AN INVESTIGATION INTO SEED GERMINATION REQUIREMENTS OF SOME OF THE NUT-FRUITED RESTIONACEAE

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I dedicate this thesis to my Grandmother
Iris Bruyns
Who instilled in me at a young age
A great love of our magnificent flora

PREFACE

The experimental work described in this dissertation was carried out in the Department of Botany, University of Cape Town, under the supervision of Assoc. Prof. J.M. Farrant and Prof. W.J. Bond.

I hereby declare that this thesis, submitted for the degree of Master of Science, is the original work of the author, and has not been submitted in any form to any other institution. Where use was made of the work of others, it has been duly acknowledged in the text.

Signed by candidate

Signature Removed

Rosemary Newton
March 2000

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ABSTRACT

The nut-fruited Restionaceae are a group of plants with mostly ant-dispersed (myrmecochorous) seeds that are endemic to the fynbos region of South Africa. Prior to this study, germination trials largely based on an ecological approach had not elucidated methods of improving seed germination in these species. Investigations of seed morphology and development, seed collection and sorting methods, and seed viability were conducted in species of *Willdenowia*, *Cannomois* and *Hypodiscus* to determine whether poor seed quality may have resulted in poor seed germination in previous studies. Morphological studies on *C. virgata* and *W. incurvata* using transmission electron microscopy showed the large starchy endosperm to be comprised mostly of starch grains and some protein bodies. The small and undifferentiated disk-like embryo, situated on top of the endosperm directly below the funiculus, comprised cells packed with lipid and protein bodies. Ultrastructural observations of mitochondria and nuclei showed these seeds to be typical of quiescent or dormant seeds. The seed coat was reduced to a few compact convoluted cell layers. A hard and lignified ovary wall surrounded the seed, the thickness of which varied between species. Developmental studies showed the importance of species-specific knowledge for successful seed collection. Collecting seeds naturally released from plants using the shaking or ground method yielded better quality seed collections than seeds cut from plants, however the former approach was found to be impractical in species with bony bracts. X-radiography most accurately identified full and non-fully formed seed. However removal of empty and partially formed seed from collections was most efficiently achieved by placing seed in water and discarding floaters. Weighing was a less accurate method of seed sorting. Electrolyte leakage was an unsuitable method of separating viable and non-viable seed. Seed viability (as revealed by tetrazolium tests) in sorted seed collections with fully developed seed was generally high, indicating that lack of seed germination could not be ascribed to poor seed quality. Poor germination therefore must have been a consequence of seed dormancy.

The approach in investigating seed dormancy was mainly a physiological one, in which experiments systematically tested for coat-imposed (physical, mechanical and chemical) and embryo (morphological and physiological) dormancy. Water uptake experiments suggested a lack of physical dormancy in species tested. It was unclear from embryo culture whether mechanical or chemical dormancy was operational in these seeds. The fire-prone *C. virgata* behaved differently to species of *Willdenowia*, and it appears as if they have different dormancy mechanisms. *C. virgata* seed appeared to require a period of soil storage, with wetting and drying and temperature fluctuation, during which embryo expansion appeared to occur. Germination in these seeds was subsequently enhanced by smoke treatment that would ecologically occur following a fire. Predictions of seed survivorship suggest that these seeds may remain viable in the soil, with a low percentage germinating during favourable winter conditions, until the occurrence of a fire, following which the majority of seeds germinate. The *Willdenowia* species used in this study inhabit environments that typically do not experience fire. Embryos of *W. incurvata* and *W. teres*, unlike *C. virgata*, imbibe to a lower water content when placed in water, which is thought to be too low for germination to proceed. These seeds might require, as part of the dormancy release mechanism, relief from mechanical restraint to allow further water uptake into the embryo. Scanning electron microscopy showed slight degradation of the ovary wall during soil storage, which over longer periods might relieve mechanical dormancy. During soil storage these species also exhibited embryo expansion, which may suggest a morphological dormancy, as was observed in *C. virgata*. A small percentage of seed germinated during each "winter" (laboratory simulation experiments) season in *W. incurvata*. Seed survivorship *W. incurvata* and *W. teres* when buried in the soil was less than half that of *C. virgata*. Smoke and heat did not improve seed germination. The strategy of *W. incurvata* appears to be one of gradual recruitment due to a loss of physiological and / or morphological and / or mechanical dormancy during the favourable germination conditions of winter, with seed germination unrelated to fire. Dormancy mechanisms in *W. teres* are still unknown.

ABBREVIATIONS

ABA	abscisic acid
ANOVA	analysis of variance
ca.	*circa*
df	degrees of freedom
DMB	dry mass basis
F	F statistic / F value
g	gram
GA	gibberellic acid
h	hour
H	Kruskal-Wallis test statistic
HgCl	mercuric chloride
H_2O_2	hydrogen peroxide
ISTA	International Seed Testing Association
kV	kilovolt
l	litre
m	metre
M	molar
mA	milliampere
min	minute
mol	mole
n	sample size
NaOCl	sodium hypochlorite
NaOH	sodium hydroxide
$NaPO_4$	phosphate buffer
NO_2	nitrogen dioxide
p	probability
rpm	revolutions per minute
s	second
S	siemen (unit of conductivity)
SD	standard deviation
SEM	scanning electron microscopy
TEM	transmission electron microscopy
WMB	wet mass basis
χ^2	Chi-squared value
°C	degree celcius

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CHAPTER 1

INTRODUCTION

The problem of understanding the mechanisms of seed dormancy release in a species is usually investigated using primarily either an ecological or physiological approach. The ecological approach involves applying known germination cues from species growing in similar environmental conditions or suspected cues that mimic environmental conditions preceding natural seedling appearance. The physiological approach attempts to identify and locate barriers to germination that may exist in the coat and / or embryo. Once barriers to seed germination have been identified, appropriate dormancy-breaking treatments (relating to the type(s) of dormancy in the seed) can be tested.

Although this division is largely artificial, past studies of the nut-fruited Restionaceae have generally used an ecological approach to seed germination, without much success. Morphological and physiological knowledge of seeds in general and their germination requirements has grown enormously over the past few decades. However little is known about physiological aspects of nut-fruited Restionaceae dormancy and germination. For the above reasons, this study adopted a physiological approach in investigating seed germination cues and possible dormancy mechanisms of some of the nut-fruited Restionaceae. The more specific objectives of this study included the examination of physiological and morphological aspects of seed development, seed collection, seed quality, seed storage / ageing and seed dormancy, and attempted to relate these findings to seed ecology.

This chapter will provide an overview of:

a) basic seed morphology, development and germination;
b) the physiological approach: seed dormancy;
c) the ecological approach: seed recruitment patterns and germination strategies in the fynbos;

d) seed dormancy breaking treatments: natural and artificial;
e) taxonomy, biology and germination of the Restionaceae; and
f) the motivation and study approach.

1.1) Basic seed morphology, development and germination

Seeds may be broadly classified as orthodox or recalcitrant, depending on their ability to dry down to low moisture contents and survive (Roberts 1973). This classification is not always clear cut (Berjak & Pammenter 1994), as the degree to which a seed is recalcitrant or orthodox varies between species and with seed characteristics, and is consequently more correctly viewed as a continuum (Farrant *et al.* 1988). However generally speaking, orthodox seeds are tolerant of desiccation and can survive in the dehydrated state for a length of time, while recalcitrant seeds are sensitive to desiccation and cannot be effectively stored (Pammenter & Berjak 1999). Restionaceae seeds are typically orthodox and therefore recalcitrant seed characteristics will not be discussed further.

According to Boesewinkel and Bouman (1995), a seed is comprised of the following structures:

a) *embryo*, arising from the fertilisation of the egg cell by one of the male pollen tube nuclei;
b) *endosperm*, originating from the fusion of two polar nuclei in the embryo sac with the other pollen tube nucleus; and,
c) *testa or seed coat*, formed from one or both of the integuments surrounding the ovule.

The developing seed is surrounded by the ovary wall, and is connected to the placenta of the parent plant by the funiculus or funicle (ovule stalk). On seed release, the funiculus (whole or in part) abscises from the ovule, and leaves a scar termed the hilum. If the ovary wall remains attached to the seed following release (as in the case of the nut-fruited Restionaceae), the dispersal propagule is termed a fruit (Abercrombie *et al.* 1987 and Bewley & Black 1994).

Seed development and maturation involves a number of important morphological, cellular, and biochemical processes that result in embryogeny. Embryogeny includes histodifferentiation, storage tissue formation and reserve accumulation (growth) and embryo maturation (Kermode 1995). These processes, which are controlled both genetically and hormonally, in orthodox seeds culminate in the process of maturation drying, which is the normal terminal event in seed development (Vertucci & Farrant 1995). Following maturation drying, such seeds reach a metabolically quiescent state, and it is at this stage that seeds are often released from the plant (Bewley & Black 1994).

According to Ellis *et al.* (1985), an orthodox seed collection may comprise four types of seed:

a) viable, non dormant seeds, which germinate under a wide range of conditions;
b) viable, dormant seeds, which only germinate under specific environmental conditions;
c) non-viable seeds; and,
d) empty seeds.

It is crucial that germination experiments are conducted on seed lots that do not contain seed in the latter two categories, as inclusion of such seed would result in erroneously low germination values and incorrect conclusions about the viability of the seed lot. Methods of seed collection (requiring a knowledge of seed development) and seed sorting are therefore important to ensure that seed lots used in germination trials consist of a high number of viable seeds. The quality of a seed collection can be established through seed germination or other seed viability tests.

However, the fact that a seed is viable does not ensure that germination will take place, as germination is also dependent on the prevalence of favourable chemical and physical environmental conditions (external controls of seed germination). Yet, when all the environmental requirements for germination have been met, some viable seeds still fail to germinate. Such seeds possess internal controls of germination, and are considered to be dormant (Bewley & Black 1994).

According to Bewley and Black (1994), germination is defined as the period which "...*begins with water uptake by the seed (imbibition) and ends with the start of elongation by the embryonic axis, usually the radicle*". Germination encompasses a number of processes and events, comprising *inter alia* subcellular structural changes, respiration, macromolecular synthesis and cell elongation. Although germination *sensu stricto* does not include mobilisation of storage reserves or seedling growth and emergence, "germination" is often used loosely to include these additional processes by seed technologists or ecologists when plant establishment is of importance (Bewley & Black 1994). In this instance, "visible germination" is a more practical term that should be used to refer to radicle emergence (Bewley 1997).

The understanding of the complex process of seed germination is enhanced by knowledge of the physical, physiological and morphological state of the seed at maturity, the changes in these states that precede germination and the environmental conditions required for these changes to take place (Baskin & Baskin 1998). Successful seed germination therefore requires a knowledge of the ecology of the species in question, to enable collection of mature seed, and application of appropriate pre-treatments or germination conditions to break any dormancy that may be present within the seeds.

1.2) Physiological approach: seed dormancy

1.2.1) Seed dormancy

Seed dormancy is "*fundamentally the inability of the embryo to germinate because of some inherent inadequacy...*" (Bewley & Black 1994). Dormancy resulting from an inherent inadequacy in the seed is known as organic dormancy (Baskin & Baskin 1998).

The first comprehensive classification of organic seed dormancy was developed by Nikolaeva (1977) *loc. cit.* Baskin and Baskin (1998), in which she divided seed dormancy into two basic types: exogenous (coat-imposed) dormancy and endogenous (embryo) dormancy. Exogenous dormancy control encompasses a physical, mechanical or chemical restriction of germination by the seed coat or equivalent structures surrounding the embryo. Endogenous dormancy control includes physiological or morphological dormancy within the embryo, or a combination of these controls (Baskin & Baskin 1998).

A second classification of seed dormancy, which is not mutually exclusive of the first, concerns the time during which dormancy is initiated. Primary dormancy is initiated during seed development, resulting in seeds being dormant when released from the parent plant. Secondary dormancy, which is reversible, is initiated after seed dispersal when non-dormant seeds are placed in environmental conditions unsuitable for germination (Hilhorst 1995). Conditional dormancy is a dormancy state between fully dormant and non-dormant seeds in which germination occurs only over a specific, narrow range of environmental conditions (Baskin & Baskin 1998).

1.2.1.1) Coat-imposed / exogenous dormancy

Exogenous or coat-imposed dormancy is a dormancy in which the seed coat (testa) and / or surrounding structures (including the endosperm, pericarp or extrafloral organs) prevent seed germination (Bewley & Black 1994). According to Baskin and Baskin (1998), a seed coat or tissues enclosing an embryo may have three main possible effects which prevent germination: physical dormancy, mechanical dormancy and chemical dormancy. Physical dormancy includes interference with water uptake and gas exchange, mechanical dormancy involves mechanical restraint of the embryo, and chemical dormancy encompasses germination inhibitors originating from the seed coat or tissues surrounding the embryo (Bewley & Black 1994).

Physical dormancy

Physical dormancy is characteristic of seeds with hard impermeable seed coats, which delay germination primarily by preventing water entry (Baskin & Baskin 1998). Seed germination cannot take place until the coat becomes permeable to water, as water supply to the embryo is crucial for germination (Bradford 1995). Germination may also be inhibited by the surrounding structures of the embryo preventing oxygen supply to the embryo, which is necessary for cellular respiration (Corbineau & Côme 1995). Release from physical dormancy involves the impermeable seed coat or a specific portion of the seed coat (e.g. chalaza, hilum or strophiole) becoming permeable (Baskin & Baskin 1998).

Mechanical dormancy

Dormancy is imposed in some seeds by mechanical resistance to the emergence of the embryo. In these seeds, weakening of these restraining structures, either mechanically (Bewley & Black 1994) or physiologically by enzymes originating from the embryo (Black 1996), is necessary before germination can take place. Physiological changes leading to mechanical dormancy release may result in the breakdown of the restraining tissues (e.g. Jacobsen *et al.* 1976, Finch-Savage & Clay 1997, Toorop *et al.* 1998 and Welbaum *et al.* 1998) or an increase of the growth potential of the embryo to push through the restraining tissues (e.g. Hilhorst 1997).

Chemical dormancy

Chemical dormancy may be attributed to chemical inhibitors of seed germination that originate from the seed coat or structures surrounding the embryo. A wide variety of chemical inhibitors have been identified; some of the more common ones being abscisic acid (ABA), coumarin, catechin, tannins, inorganic ions and phenolic compounds (Bewley & Black 1994). According to Baskin and Baskin (1998), chemical dormancy may be broken by the removal or inactivation of the inhibitor(s), by reduced sensitivity of the embryo to the inhibitor(s) or by the counteraction of the effects of the inhibitor(s) by germination promoting chemicals.

1.2.1.2) Embryo / endogenous dormancy

If the block to germination lies within the embryo itself, it is known as embryo dormancy. Endogenous dormancy may be morphological or physiological, or a combination of these controls (Nikolaeva 1977 *loc. cit.* Baskin & Baskin 1998).

Morphological dormancy

Morphological dormancy occurs in seeds with rudimentary embryos, in which the embryo may be only 1% of the size of the seed (Baskin & Baskin 1998). These underdeveloped embryos require a maturation period of growth and / or differentiation before germination can take place (e.g. Rizzini 1973, Lush *et al.* 1984, Baskin & Baskin 1986 and Baskin & Baskin 1990). Embryo growth occurs after release from the parent plant, and requires favourable conditions such as moisture, suitable temperatures, and sometimes specific light / dark requirements (Baskin & Baskin 1998).

Physiological dormancy

Physiological dormancy is caused by a physiological inhibiting mechanism of the embryo that prevents radical emergence (Baskin & Baskin 1998). In other words, the embryo is metabolically deficient in some way (Bewley & Black 1994). Physiological dormancy is often associated with dormancy cycling, in which the dormancy status of a seed changes between dormant and non-dormant according to environmental conditions, such as temperature (Baskin & Baskin 1998). Physiological dormancy induction and release is usually a gradual process in which the environmental conditions favourable for seed germination can narrow or widen (Vleeshouwers *et al.* 1995).

Physiological dormancy may be due to a lack of some essential component(s) which are necessary for germination, or the presence of germination inhibitors in the embryo, such as ABA (Bewley & Black 1994). ABA is a well-known growth regulator or hormone, which *inter alia*, is involved with dormancy induction, especially during seed development (Hilhorst 1995). Gibberellic acid (GA) is a hormone that frequently controls seed dormancy along with ABA, however it is known for inducing rather than inhibiting germination (Hilhorst & Karssen 1992).

Physiological dormancy control may reside in the state of the cellular membranes within the embryo. According to Hilhorst (1998), membranes have been proposed to be the primary target for temperature perception, and alterations in cell membrane properties are involved in seed dormancy regulation. Cell membranes can undergo changes from a crystalline or gel phase to a liquid-crystalline or fluid phase. These changes may be associated with a change in temperature, change in hydration, changes in membrane proteins or fatty acid saturation of membrane phospholipids (homeoviscous adaptation) or interaction with chemical compounds such as alcohols (Hoekstra *et al.* 1997 and Hilhorst 1998). The characteristics and functioning of membranes can be dramatically altered if the membrane phase is changed (Hoekstra *et al.* 1997). As membranes control solute movements, their state can profoundly influence cell physiology, and in this way control seed germination (Bewley & Black 1994).

Although the mechanisms that underlie dormancy breakage are still not well understood, environmental physiological dormancy breaking treatments may lead to changes at a physiological level that result in dormancy release (Harada 1997). For example, afterripening (Groot & Karssen 1992 and Bianco *et al.* 1994) and light (Toyomasu *et al.* 1994 *loc. cit.* Hilhorst 1997) may lead to degradation of ABA and hence seed dormancy release. Dormancy release may be brought about by the same environmental factors producing different changes at a physiological level. For example, irradiation, stratification, afterripening and high temperatures have been linked to the production of GA, resulting in seed dormancy release (Khan 1997).

Knowledge of physiological and biochemical controls and the genetic basis of seed dormancy is rapidly increasing with advances in molecular biology. This research has begun to elucidate some of the hormonal and molecular changes that occur in the embryo during physiological dormancy breaking (Bewley 1997, Khan 1997 and Foley & Fennimore 1998). To best detect this type of dormancy, molecular studies involving, for example, comparison of gene expression and nature of protein synthesis in dormant and non-dormant seeds, is required (Bewley & Black 1994). This avenue of potential research, although beyond the scope of this study, is an exciting one that warrants further investigation.

The following section examines seed recruitment and germination strategies in plants in the Cape Floral Kingdom, of which the nut-fruited Restionaceae form an integral part.

1.3) Ecological approach: seed recruitment patterns and germination strategies in the fynbos

Fynbos is an important and unique vegetation type within the Cape Floral Region of South Africa, which is dominated by sclerophyllous evergreen shrub communities. The fynbos is essentially characterised by the presence of wiry evergreen aphyllous restioids, overstory proteoid shrubs with large leaves and small-leafed ericoid shrubs. Fynbos occurs between latitudes 31°S - 35°S and longitudes 18°E - 26°E in the south-western Cape, and is well known for its richness in plant species (8700 species) and high endemicity (Goldblatt 1978, Taylor 1978, Kruger 1979, Bond & Goldblatt 1984, Campbell 1985 and Rebelo 1996).

Important environmental factors that characterise the fynbos are the Mediterranean winter-rainfall climate with summer drought, nutrient poor soils and recurrent disturbance by periodic fires (Taylor 1978, Bond & Goldblatt 1984 and Kruger & Bigalke 1984). Vegetation types in other parts of the world experience similar environmental conditions, and perhaps due to convergent evolution, share similarities with the fynbos vegetation of the south-western Cape (Cody & Mooney 1978 and Parker & Kelley 1989). These vegetation types include Californian chaparral (Keeley 1991), central Chilean mattoral (Arroyo *et al.* 1994), macchia, maquis and garrique around the Mediterranean Sea (Naveh

1974 and Papió & Trabaud 1991) and south-western Australian mallee-heath (Bell *et al.* 1993).

Fire plays an important role in these ecosystems, directly influencing plant growth, survival and reproduction. Fire also impacts seed and seedling dynamics in these vegetation types (Bond & van Wilgen 1996). The characteristics of a fire, such as fire intensity (Bond *et al.* 1990), season of burn (Bond 1984, van Wilgen & Viviers 1985, Le Maitre 1988a and Le Maitre 1988b) and fire frequency (van Wilgen 1981) all influence the dynamics of vegetation regeneration (Kruger & Bigalke 1984). The survival strategy of a plant following a fire may be a vegetative and / or reproductive one. Vegetative survival essentially involves resprouting of the plant from buds protected by the fire (Bond & van Wilgen 1996). Reproductive responses to fire are more complex, and attempts have been made to correlate reproductive strategies of plants with life history strategies (Keeley 1994 and Keeley & Bond 1997).

Keeley (1994) identified three broad groups of plant recruitment in Mediterranean-climate regions, based on seed germination behaviour and life history strategies; namely disturbance-free recruitment, delayed disturbance-dependent recruitment or immediate post-fire recruitment. This classification was later refined for the fynbos region (Bond & van Wilgen 1996 and Keeley & Bond 1997).

According to Keeley and Bond (1997), the fynbos flora can initially be divided into plants that function independently of fire and plants that are dependent on fire cues at some stage in their life cycle. There are three main groups of plants that respond to fire-cues. The first are plants that exhibit a fire-cued seed release, which includes all the serotinous species with canopy stored seeds. The second group includes plants that have fire-stimulated flowering. The third is those plants which have fire-cued seed germination. This latter category can be further sub-divided into plants that have seeds that respond to physical germination cues, such as heat shock, and plants that have seeds that respond to chemical germination cues, such as smoke. Seeds sometimes require multiple germination cues, which may be a combination of physical and chemical signals.

1.3.1) Plants independent of fire-cues

The group of species that function independently of fire-cues is fairly small, and consists of some shrubs and herbaceous perennials. Some of these species avoid fire, as they are restricted to moist ravines, while other species may resprout after a fire. These woody species possess non-refractory seeds (seeds independent of fire cues for germination), typically form transient seed banks, and germinate and establish independently of fire. These species usually do not have special germination requirements. However alternating temperatures induce germination of some species, while others are triggered by light changes. Species exhibiting this type of recruitment include *Heeria* spp. and *Rhus tomentosa* (Anacardiaceae), *Petrorhagia prolifera* (Caryophyllaceae), *Maytenus* spp. (Celastraceae), *Cunonia capensis* (Cunoniaceae), *Diospyros* spp. (Ebenaceae), *Kiggelaria africana* (Flacourtiaceae), *Montinia caryophyllacea* (Montiniaceae), *Olea europaea* (Oleaceae) and *Anthospermum spathulatum* (Rubiaceae) (Keeley 1994, Bond & van Wilgen 1996 and Keeley & Bond 1997).

1.3.2) Plants dependent on fire-cues

1.3.2.1) Fire-cued seed release

Serotinous species, which exhibit fire-stimulated seed release, consist of suffrutescents and shrubs. These species have seeds that are retained in protective cones for a variable length of time, with seed release occurring mainly after a fire (Lamont *et al.* 1991 and Keeley 1994). Seeds are characteristically non-refractory, germinating readily in the first rainy season after shedding (Bond 1985 and Keeley 1994). These species therefore have a persistent seed bank in the canopy for the life of the parent, but a transient seed bank in the soil, as seeds often do not survive for more than one season once released (Bond & van Wilgen 1996).

Germination is delayed until the commencement of the rainy season, by either a high temperature-induced dormancy or a low temperature germination requirement (Deall & Brown 1981 and Bond 1984). Species that display this recruitment strategy include; *Helipterum* spp., *Phaenocoma* spp. and *Syncarpha eximia* in the Asteraceae, *Berzelia* spp., *Brunia* spp. and *Nebelia* spp. in the Bruniaceae, *Widdringtonia nodiflora* in the Cupressaceae, *Erica sessiliflora* in the Ericaceae and *Aulax* spp., *Leucadendron* spp. and *Protea* spp. in the Proteaceae (Deall & Brown 1981, Lamont *et al.* 1991, Mustart & Cowling 1991, Keeley 1994, Bond & van Wilgen 1996 and Keeley & Bond 1997).

1.3.2.2) Fire-stimulated flowering

Species that have a fire-stimulated flowering requirement include most of the geophytes and some herbaceous perennials (Keeley 1994). These plants characteristically resprout, and have fire-stimulated flowering in the first year, followed by seedling establishment in the second and possibly subsequent years (Bond & van Wilgen 1996). Seeds of geophytes form transient seed banks, and tend to germinate well in favourable conditions (Keeley 1994). Examples of families which have members with fire-stimulated flowering are the Amaryllidaceae (*Brunsvigia orientalis*, *Cyrtanthus angustifolius*, *C. ventricosus* and *Haemanthus canaliculatus*), Geraniaceae, Haemodoraceae (*Wachendorfia paniculata*), Iridaceae (*Bobartia spathacea*, *Geissorhiza*, *Moraea* and *Watsonia pyramidata*), Liliaceae (*Androsymbirium leucanthum*), Orchidaceae and Oxalidaceae (Le Maitre & Midgley 1992 and Keeley 1994).

1.3.2.3) Fire-cued seed germination

Species in which seed germination is directly or indirectly stimulated by the occurrence of a fire are prominent in the fynbos (Brown & Botha 1997). These species typically have soil-stored seeds that are refractory (require a fire cue for germination), are thought to be long-lived, and consequently maintain a persistent seed bank (Keeley 1994 and Bond & van Wilgen 1996). Fire-linked seed germination cues signal a favourable environment for seed germination (Brown & Botha 1997). Similar fire-linked germination cues have been found in plants in the Australian shrublands (Hughes & Westoby 1992, Bell *et al.* 1993 and Dixon *et al.* 1995), Californian chaparral (Keeley *et al.* 1985, Keeley 1987, Keeley 1991 and Keeley 1994) and Mediterranean shrublands (Thanos & Georghiou 1988, Parker & Kelly 1989, Roy & Sonié 1992 and Keeley 1994). These environmental germination cues may be physical (such as heat-shock, temperature fluctuation or light changes) or chemical (for example smoke and charate treatment). These germination cues, and examples of fynbos species that respond to these cues, are discussed in the following section.

1.4) Seed dormancy breaking treatments: natural and artificial

The ecological approach to seed dormancy involves the selection of treatments that closely imitate the environmental conditions that naturally result in seed dormancy release. This requires an intimate knowledge of the environmental conditions to which a seed is subjected prior to seedling emergence. The physiological approach to seed dormancy involves the selection of artificial treatments that mimic environmental conditions or their dormancy-breaking effects. Successful use of artificial treatments requires an understanding of the type of seed dormancy and the mechanisms or process resulting in seed dormancy release. Under the dormancy breaking treatment headings below, both artificial treatments resulting in seed dormancy release as well as natural germination cues that operate in the fynbos are discussed, with examples.

1.4.1) Scarification

Scarification is mainly an artificial laboratory treatment that breaks physical dormancy, and to a lesser extent, mechanical dormancy. Scarification may be achieved by acidic (e.g. Mackay *et al.* 1995, Brits 1996, Fu *et al.* 1996 and Baskin *et al.* 1998) or mechanical (e.g. Piotto 1995, Prasad & Nautiyal 1996 and González-Melero *et al.* 1997) treatments. In the fynbos scarification ecologically mimics the heat effects of a fire by causing the cracking or fracturing of the seed coat, resulting in the seed coat becoming permeable to water and gases. Scarification has improved germination in *Leucadendron* spp. of the Proteaceae (Brown & van Staden 1973a and Brown & Dix 1985).

1.4.2) Heat shock

Heat shock is a cue that primarily breaks coat-imposed physical and mechanical dormancy (Baskin & Baskin 1998). Both wet heat (e.g. Prasad & Nautiyal 1996, Jhurree *et al.* 1998, Nan *et al.* 1998 and Sacheti & Al-Rawahy 1998) and dry heat (e.g. Musil & de Witt 1991, Cocks & Stock 1997, Baskin *et al.* 1998 and Morrison *et al.* 1998) have been successfully employed in dormancy breakage. Heat from a fire may also break physiological dormancy by accelerating embryo afterripening (Baskin & Baskin 1998). Ecologically heat shock occurs at high temperatures, such as experienced in a fire (Bond *et al.* 1990).

Fynbos species that display heat shock-stimulated germination are mainly shrubs and suffrutescents (Keeley & Bond 1997). In these seeds, germination is enhanced by structural changes to the hard impermeable seed coat, caused by a fire. Cells of the seed coat or pericarp loosen in certain regions on exposure to heat. Cracking of the coat by heat and subsequent desiccation releases any mechanical restriction on the embryo and allows water and oxygen to enter, and germination to proceed (Brits *et al.* 1993).

Fynbos species requiring heat shock-simulation for germination are: *Elytropappus rhinocerotis* (Levyns 1929) and *Metalasia muricata* (Musil 1991) in the Asteraceae, *Aspalathus* spp. (Cocks & Stock 1997) and *Podalyria calyptrata* (Jeffery *et al.* 1988) in the Fabaceae, *Phylica ericoides* (Keeley 1992 and Kilian & Cowling 1992) and *Phylica stipularis* (Musil 1991) in the Rhamnaceae, *Agathosma* spp. (Blommaert 1972) in the Rutaceae and *Hermannia* spp. (Keeley & Bond 1997) in the Sterculiaceae.

1.4.3) Temperature fluctuation

Large temperature fluctuations, indicative of a gap in the vegetation, are known to break physiological dormancy (Brits 1986a and Pierce & Moll 1994). The removal of shade-providing plants by fire, and subsequent soil denudation, results in an increase in daily temperature fluctuation near the soil surface (Kruger & Bigalke 1984 and Brits 1986a). This indirect fire cue has been found to be important in breaking dormancy in species of *Leucospermum* (Brits 1986a, Brits & van Niekerk 1986 and Brits 1987) in the Proteaceae and in *Muraltia squarrosa* in the Polygalaceae and *Passerina vulgaris* in the Thymelaeaceae (Pierce & Moll 1994). Many fynbos seeds, once dormancy is broken, have a germination requirement of low alternating temperatures that correspond to the arrival of the autumn rains.

