Cape Peninsula
University of Technology

BREAKING THE DORMANCY OF SELECTED ASTERACEAE ANNUALS IN THE WINTER-RAINFALL REGION OF SOUTH AFRICA

by

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DECLARATION

I, Roger Clive Oliver, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

July 2021

Signed **Date**

ABSTRACT

The aim with study was to investigate whether the germination of *Arctotis hirsuta, Cotula duckittiae* and *Oncosiphon suffruticosum* are three Asteraceae annuals can be improved by determining its individual temperature- and light-condition requirements and subjecting the achenes to pre-germination treatment such as scarification, GA_3 and after-ripening at high temperatures. Experiments were conducted between 2019 and 2020. The first experiments evaluated the temperature and light required per species. Treatments consisted of inducing germination at 5 different temperatures namely 7 °C, 12 °C, 17 °C, 22 °C, 27 °C and alternate temperatures at 12 °C/22 °C and 17 °C/27 °C in conditions of light, darkness and alternate lightning (12 hours each of light and darkness). In the second experiment, pre-germination treatments consisted of scarification (pericarp-pricking) and the soaking of seed over 4 periods in 4 different gibberellic-acid (GA_3) concentrations. Seeds of all species were then subjected to pre-determined light and temperature conditions as follows: *A. hirsuta* (collection year 2016) [22 °C in light]*, C. duckittiae* (collection year 2017) [22 °C in light], *O. suffruticosum* (collection year 2016) [12 °C in light] and *O. suffruticosum* (collection year 2017) [22 °C in dark] to ensure optimum germination. The third experiment, after-ripening, consisted of two different treatments over two periods (30 °C & 45 °C for 4 and 8 weeks). Seeds of all species were then subjected to pre-determined light and temperature conditions as follows: *A. hirsuta* (collection year 2016) [22 °C in light]*, C. duckittiae* (collection year 2017) [22 °C in light], *O. suffruticosum* (collection year 2016) [12 °C in light] and *O. suffruticosum* (collection year 2017) [22 °C in dark] to ensure optimum germination.

Chapter 2 reviewed the importance to alleviate the germination-challenges for the three species when grown from seed of wild plants. It was found that when incubated at specific temperature- and light-requirements, some winter-growing annuals can improve their germination. The utilization of these pre-determined light- and temperature-requirements, in conjunction with other pre-germination treatments such as scarification, soakings in GA_3 and after-ripening at elevated temperatures have been deemed very effective to counter challenges with germination for some species of Asteraceae-annuals.

Chapter 3 indicated that germination of *A. hirsuta* achenes is the result of a temperature and light interaction for temperatures between the 22 °C and 27 °C in the light. The impact of the interaction between temperature and light/dark phases on the germination of *C. duckittiae* (collection year 2016) were insignificant through a range of temperatures

and light- or dark-conditions. This could also be observed from the interaction of alternating temperature under cyclic light/dark conditions on the germination of *C. duckittiae* (collection year 2016) at 12 °C/22 °C compared to 17 °C/27 °C. Achenes of *C. duckittiae* (collection year 2017) achieved optimum germination in the dark at 22 °C, but very low to no germination in all the other treatment combinations. In *O. suffruticosum* (collection year 2016) the best germination was achieved at 12 °C in the light, however this did not differ very much from 17° C, (in light), 22° C (in light) and 7° C (in dark). *O. suffruticosum* (collection year 2017) recorded very low germination over all the treatments.

In chapter 4 the pre-germination treatments, scarification and the GA_3-treatment, had a significant impact on the germination of *A. hirsuta* (collection year 2016), but could not effect improved germination for *C. duckittiae* (collection year 2017) and *O. suffruticosum* (collection years 2016 & 2017). These results suggested that the use of GA_3 or scarification as a single pre-treatment application are not effective in improving germination for *C. duckittiae* (collection year 2017) and *O. suffruticosum* (collection years 2016 & 2017).

Chapter 5 investigated whether the impact of accelerated high temperature after-ripening treatments of 30 °C and 45 °C over 4 and 8 weeks may alleviate dormancy and result in improved germination. Findings from this experiments indicated that none of the treatments could improve germination.

Overall this study has found that some species stored (after-ripened) at controlled conditions in a seed room, will have no specific requirements in order to alleviate dormancy as was the case of *C. duckittiae* (collection year 2016) when stored for between 28 - 32 months. Good germination was obtained over several temperature-ranges in light or dark conditions. *A. hirsuta* achieved optimum germination and only exhibited a mild form of dormancy between 22 °C - 27 °C. Pre-treatments with scarification and GA_3 yielded very average results for this species. After-ripening, at the temperatures of 30 °C and 45 °C, as well as scarification and a GA_3-treatment, did not improve germination for *C duckittiae* (collection year 2017), *O. suffruticosum* (collection year 2016) and *O. suffruticosum* (collection year 2017).

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GENERAL INTRODUCTION, STRUCTURE OF THESIS, PROBLEM STATEMENT, AIMS, HYPOTHESES AND OBJECTIVES

1.1 GENERAL INTRODUCTION

There is limited research available on the temperature or light requirements, the pre-treatment of seeds and the effects of after-ripening at different regimes on seed dormancy of the three winter annuals *Arctotis hirsuta* (Harv.) Beauvard, *Cotula duckittiae* (L. Bolus) K. Bremer & Humphries and *Oncosiphon suffruticosum* (L.) Källersjö. This is a gap in the existing body of knowledge on the germination ecology of Asteraceae winter annuals in South Africa.

The specific objectives are: 1) to determine whether exposure to different ranges of temperature and light, 2) different seed pre-treatments (scarification and soaking with GA_3) and 3) different after-ripening conditions (periods and temperatures) will improve the breaking of seed dormancy of this winter annuals.

The experiments will be conducted in the laboratory of Kirstenbosch Research Centre and will be laid out in a randomized complete block design. Germination tests will be done on freshly matured seed and one-year old seed, depending on seed availability. The treatments will include incubation of seed at five constant temperatures in light and dark conditions for 24 hours and two alternating temperatures at a combination of light and dark conditions at 12 hours each. This will result in determining optimum temperature and light conditions that will be used to incubate seed for all other treatments to be conducted further. The pre-treatment of seed through scarification will involve the pricking of the seed coat. The scarified seed and the control of unscarified seed will then be incubated at the optimum light and temperature conditions determined earlier.

Pre-treating the seed with GA_3 will be done at durations of one and 2 hours respectively using 250mg/l and 500 mg/l as concentrations for both periods. The treated seed will then be incubated at the optimum light and temperature conditions determined earlier. The control will consist of untreated seed.

In assessing the effect of after-ripening, the seed will be after-ripened over a period of 1 and 2 months at the three different temperatures of 15 ˚C, 30 ˚C and 45 ˚C. Seed will then be incubated under the optimum temperature and light conditions. Data will be collected as planned and will include: germination percentage.

The statistical analyses will be done using one- or two-way analysis of variance (ANOVA). These computations will be done with the software program Minitab. Tukey's Pairwise Comparison and Fisher's Least Significance Test (LSD) will be used to compare treatment means at $p \leq 0.05$ level of significance

1.2 STRUCTURE OF THE THESIS

The thesis is drafted differently to the alternative of a traditional format for a thesis. The article-format thesis examples of published, co-published and/or "ready-for-publication" articles was prepared during candidature and applies to the format prescribed by CPUT for 100% master's studies which complies to the following principles:

1. The overriding principle of the thesis is that it remains an original contribution to the discipline or field by the candidate.
2. Chapters containing the journal articles form a coherent and integrated body of work, which focused on a single project or set of related questions or propositions. All journal articles form part of the sustained thesis with a coherent theme.
3. The study does not include work published prior to commencement of the candidature.
4. The number of articles included depending on the content and length of each article and take full account of the university's requirements for the degree as well as the one article already published or "ready-for-publication" expected for a master's degree in this discipline.
5. The thesis should be examined in the normal way and according to the normal requirements as set out by the "Guidelines for Examiners of Dissertations and Theses" (using form HDC 1.7).

The thesis consists of the following chapters which are concisely discussed as:

Chapter One: This chapter provides an overview of the research into the dormancy-breaking of three Asteraceae annuals of the winter-rainfall region of South Africa.

Chapter Two: This chapter provides a review of the effect of germination-ecology on the germination of three Asteracea annuals in the winter-rainfall region of South Africa.

Chapter Three: This chapter provides an investigation into the influence of light and temperature on the germination of three Asteraceae annuals in the winter-rainfall region of South Africa.

Chapter Four: This chapter provides an investigation into the influence of seed pre-treatments on the germination of three Asteraceae annuals in the winter-rainfall region of South Africa.

Chapter Five: This chapter provides an investigation into the influence of after-ripening on the germination of three Asteraceae annuals in the winter-rainfall region of South Africa.

Chapter Six: General discussion, conclusions and recommendations.
This chapter deals with the general discussion which connects the previous chapters and is followed by the conclusions of the study. Recommendations are made for further work; to introduce future research topics.

Chapter Seven: References

1.3 BACKGROUND TO THE RESEARCH PROBLEM

Arctotis hirsuta (Harv.) Beauvard, *Cotula duckittiae* (L. Bolus) K. Bremer & Humphries and *Oncosiphon suffruticosum* (L.) Källersjö are three winter annuals that horticulturists at the Kirstenbosch National Botanical Garden have been struggling to introduce as bedding plants from fresh seed collected in the wild. In addition to this, *Cotula duckittiae* is listed as a vulnerable species in a threatened ecosystem. This threat becomes greater every day. *Oncosiphon suffruticosum* is a much revered medicinal plant from the Western and Northern Cape. The economic potential of this species is stifled by poor germination.

There is limited research on the temperature or light requirements, the pre-treatment of seeds and the effects of after-ripening at different regimes on seed dormancy of the three winter annuals. This is a gap in the existing body of knowledge on the germination ecology of Asteraceae winter annuals in South Africa.

Investigating the effect of temperature, light, different seed pre-treatments and after-ripening under different regimes will contribute to the development of optimal propagation protocols of the three winter annuals.

1.4 STATEMENT OF THE RESEARCH PROBLEM

Investigation of specific temperature and light requirements, seed pre-treatments and the after-ripening conditions are likely to break the dormancy of three Asteraceae annuals *A. hirsuta*, *C. duckittiae* and *O. suffruticosum* of the winter rainfall region of South Africa. This research therefore seeks to address the following research question, “Do specific temperature and light

requirements, pre-treatments or after-ripening improve the breaking of seed dormancy of selected Asteraceae annuals of the South African winter-rainfall region?"

1.5 AIMS

The study aims to assess the effects of light, temperature, pre-germination treatments and after-ripening on breaking dormancy in order to improve germination of selected South African winter-rainfall annuals of the Asteraceae family.

1.6 HYPOTHESES

It is hypothesised that different ranges of temperature will have varying effects on the germination percentage and germination rate of selected South African winter-rainfall annuals of the Asteraceae family.

It is hypothesised that different ranges of light will have varying effects on the germination percentage and germination rate of selected South African winter-rainfall annuals of the Asteraceae family.

It is hypothesised that pre-treating seed through scarification will have varying effects on the germination percentage and germination rate of selected South African winter-rainfall annuals of the Asteraceae family when incubated at light and temperature conditions determined in specific objective 1 and 2.

It is hypothesised that pre-treating seed with gibberellic acid will have varying effects on the germination percentage of selected South African winter-rainfall annuals of the Asteraceae family when incubated at light and temperature conditions determined in specific objective 1 and 2.

It is hypothesised that different ranges of periods of after-ripening will have varying effects on the germination percentage of selected South African winter-rainfall annuals of the Asteraceae family when germinating under optimum temperature and light conditions from data gathered in specific objective 1 and 2.

It is hypothesised that different ranges of temperatures during after-ripening will have varying effects on the germination percentage of selected South African winter-rainfall annuals of the Asteraceae family when germinating under optimum temperature and light conditions from data gathered in specific objective 1 and 2.

It is hypothesised that results from hypotheses one to seven will assist in determining which method, treatment or combination thereof is optimal in breaking dormancy and improving germination of selected South African winter-rainfall annuals of the Asteraceae family.

1.7 OBJECTIVES

1.7.1 Main objective

To investigate the required germination conditions of selected South African Asteraceae annuals in order to establish an optimal propagation protocol.

1.7.2 Specific objectives

1.7.2.1 The purpose of this investigation is to determine the germination percentage of three South African winter-rainfall annuals of the Asteraceae family in response to various temperature ranges in order to establish an optimal propagation protocol.

1.7.2.2 The purpose of this investigation is to determine the germination percentage of three South African winter-rainfall annuals of the Asteraceae family in response to various light ranges in order to establish an optimal propagation protocol.

1.7.2.3 The purpose of this investigation is to determine the germination percentage of three South African winter-rainfall annuals of the Asteraceae family in response to scarification prior to germination tests at optimum temperature and light conditions from data gathered in specific objective 1 in order to establish an optimal propagation protocol.

1.7.2.4 The purpose of this investigation is to determine the germination percentage of three South African winter-rainfall annuals of the Asteraceae family in response to a pre-treatment with gibberellic acid prior to germination tests at optimum temperature and light conditions from data gathered in specific objective 1 and 2 in order to establish an optimal propagation protocol.

1.7.2.5 The purpose of this investigation is to determine the germination percentage of selected South African winter-rainfall annuals of the Asteraceae family at optimum temperature and light conditions gathered from data in specific objective 1 and 2 in response to various periods of after-ripening in order to establish an optimal propagation-protocol.

1.7.2.6 The purpose of this investigation is to determine the germination percentage of four South African winter-rainfall annuals of the Asteraceae family at optimum temperature and light

conditions gathered from data in specific objective 1 and 2 in response to after-ripening at various ranges of temperature in order to establish an optimal propagation-protocol.

1.7.2.7 The purpose of this investigation is to determine the optimal germination response of four South African winter-rainfall annuals of the Asteraceae family from data gathered in specific objectives 1–6 in order to establish an optical growing protocol by breaking dormancy and improving germination.

CHAPTER TWO

GERMINATION-ECOLOGY OF THREE ASTERACEAE ANNUALS- ARCTOTIS HIRSUTA, ONCOSIPHON SUFFRUTICOSUM AND COTULA DUCKITTIAE IN THE WINTER-RAINFALL REGION OF SOUTH AFRICA: A REVIEW

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2.1 ABSTRACT

Asteraceae annuals from South Africa's winter-rainfall region often exhibit poor germination and it is a challenge to establish a garden-display using fresh seed from the wild. *Arctotis hirsuta* (Harv.) Beauvard is a popular ornamental, *Oncosiphon suffruticosum* (L. Bolus) K. Bremer & Humphries is important in traditional medicine and the Red Listed Vulnerable *Cotula duckittiae* (L. Bolus) K. Bremer & Humphries has a Vulnerable (VU) status on the Red List of South African plants. *C. duckittiae* is barely hanging on in a few localities in severely threatened ecosystems due to continued pressure on land for housing developments and invasive aliens. The successful propagation of this species may enable it to increase numbers in the wild and at some botanical gardens from wildly harvested seed. This is also applicable to the medicinally valuable *O. suffruticosum* and the aesthetic *A. hirsuta*. At present there is no knowledge of *O. suffruticosum* being cultivated exclusively for its medicinal properties. The successful cultivation of this species may allow it to fulfil not only a more acute medicinal role in the society, but also in the economy to create precious job-opportunities. The potential to develop or improve certain breeding lines of *A. hirsuta* commercially, besides just normal wild forms of these species at the KNBG, are huge. This, in addition to the ongoing pressure exerted on wild populations of *C. duckittiae* warrants investigations into aspects of germination-ecology of this vulnerable species of the West Coast. This review focuses on measures required to ensure improved germination of the 3 species. These measures will include temperature- and light requirements and the responses to seed pre-germination treatments such as scarification, gibberellic acid and after-ripening.

Keywords: dormancy, Kirstenbosch NBG, red-listed, temperature, light, scarification, gibberellic acid, after-ripening

2.2 INTRODUCTION

Winter annuals are plants that complete a full life-cycle in one year. Germination occurs in the autumn, growing is completed in the winter; the plant flowers during the late winter or spring and sheds seed during spring or early summer (Pemadasa & Lovell, 1974; Magee, 2011). This group of plants normally grows rather quickly and after planting they are able to bring color into a part of the garden the soonest (Oliver, 2011).

The ornamental display of spring-annuals at the Kirstenbosch National Botanical Garden (KNBG) have been displayed since the Garden's inception in 1913 (Velembo, pers. comm. 2018). This collection is one of the core display-collections of the Garden, but also one with its own set of challenges. *Arctotis hirsuta*, *Cotula duckittiae* and *Oncosiphon suffruticosum* are three South African winter-rainfall annuals of the Asteraceae family. This family shows remarkable variation in growth form and general morphology because it occurs in so many different localities and habitats (Herman, 2004). This ability allows humans to benefit from the genetic diversity, as seeds offer the potential to introduce an exciting range of plants with new floral or habit forms (Toogood, 2011).

Despite the economic value it may offer, the full potential of South African Asteraceae species for gardening has not been fully utilized (Herman, 2004) as poor germination is limiting the cultivation of many Asteraceae species (Bunker, 1994). *A. hirsuta*, *C. duckittiae* and *O. suffruticosum* are three annual-species that horticulturists at the KNBG have been struggling to introduce as bedding plants from fresh wild-collected seed.

The regulation of seasonal changes in the temperature often ensures some changes in the level of seed dormancy (Fenner, 1985). The seed of wild plants display immense variability in their light-requirements. This important aspect has proven to contribute to the breaking of seed dormancy since a specific light-requirement influence the timing of germination in the wild (Fenner, 1985; Pons, 1992).

The pre-treament of seeds can result in the breaking of dormancy of several native herbaceous plants. Pretreatments with scarification and chemical treatments with gibberellic acid all resulted in breaking seed dormancy of winter annuals (Hartmann, *et al*., 1997). Gibberellic acid has proven to be the most effective of all the gibberellins in breaking seed dormancy of many Asteraceae winter annuals (Bunker, 1994).

The process of after-ripening has resulted in the breaking of seed dormancy of many arid zone species, including Asteraceae (Schütz *et al.,* 2002). After-ripening under ambient conditions is a slow process which can be detrimental to seed stockists, researchers or growers. Several winter annuals responded favourably to after-ripening at a high temperature (Peishi *et al.,* 1999). In Australia the breaking of dormancy in various Asteraceae winter annuals was

achieved through an increase in temperature and over different periods of after-ripening (Plummer *et al.,* 2006).

There is limited research on the temperature or light requirements, the pretreatment of seeds and the effects of after-ripening at different regimes on seed dormancy of the three species. This is a gap in the existing body of knowledge on the germination ecology of Asteraceae annuals of the winter-rainfall region in South Africa. Investigating the effect of temperature, light, different pre-treatments and after-ripening under different regimes will contribute to the development of optimal propagation protocols of the three annual-species.

2.2.1 The family *Asteraceae*

Asteraceae represents the largest plant family in the world (Herman, 2004, Pienaar & Smith, 2011; Koekemoer *et al.,* 2013.) The family consists of an estimated 1200 genera and 21 400 species worldwide. In southern Africa, where it is also the largest family, it is represented by 2481 species where this large concentration of Asteraceae-species can be found in the winter-rainfall region (Jackson, 1980; Koekemoer *et al.,* 2013). The family is known to be used for various purposes, which include as medicines, foods, herbs, cut flowers and ornamentals [bedding and potted plants] (Herman, 2004; Koekemoer *et al.,* 2013). The family is further represented by only a handful of trees (Weier *et al.,* 1974; Herman, 2004), various forms of shrubs, herbaceous perennials, biennials, annuals, and aquatics (Jackson, 1980; Herman, 2004). In the beautification of both the natural and artificial landscape, several species are crucial role players (Langkamp, 1987; Simpson, 2009; Pienaar & Smith, 2011; Koekemoer *et al.,* 2013). This is exemplified in some natural areas of South Africa, especially the West Coast and Namaqualand, where several annual species, including *A. hirsuta, O. suffruticosum* and *C. duckittiae* are just some of the species responsible for the kaleidoscope of colour during late winter to early spring (Magee, 2011; Oliver, 2011; Pienaar & Smith, 2011; Oliver, 2014). In some areas annuals also show immense potential for landscape-rehabilitation (Langkamp, 1987).

2.2.2 The genus *Arctotis*

This exclusively African genus of 63 species consist mainly of annuals, perennials and shrubs and is found throughout southern Africa towards Angola. The highest concentration of species is confined to Namaqualand and the Western and Eastern Cape of South Africa. Some of the species are easy to grow and make superb garden plants (Van der Walt, 2006; Notten, 2008).

2.2.3 The genus *Cotula*

This group of plants contain ± 55 species, mainly annuals and perennials, and is predominantly confined to southern Africa. Several other species can also be found in Tristan da Cunha,

Australia and South to Central America (McQuillan, 2010; Jakoet & Magee, 2014; Jakoet, 2020)

2.2.4 The genus *Oncosiphon*

The genus *Oncosiphon* is endemic to southern Africa and consists of 7 species which are mainly found in Namibia and the Western and Northern Cape (Leistner, 2000; Magee, 2011).

2.3 RESEARCH METHODOLOGY

- Librarian-requests: Harry Molteno Library at Kirstenbosch Research Centre for Journal-articles and books.
- Library: Kirstenbosch National Botanical Garden (KNBG) + Harry Molteno for books/articles
- Desktop-search (Internet/J-Stor etc.)
- KNBG Plant-recording database
- KNBG Annual-collection propagation records.
- 138 articles were reviewed
- 92 articles have been cited

2.4. ECOLOGY AND BOTANICAL DESCRIPTION OF THREE IMPORTANT ANNUAL ASTERACEAE SPECIES

2.4.1 *Arctotis hirsuta*

Arctotis hirsuta is a robust, spreading annual growing to 50 cm x 50 cm with orange, yellow or cream-coloured solitary flower heads. The habitat is sandy slopes and flats from Elandsbaai to the Agulhas Plain in the Western Cape (Joffe, 1993; Manning & Goldblatt, 2012). This species has a Least Concern (LC) conservation status (Victor & McKenzie, 2007; Raimondo *et al.,* 2009).

