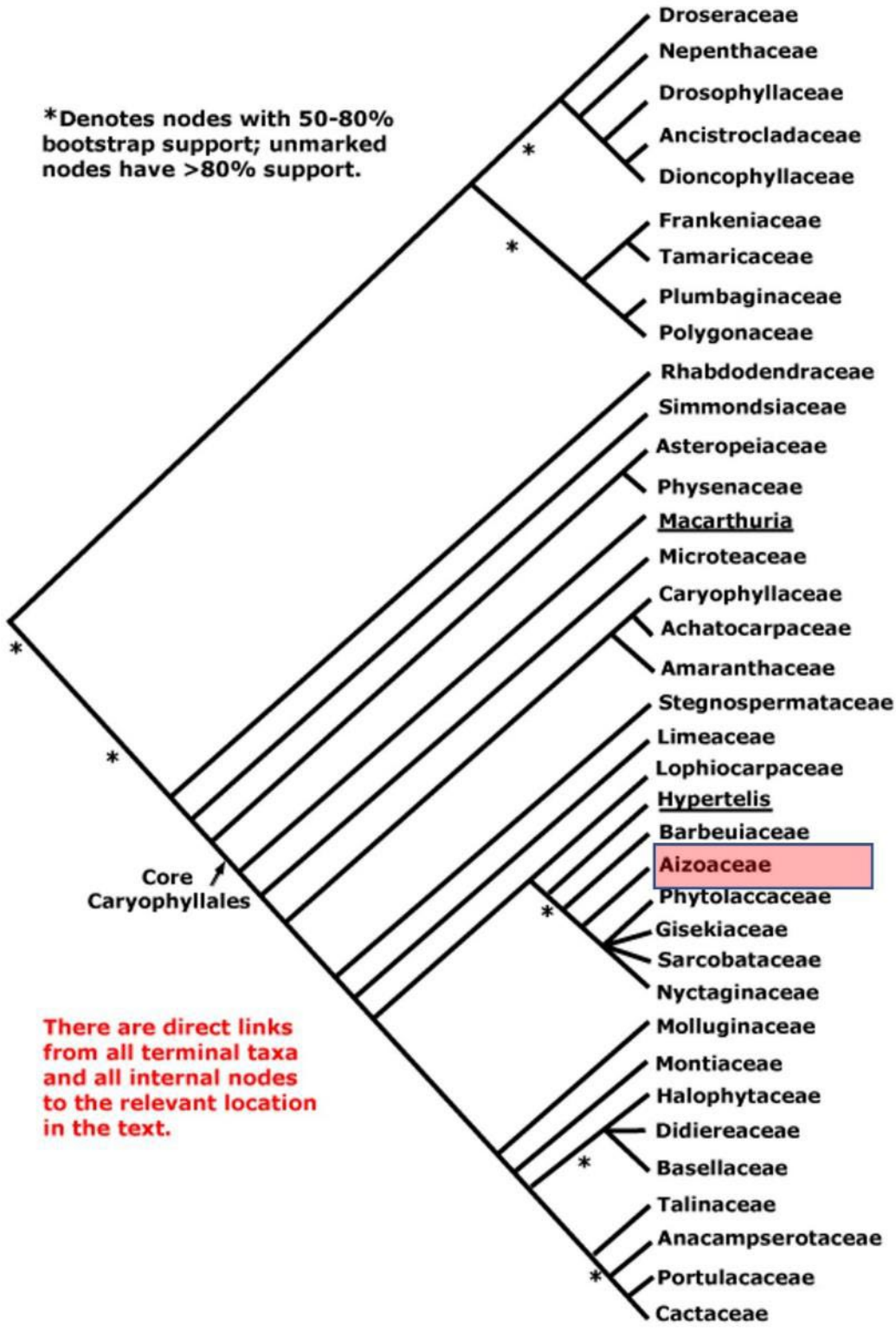

Figure 16: Phylogenetic tree of the Caryophyllales (from the Angiosperm Phylogeny Website, see: https://www.mobot.org/MOBOT/research/APweb/).

Table 1: Proposed test plant list. Status is defined as: (I) an introduced plant species to the UK/Europe; (O) an ornamental species; (N) a native species (or archaeophyte) in the RA region (UK); (NE) native in mainland Europe, and (E) an economically important plant species to the UK/Europe. Species are prioritised 1 – 3 in order of importance (a balance of ecological, economic or horticultural value).