1.4.4) Dehydration and hydration cycles

Dehydration and hydration cycles in the soil, in which seeds are exposed to alternate wetting and drying, have been reported to break physical and mechanical seed dormancy, by gradual weakening of the seed coat during desiccation (Brits *et al.* 1993) or by microbial (fungal) action (Baskin & Baskin 1998). The process of wet-dry cycling (also referred to as hardening) of seeds (Rehman *et al.* 1998) may also break morphological or physiological dormancy (e.g. Griswold 1936 and Maynard & Gates 1963). Repeated wetting and drying of seeds often results in an increased germination rate in subsequent wetting (Vincent & Cavers 1978 and Baskin & Baskin 1982). Wet-dry cycles have also been reported to be beneficial to seed viability as cellular repair of hydrated dormant seeds often occurs during wetting (Priestley 1986). In the fynbos, desiccation treatments have improved seed germination in some species of *Leucospermum* (Proteaceae), where the impermeable seed coat inhibits gas exchange (Brits *et al.* 1993).

1.4.5) Afterripening

Afterripening is a process in which dry dormant seeds slowly lose dormancy over time (Quick & Hsiao 1984, Leopold *et al.* 1988, Esashi *et al.* 1993, Christensen *et al.* 1996 and Walck *et al.* 1997a). This process may occur in as little as a few weeks, or may take up to five years (Bewley & Black 1994). The efficacy of afterripening depends on the environmental conditions of moisture and temperature, and to a lesser extent oxygen (Bewley & Black 1994). If the prevailing conditions are not optimal (i.e. around 5 - 20% seed water content, 20 - 50°C and adequate oxygen supply) for afterripening to occur, afterripening may be prevented or occur at a slow rate (Bewley & Black 1994 and Joel *et al.* 1995). Under sub-optimal conditions for afterripening, or following afterripening, seed ageing or deterioration (which are also dependent on the interaction of time, temperature and humidity) may take place (Priestley 1986 and Campbell & Nicol 1997). Afterripening is associated with morphological or physiological dormancy breakage (Bewley & Black 1994 and Baskin & Baskin 1998). In the fynbos, afterripening might be important in canopy- and soil-stored seeds, in which seeds accumulate for several years prior to fire-cued release and germination. However the importance of afterripening in fynbos seeds is yet to be quantified.

1.4.6) Stratification

Stratification is a process in which dormancy in hydrated seeds is broken by a period of chilling (Deall & Brown 1981, Poulsen 1996, Jones *et al.* 1997, Ceccherini *et al.* 1998 and Downie *et al.* 1998). The length of chilling time and the optimal chilling temperature (usually between 1°C and 10°C) required for dormancy release varies depending on the species (e.g. Edwards & El-Kassaby 1995, Skordilis & Thanos 1995 and Jones *et al.* 1997). Stratification has been associated with the relief of physiological dormancy (Baskin & Baskin 1998) and mechanical dormancy in which the restraining tissues surrounding the embryo are weakened enzymatically (Downie & Bewley 1996). Germination of seeds with a requirement of stratification as a dormancy breaking treatment does not typically occur during these low temperatures. Rather seed germination occurs with warmer temperatures that ecologically signal the arrival of favourable germination conditions of spring (Bewley & Black 1994). In contrast, fynbos species typically have a low temperature requirement for germination, as seed germination occurs during cooler winter temperatures. Some species for which this low temperature requirement has been observed are *Leucadendron daphnoides*, *Protea compacta* (Brown & van Staden 1973b), *Protea magnifica* (Deall & Brown 1981) and *Leucospermum cordifolium* (Brits & van Niekerk 1986) of the Proteaceae and *Erica junonia* (Small *et al.* 1982) of the Ericaceae.

1.4.7) Light changes

Germination in some seeds may be inhibited by exposure to light (Koller 1964 and Baskin & Baskin 1998), however more often seeds have a light requirement for dormancy breakage (Giba *et al.* 1995, Dixit & Amritphale 1996, Afolayan *et al.* 1997 and Walck *et al.* 1997b). Both photoperiod (Joel *et al.* 1995) and light quality (Botto *et al.* 1998) are important factors that affect the dormancy status of the seed. Phytochrome is a photoreceptor pigment that exists as two reversible forms, and is involved in the perception of light (Devlin *et al.* 1997). The quantity (ratio) of these two reversible pigment forms is influenced by light quality, which is determined by the ratio of far red light to red light. In this way light, via phytochrome, controls seed dormancy (Bewley & Black 1994 and Baskin & Baskin 1998).

Phytochrome receptors are almost always located in tissues surrounding the embryo, and therefore should be classified as coat-imposed dormancy (Bewley & Black 1994). However changes in phytochrome on exposure to or shielding from light often result in physiological changes in the embryo (e.g. Casal & Sánchez 1998 and Roth-Bejerano *et al.* 1999) and for this reason it is often included with physiological dormancy. In addition light treatment can also relieve mechanical dormancy by enzymatically weakening the restraining tissues surrounding the embryo (Black 1996). Ecologically speaking, an increase in light exposure is characteristic of the post-burn environment, and may stimulate germination (Keeley 1987 and Bond & van Wilgen 1996). Light has been found to stimulate germination, as in *Metalasia muricata* (Pierce & Moll 1994) in the Asteraceae and *Protea cynaroides* (van Staden 1966) in the Proteaceae.

1.4.8) Leaching

Chemical inhibitors in the seed coat are a well-known dormancy mechanism in seeds (Bewley & Black 1994). Breaking of chemical dormancy may occur ecologically by leaching of inhibitors from the structures surrounding the embryo (Koller 1964, Forsyth & Brown 1982 and Thanos *et al.* 1995). This leaching effect can be achieved artificially by presoaking or rinsing seed (Duan &

Burris 1997 and Ren & Kermode 1999). Although this process has not been documented as a dormancy mechanism in fynbos seeds, it may be important, especially in soil-stored seeds that may experience several rainy seasons prior to germination.

1.4.9) Gases

Various gases have been linked to seed dormancy breaking. An increase in oxygen concentration or availability may directly break physiological seed dormancy, or may indirectly result in increased ethylene (Corbineau & Côme 1995), a growth regulator that is known to stimulate seed germination (Sutcliffe & Whitehead 1995 and Kepczynski & Kepczynska 1997). Increases in carbon dioxide also tend to promote seed germination, most likely due to its effects of enhancing ethylene biosynthesis (Corbineau & Côme 1995). If oxygen supply to the embryo is physically restricted by structures surrounding the embryo (e.g. van Staden & Brown 1977) or chemically restricted by inhibitors (e.g. phenolics) in the seed coat (refer to section 1.2.1.1) then seed dormancy is coat-imposed (Corbineau & Côme 1995). Dormancy due to a lack of oxygen may be relieved artificially by seed incubation in elevated oxygen concentrations (e.g. Brown & van Staden 1973a and van Staden & Brown 1973a) or imbibition in dilute hydrogen peroxide (e.g. Brown & Dix 1985, Brits 1986b and Brits & van Niekerk 1986).

In the fynbos, gaseous components of combustion that occur after a fire can alter the gaseous environment of the seeds and may be directly responsible for breaking seed dormancy. These components include ethylene, ammonia and nitrogen dioxide, which have been shown to enhance seed germination in *Erica hebecalyx* of the Ericaceae and a fire-stimulated chaparral species, *Emmenanthe penduliflora* (van der Venter & Esterhuizen 1988 and Keeley & Fotheringham 1997). Furthermore, oxygen concentrations increase in the soil after a fire, as a result of the reduced vegetation and organisms in the soil, and could consequently function as a germination cue for soil-stored seed (Brits 1986b).

A number of species of *Leucadendron* have been shown to respond to H_2O_2 and pure oxygen treatments (Brown & van Staden 1973a and Brits 1986b).

1.4.10) Organic and inorganic chemicals

According to Bewley and Black (1994) and Baskin and Baskin (1998), organic or inorganic chemicals have been successfully employed in physiological dormancy breakage.

1.4.10.1) Smoke and charate

Some seeds are stimulated by chemical changes in the environment following a fire (de Lange & Boucher 1990 and Brown 1993a). Wicklow (1977) first reported a germination response in refractory chaparral seeds to the presence of charred wood (charate). The positive effects of smoke on the germination of a fynbos species (*Audouinia capitata* of the Bruniaceae) were reported by de Lange and Boucher (1990). Subsequent to these discoveries, smoke, charate and aqueous smoke extracts have been shown to improve germination of many fynbos species (Brown 1993a and Brown 1994). The chemical nature and properties of the active compounds in smoke that act at a physiological level to break seed dormancy are still being investigated (Brown & van Staden 1997).

Keeley and Bond (1997) noted smoke-stimulated seed germination occurred in a large number of annuals, as well as some shrubs, suffrutescents and herbaceous perennials. Examples of fynbos species that respond to smoke include: *Euryops* spp., *Elytropappus* spp. (Keeley 1994), *Eriocephalus africanus*, *Helichrysum tinctum* (Keeley & Bond 1997) *Metalasia densa*, *Senecio grandiflorus* and *Syncarpha* spp. (Brown 1993a and Brown 1993b) in the Asteraceae, *Heliophila* spp. (Keeley & Bond 1997) in the Brassicaceae, *Erica* spp. (Brown 1993a and Brown *et al.* 1993) in the Ericaceae, *Penaea* sp. (Keeley & Bond 1997) in the Penaeaceae, *Protea compacta* and *Serruria phylicoides* (Brown 1993a) in the Proteaceae, *Chenopodiopsis* spp., *Dischisma capitatum*, *Hebenstreitia paarlensis* and *Nemesia* spp. (Keeley & Bond 1997) in the Scrophulariaceae and *Cliffortia* spp. (Keeley 1994) in the Rosaceae. Many Restionaceae have also been found to respond to smoke (Brown *et al.* 1994), and this is discussed in more detail in the next section.

1.4.10.2) Soil nutrients and allelochemicals

Heat (Cass *et al.* 1984) and the addition of smoke or charate (Stock & Lewis 1986) from a fire affects the nutrient status of the soil, and can lead to nutrient mobilization (Cass *et al.* 1984). Changes in the nutrient status can promote seed germination. For example, Thanos and Rundel (1995) found seed germination in three chaparral species was triggered by the addition of nitrogenous compounds, which increase in the soil following a fire. Reduction in soil allelochemicals (inhibitory leachates from plants that are detrimental to other plants, preventing seed germination or plant establishment) may also lead to dormancy release following vegetation removal after a fire (Chou & Muller 1972 and Pierce & Moll 1994).

1.4.10.3) Artificial methods

Artificial methods of breaking physiological dormancy include hormone applications, such as gibberellic acid (GA) (Small *et al.* 1982, Khan & Ungar 1998 and Kochankov *et al.* 1998) and cytokinins (Brown & Mitchell 1984 and Bewley & Black 1994).

Chemical applications of various elements, acids, aldehydes, alcohols and esters (Cohn 1996, Hou & Romo 1998 and Naredo *et al.* 1998) have been shown to break physiological dormancy. Nitrogenous compounds (e.g. nitrate, nitrite and thiourea), sulfhydryl compounds (dithiothreitol and 2-mercaptoethanol) and oxidants (such as hypochlorite) are other chemical compounds that are also known to improve seed germination (Bewley & Black 1994, Joel *et al.* 1995 and Wang *et al.* 1998).

1.4.11) pH

Smoke or charate can also influence soil pH (Cass *et al.* 1984). The general effect of fire is an increase in soil pH, due to the addition of basic cations to the soil (Moore 1996), however the magnitude of this increase can vary from negligible to highly significant (Cass *et al.* 1984). Brown and van Staden (1997) found that germination in smoke-stimulated seeds was not affected by varying the pH (pH 2.3 to pH 9.3) of the smoke extract (normal pH = 3.3). However pH is known to influence seed germination (Choudhury & Gupta 1998), and should not be discarded as a possible germination stimulant (Keeley & Fotheringham 1998a).

1.4.12) Embryo culture

Embryo culture or removal of the seed coat is an artificial technique that may be used for relief from physical and mechanical dormancy (e.g. Negi & Todaria 1995, Thapliyal & Naithani 1996 and Cantos *et al.* 1998) and also chemical dormancy (Stabell *et al.* 1996, Thapliyal & Naithani 1996 and Wang 1997).

1.4.13) Biotic action

Biotic factors that can lead to physical dormancy breakage include microbial action during soil storage (Brits 1996 and Morpeth *et al.* 1997) and acid or mechanical scarification in the digestive tract of animals (Baskin & Baskin 1998). No examples of seed scarification in the digestive tract of an animal to my knowledge have been published for fynbos seeds.

1.4.14) Multiple germination cues

Often cues do not act in isolation to break dormancy, as in some species more than one type of dormancy and / or germination requirement may be in operation. For example, in *Protea compacta* (Brown & van Staden 1973a) and *Leucadendron tinctum* (Brown & Dix 1985) of the Proteaceae, there is a seed coat-imposed dormancy coupled with a low temperature requirement for seed germination. In these species scarification and low temperature treatments both improve seed germination. *Erica hebecalyx* of the Ericaceae also responds to the multiple cues of heat and ammonia or ethylene gas which are released during a fire (van der Venter & Esterhuizen 1988). Multiple germination cues are also found in other fire-driven ecosystems: for example, in the chaparral plants

Adenostoma fasciculatum and *Eriodictyon crassifolium*, heat shock and smoke together promote germination (Keeley 1987).

1.5) Taxonomy, biology and germination of the Restionaceae

1.5.1) Taxonomy of the Restionaceae

The Restionaceae is a family of evergreen rush-like monocotyledonous plants occurring almost exclusively in the Southern Hemisphere (Hutchinson 1973 and Linder 1984). The richest concentration of members of this family is undoubtedly in the Cape Floristic Region, which contains 314 species (Linder 1985), of which 300 are endemic to the region (Brown *et al.* 1998). Of the remaining species in the family, one hundred are found in Australia, three in New Zealand, one in Malaysia and south-east Asia, and one in Chile (Brown *et al.* 1994). This distribution pattern indicates that the Restionaceae are likely to be a Gondwanan relic (Linder 1987).

A cladogram for the genera of the South African Restionaceae, modified from Linder (1991), is shown in Figure 1.1. The evolution of a woody nut occurred at node 5.2, including the genera *Anthochortus*, *Mastersiella*, *Cannomois*, *Hypodiscus*, *Ceratocaryum* and *Willdenowia* in the group of nut-fruited Restionaceae. Myrmecochory (ant-dispersal) evolved at node 6.3, marked by the appearance of elaiosomes. Elaiosomes are present on most species of *Mastersiella*, *Cannomois*, *Hypodiscus*, *Ceratocaryum* and *Willdenowia.* There has however been a loss of both elaiosomes and ant-dispersal in some species of this group (Swift 1994 and Linder, personal communication).

1.5.1.1) <u>Seed morphology</u>

Organs surrounding the developing seed include the outer bracts, perianth, ovary wall and inner seed coat. These organs may function in protecting the developing seed, or assist in seed dispersal (Linder 1991). It should be noted that the correct terminology for the dispersal propagule in the Restionaceae is a fruit. However for reasons of simplicity, "fruit" has been, and will be, referred to as "seed" in the text.

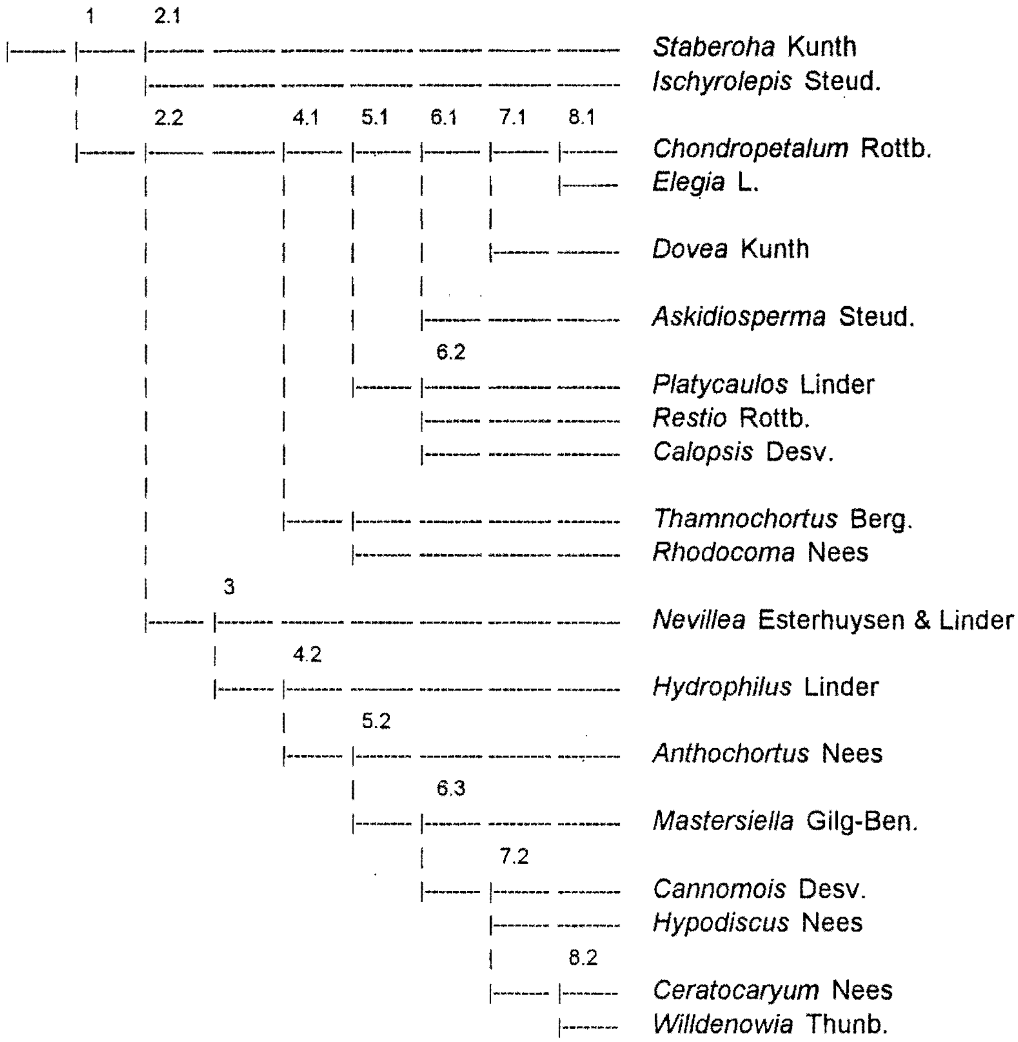

Figure 1.1. A cladogram, modified from Linder (1991), showing the evolutionary relationships between the African genera in the Restionaceae. Nuts evolved at node 5.2 and myrmecochory (ant-dispersal) evolved at node 6.3.

In the nut-fruited Restionaceae, the bracts function to protect the young flowers. These bracts are prominent in the myrmecochorous species – for example, the bracts of *Cannomois* can be bony and very difficult to separate until the seed is mature. In contrast, the perianth is reduced to small membranous scales. An important characteristic of the nut-fruited Restionaceae is that the fruit is indehiscent. The evolution of this characteristic resulted in the function of protection to be shifted from the seed coat to the ovary wall. The thick and heavily lignified ovary wall of the nut-fruited Restionaceae is remarkably hard, and is believed to be an adaptation for defence (Linder 1991). The ovary wall, which may be smooth or pitted (Linder 1984), protects both developing and mature seeds. As a result of the thickening of the ovary wall, the protective function of the seed coat has been reduced. The seed coat in nut-fruited Restionaceae is very thin, and does not contain tannins (Linder 1991).

1.5.2) Biology of the Restionaceae

Knowledge about the biology of Restionaceae species is still very poor, and therefore strategies and functions have, in some instances, been inferred from morphology and sparse field observations (Linder 1991).

1.5.2.1) Habitat

According to Bond (1981), Restionaceae tend to dominate areas of seasonal drought, as well as waterlogged areas in the fynbos (Linder 1991). Members of the Restionaceae are known to exhibit a greater habitat-specificity than other dominant elements of the fynbos flora (Milewski & Esterhuysen 1977). The nut-fruited Restionaceae as a group do not appear to be restricted to a specific habitat. Species occur from coastal plains (e.g. *Cannomois parviflora*, *Hypodiscus alternans* and *Willdenowia teres*) to rocky mountainous fynbos (e.g. *Cannomois aristata*, *Hypodiscus montanus*, *Mastersiella purpurea* and *Willdenowia stokoei*) growing on well-drained nutrient-poor sandy soils (e.g. *Cannomois nitida*, *Hypodiscus aristatus* and *Willdenowia incurvata*) to occasionally wet clays or marshy sands (e.g. *Hypodiscus rugosus* and *Willdenowia purpurea*) (Linder 1985).

1.5.2.2) Reproductive biology and seed development

Many gaps still exist in the knowledge of the reproductive biology of the Restionaceae. The South African Restionaceae are dioecious. Members of this family are wind-pollinated (Linder 1991). Flowering occurs mainly during spring or late summer, with seed being produced six to eleven months later (Brown *et al.* 1998). Seeds range from very fine seed with approximately 10000 seeds per gram (*Chondropetalum tectorum*) to the large nut-like seeds of *Ceratocaryum argenteum* which reach up to 10 mm in diameter (Jamieson & Brown 1995).

Early embryological studies in the Restionaceae include embryo sac development in *Elegia racemosa* and *Restio dodii* (Borwein *et al.* 1949), *Thamnochortus fruticosus* (Młodzianowski 1964) and *Hypodiscus aristatus* (Krupko 1962). In his studies of the embryology of the nut-fruited *Hypodiscus aristatus*, Krupko (1962) found that there was a significant tendency for embryos in seeds to remain undeveloped. This could reduce the quality of a seed collection.

More recently Kircher (1986) published results on his investigation of the embryology of 36 species from 18 genera in the Restionaceae, in which he stated the embryology of the Restionaceae to be of the *Polygonum* type. These seeds, by virtue of their massive starchy nucellar tissue, are endospermous, with endosperm development preceding embryo formation (Boesewinkel & Bouman 1995).

Although much is known on the embryology of the Restionaceae, embryogeny (post-fertilisation seed development), and especially embryo development, is unknown (Kircher 1986 and Johri *et al.* 1992). What is known is that following fertilisation seeds of the nut-fruited Restionaceae are retained on the plant for a long period of time, during which the endosperm is liquid. Shortly before seed release, the endosperm hardens (Linder 1991). The mature embryo is small, lens-shaped, and not fully differentiated (Hutchinson 1973 and Johri *et al.* 1992).

1.5.2.3) Seed release and dispersal

Three types of seed release and dispersal groups have been recognised in the Restionaceae. The first group is characterised by a dehiscent ovary with one to three locules, whereas the latter two groups have an indehiscent unilocular ovary (Linder 1991).

a) The plesiomorphic (ancestral) condition is a dehiscent ovary, from which the seed is released when mature. Seed may be retained in the ovary for different periods of time, as older spikelets have been observed containing seed. The mechanism of seed dispersal following release from the ovary is unknown. Genera belonging to this group include *Restio*, *Ischyrolepis*, *Askidiosperma*, *Chondropetalum*, *Dovea*, *Nevillea*, *Rhodocoma*, *Elegia* and several species of *Calopsis*.

b) Wind dispersal occurs in seeds that typically have a persistent perianth. This perianth acts as a wing for the fruit. Genera that are wind dispersed include *Thamnochortus*, *Staberoha* and *Calopsis*.

c) Ant dispersal (myrmecochory) occurs in seeds that have a fleshy oil body, or elaiosome. In myrmecochorous fruits the elaiosome is derived from flower internodes, the ovary wall, or occasionally the flower pedicel (Linder 1991). Interestingly, some species within the myrmecochorous clade lack elaiosomes (Linder 1984). The dispersal mechanism of these species is unknown, as the seeds drop to the base of the plant when mature, where they often remain (Linder 1991), or may be buried by shifting sands (personal observation). The majority of myrmecochorous species are, by definition, ant-dispersed, but those that are not include *Hypodiscus alternans* and *Willdenowia striata* (Linder 1984), *Ceratocaryum argenteum* (Linder 1991) and *Willdenowia incurvata* (personal observation).

Myrmecochory is not unique to the Restionaceae. It is believed that 20% of species in the Cape Floral Region possess ant-dispersed seed. This type of seed dispersal is common to many different families in the fynbos, e.g. Asteraceae, Bruniaceae, Cyperaceae, Fabaceae, Penaeaceae, Proteaceae, Rutaceae and Stilbaceae (Bond & Slingsby 1983). Myrmecochory is also very common in the Australian shrublands (Milewski & Bond 1982 and Westoby *et al.* 1982).

Myrmecochorous seeds are released mainly in the summer when ants are most active. When these seeds drop from the plant they are immediately collected, chiefly by *Anoplolepis* spp., for their oil-rich elaiosome (Slingsby & Bond 1981 and Bond *et al.* 1991). Researchers have showed that removal of the elaiosome, which functions to attract ants and stimulate carrying behaviour, resulted in poor seed removal (Brew *et al.* 1989). However elaiosome removal had no direct effect on seed viability (Majer 1982) or seed germination (Slingsby & Bond 1985). Following collection, seeds are buried in chambers or horizontal channels 4 - 7 cm (occasionally up to 12 cm) below ground (Bond *et al.* 1991) and germinate from this depth (Musil & de Witt 1990). The effects of soil storage on the nut-fruited Restionaceae are unknown.

In this mutualistic relationship the ants benefit from a high quality food source and the plants benefit from dispersal of their seed (Buckley 1982). Seed dispersal by ants has the advantage of a "safe site" strategy (Cowling *et al.* 1994), in which the seeds are protected from potentially detrimental surface disturbances such as fire (de Lange & Boucher 1993) and predation (Bond & Breytenbach 1985). It was evident from the many empty and broken seed coats scattered at plant bases of *Willdenowia incurvata* that seeds not actively buried by ants were susceptible to rodent predation (personal observation). A further benefit of myrmecochory to many seeds is dispersal to nutrient rich sites. However this does not appear to be the case in ecosystems with nutrient poor soils, such as the fynbos and sclerophyllous vegetation of south-western Australia (Rice & Westoby 1986 and Bond & Stock 1989). Although a disadvantage with myrmecochory is that dispersal to new localities is limited, due to short transport distances of seed from the parent plant (Slingsby & Bond 1985 and Westoby *et al.* 1991), this disadvantage outweighs the safe-site benefit provided by the ants (Andersen 1988).

1.5.2.4) Fire survival

According to Linder (1991), many of the Restionaceae resprout after fire, which occurs with a frequency of between five and forty years (Brown & Botha 1997). Resprouting is likely to be the dominant regeneration mechanism in the family. A nut-fruited Restionaceae species that resprouts after fire is *Willdenowia sulcata*. Yet not all Restionaceae plants survive fire, and some species, such as the nut-fruited *Cannomois virgata*, are killed by fire. Fire survival of plants is related to the season of burn – fire prior to new culm initiation is not as lethal as a burn after new culm initiation (Linder 1991). Seed regeneration following fire, especially in Restionaceae that do not resprout after a fire, has been observed (e.g. *Mastersiella purpurea*) by Vlok (*loc. cit.* Linder 1991). Myrmecochorous species survive the heat of fires by seed being buried deep in the soil. This deep burial implies unusual germination cues (Bond *et al.* 1991).

1.5.3) Germination of the Restionaceae

Horticulturists and researchers at Kirstenbosch Botanical Gardens have done much research into Restionaceae germination (*inter alia* Brown *et al.* 1994 and Brown *et al.* 1998). Possible factors that contribute to poor germination in these seeds include limited seed set in some species, and difficulty in determining when seeds are mature, resulting in collection of immature and potentially non-germinable seed (Brown & Botha 1997). The unusual early hardening of the ovary wall surrounding the seed gives the impression that the seed is mature and ready for collection. However this occurs long before the endosperm has solidified and embryonic tissue has matured (Jamieson, personal communication and personal observation). Immature seeds are green on dissection (Jamieson & Brown 1995). Collection of mature seed of the nut-fruited Restionaceae is further exacerbated due to the paucity of information on the timing of flowering, seed maturation and release of individual species (Clegg 1980 and Brown *et al.* 1998). Poor screening methods may also account for some of the observed poor seed germination.

According to Brown and Botha (1997), three seed treatments that have improved seed germination in some of the Restionaceae are:

a) heat treatment of seeds in *Staberoha distachya* and *Calopsis impolita* (Musil & de Witt 1991);

b) high and low diurnal temperature fluctuation; and,

c) treatment of seeds with plant derived smoke or aqueous smoke extracts in many genera, including *Askidiosperma*, *Calopsis*, *Chondropetalum*, *Dovea*, *Elegia*, *Ischyrolepis*, *Restio*, *Rhodocoma*, *Staberoha* and *Thamnochortus* (Brown *et al.* 1994 and Jamieson & Brown 1994).

Although these treatments have increased germination in some Restionaceae species tested, they have not significantly improved germination in the nut-fruited myrmecochorous species from the typical germination success rate of less than five percent. In fact, germination in this group of seeds, despite tests by different researchers of various dormancy-breaking treatments, germination requirements, and combinations thereof, has proved notoriously difficult to achieve. This is particularly the case in species belonging to the genera *Cannomois*, *Hypodiscus* and *Willdenowia*. The only exception is *Cannomois virgata*, which responded to smoke treatment with an improved germination from 2 ± 5% to 18 ± 13% (Brown *et al.* 1994). Table 1.1 documents the published and known unpublished germination tests by various researchers, none of which have resulted in significant increases in germination in the nut-fruited Restionaceae. It is interesting to note that germination requirements of myrmecochorous chaparral species are also poorly known (Keeley & Bond 1997).

Table 1.1. Unpublished and published germination tests of dormancy-breaking treatments in *Cannomois* spp., *Hypodiscus* spp. and *Willdenowia* spp. in the nut-fruited Restionaceae. No germination results are given, as these treatments did not result in significant levels of germination. Results from unpublished experiments were obtained as personal communication from the following researchers: WB = William Bond, HdL = Hannes de Lange and HJ = Hanneke Jamieson.