Figure 2.1*: Arctotis hirsuta* (Source: Roger Oliver)
1/10/2013: Kleinmond 34.3392417 S 19.0177145 E

2.4.2 *Cotula duckittiae*

C. duckittiae is a robust, soft hairy annual of 30 x 30 cm with brightly showy orange rays. It occurs on sandy coastal slopes from Yzerfontein to Bokbaai along the West Coast of South Africa (Manning & Goldblatt, 2012; Oliver, 2014). The conservation status of this species is Vulnerable (VU) due to threats from crop farming, invasive alien plants and urban encroachment (Helme, 2006; Raimondo *et al.,* 2009). According to the South African Red Data List this vulnerable species occurs in Hopefield Sand Fynbos which is in itself an endangered ecosystem. At present more than 40% of this system has already been transformed for farming practices such as grazing land and cultivation. In addition to this, invasive alien species like *Pinus, Acacia and Eucalyptus* species are encroaching and the area is shrinking. While a great portion of this Sand fynbos type is conserved in the West Coast National Park, the Hopefield and Jakkalsfontein Nature Reserves only protect an extra 2% (Raimondo *et al.,* 2009; Oliver, 2014).

Figure 2.2: *Cotula duckittiae* (Source: Roger Oliver)
30/9/2015: Kirstenbosch NBG 33.990574 S 18.43199,390 E

2.4.3 *Oncosiphon suffruticosum*

This is a robust erect aromatic annual herb with dense yellow corymb flower heads. It grows to 30 x 30 cm and is found on sandy flats and slopes from southern Namibia and Western Karoo to Gansbaai (Manning & Goldblatt, 2012). The seed is a yellowish-brown cypsela (Leistner, 2000). The conservation status of *O. suffruticosum* is Least Concern (LC) with no serious threats (Foden & Potter, 2005; Raimondo *et al.,* 2009).

Figure 2.3*: Oncosiphon suffruticosum* (Source: Anthony Magee)

2.5. WINTER ANNUALS AND THEIR VALUES AS MEDICINAL, ORNAMENTAL AND AGRICULTURAL CROPS

2.5.1 Medicinal values

2.5.1.1 *Arctotis hirsuta*

There is currently no medicinal use for this species recorded (Oliver, 2011.)

*2.*5.1.2 *Cotula duckittiae*

No form of medicinal usage, in modern medical science or traditional medicines, has been reported for the Bokbaai buttons (Van Wyk & Gericke, 2007; Oliver, 2014)

2.5.1.3 *Oncosiphon suffruticosum*

This species is extensively used for its medicinal value and was critical in several remedial applications used by the Khoi indigenous tribe of South Africa (Hulley *et al.,* 2010). A mixture of fresh plant material gets crushed with *Carpobrotus edulis* and *Exomis microphylla*, or *Ruta graveolens* to relieve convulsions in infants (Van Wyk & Gericke, 2007). A leaf-poultice is also applied topically and is effective in relieving the pain associated with inflammation and scorpion stings (Van Wyk *et al.,* 2009). Small quantities of juice from the leaves is often added to mother's milk and acts as gripe-water for infants and cramps. An infusion of the entire plant can also often be orally consumed to treat various ailments associated with stomach pain, intestinal worms, several types of fever and respiratory illnesses (Magee, 2011).

2.5.2 Ornamental values

2.5.2.1 *Arctotis hirsuta*

This species can be planted *en masse* in mixed plantings for the small or bigger garden. Flowering is from winter to early spring (Pienaar & Smith, 2011; Oliver, 2011)

2.5.2.2 *Cotula duckittiae*

The species is suited to mix plantings; works wonders as a seasonal border plant or mass plantings. In high rainfall areas, *C. duckittiae* is preferably displayed in well-drained areas like rockeries or along slopes. Plant in full sun (Oliver, 2014).

2.5.2.3 *Oncosiphon suffruticosum*

This widespread species favours disturbed areas where it covers large tracts. It thrives in coastal areas and due to it scraggly habit is more suited to mass plantings with other species. Optimum flowering is achieved in full sun (Magee, 2011).

2.5.3 Agricultural values

2.5.3.1 *Arctotis hirsuta*

There is no information available on such a role, although there are seed-stockists making seed available to the public (Silverhill Seeds, 2020). Breeding can contribute towards the development of superior forms (Capon, 2005).

2.5.3.2 *Cotula duckittiae*

No records exist of where *C. duckittiae* is being used as a crop plant (Oliver, 2014). Lakhdar (2018) indicated that *C. sericea* Del., a popular African annual, is being extensively utilized in traditional African medicine.

2.5.3.3 *Oncosiphon suffruticosum*

There is no record of this species being actively utilized as an agricultural crop. Its myriad functions as a medicinal plant makes it an excellent prospect as a seasonal crop in disturbed areas of South Africa's winter-rainfall region. Research from Australia, where this species is considered a weed, indicate that some of the undesirable factors of *O. suffruticosum* are that plant communities may lower production-yield of cereal crops, pasture plants are being displaced, is unpalatable to stock and contribute to the tainting of milk and meat products (Landscape South Australia, 2019).

2.6. GERMINATION ECOLOGY OF ANNUALS

2.6.1. Seed anatomy

A. hirsuta

The seed are tiny achenes (to 2.75 mm long) with 2 ovate-linear abaxial cavities, uniseriate trichomes, no coma is present, and very small pappus scales are present (Leistner, 2000; McKenzie *et al.,* 2005). The median abaxial wing exceeds that of the lateral abaxial wings. The cavities have a rounded to obtuse appearance at the apex and base. Lower section of the 3 abaxial wings are fused above the carpopodium-section. The base of the cypsela is constricted and short. The abaxial wings are transversely (barely to strongly) rugose. These wings can bear short, obtuse teeth when not entire. Dense presence of clavate trichomes on the smooth adaxial surface. Very few clavate trichomes can be found on the abaxial area. A uniseriate

pappus containing 8-9 rounded scales (0.15 mm in length) can be found on the achene. Those present on the adaxial surface are often longer (Mckenzie *et al.,* 2015)

C. duckittiae

Achenes in the genus *Cotula* generally appear oblong, obovoid to terete with variable (either 2-3 or 3-19) ribs. Myxogenic cells and/or ribs containing resin canals are features in several species. Achene in some species are either papillose or hairy. In *Cotula* the embryo sac development is monosporic (Oberprieler *et al*., 2007).

O. suffruticosum

The obovoid-shaped achenes are between 1-2 mm long, cut-off at the top to have a 3-angled appearance. Gland-dots can be found between the hairy ribs (Thompson, 2007; Landscape, South Australia, 2019). The fruits are non-myxogenic (no mucus formed when wet) (Oberprieler *et al*., 2007; Magee, 2011).

2.6.2 Seed colour

A. hirsuta

The wild-collected achenes are of a black colour. In seed harvested from cultivation plants, achenes with a yellowish-brown and darker brown colour can be distinguished (Pers. obs. 2020).

C. duckittiae

Achenes with two colours, light brown and yellowish-brown, are produced. The light brown achenes are produced in abundance ass oppose to the other colour. The yellowish- brown achenes appeared slightly winged (Pers. obs. 2020).

O. suffruticosum

This species only produced yellowish-brown achenes (Magee, 2011)

2.6.3 Seed weight (mg)

The impact of seed mass is of considerable importance for Asteraceae in unpredictable environments and this will impact on seedling characteristics such as emergence, timing of germination and seedling size (Venable & Brown, 1988). Research on some annuals with a mass less than 0.5 mg indicated that such seeds has a light-requirement for germination. Heavier seeds do not have the same requirement (Schütz *et al.,* 2000). Weight is pivotal to

the dispersal-method as some achene weigh less and is equipped with a pappus (Cruz-Mazo *et al.,* 2010).

Table 2.1: The determined weight of achenes for the three winter-rainfall annuals at KNBG.

Species	Weight (mg)
Arctotis hirsuta	0.2 mg
Cotula duckittiae	0.1 mg
Oncosiphon suffruticosum	0.07 mg

*2.*6.4 Seed coat thickness and structure

Arctotis hirsuta

Achenes of the genus *Arctotis* and other members of the Asteraceae clade Arctotidinae often all possesses lignified, yet well-developed pericarp which surrounds the embryo. This centrally developed pericarp is several cell layers thick. In areas along the ribs or ridges, some sections may even be thicker. The pericarp is also marked by the presence of oblong sclerified cells found within one or subepidermal layers (Oberprieler *et al*., 2007).

Cotula duckittiae & Oncosiphon suffruticosum

Both species form part of the clade Anthemideae and share several similarities at a genus-level. The species have a centripetally well-developed pericarp of a few layers thick. The pericarp also contain are oblong sclerified cells in one or two subepidermal layers. There is also a persistent testa epidermis present with varied reinforcement patterns (Oberprieler *et al*., 2009).

2.6.5 Seed dispersal

Arctotis hirsuta

In the section Arctotidinae, the annual species in the *Arctotis* clades all have tiny achenes in which the pappus can be either lost or highly reduced (McKenzie *et al.*, 2005). The lack of a pappus greatly reduces the chance of *A. hirsuta* to be dispersed by wind and achenes may mostly end up around or close to the mother plant (Van Rheede Van Oudtshoorn & Van Rooyen, 1999). The achene has a uniseriate pappus of 8 or 9 rounded scales to 0.15 mm long, which are often longer on the adaxial surface (McKenzie *et al.*, 2005).

Cotula duckittiae

Jakoet & Magee (2014) postulated that the presence of an apically inflated peduncle in the genus *Cotula* may be significant in seed dispersal through a shaking-process and thereby

dispersing seed in the wind. During fruiting this inflation is at its largest. This phenomenon is present in all *Cotula*-species.

Oncosiphon suffruticosum

At the end of the flowering season, flowering stems are being broken off and seed gets spread through wind-movement. Some seeds also remain within the flower head. A tiny small crown of white scales (1 mm in length) represent the pappus (Magee, 2011; Herbiguide, 2019).

2.6.6 Seedling emergence

Arctotis hirsuta

No records could be found for plants grown from seed harvested from cultivated or wild plants.

Cotula duckittiae

Seed harvested from cultivated plants in the Annual-collection at KNBG took 8 days to germinate when sown at the beginning of March. Seeds were sown in open conditions (outside) without any temperature- or light-control.

Oncosiphon suffruticosum

No records could be found for plants grown from seed harvested from cultivated or wild plants.

2.6.7 Conditions affecting seed germination

2.6.7.1 *Sowing depth*

The depth at which seed are buried in the soil can influence the germination and germination-rate (Anderson, 1996). Most seedlings of *Parthenium hysterophus* only emerged in a depth of less than 5mm. No emergence was observed at depths exceeding 5 mm (Tamado *et al.,* 2002). During times when the seeds *A. hirsuta* and *C. duckittiae* were covered with a soil-layer exceeding 5mm during sowing, germination exceeded the 6 & 8 days mentioned above under seedling emergence (Pers. obs. 2020). No records are available for *O. suffruticosum.*

2.6.7.2 *Temperature*

Temperature is integral to the success or failure of plant establishment and impacts on germination and the dormancy status of the seed (Probert, 1992; Bewley & Black, 1994). The seeds of annual species are conditioned to germinate at a specific period of the year. The regulation of seasonal changes in the temperature often ensures some changes in the level of dormancy. The breaking of dormancy is determined through fluctuations of the temperature range for germination (Fenner, 1985).

The ability to germinate over a wide spectrum of temperatures is a common characteristic of many species of *Asteraceae* (Martinez-Garcia *et al.,* 2012). Seeds of winter annuals are normally in a state of conditional dormancy following dispersal and germination is restricted to a limited range of low temperatures (Baskin & Baskin, 1976; Fenner, 1985). The majority of seeds, including some winter annuals, from the Namaqualand region of South Africa germinated best at temperatures between 12 ˚C–22 ˚C (Beneke *et al.,* 1993; Visser, 1993). The optimum germination temperature for *Arctotis fastuosa* varied from other species of this region and was achieved at 32 ˚C (Beneke *et al.*, 1993). The annual *Senecio coincyi* achieved 90% germination between 15 ˚C – 30 ˚C. This germination rate decreased to less than 20% at a temperature below 10 ˚C (Martinez-Garcia *et al*., 2012). The results from several researchers indicate that there definitely exists a specific temperature at which the impact of dormancy can be reduced, and optimum germination can be obtained. The need to investigate the role of temperature on the dormancy and germination of these three species will therefore be crucial if dormancy wants to be overcome and the species successfully grown at KNBG.

2.6.7.3 *Light*

Among cultivated plants there is very little evidence for light as a factor influencing germination and seed usually germinate equally well in the dark and in the light (Fenner, 1985). In contrast, among wild plants much variability in the behaviour toward light in their requirement for light is observed and light is a major factor in the breaking of dormancy as the response of seeds to light is a critical control-mechanism in the timing of germination in the wild (Fenner, 1985; Pons, 1992).

Seeds may be divided into those which germinate only in the dark, seed which germinate only in continuous light, seed which germinate after being given brief illumination and seed which are indifferent to the presence or absence of light during germination. Under natural conditions seeds may be shed so as to fall on the soil or enter the soil or be covered by leaf litter, thus creating different conditions of light during germination.

Exposure to light, fluctuations in temperature, or combinations of these factors may be needed to relieve residual and induced dormancy at times of low dormancy (Fenner, 1985). The seed of some species may require light at one temperature, but no light at another. *Lactuca sativa* is an example of a species in which light is not a requirement for germination at low temperatures. However, light is required for germination at higher temperatures (Pons, 1992). Research indicates that optimum germination for South African winter annuals *Felicia australis*, *Ursinia anthemoides, Dimorphotheca sinuata* and *Arctotis fastuosa* occurs in light, whereas *Dimorphotheca polyptera* germinates best in dark (Beneke *et al*., 1993; Visser, 1993; Schütz

et al., 2002). Very few Asteraceae species germinate equally well in both light and dark conditions. In species where the light-requirements could be determined and met with certainty, the dormancy-level could be reduced and the germination-percentages increased. Therefore, in order for dormancy to be overcome, determining the light-requirements of the three wild species will be a crucial step towards the successful propagation of these annuals.

2.6.7.4 *Scarification of seeds*

Seed coats can also influence a physical restraint on the maturing embryo. However, should the amount of thrust developed through inhibition and growth be inadequate, the embryo will not puncture the seed coat resulting in no germination. This dormancy-type can be broken by some form of an abrasion or decay of the hard seed coat (Mayer & Poljakoff-Mayber, 1982). Research indicates that scarification through puncturing the pericarp and testa is a successful technique that results in the breaking of seed dormancy of several Asteraceae-species (Visser, 1993; Bunker, 1994). Scarified seeds of *Foveolina albida* and *Oncosiphon grandiflorum* showed a higher uptake of water compared to seeds not scarified. The low uptake of water in the seeds suggests a water-impermeable seed coat that results in seed coat dormancy (Visser, 1993). The seeds of annuals are adapted to not germinate at once through a combination of aspects such as a restrictive seed coat or a physical restraint on the maturing embryo by the seed coat. The soul aim being to ensure that a good proportion of seeds remain in the soil as a seed bank (Venable & Lawlor, 1980). The gradual degradation of the seed coat may also involve a lengthy period of time. However, scarification by puncturing the seed coat is a method employed very successfully on several annuals-species in the Asteraceae-family. This could prove to be a very efficient method to break dormancy of the three annuals and result in the successful germination and mass production of the species at KNBG.

2.6.7.5 *Application of a growth hormone such as Gibberellic acid*

The process of germination often involves plant hormones and the presence of gibberellins, specifically gibberellic acid (GA_3), in developing seeds is instrumental in facilitating germination and the breaking of dormancy in many species (Hartman *et al.,* 1997). In the Asteraceae genus *Lactuca* some species encounter challenges with germination since micropylar endosperm and testa tissue prevent the protrusion of the radicle. The removal of the micropylar and testa tissue enables protusion of the radicle and results in improved germination. However, in some species pretreating with GA_3 can result in enervating the micropylar endosperm through limiting the restrictiveness of the seed coverings and therefore break dormancy (Bradbeer, 1988; Kucera *et al*., 2005). Several other workers have indicated experimental successes in germinating Asteraceae species with GA_3 (Van Auken, 2001; Cochrane & Probert, 2006; Ha, 2014). Pretreatment with a combination of scarification followed by a soaking in GA_3 achieves optimum germination some Australian annuals *species* (Bunker, 1994). Various concentrations

of GA_3 can be applied and this varies according to species. Germination has improved in several species by soaking in a concentration of 500 ppm (Bunker, 1994; Abdalla & McKelvie, 1980; Puttha *et al*., 2014). In some species a concentration of 300 ppm can result in improved germination (Plummer *et al*., 2006; Farimani *et al.,* 2011.) There is clear evidence of the proven ability of GA_3 to optimize germination through a reduction of the dormancy-levels of various Asteraceae-species. This warrant an investigation into the soaking of seeds in GA_3 using different strengths over different periods to determine whether it can improve germination of the three annual species.

2.6.7.6 *After-ripening treatment*

Winter annuals may cover various open spaces that experience unfavourable conditions from late spring to early autumn. A survival mechanism for many winter annual species not adapted to the seasonally arid conditions is to become dormant and survive this unfavourable period as seed (Baskin & Baskin, 1976; Le Roux & Schelpe, 1997)**.** Research indicates that after-ripening plays a vital role in the break of seed dormancy of many arid zone species (Visser, 1993; Schütz *et al.,* 2002; Commander, 2008), including Asteraceae (Forsyth & Brown, 1982; Schütz *et al*., 2002; Plummer *et al.,* 2006; Aleman *et al.,* 2011; Qaderi *et al.,* 2012). Winter annuals may require after-ripening which involves a high temperature during summer (Baskin and Baskin, 1976). The literature suggests further that conditions of high temperature and low humidity during after-ripening is required to break seed dormancy (Mott, 1972, Peishi *et al*., 1999). Species from Mediterranean climates after-ripen better at high temperatures compared to that of low temperatures (Mott, 1972, Bell, 1999; Commander, 2008). However, research has shown that after-ripening of some species of Asteraceae can be achieved at high and low temperatures (Plummer *et al*, 2006; Aleman *et al*., 2011). The after-ripening period is species-dependant (Aleman *et al.,* 2011). In two everlasting annuals from Western Australia, *Schoenia filifolia* subsp. *filifolia* and *Rhodanthe cholorocephala,* the germination rate exceeded 85% after 3 months of after-ripening. This germination was obtained from seeds stored at 25 ˚C, 30 ˚C and 40 ˚C. *R. chorochephala* maintained a germination rate of more than 90% after storing for three months at temperatures of 15 ˚C, 25 ˚C, 30 ˚C, 40 ˚C and 55 ˚C. Research on winter-annuals from Mediterranean-areas or semi-arid areas indicate that the exposure of seed to after-ripening may reduce dormancy in some species. This form of pre-treatment may provide important information on how the species respond to after-ripening and whether it should be considered the most effective pre-treatment method to overcome dormancy in some of the species.

2.6.7.7 *Changes in climate parameters*

The winter-rainfall regions of both the Fynbos and Succulent Karoo biome are predominantly subjected to Mediterranean climatic conditions (Mucina *et al.,* 2006; Rebelo *et al.,* 2006; Manning & Goldblatt, 2012; Van Deventer *et al.,* 2013). According to Dallman (1988), a section of the Western Cape Province in South Africa, can be regarded as forming part of a Mediterranean ecosystem. Climates in these particular regions are driven by mild, wet winters and followed by dry, hot summers (Dallman, 1988; Rundel & Cowling, 2013). The largest part of the Fynbos-biome receives winter rainfall in the west and further east rainfall peaks are experienced in autumn and spring (Manning & Paterson-Jones, 2004; Manning & Goldblatt, 2012). Annuals can be regarded as poorly represented in the fynbos as the majority of this plant-type are particularly confined to areas of strandveld, sand fynbos, renosterveld-types and asteraceous fynbos (Rebelo *et al.,* 2006). The Succulent Karoo biome can be regarded as a desert-like region found in the Western and Northern Cape of South Africa. Rainfall is mainly in winter and less than 200 mm per year and the summer is a period of extreme aridity (Desmet, 2007; Esler *et al.,* 2015). The presence of the Atlantic Ocean contributes significantly to cooler conditions during the sweltering summer months through the formation of moisture that eventually reach the land as either fog or a light rain (Cowling & Pierce, 1999; Manning & Paterson-Jones, 2004; Mucina *et al.,* 2006). Annuals, represented by 390 species, are responsible for the majestic late winter and spring displays (Cowling & Pierce, 1999).

2.6.7.8 *Seed dormancy*

Seed dispersal in winter annuals during unfavorable dry conditions of summer may result in the postponement of germination until the more favorable conditions of autumn. This process of suspending germination is termed dormancy and may affect the seed embryo, seed coat or seed covering (Baskin & Baskin, 2001; Schütz *et al.,* 2002). Dormancy is a common phenomenon in the daisy family, in particular in areas experiencing a Mediterranean and arid or semi-arid climate (Bell, 1999). Plant species exhibit diverse forms of seed dormancy which can be classed into primary and secondary dormancy. Three varieties of primary dormancy can be distinguished namely exogenous, endogenous and combinational (Geneve, 2013; Tiwari *et al.,* 2016). Endogenous dormancy comprises of factors inside the embryo and exogenous dormancy relates to dormancy outside the embryo. Combinational dormancy consists of both endogenous and exogenous dormancies. Secondary dormancy is the result of germination failure in non-dormant seeds due to *unsuitable* germination-conditions (Hartmann *et al.,* 1997).