Order	Family	Species	Common Name	Status	Priority rating
Caryophyllales	Aizoaceae	***Carpobrotus edulis***	Cape Fig	Target	1
Caryophyllales	Aizoaceae	***Carpobrotus acinaciformis***	Sally-my-handsome	I, O	1
Caryophyllales	Aizoaceae	***Carpobrotus glaucescens***	Angular Sea-fig	I, O	1
Caryophyllales	Aizoaceae	***Delosperma cooperi***	Trailing Iceplant	I, O	1
Caryophyllales	Aizoaceae	***Disphyma crassifolium***	Purple Dewplant	I, O	1
Caryophyllales	Aizoaceae	*Drosanthemum floribundum*	Pale Dewplant	I, O	2
Caryophyllales	Aizoaceae	***Lampranthus falciformis***	Sickle-leaved Dewplant	I, O	1
Caryophyllales	Aizoaceae	*Oscularia deltoidea*	Deltoid-leaved Dewplant	I, O	2
Caryophyllales	Aizoaceae	*Ruschia caroli*	Shrubby Dewplant	I, O	2
Caryophyllales	Aizoaceae	***Cleretum* (syn. *Dorotheanthus*) *bellidiforme***	Livingstone Daisy	I, O	1
Caryophyllales	Aizoaceae	***Mesembryanthemum* (syn. *Aptenia*) *cordifolium***	Heart-leaf Iceplant	I, O	1
Caryophyllales	Aizoaceae	*Mesembryanthemum nodiflorum*	Slender Iceplant	NE	2
Caryophyllales	Aizoaceae	*Mesembryanthemum crystallinum*	Iceplant	NE	2

Order	Family	Species	Common Name	Status	Priority rating
Caryophyllales	Aizoaceae	***Aizoanthemum hispanicum***	Aspanish Aizoon	NE	2
Caryophyllales	Aizoaceae	***Tetragonia tetragonioides***	New Zealand Spinach	I	1
Caryophyllales	Nyctaginaceae	*Mirabilis peruviana*	Marvel-of-Peru	I, O	2
Caryophyllales	Montiaceae	***Montia fontana***	Blinks	N	1
Caryophyllales	Molluginaceae	*Mollugo cerviana*	Threadstem Carpetweed	NE	2
Caryophyllales	Portulacaceae	*Portulaca oleracea*	Common Purslane	I	2
Caryophyllales	Basellaceae	*Basella alba*	Malabar Spinach	I, O	2
Caryophyllales	Amaranthaceae	*Amaranthus caudatus*	Love-lies-bleeding	I, O	2
Caryophyllales	Amaranthaceae	***Oxybasis* (syn. *Chenopodium*) *rubra***	Red Goosefoot	N	1
Caryophyllales	Amaranthaceae	***Chenopodium album***	Fathen	N	1
Caryophyllales	Amaranthaceae	***Spinacia oleracea***	Spinach	I, E	1
Caryophyllales	Amaranthaceae	***Atriplex glabruiscula***	Babington's Orache	N	1
Caryophyllales	Amaranthaceae	*Atriplex pedunculata*	Pedunculate Sea-purslane	N	3
Caryophyllales	Amaranthaceae	*Atriplex portulacoides*	Sea-purslane	N	2
Caryophyllales	Amaranthaceae	***Beta vulgaris* subsp. *maritima***	Sea Beet	N	1
Caryophyllales	Amaranthaceae	***Beta vulgaris* subsp. *vulgaris***	Root Beet, Beetroot, Sugar Beet	I, E	1

Order	Family	Species	Common Name	Status	Priority rating
Caryophyllales	Amaranthaceae	*Sarcocornia perennis*	Perennial Glasswort	N	2
Caryophyllales	Amaranthaceae	*Salicornia europaea*	Common Glasswort	N	2
Caryophyllales	Amaranthaceae	*Suaeda vera*	Shrubby Sea-blite	N	2
Caryophyllales	Amaranthaceae	*Salsola kali*	Prickly Saltwort	N	2
Caryophyllales	Caryophyllaceae	*Honckenya peploides*	Sea Sandwort	N	2
Caryophyllales	Caryophyllaceae	*Spergularia marina*	Lesser Sea-spurrey	N	2
Caryophyllales	Caryophyllaceae	*Silene uniflora*	Sea Campion	N	2
Caryophyllales	Polygonaceae	*Polygonum maritimum*	Sea Knotgrass	N	2
Caryophyllales	Polygonaceae	*Rumex acetosella*	Sheep's Sorrel	N	2
Caryophyllales	Polygonaceae	*Rumex rupestris*	Shore Dock	N	2
Caryophyllales	Plumbaginaceae	*Limonium binervosum*	Rock Sea-lavendar	N	2
Caryophyllales	Plumbaginaceae	*Armeria maritima*	Thrift	N	2
Caryophyllales	Plumbaginaceae	*Plumbago auriculata*	Cape Leadwort	I, O	2
Caryophyllales	Tamaricaceae	*Tamarix gallica*	Tamarisk	I, O	2
Caryophyllales	Frankeniaceae	*Frankenia laevis*	Sea-heath	N	2
Ericales	Primulaceae	*Lysimachia* (syn. *Glaux*) *maritima*	Sea Milkwort	N	3
Solanales	Convolvulaceae	*Calystegia soldanella*	Sea Bindweed	N	3