Species	Dormancy-breaking treatment	Germination conditions	Researcher
C. parviflora	Smoke: Seeds sown in trays, then exposed to smoke for 2 h in a smoke tent.	Seeds in sterilised soil, incubated at 10°C (night) and 25°C (day) in open shade.	Brown *et al.* 1994
C. virgata	None.	Incubation of aseptic embryos & whole seeds in culture medium following methods in Meney and Dixon (1988).	Brown *et al.* 1994
C. spp.	Dry and wet heat: Various treatments.	Petri dish in incubator; diurnal winter temperatures.	WB
H. aristatus	Dry and wet heat: Various treatments.	Petri dish in incubator; diurnal winter temperatures.	WB
H. neesii	Smoke: Seeds sown in trays, then exposed to smoke for 2 h in a smoke tent.	Seeds in sterilised soil, incubated at 10°C (night) and 25°C (day) in open shade.	Brown *et al.* 1994
H. striatus	Smoke: Seeds sown in trays, then exposed to smoke for 2 h in a smoke tent.	Seeds in sterilised soil, incubated at 10°C (night) and 25°C (day) in open shade.	Brown *et al.* 1994
W. incurvata	Smoke: Seeds sown in trays, then exposed to smoke for 2 h in a smoke tent.	Seeds in sterilised soil, incubated at 10°C (night) and 25°C (day) in open shade.	Brown *et al.* 1994
W. incurvata	Smoke: 100 seeds soaked in 1:2, 1:20 and 1:200 dilutions of smoke extract for 24 h.	Petri dish in growth chamber (6°C 16 h dark / 24°C 8 h light) & under autumn temperatures in fybos mix.	HdL & HJ
W. incurvata	Activated charcoal: 100 seeds soaked in 1% activated charcoal for 24 h (Petri dishes), & seeds covered with activated charcoal (fynbos mix).	Petri dish in growth chamber (6°C 16 h dark / 24°C 8 h light) & under autumn temperatures in fybos mix.	HdL & HJ
W. incurvata	Folic acid: 100 seeds treated with various concentrations of folic acid.	Petri dish in growth chamber (6°C 16 h dark / 24°C 8 h light) & under autumn temperatures in fybos mix.	HdL & HJ

Table 1.1. (continued).

W. incurvata	None.	Incubation of aseptic embryos & whole seeds in culture medium following methods in Meney and Dixon (1988).	Brown *et al.* 1994
W. incurvata	Gibberellic acid: 100 seeds soaked in 10 mg l^{-1}, 50 mg l^{-1} and 500 mg l^{-1} of GA_3 for 24 h.	Petri dish in growth chamber (6°C 16 h dark / 24°C 8 h light) & under autumn temperatures in fybos mix.	HdL & HJ
W. incurvata	Ethrel: 100 seeds soaked in 0.01 mg l^{-1}, 0.1 mg l^{-1} and 1.0 mg l^{-1} of ethrel for 24 h.	Petri dish in growth chamber (6°C 16 h dark / 24°C 8 h light) & under autumn temperatures in fybos mix.	HdL & HJ
W. incurvata	Heat: 100 seeds agitated in a heated tin on a hot plate for approximately 20 s.	Petri dish in growth chamber (6°C 16 h dark / 24°C 8 h light) & under autumn temperatures in fybos mix.	HdL & HJ
W. incurvata	Heat & smoke: 100 seeds agitated in a heated tin on a hot plate for ± 20 s, then soaked in smoke extract for 24 h.	Petri dish in growth chamber (6°C 16 h dark / 24°C 8 h light) & under autumn temperatures in fybos mix.	HdL & HJ
W. incurvata	Cold: 100 seeds soaked in water until imbibed, then kept at 4°C for 3 weeks in an equal mixture of sand, perlite and moist peat moss.	Petri dish in growth chamber (6°C 16 h dark / 24°C 8 h light) & under autumn temperatures in fybos mix.	HdL & HJ
W. incurvata	Stratification: 100 seeds soaked in water until imbibed, then kept at 6°C 16 h / 24°C 8 h for 3 weeks in an equal mixture of sand, perlite and moist peat moss.	Petri dish in growth chamber (6°C 16 h dark / 24°C 8 h light) & under autumn temperatures in fybos mix.	HdL & HJ
W. incurvata	Warmth & moisture: 100 seeds.	Seeds sown in fynbos mix, placed on bottom heat and kept moist.	HdL & HJ
W. spp.	Dry and wet heat: Various treatments.	Petri dish in incubator; diurnal winter temperatures.	WB

1.6) Motivation and study aims

1.6.1) Motivation

Plants of the Restionaceae family have a number of economic uses, including thatching (Linder 1990), horticulture and soil binding, and to a lesser degree, grazing, house building and broom making (Linder 1991). The horticultural potential of the Restionaceae is large (Clegg 1980), as some species are used in the cut flower industry (Jamieson & Brown 1994) along with members of the Proteaceae and Ericaceae (Jamieson & Brown 1995). As most of the myrmecochorous Restionaceae have attractive sculptural forms (Brown *et al.* 1996), it would be horticulturally important if the mechanisms of dormancy breaking and germination in these species could be determined.

1.6.2) Study aims and approach

The objectives of my study are to address the following questions:

1) Is poor seed germination in previous studies a result of poor seed quality?

To answer this question I investigated seed morphology and development, seed collection and sorting methods, and seed viability in the nut-fruited Restionaceae.

2) What type(s) of seed dormancy exist in the nut-fruited Restionaceae?

To answer this question I examined some potential coat-imposed physical, mechanical and chemical barriers, and possible morphological and physiological barriers inherent in the embryo, to seed germination. Thus by a logical and systematic process of barrier elimination, this study attempted to identify the dormancy mechanism(s) operational in these intractable seeds.

Since my study used a physiological approach for elucidating barriers to seed germination in the nut-fruited Restionaceae, as opposed to the ecological approach of past studies, it provided an opportunity to compare the merits of these different approaches in seed dormancy research.

CHAPTER 2

SEED MORPHOLOGY & DEVELOPMENT, SEED COLLECTION & STORAGE, AND SEED QUALITY

2.1) Introduction

One of the problems that can affect seed germination trials is the quality of seeds used in germination tests. It is of vital importance that seeds used in germination tests are mature and viable. To ensure valid seed germination tests, a knowledge of suitable seed collection and sorting methods are necessary to yield a fully formed, mature and viable batch of seed (Ellis *et al.* 1985).

Knowledge of the timing of seed collection is very important to ensure successful seed collection, as most seeds undergo maturation drying prior to shedding (Bewley & Black 1994). Seeds collected before this stage are immature and would therefore be unlikely to be able to germinate. Collection of immature seeds can result in poor germination percentages (e.g. Blommaert 1972). Consequently, information about the biology of the species from which seed is to be collected is vital for successful seed collection (Clegg 1980).

Following seed collection, appropriate methods of seed sorting may be required prior to germination testing, as lack thereof can result in poor germination percentages (e.g. Horn 1962 and van Staden & Brown 1977). Seed sorting is necessary for the removal of empty, immature, damaged or deformed seed from a collection (Doussi & Thanos 1997, Tekrony & Egli 1997, Tillman-Sutela 1997, Cantos *et al.* 1998, Ceccherini *et al.* 1998 and Fuentes & Schupp 1998). It is therefore important that sorting methods are carefully chosen to eliminate this problem.

As little is known on seed morphology and development in the nut-fruited Restionaceae, some of these basic aspects were investigated in the first section of this chapter. The second part evaluates the different types of seed collection methods that have been used in these seeds, and details storage conditions of seeds used in subsequent experiments. In the third section seed quality as a potential cause of poor seed germination was investigated. Methods of seed sorting were evaluated, with specific reference to the applicability of these methods to the nut-fruited Restionaceae. Different viability tests were used to assess the quality of seed collections.

2.2) Materials and methods

2.2.1) Species and study sites

Seeds from the following species were used in this study: *Cannomois virgata* (Rottb.) Steud., *Hypodiscus aristatus* (Thunb.) Krauss, *Hypodiscus neesii* Mast., *Willdenowia incurvata* (Thunb.) Linder, *Willdenowia sulcata* Mast. and *Willdenowia teres* Thunb.. Collection details of seed used in subsequent experimentation are given in Table 2.1. Due to difficulty in collecting large quantities of seed, particularly at the commencement of the study, investigations were not performed consistently on all the species listed above.

2.2.2) Seed morphology and development

2.2.2.1) Seed morphology

Gross morphology

Seed morphology was studied by cracking the ovary wall longitudinally in a vice, and viewing the seeds under a dissecting microscope (Cambridge Instruments, Model Z30E, China). Preliminary investigations revealed that wax-embedding and freeze microtoming yielded unsuitable preparations for examining seed morphology.

Seed ultrastructure

Transmission electron microscopy (TEM) was used to examine the ultrastructure of *W. incurvata* 2 and *C. virgata* 4 seeds. Small pieces (*ca.* 3 mm^2) of tissue containing embryo, endosperm and seed coat were fixed in 2.5% gluteraldehyde (in phosphate buffer [0.1 M $NaPO_4$, pH 7.4], containing 0.5% caffeine) for 18 h.

Table 2.1. Collection date(s), locality, external collector(s) and method(s) of collection of seed material used in experiments in following chapters are detailed below. Key to external collectors: HJ = Hanneke Jamieson [1], RL-O = Richard Lechmere-Oertel [2] and RS & RS = Rod & Rachel Saunders [3]. Collections of the same species have been allocated numbers in the table, for ease of reference in the text. As *C. virgata* seed exists in two forms, the woody large-seeded form and brittle small-seeded form are indicated in the table as (wls) and (bss) respectively.

Species	**Collection dates**	**Locality**	**Latitude & longitude co-ordinates**	**Collection method**	**External collector**
C. virgata 1 (wls)	6/11/95	Kammanassie	33°37' S, 22°50' E	Cutting	RS & RS
C. virgata 2 (wls)	13/11/97	Garcias Pass	33°58' S, 21°15' E	Cutting	RS & RS
C. virgata 3 (wls)	10/11/97	Swartberg Pass	33°20' S, 22°05' E	Cutting	RS & RS
C. virgata 4 (wls)	3/1/98	Swartberg Pass	33°20' S, 22°05' E	Ground	RS & RS
C. virgata 5 (bss)	30/11/97	Pakhuis Pass, Cederberg	32°09' S, 19°02' E	Cutting	
H. aristatus	Detailed in text	Silvermine Nature Reserve	34°05' S, 18°25' E	Cutting	
H. neesii	9-10/12/97	Ezelsbank, Cederberg	32°22' S, 19°16' E	Cutting	
W. incurvata 1	12/12/96	Truitjes Kraal, Cederberg	32°31' S, 19°19' E	Shaking	RL-O
W. incurvata 2	22-23/11/97	Truitjes Kraal, Cederberg	32°31' S, 19°19' E	Shaking & Ground	
W. incurvata 3	2/11/97	Atlantis	33°37' S, 18°26' E	Shaking	
W. incurvata 4	8-10/11/97	Melkbos / Duynefontein	33°42' S, 18°27' E	Shaking	
W. incurvata 5	12/11/97	Yzerfontein turnoff	33°20' S, 18°14' E	Shaking & Ground	
W. incurvata 6	7-8/11/98	Melkbos / Duynefontein	33°42' S, 18°27' E	Shaking & Ground	
W. incurvata 7	9/11/98	Yzerfontein turnoff	33°20' S, 18°14' E	Shaking & Ground	
W. sulcata	13/11/96	Mamre	33°29' S, 18°28' E	Cutting	HJ
W. teres	19 & 25/11/97	Cape Flats Nature Reserve	33°56' S, 18°37' E	Shaking	

[1] National Botanical Institute, Private Bag X7, Claremont, 7735, Republic of South Africa

[2] Department of Botany, University of Cape Town, Private Bag, Rondebosch, 7701, Republic of South Africa

[3] Silverhill Seeds, 18 Silverhill Crescent, Kenilworth, 7700, Republic of South Africa

Fixed samples were washed three times in phosphate buffer, postfixed in 1% osmium tetroxide for 1 h, then re-washed three times in the phosphate buffer. Samples were dehydrated in a graded ethanol series over a period of 1.5 h, with a final dehydration in 100% ethanol twice. Samples were then transferred to a 100% acetone solution for 20 min. Thereafter they were placed into solutions of increasing proportions of epoxy resin : acetone (Spurr 1969) over two days, with a final placement into 100% resin twice. Resin containing seed tissue was polymerised for 16 h at 60°C, and then sectioned (90 μm) with an ultramicrotome (Reichert Ultracut-S, Leica Instruments, Austria). Tissue sections were stained according to Reynolds (1963) in uranyl acetate for 10 min, washed in distilled water, re-stained in a solution of lead citrate for 10 min, and re-washed in distilled water. A JEOL 200 CX transmission electron microscope (Akishma, Japan) was used to view the cellular ultrastructure of the seed coat, embryo and endosperm.

2.2.2.2) Seed development

Seed water contents of *H. aristatus* and *W. incurvata* 6 (refer to Table 2.1) were investigated over a period of seed development. This was done in order to gain an understanding of the time scale of seed maturation. Thirty seeds were collected every month, ten of which were used to determine the water content of the whole seed. The remaining seeds were dissected into the ovary wall and true seed (seed coat + endosperm + embryo) to ascertain water contents for these two regions. Whole and separated seeds were weighed, dried in a convection oven at 100°C for 24 h, and cooled in a silica gel desiccator for 30 min prior to re-weighing. Water content was expressed on a fresh mass basis. Lack of a suitable population of *C. virgata* close to the laboratory prevented a detailed study of seed development in this species.

2.2.3) Seed collection and storage

2.2.3.1) Seed collection

Two seed collection methods were used once the timing of seed maturity had been established. The first collection method involved cutting seed heads from the plant ("cutting method"). Care was taken to select only the mature old seed heads that were opening. The second seed collection method involved gently shaking the stems with mature seed heads over a bucket ("shaking method"). In a couple of instances, when most of the seed had been released from plants, the naturally released seed was collected from the ground ("ground method"). Seeds collected using the cutting method were dried in a warm room or in the sun for a few days prior to storage.

A Microlepidopteran, probably Gracillariidae (Picker, personal communication), had parasitised a significant proportion of the seeds collected from *W. incurvata*. These seeds were easily separated from unparasitised seed by examining the hilum for a distinctive small hole, often through the funiculus (vasculature that connected the seed to the parent plant). Seeds in which the funiculus was missing were almost always parasitised, and often when the seed was cracked open, a white worm was found within. Separation of parasitised seed from collections was completed prior to seed storage or seed testing. Figure 2.1 shows healthy and parasitised *W. incurvata* seeds.

The cutting and shaking seed collection methods were evaluated by x-radiography. X-radiographs of a random seed sample (n = 100) from 13 different seed collections were examined, and the quality of the seed collection assessed by noting the number of full versus empty seeds.

2.2.3.2) Seed storage

Unless otherwise stated, seed was stored in brown paper bags under ambient conditions in the laboratory prior to experimentation.

Figure 2.1. Healthy seeds of *W. incurvata* (upper row), with intact funiculus. Parasitised seed of *W. incurvata* (lower row) identified because of the missing funiculus (centre) or a small (black) penetration hole through the hilum and funiculus (left) (X 2.5).

Seed burial conditions

Seeds of *C. virgata* 1, *W. incurvata* 1 and *W. sulcata* were divided into three lots. One third of the seed were stored in the laboratory in paper bags under ambient conditions ("laboratory-stored seed"). One third were buried in one of two metal containers (1 m X 1 m X 0.15 m) in the laboratory at ambient temperatures (*ca.* 10 - 20°C) in soil obtained from Silvermine Nature Reserve ("soil-stored laboratory seed"). These seeds were wet thoroughly with 10 l of water once a month, by which time the soil had completely dried out from the previous watering. The final third was buried at a depth of 10 cm in the field at Silvermine Nature Reserve at the onset of winter ("soil-stored field seed"). Seeds buried in the field were evenly divided into 12 lots, and placed into bags made of plumbers' mesh, which allowed seeds to be exposed to the surroundings. Soil from the site was added to the bag prior to burial. The 12 bags were separated from one another by a minimum of three metres, and buried along a downhill gradient. Two and three months after burial the bags were lifted to check for, and remove, any germinating seeds. All remaining seeds were removed from the soil six months after burial.

Soil characterisation

Silvermine Nature Reserve soil used in seed burial and germination experiments is derived primarily from quartzitic sandstone of the Table Mountain Group (Geological Survey Map 1990). Soil was characterised according to texture, pH, conductivity and soil organic matter content.

Soil texture

Soil texture was determined using a modified version of the hydrometer method of particle size analysis (Bouyoucos 1962). Soil organic matter was removed from the soil prior to analysis by boiling in hydrogen peroxide. The soil was dried in a convection oven at 110°C. A 50 ml Calgon solution of 10% (mass / volume) sodium hexametaphosphate (adjusted to pH 9 with 20% NaOH) was added to three replicates of 50 g of soil and allowed to stand for 30 min. The pH was readjusted to pH 9 prior to the addition of 150 ml of tap water. The solution was stirred at 1300 rpm for 5 min and allowed to stand overnight in a 1 l cylinder. A Model D4050 280 mm soil hydrometer (Zeal, England) was used for silt and clay determination. The combined proportion of silt and clay was calculated by multiplying a hydrometer reading by a factor of two, 7 min after inverting the cylinder a few times to disperse the soil. The clay proportion was similarly calculated after 7 h. The proportion of sand was determined by the dry weight of the fraction that did not pass through a 0.05 mm sieve. Proportions were plotted on a textural triangle (Miller & Donahue 1990) to determine soil texture.

Soil pH

Following Rowell (1994), soil pH was determined in four replicates by agitating a suspension of 20 g (2 mm sieved) soil in 50 ml 0.01 M calcium chloride on a shaker for 30 min prior to pH measurement. Soil pH was measured with a Model 9210N ATC pH meter (Hanna Instruments, Portugal).

Soil conductivity

Soil conductivity was measured using methods modified from Rowell (1994) and Miller and Donahue (1990). A known quantity of distilled water was added to 50 ml of soil to form a saturated paste, which was allowed to stand for 4 h to wet thoroughly. The paste was then made up to a 1 : 1 (volume / volume) soil water suspension. This was filtered through Whatman No 50 filter paper fitted to a Buchner funnel to which a vacuum was applied. Soil conductivity (n = 4) was measured with a Jenway Model 4070 conductivity meter (Jenway Inc., Princetown, New Jersey, USA), and converted to a paste extract conductivity using a multiplication factor of 4 for sandy soil (Miller & Donahue 1990).

Soil organic matter content

For soil organic matter determination, the mass of four replicates of 10 g oven dried (105°C) soil samples in crucibles was noted prior to their placement in a furnace (Kiln Contracts, Cape Town, South Africa) at 450°C for 16 h. Samples were removed from the furnace and placed in a silica gel desiccator for 30 min prior to reweighing. Organic matter content was calculated as a percentage of the oven dried soil (Rowell 1994).

Effects of soil storage

The effect of soil storage on the ovary wall and funiculus was investigated using scanning electron microscopy (SEM). Portions of the ovary wall (including the hilum region) of laboratory-stored and soil-stored field seed of *C. virgata* 1, *W. incurvata* 1 and *W. sulcata* were mounted on aluminium stubs, and sputter-coated with gold-palladium. The funiculus and outer surface of the ovary wall were photographed with a Cambridge S200 scanning electron microscope (LEO Electron Microscopy Ltd, Cambridge, England). For examination of the internal structure of the ovary wall, portions were freeze-fractured in liquid nitrogen prior to sputter-coating, to expose the internal cellular region.

2.2.4) Seed quality

2.2.4.1) Seed sorting

A random sample of 50 seeds from different seed collections were individually placed through a screening process of x-radiography, weighing and floating tests. Comparisons of weighing and floating results with seed x-radiographs for each seed enabled the evaluation of the effectiveness of these three seed sorting techniques in the nut-fruited Restionaceae.

X-radiography

X-radiography studies were undertaken in a Softex type EMB x-ray machine (Softex Co. Ltd, Tokyo, Japan), at the Department of Forestry, University of Stellenbosch, South Africa. Small to medium sized seed samples were placed in plastic holders, and larger seeds were stuck onto strips of cellotape. The seed samples were then placed onto Kodak X-Omat MA ready pack (envelope-wrapped) film. The seeds and film were exposed for 1 min at 9 mA, at a focus-film distance of 69.5 cm. The kilovoltage, which ranged between 14 kV and 17 kV, was adjusted for different seed sizes, to maximise the contrast between empty and full seeds. X-radiographic film was developed in Agfa G150 developer in a dark room at 20°C for 4 min. Development was stopped in a bath of 3% acetic acid for 30 s, before fixing in Agfa G334 fixative at 20°C for twice as long as it took for the film to darken. The film was then rinsed clean in a running water bath for 20 min, and hung up to dry.

Weighing and floating

The x-rayed seeds were individually weighed and then tested for floating in water. Any air bubbles that formed on the seeds when placed in the water were removed. The seed was recorded as a "floater" if, after 1 min, the seed remained floating.

2.2.4.2) Seed viability

Topographical tetrazolium test

Embryos were tested for viability following the ISTA (International Seed Testing Association) guidelines (ISTA 1999) of the Topographical Tetrazolium Test developed by Lakon (1942). All seeds tested for viability were less than a year old. Prior to tetrazolium testing, small seeds (such as *W. sulcata* and the small brittle form of *C. virgata*) were imbibed for 24 h, while larger seeds (for example *W. incurvata*) were imbibed for 48 h. Imbibition of the seed prior to the removal of the hard outer ovary wall was necessary, as dry seeds cracked in a vice often shattered, resulting in considerable damage to the seed.

The ovary wall was removed from the seed by cracking it in a vice, and carefully prying it away. The small disk-like embryo was dissected carefully from the seed onto Whatman No 1 filter paper in a Petri dish. A 5 ml solution of 1% 2,3,5 triphenyl tetrazolium chloride was added to the Petri dishes, and incubated at ambient temperatures in the dark (as the chemical is light sensitive). After 18 h embryos were checked under a Meiji binocular microscope (Labax Co., Ltd., Tokyo, Japan) for a red stain, which is indicative of the presence of living tissue, irrespective of seed dormancy (Lakon 1949, Heydecker 1974 and Don 1979).

Interpretation of tetrazolium staining was difficult, as these results could not be correlated with standard germination tests. Embryos that stained pink were recorded as viable, while embryos that did not stain or were very pale were recorded as non-viable. Most embryos exhibited uniform staining, however embryos in which stain intensity or positioning varied were not quantified or recorded, as embryos were small and undifferentiated. In the few seeds in which staining varied, those embryos that exhibited less than 50% of healthy stained tissue were interpreted to be non-viable.

Electrolyte leakage

The electrical conductivity test (Matthews & Bradnock 1967) is based on observations that dead seeds and seeds of low vigour are leaky (Moore 1969), and exude high quantities of sugar (Takayanagi & Murakami 1968) and electrolytes (Matthews & Bradnock 1967) when soaked in distilled water (Heydecker 1974). This non-destructive technique assesses the ability of cell membranes to prevent the leaching out of electrolytes, and therefore is indicative of seed vigour and viability. Rates of electrolyte leakage from seeds provide an index of cell membrane integrity (Simon 1974), and thus embryo damage (Bewley & Black 1994). Electrolyte leakage was tested as a non-destructive method of evaluating seed viability in the nut-fruited Restionaceae. *W. incurvata* 1 seeds were divided equally into four categories or treatments:

a) seeds with funiculus removed;
b) seeds cracked in a vice;
c) seeds autoclaved, then funiculus removed; and,
d) seeds autoclaved, then cracked in a vice.

Some of the seeds were autoclaved at 121°C at a pressure of 1 kg cm^2, as the high temperatures and pressures during the autoclaving process kills the seeds and disrupts cell membrane integrity (Bhattacharjee & Mukherjee 1998). Autoclaved seeds therefore exhibit the maximum possible electrolyte leakage when placed in water, which provides a useful upper limit of leakage for comparison with the electrolyte leakage of untreated seeds. Healthy seeds usually exude only a small amount of solutes, whereas seeds that have lost viability (dead seeds) leak a much larger amount of solutes (Simon 1974), similar to autoclaved seeds.

Seeds were autoclaved in sealed containers, to keep them dry and prevent electrolyte loss during the autoclaving process. Embryos of 10 autoclaved seeds not used for electrolyte leakage were dissected and checked for viability using tetrazolium, to confirm that seed death had occurred during the autoclaving process. Seeds were either cracked in a vice, or the funiculus was removed by scraping it carefully out with a blunt needle. This was done to allow for free water movement in and out of the seed, to allow for conductivity assessment. Cracking of seeds in the vice did not damage the embryo, but occasionally damaged the endosperm.

All seeds tested for electrolyte leakage were weighed and placed into glass tubes that had been thoroughly rinsed with ultrapure water (de-ionised distilled and filtered through 0.22 µm cellulose acetate mesh), to which 5 ml of ultrapure water was then added. Conductivity measurements were taken with a Jenway Model 4070 conductivity meter (Jenway Inc., Princetown, New Jersey, USA) over a 160 h period. Regressions of conductivity increase against time were computed for the first 12 h of electrolyte leakage. The rate of leakage per seed was calculated from the gradients of the regressions and corresponding dry seed masses.

2.2.5) Statistical analyses

Statistical analyses were performed using Statistica version 5.5 for Windows (1999). A Kruskal-Wallis ANOVA tested for differences in electrolyte leakage rates between treatments. A one-way fixed-effects ANOVA (data checked for homogeneity of variances using Bartlett's test, $p > 0.05$) was used to test for significant differences in maximum electrolyte leakage at 160 h between different treatments. Significantly different means were separated using a Tukey test at $p < 0.05$ (Zar 1996).

2.3) Results

2.3.1) Seed morphology and development

2.3.1.1) Seed morphology

Gross morphology

Figure 2.2 illustrates the typical structure of the mature dispersal propagule (commonly referred to as a seed in the text) of *C. virgata* and *W. incurvata*. The funiculus is the region of vasculature that connected the developing seed to the parent plant. The small disk-like embryo is positioned on top of the massive endosperm, just under the membranous seed coat, directly below the funiculus. Germination of the embryo takes place through the funiculus region. The overall structure of all species examined was similar, however the amount of endosperm relative to the embryo varied between species, with a larger proportion of endosperm in large-seeded species. Ovary wall thickness varied both within and between species.

Seed ultrastructure

Figure 2.3 shows cellular detail of the embryo (Figure 2.3i), endosperm (Figure 2.3ii) and seed coat (Figure 2.4) of *C. virgata* and *W. incurvata* viewed with a transmission electron microscope. The seed coat appears to be comprised of several compact collapsed cell layers, some of which are folded. During sectioning the seed coat with a single layer of cells pulled away from the embryo. Endosperm tissue consisted mostly of clustered starch grains and some protein bodies within cytoplasmic regions of undefined composition. No organellar detail was discernable. Due to the dense packing of these reserves, the tissue, although well fixed, had a fractured appearance. Cell walls were sometimes visible in the endosperm tissue, but appeared disrupted in some areas.

Embryos of *C. virgata* and *W. incurvata* (Figure 2.3i) were undifferentiated and no radicle meristem was evident. Embryo cells of both species were typically filled with lipid and protein bodies. Starch grains were not present in embryo tissue. Protein bodies consisted of a protein matrix with frequent inclusions. Lipid bodies, which varied in size, lined protein bodies and

Figure 2.2. Photograph of the large woody-seeded form of *C. virgata* (top) and seed of *W. incurvata* (bottom), in which the ovary walls have been cracked and separated to show the true seed. In both species the surrounding thick brown ovary wall and funiculus (F) is visible. The white starchy endosperm (E) is visible in *W. incurvata* (bottom left) where the seed coat has torn away. The seed coat is visible in the ovary wall of *W. incurvata* (bottom right). In *C. virgata* the seed coat is intact (top left) (X 4).

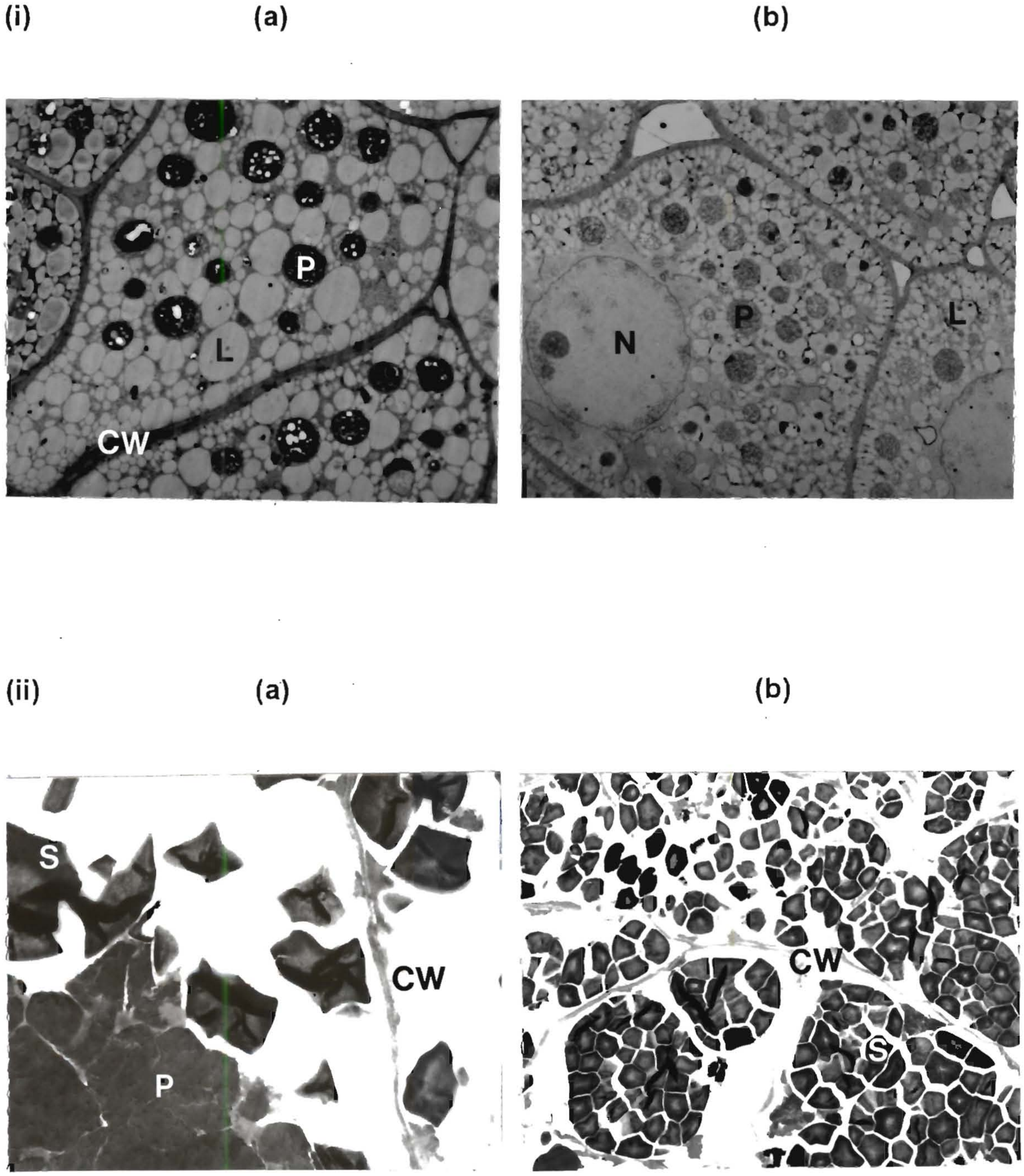

Figure 2.3. Transmission electron micrographs showing cellular detail of **(i)** the embryo of **(a)** *C. virgata* 4 (X 3400) and **(b)** *W. incurvata* 2 (X 3400) and **(ii)** endosperm of **(a)** *C. virgata* 4 (X 7300) and **(b)** *W. incurvata* 2 (X 3000). Indicated on the micrographs are starch grains (S), cell walls (CW), nucleus (N), lipid (L) and protein bodies (P).