Some Asteraceae-genera which display exogenous dormancy include *Echinacea*, *Koelpinia* and *Epilasia* species. This form of dormancy is the result of external factors and excludes any

limitation imposed by the embryo (Kosma *et al.,* 2014). The most frequent embodiment of this dormancy-type involves seed with hard coats that become suberized and impenetrable to water (Kosma *et al.,* 2014; Tiwari *et al.,* 2016). Alterations to seed coverings affect the outer integument layer of the seed and may result in the seed coat becoming hard, fibrous or mucilaginous during dehydration and ripening. In Asteraceae the semipermeable seed coat fuses with remnant layers of the endosperm. The layers of the integument, remnants of the endosperm and nucellus maintain a physiological activity during ripening and for a period after dispersal. All these active layers contribute towards limited restriction of aeration and inhibitor movement (Hartmann *et al.,* 1997).

Dormancy limits the introduction of various species into horticulture (Cochrane & Probert, 2006). However, it is possible to induce germination of dormant seed by means of a single or multiple action (Bell *et al.,* 1993; Bell, 1999; Cochrane & Probert, 2006). Fluctuations in temperature and light can contribute towards the breaking of seed dormancy (Fenner, 1985). Seed dormancy of various Asteraceae-species can also be broken by after-ripening (Forsyth & Brown, 1982; Plummer *et al.,* 2006). According to Bunker (1994) dormancy in several Asteraceae genera can be overcome by individual or combinational applications of scarification and GA_3.

2.7. POTENTIAL STRATEGIES TO INCREASE GERMINATION

2.7.1 The importance of annual displays in botanical gardens and the need to introduce measures to improve germination

Annual displays can fulfil various functions in a botanical garden. They can be the centre-stage and have garden-beds just exclusively dedicated to it to enable visitors to marvel at the combinations of colour, leaf-textures or shape of the flowers. Annuals can also play an auxiliary function where it can be used to fill in gaps where perennials, shrubs or mesembs are slow-growing (Oliver, 2011). This group of plants can also be exhibited as part of the medicinal or food crops (Magee, 2011; Van Jaarsveld, 2019). According to Adams (2014) the KNBG has been instrumental in promoting and encouraging gardening with indigenous plants.

In order to maintain the plants on display at a botanical garden, it is of critical importance to have a sound understanding of sustainable horticultural practices (Krishan & Novy, 2016). This is also true about species that has never been grown before or those that encounter germination-challenges. This role is expected to increase given the threats biodiversity is facing in dwindling ecosystems and the impact of climate change. Botanical gardens will become vital catalysts for the introduction of new food and medicinal crops as well as

germplasm of ornamentals (Krishan & Novy, 2016). This role can be further extended to ex situ conservation and research on climate change aspects (Donaldson, 2009).

Studies into the propagation of indigenous species and the transplanting of some species contributed significantly to the successful introduction into the wild. Results from such studies is of critical importance as it enabled horticulturists from KNBG to propagate, cultivate and restore some plant species successfully (Adams, 2014). The inability by seed of the three species to germinate will not enable it to be showcased in the KNBG display of Annuals. Like several other species, the inability to grow plants successfully will compromise the ability to promote the use of indigenous species in gardening. This will also impact on the variety or the diversity of species that can be utilized in an annuals-display. From a conservation-perspective, not being able to grow some of the species successfully may also place additional pressure on red-listed species due to a lack of understanding of the germination-ecology of the species. A knowledge of not only how to successfully propagate, but also the implementation of measures to sustain the species going forward, will be critical in order for this species to form part of the annuals-display and to be grown for economic or conservation-initiatives.

2.7.2 The development of a management protocol

Seed harvesting

Keep records of the state of the mother plants during the harvesting process. Seeds can also be stored separately. After several years of seed-viability and germination-tests, and also taking the prevailing climatic conditions at KNBG into consideration, standards for seed harvesting can be determined. Species must be observed closely with onset of the seed-maturation process. Monitor whether seeds collected from different localities, exhibit different levels of dormancy (Gutterman & Heydecker, 1973; Anderson & Milberg, 1998; Meyer, 2006)

Seed-type

Collect all seed, irrespective of type or colour of the achenes. During the cleaning-process, seed must be separated to keep better track of the seed-viability of the different seed-type and its dormancy-level or lack thereof (Carpenter & Ostmark, 1992; Meyer, 2006; Cruz-Maso *et al.,* 2010)

Timing of sowing

This can be dictated by the research-results, although once dormancy has been overcome, some seeds might then be able to germinate over a wider germination-window of temperatures. The most critical aspect during sowing for winter-annuals, apart from the

initiation of germination during a period of lower temperatures, will then be the regular availability of moisture (Wheeler *et al.,* 2000).

Climatic conditions

Measure the soil-moisture encountered by the mother plants and adjust irrigation where deemed necessary. When there are significant differences, do germination-tests (Fenner, 1991; Meyer, 2006).

Pre-germination treatments

In some cases, where dormancy has totally been overcome, there might be no requirement for any of the seeds to be subjected to a pre-germination treatment going forward (Fenner, 1985).

Seed-covering methodology

The seed of several annual-species are quite light and small. In order to prevent them from blowing away, especially when sown outdoor, seeds are covered with either coarse river sand, fine bark or compost. If this cover-layer impedes germination due to a light requirement, another possibility could be to sow seed under a roofed-structure where seed can just be scattered on top of the sowing-medium without any cover (Oliver, 2011).

After care

This can involve the storing of seed under controlled or ambient conditions. Regular viability tests can be conducted to test or determine the ideal storage-requirement per species (Carpenter & Ostmark, 1992; Meyer, 2006).

2.7.3 Mitigation and improved adaption strategies

Given the uncertainty around climate change, it cannot be said with certainty which species will thrive at KNBG and which not. This will not only provide the opportunity to determine germination requirements, but necessitate such action, to find and ultimately, if possible make a selection (using species from different localities) of what species will adapt to form part of the ornamental-display at KNBG. Where this is not possible, germination tests- can still be performed on other winter-annuals to ensure their germination- and cultivation requirements are being met (Meyer, 2006). Trials can be done in artificial conditions under roof where there is the need to produce or bulk up on seed. This will also enable the restoration of threatened species to be restored to fragmented habitats or form part as a collective group of plants used in restoration. Such species can also be grown at other National Botanical Gardens where climatic conditions are more suitable to be displayed as ornamentals (Donaldson, 2009)

The time of harvesting will have to be investigated and recorded in order to ensure the best seed is being collected (Aronson *et al.,* 1992; Anderson & Milberg, 1998; Mattana *et al.,* 2010). Choices will have to be made as whether to wild-collected seed will be collected in periods of extreme drought (Aronson *et al.,* 1992). From a research perspective though, it may be good to collect drought-subjected seeds to investigate the impact of drought on the germination, the seed-anatomy, how and what (possible) pre-germination treatment or combinations thereof will influence dormancy. Climatic conditions during the seed-maturation will have to be strictly monitored, once a collection of the three species have been established (Mott, 1973). This is to minimize the presence of dormancy during germination of certain years (Meyer, 2006). The state of the mother plant during seed-harvesting is another critical element (Anderson & Milberg, 1998). A severe degree of dormancy can be expected in senescent mother plants. Where possible, regular investigations, need to be made to ascertain the conditions of the mother plant during the growing season (Koller, 1957).

2.7.4 Synergies in adaption and mitigation

Once incorporated into the existing Annuals Living Collection of the KNBG, these three species and others in the future, can be used in climate change studies to monitor phenology of winter-annuals. This ongoing research can provide a platform from which informed decisions can be made to adjust management strategies (Angert *et al*., 2007).

Increased pressure from climate change, invasive aliens and pressure on the lack of land will put pressure on areas where plant sub-populations are confined to such habitats (Cowling *et al.,* 2003; Rebelo *et al.,* 2006). Overcoming dormancy in red-listed species such as *Cotula duckittiae* can potentially enable material to be restored back into the wild. Given the threats it faces in its ever shrinking habitat, restoring plants (best method must still be determined) could not only result in increasing some of the sub-populations, but the species may find it in a better position to attract more pollinators once it habitat gets increased. Small populations of plants may experience limited seed-set due to a lack of pollinators. Through restoration the population-numbers can be increased by enabling more seed set through higher pollinator numbers (Mattana *et al.,* 2010).

Seed can also be exchanged to other local National Botanical Gardens and other botanical gardens displaying Mediterranean garden-themes (Donaldson, 2009).

Records need to be kept of the different environmental conditions and state of the mother plants when seed is harvested. In years of low precipitation, it is possible to irrigate plants when seeds are maturing on the mother plant. Research can also be initiated to determine the ideal combination of temperature and moisture needed to produce seeds with a low level of dormancy per species (Mott, 1973)

Seed-viability needs to be done regularly to determine under which conditions and for how long seeds can be stored. This is applicable to both central and peripheral seeds (Meyer, 2006). It is therefore imperative to constantly ensure a balance between optimum germination and management strategies.

2.8 CONCLUSION

Asteraceae is a cosmopolitan, extremely diverse plant family and known for several reasons such as garden-ornamentals, as well as food and medicinal plants. The majority of South Africa's rural population depends on traditional medicine as their primary source of healthcare. In southern Africa more than 4000 species constitute traditional medicinal species, with more than 3000 of these utilized almost constantly. In a country like South Africa, scourged by a high unemployment rate, the cultivation of a species like *O. suffruticosum* can be beneficial two-fold through becoming more pronounced in its use as a traditional medicine, but also at the same time potentially provide much-needed economic-relief opportunities to communities ravaged by poverty. The growing of *A. hirsuta* from freshly harvested seed may enhance the value of this species as an ornamental. The successful propagation of *C. duckittiae* may lead to an increase in the size of wild populations through restoration-efforts and in the process also contribute towards popularizing the species as a garden-ornamental. Therefore, in order to pre-empt the possible extinction of *C. duckiittiae,* improve the medicinal or economic value of *O. suffruticosum* and the ornamental stature and breeding lines for *A. hirsuta,* urgent research is required to investigate the germination-ecology and the development of propagation-protocols for these species.

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CHAPTER THREE

THE INFLUENCE OF TEMPERATURE AND LIGHT ON THE GERMINATION OF THREE SELECTED ASTERACEAE ANNUALS FROM THE WINTER-RAINFALL REGION OF SOUTH AFRICA

THE INFLUENCE OF TEMPERATURE AND LIGHT ON THE GERMINATION OF THREE SELECTED ASTERACEAE ANNUALS FROM THE WINTER-RAINFALL REGION OF SOUTH AFRICA

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3.1 ABSTRACT

Arctotis hirsuta (Harv.) Beauvard*, Cotula duckittiae* (L. Bolus) K. Bremer & Humphries and *Oncosiphon suffruticosum* (L.) Källersjö are three Asteraceae annuals from South Africa's winter-rainfall region. *A. hirsuta* is a popular garden ornamental from the Western and Northern Cape, the red-data listed Vulnerable *C. duckittiae* is limited to the Bokbaai/ Darling-area along the West Coast in the Western Cape and *O. suffruticosum* is a very popular traditional medicinal plant from the Northern and Western Cape. The three selected annuals are species which present germination-challenges and as such could not be introduced into the Kirstenbosch National Botanical Garden's annuals-display when propagated from fresh wild-collected seed. The purpose of this study was to investigate methods to overcome dormancy of the three selected species. Seed were collected from the wild, Hantam National Botanical Garden or sourced from Silverhill Seeds. Seven treatments were evaluated with 4 sample replicates. The first experiments evaluated the temperature and light required per species. Treatments consisted of inducing germination at 5 different temperatures namely 7 °C, 12 °C, 17 °C, 22 °C, 27 °C and alternate temperatures at 12 °C/22 °C and 17 °C/27 °C in conditions of light, darkness and alternate lightning (12 hours each of light and darkness). Results found that germination of *A. hirsuta* achenes is the result of a temperature and light interaction for temperatures between the 22 °C and 27 °C in the light. The achenes of *C. duckittiae* (2016) recorded germination ≥ 60% for the temperature-ranges 7 °C - 17 °C and the alternating temperature of 12 °C/ 22 °C. *C. duckittiae* (collection year 2017) achieved optimum germination in the dark at 22 °C, but negligible germination in all the other treatment combinations. In *O. suffruticosum* (collection year 2016) the best germination was achieved at 12 °C in the light, however this did not differ very much from 17° C, (in light), 22° C (in light) and 7° C (in dark). *O. suffruticosum* (collection year 2017) recorded very low germination over all the treatments.

Key words: dormancy, germination-challenges, ornamental, red-listed, medicinal, annuals-display

3.2 INTRODUCTION

3.2.1 *Arctotis hirsuta* (Harv.) Beauvard

Arctotis hirsuta (Harv.) Beauvard is an annual growing to 50 cm x 50 cm with flowers ranging from orange, yellow or cream-coloured solitary flower heads. The habitat is sandy slopes and flats in the Northern and Western Cape (Joffe, 1993; Pienaar & Smith, 2011; Manning & Goldblatt, 2012).

3.2.2 *Cotula duckittiae* (L. Bolus) K. Bremer & Humphries

Cotula duckittiae (L. Bolus) K. Bremer & Humphries is a soft hairy annual of 30 x 30 cm with brightly showy orange rays. It occurs on sandy coastal slopes along the West Coast of South Africa (Manning & Goldblatt, 2012; Oliver, 2014).

3.2.3 *Oncosiphon suffruticosum* (L.) Källersjö

Oncosiphon suffruticosum (L.) Källersjö is an aromatic annual herb with dense yellow corymb flower heads. It grows to 30 x 30 cm and is found on sandy flats and slopes from southern Namibia to the Western Cape (Manning & Goldblatt, 2012).

3.2.4 Dormancy and germination

The importance of breaking seed dormancy is instrumental in realizing the horticultural potential of various native species around the world (Wrigley and Fagg, 1986; Bunker, 1994) as sound knowledge of local seed germination ecology also has major implications to forestry, horticulture and conservation practices (Dyer, 1995; Commander, 2008). The germination-process is essential in contributing towards species-survival and is one of the primary physiological responses regulated by environmental conditions experienced in the habitat of the species. Therefore, germination will not take place if dormancy is not overcome or under unfavorable environmental conditions that may result in a low seed production yield (Mayer & Poljakoff-Mayber, 1982; Vleeshouwers, *et al.,* 1995; Voesenek & Blom, 1996; Le Roux & Schelpe, 1997).

Seed dispersal in winter annuals during unfavourable dry conditions of summer may result in the postponement of germination until the more favourable conditions of autumn. This process of suspending germination is termed dormancy and may affect the seed embryo, seed coat or seed covering (Baskin & Baskin, 2001; Schütz *et al.,* 2002). Dormancy is a common phenomenon in the daisy family, in areas experiencing a Mediterranean and arid or semi-arid climate (Le Roux & Schelpe, 1997; Bell *et al.,* 1993; Bell, 1999).

3.2.5 The temperature-requirement for germination

Temperature is one of the most important influences on seed germination (Mott, 1972; Zarghani, *et al.,* 2014) through regulating the timing, rate and percentage of germination, overcoming dormancy as well as the germination process (Bouwmeester & Karssen, 1992). The responses by seed to temperature is critical as this element facilitate the most suitable germination conditions for seedling establishment (Probert, 2000; Taab & Anderson, 2009; Aleman *et al.,* 2011). In natural environments the seeds of winter annuals are normally in a state of conditional dormancy following dispersal during the warmer spring and summer months (Baskin & Baskin, 1976; Fenner, 1985). High summer temperatures often enable the seed of winter annuals to break dormancy (Baskin & Baskin, 1976; Bouwmeester & Karssen, 1992; Probert, 2000). Winter annuals from semi-arid to arid regions are often likely to encounter near-death conditions during the vegetative stage (Clauss & Venable, 2000) as rain may arrive too late, are too little or none are received at all (Van Rheede Van Oudtshoorn & Van Rooyen, 1999). In such areas subjected to seasonal climates, the most critical environmental factor is the amount of rain received and its distribution range (Boeken & Gutterman, 1990; Gutterman, 1994; Gutterman & Gozlan, 1998).

Temperature adaptation to ensure germination occur during the most favourable environmental conditions, may result in enhanced germination during the winter-rainfall period (Mott, 1972; Lodge, 1981; Lodge & Whalley, 1981; Fenner & Thompson, 2005; Taab & Anderson, 2009). The seeds of annual species are conditioned to germinate at a specific period of the year (Fenner, 1985; Footitt, *et al.,* 2014) when there is an overlap of field temperature and temperature range. This is due to the fact that soil-water is in good supply and an overall reduction of evaporation due to cool conditions (Baskin & Baskin, 1976; Baskin *et al.,* 1986; Bellairs & Bell, 1990; Bell *et al.,* 1993; Gutterman & Gozlan, 1998).

In Asteraceae there are low optima (12°-20 °C) recorded for several winter annual species. These temperature conditions are more in line with conditions during the winter rainfall season (Mott, 1972; Willis & Grove, 1991; Gutterman, 1994). However, the ability to germinate over a wide spectrum of temperatures is a common characteristic of many species of Asteraceae (Zheng *et al.,* 2005; Martinez-Garcia *et al.,* 2012). In many species this enable germination to be stretched over a longer period (Martinez-Garcia *et al.,* 2012). Overcoming dormancy is determined through fluctuations of the temperature range for germination (Vegis, 1964; Fenner, 1985; Zheng *et al.,* 2005).

3.2.6 The light-requirement for germination

Light is an integral environmental factor to seed germination (Pons, 1992; Scopel *et al.,* 1994; Botta *et al.,* 1998; Toyamashu *et al.,* 1998; Ballare & Casal, 2000; Probert, 2000) and in

conjunction with temperature triggers the germination process and regulate the germination percentage (Bewley & Black, 1994, Casal & Sanchez; 1998; Qaderi *et al.,* 2012).

Among cultivated plants there is very little evidence for light as a factor influencing germination and seed usually germinate equally well in the dark and in the light (Fenner, 1985). In contrast, among wild plants much variability in the behaviour toward light in their requirement for light is observed and light is a major factor in the breaking of dormancy as the response of seeds to light is a critical control-mechanism in the timing of germination in the wild (Fenner, 1985; Pons, 1992; Jackson, 2009).

Seeds may be divided into those which germinate only in the dark, seed which germinate only in continuous light, seed which germinate after being given brief illumination and seed which are indifferent to the presence or absence of light during germination. Under natural conditions seeds may be shed so as to fall on the soil or enter the soil or be covered by leaf litter, thus creating different conditions of light intensity during germination (Fenner, 1985).

Exposure to light, fluctuations in temperature, or combinations of these factors may be needed to relieve residual and induced dormancy at times of low dormancy (Fenner, 1985). The seed of some species may require light at one temperature, but no light at another. Light is not a requirement for germination at low temperatures for the species *Lactuca sativa*. However, light is required for germination at higher temperatures (Pons, 1992).

Some annuals exhibit higher germination in darkness than in light, for example the two daisies *Ursinia anthemoides* and *Podotheca gnaphaloides* (Schütz *et al.,* 2002). Similar results have been obtained with the winter annual *Dimorphotheca polyptera* germinates (Beneke *et al.,* 1993; Visser, 1993).

It is generally regarded that a light requirement prohibits germination of seeds buried too deep for seedlings to emerge (Pons, 1992). Grime *et al.* (1981) studied 271 species from a range of families and habitats and found that the presence of light promoted germination in most species, including the majority of Asteraceae-species. This light stimulation of species within the Asteraceae-family was confirmed by several authors (Mott, 1972; Atwater, 1980; Willis & Grove, 1991; Beneke *et al.,* 1993; Visser, 1993; Plummer & Bell, 1995; De Clavijo, 2005). The germination of the winter-annuals *Rhodanthe humboldiana* and the *Schoenia* species revealed that most seed germinated equally well in light or dark, indicating an absence of light-imposed dormancy (Mott, 1972; Aleman *et al.,* 2011.)

The purpose of the study was therefore to determine what influence temperature and light has on the germination of wild-collected seed of the three species. Results from this study can contribute to the body of knowledge regarding propagation of *A. hirsuta*, *C. duckittiae* and *O. suffruticosum*.

3.3 MATERIAL AND METHODS

3.3.1 Growth chamber experiment

The experiments were conducted from February to May 2019 at the growth chambers of the tissue culture facilities at the Kirstenbosch Research Centre of the South African National Biodiversity Institute (SANBI) at the Kirstenbosch National Botanical Garden (KNBG), Newlands, Cape Town.

Seed of the three species were incubated in a GC-550R growth chamber with 4000-8000 LUX illumination. This chamber is equipped with a timer-device that allows regulation of the temperature and light required over a 24-hour period.

3.3.2 Seed preparation

Achenes of the various species have been collected in the winter-rainfall areas of the Western Cape and Northern Cape Provinces of South Africa by the researcher or sourced from Silverhill Seeds during 2016 and 2017.

Achenes of all three species have been collected during October- November 2016. *A. hirsuta* was collected at the Hantam National Botanical Garden. *O. suffruticosum* and *C. duckittiae* were sourced from Silverhill Seeds. The seed of *C. duckittiae* (L. Bolus) K. Bremer & Humphries and *O. suffruticosum* L.) Källersjö were collected by the researcher during October and November 2017, respectively.

Following collection, seeds were stored in paper envelopes at 15 °C and 15% relative humidity (RH) in a cold room of the seed room at KNBG until the start of the experiments.

Table 3.1: The three winter-rainfall species according to the year collected**.**

Species	Collection-year
Arctotis hirsuta	2016
Cotula duckittiae	2016
Cotula duckittiae	2017
Oncosiphon suffruticosum	2016
Oncosiphon suffruticosum	2017

3.3.3 Experimental treatments

Temperature & Light

Achenes were surface sterilized in 1% liquid sodium hypochlorite for 2 minutes, rinsed with sterilized deionised water and then placed on two moist layers of Whatman no. 3 filter paper discs (5 ml sterilized deionized water was used to moisten the filter discs) in plastic Petri dishes (90 mm x 15 mm). All petri dishes were sealed with parafilm. During the incubation period, some of the filter paper discs were re-moistened with sterilized deionized water to prevent complete drying out. Experiments involving the *A. hirsuta* consistently got infected and it was decided to increase the liquid sodium hypochlorite concentration to 2%.

Every treatment, which consisted of 20 seeds per Petri-dish, were repeated four times in constant light or dark conditions (all for 24 hours). For treatments in total dark conditions, the petri-dishes were wrapped in aluminum-foil.