Order	Family	Species	Common Name	Status	Priority rating
Apiales	Apiaceae	*Crithmum maritimum*	Rock Samphire	N	3
Asterales	Asteraceae	*Tripolium pannonicum* (syn. *Aster tripolium*)	Sea Aster	N	3
Saxifragales	Crassulaceae	*Sedum album*	English Stonecrop	N	3
Brassicales	Brassicaceae	*Brassica oleracea var. oleracea*	Wild Cabbage	N, E	2

Preliminary assessment of natural enemy prospects in native range

Pathology

In collaboration with researchers at the Agricultural Research Council (ARC) and Forestry and Agricultural Biotechnology Institute (FABI) in South Africa, first steps were taken to source a culture of a novel pathogen previously reported (Jami et al., 2018). The *Cytospora* species, *Cytospora carpobroti* sp. nov, had been recorded in association with stem death of *C. edulis* in its native range and could hold promise as a damaging agent; however, this group of fungal pathogens is generally more known to take advantage of plant stress rather than being virulent primary pathogens. Nonetheless, if an isolate of this *Cytospora* species can be easily obtained it would be warranted to import a culture of the pathogen to quarantine to carry out preliminary testing against *C. edulis,* and if results are promising follow this up with priority test plant species.

Following further discussions with researchers at the above institutions and lead pathologists at CABI, pathogen surveys in the native range are considered the most sensible next step, with the fungal pathogen species affecting *Carpobrotus* spp. in South Africa poorly described at present. Ideally these could be combined with invertebrate surveys.

Invertebrates

Following from the field assessment and impact study on *P. mesembryanthemi*, it could be valuable to conduct host range studies with the scale against priority test plants. If deemed suitably specific and given the relatively extensive climatic suitably for the scale of UK regions affected by *C. edulis*, a mass rearing and redistribution of the scale to new areas could be considered, to increase the geographic range and propagule pressure of the scale on *C. edulis* populations.

There are a number of additional scale species associated with *Carpobrotus* spp., including several South African natives in the Diaspididae (armoured scales) that have relatively narrow host records and could therefore hold promise as suitably specific agents. These include *Hemiberlesia mitchelli*, *Diaspidiotus mesembryanthemae* and *Entaspidiotus lounsburyi* (http://scalenet.info/catalogue/Entaspidiotus%20lounsburyi/), the latter of which has been found to be destructive on *Carpobrotus* spp. despite high parasitism rates in parts of South Africa and attacks the non-native *Disphyma crassifolium* in Italy where it has been introduced (Campoy et al., 2018). The authors of the current report have a tentatively identified record of *E. lounsburyi* from a visit to Kirstenbosch National Botanical Garden that would be a prime location to survey (Figure 17).

Figure 17: An armoured scale tentatively identified as *Entaspidiotus lounsburyi*, on *Carpobrotus edulis* at Kirstenbosch National Botanical Garden, South Africa. Photo © CABI

In addition to the scale complex attacking *Carpobrotus* spp. in South Africa, it is likely that additional damaging invertebrates would be apparent on this plant genus, which to date has had limited assessment as a target for classical biological control. As such the logical next step in progressing this research would be to conduct native range surveys. Along this line, the authors have been in discussion with scientists at Rhodes University who have extensive biocontrol and field survey experience and have shown a strong interest in collaborating on future *Carpobrotus* research. The researchers are currently investigating the potential biocontrol of two ice plant species (*Cryophytum crystallinum* and *Mesembryanthemum nodifolrum*, Aizoaceae) in collaboration with the United States Department of Agriculture (USDA) and as such have highly relevant experience that could complement the UK research into *Caprobrotus* biocontrol. Invertebrate surveys could be combined with pathogen surveys to effectively assess the full suite of *C. edulis* natural enemies.

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