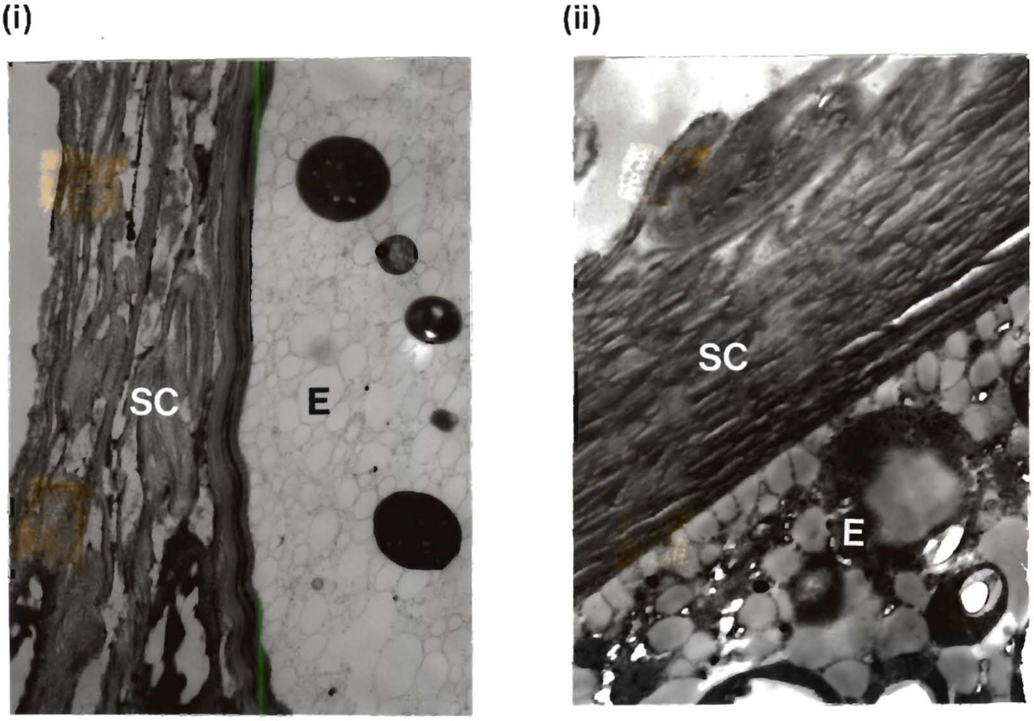

Figure 2.4. Transmission electron micrographs of the seed coat of **(i)** *C. virgata* 4 (X 10000) and **(ii)** *W. incurvata* 2 (X 10000). Indicated on the micrographs are the outer seed coat layer (SC) and embryo (E).

cell walls. Few mitochondria were evident, as embryo cells were mostly filled with storage reserves. Mitochondria that were observed did not possess well-defined cristae typical of quiescent seeds (Bewley & Black 1994). Nuclei when observed were characteristic of dry seed tissue, with clumped chromatin discernible, particularly around the nuclear membrane.

2.3.1.2) Seed development

The decreasing water content (as a percentage of fresh mass) of the true seed (endosperm + embryo + seed coat), ovary wall and whole seed (true seed + ovary wall) of *H. aristatus* and *W. incurvata* over time are shown in Figure 2.5 and Figure 2.6 respectively. Monitoring of water contents from developing seed commenced shortly after pollination, once a young seed had been formed. Included in these figures are climatic data (monthly rainfall and mean monthly maximum and minimum temperatures) from the meteorological station closest to the study site for the period during which seed development was monitored.

H. aristatus had a much longer developmental time period than *W. incurvata*. Observations in the field revealed that flowering of *H. aristatus* occurred in June. Based on the examination of herbarium specimens (Esterhuysen 34338 BOL and Esterhuysen 34573 BOL) in the Bolus Herbarium (Botany Department, University of Cape Town), flowering in this species occurs from May to August. Seeds from a flowering event are released some 18 months after pollination in January or February. Seeds of different ages are therefore present on the plant at the same time during certain times of the year, making seed collection more tricky. However the colour and condition (weathering) of the protective bracts surrounding the different-aged seeds allows them to be relatively easily distinguished from each other (Figure 2.7). When collecting seed of *C. virgata*, it was noticed that this species also exhibits this overlap of two different ages of seed on one plant during certain times of the year.

W. incurvata does not have an overlap of differently aged seed. This species flowers from May to June (Swift 1994) and seeds develop over a five-month period, with release in November of the same year. In this species the ovary wall

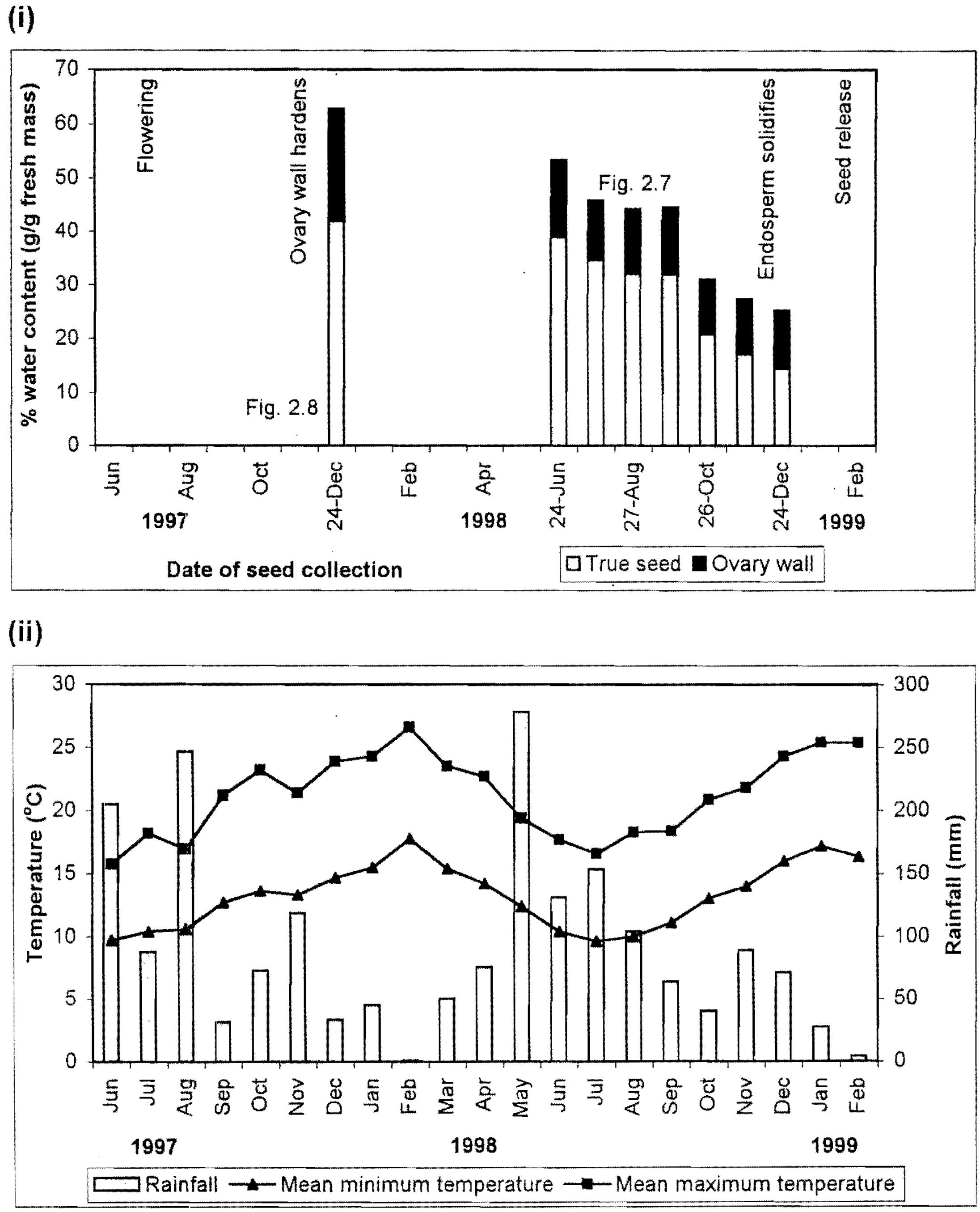

Figure 2.5. **(i)** Water contents of the true seed (n = 20) and ovary wall (n = 20) expressed as a proportion of the whole seed water content (n = 10) of developing *H. aristatus* seed. Occurrence of flowering, ovary wall hardening, endosperm solidification and seed release, as well as timing of seed developmental changes pictured in Figure 2.7 and Figure 2.8, are indicated on the graph. **(ii)** Climatic data of rainfall (Silvermine Nature Reserve) and temperatures (Kirstenbosch) over the seed developmental period (data courtesy of the Climate Information Section of the South African Weather Bureau, Pretoria, South Africa).

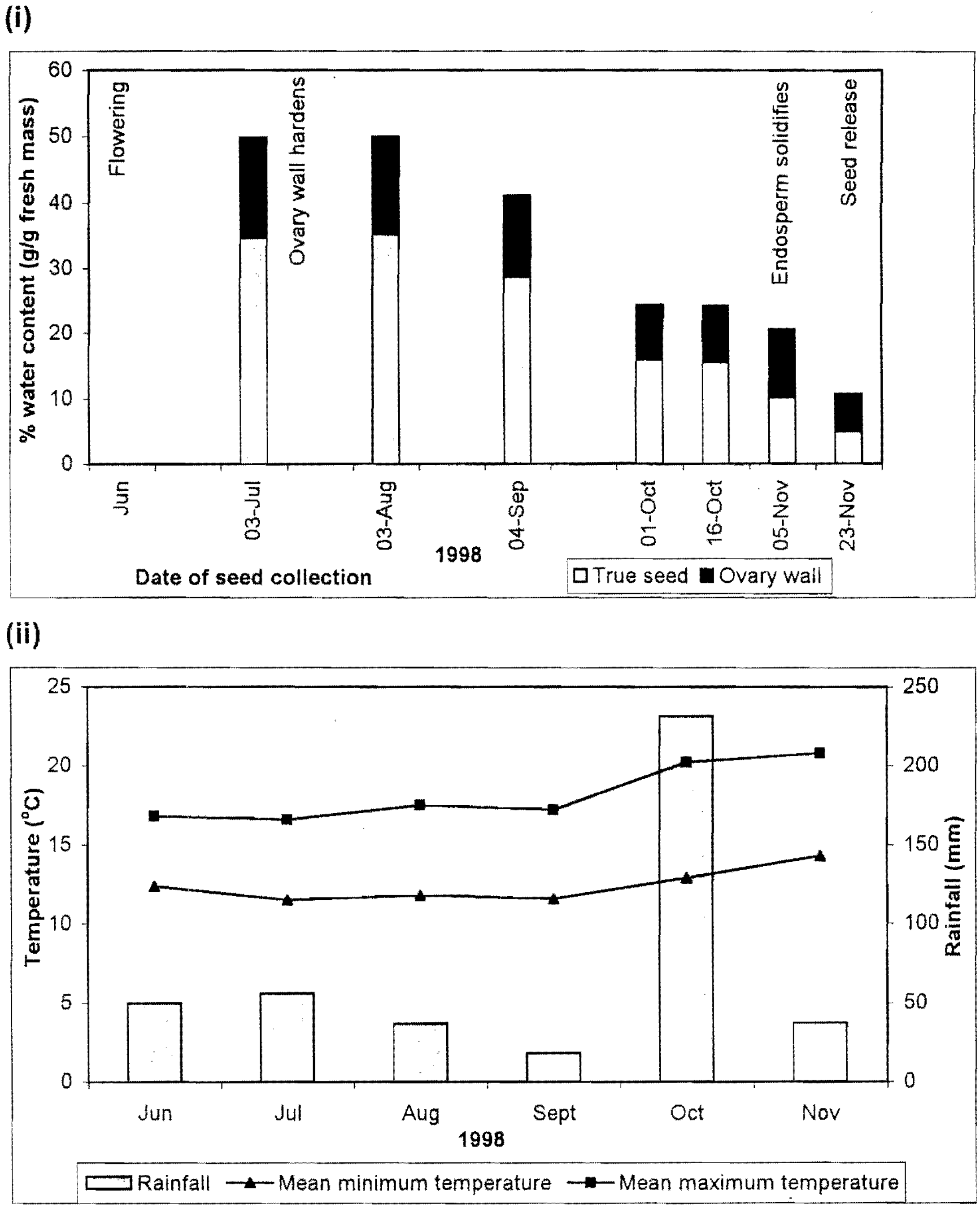

Figure 2.6. **(i)** Water contents of the true seed (n = 20) and ovary wall (n = 20) expressed as a proportion of the whole seed water content (n = 10) of developing *W. incurvata* seed. Occurrence of flowering, ovary wall hardening, endosperm solidification and seed release are indicated on the graph. **(ii)** Climatic data of rainfall and temperatures over the seed developmental period from Table Bay (data courtesy of the Climate Information Section of the South African Weather Bureau, Pretoria, South Africa).

was initially (*ca.* mid June to early July) fleshy and green in colour, but by mid July they had turned black and hardened. The endosperm during all of this time was transparent, and very watery. By August the endosperm had changed to an opaque white colour, but was still liquid. Solidification of the endosperm occurred during the last couple of weeks prior to seed dispersal. Similar developmental changes were observed for *H. aristatus* (Figure 2.8), but on a protracted time scale, in which the ovary wall hardened and darkened early in development, but endosperm solidification occurred late in seed development, a couple of months prior to seed release.

Seed release occurred over a period of approximately two weeks in *W. incurvata.* The mean seed moisture content of naturally released seed was 10.7 ± 1.9% (n = 10). Mature seeds can be visually identified by the presence of a solid, dry and crumbly endosperm on dissection (Jamieson, personal communication). Seed maturity and release may be further recognised by evidence of opening of bracts surrounding the seeds (e.g. *C. virgata*) and by recently shed seeds surrounding the base of the plant (e.g. *W. incurvata* and *W. teres*).

2.3.2) Seed collection and storage

2.3.2.1) Seed collection

Five seed collections of 100 seeds each of *W. incurvata* 1 (laboratory- and soil-stored), *W. incurvata* 3, *W. incurvata* 4 and *W. teres* gathered using the shaking method averaged 94.3 ± 4.2% full seed as evaluated by x-radiography. Seven seed collections of 100 seeds each of *C. virgata* 2, 3 and 5, *H. neesii* and *W. sulcata* (laboratory- and soil stored) collected by the cutting method contained 68.1 ± 26.7% full seed. Only one collection was made using exclusively the ground method (*C. virgata* 4), which had 94% full seed. Seed quality (the percentage of full seed) in seed collections gathered by the cutting method was much lower than seed collected with the shaking and ground methods. There was also a large variation in seed quality between collections gathered by the cutting method. In contrast, the shaking and ground methods consistently yielded high seed quality in the seed collections tested.

Figure 2.7. Photographic illustration of the outer protective bracts surrounding the dispersal propagule of an older *H. aristatus* seed (left) and a young *H. aristatus* seed (right) that were collected from the same plant at the same time in August 1997 (X 3). The mature seed can be distinguished from the young seed by the larger size and the darker, more worn bracts.

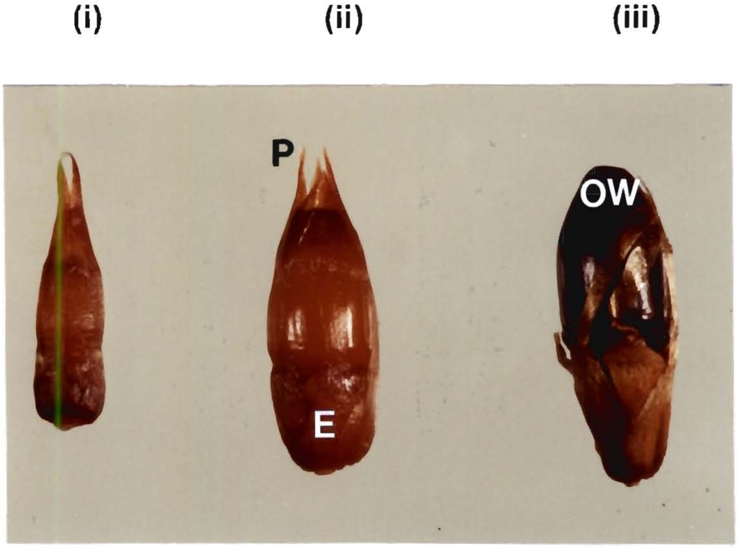

Figure 2.8. Developmental changes in the dispersal propagules of *H. aristatus* collected at **(i)** 16 months **(ii)** 15 months and **(iii)** 14 months prior to seed maturity and release, showing the membranous perianth (P), the fleshy elaiosome (E) and the ovary wall (OW) (X 3.5).

2.3.2.2) Seed storage

Soil characterisation

Silvermine soil used in germination experiments was sandy, with a pH of 4.82 ± 0.01, conductivity of 488 ± 51 $\mu S\ ml^{-1}\ ml^{-1}$ dry soil and a soil organic matter content of 1.43 ± 0.04 $g\ g^{-1}$ soil (dry mass basis). These values indicate the soil to be acidic and low in nutrients, typical of fynbos soils.

Effects of soil storage

Soil storage had varied effects on seeds. Figure 2.9 shows comparative scanning electron micrographs of the ovary wall of seeds stored in the laboratory and seeds stored in soil in the field. Degradation of the outer surface of the ovary wall ranged from being notable, in which wearing and flattening of the outer cells was visible (e.g. *W. incurvata* - Figure 2.9i), to negligible, in which no difference could be observed (e.g. *W. sulcata* in Figure 2.9ii and *C. virgata* in Figure 2.9iii). Details of the funiculus structure and its degradation are shown in Figure 2.10. Xylem vessels can be clearly seen in micrographs of the funiculus, evidence of the previous connection of the seed to the maternal plant. Figure 2.11 shows the internal structure of the freeze-fractured ovary wall of some species stored in the laboratory. It is interesting to see that many pores were visible on the surface of the cells comprising the ovary wall. These pores were present in all of the species examined.

2.3.3) Seed quality

2.3.3.1) Seed viability

Electrolyte leakage

Rates of electrolyte leakage ($\mu S\ g^{-1}\ ml^{-1}\ min^{-1}$) for *W. incurvata* 1 seeds that had been autoclaved or not autoclaved, and in which either the funiculus had been removed or the ovary wall had been cracked, are shown in Figure 2.12.

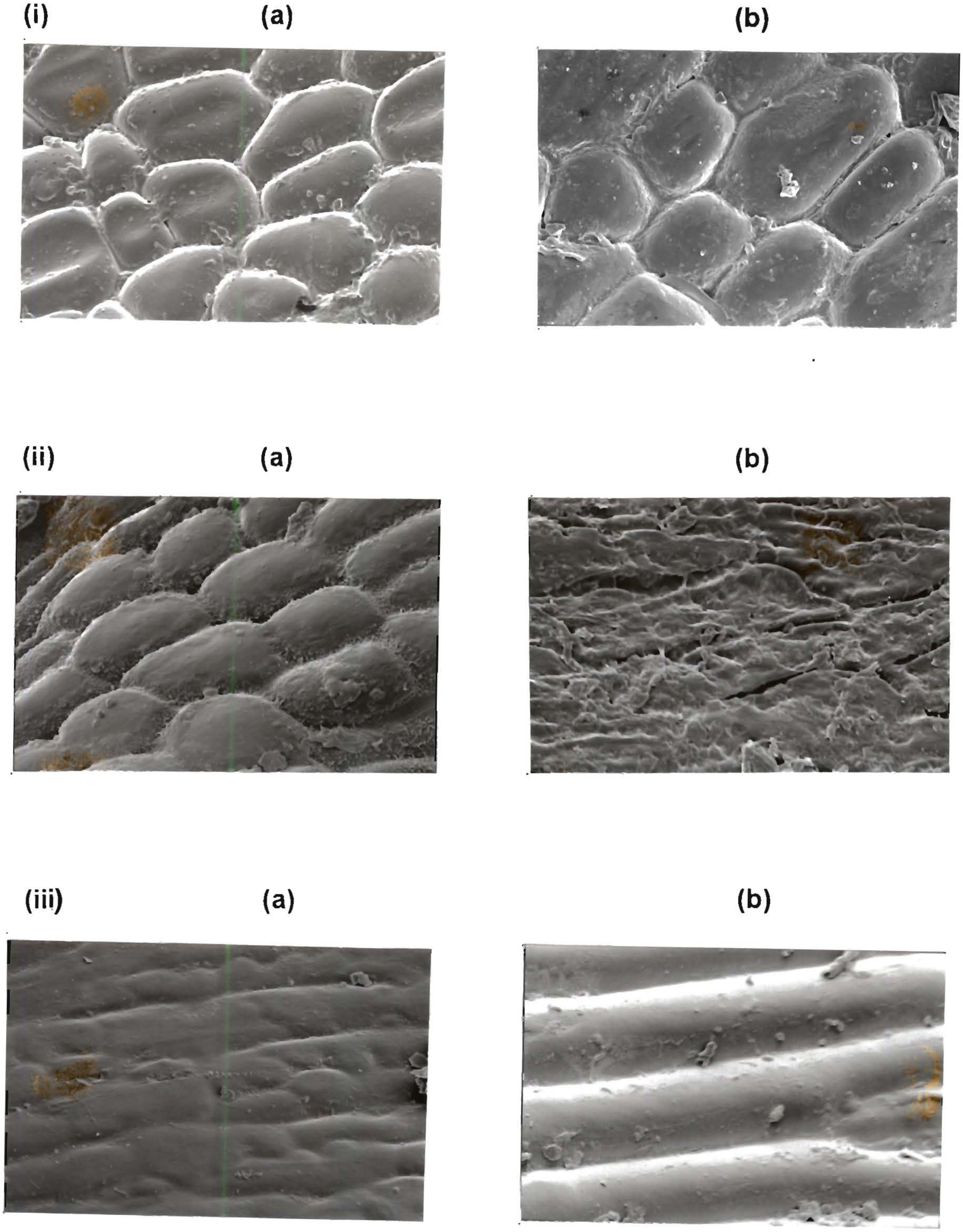

Figure 2.9. Scanning electron micrographs of the ovary wall of **(i)** *C. virgata* 1 **(a)** laboratory-stored (X 1020) and **(b)** soil-stored field (X 1090) seed; **(ii)** *W. incurvata* 1 **(a)** laboratory-stored (X 1510) and **(b)** soil-stored field (X 1510) seed and **(iii)** *W. sulcata* **(a)** laboratory-stored (X 1500) and **(b)** soil-stored field (X 1500) seed. Ovary wall degradation is visible in soil-stored *W. incurvata* seed.

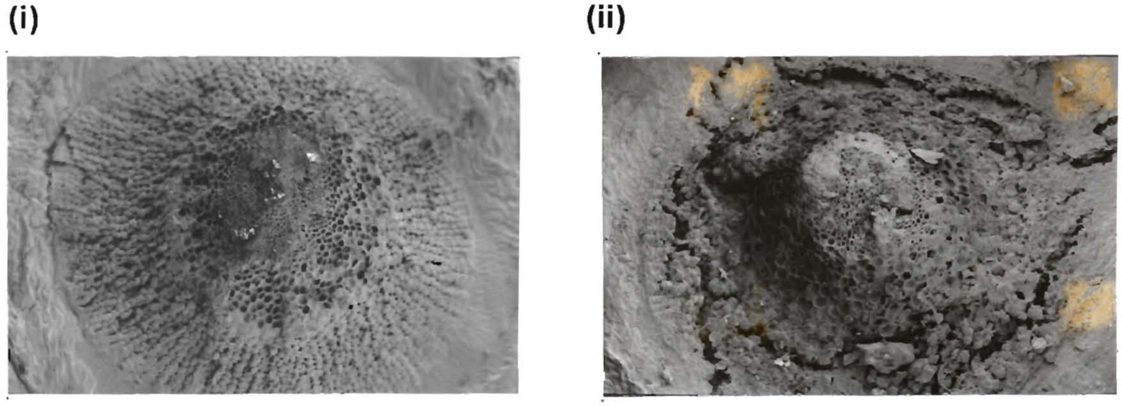

Figure 2.10. Scanning electron micrographs of the hilum and funiculus of *W. incurvata* 1 **(i)** laboratory-stored (X 930) and **(ii)** soil-stored field (X 970) seed.

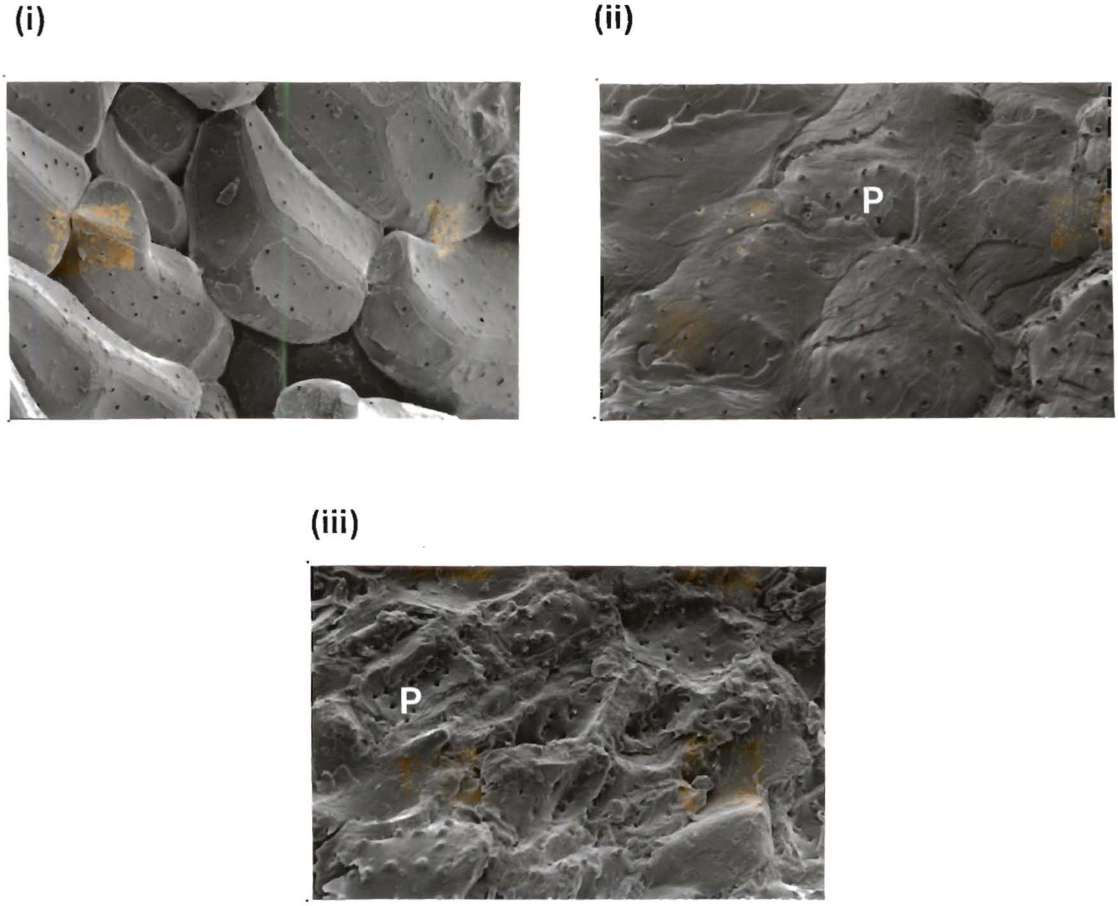

Figure 2.11. Scanning electron micrographs of the internal structure of the ovary wall of **(i)** *C. virgata* 1 (X 720); **(ii)** *H. aristatus* (young seed) (X 1080) and **(iii)** *W. sulcata* (X 1540). Note the pores (P) present in the cells of all the species.

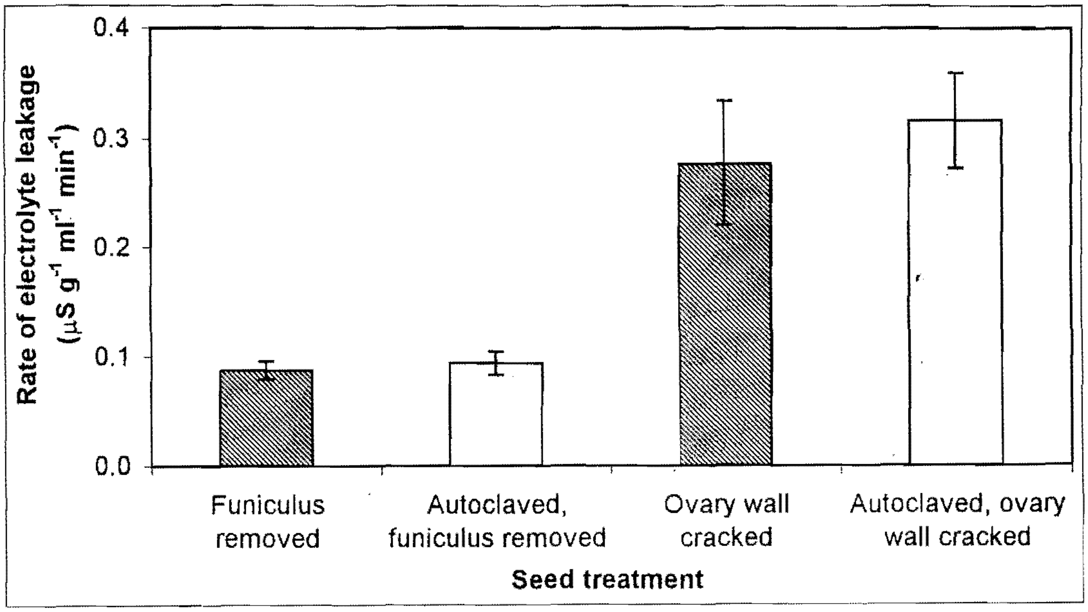

Figure 2.12. Electrolyte leakage (n = 5) in cracked *W. incurvata* 1 seed or seed in which the funiculus was removed, with and without autoclaving. Rates of electrolyte leakage were higher in cracked seeds.