Treatments for a range of 5 temperatures and light or dark periods were as follows:
T1 7 °C under constant light conditions
T2 12 °C under constant light conditions
T3 17 °C under constant light conditions
T4 22 °C under constant light conditions
T5 27 °C under constant light conditions
T6 7 °C under constant dark conditions
T7 12 °C under constant dark conditions
T8 17 °C under constant dark conditions
T9 22 °C under constant dark conditions
T10 27 °C under constant dark conditions

Alternating temperatures with cyclic light and dark conditions were as follows:
22 °C/ 12 C under cyclic 12 h light and 12 h dark conditions
27 °C/ 17 °C under cyclic 12 h light and 12 h dark conditions

Germination-counts were done either every day or second day for 21 days. Achenes subjected to dark conditions, were only counted at the end of the 21 days. For this experiment, germination was considered as the emergence of the radicle (Perez-Fernandez & Rodriguez-Echeverria, 2003).

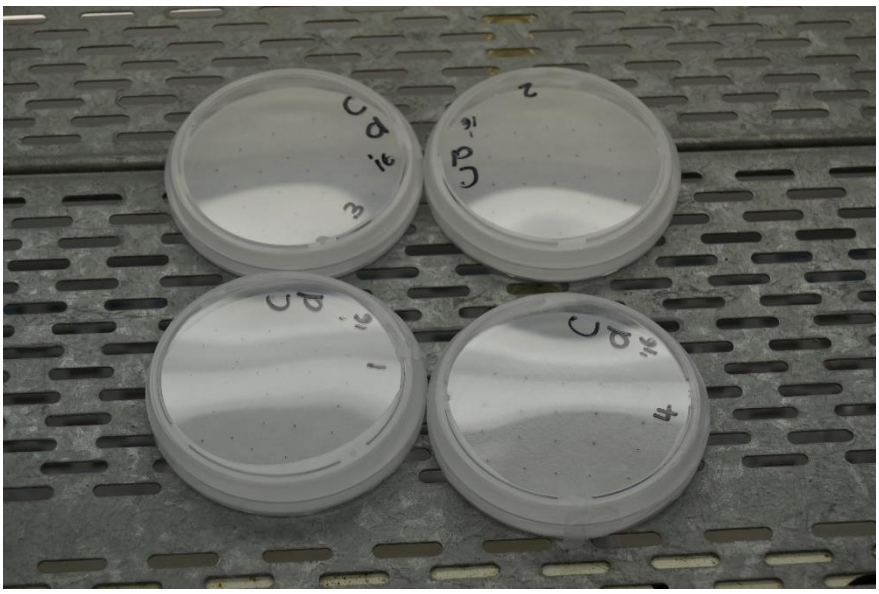

Figure 3.1: *C. duckittiae* (Cd ’16) replicates 1 - 4 subjected to light and temperature at 7 °C.

Figure 3.2: Testing germination-response of *A. hirsuta* exposed to darkness and a temperature of 12 °C.

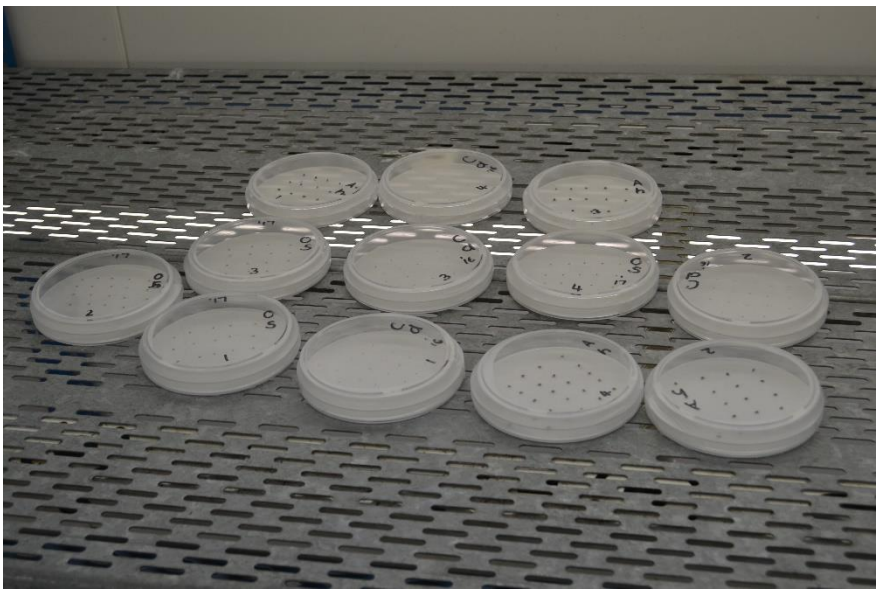

Figure 3.3: Evaluating the three species in light and temperature at 7 °C in light.

3.3.4 Statistical analysis

All data collected was statistically analysed using one-way or two-way analysis of variance (ANOVA). Treatment means were compared using Tukey's Pairwise Comparison at values of $p \leq 0.05$ (Khan, 2013; Lee & Lee, 2018). The software program Minitab was used to perform all calculations.

3.4 RESULTS

3.4.1 *Arctotis hirsuta* (collection year 2016)

3.4.1.1 Constant temperatures and light or dark conditions

Temperature

Germination of *A. hirsuta* seed tested under various temperature ranges were not statistical significant even though temperature ranges from 22 and 27 °C when linked with constant light during the germination phase interacted positively (Table 3.2 and Figure 3.4).

Light / Dark

The effects of light or darkness on the germination of *A. hirsuta* was found not statistically significant even though the temperature range of 22 to 27 °C under light proved to be

significant. Availability of light or darkness thus played no significant role during the germination period but only in combination with specific temperature ranges (Table 3.2 and Figure 3.4).

Interaction between temperature and light/dark conditions

The impact of the interaction between temperature and light on the germination of *A. hirsuta* was statistically significant at the value of $p \leq 0.05$ for all temperatures (Table 3.2).

Table 3.2: The effect of temperature and light/dark conditions on the germination of *Arctotis hirsuta* (collection year 2016) under constant temperature and light/dark conditions.

Treatments		**Germination (n)**			
Temp	**Light/Dark**	**Mean**		**Standard Error + Mean Group**	
7 °C	Light	0.056818		±0.06b	
12 °C	Light	0.000000		±0.00b	
17 °C	Light	0.193182		±0.19ab	
22 °C	Light	0.454545		±0.45a	
27 °C	Light	0.426966		±0.43a	
7 °C	Dark	0.045455		±0.05b	
12 °C	Dark	0.034091		±0.03b	
17 °C	Dark	0.011364		±0.01b	
22 °C	Dark	0.011364		±0.01b	
27 °C	Dark	0.000000		±0.00b	
Two-way ANOVA F-Statistics					
Source	DF	Adj SS	Adj MS	F-value	P-value
Temp	4	6.529	1.6322	3.47	0.008
Light/Dark	1	9.322	9.3219	19.84	0.000
Temp*Light/Dark	4	8.852	2.2131	4.71	0.001*

The mean germination values in response to five different temperatures in light or dark conditions. Mean values followed by a different letter are significantly different at $p \leq 0.05$(*) and NS (not significant) as calculated by Tukey's Pairwise Comparisons.

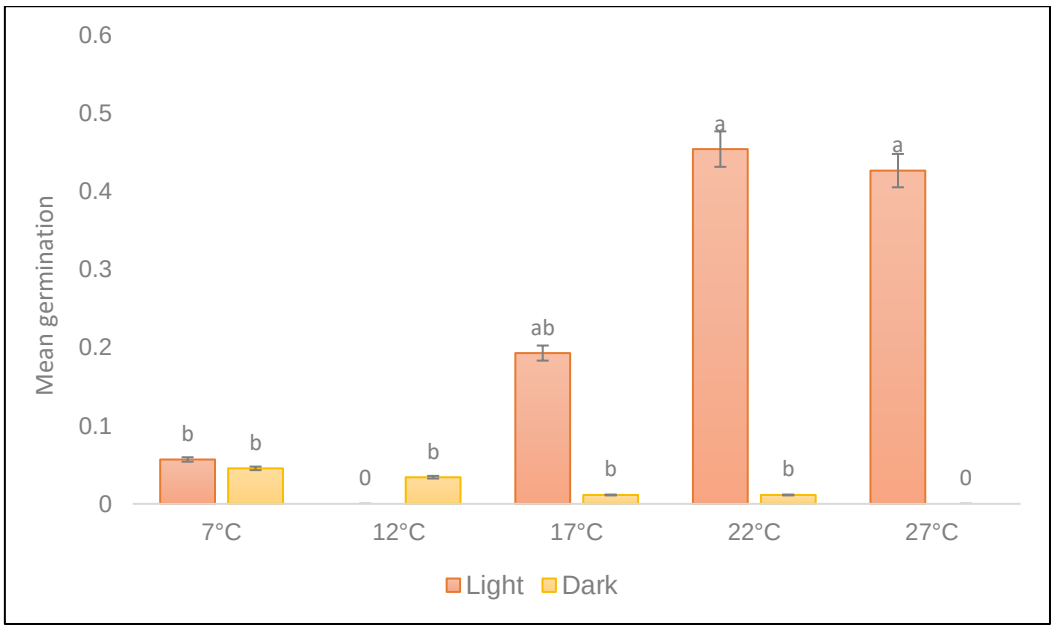

Figure 3.4: Germination percentage of *Arctotis hirsuta* (collection year 2016) at different temperatures under constant light or dark conditions.

3.4.1.2 Alternating temperatures under cyclic light and dark conditions

Interaction between alternating temperature ranges and light and dark periods

The interaction of alternating temperature on the germination of *A. hirsuta* was found insignificant at the value of $p \leq 0.05$ (See Table 3.3 and Figure 3.5). These results indicate the variation between temperatures and light and dark phases did not have a significance on the germination of *A. hirsuta* (Table 3.3 and Figure 3.5).

Table 3.3: The effect of alternating temperatures on the germination of *Arctotis hirsuta* (collection year 2016) under cyclic light and dark conditions.

Treatment		**Germination (n)**			
Alt. temp		**Mean**		**Standard Error + Mean Group**	
12 °C/27 °C		0.2045		±0.20a	
17 °C/27 °C		0.1136		±0.11a	
One-way ANOVA F-Statistics					
Source	DF	Adj SS	Adj MS	F-value	P-value
Alt. Temp	1	0.03636	0.3636	0.86	0.354*

The mean germination values in response to two different temperature ranges under cyclic light and dark conditions. Mean values followed by a different letter are significantly different at $p \leq 0.05$(*) as calculated by Tukey's Pairwise Comparisons.

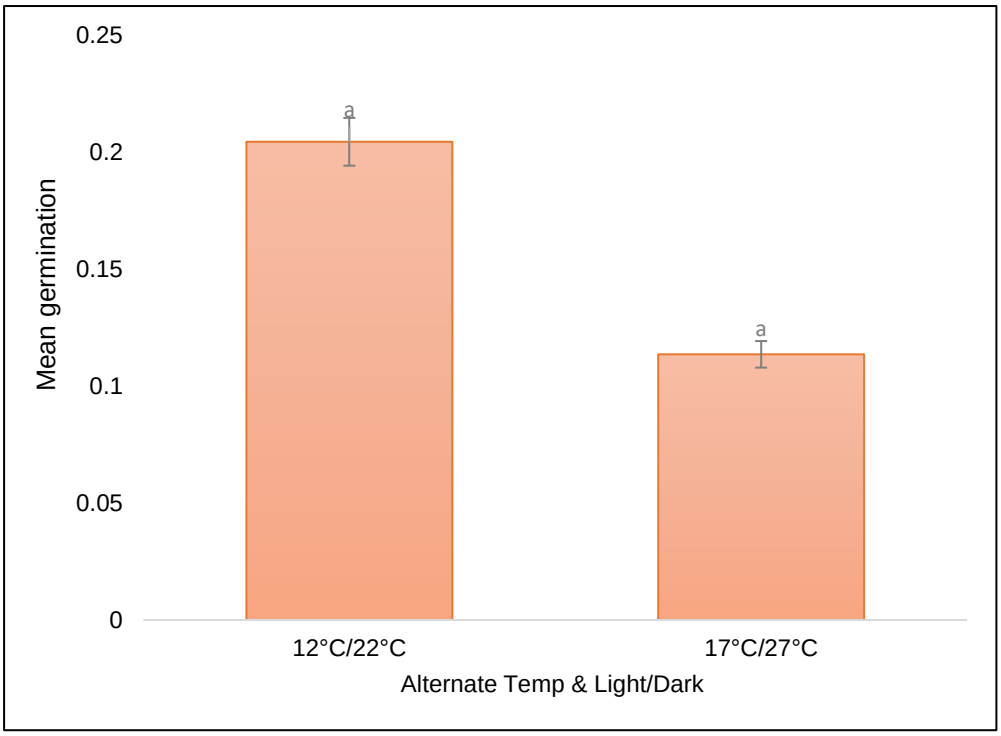

Figure 3.5: Germination percentages of *Arctotis hirsuta* (collection year 2016) at two alternating temperature ranges under cyclic light and dark conditions.

3.4.2 *Cotula duckittiae* (collection year 2016)

3.4.2.1 Constant temperatures and light or dark conditions

Temperature

The effect of various temperature ranges on the germination of *C. duckittiae* was statistically insignificant at the value of $p \leq 0.05$ (See Table 3.4).

Light / Dark

The effects of light or dark on the germination of *C. duckittiae* was statistically insignificant compare to each other (See Table 3.4 and Figure 3.6).

Interaction between temperature and light/dark conditions

The impact of the interaction between temperature and light/dark phases on the germination of *C. duckittiae* were statistically significant at the value of $p \leq 0.05$ (Table 3.4 and Figure 3.6).

Table 3.4: The effect of temperature and light/dark conditions on the germination of *Cotula duckittiae* (collection year 2016) under constant temperature and light/dark conditions.

Treatments		Germination (n)	
Temp	**Light/Dark**	**Mean (N)**	**Standard Error + Mean Group**
7 °C	Light	0.67045	±0.67a
12 °C	Light	0.625000	±0.63a
17 °C	Light	0.818182	±0.82a
22 °C	Light	0.409091	±0.41a
27 °C	Light	0.325843	±0.33a
7 °C	Dark	0.772727	±0.77a
12 °C	Dark	0.7277273	±0.73a
17 °C	Dark	0.477273	±0.48a
22 °C	Dark	0.659091	±0.66a
27 °C	Dark	0.034483	±0.03a

Two-way ANOVA F-Statistics

Source	DF	Adj SS	Adj MS	F-value	P-value
Temp	4	33.77	8.4436	1.87	0.114
Light/Dark	1	0.28	0.2779	0.06	0.804
Temp*Light/Dark	4	12.24	3.0602	0.68	0.608*

The mean germination values in response to five different temperatures in light or dark conditions. Mean values followed by a different letter are significantly different at $p \leq 0.05$(*) as calculated by Tukey's Pairwise Comparisons.

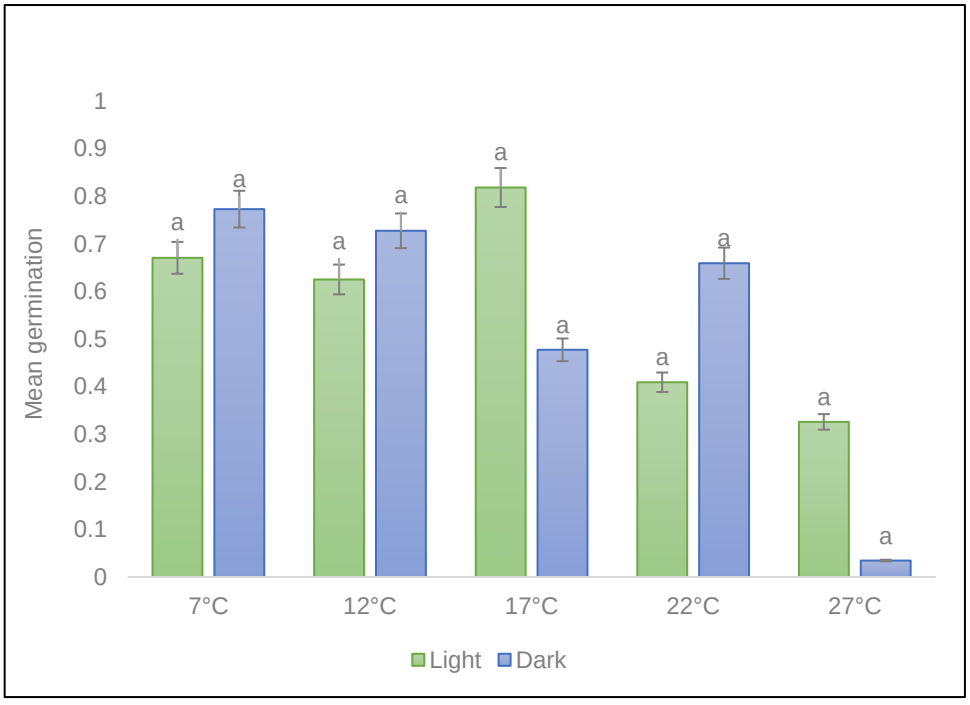

Figure 3.6: Germination percentage of *C. dukittiae* (collection year 2016) for five different constant temperatures under light or dark conditions.

3.4.2.2 Alternating temperatures under cyclic light and dark conditions

Interaction between temperature and light/dark conditions

The interaction of alternating temperature under cyclic light/dark conditions on the germination of *C. duckittiae* (collection year 2016) was statistically significant at the value of $p \leq 0.05$ at 12 °C/22 °C temperature range compared to the 17 °C/27 °C temperature range under the same cyclic light and dark conditions (See Table 3.5 and Figure 3.7).

Table 3.5: The effect of alternating temperatures on the germination of *C. duckittiae* (collection year 2016) under cyclic light and dark conditions.

Treatment	**Germination (n)**				
Alt. temp	**Mean**			**Standard Error + Mean Group**	
Alt Temp	N			Mean	
12 °C/22 °C	0.736			±0.74a	
17 °C/27 °C	0.0455			±0.05b	
One-way ANOVA F-Statistics					
Source	DF	Adj SS	Adj MS	F-value	P-value
Alt Temp	1	20.84	20.839	20.63	0.000*

The mean germination values in response to two different temperatures ranges under cyclic light and dark conditions. Mean values followed by a different letter are significantly different at $p \leq 0.05$ (*) and NS (not significant) as calculated by Tukey's Pairwise Comparisons.

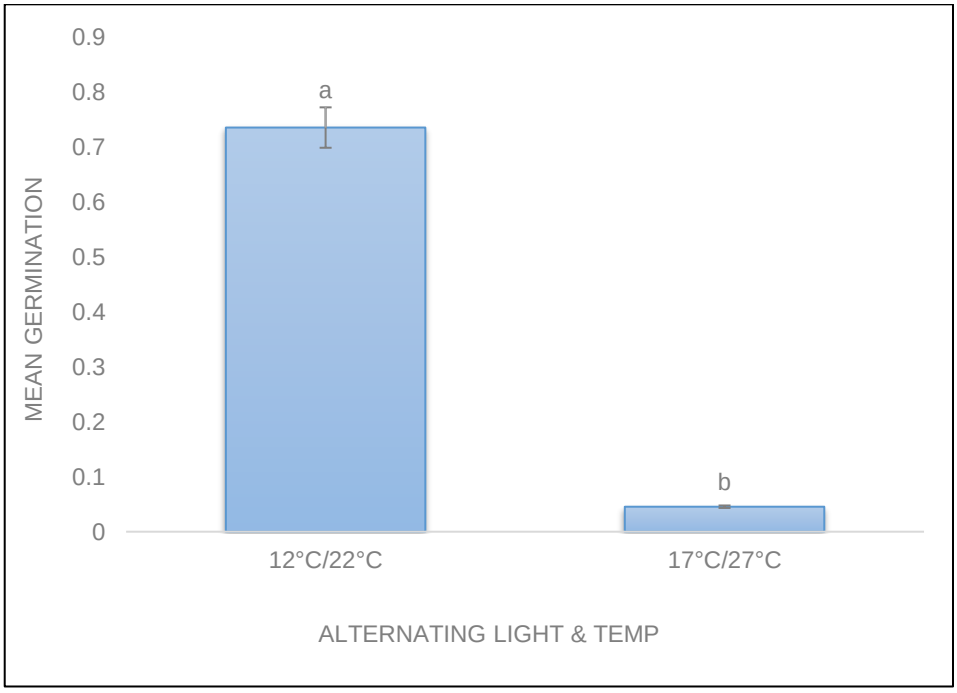

Figure 3.7: The germination percentage for *C. duckittiae* (collection year 2016) under alternating temperatures and cyclic light and dark conditions.

3.4.3 *Cotula duckittiae* (collection year 2017)

3.4.3.1 Constant temperatures and light or dark conditions

Temperature

The effect of temperature was statistically significant compare to other treatments. (Table 3.6 and Figure 3.8).

Light /Dark

The effects of the constant dark condition were not statistically significant at the value of $p \leq 0.05$ compared to light conditions of other treatments. (Table 3.6 and Figure 3.8).

Interaction between temperature and light/dark conditions

The impact of the interaction between temperature and light or dark conditions on the germination of *C duckittiae* was statistically significant (Table 3.6 and Figure 3.8)

Table 3.6: The effect of temperature and light/dark conditions on the germination of *C. duckittiae* (collection year 2017) under constant temperature and light/dark conditions.

Treatments		**Germination (n)**			
Temp	**Light/Dark**	**Mean (N)**		**Standard Error + Mean Group**	
7 °C	Light	0.000000		±0.00b	
12 °C	Light	0.011364		±0.01b	
17 °C	Light	0.011364		±0.01b	
22 °C	Light	0.000000		±0.00b	
27 °C	Light	0.000000		±0.00b	
7 °C	Dark	0.011364		±0.01b	
12 °C	Dark	0.000000		±0.00b	
17 °C	Dark	0.000000		±0.00b	
22 °C	Dark	0.397727		±0.40a	
27 °C	Dark	0.000000		±0.00b	
Two-way ANOVA F-Statistics					
Source	DF	Adj SS	Adj MS	F-Value	P-value
Temp	4	5.336	1.3341	3.43	0.009
Light/Dark	1	1.314	1.3136	3.38	0.066*
Temp*Light/Dark	4	5.664	1.4159	3.64	0.006

The mean germination values in response to five different temperatures in light or dark conditions. Mean values followed by a different letter are significantly different at $p \leq 0.05$(*) and NS (not significant) as calculated by Tukey's Pairwise Comparisons.

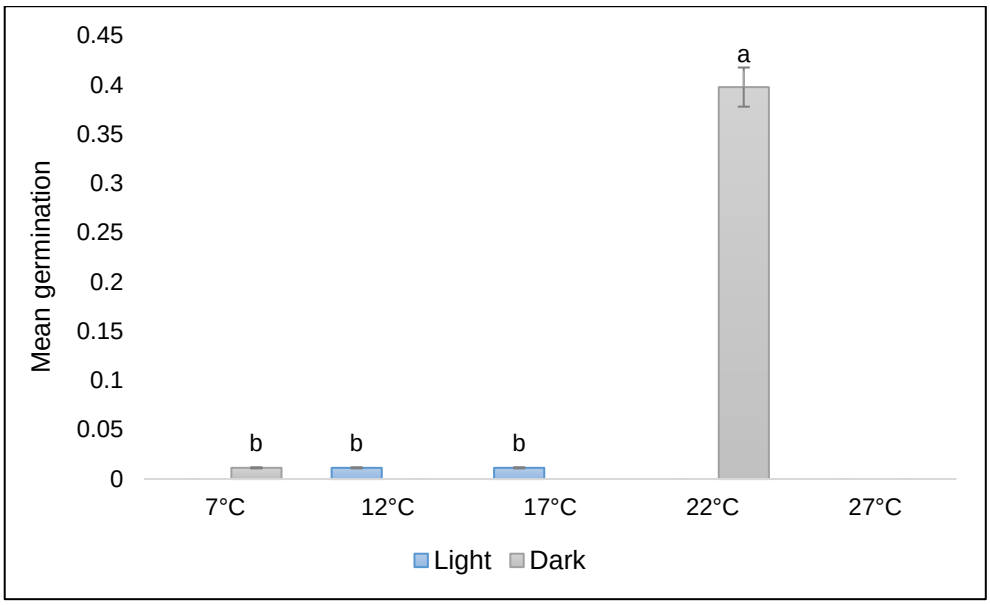

Figure 3.8: Germination percentage for *C. duckittiae* at five different temperatures which were subjected to light or dark conditions.

3.4.3.2 Alternating temperature under cyclic light and dark conditions

Interaction between temperature and light-conditions

No data was recorded for alternating light and temperature of these species due to shortage of seed supply.

3.4.4 *Oncosiphon suffruticosum* (collection year 2016)

3.4.4.1 Constant temperatures and light or dark conditions

Temperature

The effect of temperature of 12 °C (±0.35) on the germination of *O. suffruticosum* (collection year 2016) was statistically significant at the value of $p \leq 0.05$ compared to other temperatures (see Table 3.7).

Light

The effects of light on the germination of *O. suffruticosum* was statistically significant at the value of $p \leq 0.05$. (Table 3.7 and Figure 3.9)

Interaction between temperature and light /dark conditions

The impact of the interaction between temperature and light on the germination of *O. suffruticosum 2016* was statistically significant during the germination period.

Table 3.7: The effect of temperature and light/dark conditions on the germination of *Oncosiphon suffruticosum* (collection year 2016) under constant temperature and light/dark conditions.

Treatments		Germination (n)			
Temp	**Light/Dark**	**Mean (N)**	**Standard Error + Mean Group**		
7 °C	Light	0.000000	±0.00b		
12 °C	Light	0.352273	±0.35a		
17 °C	Light	0.215909	±0.22ab		
22 °C	Light	0.261364	±0.26ab		
27 °C	Light	0.000000	±0.00b		
7 °C	Dark	0.272727	±0.27ab		
12 °C	Dark	0.034091	±0.03b		
17 °C	Dark	0.034091	±0.03b		
22 °C	Dark	0.011364	±0.01b		
27 °C	Dark	0.011494	±0.01b		
Two-way ANOVA F-Statistics					
Source	DF	Adj SS	Adj MS	F-value	P-value
Temp	4	3.338	0.8345	2.29	0.058*
Light/Dark	1	1.909	19091	5.24	0.022
Temp*Light/Dark	4	10.028	2.5071	6.88	0.000

The mean germination values in response to five different temperatures in light or dark conditions. Mean values followed by a different letter are significantly different at p ≤ 0.05(*) and NS (not significant) as calculated by Tukey's Pairwise Comparisons.

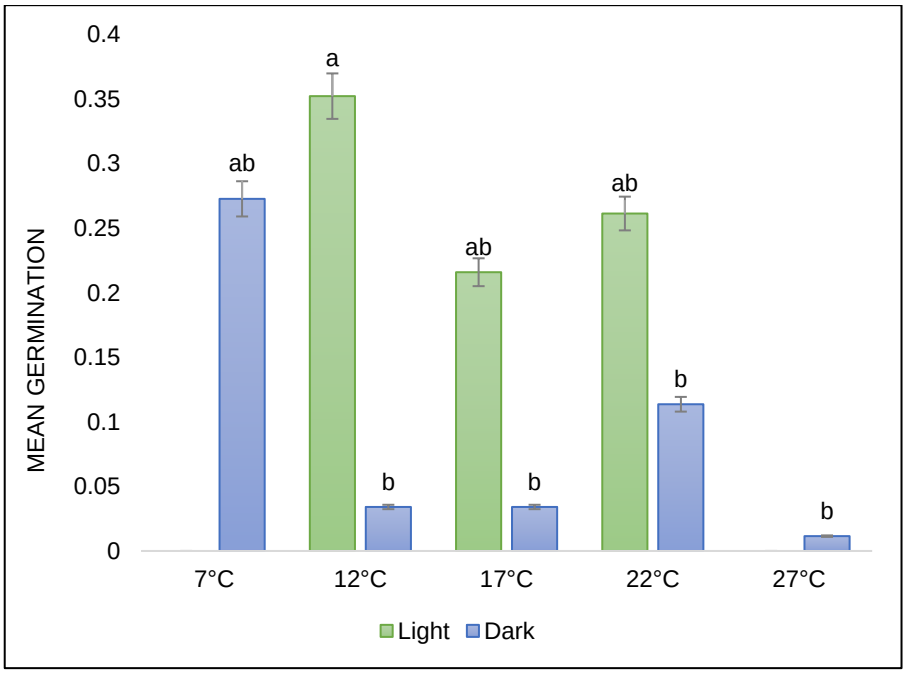

Figure 3.9: Germination percentage of *Oncosiphon suffruticosum* (collection year 2016) at five different temperatures under light or dark conditions.

3.4.4.2 Alternating temperatures under cyclic light and dark conditions

Interaction between temperature and light-conditions

The interaction of alternating temperature under alternating light and dark conditions on the germination of *O. suffruticosum 2016* was statistically insignificant at the value of $p \leq 0.05$ (see Table 3.8 and Figure 3.10).

Table 3.8 The effect of alternating temperatures on the germination of *Oncosiphon suffruticosum* (collection year 2016) under cyclic light and dark conditions.

Treatment		**Germination (n)**			
Alt. temp		**Mean**		**Standard Error + Mean Group**	
17 °C/27 °C		0.284091		±0.28a	
12 °C/22 °C		0.284091		±0.28a	
One-way ANOVA F-Statistics					
Source	DF	Adj SS	Adj MS	F-value	P-value
Alt Temp.	1	0.000	0.00000	0.00	1.000*

The mean germination values in response to five different temperatures in light or dark conditions. Mean values followed by a different letter are significantly different at $p \leq 0.05$(*) as calculated by Tukey's Pairwise Comparisons.

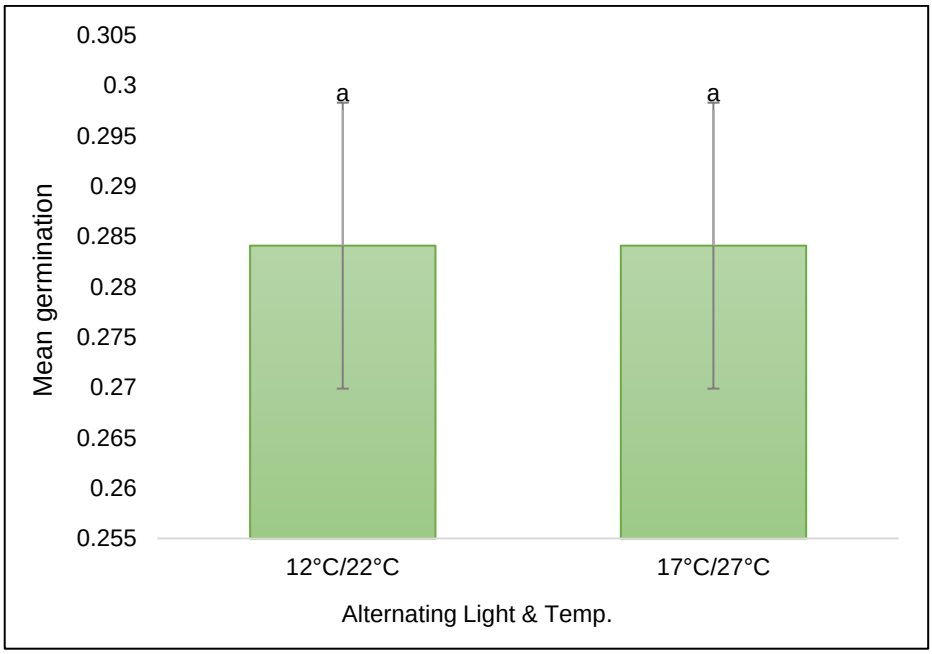

Figure 3.10: Germination percentage for *Oncosiphon suffruticosum* (collection year 2016) under alternating temperature and alternate light and dark conditions.

3.4.5 *Oncosiphon suffruticosum* (collection year 2017)

3.4.5.1 Constant temperatures and light or dark conditions

Temperature

The effect of temperature on the germination of *O. suffruticosum* was statistically insignificant at the value of $p \leq 0.05$ (Table 3.9 and Figure 3.11).

Light

The effects of light on the germination of *O. suffruticosum* was statistically insignificant at the value of $p \leq 0.05$ (Table 3.9 and Figure 3.11).

Interaction between temperature and light/dark conditions

The impact of the interaction between temperature and light on the germination of *O. suffruticosum 2017* was statistically insignificant at the value of $p \leq 0.05$ (Table 3.9 and Figure 3.11).

Table 3.9: The effect of temperature and light/dark conditions on the germination of *Oncosiphon suffruticosum* (collection year 2017) under constant temperature and light/dark conditions.

Treatments		**Germination (n)**			
Temp	**Light/Dark**	**Mean (N)**	**Standard Error + Mean Group**		
7 °C	Light	0.149425	±0.15a		
12 °C	Light	0.079545	±0.08a		
17 °C	Light	0.170455	±0.17a		
22 °C	Light	0.090909	±0.09a		
27 °C	Light	0.067416	±0.07a		
7 °C	Dark	0.000000	±0.00a		
12 °C	Dark	0.297619	±0.30a		
17 °C	Dark	0.170455	±0.18a		
22 °C	Dark	0.045455	±0.00a		
Two-way ANOVA F-Statistics					
Source	DF	Adj SS	Adj MS	F-value	P-value
Temp	4	3.378	0.844397	1.89	0.111*
Light/Dark	1	0.009	0.009445	0.02	0.885*
Temp*Light/Dark	4	3.305	0.447723	1.85	0.118*

The mean germination values in response to five different temperatures in light or dark conditions. Mean values followed by a different letter are significantly different at $p \leq 0.05$(*) as calculated by Tukey's Pairwise Comparisons.

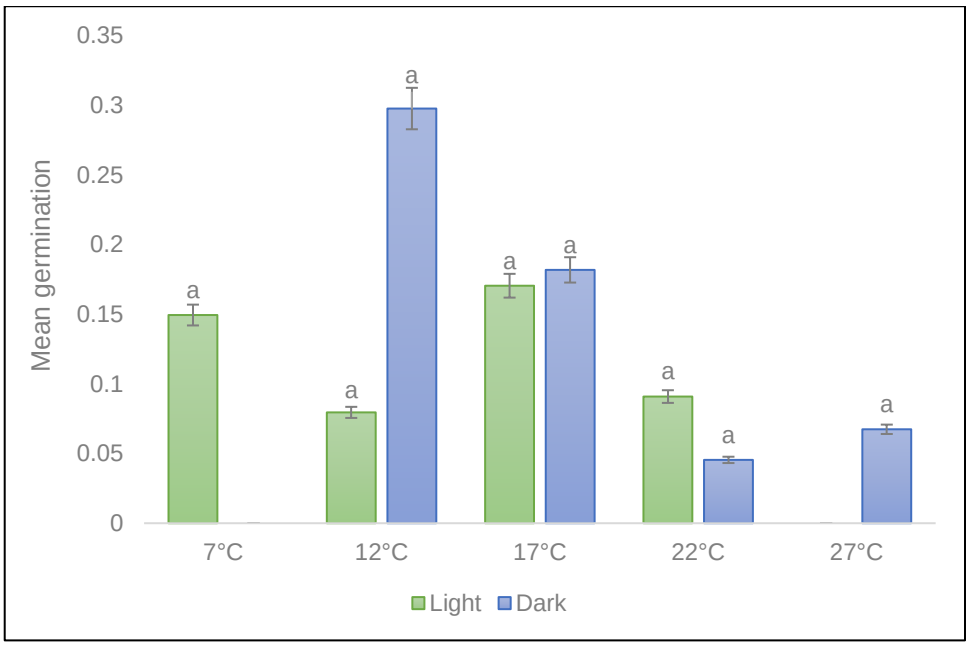

Figure 3.11: The mean germination percentage for *O. suffruticosum* (collection year 2017) under five different temperatures in light or dark conditions.

3.4.5.2 Alternating temperatures under cyclic light and dark conditions

Interaction between temperature and light-conditions

The interaction of alternating temperature under alternating light and dark conditions on the germination of *O. suffruticosum* (collection year 2017) was statistically insignificant at the value of $p \leq 0.05$ (see Table 3.10 and Figure 3.12).

Table 3.10: The effect of alternating temperatures on the germination of *Oncosiphon suffruticosum* (collection year 2017) under cyclic light and dark conditions.

Treatment		**Germination (n)**			
Alt. temp		**Mean**		**Standard Error + Mean Group**	
12 °C/22 °C		0.0682		±0.07a	
17 °C/27 °C		0.0341		±0.03a	
One-way ANOVA F-Statistics					
Source	DF	Adj SS	Adj MS	F-Value	P-value
Alt Temp	1	0.05114	0.05114	1.05	0.307*

The mean germination values in response to five different temperatures under cyclic light and dark conditions. Mean values followed by a different letter are significant at the value of $p \leq 0.05$ as calculated by Tukey's Pairwise Comparisons.

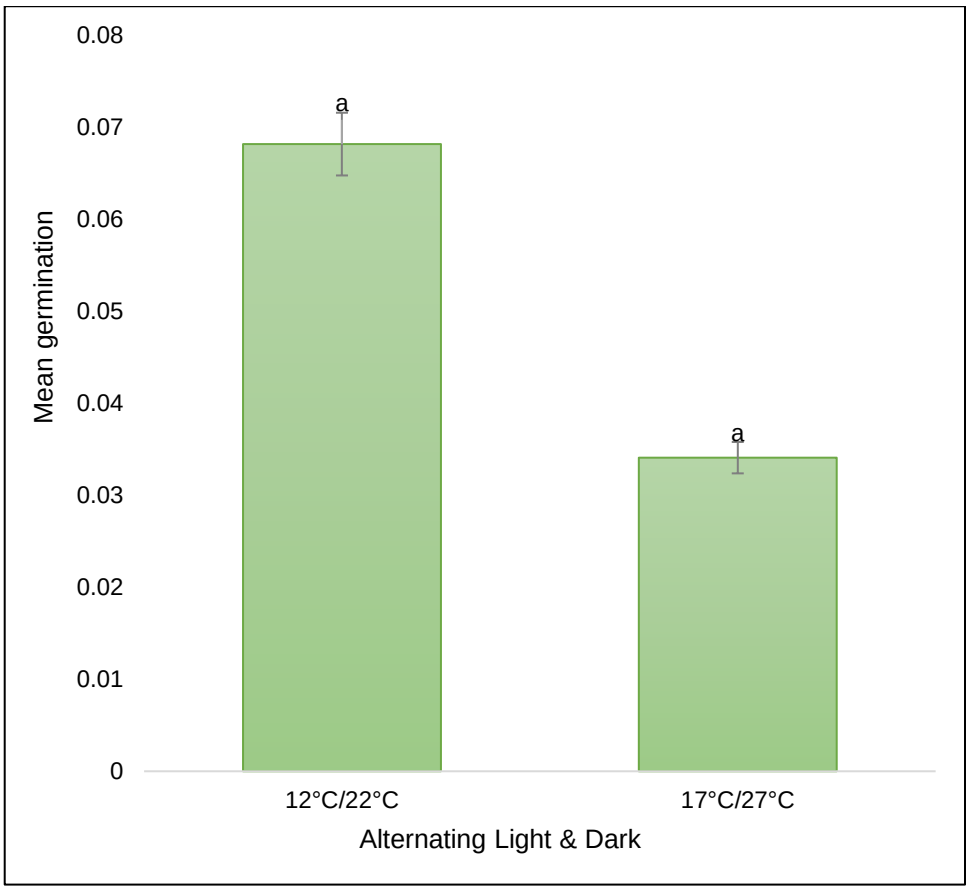

Figure 3.12: The mean germination percentage of *O. suffruticosum* (collection year 2017) at alternating temperatures and cyclic light and dark conditions.

3.5 DISCUSSION & CONCLUSION

The ability of seed to germinate hinges on a combination of environmentally and hormonal stimuli (Erwin, 1991) and include a required temperature, moisture and oxygen (De Villiers *et al.*, 2002). In the seed-germination process, the role of light is one other critical prerequisite to overcome (Grime *et al.,* 1981; Erwin, 1991; Pons, 1992; Bewley & Black, 1994; Copeland & McDonald, 1995).

*C. duckittiae (*collecting year 2016) germinated well in light and dark conditions, with the best result achieved at 17 °C in light. The germination percentage, which consistently exceeded ≥ 50%, were obtained between temperature ranges 7 °C – 22 °C in light and dark conditions, apart from 22 °C in light and 17°C in dark which recorded less than 50%. The maximum figure for germination in darkness was achieved at 7 °C.

C. duckittiae (collecting year 2017) recorded the highest germination under dark conditions at 22 °C with negligible to zero germination for all the other temperature and light or dark conditions. Results indicated that temperature is a crucial element for germination for achenes of collecting year 2017.

O. suffruticosum (collecting year 2016) germinated in light over a wide range of temperatures from 12°C - 22 °C. The highest germination was recorded at 12 °C in light. No germination registered at 7 °C and 27 °C in light. Germination in dark condition did not yield good results with very low germination between 12 °C - 27 °C.

The highest germination percentage for *O. suffruticosum* (collecting year 2017) was recorded at the temperature of 12 °C in the dark.

Achenes of *A. hirsuta* recorded poor germination under dark conditions for all temperature-ranges from 7 °C - 27 °C. In light, for temperatures 7 °C - 12 °C, a similar germination was recorded. From 17 °C, a marked increase in germination can be observed under light. Similar germination percentages were obtained at alternating temperatures and cyclic light and dark conditions 12 °C / 22° and 17 °C/ 27 °C. The best germination was recorded at 22 °C in light. Results have shown the interaction between light and temperature is essential to effect germination.

These studies revealed that temperature did not play an integral part in the germination of the achenes for both collecting years of *O. suffruticosum*. However, the most significant difference between the achenes of collecting years 2016 and 2017 is the need for a germination-requirement for light and dark between the two respective years. In collecting 2016 light and darkness played a pivotal role in germination, whereas this was not the case for collecting year 2017. This may point to the absence of a dormancy imposed by the requirement for light. This lack of light to induce germination was also found to be present in the annual *Dimorphotheca sinuata* (Beneke *et al.,* 1993). Achenes which are generally buried too deep in the soil, experienced a light-imposed dormancy (Venable & Brown, 1988; Pons, 1991). This requirement is perfectly suited to the small seeds of annuals as it prevents germination (Atwater, 1980; Schütz *et al.,* 2002), especially under unfavorable conditions (Mott, 1972; Thanos *et al.,* 1991). The failure of light-requiring achenes to germinate can be attributed to the fact that unusable phytochrome cannot be changed into an active form that can be used (Langkamp, 1987).

Results from this study indicated that the need for a light-requirement promoted optimum germination in *A. hirsuta* and *O. suffruticosum* (collecting year 2016). The need for light during the germination-process is common in Asteraceae (Mott, 1972; Atwater, 1980; Beneke *et al.,* 1993; Plummer & Bell, 1995; De Villiers 2000; Baskin & Baskin, 2001; Eddy & Van Auken, 2014).

The combination of environmental and hormonal stimuli (Erwin, 1991) are critical aspects in the germination-process and seasonal temperature is of vital importance amongst this for the establishment and development of plants (Fankhauser & Chory 1997; Zarghani *et al.,* 2014).

Optimum germination for an extensive range of winter-annuals were recorded below 20°C (Ratcliff, 1961; Mott, 1972; Willis & Grove, 1991; Beneke *et al.,* 1993; Visser, 1993; Plummer & Bell, 1995). The highest germination percentage for *Cotula duckittiae* (collecting year 2016) and *Oncosiphon suffruticosum* (collecting years 2016 & 2017) were recorded between the 12 °C and 17 °C. These results agreed with the findings that germination at these temperatures signify an association with germination during the winter-rainfall period. The ability to germinate during such a period enable the achenes to benefit from cooler temperatures due to increased moisture in the soil and reduced evaporation (Bell *et al.,* 1993).