Rates of electrolyte leakage were significantly different between treatments (Kruskal-Wallis ANOVA, $n = 20$, $H = 14.66$, $p = 0.002$). However electrolyte leakage rates from autoclaved seeds were similar to seeds that were not autoclaved, while rates of electrolyte leakage from cracked seeds were higher than from seeds in which the funiculus had been removed (Figure 2.12). These results were contrary to expectations, in which high leakage is expected in autoclaved seeds due to seed death and membrane disruption, and low leakage is expected in non-autoclaved viable seeds (Bhattacharjee & Mukherjee 1998). It is possible that these seeds, because of the thick ovary wall, were able to withstand the temperatures and even pressures of the autoclaving process, which may have resulted in unexpected results. However tetrazolium tests on similarly autoclaved seeds showed the embryos to be non-viable. Cracking of the ovary wall may have damaged the seeds, leading to higher rates of electrolyte leakage. Exposure of an increased surface area of seed tissue to water due to cracking the ovary wall may have allowed for further electrolyte exchange.

Topographical tetrazolium test

Seeds that had been used to compare the accuracy of the x-radiography, floating and weighing methods were used (after imbibition) to test for seed viability. All full seeds (identified from x-radiographs: refer to section 2.3.3.2) that were dissected contained embryos. Figure 2.13 shows a representative sample of embryos from different species following tetrazolium testing. Only embryonic tissue stained pink. Endosperm tissue did not react with the tetrazolium, remaining white in colour.

Percentages of viable embryos (stained pink following tetrazolium tests) of full seeds (identified from x-radiographs) are shown in Table 2.2. Collections of *W. sulcata* and *W. teres* had poor viability in the embryos tested, reasons for which are unknown.

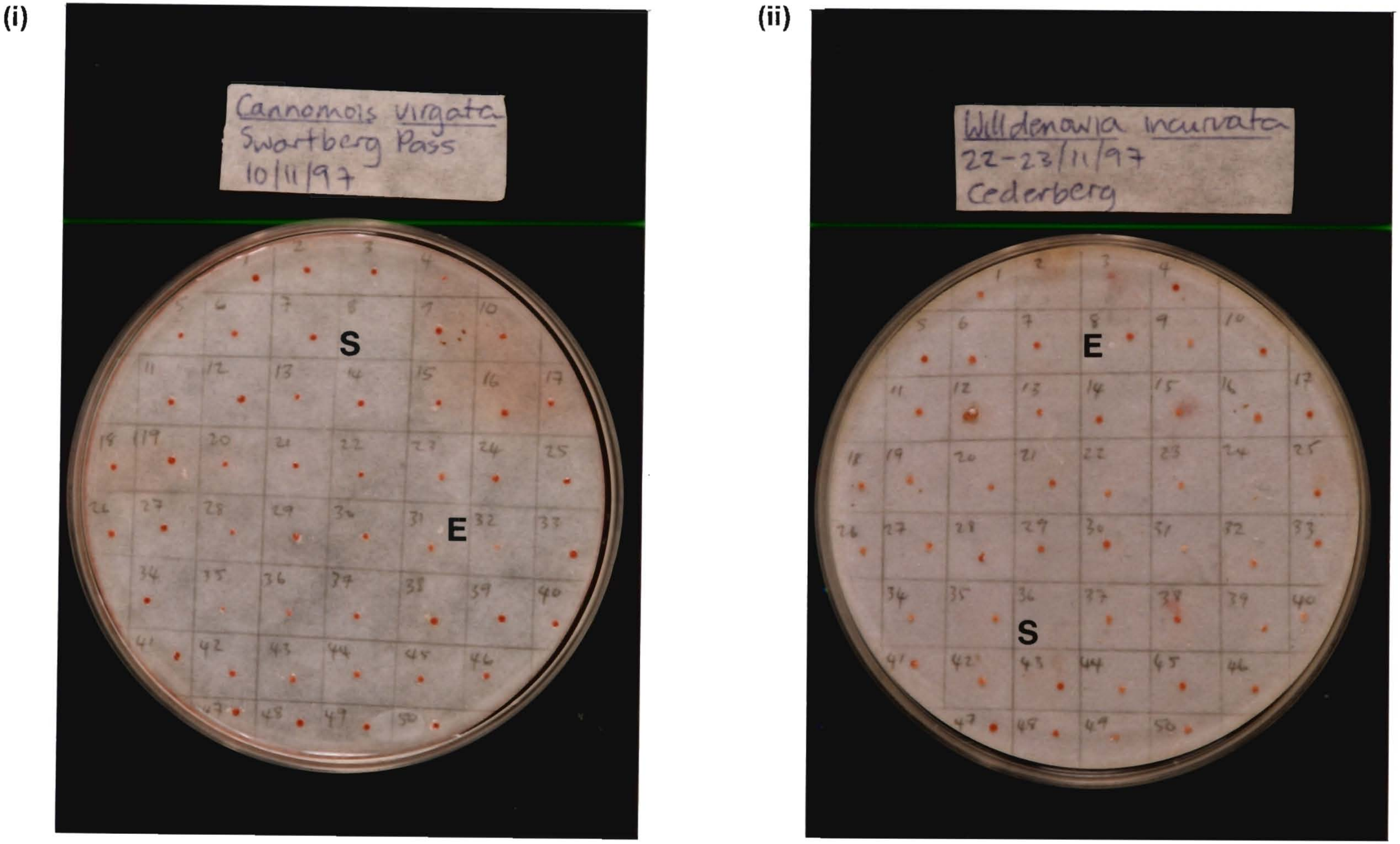

Figure 2.13. Examples of dissected embryos of **(i)** the woody large-seeded form of *C. virgata* 3 and **(ii)** *W. incurvata* 2 showing staining following tetrazolium tests. Indicated on the photographs are small, unstained pieces of endosperm (E) that adhered to the embryo during dissection. Empty spaces (S) correlated with undeveloped seeds (as seen on x-radiographs) that did not contain embryos (X 0.9).

Table 2.2. Percentage of viable embryos as determined from tetrazolium tests of full seed (identified by x-radiography) of seed collections of *C. virgata*, *H neesii*, *W. incurvata*, *W. sulcata* and *W. teres*. Note that n varied due to the number of full seeds being different for each collection (n = 50) x-rayed.

Seed collection	**n**	**Viable embryos (%)**
C. virgata 2	45	100%
C. virgata 3	49	98%
C. virgata 4	50	100%
C. virgata 5	41	67%
H. neesii	42	88%
W. incurvata 1	45	91%
W. incurvata 2	47	98%
W. incurvata 3	49	94%
W. incurvata 5	47	83%
W. sulcata (soil-stored field seed)	13	63%
W. teres	49	44%

Five collections showed a viability of 98% or more of full seeds tested, with only four of the 11 collections having less than 85% viability in full seeds. Interestingly, some seeds that appeared incompletely developed on x-radiographs were found to contain fully formed and viable embryos, in spite of a partially developed endosperm (e.g. five seeds in *H. neesii* and one seed in *C. virgata* 3).

2.3.3.2) Seed sorting

The x-radiographic film produced is a positive radiograph in which the darkest areas of the image correspond to areas that were most easily penetrated by the x-rays, while the light areas correspond to areas that were more difficult for the x-rays to penetrate (Ellis *et al.* 1985). Thus on a positive radiograph, full seeds are light, whilst empty seeds are dark.

Small embryo size in these seeds rendered it impossible to identify the presence or absence of an embryo from a x-radiograph. Seeds were therefore separated into two categories, depending on the x-radiographic image of the whole seed. Seeds that were light in all areas (fully formed) were referred to as "full", whilst seeds that were dark in greater than 20% of the seed area, or not fully developed in the region where the embryo would be, were defined as "empty". Figure 2.14 shows a representative sample of x-radiographs of different species of the nut-fruited Restionaceae.

Since x-radiography was found to be an accurate method for identifying and separating full from empty seeds during dissection of embryos for tetrazolium testing (section 2.3.3.1), x-radiography was also used to evaluate the weighing and floating methods of seed sorting.

Means and standard deviations for seed mass of full and empty seed (determined from x-radiographs) from the 13 seed collections are shown in Table 2.3. In a number of collections, few empty seeds were present, which precluded statistical assessment of the results. However, results of a two-tailed Student t-test for significant differences between the means of full and empty seeds were conducted on *C. virgata* 5, *H. neesii* and soil-stored (field) *W. sulcata* seed ($n > 10$). Only *W. sulcata* showed a significant difference ($p < 0.05$) between the mass of empty and full seed ($t = 5.471$). In seeds in which statistical analysis was not possible, the standard deviations of empty and full seeds often overlapped. The observed variation in ovary wall thickness between seeds within a collection may have contributed to or exacerbated the inaccuracy of this sorting method.

Table 2.3 also reports on the floating method for separating seeds. The accuracy of this method for separating full from empty seed (as identified on x-radiographs) is expressed as a floating error (% of seeds that are not totally filled that sank). A low floating error does not however guarantee the floating method to be a suitable seed sorting technique. The number of full seeds that floated, and were thus discarded, is also important to determine, as many good seeds may be discarded as "empty" if they float.

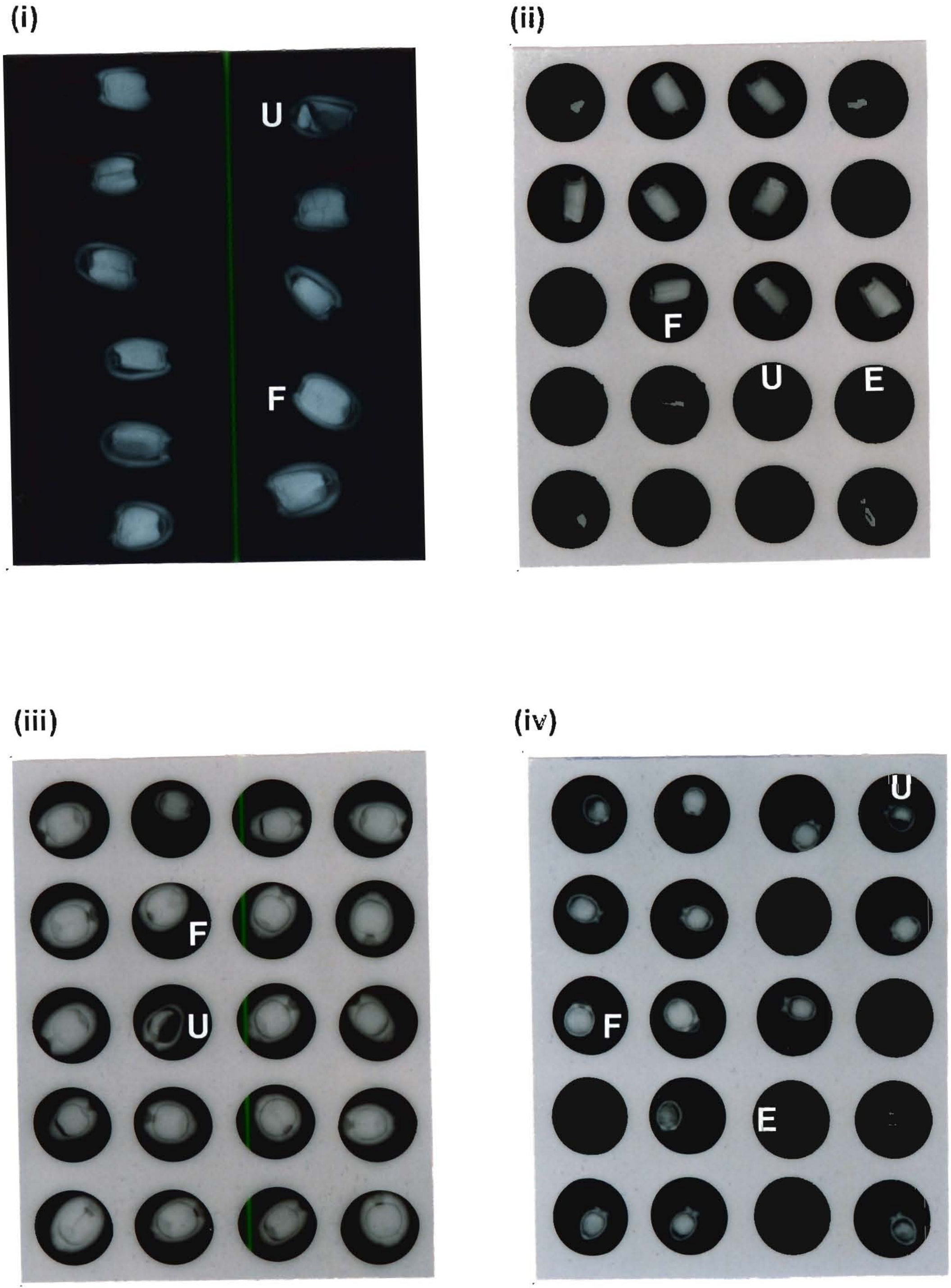

Figure 2.14. X-radiographs of (i) the woody large-seeded form of *C. virgata* 3; **(ii)** the brittle small-seeded form of *C. virgata* 5; **(iii)** *W. incurvata* 5 and **(iv)** *W. sulcata*. Indicated on the photographs are full seeds (F), and empty (E) or underdeveloped (U) seeds (X 1).

Table 2.3. Results of weighing and floating methods of seed sorting of *C. virgata*, *H neesii*, *W. incurvata*, *W. sulcata* and *W. teres* as compared with x-radiography. Mean mass (mg) and standard deviations (SD) of full and empty seed (determined from x-radiographs) are tabulated for the weighing method of seed separation. Floating errors (% of seeds not completely filled that sank) and discarding errors (% of seeds completely formed that floated) are given for the floating seed sorting method.

Species	SEED WEIGHT (mg)				FLOATING METHOD	
	Full seeds		Empty seeds		Floating error	Discarding error
	Mean ± SD	n	Mean ± SD	n		
C. virgata 2	545.7 ± 71.6	45	433.5 ± 126.4	5	0%	0%
C. virgata 3	206.3 ± 33.1	47	183.9 ± 25.4	3	0%	0%
C. virgata 4	212.5 ± 25.9	50	-	0	0%	16%
C. virgata 5	54.2 ± 5.7	24	41.6 ± 11.2	26	0%	5%
H. neesii	41.6 ± 7.5	38	37.4 ± 4.3	12	6%	0%
W. incurvata 1	153.1 ± 23.1	50	-	0	0%	0%
W. incurvata 2	150.1 ± 32.0	43	92.2 ± 22.7	7	4%	2%
W. incurvata 3	185.0 ± 29.9	49	171.6 ± 0.0	1	0%	0%
W. incurvata 4	177.9 ± 33.4	50	-	0	0%	0%
W. incurvata 5	185.4 ± 26.9	48	88.1 ± 29.9	2	0%	0%
W. sulcata (lab-stored)	35.3 ± 3.8	47	31.6 ± 3.2	3	6%	0%
W. sulcata (soil-stored field)	28.6 ± 3.5	16	20.6 ± 5.3	33	2%	0%
W. teres	102.2 ± 19.3	47	85.7 ± 6.4	3	4%	0%

Of the 13 seed collections tested, eight had a floating error of 0%. Of the remaining five collections, the highest floating error was 6%, in *W. sulcata* and *H. neesii*. The discarding error was negligible in ten out of the 13 collections. The other three collections had discarding errors of 2%, 5% and 16% respectively. It should be noted that the discarding error of 16% in *C. virgata* 4 dropped to zero after 1 h in water, when the seeds sank.

2.4) Discussion

Seeds of the nut-fruited Restionaceae examined in this study typically consisted of a hard, often woody ovary wall that enclosed the true seed. The seed coat is membranous (Linder 1991), and at the ultrastructural level was comprised by several compact layers. The tiny embryo is positioned on top of a large starchy endosperm. In the species examined, the endosperm was comprised of many starch grains and protein bodies in a cytoplasmic matrix. According to Bewley and Black (1994), the majority of endosperm cells are non-living at maturity, as the cytoplasmic contents are replaced by storage reserves during development, mainly carbohydrates in the form of starch in the nut-fruited Restionaceae investigated. In species examined, embryo tissue was undifferentiated, and cells were packed with lipid and protein reserves. Mitochondria and nuclei were typical of dormant or quiescent seeds (Bewley & Black 1994).

The nut-fruited Restionaceae examined here have a seed development that differs markedly between species in time. This variation in the length of seed development and the timing of seed maturation and release, followed by the rapid burial of seed in the soil by ant or wind action, highlights the importance of knowledge of the timing of events for successful seed collection of individual species. Seed development is typically triggered in a precise temporal fashion irrespective of environmental conditions (Kermode 1995), which was evident in *W. incurvata*, the development of which was unaffected by the high October rainfall (Figure 2.6). In the species examined, there was no relationship between seed size and length of seed development.

Effects of soil storage, examined by comparing soil- and laboratory-stored seeds using scanning electron microscopy, revealed signs of degradation of the protective ovary wall and funiculus in some nut-fruited Restionaceae seeds. The effects of soil storage on seed coats of other hard-seeded species (e.g. *Leucospermum* spp.) may be pronounced, and coat degradation can even lead to seed dormancy breakage (Brits 1996). Although the damage caused by temperature fluctuation and desiccation did not visibly disrupt the integrity of the ovary wall in the nut-fruited Restionaceae, the length of time the seeds were buried in the soil was six months. In contrast, the burial time for *Leucospermum* seeds in Brits' study, in which substantial coat deterioration was recorded, was four years (Brits 1996). Thus although the effects of six months of soil storage on nut-fruited Restionaceae seeds appear negligible, the effects of longer storage periods is unknown.

Of the seed collection methods that were evaluated, the shaking and ground methods produced a consistently higher quality of seed in a collection than the cutting method. This could be attributed to the fact that seeds on a plant mature and are released over a few weeks, yet only seeds ready for release shake off the plant, while immature seeds remain on the plant. This also explains the lower seed quality in the cutting method, as some seeds cut from the plant were unlikely to be completely mature. Thus the higher proportion of incompletely filled seed observed in seed collected using the cutting method is indicative of the harvesting of immature seed. Early cutting of seed heads from plants has been found to lead to poor germination due to seed being immature in other species (van Staden 1978, Jett & Welbaum 1996 and Tekrony & Egli 1997).

The main advantage of the cutting method in the nut-fruited Restionaceae is that it allows a large number of seeds to be collected in a short space of time, thus markedly reducing the time spent collecting seed in the field. However a large proportion of this seed may need to be discarded due to seed immaturity during seed sorting, which is necessary to ensure that the collection consists of fully formed mature seed. This would not only be time consuming, but could also yield a far smaller collection of seed than expected.

The ground collection method yielded a high proportion of mature seed. This seed collection method has been documented to be suitable in other species for achieving optimum seed germination (Thomsen 1997 and Upreti & Dhar 1997). However in the nut-fruited Restionaceae, this method would be suitable in opportunistic instances only. There would undoubtedly be low yields in myrmecochorous species, as seed released from the plant is usually collected and buried immediately by ants (Bond *et al.* 1991). In non-myrmecochorous species, this method would be suitable if seed collection coincided with the short period between seed release and seed burial by shifting sand or seed predation by rodents.

The shaking method, although slower than the cutting method during seed collection, yielded a better quality seed lot that reduced the time spent sorting the seed. The shaking method of seed collection has been used successfully in other species (Nowag *et al.* 1997 and Sunilkumar & Sudhakara 1998), and based on experimental results is the recommended collection method for the nut-fruited Restionaceae seed. This method works particularly well in plants that carry many seeds held in a loose bract, such as *W. incurvata* and *W. teres.* Species of *Cannomois* are an exception to this recommendation, as fewer seeds are generally produced per plant, and seeds are held more tightly in the surrounding bony bracts. Use of the shaking method for collecting seed in these types of plants is impractical, and in such cases the cutting method of seed collection would be most suitable.

X-radiography is a quick, non-destructive and reliable method for differentiating between filled, incompletely formed, empty, insect- and physically-damaged seed (Kamra & Simak 1965, Vozzo 1988, Jones *et al.* 1997, Tryka *et al.* 1997, Cicero *et al.* 1998 and ISTA 1999). X-radiography was found to be suitable for distinguishing the empty seed fraction in the nut-fruited Restionaceae. However it is also an expensive and time-consuming method, and so it was used to evaluate the more affordable weighing and floating methods of seed separation.

The weighing method was not a very reliable method for seed sorting in the nut-fruited Restionaceae, as there was overlap in full and empty seed weight in a collection. This overlap may have been exacerbated by variations in ovary wall thickness. A further disadvantage of the weighing method is that it is very time consuming, as each seed needs to be individually weighed.

The floating method was found to be more reliable seed separating technique than the weighing method as both floating and discarding errors were consistently low. Advantages of the floating method are that it is a cheap and relatively quick seed sorting method. Other species have also been sorted using the floating method (van Staden & Brown 1973b, Forsyth & van Staden 1983 and Poulsen 1995). It would be important to empirically determine the optimum floating time for different species, as an extended floating time was required to reduce the discarding error in the large-seeded *C. virgata*.

Seed separation in the nut-fruited Restionaceae is probably best achieved firstly by floating, and if necessary, x-radiography can be used to check questionable seeds. The floating method, although not as accurate as x-radiography for detecting empty seeds, has an acceptable degree of accuracy, is relatively quick and inexpensive, and is the method I would recommend to separate the empty fraction of seeds from fully developed ones.

Viability of full seeds was assessed using tetrazolium salts (a destructive method of seed viability determination). Electrolyte leakage was investigated as a non-destructive method for assessing seed viability. Electrolyte leakage was not a good predictor of seed viability in nut-fruited Restionaceae, as autoclaved seeds did not exhibit a consistently rapid electrolyte leakage. Instead, seeds in which the ovary wall was cracked exhibited rapid electrolyte leakage, while seeds in which the funiculus had been removed showed a much slower rate of electrolyte loss. The higher rates of electrolyte loss were most likely due to an increase in ovary wall permeability in cracked seeds. This increase in permeability would result in increased water movement into and out of the seed. A hard seed coat (Verma & Ram 1987) or seed coat integrity or permeability (Beresniewicz *et al.* 1995 and Taylor *et al.* 1995) have been shown to interfere with rates of electrolyte leakage during imbibition in other species, and similarly this may explain the higher leakage observed in cracked Restionaceae seeds.

Another explanation for this observed pattern of electrolyte loss is that the surface area of the seed exposed to water in cracked seeds initially was much greater and would therefore have resulted in higher leakage rates. Furthermore, possible damage to the seed coat and endosperm when cracked in the vice may have increased electrolyte leakage in these treatments. An additional confounding factor of using electrolyte leakage to assess viability in these seeds may be that of the relative size of the tiny embryo compared to the massive endosperm. Seed viability is assessed on the viability of the embryo (ISTA 1999), and electrolyte leakage from endosperm tissue, especially in cracked seeds in which the endosperm was damaged, would no doubt mask any subtle leakage differences from the embryo.

As there was no distinct difference between electrolyte leakage rates of healthy and autoclaved seeds, electrolyte leakage does not appear to be a suitable tool for separating viable from dead seed in nut-fruited Restionaceae. Although some studies have shown this technique to be a good predictor of seed germinability and vigour (Siddique & Goodwin 1985, Fernandez & Johnston 1995 and Thapliyal & Connor 1997), other researchers have found electrolyte leakage unreliable in measuring germination potential or in separating dead from live seeds (Hepburn *et al.* 1984 and Davies & Prichard 1998).

Tetrazolium tests for seed viability indicated that, for the most part, full seeds contained a high proportion of viable embryos. The variation in intensity of colour staining observed between embryos may be indicative of tissue ageing (Don

1979) and different seed vigour (Moore 1970). Generally, these biochemical test results indicate that, given optimal germination conditions and correct dormancy breaking treatments, seed germination in the species tested should be successful. Although the majority of collections had a high percentage of viable seed following sorting, it is recommended that a tetrazolium test for viability be routinely run in conjunction with any germination experiments to confirm the collection consists of a high proportion of viable seed.

Tetrazolium testing has shown that, with careful seed collection and suitable seed sorting techniques, a high proportion of mature and viable seeds is readily obtained in collections of nut-fruited Restionaceae seeds used in this study. This suggests that poor germination in such seed collections is not a result of poor seed quality, but rather of seed dormancy.

CHAPTER 3

SEED DORMANCY

3.1) Introduction

Successful seed germination is dependent on a number of factors. In the previous chapter seed quality was investigated as a cause of poor seed germination in the nut-fruited Restionaceae. Since it was found that seed quality is generally high with suitable collection and sorting techniques, poor seed germination must be due to seed dormancy. The approach in this study, as stated in the introduction, was mainly a physiological one. As far as possible, repetition of unpublished experiments of other researchers (Table 1.1) was avoided. Experiments in this study were designed to systematically test for the possibility of the different types of coat-imposed (physical, mechanical and chemical) and embryo (morphological and physiological) dormancy acting in these seeds. Some of the experiments conducted tested for more than one type of dormancy. However for ease of reference, experiments have been placed under headings indicating testing for a specific type of dormancy only. All experiments were conducted on seed that had been sorted by the floating method (section 2.2.4.1).

3.2) Materials and methods

3.2.1) Water and dye uptake

The possible existence of a barrier to water movement into nut-fruited Restionaceae seeds was tested with water and dye uptake experiments. These experiments tested for coat-imposed physical dormancy in the nut-fruited Restionaceae.

3.2.1.1) Water uptake

Six different treatments were investigated in water uptake:

a) laboratory-stored *W. incurvata* 1 (refer to Table 2.1) seed in which the ovary walls were cracked in a vice prior to testing;

b) laboratory-stored *W. incurvata* 1 seed in which the funiculus was removed;
c) laboratory-stored *W. incurvata* 1 seed left intact;
d) laboratory stored *W. incurvata* 1 seed in which Vaseline petroleum jelly (vaseline) was placed over the funiculus;
e) field soil-stored *W. incurvata* 1 seed (soil storage detailed in section 2.2.3.2); and,
f) freshly picked *W. incurvata* 2 seed.

With each treatment 10 seeds were weighed, immersed in distilled water, and re-weighed regularly over a 240 h period. Prior to weighing, excess surface water on each seed was removed by blotting with tissue paper. Care was taken not to remove the vaseline from the funiculus of vaseline-treated seeds. Water was replaced every 48 h with fresh distilled water. At the end of the experiment, seeds were dried in a convection oven at 100°C for 24 h, and cooled in a silica gel desiccator for 30 min prior to weighing. This enabled water uptake of the seeds to be calculated on a dry mass basis. Significant differences in mean final moisture content of seeds at the end of the experiment were tested for using a Kruskal-Wallis ANOVA by ranks.

The partitioning of water in the ovary wall, true seed (seed coat + endosperm + embryo) and embryo was then investigated in *W. incurvata* 4, *C. virgata* 4 and *W. teres* following water uptake. Seeds were imbibed for 240 h as in the water uptake experiment described above. Final water contents were expressed on a dry and wet mass basis. Embryo water contents were determined from an average of three replicates of 10 pooled embryos. Final embryo water contents were tested for differences using a single factor ANOVA (data checked for homogeneity of variances using Bartlett's test, $p > 0.05$). Significantly different values were separated using Scheffé's multiple range tests (Zar 1996).

3.2.1.2) Dye uptake

Twenty seeds of *C. virgata* 2, *W. teres* and *W. incurvata* 1 respectively were submersed in a 1% (mass / volume) solution of eosin ($C_{20}H_8N_2O_9Br_2$) dye. This solution was replaced with a fresh solution on a weekly basis. Seeds were rinsed to remove surface dye under running water prior to dissection. Dye penetration was checked using a Model Z30E dissecting microscope (Cambridge Instruments, China) at 240 h and 480 h after immersion, by examining longitudinally bisected seeds following ovary wall removal.

3.2.2) Seed and embryo culture

Culturing whole seeds and embryos, following removal of the ovary wall, seed coat and surrounding endosperm, tested for coat-imposed mechanical and / or chemical dormancy. Whole seeds (with only the ovary wall removed) and excised embryos were cultured under either continuous light (maximum intensity of 75 µmol m^{-2} s^{-1}) or dark with a variety of temperature regimes, hormone concentrations and surface sterilisation (Table 3.1). As Brown *et al.* (1994) reported lack of germination of *C. virgata* embryos and whole seeds using the embryo culture conditions by Meney and Dixon (1988) for Australian Restionaceae (see Table 1.1), variations of the composition of their culture media were tested.

Table 3.1. Culture conditions of excised embryos and whole seeds of *C. virgata*, *W. incurvata*, *W. sulcata* and *W. teres*. Details of surface sterilisation are given in the text.

	Hormones	Temperature	Lighting	Surface sterilization
1	0.5 µM GA_3 & 1 µM kinetin	20°C	Light	NaOCl and Tritron X-100
2	0.5 µM GA_3 & 1 µM kinetin	20°C	Dark	Ethanol, NaOCl and Tritron X-100
3	5 µM GA_3 & 1 µM kinetin	20°C	Dark	Ethanol, NaOCl and Tritron X-100
4	5 µM GA_3 & 1 µM kinetin	10°C 16 h / 20°C 8 h	Dark	HgCl, ethanol, NaOCl and Tritron X-100
5	0.5 µM GA_3 & 1 µM kinetin filter-sterilised	10°C 16 h / 20°C 8 h	Dark	HgCl, ethanol, NaOCl and Tritron X-100
6	0.5 µM GA_3 & 1 µM kinetin filter-sterilised, smoke extract added	10°C 16 h / 20°C 8 h	Dark	HgCl, ethanol, NaOCl and Tritron X-100

To minimise fungal infection, seeds were surface sterilised in a 0.01 mg ml^{-1} solution of mercuric chloride (HgCl) for 1 min and rinsed in sterile distilled water prior to imbibition. Seeds were imbibed in autoclaved water in sterilised Petri dishes for 36 - 48 h prior to ovary wall removal by cracking in a vice. Following removal of the ovary wall, the seeds or embryos were placed in 70% ethanol for 1 min and then rinsed in autoclaved distilled water for 5 min. They were further sterilised for 5 min in a solution of 0.88% (mass / volume) sodium hypochlorite (NaOCl) with Tritron X-100 (wetting agent), re-rinsed, and then placed aseptically into embryo culture.

Species cultured under tabulated test conditions listed above included *W. incurvata* 1 (soil-stored laboratory seed), *W. sulcata* (both soil-stored field and laboratory-stored seed) and freshly harvested *C. virgata* 5, *W. incurvata* 2 and *W. teres*. A minimum of five seeds and five embryos per species were used in each treatment and were cultured for eight weeks in full-strength Murashige and Skoog medium (Murashige & Skoog 1962) supplemented with 8 g l^{-1} of agar and 20 g l^{-1} of sucrose.

3.2.3) Hydrogen peroxide treatment

The possibility of physiological embryo dormancy due to oxygen limitation was tested as a dormancy mechanism by treating 50 seeds with hydrogen peroxide (H_2O_2) prior to germination. This was tested in both *W. incurvata* 1 (collected in 1996) and *W. incurvata* 2 (collected in 1997). The four seed treatments were:

a) seed with funiculus removed, soaked in 1% H_2O_2;
b) seed with funiculus removed (control);
c) intact seed soaked in 1% H_2O_2; and,
d) intact seed (control).

Seeds soaked in H_2O_2 were left for 50 h to allow for complete imbibition, and then rinsed in distilled water prior to sowing. Seeds were sown in soil collected from Silvermine Nature Reserve (soil characteristics detailed in section 2.3.2.2). All seedling trays were placed in a black plastic bag, and smoke from burning a mixture of dry and fresh *Passerina vulgaris* Thoday (Thymelaceae) in a bee smoker was pumped into the bag. The bag was then thoroughly sealed to prevent the smoke from escaping, and left for 24 h. Seedling trays were placed in a growth chamber at (10°C 16 h dark / 20°C 8 h light photoperiod). The maximum light intensity during the light phase, measured with a Model SKP200 light meter (Skye Instruments Ltd., Powys, UK) was 420 μmol m^{-2} s^{-1}. Seed germination was monitored over a period of 10 weeks. A Fisher exact test was used to check for significant differences in seed germination (Zar 1996).

3.2.4) Afterripening and low temperature treatment (condensed seasons experiment)

The possibility of physiological embryo dormancy relieved by moisture and temperature cycling was tested as a dormancy mechanism in the nut-fruited Restionaceae. This experiment investigated the effect of alternating afterripening (summer conditions: hot and dry) and low temperature treatment (winter conditions: cold and wet) on seed dormancy and germination.

The effects of afterripening and low temperature treatment were tested over an artificial '3-year' period in a phytotron, in which the season time was shortened, with 'one year' equivalent to four months in real time. The reason for condensing the seasons was because of the time constraints of this study. Conditions to which seeds were subjected for the different seasons are detailed in Table 3.2. As seasons were artificially condensed, temperatures were chosen to clearly signal the onset of "winter" conditions with lower-than-normal "autumn" temperatures. Differences in maximum and minimum temperatures during "autumn" were also kept low to approximate soil shaded with mature fynbos vegetation. The onset of "summer" was preceded with higher-than-normal "spring" temperatures, to clearly signal the end of "winter".

A minimum of three replicates of seeds ($n > 500$) were buried in trays containing soil collected from Silvermine Nature Reserve (refer to section 2.3.2.2 for soil characteristics). Sodium metal halide lighting (420 μmol m^{-2} s^{-1}, measured using a Model SKP200 light meter, Skye Instruments Ltd., Powys, UK) was supplied during the "winter" season, to allow germinants to photosynthesise. The experiment commenced in March 1998 (near the end of summer) with the "autumn" season. No other dormancy breaking treatments were applied to these seeds. In the third "winter", technical problems in the phytotron led to a failure in the cooling system, resulting in about four warm days approximating "summer" temperatures in the fourth week of "winter".

Table 3.2. Seasonal conditions in the phytotron for the condensed seasons experiment. Seed trays were watered to field capacity with a fine-spray hose with frequencies indicated below.

Season	**Alternating temperatures**	**Duration**	**Watering**
"Autumn"	5°C 12h / 15°C 12h	2 weeks	Four times in 2 weeks
"Winter"	10°C 16h / 20°C 8h	8 weeks	Four times every 2 weeks
"Spring"	20°C 14h / 30°C 10h	2 weeks	Once a week
"Summer"	20°C 10h / 30°C 14h	4 weeks	Single wetting at the beginning of the 3rd week

After the second week of each "spring", germinating seeds (identified by radicle protrusion) were removed from the soil. Deteriorated seeds, that were identified by visual inspection and floating, were discarded. Often a white fungus was visible around the hilum and in the funiculus of deteriorated seeds. This could also be identified by clumped sand around the hilum. When the sand was removed from the hilum region, often the funiculus would dislodge, leaving the seed exposed. Following visual inspection, seeds were placed in water, and floaters removed (see section 2.2.4.1). Seeds that floated were found to be shrivelled or deteriorated when cracked open. The remaining seeds were then replaced in the soil, and the trays returned to the phytotron that was set to "summer" season conditions. Tetrazolium tests (refer to section 2.2.4.2) were conducted at the end of the experiment on a sub-set of seeds ($n = 20$) to check seed viability.

Since seed that deteriorates lessens the germinable portion of seed in the soil, seed deterioration and survivorship were monitored in soil-stored seed. Seed survivorship in the soil was estimated from deterioration data using the following equation (Roberts 1974):

$$S = S_0 e^{-g t}$$

where S is the number of viable seeds in the soil at time t, S_0 is the number of seeds in the initial population, and g is the annual rate of deterioration for the species and burial environment.

3.2.5) Embryo size

Embryo sizes of freshly harvested seed, afterripened seed (dry laboratory storage) and afterripened and low temperature treated seed (condensed seasons seed) were compared to test for the possibility of morphological embryo dormancy. A minimum of thirty seeds of freshly harvested *W. incurvata* 2, laboratory-stored *C virgata* 4, *W. incurvata* 1 and *W. teres* and condensed seasons-stored *C virgata* 4, *W. incurvata* 2 and *W. teres* were hydrated for 48 h (to prevent damage to the embryo during dissection). Following dissection, the mean diameter of each embryo was calculated from two perpendicular measurements taken with a graduated eyepiece while viewing the embryo under a Model CH Olympus compound microscope (Olympus Optical Co., Ltd, Japan). A Chi-squared goodness of fit test was used to test for differences in embryo diameters of seeds of different treatments (Zar 1996).

3.2.6) Fire treatment

This experiment attempted to simulate fire conditions experienced by soil-stored seeds, to test the role of fire as a seed dormancy breaking mechanism. The heat and / or smoke and gaseous components from the fire were tested separately and combined for breaking physiological dormancy. Three hundred seeds per seed tray from the condensed seasons experiment, and similar quantities of laboratory-stored seed of the same seed collections, were selected for a fire experiment. One third of the seed was placed in small bags of plummers' mesh. These bags were buried 4 cm below the surface in a metal container (1 m X 1 m X 0.15 m) containing Silvermine soil. Approximately 0.14 m^3 of dead brush and live fynbos vegetation consisting mostly of *Passerina vulgaris* Thoday (Thymelaceae) and *Restio triticeus* Rottb. (Restionaceae) was collected as fuel. The brush was spread out evenly over the soil surface. Once the fire was lit, surface and soil temperatures were monitored until temperatures returned to ambient levels. Probes of a K-type Fluke Thermocouple (Fluke, Melrose, Massachusetts, USA) to measure soil temperature were buried in the soil at the same depth as the seeds. A Raynger 3I series Raytec Infrared Thermometer (EPD Technology Corp., Elmsford, New York, USA), set to an emissivity of 0.95, was used to measure the surface temperature of the fire.

Seeds were removed from the soil some hours after the fire treatment, and soil (including the surface charate) was placed into germination trays, into which the seeds exposed to the fire (seeds exposed to heat + smoked soil) were buried. One third of seed set aside prior to the experiment was buried in soil that had been exposed to the fire (seeds exposed to smoked soil). The remaining seed was buried in untreated soil (control). Trays were placed in a phytotron for one week without watering at 10°C 12h / 30°C 12h to simulate "autumn" temperatures following vegetation removal by fire. The phytotron was then set to "winter" conditions as used in the condensed seasons experiment (Table 3.2). Due to an unforeseen temperature spike at the end of the second week of "winter", during which the temperature rose to 36°C for about 18 h, the length of this season was extended by two weeks, as seed emergence appeared to be delayed by this event.

At the end of the experiment, germinants and visibly deteriorated seed (identification described in section 3.2.4) were recorded, and a subset of the remaining seed tested for viability using tetrazolium (refer to section 2.2.4.2). Soil pH and conductivity were determined (refer to section 2.2.3.2 for methods of soil characterisation) for control and fired soil, and a one-way fixed effects ANOVA (data checked for homogeneity of variances using Bartlett's test, $p > 0.01$) tested for significant differences. A three-way fixed-effects ANOVA (data checked for homogeneity of variances using Bartlett's test, $p > 0.05$) was used to test for significant differences in arc-sine transformed germination and deterioration. Significantly different values were separated using Scheffé's multiple range tests (Zar 1996).

3.3) Results

3.3.1) Coat-imposed physical dormancy

3.3.1.1) Water uptake

Water uptake occurred in all seeds. Water uptake was initially rapid, but levelled off after three days, and remained constant until the end of the experiment (Figure 3.1). Water uptake was most rapid in seeds in which the ovary wall was cracked in a vice. All other treatments imbibed water at a similar rate. As water uptake occurred in seeds in which the hilum region was covered in vaseline, water movement must have occurred through the ovary wall as well as the funiculus. The mean final moisture content of seeds at the end of the experiment was not significantly different (Kruskal-Wallis ANOVA, $n = 59$, $H = 12.97$, $p > 0.01$).

Whole seed water contents were low (Table 3.3). However as this overall water content may not reflect individual water contents of different seed components, water contents of the ovary wall, true seed (seed coat + endosperm + embryo) and embryo were determined following water uptake (Table 3.4).

Table 3.3. Mean initial and final moisture contents, expressed as a percentage of dry mass, of *W. incurvata* seeds used in water uptake experiments.

Seed collection	**Seed storage**	**Additional treatment**	**Water content (% DMB)**		
			n	**Initial**	**Final**
W. incurvata 1	laboratory-stored	cracked	10	14.2 ± 1.7	35.8 ± 2.4
W. incurvata 1	laboratory-stored	funiculus removed	10	12.9 ± 1.8	33.4 ± 2.0
W. incurvata 1	laboratory-stored	-	10	13.4 ± 1.3	34.5 ± 3.7
W. incurvata 1	laboratory-stored	funiculus vaselined	9	12.6 ± 2.9	34.5 ± 7.7
W. incurvata 2	freshly harvested	-	10	12.9 ± 2.8	34.2 ± 4.9
W. incurvata 1	soil-stored field	-	10	13.6 ± 0.6	30.9 ± 1.8

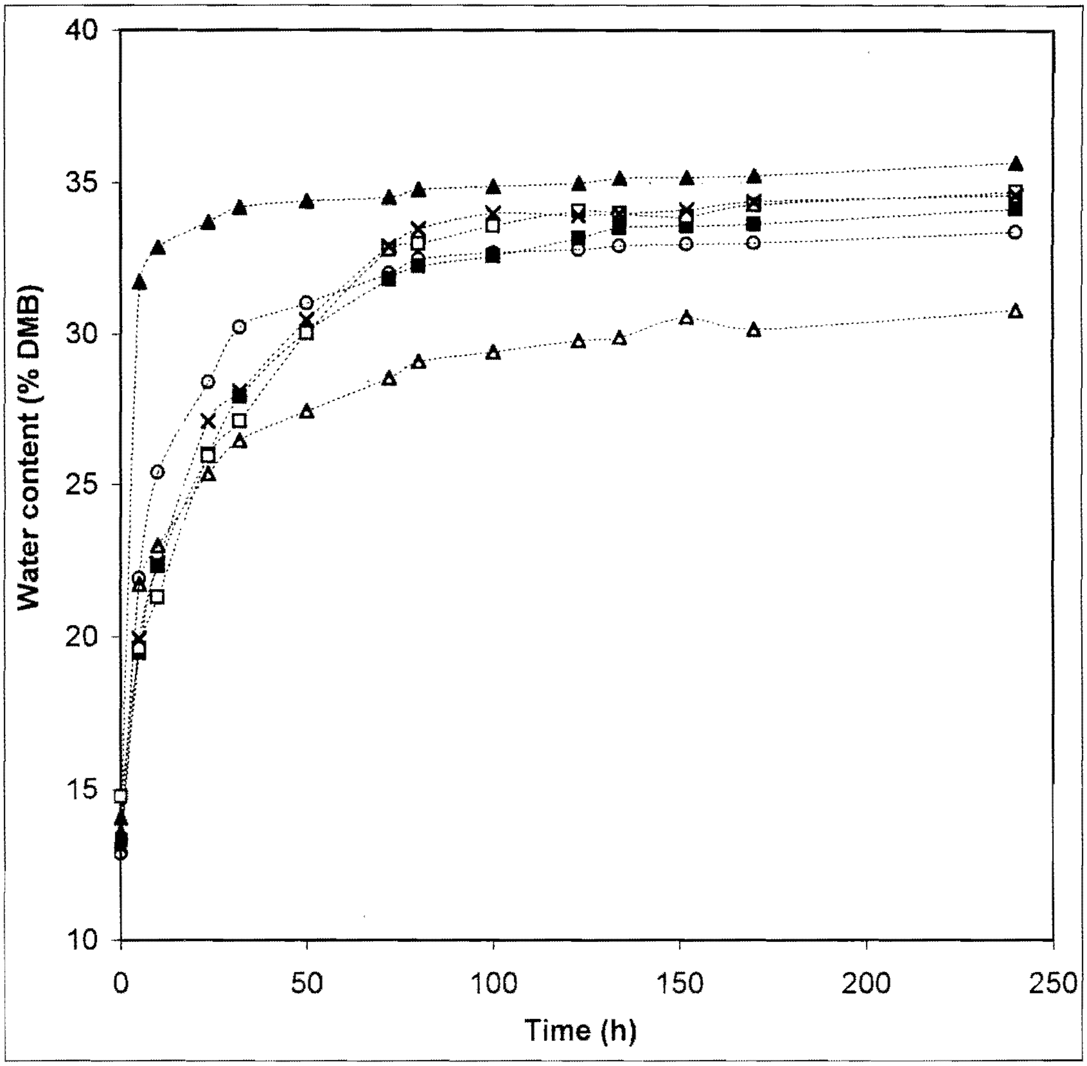

Figure 3.1. Rate of water uptake (n = 10) in **(i)** laboratory-stored, ovary wall cracked in vice [▲], **(ii)** laboratory-stored with funiculus removed [o], **(iii)** laboratory stored [■], **(iv)** laboratory-stored with funiculus coated in vaseline [□], **(v)** field soil-stored [△] and **(vi)** freshly harvested [**x**] *W. incurvata* seeds.

Water contents of the true seed were higher than water contents of the ovary walls of the same species. Mean final embryo water contents were higher than true seed water contents (Kruskal-Wallis one-way ANOVA) in *C. virgata*, (n = 23, H = 12.75, p = 0.0004), *W. incurvata* (n = 24, H = 9.60, p = 0.0019) and *W. teres* (n = 30, H = 11.48, p = 0.0007). *C. virgata* embryos hydrated to a significantly higher water content than *W. incurvata* and *W. teres* embryos (one-way fixed effects ANOVA, F = 23.54, p < 0.0001). Initial embryo water contents were not determined, as the tissue was too dry to separate embryos from the endosperm.

Table 3.4. Mean initial and final moisture contents (expressed as a percentage of both wet and dry mass) of seeds following water uptake in laboratory-stored *W. incurvata*, *C. virgata* and *W. teres*. Water contents of the seed (ovary wall + true seed), ovary wall, true seed (seed coat + endosperm + embryo) and embryo are displayed.

Species	**Initial water content (% DMB)**					
	n	**Seed**	**Ovary wall**	**True seed**		
W. incurvata 4	15	10.1 ± 0.4	10.5 ± 0.5	9.1 ± 0.6	-	
W. teres	14	10.6 ± 0.6	11.0 ± 0.6	9.5 ± 1.6	-	
C. virgata 4	14	9.3 ± 0.9	9.6 ± 1.0	8.3 ± 1.3	-	
	Final water content (% DMB)					
	n	**Seed**	**Ovary wall**	**True seed**	**n**	**Embryos**
W. incurvata 4	15	30.4 ± 3.3	27.5 ± 4.4	38.8 ± 2.3	4	80.0 ± 16.6
W. teres	15	33.2 ± 2.8	31.8 ± 2.5	37.0 ± 5.1	10	83.3 ± 39.3
C. virgata 4	15	37.1 ± 3.1	33.5 ± 2.0	46.9 ± 8.5	6	236.5 ± 65.3
	Final water content (% WMB)					
	n	**Seed**	**Ovary wall**	**True seed**	**n**	**Embryos**
W. incurvata 4	15	23.3 ± 1.8	21.5 ± 2.4	28.0 ± 1.2	4	44.3 ± 4.7
W. teres	15	24.9 ± 1.5	24.1 ± 1.4	26.9 ± 2.5	10	43.2 ± 12.2
C. virgata 4	15	27.0 ± 1.7	25.1 ± 1.1	31.7 ± 3.7	6	69.3 ± 5.3

3.3.1.2) Dye uptake

Eosin dye penetrated through the ovary wall, staining the outside of the membranous seed coat a red colour. However the dye did not penetrate through the seed coat to the embryo and endosperm in any of the species (*C. virgata*, *W. incurvata* and *W. teres*) examined after 20 days imbibition in the solution (data not shown). As water was able to penetrate the seed coat, it is possible that the eosin dye molecules were restricted physically by crossing the seed coat barrier due to their large molecular size ($C_{20}H_8N_2O_9Br_2$) compared to water (H_2O).

3.3.2) Coat-imposed mechanical and chemical dormancy

3.3.2.1) Seed and embryo culture

None of the seed culture experiments resulted in germination. A single excised *W. incurvata* embryo from treatment 5 germinated over the three-month period during which seeds and embryos were cultured (10% germination). This embryo germinated on a culture medium, in which 0.5 µM GA_3 & 1 µM kinetin had been filter-sterilised into the culture medium, and was kept in the dark at a temperature regime of 10°C (16 h) / 20°C (8 h). Germination under these conditions might mean that relatively low filter-sterilised GA_3 and dark conditions are a requirement for germination. However since only one embryo germinated, and the history of that embryo is unknown, other factors or a lack of dormancy may have predisposed it to respond to the tissue culture conditions.

3.3.3) Morphological embryo dormancy

3.3.3.1) Embryo size

The proportion of larger size embryos in a sample tended to increase with both dry afterripening (Figure 3.2i) and alternating dry afterripening and wet low temperature treatment of condensed seasons-stored seed (Figure 3.2ii-iv). A Chi-squared goodness of fit test on the original values showed this observed trend to be significant in condensed seasons-stored *W. incurvata* 2 (df = 6, $\chi^2 = 13.110$, $p < 0.05$). This trend was not significant in condensed seasons-stored *C. virgata* 4 (df = 6, $\chi^2 = 2.105$, $p > 0.05$) and *W. teres* (df = 4, $\chi^2 = 6.105$, $p > 0.05$) and in dry afterripened laboratory-stored *W. incurvata* 1 seed (df = 5, $\chi^2 = 3.522$, $p > 0.05$).

3.3.4) Physiological embryo dormancy: gases

3.3.4.1) Hydrogen peroxide treatment

Table 3.5 details the germination response of seeds of *W. incurvata* treated with hydrogen peroxide. From the table it can be seen that hydrogen peroxide treatment did not enhance germination in this species. Interestingly, funiculus removal significantly enhanced germination in the control (two-tailed Fisher's exact test, $p = 0.031$). However this was a single observation in a single species,

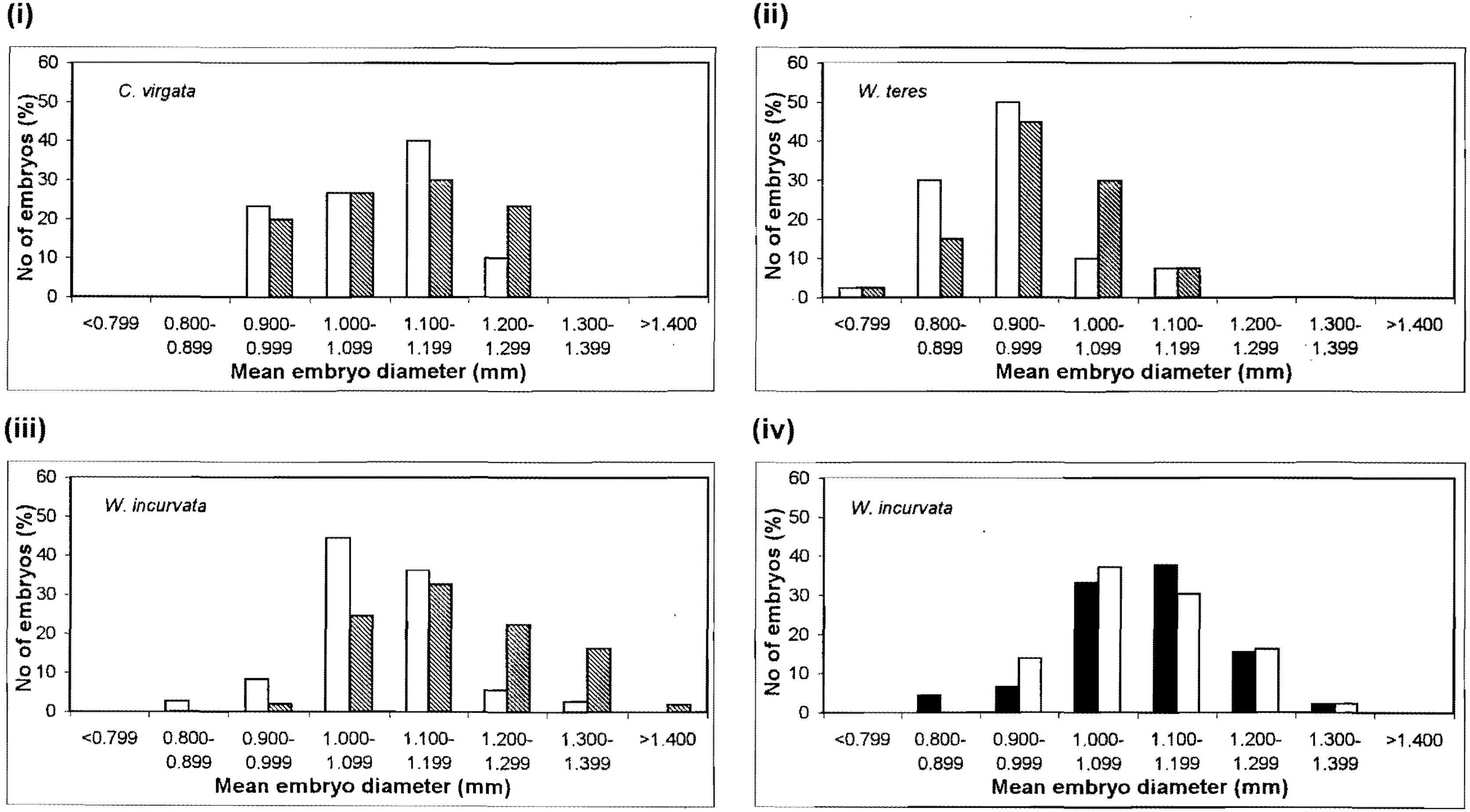

Figure 3.2. Frequency distributions (converted to percentages for comparison) of the embryo diameters (n >= 30) of laboratory-stored (open bars) and condensed seasons-stored (hatched bars) **(i)** *C. virgata* 4, **(ii)** *W. teres* and **(iii)** *W. incurvata* 2 and **(iv)** freshly harvested *W. incurvata* 2 (solid bars) and laboratory-stored *W. incurvata* 1 (open bars).

and this observation would require further testing before any role of funiculus removal in dormancy could be inferred.

Table 3.5. Germination percentages of *W. incurvata* seeds (n = 50) with and without hydrogen peroxide (H_2O_2) treatment and funiculus removal.

Species	**Treatment**	**Control**	**H_2O_2 treated seeds**
W. incurvata 1	-	0%	0%
W. incurvata 1	funiculus removed	0%	0%
W. incurvata 2	-	2%	0%
W. incurvata 2	funiculus removed	16%	2%

3.3.5) Physiological embryo dormancy: temperature and moisture

3.3.5.1) Afterripening and low temperature treatment (condensed seasons experiment)

Figure 3.3 shows the germination observed over the three-year condensed seasons experiment, during the first, second and third "winter" in *C. virgata* 3, 4 and 5 (data combined), *W. incurvata* 3, 4 and 5 (West coast), *W. incurvata* 2 (Cederberg) and *W. teres*. *C. virgata* data was combined as germination and deterioration values were similar in the brittle small-seeded and woody large-seeded form (data not shown). *W. incurvata* (West Coast) was not combined with *W. incurvata* (Cederberg) data as the two groups naturally experience different fire regimes, the former group occurring in dune thicket (Lubke 1996) while the latter occurs in mountain fynbos (Rebelo 1996) and is therefore more at risk of burning. Germination was low (< 8%) in all species and seasons. Of the four species, *W. incurvata* exhibited the highest germination, while *W. teres* had practically no germination. Each "winter" a small percentage of seeds germinated, however this decreased with each season in *W. incurvata*. *C. virgata* exhibited greatest germination in the second "winter". However this germination value was still low.

The percentage of non-viable seeds at the end of the condensed seasons experiment (determined from combining visibly deteriorated seeds and non viable seeds identified by tetrazolium tests) is shown in Table 3.6. Floating was not as accurate for separating deteriorated seed from viable seed (Table 3.6) as it was

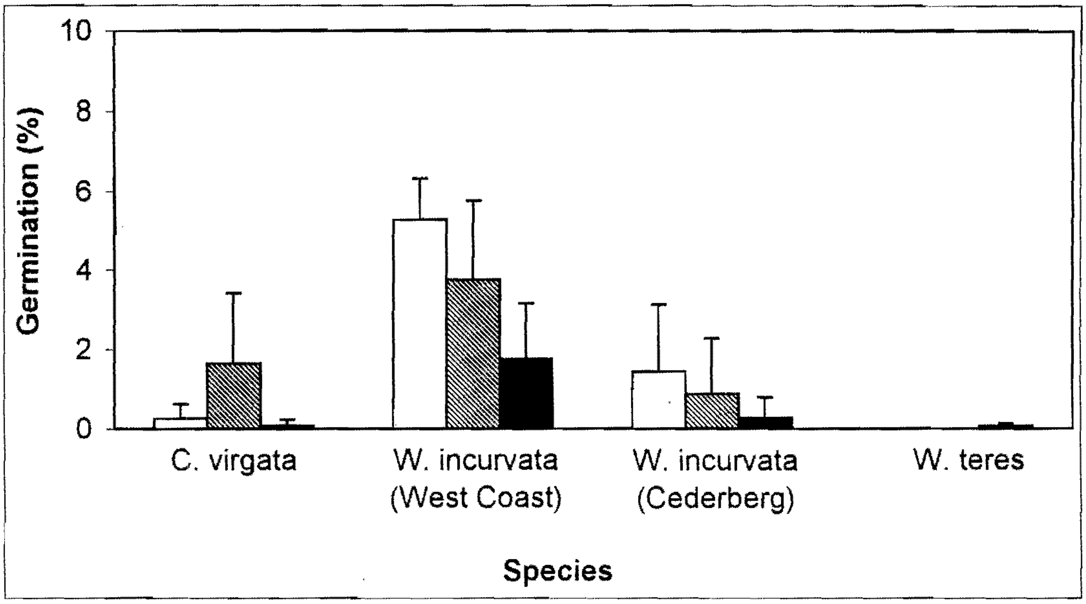

Figure 3.3. Percentage germination of a minimum of three replicates of *C. virgata*, *W. incurvata* (West coast), *W. incurvata* (Cederberg) and *W. teres* seed (n > 500 per replicate) during "winter 1" (open bars), "winter 2" (hatched bars) and "winter 3" (solid bars) of the condensed seasons experiment. Only the upper error bar is displayed.

in seed quality testing where it was used to separate full from empty seed (section 2.3.3.2). Tetrazolium testing in addition to visible inspection and floating was necessary. The half-life of seeds in the soil (i.e. the time taken for 50% of the seeds to lose viability) was calculated (refer to section 3.2.4) for each species (Table 3.6). Seed survivorship in the soil was predicted to be higher in *C. virgata* than in *W. incurvata* and *W. teres*.

Table 3.6. Mean seed deterioration of a minimum of three replicates (seed trays) per 'year' of *C. virgata*, *W. incurvata* and *W. teres* as determined from visual inspection and floating (all seeds) and tetrazolium seed viability testing (n = 20 per seed tray). Predicted seed half-life was calculated from deterioration data using Roberts' (1974) seed survivorship equation (refer to section 3.2.4).

Species	**Seed deterioration per 'year'**			**Predicted seed half-life (years)**
	Visible	**Tetrazolium**	**Total**	
C. virgata	3.8%	0%	3.8 ± 1.5%	18.2
W. incurvata (West coast)	4.3%	4.2%	8.5 ± 2.4%	8.2
W. incurvata (Cederberg)	2.2%	6.0%	8.2 ± 2.7%	8.4
W. teres	1.0%	8.0%	9.0 ± 1.6%	7.7

3.3.6) Physiological embryo dormancy: organic and inorganic chemicals

3.3.6.1) Fire treatment

Characteristics of the fire

Surface temperatures (n = 5) and soil temperatures 4 cm below the surface (n = 3) during the fire are shown in Figure 3.4. The maximum average surface temperature was in excess of 700°C, however these high surface temperatures only persisted for about 10 min. The soil temperatures at the level at which the seeds were buried rose to a maximum average temperature of 83°C, and returned to the original levels 4 h after the fire was lit.