The best germination percentage for *A. hirsuta* was recorded at temperatures above 20 °C [22 °C - 27 °C]. In the event of such germination during sporadic rainfall, the lack of continuous moisture and low temperatures may result in mass death of seedlings (Elner & Shmida, 1981). Despite the competitive advantage that this germination may offer (Plummer & Bell, 1995), the lack of enough rain and the potential seedling fecundity or death is capable of the depletion of the seed bank (Venable & Brown 1988; Beneke *et al.,* 1993).

The achenes of *C. duckittiae* and *O. suffruticosum* (both collecting year 2016) displayed the ability to germinate over a large proportion of temperature ranges. *O. suffruticosum* (collecting year 2016), with the exception of 7 °C, displayed the ability germinate at almost a similar level of germination from 12° - 22 °C. The same percentage were also recorded for the alternating temperatures of 12 °C/ 22 °C and 17 °C/ 27 °C. The achenes of *C. duckittiae* (2016) recorded germination ≥ 60% for the temperature-ranges 7 °C - 17 °C and the alternating temperature of 12 °C/ 22 °C.

Another factor to be considered, is the period some achenes were subjected to during after-ripening under controlled conditions of 15 °C and 15% RH at the Seedroom of the KNBG. Germination-results indicated that whether under constant or alternating temperature or light-conditions, achenes germinated optimally from achenes collected in the year 2016. These achenes, oppose to those collected in the year 2017, were stored for between 28 – 32 months and resulted in a longer period for the embryo to develop.

To conclude, results indicated that germination can be enhanced by storing achenes at the after-ripening conditions at KNBG for a minimum period of two-years for some species (*C. duckittiae* [collected in 2016]; *A. hirsuta*, [collected in 2016]). In the case of species collected in year 2016, achenes were stored for between 28 – 32 months. Achenes stored for less than two-years did not germinate well at all and in most of them exhibited a level of dormancy. For *C. duckittiae*, where conservation-work is to be considered, seed must be stored for a minimum of two years for optimum germination. In some species investigations could be done to look determine the optimum storage-conditions like temperature or period of storage.

3.6 ACKNOWLEDGEMENTS

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CHAPTER FOUR:

THE INFLUENCE OF SCARIFICATION AND GIBBERELLIC ACID ON THE GERMINATION OF THREE SELECTED ASTERACEAE ANNUALS FROM THE WINTER-RAINFALL REGION OF SOUTH AFRICA

THE INFLUENCE OF SCARIFICATION AND GIBBERELLIC ACID ON THE GERMINATION OF THREE SELECTED ASTERACEAE ANNUALS FROM THE WINTER-RAINFALL REGION OF SOUTH AFRICA

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4.1 ABSTRACT

Asteraceae annuals often provide germination-challenges and as such cannot be introduced *en masse* into the Kirstenbosch National Botanical Garden (KNBG) annuals-display when propagated from fresh wild-collected seed. *Arctotis hirsuta* (Harv.) Beauvard*, Cotula duckittiae* (L. Bolus) K. Bremer & Humphries and *Oncosiphon suffruticosum* (L.) Källersjö are three Asteraceae annuals from South Africa's winter-rainfall region. *A. hirsuta* is a garden ornamental from the Western and Northern Cape, the red-data listed Vulnerable *C. duckittiae* is endemic to West Coast in the Western Cape and *O. suffruticosum* is a traditional medicinal plant from the Northern and Western Cape. The purpose of this study was to investigate methods to alleviate dormancy of the three selected species. Pre-germination treatments consisted of scarification (pericarp-pricking) and the soaking of seed over 4 periods in 4 different gibberellic-acid (GA_3) concentrations. Seeds of all species were then subjected to pre-determined light and temperature conditions as follows: *A. hirsuta* (collection year 2016) [22 °C in light]*, C duckittiae* (collection year 2017) [22 °C in light], *O. suffruticosum* (collection year 2016) [12 °C in light] and *O. suffruticosum* (collection year 2017) [22 °C in dark] to ensure optimum germination. Results indicated that scarification and the GA_3-treatment had no impact on the germination of *A. hirsuta* (collection year 2016). None of the two pre-treatments improved germination for *C. duckittiae* (collection year 2017) and *O. suffruticosum* (collection years 2016 & 2017) and results suggested using GA_3 or scarification as a single pre-treatment application under these condition are not effective in improving germination.

Key words: wild-collected seed, germination-challenge, dormancy, red-data listed, endemic, medicinal, ornamental

4. 2 INTRODUCTION

4.2.1 Winter-annuals from semi-arid to arid regions (in South Africa)

Arctotis hirsuta, Cotula duckittiae and *Oncosiphon suffruticosum* are three Asteraceae annuals from South Africa's winter-rainfall region. *C. duckittiae* is an endemic of the West Coast. This 30 x 30 cm orange-flowering species is of conservation concern and has been accorded a red-listed status of Vulnerable (Helme, 2006; Manning & Goldblatt, 2012). *O. suffruticosum* is a yellow-flowering aromatic herb that is well-known for its medicinal properties (Magee, 2011). The distribution range for this species is from the Northern to the Western Cape (Manning & Goldblatt, 2012). *A. hirsuta* with its bright-coloured cream, orange and yellow flower heads is widely distributed throughout the Western and Northern Cape is an impressive garden-ornamental (Joffe, 1993; Pienaar & Smith, 2011; Manning & Goldblatt, 2012).

Winter-rainfall regions in South Africa, in particular the Fynbos & Succulent Karoo biomes, experience mostly Mediterranean climatic conditions (Mucina *et al.,* 2006; Rebelo *et al.,* 2006; Manning & Goldblatt, 2012). The result of this means the climate of such areas are subjected to mild and wet winters. This is usually followed by hot and dry summers (Dallman, 1988; Rundel & Cowling, 2013). This type of climate can place challenging demands on some plants, especially annuals which disperse their seed towards the end of the rainy season. One such counter-measure by winter-annuals is to ensure germination is limited only to conditions deemed favorable to ensure the survival of the species through any unfavorable climatic periods (Le Roux & Schelpe, 1997). In response to this the winter-rainfall annuals experience seed dormancy as an environmental-adaptation to survive warm, dry summer conditions after the seeds has been dispersed in spring from the mother plant in order to prevent germination (Baskin & Baskin, 1976; Le Roux & Schelpe, 1997, Fenner & Thompson, 2005;). This dormancy-mechanism present in winter annuals from the semi- to arid areas is beneficial to the survival of such species by ensuring that there remains a viable seed bank in the soil (Venable & Lawlor, 1980; Freas & Kemp, 1983; Visser, 1993).

4.2.2 Influence of scarification and gibberellic acid on germination

Germination is a critical phase in the survival of species and is the first of the physiological responses under the influence of the environment (Mayer & Poljakoff-Mayber, 1982). Seeds may fail to germinate despite being exposed to suitable environmental conditions (Langkamp, 1987). For germination to occur, the seed must not be in a state of dormancy and the environmental requirements for germination of that seed must be met (Vleeshouwers *et al.,* 1995). The 'freshly harvested' seed of several wild Asteraceae species experience a low germination and high dormancy (Maiti *et al.,* 2006; Seiler, 2010).

Dormancy is considered to be a seed characteristic and variations in the degree of dormancy defines the environmental conditions that must be met to allow the seed to germinate (Vleeshouwers *et al*., 1995). Seed dormancy manifests itself in many ways and may include special requirements for temperature or light and the presence of inhibitors that need leaching (Tran & Cavanagh, 1984). Dormancy may also be influenced by impermeability of the seed coat and gases, immaturity of the embryo, and mechanical restriction to embryo growth and development (Kozlowski & Gunn, 1972; Bell, 1999; Copeland & McDonald, 2001; Jusaitisa *et al.,* 2004). In the natural environment seeds possessing hard seed coats get damage or become softer over time through fires or scarification to overcome dormancy (Rolston, 1978). According to Tran and Cavanagh (1984) dormancy in each case may be due to only one cause, or to even several of the above-mentioned factors operating in tandem. The presence of an enlarged and lignified outer epidermis of the pericarp, plentiful tanniniferous substances as well as a broad sclerenchymatous zone in the mesocarp and cuticles often constitute impediments to germination due to dormancy in several Asteraceae annual species of the winter-rainfall region in South Africa (Beneke *et al.,* 1992).

There is huge potential amongst daisies as bedding plants, pot plants or medicine (Bunker, 1994; Plummer & Bell, 1995; Herman, 2004; Magee, 2011; Oliver, 2011; Pienaar & Smith, 2011; Oliver, 2014). Despite the ability of some species to be grown for ecological restoration or re-vegetation projects (Norcini & Aldrich, 2007), there is still a demand for specific ecotype seeds or research on such seeds as native plants are often exclusively grown by a handful of growers (Capon, 2005), in part due to wild collected seed being more dormant than domesticated seed (Copeland, 1976). In order to realize the horticultural potential of these native winter-rainfall annual composites, it is imperative that seed dormancy be overcome (Wrigley & Fagg, 1986; Bunker, 1994) as the availability of seed with a consistently high germination is a limiting factor (Nasreen *et al.,* 2015).

Dormancy can be overcome by exposing seed to a certain set of conditions or treatments (Langkamp, 1987). In Asteraceae this may include the application of different concentrations of GA_3 in which seeds can be soaked for a specific period of time (Abdalla & McKelvie, 1980; Bunker, 1994; Van Auken, 2001; Cochrane & Probert, 2006; Plummer *et al.,* 2006; Farimani *et al.,* 2011; Ha, 2014; Puttha *et al.,* 2014) and scarification through pricking of the seed coat (Mayer & Poljakoff-Mayber, 1982; Visser, 1993; Bunker, 1994).

The objectives of this study were therefore to evaluate the impacts of pre-germination treatments on the germination of wild-collected seed of winter-annuals. The results of this investigation can contribute to the understanding of the propagation of these selected species and also support conservation-efforts, including restoration of rare and endangered species.

4.3 MATERIAL AND METHODS

4.3.1 Growth chamber experiment

The experiments were conducted from June to July 2019. It was located in the growth chambers of the tissue culture facilities at the Kirstenbosch Research Centre of the South African National Biodiversity Institute (SANBI) at the Kirstenbosch National Botanical Gardens (KNBG), Newlands, Cape Town.

4.3.2 Seed preparation

Seeds of the various species have been collected in the winter-rainfall areas of the Western Cape and Northern Cape Provinces of South Africa by the researcher or sourced from Silverhill Seeds during 2016 and 2017. Seed collected over the two periods are referred to as A (2016) and B (2017) respectively. Seed of all three species have been collected during October 2016. Only one collection of *A. hirsuta* has been made in 2016, hence *C. duckittiae* (A), *O. suffruticosum* (A). In 2017 seed of *Cotula duckittiae* and *Oncosiphon suffruticosum* were collected during October and November respectively. This seed will be referred to as *C. duckittiae* (B) and *O. suffruticosum* (B). No collection of *A. hirsuta* was made for this year. Following collection, seeds were stored in paper envelopes at 15 °C and 15 % relative humidity in a cold room at KNBG until the start of the experiments.

4.3.3. Experiments with pretreatments of scarification and GA_3

4.3.3.1 Scarification

Seeds were pricked with a dissecting needle under a light microscope to cut through the seed coat in order to allow penetration of the seed coat. Achenes (unscarified used as the control and scarified) were then surface sterilized in 1 % liquid sodium hypochlorite for 2 minutes, rinsed with sterilized deionised water and then placed on two moist layers of Whatman no. 3 filter paper discs (5 ml sterilized deionized water was used to moisten the filter discs) in plastic Petri dishes (90 mm x 15 mm). All petri dishes were sealed with parafilm. During the incubation period, some of the filter paper discs were re-moistened with sterilized deionized water to prevent complete drying out. Experiments involving the *A. hirsuta* consistently got infected and it was decided to increase the liquid sodium hypochlorite concentration to 2 %.

Every treatment, which consisted of 20 seeds per Petri-dish, were repeated four times in constant light or dark conditions (all for 24 hours). For treatments in total dark conditions, the petri-dishes were wrapped in aluminum-foil.

4.3.3.2 Gibberellic acid

Achenes were surface sterilized in 1% liquid sodium hypochlorite for 2 minutes and rinsed with sterilized deionised water. Achenes were then soaked into two concentrations of 250 ppm and 500 ppm of GA_3 over 1 hour and 2 hours. Treatments of untreated seed (the control) have been used to compare against the various concentrations. All seed were treated as in 4.3.3.1.

Every treatment consisted of 20 seeds per Petri-dish and has been repeated four times under the optimal temperature and light conditions (See Table 4.1) of the three species.

Table 4.1: The three winter-annuals with the temperature and light-conditions needed for optimum germination

Species	Temperature	Light-condition
Arctotis hirsuta (collection year 2016)	22 °C	Light
Cotula duckittiae (collection year 2017)	22 °C	Light
Oncosiphon suffruticosum (collection year 2016):	12 °C	Light
Oncosiphon suffruticosum (collection year 2017):	22 °C	Dark

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Experiments for the range of pre-germination treatments were as follows:
T1: Untreated (control)
T2: Scarification
T3: 250 ppm GA_3/ 1H soaking
T4: 250 ppm GA_3/ 2H soaking
T5: 500 ppm GA_3/ 1H soaking
T6: 500 ppm GA_3/ 2H soaking

Achenes were incubated in a GC-550R growth chamber with 4000-8000 LUX illumination. This chamber is equipped with a timer-device that allows regulation of the temperature and light required over a 24-hour period. Germination-counts were done either every day or second day for 21 days. Achenes subjected to dark conditions, were only counted at the end of the 21 days. For this experiment, germination was considered as the emergence of the radicle (Perez-Fernandez & Rodriguez-Echeverria, 2003).

Figure 4.1: Achenes of *C. duckittiae* at 22 °C and light after being pre-treated by pricking and a GA_3-soak in different concentrations over different periods.

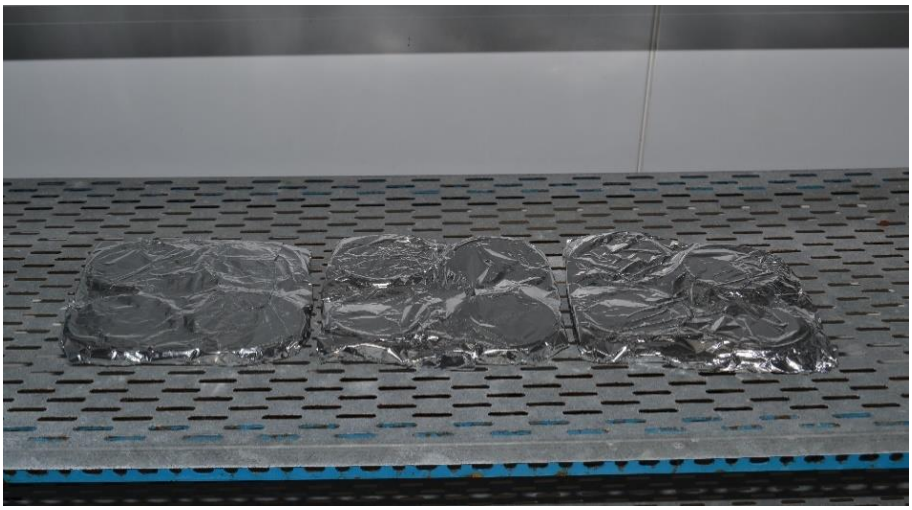

Figure 4.2: Achenes of *O. suffruticosum* (collecting year 2017) subjected to 22 °C and darkness after a pre-treatment of scarification and GA_3-applications.

4.4 STATISTICAL ANALYSIS

All data collected was statistically analysed using one-way analysis of variance (ANOVA). Treatment means were compared using Fishers Least Significant Difference at values of $p \leq 0.05$ (Steel & Torie, 1980). The software program Minitab was used to perform all calculations.

4.5 RESULTS

4.5.1 *Arctotis hirsuta* (collection year 2016)

Scarification

The impact of scarification on the germination of *A. hirsuta* was statistically insignificant at the value of $p \leq 0.05$ (Table 4.2; Figure 4.3).

Gibberellic acid

All treatments, with the exception of 500ppm GA_3 /2H, differed significantly compared to the untreated control (See Table 4.2 and Figure 4.3). Germination following a GA_3-soaking in different concentrations over different periods for *Arctotis hirsuta* species was statistically significant at $p \leq 0.05$ for all treatments (See Table 4.2).

Table 4.2: The effect of pre-treating achenes with scarification and GA_3-soaking with varying concentrations over varying periods on *Arctotis hirsuta* (collection year 2016).

Treatment	**Germination(n)**	
	Mean	**Standard Error + Mean Group**
Control	0.465909	±0.47a
Scarification	0.397727	±0.40a
250ppm GA_3 /1H	0.352273	±0.35ab
250ppm GA_3 /2H	0.217391	±0.22ab
500ppm GA_3 /1H	0.193182	±0.19ab
500ppm GA_3 /2H	0.083333	±0.08b

One-way ANOVA F-Statistics

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatments	5	8.975	1.7949	2.04	0.071*

Mean values representing the effect of pretreating *A. hirsuta* (collection year 2016) with different concentrations of GA_3 over different periods and scarification are shown in columns. Means that do not share a letter are significantly different at $p \leq 0.05$ as calculated by Fishers least significant difference

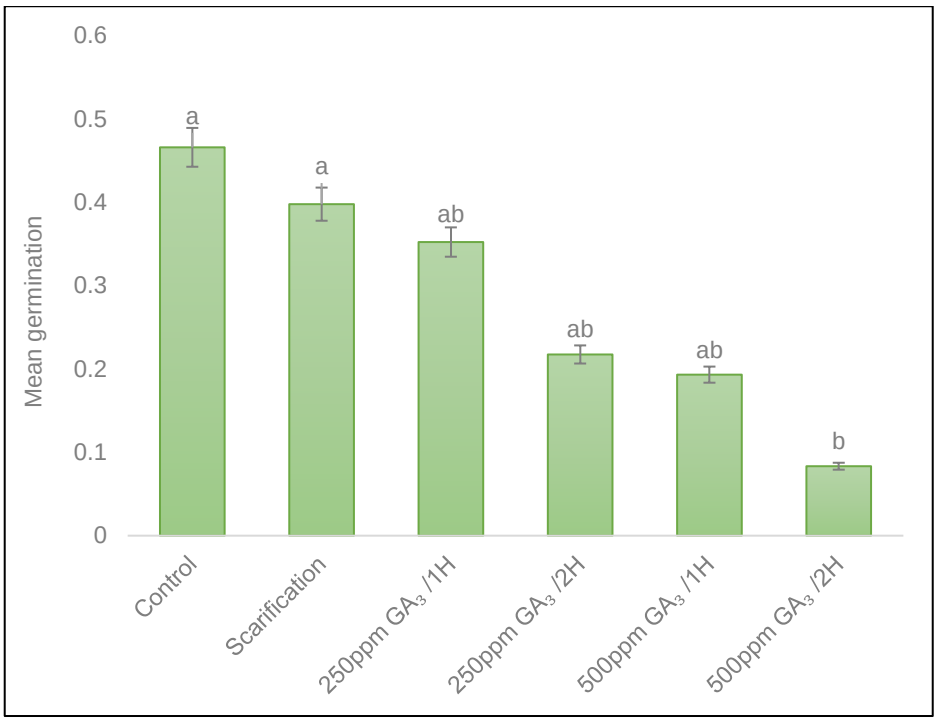

Figure 4.3: The mean germination percentage for *A. hirsuta* when pretreated with different GA_3-concentration and scarification. Mean values represented by different letters differ significantly at $p \leq 0.05$ as calculated by Fishers least significant difference.

4.5.2 *Cotula duckittiae* (collection year 2017)

Scarification

The scarified achenes obtained an inferior germination percentage (Table 4.3; Figure 4.4) in comparison to the untreated control.

Gibberellic acid

None of the four GA_3-treatments could positively affect the germination-percentage and result in the breaking of dormancy. Therefor no conclusion could be made regarding the interaction of GA_3 and its impact on dormancy.

Germination of pre-treated achenes through scarification and soaking in different strengths of GA_3 for this species was significant at $p \leq 0.05$ (See Table 4.3)

Table 4.3: The effect of pre-treating achenes with scarification and GA_3-soaking with varying concentrations over varying periods on *Cotula duckittiae* (collection year 2017).

Treatment	Germination(n)	
	Mean	**Standard Error + Mean Group**
Control	0.477273	±0.48a
Scarification	0.011364	±0.01b
250ppm GA_3 /1H	0.00000	±0.00b
250ppm GA_3 /2H	0.00000	±0.00b
500ppm GA_3 /1H	0.00000	±0.00b
500ppm GA_3 /2H	0.00000	±0.00b

One-way ANOVA F-Statistics

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatments	5	16.55	3.3110	23.06	0.000

Mean values representing the effect of pretreating *C. duckitiae* with different concentrations of GA3 and scarification are shown in columns. Means that do not share a letter are significantly different at $p \leq 0.05$ as calculated by Fishers least significant difference.

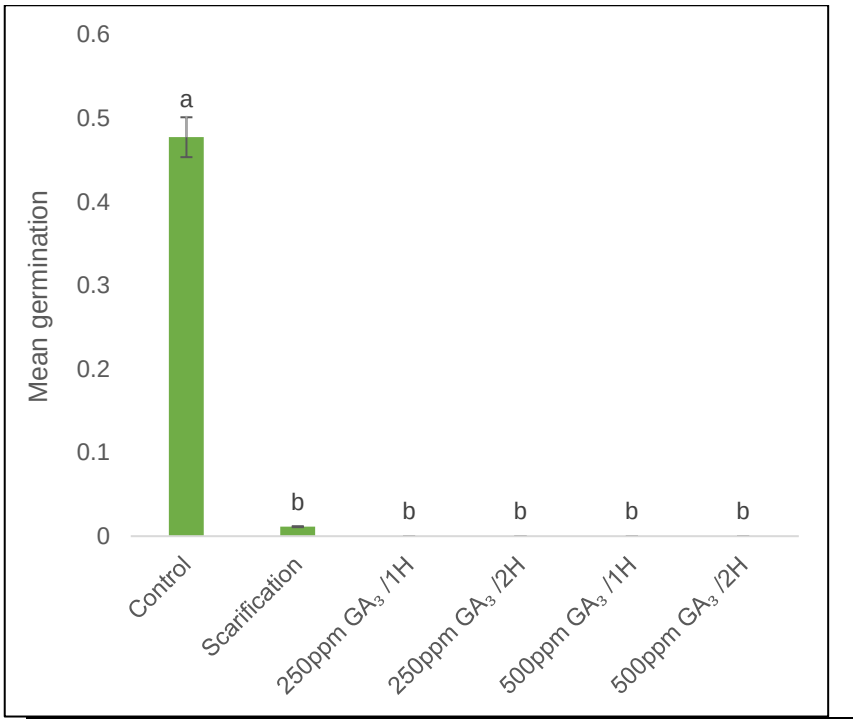

Figure 4.4: The mean germination percentage for *C. duckittiae* (collection year 2017) when pretreated with GA_3 and scarification. Mean values represented by different letters differ significantly at $p \leq 0.05$ as calculated by Fishers least significant difference.

4.5.3 *Oncosiphon suffruticosum* (collection year 2016)

Scarification

Results from the germination-experiment indicated that the scarification-method did not overcome dormancy to improve the germination-percentage. Despite being statistically

significant, scarification differed statistically compared to the untreated control (See Figure 4.5).

Gibberellic acid

None of the GA_3-treatments resulted in an increase in germination. The effect of soaking in different strengths over different periods of GA_3 on germination for this species was statistically significant at $p \leq 0.05$ (See Table 4.4) for all treatments.

Table 4.4: The effect of pre-treating achenes with scarification and GA_3- soakings with varying concentrations over varying periods on *Oncosiphon suffruticosum* (collection year 2016)

Treatment	Germination(n)	
	Mean	Standard Error + Mean Group
Control	0.375000	±0.38a
Scarification	0.136364	±0.14b
250ppm GA_3 /1H	0.095238	±0.10bc
250ppm GA_3 /2H	0.056818	±0.06bc
500ppm GA_3 /1H	0.000000	±0.00c
500ppm GA_3 /2H	0.000000	±0.00c

One-way ANOVA F-Statistics

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatments	5	8.686	1.7372	9.55	0.000

Mean values represented by different letters differ significantly at $p \leq 0.05$ as calculated by Fishers least significant difference.

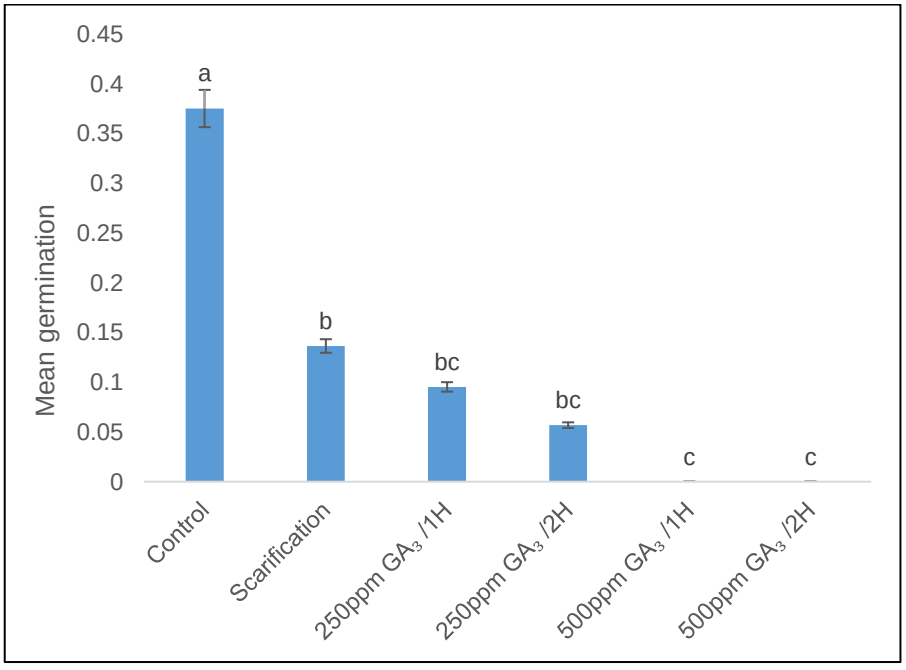

Figure 4.5: The mean germination percentage for *C. duckittiae* (collection year 2017) when pretreated with GA_3 and scarification. Mean values represented by different letters differ significantly at $p \leq 0.05$ as calculated by Fishers least significant difference.

4.5.4 *Oncosiphon suffruticosum* (collection year 2017)

Scarification

This pre-treatment measure did not manage to reduce the impact of seed-dormancy and resulted in a lower germination percentage than the achenes of the untreated control (See Figure 4.6).

Gibberellic acid

Pre-treatment of the achenes by means of GA_3-soakings was not effective and did not result in improved germination. Three of the four treatments, 250 ppm (H), 250 ppm (2 H) and 500 ppm (H) yielded very low germination (See Figure 4.6). No germination was recorded for the treatment of 500 ppm (2 H). The germination for all form of pre-treatments were statistically insignificant for $p \leq 0.05$.

Table 4.5: The effect of pre-treating achenes with scarification and GA_3- soaking with varying concentrations over varying periods on *Oncosiphon suffruticosum* (collection year 2017).

Treatment	Germination(n)	
	Mean	Standard Error + Mean Group
Control	0.284091	±0.28a
Scarification	0.056818	±0.06b
250ppm GA_3 /1H	0.034091	±0.03b
250ppm GA_3 /2H	0.021739	±0.02b
500ppm GA_3 /1H	0.011364	±0.01b
500ppm GA_3 /2H	0.000000	±0.00b

One-way ANOVA F-Statistics

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Treatments	5	5.089	1.0178	3.01	0.011

Mean values representing the effect of pretreating *O. suffruticosum* (collection year 2017) with different concentrations of GA_3 and scarification are shown in columns. Means that do not share a letter are significantly different at $p \leq 0.05$ as calculated by Fishers least significant difference.

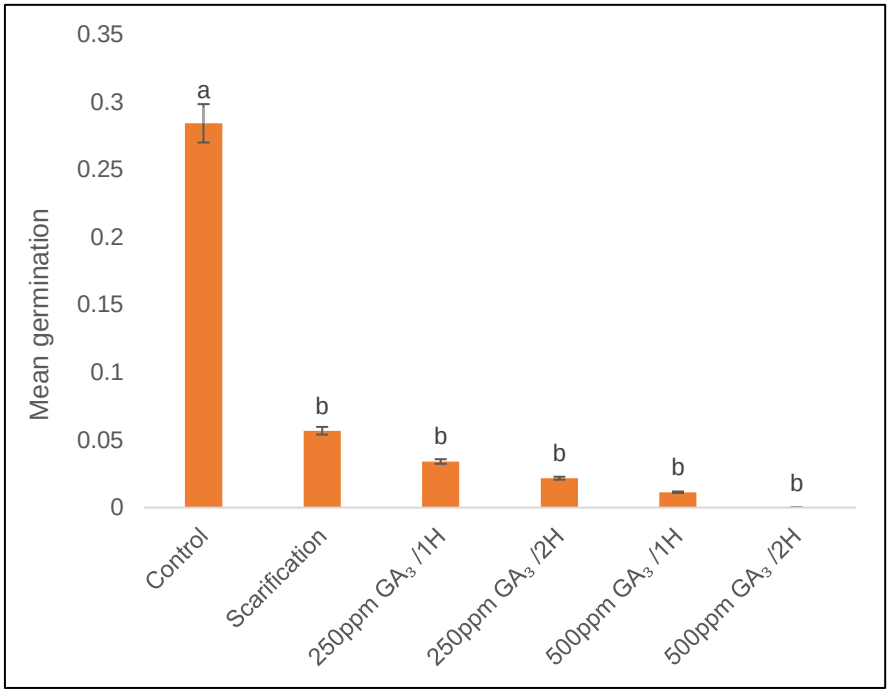

Figure 4.6: The mean germination percentage for *O. suffruticosum* (collection year 2017) when pretreated with different GA_3- concentrations over different periods and scarification. Mean values represented by different letters differ significantly at $p \leq 0.05$ as calculated by Fishers least significant difference.

4.6 DISCUSSION & CONCLUSION

4.6.1 Scarification

Although this method resulted in some germination for *A. hirsuta*, dormancy was not totally overcome and the seed still exhibited some form of dormancy. Dormancy does not appear to involve a hard seed coat, as germination is not improved by scarification to the extent that it significantly reduced or nullified the effect of dormancy (Bunker, 1994). This result agreed with the findings of improved germination for Asteraceae-annuals through a scarification pre-treatment (Atwater, 1980; Visser, 1993; Bunker, 1994; De Villiers, 2000). Visser (1993) found that scarification improved germination of three winter-annual species without totally overcoming dormancy.

Scarification is not always effective with all species of Asteraceae annuals, as the findings of extremely low germination percentages in *C. duckittiae* (collection year 2017) and *O. suffruticosum* (collection years 2016 & 2017) attest to. Bunker (1994) reported zero germination for several Asteraceae winter-annuals, despite the success of a pericarp-scarification pre-germination treatment advocated by several researchers (Hartmann & Kester, 1997; Copeland & McDonald, 2001).

Germination-limitations encountered by the species can be attributed to low oxygen permeability, and/or mechanical restriction by the pericarp (De Villiers *et al.,* 2002b). In nature this feature is a crucial element in the survival of species since it regulates the maintenance of a healthy seed bank (Fountain & Outred, 1991).

To conclude, in order to increase the germination percentage, further tests would be needed to determine how other pre-germination treatments can improve germination. Germination-results may signify that some species may exhibit more than just a single form of germination-impediment. Research by De Villiers *et al*. (2002b) indicated that a high germination percentage can be obtained by subjecting South African winter-rainfall annuals to several different treatments (leaching, hydration/dehydration or the combination of scarification and leaching).

4.6.2 Gibberellic-acid treatment

None of the various concentrations influenced germination in a way to successfully mitigate dormancy. Experiments involving *A. hirsuta* revealed that germination with the untreated control exceeded that of the four different GA_3 -treatments. Results of the GA_3-treatments carried out with *C. duckittiae* (collection year 2017) & *O. suffruticosum* (collection years 2016 & 2017) yielded very poor to no germination. This is not in agreement with the findings that applications of GA_3 can overcome dormancy by improving germination of Asteraceae (Chaharsoghi & Jacobs, 1998; Ha, 2014), and in particular winter-annuals (Bunker, 1994; Plummer & Bell, 1995; Hoyle *et al*., 2008).

In achenes of *O. suffruticosum* (collection year 2017) none of the GA_3-applications improved germination in the dark. This is in contrast with the results of several researchers which indicated such applications can aid seed germination in the absence of light (Bunker, 1994; Lopez del Egido *et al*., 2019).

Storage-conditions of 15°C and 15 RH at the Kirstenbosch NBG until the experiments were conducted, were perhaps not the ideal conditions or long enough to enable after-ripening of the different species. In several species of Asteraceae, where a specific after-ripening requirement is considered a prerequisite for optimum germination, treatments with GA_3 may not induce germination (Willis & Grove, 1991).

In conclusion, the parameters at which the experiments were evaluated, are perhaps not the most suitable for the species to ensure optimum germination, especially with regards to the application of GA_3. This is because optimum germination is not always guaranteed when

achenes are pre-treated with a GA_3-application. Germination may be improved with the testing of several different concentrations applied together with the optimum temperature and light-condition for a species. This is to determine the most efficient GA_3-concentration to improve germination.

Further studies would be required to determine germination if scarification and GA_3 are applied as a combinational pre-treatment. In addition to this, treatment-applications like leaching, administered prior to scarification, must also be considered. Studies can also be conducted into the most effective storage-conditions (per individual species) which will ensure optimum germination.

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CHAPTER FIVE

THE INFLUENCE OF AFTER-RIPENING ON THE GERMINATION OF THREE SELECTED ASTERACEAE ANNUALS FROM THE WINTER-RAINFALL REGION OF SOUTH AFRICA

THE INFLUENCE OF AFTER-RIPENING ON THE GERMINATION OF THREE SELECTED ASTERACEAE ANNUALS FROM THE WINTER-RAINFALL REGION OF SOUTH AFRICA

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5.1 ABSTRACT

Germination-challenges prevent several Asteraceae annuals from South Africa's winter-rainfall region from being introduced *en masse* as display-ornamentals at the Kirstenbosch National Botanical Garden (KNBG). Poor germination also stymies the cultivation of rare and threatened species on the Red Data list for South African species. *Cotula duckittiae* (L. Bolus) K. Bremer & Humphries*, Oncosiphon suffruticosum* (L.) Källersjö and *Arctotis hirsuta* (Harv.) Beauvard are species from the Fynbos- & Succulent Karoo Biome which struggle to germinate when grown from fresh wild-collected seed at KNBG. The red listed Vulnerable (VU) *C. duckittiae* has potential as an ornamental like the popular *A. hirsuta, whereas O. suffruticosum* is popular in traditional medicinal. The purpose of this study was to investigate methods to improve germination of the three species. Pre-germination treatments consisted of two different after-ripening treatments over two periods (30 °C & 45 °C for 4 and 8 weeks). Seeds of all species were then subjected to pre-determined light and temperature conditions as follows: *A. hirsuta* (collection year 2016) [22 °C in light]*, C duckittiae* (collection year 2017) [22 °C in light], *O. suffruticosum* (collection year 2016) [12 °C in light] and *O. suffruticosum* (collection year 2017) [22 °C in dark] to ensure optimum germination. None of the pre-treated achenes germinated compared to untreated control.

Key words: wild-collected seed, germination-challenge, cultivation, red-data listed, medicine, temperature, ornamental,

5. 2 INTRODUCTION

5.2.1 Seed dormancy in South African winter-rainfall annuals

Seed dormancy in several plant species is a seasonal adaptation (Milberg & Anderson, 1997) that is linked directly to seasonal temperature (Atwater, 1980). This reflects the immense unpredictable nature of arid and semi-arid regions where the seeds of many winter-rainfall plant species are dormant during dispersal by the parent plant. This is due the germination phenology of winter-rainfall annuals that is highly linked to the seed's responsiveness to the seasonal temperature cycle (Baskin & Baskin, 2001; Willis *et al.,* 2014).

The prostate *Arctotis hirsuta* (bright orange, cream or yellow flowers)*,* slightly erect *Cotula duckittiae* (orange) and the erect *Oncosiphon suffruticosum* (yellow-flowers) are three Asteraceae annuals from South Africa's winter-rainfall region. Flowering for all species is mainly from August to October and the trio of species starts dispersing seed from middle to late spring (Magee, 2011; Oliver, 2011; Oliver, 2014). In order to survive the unfavorable conditions of the summer season, these three winter annuals die and survive the harsh environment as dormant seed (Capon, 2005) to mitigate against adverse environmental conditions during summer which can result in its extinction (Tran & Cavanagh, 1984; Capon, 2005). Dormancy facilitates the reduction of the physiological activities within the seed to the lowest level required for survival (Capon, 2005; Tarasoff *et al.,* 2007; Bentsink & Koorneef, 2008). However, despite it being paramount that winter annuals survive unfavorable, harsh summers as seed, the very same harsh conditions are often enabling the breaking of seed dormancy through persistent intervals of high temperature (Mott & Groves, 1981; Langkamp, 1987; Peishi *et al.,* 1999).

5.2.2 Influence of after-ripening on germination

In various species, seed dormancy is naturally lost in storage through a physiological process namely after-ripening (Copeland & Macdonald, 2001; Bentsink & Koorneef, 2008), because in a natural habitat the seeds in the soil seed bank gets exposed to environmental conditions (Iglesias-Fernandez *et al.,* 2011) which enhances the breaking of dormancy (Commander, 2008). After-ripening in winter-annuals occurs during a period of dry-storage of freshly harvested seeds (Copeland & Macdonald, 2001; Finch & Savage, 2006), when the seeds are characteristically dormant at maturity, requiring after-ripening during summer (Probert, 1992). This is a very effective means of breaking dormancy in several species (Tothill, 1977; Baskin & Baskin, 1976; Bell, 1999; Commander *et al.,* 2009; Turner *et al.,* 2009; Aghillian *et al.,* 2014; Erickson *et al.,* 2016, Ma *et al.,* 2018; Wang *et al.,* 2019), and specifically in the family Asteraceae (Mott & Groves, 1981; Peishi *et al.,* 1999; Schütz *et al.,* 2002; Aleman *et al.,* 2011).

After-ripening (allows for the expansion of a new germination-window within which seeds are now able to germinate (Qaderi *et al.,* 2012). Seeds that have been subjected to an after-ripening period, are not only able to germinate quicker (Tothill, 1977; Rubio de Casa *et al.,* 2014) and also improves germination (Tothill, 1977; Daehler & Goergen, 2005; Moyo *et al.,* 2009; Mira *et al.,* 2011). The progressive loss of dormancy in mature dry seeds may take from a few days to a few years (Gutterman, 2000; Schütz *et al.*, 2002; Shi-Zeng *et al.,* 2013; Mira *et al.,* 2011) and varies from species to species (Aleman *et al.,* 2011; Iglesias-Fernandez; 2011). In several winter-growing annuals composites it was found that long periods of high temperatures is needed to overcome dormancy (Mott & Groves, 1981; Peishi *et al.,* 1999).

Given the dearth of knowledge, it is not sure whether the fresh wild-collected seed will after-ripen in storage conditions that will facilitate the breaking of dormancy. Seed intended for restoration-purposes can be utilized after 1-2 year after being stored in conditions not optimum (e.g. 5 or -18 °C) for after-ripening over a short period (Merrit *et al.,* 2004; Commander *et al.,* 2009). The ability to manage an accelerated form of after-ripening can contribute to the understanding of propagation of several species and aid conservation-efforts such as restoration (Commander, 2008). Although the after-ripening process is a known method to overcome seed dormancy in several species, storage conditions (period and germination) for the after-ripening of these winter rainfall annuals of South Africa has not yet being determined. The purpose of this study was to determine whether an accelerated from of after-ripening can be a successful intervention to improve germination.

5.3 MATERIAL AND METHODS

5.3.1 Growth chamber experiment

The experiments were conducted from June to August 2020. It was located in the growth chambers of the tissue culture facilities at the Kirstenbosch Research Centre of the South African National Biodiversity Institute (SANBI) at the Kirstenbosch NBG, Newlands, Cape Town.

5.3.2 Seed preparation

Seeds of the various species have been collected in the winter-rainfall areas of the Western Cape and Northern Cape Provinces of South Africa by the researcher or sourced from Silverhill Seeds during 2016 and 2017. Seed collected over the two periods are referred to as A (2016) and B (2017) respectively. Seed of all three species have been collected during October 2016. Only one collection of *A. hirsuta* has been made in 2016, hence *C. duckittiae* (A), *O. suffruticosum* (A). In 2017 seed of *Cotula duckittiae* (L. Bolus) K. Bremer & Humphries and *Oncosiphon suffruticosum* L.) Källersjö were collected during October and November

respectively. This seed will be referred to as *C. duckittiae* (B) and *O. suffruticosum* (B). No collection of *A. hirsuta* was made for this year. Following collection, seeds were stored in paper envelopes at 15 °C and 15% relative humidity in a cold room at Kirstenbosch (KNBG) until the start of the experiments.

5.3.3. Experiments with after-ripening at high temperatures

5.3.3.1 After-ripening

Achenes were hermetically sealed in aluminium foil bags in growth chambers set at temperatures of 30° and 45° over four and eight weeks. Achenes (untreated used as the control) were then surface sterilized in 1% liquid sodium hypochlorite for 2 minutes, rinsed with sterilized deionised water and then placed on two moist layers of Whatman no. 3 filter paper discs (5 ml sterilized deionized water was used to moisten the filter discs) in plastic Petri dishes (90 mm x 15 mm). All petri dishes were sealed with parafilm. During the incubation period, some of the filter paper discs were re-moistened with sterilized deionized water to prevent complete drying out. Experiments involving the *A. hirsuta* consistently got infected and it was decided to increase the liquid sodium hypochlorite concentration to 2%.

Every treatment, which consisted of 20 seeds per Petri-dish, were repeated four times in constant light or dark conditions (all for 24 hours). For treatments in total dark conditions, the petri-dishes were wrapped in aluminum-foil.

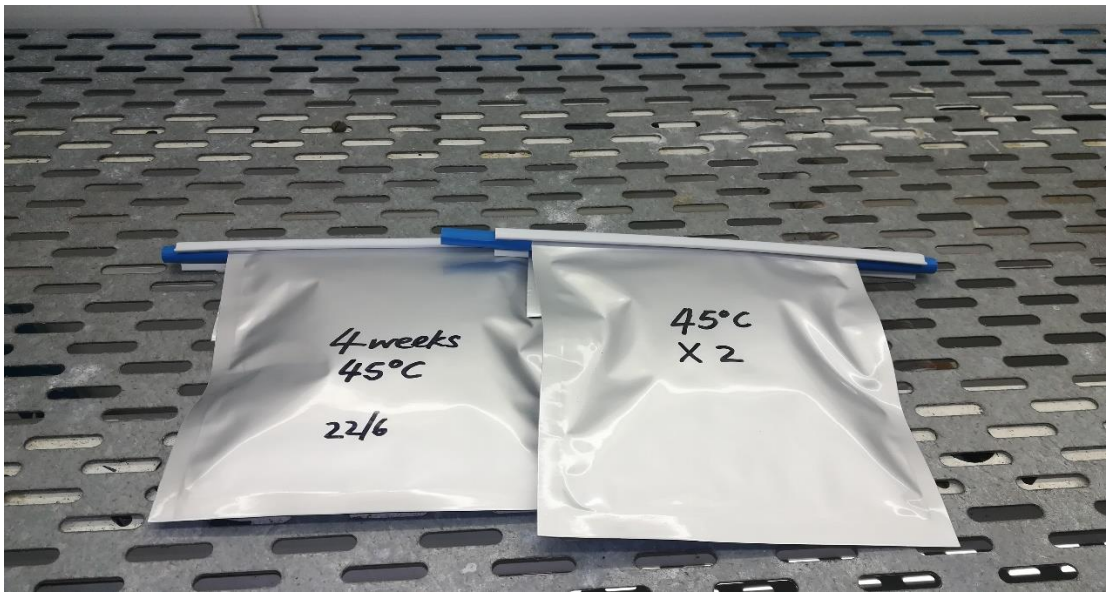

Figure 5.1: Some of the hermetically sealed achenes stored at 45 °C at 4 and 8 weeks.