Characteristics of the soil

Soil conductivity and pH of the control soil, fired soil, and fired soil in which seeds had been germinated (which had been exposed to watering for two months) was determined (Figure 3.5). Soil pH was significantly less acidic in fired soil that had

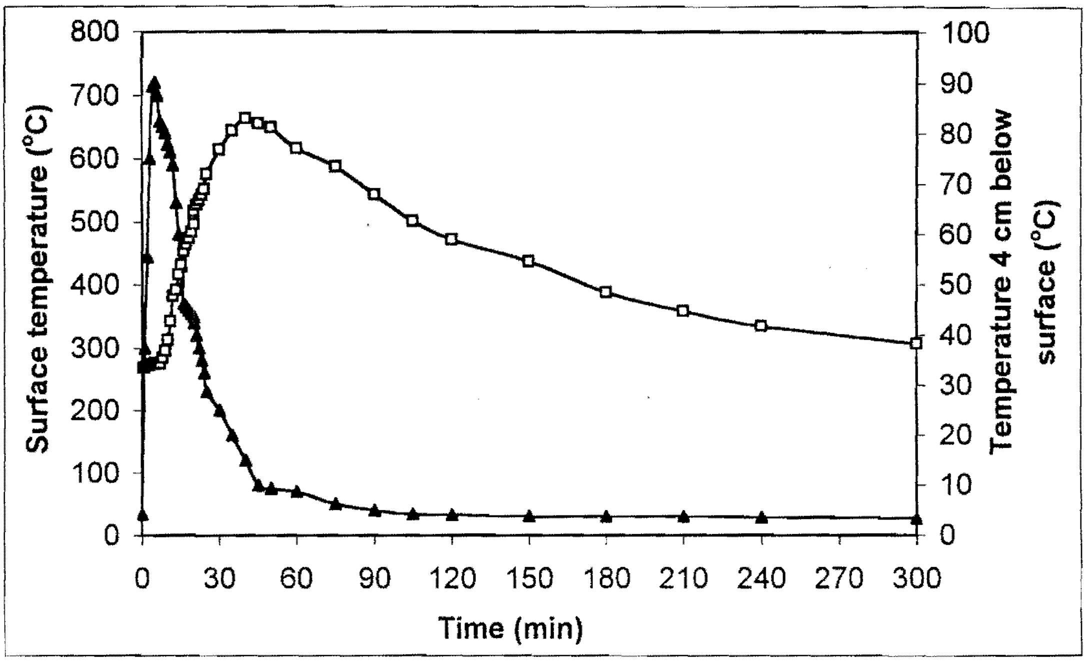

Figure 3.4. Surface temperatures (solid triangles – temperature axis on the left) and soil temperatures 4 cm below the surface (open squares – temperature axis on the right) during the fire experiment.

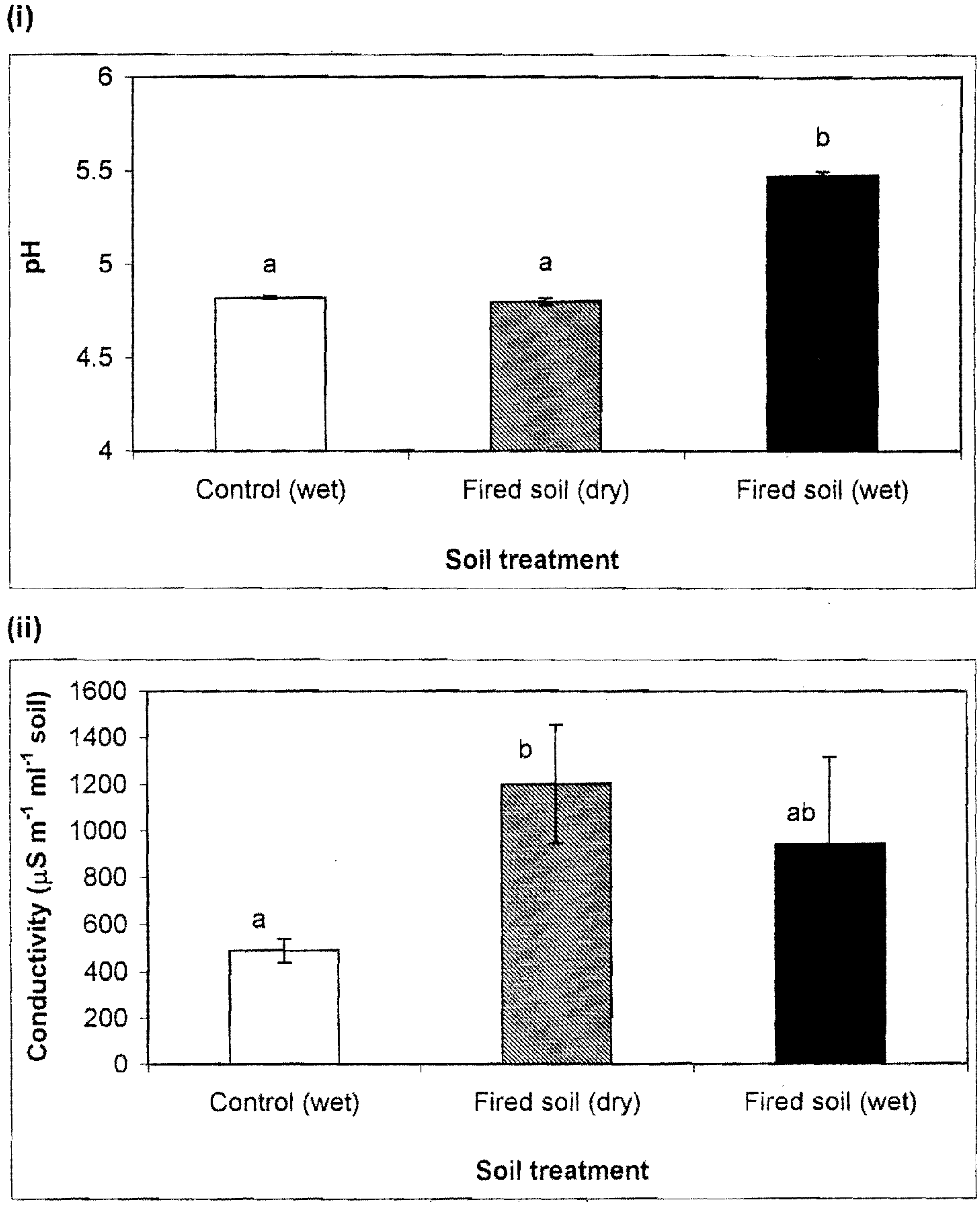

Figure 3.5. **(i)** pH and **(ii)** conductivity of four replicates of **(a)** control soil in which seeds were germinated (open bar), **(b)** soil exposed to the fire but not wet (hatched bar) and **(c)** soil exposed to the fire in which seeds were germinated (solid bar). Letters represent the means separation by Scheffé's multiple range test. Values with the same letter are not significantly different ($p < 0.05$).

been wet. Soil conductivity increased in soil exposed to fire: dry fired soil had a significantly higher conductivity than control soil.

Seed germination and deterioration

Figure 3.6 shows germination in a minimum of three replicates of 100 seeds per replicate of the control (untreated), smoked (unheated seeds germinated in smoked soil) and fired (exposed to heat of fire + smoked soil) seeds of *C. virgata*, *W. incurvata* (West Coast), *W. incurvata* (Cederberg) and *W. teres*. Results of a three-way ANOVA on arcsine transformed germination percentages are presented in Table 3.7. It can be seen from this table that seed germination was significantly higher in *C. virgata* compared to the other species. Condensed seasons-stored seed had a significantly higher germination percentage than laboratory-stored seed. Smoke and fire significantly improved germination from the control.

Table 3.7. Results (F values and significance levels) of a three way ANOVA on arcsine transformed germination percentages of *C. virgata*, *W. incurvata* and *W. teres* seed following exposure to fire. Seed germination was calculated from a minimum of three replicates of 100 seeds per seed tray. Within a specific effects Scheffé's multiple range test different letters indicate significantly different means ($p < 0.05$).

SPECIFIC EFFECTS	**Mean germination**	**Value**	**F value**	**Significance level**
Species				
C. virgata	29.07	a	175.51	p < 0.001
W. incurvata (West coast)	5.77	b		
W. incurvata (Cederberg)	3.22	bc		
W. teres	0.32	c		
Storage				
Laboratory-stored	3.57	a	155.41	p < 0.0001
Condensed seasons-stored	15.62	b		
Treatment				
Control	6.40	a	12.26	p < 0.0001
Smoke	12.16	b		
Fire (smoke + heat)	10.23	b		

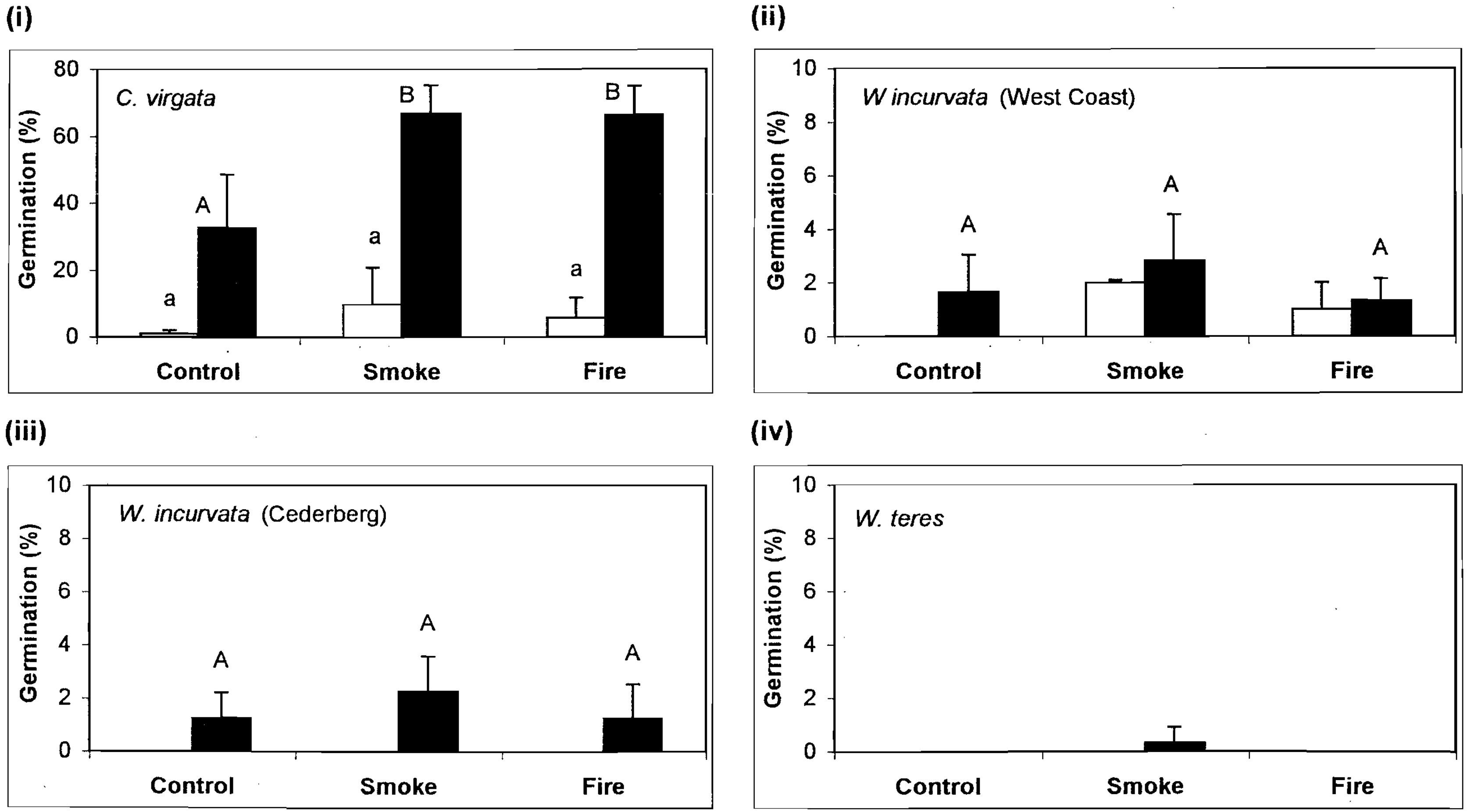

Figure 3.6. Percentage germination of three or more replicates of seed (n = 100) of laboratory-stored (open bars) and condensed seasons-stored (solid bars) **(i)** *C. virgata* **(ii)** *W. incurvata* (West coast) **(iii)** *W. incurvata* (Cederberg) and **(iv)** *W. teres* following exposure to fire. Only the upper error bar is displayed. Note that the y-axis is scaled differently in *C. virgata*. Letters represent the means separation showing comparisons within laboratory-stored (lower case lettering) and condensed seasons-stored (upper case lettering) treatments independently ($p < 0.05$) for those data for which analyses could be performed.

Results of a three-way ANOVA on arcsine transformed deterioration percentages are presented in Table 3.8. *C. virgata* exhibited a significantly lower percentage of deterioration than the other species. There were no significant differences in deterioration between condensed seasons-stored and laboratory-stored seed. There was a significantly higher deterioration in seeds that had been exposed to the heat of the fire than seeds that had not been exposed to the fire.

Table 3.8. Results (F values and significance levels) of a three way ANOVA on arcsine transformed deterioration percentages of *C. virgata*, *W. incurvata* and *W. teres* seed following exposure to fire. Seed deterioration was calculated from a minimum of three replicates of 100 seeds per seed tray. Within a specific effects Scheffé's multiple range test different letters indicate significantly different means ($p < 0.05$).

SPECIFIC EFFECTS	**Mean deterioration**	**Value**	**F value**	**Significance level**
Species				
C. virgata	24.53	a	12.63	p < 0.0001
W. incurvata (West coast)	35.57	b		
W. incurvata (Cederberg)	35.67	b		
W. teres	37.05	b		
Storage				
Laboratory-stored	34.63	a	3.03	p < 0.086
Condensed seasons-stored	31.78	a		
Treatment				
Control	29.01	a	14.22	p < 0.0001
Smoke	31.37	a		
Fire (smoke + heat)	39.24	b		

Table 3.9 reports the percentages of germinated, deteriorated and remaining viable seed that did not germinate or deteriorate in the fire experiment. Lack of germination in the remaining viable seeds is ascribed to seed dormancy.

Table 3.9. Percentages (calculated from a minimum of three replicates of 100 seeds) of germinated, deteriorated and remaining viable seed used in the fire experiment. Lack of germination in viable seed is ascribed to seed dormancy.

SPECIES	**Laboratory-stored seed**			**Condensed seasons-stored seed**		
Control	**% germination**	**% deterioration**	**% viable**	**% germination**	**% deterioration**	**% viable**
C. virgata	1 ± 1%	6 ± 5%	93 ± 5%	33 ± 16%	7 ± 4%	60 ± 17%
W. incurvata (West coast)	0 ± 0%	49 ± 6%	51 ± 6%	2 ± 1%	35 ± 12%	63 ± 13%
W. incurvata (Cederberg)	0 ± 0%	31 ± 7%	69 ± 7%	1 ± 1%	27 ± 5%	72 ± 5%
W. teres	0 ± 0%	29 ± 4%	71 ± 4%	0 ± 0%	20 ± 3%	80 ± 4%
Smoke						
C. virgata	10 ± 11%	30 ± 23%	60 ± 24%	67 ± 8%	17 ± 6%	16 ± 5%
W. incurvata (West coast)	2 ± 0%	19 ± 10%	79 ± 10%	3 ± 2%	27 ± 7%	70 ± 7%
W. incurvata (Cederberg)	0 ± 0%	31 ± 23%	69 ± 23%	2 ± 1%	32 ± 11%	66 ± 11%
W. teres	0 ± 0%	33 ± 18%	67 ± 18%	1 ± 1%	36 ± 18%	63 ± 17%
Fire						
C. virgata	6 ± 6%	40 ± 3%	54 ± 3%	66 ± 9%	17 ± 9%	17 ± 7%
W. incurvata (West coast)	1 ± 1%	39 ± 7%	60 ± 6%	1 ± 1%	39 ± 11%	60 ± 11%
W. incurvata (Cederberg)	0 ± 0%	63 ± 20%	37 ± 20%	1 ± 1%	37 ± 11%	62 ± 12%
W. teres	0 ± 0%	36 ± 20%	64 ± 20%	0 ± 0%	54 ± 8%	46 ± 8%

3.4) Discussion

Water uptake occurred in all nut-fruited Restionaceae seeds tested in this study (Table 3.4). Interestingly, water uptake also took place in seeds in which the funiculus was sealed with vaseline (Table 3.3). This indicates that water uptake occurs through the funiculus and the ovary wall, unlike in the leguminous *Sesbania punicea*, in which water movement was through the lens of the seed coat only (Manning & van Staden 1987). The finding that water uptake occurs through the ovary wall is supported by the presence of pores in cells of the ovary wall (refer to Figure 2.11), which presumably allow for water movement into and through the ovary wall. This is further supported by the fact that the ovary wall is able to take up water during imbibition (refer to Table 3.4).

The rate of water uptake in *W. incurvata* was faster in seeds that had been cracked in a vice (Figure 3.1). This is hardly surprising, as increased rates of water uptake in *Prosopis* (Villagra 1995) and *Zinnia* (Miyajima 1996) seeds have similarly been achieved by coat disruption. However seed coat degradation that was observed in *W. incurvata* (see Figure 2.9i) did not significantly affect imbibition rates in soil-stored field seed.

The pattern of water uptake in nut-fruited Restionaceae species examined reflected the first two phases of the triphasic pattern of water uptake (see Figure 3.1), which is typical of seeds placed in non-limiting water conditions (Bewley & Black 1994). Imbibition was initially rapid (phase I), as a consequence of the large difference in water potential. Water uptake then slowed, levelling off after the third day (phase II). Phase II is the activation phase during which seed water content remains constant or increases slightly. Important metabolic events take place during this period that prepare the seed for phase III of water uptake, which is associated with the initiation of growth and radicle emergence (Bradford 1995). None of the seeds entered into Phase III for the duration of the experiment.

According to Bewley and Black (1994), dormant seeds do not proceed beyond phase II of water uptake. Thus a dormant seed may achieve virtually all of the metabolic steps necessary for germination, yet for some unknown reason, the radicle fails to elongate (Bewley 1997). Imbibed seeds can remain in phase II for months or years before completing germination (Powell *et al.* 1984).

Final water contents of whole seeds (Table 3.3) of *W. incurvata*, irrespective of treatment, reached a similar percentage. The ovary wall therefore appears not to restrict water uptake, unlike seeds of the wild nutmeg, in which, although the intact seed coat was permeable, testa removal increased both the amount of water uptake as well as percentage seed germination (Cunha *et al.* 1995). Furthermore, germination in this group of seeds does not result in breakage of the ovary wall at any point (personal observation), as germination takes place through the funiculus, which presumably is pushed out ahead of the radicle.

Final water contents of whole seeds (Table 3.3) of *W. incurvata* were apparently too low for starch hydrolysis (Obroucheva 1997) and germination to proceed (Bai *et al.* 1999). As water contents are known to vary between different parts of the seed (McDonald *et al.* 1994 and Bai *et al.* 1999), often due to their chemical composition (Stahl & Steiner 1998), water contents of the ovary wall, true seed and embryo were determined (Table 3.4). Embryos of all species were at higher water contents than the true seed. It is interesting that the water contents of *W. teres* and *W. incurvata* embryos were significantly lower than *C. virgata* embryos. The critical water content (the seed water content below which germination is unable to proceed due to a deficiency of water) varies widely between species (Bradford 1995 and Egli & TeKrony 1997). It is however possible that the embryo moisture contents of the majority of *W. teres* and *W. incurvata* were too low for germination (Bai *et al.* 1999), while *C. virgata* embryos may have been sufficiently hydrated for germination to occur (Gray *et al.* 1990 and Bai *et al.* 1999). If this is the case, another type of dormancy must have prevented *C. virgata* germination.

Water uptake experiments revealed that the ovary wall is permeable to water, which suggests that these seeds do not have coat-imposed physical dormancy. Seeds that survive in soil for long periods tend to fall into two groups: the first comprising hard seeds that do not imbibe until coat resistance is overcome, and the second consists of seed that survives in a fully or partially hydrated state under conditions of restrained metabolism (Priestley 1986). It appears that the nut-fruited Restionaceae fall into the second group. The role of the hard ovary wall in nut-fruited Restionaceae is therefore unlikely to be one of physical dormancy.

Although the ovary wall and seed coat were found to be permeable to water, eosin dye was unable to penetrate the membranous seed coat. Dye uptake studies (Kelly & van Staden 1985 and van Rensburg 1995) are usually performed to confirm water penetration into the seed, and more importantly the embryo. However Keeley and Fotheringham (1997, 1998b) found that eosin dye was indicative of a solute impermeable barrier just inside the seed coat of *Emmenanthe penduliflora*, *Phacelia grandiflora*, *Romneya coulteri* and *Dicentra chrysantha* seeds. They found that the permeability of this layer was altered on exposure to smoke. Some vegetable seeds also have a semipermeable layer in their seed coats preventing solute movement (Beresniewicz *et al.* 1995). Transmission electron microscopy of the seed coat of *C. virgata* (see section 2.3.1.1) showed it was composed of several sometimes folded compact layers, which probably restricted the dye movement. Although the effect of smoke on seed coat permeability was not tested due to time constraints, the finding that large molecules of eosin dye were unable to penetrate through the seed coat, while water crosses this barrier, is interesting. If the semi-permeable seed coat plays a role in seed dormancy, increased permeability to large molecules may lead to physiological changes that in turn could result in dormancy release. Further investigations would however be required to substantiate this.

Seed and embryo culture experiments testing for coat-imposed mechanical and chemical dormancy in the nut-fruited Restionaceae did not result in germination. Unfortunately this negative result cannot exclude the possibility of a mechanical restriction of the embryo by the surrounding seed structures or a chemical imposition of seed dormancy, as the lack of germination may have been due to factors unrelated to those being tested. According to Razdan (1993), immature embryos that fail to germinate require a complex nutrient medium. Furthermore, concentrations of nutritional components (macronutrients and micronutrients), vitamins and growth regulators (auxins, cytokinins, gibberellins and abscisic acid) promoting optimal growth or germination vary with respect to the particular species. The importance of determining optimal species-specific media conditions for *in vitro* culture has been demonstrated in various studies (e.g. Eeuwens 1978, Meyer & van Staden 1986 and Ho *et al.* 1995). It is unclear whether embryo dormancy or unsuitable culture conditions resulted in the lack of germination in Restionaceae embryo and seed culture experiments.

Another experiment did however suggest the possibility of mechanical coat-imposed dormancy in the nut-fruited Restionaceae. Funiculus removal in *W. incurvata* improved germination significantly (Table 3.5). This may suggest a possible release of mechanical embryo restraint, however further investigation is required as the sample size was small. Although the funiculus was not visibly broken down during six months of soil storage (Figure 2.10), it is possible that seeds remain viable in the soil for much longer (Table 3.6). Microbial action and / or soil scarification (Brits 1996) has been shown to break down the exotesta of *Leucospermum cordifolium*, resulting in dormancy release. Thus over a longer period of time, soil storage might result in funiculus degradation and the breaking of a mechanical restraint-type dormancy in *W. incurvata* and possibly some of the other nut-fruited Restionaceae seeds.

Hydrogen peroxide has been used successfully to stimulate seed germination in fynbos species with dormancy resulting in a lack of oxygen supply to the embryo (Brown & Dix 1985, Brits 1986b and Brits & van Niekerk 1986). Hydrogen peroxide treatment however did not promote germination in *W. incurvata*. This suggests that oxygen deficiency due to coat-imposed dormancy is unlikely to be a dormancy mechanism in these seeds. As the effect of hydrogen peroxide was tested only on *W. incurvata*, the possibility of oxygen exclusion in other nut-fruited Restionaceae species cannot be excluded.

The above results suggest that physical dormancy does not operate as a dormancy mechanism in the species examined, however a mechanical or chemical dormancy cannot be ruled out. What is clear though, is that if coat-imposed dormancy is present in these seeds, then these seeds must possess a complex dormancy involving both the embryo and the coat or tissues surrounding the embryo. The role of the ovary wall in these species appears to primarily be in maintaining viability of ungerminated seeds by providing protection against harmful micro-organisms, unfavourable environmental conditions and field weathering (Mohamed-Yassen *et al.* 1994 and Longer & Degago 1996). Seed survival following the high temperatures experienced during the fire experiment (Table 3.9) supports this protective function of the ovary wall. However whether the ovary wall or seed coat plays a secondary role in seed dormancy remains to be determined.

Investigation into morphological embryo dormancy by measurement of embryo size of laboratory-stored and condensed seasons-stored seed revealed an interesting trend of increased size in condensed seasons-stored seeds, especially in *W. incurvata*. It is likely that the repeated wetting and drying during season alternation in the condensed seasons experiment resulted in embryo enlargement. Embryo enlargement following wetting has been noted in other species (Rizzini 1973, Lush *et al.* 1984 and Chien *et al.* 1998). Wetting and drying also appears to prime some seeds, resulting in increased and more rapid germination (Griswold 1936, Maynard & Gates 1963 and Lush *et al.* 1981). Whether embryo enlargement was due to cell expansion or cell division was not determined, however no evidence of meristem formation was observed during ultrastructure studies, indicating that this increase might have resulted from cell enlargement during wet periods.

Afterripening and low temperature treatment (condensed seasons experiment) tested for the relief of physiological dormancy by alternating regimes of temperature and moisture. Although germination percentages were low in all species, it is interesting that a small proportion (2 - 5%) of seed germinated during each "winter" in *W. incurvata* (West coast), while germination in other species was virtually negligible. Other fynbos seeds exhibit extended germination over successive winter seasons. Brits (1996), for example, found that seed of the fynbos species *Leucosperm cordifolium* buried in soil germinated over consecutive winter seasons, with older soil-stored seed germinating more readily than young intact seed. The decline in seed germination during successive "winters" might suggest that these seeds are entering conditional or secondary dormancy in the soil.

A predicted seed half-life for soil-stored *W. incurvata* and *W. teres* estimated using deterioration data was eight years, while for *C. virgata* this value was 18 years. It was impossible to determine whether buried seeds that deteriorated due to pathogens were intact dormant or imbibed germinating seeds. These results were supported by observations of seed deterioration in the fire experiment, where significantly fewer *C. virgata* seeds deteriorated than did seeds of *Willdenowia* spp. It should be noted that as these deterioration values are calculated from an artificial condensed seasons laboratory study, the actual deterioration in real time may differ from the estimated seed survival values. The thickness of the ovary wall did not appear to be related to seed survival in the soil, as the brittle small-seeded form of *C. virgata* with a thin ovary wall showed similar deterioration patterns to the woody large-seeded form with a thick ovary wall.

A subset of condensed seasons-stored seed and similar quantities of laboratory seed were selected for a fire experiment to test for physiological seed dormancy. Soil temperatures during the fire reached an average maximum surface temperature in excess of 700°C, with an average maximum temperature of 83°C at the level at which the seeds were buried. These temperatures are representative of an intense hot fynbos burn (Kruger & Bigalke 1984 and de Lange & Boucher 1993). Soil changes as a result of the fire included an increase in soil conductivity, which may be due to the release of nutrient elements by fire (Cass *et al.* 1984). pH also increased, which might be a result of increased basic cations in the soil that would neutralise soil acidity (Cass *et al.* 1984).

There was a higher percentage of deteriorated seed (*ca.* 10%) exposed to the fire than not exposed to the fire (Table 3.7). Heat is the most likely factor to have been the cause of seed deterioration (Jeffery *et al.* 1988), even though seeds of other species retain viability at temperatures higher than soil temperatures recorded in this study (e.g. Musil & de Witt 1991 and de Lange & Boucher 1993). Seed deterioration did not differ between laboratory-stored and condensed seasons-stored seed. This suggests that the high soil moisture contents experienced in the condensed-seasons experiment, that would normally be considered unsuitable for traditional orthodox seed storage (Roberts 1974), were not detrimental in the short term. This anomaly may be interpreted as evidence of repair processes operating in the imbibed soil-stored seed (Priestley 1986). Seeds of the soil-stored *Leucospermum cordifolium* were found to maintain viability in the soil for a minimum of four years (Brits 1996).

Seed germination in the fire experiment was enhanced in fire treated seeds as well as seeds germinated in smoked soil (Table 3.7). Furthermore, seed germination was enhanced in condensed seasons-stored seed compared to laboratory-stored seed. Condensed seasons-stored *C. virgata* responded most strongly to these cues (Figure 3.6), with a germination percentage above 65%. Thus in *C. virgata*, germination appeared to be enhanced by soil storage in which fluctuating temperatures and wetting and drying regimes were applied. Germination was then further stimulated in these seeds by smoke treatment. The lack of *C. virgata* germination in embryo culture in which smoke extract was added to the mixture may have been due to the absence of wetting and drying pre-treatment of seeds. Alternatively the dormancy-breaking component in smoke may have been lost or altered when extracted in water or transferred to the germination medium. Keeley and Fotheringham (1998b) have demonstrated a similar requirement for germination in the strict post-fire germinating chaparral species *Dicentra chrysantha*, in which a year of soil storage followed by brief smoke treatment was necessary to induce germination.

Willdenowia species, unlike *C. virgata*, did not respond to smoke or heat. Germination was higher in condensed seasons-stored seed compared to laboratory-stored seed in *W. incurvata.* In both types of seed storage seed germination was low, implying that only some had experienced full dormancy release cues and were physiologically capable of responding to them. *W. teres* exhibited deep dormancy which was not broken by any treatments tested. Different dormancy requirements must therefore be met in these species before germination can take place.

Seed germination behaviour of *W. incurvata* (West Coast) was similar to that of *W. incurvata* (Cederberg), in spite of their occurrence in vegetation types experiencing different fire regimes. According to Linder (personal communication) vegetation dominated by *W. incurvata* usually does not burn while *C. virgata* and *W. teres* occur in fire-prone environments. However the reproductive strategies of *W. incurvata* and the woody large-seeded form of *C. virgata* are similar, in that they are killed by fire and are primarily reseeders, while *W. teres* coppices after fire.

Thus for *C. virgata*, it appears that the soil seed bank is built up by ant-dispersal during non-fire years. The predicted longevity of soil-stored seeds suggests that the majority of seeds are able to remain viable until the occurrence of a fire. During non-fire years, embryos are likely to be sufficiently hydrated to commence germination under moist cool winter conditions. A physiological block therefore prevents seed germination. Repeated wetting and drying of seeds does not appear to accelerate natural seed deterioration. Conditions during soil-storage instead seem to prime the seeds for germination. This priming may result in a release from a morphological and / or physiological dormancy, in the former case resulting in embryo expansion. Following a fire, at the onset of winter rains, an unknown component in smoke is thought to break the remaining physiological dormancy in these seeds, resulting in mass seed germination.