Every treatment consisted of 20 seeds per Petri-dish and has been repeated four times under the optimal temperature and light conditions (See Table 4.1) of the three species as determined from data captured from experiments in 3.4 of Chapter 3.

Table 5.1: The three winter-annuals with the temperature and light-conditions needed for optimum germination.

Species	Temperature	Light-condition
Arctotis hirsuta (collection year 2016)	22 °C	Light
Cotula duckittiae (collection year 2017)	22 °C	Light
Oncosiphon suffruticosum (collection year 2016):	12 °C	Light
Oncosiphon suffruticosum (collection year 2017):	22 °C	Dark

Experiments for the range of pre-germination treatments were as follows:
T1: Untreated (control)
T2: 30 °C/ 4 weeks
T3: 45 °C/ 8 weeks
T4: 30 °C/ 4 weeks
T5: 45 °C/ 8 weeks

Achenes were incubated in a GC-550R growth chamber with 4000-8000 LUX illumination. This chamber is equipped with a timer-device that allows regulation of the temperature and light required over a 24-hour period. Germination-counts were done either every day or second day for 21 days. Achenes subjected to dark conditions, were only counted at the end of the 21 days. For this experiment, germination was considered as the emergence of the radicle (Perez-Fernandez & Rodriguez-Echeverria, 2003).

Figure 5.2: Achenes of *O. suffruticosum (*collection year 2017) at 22 °C in darkness after being exposed 30 °C & 45 °C for varying periods.

Figure 5.3: Achenes of *C. duckittiae* (collection year 2017) subjected to temperatures of 22 °C in light following after-ripening treatments of 30 °C and 45 °C for varying periods.

5.4 STATISTICAL ANALYSIS

All data collected was statistically analysed using one-way analysis of variance (ANOVA).

5.5 RESULTS

5.5.1 *Arctotis hirsuta* (collection year 2016)

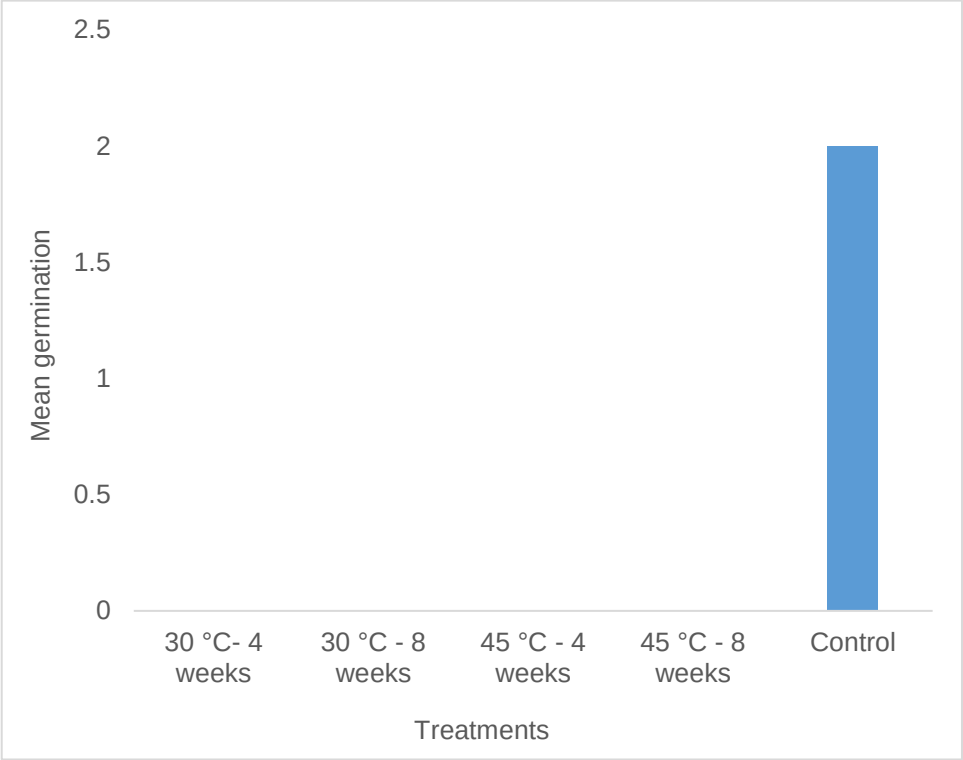

Figure 5.4: The effect of an after-ripening pre-treatment at temperatures of 30 °C & 45 °C over varying periods for *A. hirsuta* (collection year 2016)

No germination was possible for any of the 4 different treatments.

5.5.2 *Cotula duckittiae* (collection year 2017)

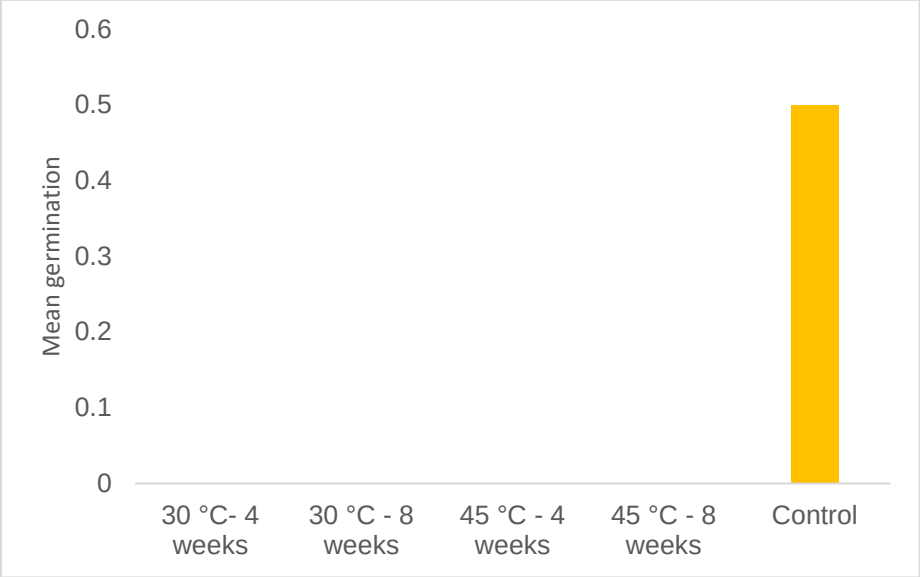

Figure 5.5: The effect of an after-ripening pre-treatment at temperatures of 30 °C & 45 °C over varying periods for *C. duckittiae* (collection year 2017)

No germination was recorded for any of the 4 different treatments.

5.5.3 *Oncosiphon suffruticosum* (collection year 2016)

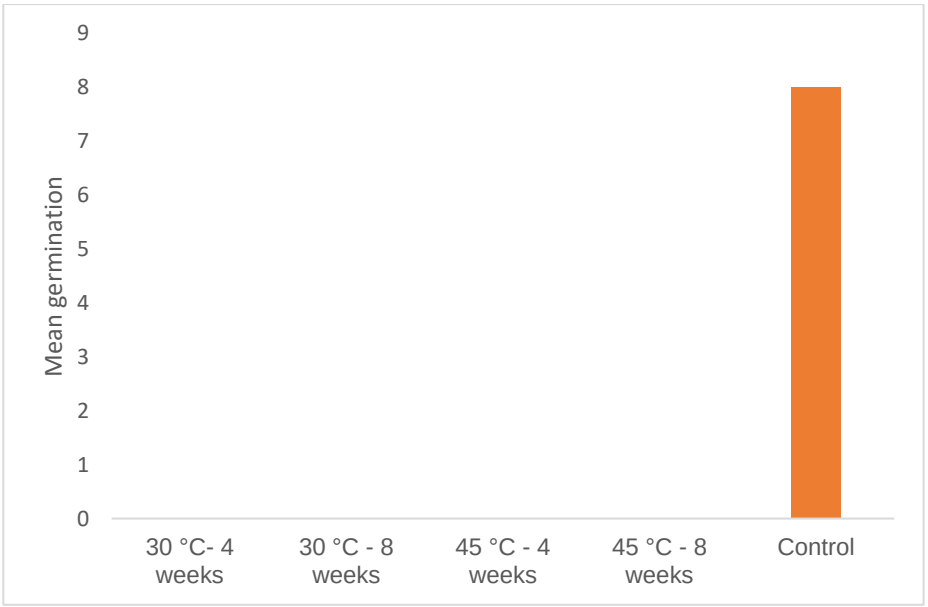

Figure 5.6: The effect of an after-ripening pre-treatment at temperatures of 30 °C & 45 °C over varying periods for *O. suffruticosum* (collection year 2016)

No germination was possible for any of the 4 different treatments.

5.5.4 *Oncosiphon suffruticosum* (collection year 2017)

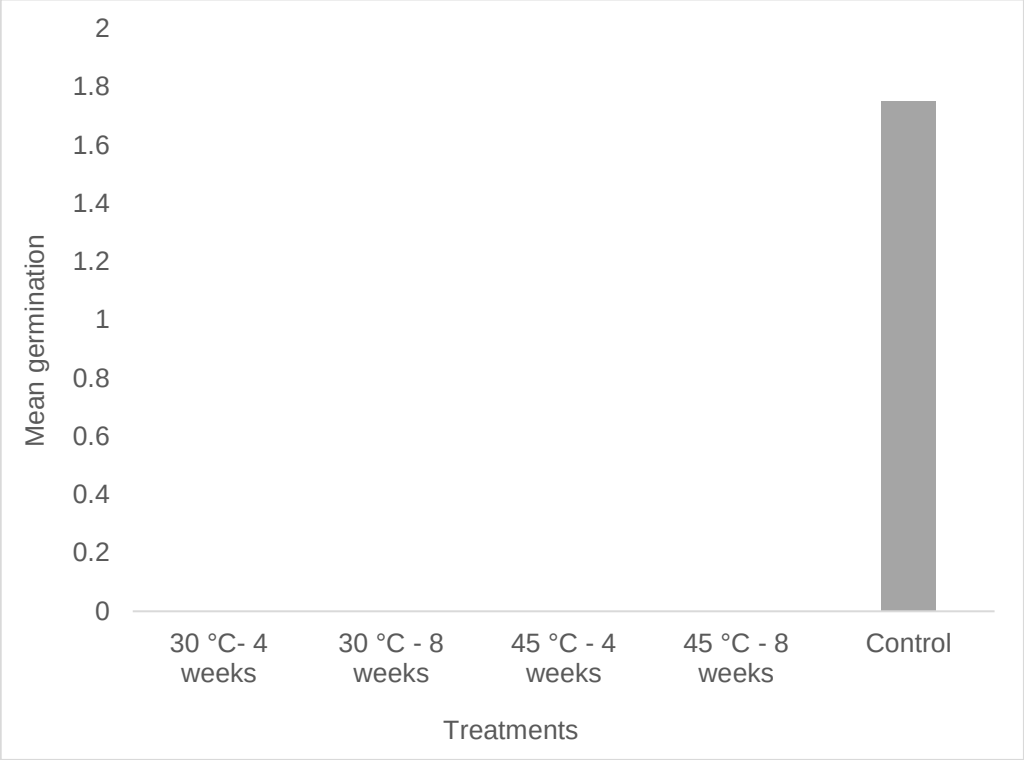

Figure 5.7: The effect of an after-ripening pre-treatment at temperatures of 30 °C & 45 °C over varying periods for *O. suffruticosum* (collection year 2016)

None of the various after-ripening pre-treatments resulted in germination.

5.5.5 Combined graph

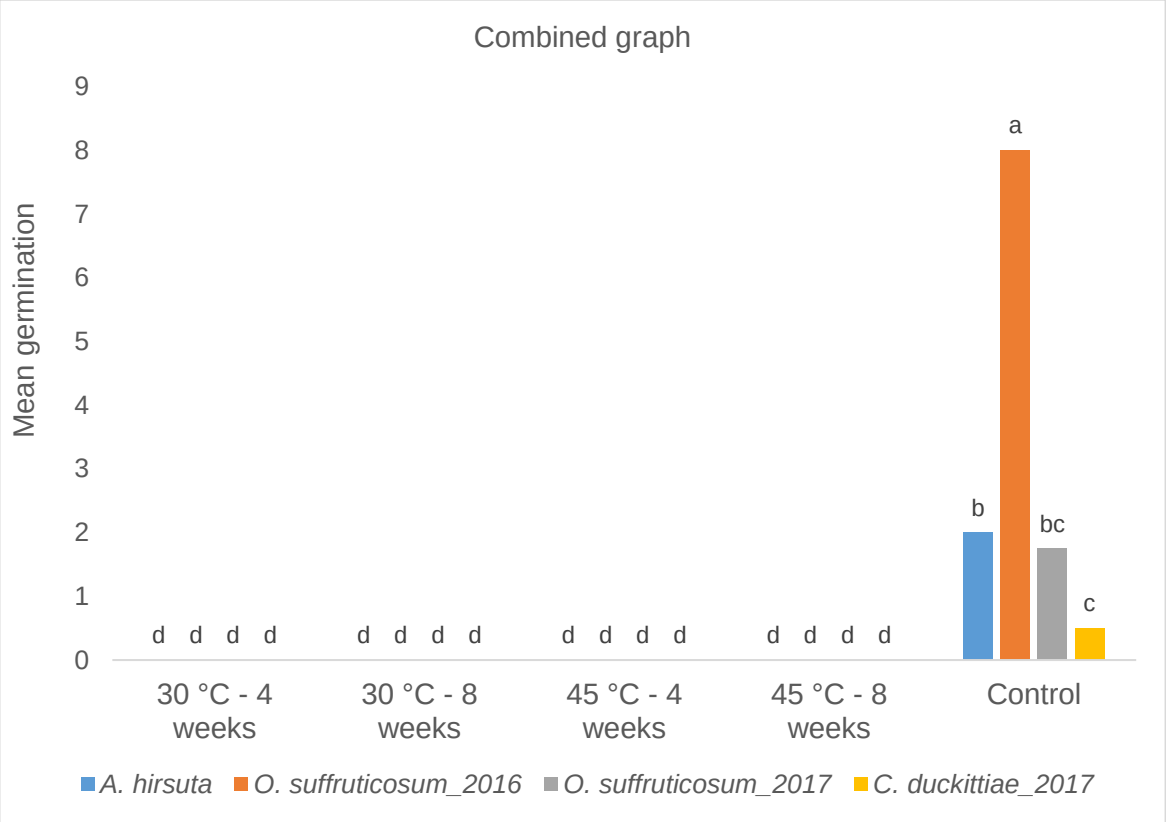

Figure 5.8: A combined graph reflecting mean germination (untreated vs treated) of all species used in the experiments.

5.6 DISCUSSION & CONCLUSION

None of the accelerated high temperature after-ripening treatments resulted in improved germination. Results did not agree with the findings that germination improved in several winter dormant annuals over the after-ripening period (Baskin & Baskin, 1976; Mott & Groves, 1981; Peishi *et al.,* 1999; Aleman *et al.,* 2011).

Visser (1993) argued that the period of after-ripening can differ in some species. The dormancy-mechanism, and perhaps more importantly, the survival of the species (Capon, 2005), in winter-rainfall annuals seeds is geared toward facilitating a germination-period in which water is not a limiting commodity (Langkamp, 1987), especially in areas of dry summer heat (Le Roux & Schelpe, 1997; Willis *et al.,* 2014). The probability for seedling survival and establishment must be high (Baskin & Baskin, 1976; Bewley & Black, 1994). Seeds may fail to germinate even though they may have been exposed to suitable environmental conditions as some seeds have more complex requirements (Toogood, 2011).

Temperature during after-ripening is another species-specific aspect which can impact germination (Commander *et al.*, 2009, Baldos *et al.*, 2014). There are species which require low temperatures over a long period of after-ripening (Schütz *et al.*, 2002). Peishi *et al.* (1999) found some annual species require higher temperatures over variable periods of after-ripening to improve germination. For this experiment where the control represented a storage-temperature of 15° C compared to after-ripening temperatures of 30° C and 45°C over periods of four and eight weeks, some germination were registered for the control compared to zero germination for the two other temperatures. This may suggest the three species may require lower temperatures of after-ripening to effect improved germination. The exposure to after-ripening at the two higher temperatures could therefor have contributed to the zero germination. According to Peishi *et al.* (1999) storage life may also impact on the germinability of winter-annuals. In the two Australian winter-annuals, *Schoenia filifolia* subsp *subufolia* and *Rhodanthe chlorocephala* the germination were lower after storage periods of 18 and less than 24 months respectively. This after-ripening experiment for the 3 South African winter-rainfall annuals were all done on seeds stored for a period of more than 24 months.

In conclusion it would be worthwhile to consider specific temperature-requirements or periods for after-ripening per species. This is in line with findings that after-ripening at lower-temperatures can be beneficial some species (Finkelstein *et al.,* 2008; Iglesias-Fernandez *et al.,* 2011; Santo *et al.,* 2014). Such an application also resulted in improved germination for Asteraceae winter-annuals (Schütz *et al.*, 2002).

Future experiments can also investigate the impact of subjecting achenes to varying temperature after-ripening treatments immediately following dispersal.

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CHAPTER SIX

GENERAL DISCUSSION AND CONCLUSION AND RECOMMENDATIONS

6.1 GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

Chapter 2 focused on the potential of the three annuals *Arctotis hirsuta* (Harv.) Beauvard, *Cotula duckittiae* (L. Bolus) K. Bremer & Humphries and *Oncosiphon suffruticosum* (L.) Källersjö as garden-ornamentals, a medicinal plant and a Vulnerable Red-listed species. This chapter confirmed that there is a gap in the existing body of knowledge on the germination ecology of the three Asteraceae winter annuals in South Africa. Further research are therefore required to ensure germination-challenges can be overcome in their propagation at the Kirstenbosch NBG

Chapter 3 found that germination of *A. hirsuta* achenes is the result of a temperature and light interaction for temperatures between the 22 °C and 27 °C in the light. The impact of the interaction between temperature and light/dark phases on the germination of *C. duckittiae* (collection year 2016) were insignificant through a range of temperatures and light- or dark-conditions. This could also be observed from the interaction of alternating temperature under cyclic light/dark conditions on the germination of *C. duckittiae* (collection year 2016) at 12 °C/22 °C compared to 17 °C/27 °C. Achenes of *C. duckittiae* (collection year 2017) achieved optimum germination in the dark at 22 °C, but very low to no germination in all the other treatment combinations. In *O. suffruticosum* (collection year 2016) the best germination was achieved at 12 °C in the light, however this did not differ very much from 17° C, (in light), 22° C (in light) and 7° C (in dark). *O. suffruticosum* (collection year 2017) recorded very low germination over all the treatments.

Results for all species, with exception of *A. hirsuta* which was not collected in 2017, indicated that better germination was obtained by achenes collected in 2016. Achenes of species collected in 2017 all spent less time in storage than those collected in 2016. Further studies must focus whether there is a need for a specific period and/or specific temperature at which dry storage must take place per species in order to achieve optimum germination.

Chapter 4 found that scarification and the GA_3-treatment had no impact on the germination of *A. hirsuta* (collection year 2016). None of the two pre-treatments improved germination for *C. duckittiae* (collection year 2017) and *O. suffruticosum* (collection years 2016 & 2017) and results suggested using GA_3 or scarification as a single pre-treatment applications under these condition are not effective in improving germination. The parameters at which the experiments were evaluated, are perhaps not the most suitable for the species to ensure optimum germination, especially with regard to the applications of GA_3. This is because optimum germination is not

always guaranteed when achenes are pre-treated with a GA_3-application. Germination may be improved with the testing of several different concentrations applied together with the optimum temperature and light-condition for a species. This is to determine the most efficient GA_3-concentration to improve germination.

In order to increase the germination percentage, further tests would be needed to determine how other pre-germination treatments can counter dormancy-mechanisms. Germination-results may signify that some species may exhibit more than just a single form of dormancy. One method worth investigating is to subject the annuals species to several different treatments which may include: leaching, hydration/dehydration or the combination of scarification and leaching).

Further studies would be required to determine germination if scarification and GA_3 are applied as a combinational pre-treatment. In addition to this, treatment-applications like leaching, administered prior to scarification, must also be considered. Studies can also be conducted into the most effective storage-conditions (per individual species) which will ensure optimum germination.

Chapter 5 found that none of the accelerated high temperature after-ripening treatments at 30 °C and 45 °C could effect improved germination over the 4 and 8 weeks. It would be worthwhile to consider specific temperature-requirements or periods for after-ripening per individual species. Future experiments can also investigate the impact of subjecting achenes to varying temperatures after-ripening treatments immediately following dispersal. Experiments which compare the results of after-ripening at ambient temperature to those of the controlled conditions at the Kirstenbosch Seed room (15 °C at 15 RH) are also worth pursuing.

Overall this study has found that some species stored (after-ripened) at controlled conditions in a seed room, will have no specific requirements in order to alleviate dormancy as was the case of *C. duckittiae* (collection year 2016) when stored for between 28-32 months. Good germination was obtained over several temperature-ranges in light or dark conditions. *A. hirsuta* achieved optimum germination between 22 °C - 27 °C. Pre-treatments with scarification and GA_3 yielded very average results for this species. After-ripening, at the temperatures of 30 °C and 45 °C, as well as scarification and a GA_3-treatment, did not improve germination for *C. duckittiae* (collection year 2017) *O. suffruticosum (collection* year 2016) and *O. suffruticosum (collection year 2017)* germinated.

CHAPTER SEVEN

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