Germination of *W. incurvata* seed, on the other hand, is quite different from *C. virgata*. Unlike *C. virgata* this species typically does not experience fire, however like *C. virgata* it does regenerate from seed. Seed longevity in the soil is less than *C. virgata*. However the massive annual seed production (personal observation) may compensate for both lower seed survival in the soil and higher seed predation due to the absence of ant dispersal in this species. A low percentage of seeds germinated during each winter. As in *C. virgata*, embryo expansion occurred following wetting and drying, which suggests a possible release from morphological dormancy or a requirement of priming prior to seed germination. Embryos may also require a possible release from mechanical restraint, allowing further water uptake, and likely possess a physiological dormancy which is yet to be determined. *W. incurvata* seeds may have a nondeep physiological dormancy, and participate in dormancy cycling, in which the physiological state of the seed fluctuates between dormancy and non-dormancy in response to environmental factors (Baskin & Baskin 1998). Furthermore, as *W. incurvata* seed germination in the natural environment is sporadic (Linder, personal communication), it is possible that seeds released from the plant might have different degrees of dormancy, which would lead to a distribution of seed germination in time. The production of seeds with differing degrees of dormancy within a species or even an individual plant is well known (reviewed by Baskin & Baskin 1998).

Seeds of *W. teres* exhibit a deep dormancy that is unlike that observed in both *C. virgata* and *W. incurvata*. There are likely to be multiple dormancy mechanisms operating in these seeds. The regeneration strategy of *W. teres* following a fire is primarily vegetative resprouting rather than reseeding. Thus, ecologically, seed recruitment may not be as important in *W. teres* as it is in *W. incurvata* and *C. virgata*. However the deep dormancy of this species is a puzzle that remains to be solved.

CHAPTER 4

GENERAL DISCUSSION AND CONCLUSIONS

Due to the paucity of knowledge about even some of the most basic aspects of the seed biology of the nut-fruited Restionaceae, and the many possible avenues of research which could have been explored, my study has, out of necessity, been broad and in some areas possibly superficial. However it compliments the existing knowledge of the nut-fruited Restionaceae, providing a basic understanding of the complex and intriguing seed biology of this group. It has involved an integration of several fields of study, ranging from seed structure and development, and seed physiology, through to dormancy mechanisms and germination requirements. I have also attempted to relate study findings to the ecology of these seeds.

The objectives of my study (outlined in section 1.6.2) were to address two main questions regarding the problem of seed germination in the nut-fruited Restionaceae. The first question was whether poor seed germination in previous studies may have been due to poor seed quality. To answer this question I investigated seed morphology and development, seed collection and sorting methods, and seed viability in some species of the nut-fruited Restionaceae. My findings can be summarised as follows:

a) Seed morphology, both at a macro and ultrastructural level, appear to be similar in the species examined. Seed development patterns are also similar. The length of seed development and timing of seed release however vary markedly between species. This makes seed collection difficult if species-specific seed development timing is not known.

b) The shaking method of seed collection results in a higher quality seed collection and is therefore the recommended collection method. However in species in which fewer seed are produced per plant, and seeds are held tightly in surrounding bony bracts, this method is impractical, and the cutting method is recommended. However further seed sorting is required, especially in seed harvested using the cutting method.

c) Separation of full seed may be achieved quickly, cheaply, and with an acceptable degree of accuracy using the floating method of seed sorting. If necessary, x-radiography can be used to check seed quality. Seed weight is not recommended for seed separation.

d) Seed viability generally appears to be high in the seeds tested. However viability testing using tetrazolium tests preceding germination tests is recommended. Electrolyte leakage is not a reliable method for evaluating viability.

Thus the answer to the first question is that poor seed germination in previous studies may have been due to poor seed quality if:

1) seed development and seed release dates of the species were not known, leading to collection of immature seed; or,
2) seed collection and sorting methods were not rigorous; or,
3) seed viability was not tested on a subset of seeds used in germination tests.

However seed quality was generally high with suitable collection and sorting techniques, thus leading me to conclude that poor seed germination in good seed collections must be due to seed dormancy. The second question investigated what type(s) of seed dormancy might exist in the nut-fruited Restionaceae. To answer this question I used a primarily physiological approach to seed germination to examine some potential coat-imposed physical, mechanical and chemical barriers, and possible morphological and physiological barriers inherent in the embryo. My findings in relation to the second question can be summarised as follows:

In all species of the nut-fruited Restionaceae tested:

a) The ovary wall and seed coat were not a barrier to seed imbibition, as water uptake occurred in all nut-fruited Restionaceae species tested. These structures may however mechanically restrict germination by preventing radicle protrusion or embryo expansion.

b) Eosin dye uptake studies revealed the presence of a solute impermeable barrier in all species tested at the level of the seed coat, which prevented dye movement into the embryo and endosperm tissue.

c) Embryos of seeds subjected to wetting and drying treatments showed a trend of increased size (i.e. were larger) following this treatment.

These results suggested that coat-imposed physical dormancy is not a mechanism in the nut-fruited Restionaceae. However the solute impermeable seed coat may be involved in physiological dormancy of these seeds. There also appears to be some embryo enlargement prior to germination, which may suggest a morphological dormancy, or possible priming of the embryo occurring during soil storage. The possibility of mechanical and chemical dormancy in these seeds was not eliminated in this study, however it is clear that if the ovary wall and seed coat function in seed dormancy, they are not the only dormancy mechanism operating in these seeds.

There were marked differences between seed behaviour in *Willdenowia* species and *C. virgata* in the following tests. These results indicate that the germination strategies for these three species tested is both very different and complex:

a) Final embryo water contents were low in *W. incurvata* and *W. teres* (*ca.* 80% DMB), possibly too low for germination, but significantly higher in *C. virgata* (*ca.* 240%).

b) Predicted seed longevity in the soil was significantly higher in *C. virgata* than in *W. incurvata* and *W. teres.*

c) High levels of germination of *C. virgata* seed were achieved in wet-dry cycled seeds that were subjected to a smoke cue following this treatment. *W. incurvata* did not respond to smoke, but instead showed a pattern of low germination during favourable winter conditions, which appeared to be promoted by wet-dry cycling. *W. teres* exhibited a deep dormancy which was not broken by any treatments.

It seems plausible that these species-specific germination strategies may be linked to the occurrence of fire as a natural component of the fynbos ecosystem and the ecological restraints of regeneration in each species. *C. virgata*, for example, does not germinate between fires (Linder, personal communication), the smoke requirement most likely preventing germination. Mature *C. virgata* plants are killed by fire, regeneration by seed being crucial for the continuance of the species. Experiments suggest that seeds in the soil germinate readily in the winter following a fire, having undergone soil storage followed by a smoke cue.

W. incurvata, on the other hand, usually does not experience fire. Seed recruitment in this species is independent of fire and tends to occur in mature fynbos. Seed germination is likely to be patchy both over time and space. The production of seeds with differing degrees of dormancy within a species or even individual plant may account for this patchy germination. However investigation is necessary to confirm whether dormancy differs within seeds of a single plant. The slow release of dormancy that appears to typify these seeds may allow for a proportion of individual seeds to be physiologically ready for germination at any time, given favourable environmental conditions.

Since I used a physiological approach for elucidating barriers to seed germination in the nut-fruited Restionaceae, as opposed to the ecological approach of past studies, this study provided an opportunity to compare the merits of these different approaches in seed dormancy research. Some of the experiments I conducted successfully eliminated a dormancy type (e.g. water uptake showed physical dormancy not to operate in these seeds), or suggested dormancy mechanisms (e.g. embryo size increases suggested a possible morphological dormancy) in the nut-fruited Restionaceae. These investigations however did not lead to successful dormancy identification. Thus the physiological approach in the limited time scale of this study was unable to pinpoint dormancy mechanisms in these seeds. My final experiment, the design of which drew from both ecological and physiological knowledge, was condensed seasons treatment of seeds followed by a fire. Ultimately this was the experiment that resulted in germination success in *C. virgata.* This highlights the importance of integration and consideration of both approaches when faced with a seed of unknown dormancy.

The findings of my study raise some interesting questions and areas in which I believe that future research should concentrate:

a) In trial studies, removal of the funiculus in *W. incurvata* promoted germination to a small extent in this species. The effect of funiculus removal on seed dormancy and germination warrants further investigation.

b) Investigation into the effect of smoke or components of smoke on the solute impermeable seed coat barrier, to determine whether smoke affects this permeability as it does in other species (Keeley & Fotheringham 1997, 1998b) would be interesting.

c) A large gap in this investigation, due to time constraints, was the lack of investigation at a molecular level (apart from the hormonal additions tested in embryo and seed culture). Investigations into the physiological control and hormonal involvement during soil storage and smoke treatment in *C. virgata* may enhance the understanding of the processes resulting in seed dormancy release.

d) Further investigation into the complex dormancy of *W. teres* is needed.

e) Time constraints prevented examination of the conditions of ant nests or the soil in which seeds are buried. Investigation into conditions specific to the immediate surroundings of buried seed may lead to further insight of seed dormancy release, particularly in the myrmecochorous Restionaceae.

f) Further examination of the observed embryo enlargement following wetting and drying, mechanisms of enlargement and consequences of this to both dormancy and germination is needed.

g) It would be interesting to complement this study with investigations into dormancy mechanisms of other nut-fruited Restionaceae species. Patterns of different germination strategies related to seed ecology may emerge with a wider base of information regarding seed dormancy release.

h) Investigations into artificial methods (e.g. by hormone application) of breaking dormancy in *C. virgata* would be useful to horticulture, to identify an easy and reliable dormancy breaking treatment to allow these attractive plants to be more widely available for cultivation.

CHAPTER 5

REFERENCES

ABERCROMBIE M., HICKMAN C.J. & JOHNSON M.L. 1987. *The Penguin Dictionary of Biology.* Seventh Edition. Penguin Books, London.

AFOLAYAN A.J., MEYER J.J.M. & LEEUWNER D.V. 1997. Germination in *Helichrysum aureonitens* (Asteraceae): effects of temperature, light, gibberellic acid, scarification and smoke extract. *South African Journal of Botany* **63**:22-24.

ANDERSEN A.N. 1988. Dispersal distance as a benefit of myrmecochory. *Oecologia* **75**:507-511.

ARROYO M.T.K., CAVIERES L., MARTICORENA C. & MUNOZ-SCHICK M. 1994. Convergence in the mediterranean floras in central Chile and California: insights from comparative biogeography. In: *Ecology and Biogeography of Mediterranean Ecosystems in Chile, California, and Australia.* Eds: M.T.K. Arroyo, P.H. Zedler & M.D. Fox, pp. 43-88. Springer-Verlag, New York.

BAI Y., BOOTH D.T. & ROMO J.T. 1999. Imbibition temperature affects winterfat (*Eurotia lanata* (Pursh) Moq.) seed hydration and cold-hardiness response. *Journal of Range Management* **52**:271-274.

BASKIN C.C. & BASKIN J.M. 1998. *Seeds: Ecology, Biogeography, and Evolution of Dormancy and Germination.* Academic Press, San Diego.

BASKIN J.M. & BASKIN C.C. 1982. Effects of wetting and drying cycles on the germination of seeds of *Cyperus inflexus. Ecology* **63**:248-252.

BASKIN J.M. & BASKIN C.C. 1986. Germination ecophysiology of the mesic deciduous forest herb *Isopyrum biternatum. Botanical Gazette* **147**:152-155.

BASKIN J.M. & BASKIN C.C. 1990. Seed germination ecology of poison hemlock, *Conium maculatum. Canadian Journal of Botany* **68**:2018-2024.

BASKIN J.M., NAN X. & BASKIN C.C. 1998. A comparative study of seed dormancy and germination in an annual and a perennial species of *Senna* (Fabaceae). *Seed Science Research* **8**:501-512.

BELL D.T., PLUMMER J.A. & TAYLOR S.K. 1993. Seed germination ecology in southwestern western Australia. *The Botanical Review* **59**:24-73.

BERESNIEWICZ M.M., TAYLOR A.G., GOFFINET M.C. & TERHUNE B.T. 1995. Characterization and location of a semipermeable layer in seed coats of leek and onion (Liliaceae), tomato and pepper (Solanaceae). *Seed Science and Technology* **23**:123-134.

BERJAK P. & PAMMENTER N.W. 1994. Recalcitrance is not an all-or-nothing situation. *Seed Science Research* **4**:263-264.

BEWLEY J.D. 1997. Seed germination and dormancy. *The Plant Cell* **9**:1055-1066.

BEWLEY J.D. & BLACK M. 1994. *Seeds: Physiology of Development and Germination.* Second Edition. Plenum Press, New York.

BHATTACHARJEE S. & MUKHERJEE A.K. 1998. The deleterious effects of high temperature during early germination on membrane integrity and subsequent germination of *Amaranthus lividus. Seed Science and Technology* **26**:1-8.

BIANCO J., GARELLO G. & LE PAGE-DEGIVRY M.T. 1994. Release of dormancy in sunflower embryos by dry storage: involvement of gibberellins and abscisic acid. *Seed Science Research* **4**:57-62.

BLACK M. 1996. Liberating the radicle: a case for softening-up. *Seed Science Research* **6**:39-42.

BLOMMAERT K.L.J. 1972. Buchu seed germination. *Journal of South African Botany* **38**:237-239.

BOESEWINKEL F.D. & BOUMAN F. 1995. The seed: structure and function. In: *Seed Development and Germination.* Eds: J. Kigel & G. Galili, pp. 1-24. Marcel Dekker Inc., New York.

BOND P. & GOLDBLATT P. 1984. Plants of the Cape Flora. A descriptive catalogue. *Journal of South African Botany* **13(S)**:1-455.

BOND W.J. 1981. *Vegetation Gradients in Southern Cape Mountains.* M.Sc Thesis, University of Cape Town.

BOND W.J. 1984. Fire survival of Cape Proteaceae - influence of fire season and predators. *Vegetatio* **56**:65-74.

BOND W.J. 1985. Canopy-stored seed reserves (serotiny) in Cape Proteaceae. *South African Journal of Botany* **51**:181-186.

BOND W.J. Personal communication. Department of Botany, University of Cape Town, Private Bag, Rondebosch, 7701, Republic of South Africa.

BOND W.J. & BREYTENBACH G.J. 1985. Ants, rodents and seed predation in Proteaceae. *South African Joumal of Zoology* **20**:150-154.

BOND W.J. & SLINGSBY P. 1983. Seed dispersal by ants in shrublands of the Cape Province and its evolutionary implications. *South African Journal of Science* **79**:231-233.

BOND W.J. & STOCK W.D. 1989. The costs of leaving home: ants disperse myrmecochorous seeds to low nutrient sites. *Oecologia* **81**:412-417.

BOND W.J. & VAN WILGEN B.W. 1996. *Fire and plants.* Population and Community Biology Series 14. Chapman & Hall, London.

BOND W.J., LE ROUX D. & ERNTZEN R. 1990. Fire intensity and regeneration of myrmecochorous Proteaceae. *South African Journal of Botany* **56**:326-330.

BOND W.J., YEATON R. & STOCK W.D. 1991. Myrmecochory in Cape fynbos. In: *Ant-plant interactions.* Eds: C.R. Huxley & D.F. Cutler, pp. 448-462. Oxford University Press, Oxford.

BORWEIN B., COETSEE M.L. & KRUPKO S. 1949. Development of the embryosac of *Restio dodii* and *Elegia racemosa*. *Journal of South African Botany* **15**:1-11.

BOTTO J.F., SÁNCHEZ R.A. & CASAL J.J. 1998. Burial conditions affect light responses of *Datura ferox* seeds. *Seed Science Research* **8**:423-429.

BOUYOUCOS G.J. 1962. Hydrometer method improved for making particle size analyses of soils. *Agronomy Journal* **54**:464-465.

BRADFORD K.J. 1995. Water relations in seed germination. In: *Seed Development and Germination*. Eds: J. Kigel & G. Galili, pp. 351-396. Marcel Dekker Inc., New York.

BREW C.R., O'DOWD D.J. & RAE I.D. 1989. Seed dispersal by ants: behaviour-releasing compounds in elaiosomes. *Oecologia* **80**:490-497.

BRITS G.J. 1986a. Influence of fluctuating temperatures and H_2O_2 treatment on germination of *Leucospermum cordifolium* and *Serruria florida* (Proteaceae) seeds. *South African Journal of Botany* **52**:286-290.

BRITS G.J. 1986b. The effect of hydrogen peroxide treatment on germination in Proteaceae species with serotinous and nut-like achenes. *South African Journal of Botany* **52**:291-293.

BRITS G.J. 1987. Germination depth vs. temperature requirements in naturally dispersed seeds of *Leucospermum cordifolium* and *L. cuneiforme* (Proteaceae). *South African Journal of Botany* **53**:119-124.

BRITS G.J. 1996. *Ecophysiology of Leucospermum R. Br. Seed Germination in Fynbos.* PhD Thesis, University of Cape Town.

BRITS G.J. & VAN NIEKERK M.N. 1986. Effects of air temperature, oxygenating treatments and low storage temperature on seasonal germination response of *Leucospermum cordifolium* (Proteaceae) seeds. *South African Journal of Botany* **52**:207-211.

BRITS G.J., CALITZ F.J., BROWN N.A.C. & MANNING J.C. 1993. Desiccation as the active principle in heat-stimulated seed germination of *Leucospermum* R. Br. (Proteaceae) in fynbos. *New Phytologist* **125**:397-403.

BROWN N.A.C. 1993a. Promotion of germination of fynbos seeds by plant-derived smoke. *New Phytologist* **123**:575-583.

BROWN N.A.C. 1993b. Seed germination in the fynbos fire ephemeral, *Syncarpha vestita* (L.) B.Nord. is promoted by smoke, aqueous extracts of smoke and charred wood derived from burning the ericoid-leaved shrub, *Passerina vulgaris* Thoday. *International Journal of Wildland Fire* **3**:203-206.

BROWN N. 1994. First the gas, now instant dehydrated smoke. *Veld & Flora* **80**:72-73.

BROWN N. & BOTHA P. 1997. Smoking them out. *The Horticulturalist* **6**:2-6.

BROWN N.A.C. & DIX L. 1985. Germination of the fruits of *Leucadendron tinctum*. *South African Journal of Botany* **51**:448-452.

BROWN N.A.C. & MITCHELL J.J. 1984. Germination of the polymorphic fruits of *Bidens bipinnata*. *South African Journal of Botany* **3**:55-58.

BROWN N.A.C. & VAN STADEN J. 1973a. The effect of scarification, leaching, light, stratification, oxygen and applied hormones on germination of *Protea compacta* R.Br. and *Leucadendron daphnoides* Meisn. *Journal of South African Botany* **39**:185-195.

BROWN N.A.C. & VAN STADEN J. 1973b. The effect of stratification on the endogenous cytokinin levels of seed of *Protea compacta* and *Leucadendron daphnoides*. *Physiologia Plantarum* **28**:388-392.

BROWN N.A.C. & VAN STADEN J. 1997. Smoke as a germination cue: a review. *Plant Growth Regulation* **22**:115-124.

BROWN N.A.C., JAMIESON H. & BOTHA P.A. 1994. Stimulation of seed germination in South African species of Restionaceae by plant-derived smoke. *Plant Growth Regulation* **15**:93-100.

BROWN N., JAMIESON H. & BOTHA P. 1998. *Grow restios*. Kirstenbosch Gardening Series, National Botanical Institute, Kirstenbosch.

BROWN N.A.C., JAMIESON H. & HITCHCOCK A. 1996. Conservation through cultivation. *The Garden* **121**:265-267.

BROWN N.A.C., KOTZE G. & BOTHA P.A. 1993. The promotion of seed germination of Cape *Erica* species by plant-derived smoke. *Seed Science and Technology* **21**:573-580.

BUCKLEY R.C. 1982. Ant-plant interactions: a world review. In: *Ant-Plant Interactions in Australia*. Ed: R.C. Buckley, pp. 111-141. Dr W. Junk Publishers, The Hague.

CAMPBELL B.M. 1985. A classification of the mountain vegetation of the fynbos biome. *Memoirs of the Botanical Survey of South Africa* **50**:1-115.

CAMPBELL M.H. & NICOL H.I. 1997. Effect of age on the germination of *Cassinia arcuata* seeds in storage and buried in the soil. *Weed Research* **37**:103-109.

CANTOS M., CUERVA J., ZÁRATE R. & TRONCOSO A. 1998. Embryo rescue and development of *Juniperus oxycedrus* subsp. *oxycedrus* and *macrocarpa*. *Seed Science and Technology* **26**:193-198.

CASAL J.J. & SÁNCHEZ R.A. 1998. Phytochromes and seed germination. *Seed Science Research* **8**:317-329.

CASS A., SAVAGE M.J. & WALLIS F.M. 1984. The effect of fire on soil and microclimate. In: *Ecological Effects of Fire in South African Ecosystems*. Eds: P. de V. Booysen & N.M. Tainton, pp. 311-325. Springer-Verlag, Berlin.

CECCHERINI L., RADDI S. & ANDRÉOLI C. 1998. The effect of seed stratification on germination of 14 *Cupressus* species. *Seed Science and Technology* **26**:159-168.

CHIEN C.-T., KUO-HUANG L.-L. & LIN T.-P. 1998. Changes in ultrastructure and abscisic acid level, and response to applied gibberellins in *Taxus mairei* seeds treated with warm and cold stratification. *Annals of Botany* **81**:41-47.

CHOU C.-H. & MULLER C.H. 1972. Allelopathic mechanisms of *Arctostaphylos glandulosa* var. *zacaensis*. *The American Midland Naturalist* **88**:324-347.

CHOUDHURY S. & GUPTA K. 1998. Studies on the germination mechanism of *Catharanthus roseus* (L.) G. DON cv. *alba* seeds: effect of promoters and pH. *Seed Science and Technology* **26**:719-732.

CHRISTENSEN M., MEYER S.E. & ALLEN P.S. 1996. A hydrothermal time model of seed after-ripening in *Bromus tectorum* L. *Seed Science Research* **6**:155-163.

CICERO S.M., VAN DER HEIJDEN G.W.A.M., VAN DER BERG W.J. & BINO R.J. 1998. Evaluation of mechanical damage in seeds of maize (*Zea mays* L.) by X-ray and digital imaging. *Seed Science and Technology* **26**:603-612.

CLEGG A.D. 1980. Restionaceae - their potential. *Veld & Flora* **66**:16-17.

COCKS M.P. & STOCK W.D. 1997. Heat stimulated germination in relation to seed characteristics in fynbos legumes of the Western Cape Province, South Africa. *South African Journal of Botany* **63**:129-132.

CODY M.L. & MOONEY H.A. 1978. Convergence versus nonconvergence in Mediterranean-climate ecosystems. *Annual Review of Ecology and Systematics* **9**:265-321.

COHN M.A. 1996. Chemical mechanisms of breaking seed dormancy. *Seed Science Research* **6**:95-99.

CORBINEAU F. & CÔME D. 1995. Control of seed germination and dormancy by the gaseous environment. In: *Seed Development and Germination*. Eds: J. Kigel & G. Galili, pp. 397-424. Marcel Dekker Inc., New York.

COWLING R.M., PIERCE S.M., STOCK W.D. & COCKS M. 1994. Why are there so many myrmecochorous species in the Cape fynbos? In: *Plant-Animal Interactions in Mediterranean-type Ecosystems*. Eds: M. Arianoutsou & R.H. Groves, pp. 159-168. Kluwer Academic Publishers, Netherlands.

CUNHA R., EIRA M.T.S. & RITA I. 1995. Germination and desiccation studies on wild nutmeg seed (*Virola surinamensis*). *Seed Science and Technology* **23**:43-49.

DAVIES R.I. & PRICHARD H.W. 1998. Seed storage and germination of the palms *Hyphaene thebaica*, *H. petersiana* and *Medemia argun*. *Seed Science and Technology* **26**:823-828.

DEALL G.B. & BROWN N.A.C. 1981. Seed germination in *Protea magnifica* Link. *South African Journal of Science* **77**:175-176.

DE LANGE J.H. Personal communication. National Botanical Institute, Private Bag X7, Claremont, 7735, Republic of South Africa.

DE LANGE J.H. & BOUCHER C. 1990. Autecological studies on *Audouinia capitata* (Bruniaceae). I. Plant-derived smoke as a seed germination cue. *South African Journal of Botany* **56**:700-703.

DE LANGE J.H. & BOUCHER C. 1993. Autecological studies on *Audouinia capitata* (Bruniaceae). 8. Role of fire in regeneration. *South African Journal of Botany* **59**:188-202.

DEVLIN P.F., HALLIDAY K. & WHITELAM G.C. 1997. The phytochrome family and their roles in the regulation of seed germination. In: *Basic and Applied Aspects of Seed Biology*. Eds: R.H. Ellis, M. Black, A.J. Murdoch & T.D. Hong, pp. 159-171. Kluwer Academic Publishers, Dordrecht.

DIXIT S. & AMRITPHALE D. 1996. Very low fluence and low fluence response in the induction and inhibition of seed germination in *Celosia argentea*. *Seed Science Research* **6**:43-48.

DIXON K.W., ROCHE S. & PATE J.S. 1995. The promotive effect of smoke derived from burnt native vegetation on seed germination of Western Australian plants. *Oecologia* **101**:185-192.

DON R. 1979. The tetrazolium test – a biochemical test for viability. In: *Seed technology for Genebanks*. International Board for Plant Genetic Resources, Rome.

DOUSSI M.A. & THANOS C.A. 1997. Ecophysiology of seed germination in composites inhabiting fire-prone Mediterranean ecosystems. In: *Basic and Applied Aspects of Seed Biology*. Eds R.H. Ellis, M. Black, A.J. Murdoch & T.D. Hong, pp 641-649. Kluwer Academic Publishers, Dordrecht.

DOWNIE B. & BEWLEY J.D. 1996. Dormancy in white spruce (*Picea glauca* [Moench.] Voss.) seeds is imposed by tissues surrounding the embryo. *Seed Science Research* **6**:9-15.

DOWNIE B., COLEMAN J., SCHEER G., WANG B.S.P., JENSEN M. & DHIR N. 1998. Alleviation of seed dormancy in white spruce (*Picea glauca* [Moench.] Voss.) is dependent on the degree of hydration. *Seed Science and Technology* **26**:555-569.

DUAN X. & BURRIS J.S. 1997. Film coating impairs leaching of germination inhibitors in sugar beet seed. *Crop Science* **37**:515-520.

EDWARDS D.G.W. & EL-KASSABY Y.A. 1995. Douglas-fir genotypic response to seed stratification. *Seed Science and Technology* **23**:771-778.

EEUWENS C.J. 1978. Effects of organic nutrients and hormones on growth and development of tissue explants from coconut (*Cocos nucifera*) and date (*Phoenix dactylifera*) palms cultured *in vitro*. *Physiologia Plantarum* **42**:173-178.

EGLI D.B. & TEKRONY D.M. 1997. Species differences in seed water status during seed maturation and development. *Seed Science Research* **7**:3-11.

ELLIS R.H., HONG T.D. & ROBERTS E.H. 1985. *Handbook of Seed Technology for Genebanks. Volume 1: Principles and Methodology*. Department of Agriculture and Horticulture, University of Reading, UK.

ESASHI Y., OGASAWARA M., GÓRECKI R. & LEOPOLD A.C. 1993. Possible mechanisms of afterripening in *Xanthium* seeds. *Physiologia Plantarum* **87**:359-364.

FARRANT J.M., PAMMENTER N.W. & BERJAK P. 1988. Recalcitrance – a current assessment. *Seed Science and Technology* **16**:155-166.

FERNANDEZ G. & JOHNSTON M. 1995. Seed vigour testing in lentil, bean, and chickpea. *Seed Science and Technology* **23**:617-627.

FINCH-SAVAGE W.E. & CLAY H.A. 1997. The influence of embryo restraint during dormancy loss and germination of *Fraxinus excelsior* seeds. In: *Basic and Applied Aspects of Seed Biology*. Eds: R.H. Ellis, M. Black, A.J. Murdoch & T.D. Hong, pp. 245-253. Kluwer Academic Publishers, Dordrecht.

FOLEY M.E. & FENNIMORE S.A. 1998. Genetic basis for seed dormancy. *Seed Science Research* **8**:173-182.

FORSYTH C. & BROWN N.A.C. 1982. Germination of the dimorphic fruits of *Bidens pilosa* L. *New Phytologist* **90**:151-164.

FORSYTH C. & VAN STADEN J. 1983. Germination of cycad seeds. *South African Journal of Science* **79**:8-9.

FU S.M., HAMPTON J.G., HILL M.J. & HILL K.A. 1996. Breaking hard seed of yellow and slender serradella (*Ornithopus compressus* and *O. pinnatus*) by sulphuric acid scarification. *Seed Science and Technology* **24**:1-6.

FUENTES M. & SCHUPP E.W. 1998. Empty seeds reduce seed predation by birds in *Juniperus osteosperma*. *Evolutionary Ecology* **12**:823-827.

GEOLOGICAL SURVEY MAP. 1990. 1:250000 *Geological Series Map 3318 of Cape Town*. Government Printer, Pretoria.

GIBA Z., GRUBIŠIC D. & KONJEVIC R. 1995. The involvement of phytochrome in light-induced germination of blueberry (*Vaccinium myrtillus* L.) seeds. *Seed Science and Technology* **23**:11-19.

GOLDBLATT P. 1978. An analysis of the flora of southern Africa: its characteristics, relationships and origins. *Annals of the Missouri Botanical Garden* **65**:369-436.

GONZÁLEZ-MELERO J.A., PÉREZ-GARCÍA F. & MARTÍNEZ-LABORDE J.B. 1997. Effect of temperature, scarification and gibberellic acid on the seed germination of three shrubby species of *Coronilla* L. (Leguminosae). *Seed Science and Technology* **25**:167-175.

GRAY D., STECKEL J.R.A. & HANDS L.J. 1990. Responses of vegetable seeds to controlled hydration. *Annals of Botany* **66**:227-235.

GRISWOLD S.M. 1936. Effect of alternating moistening and drying on germination of seeds of western range plants. *Botanical Gazette* **98**:243-269.

GROOT S.P.C. & KARSSEN C.M. 1992. Dormancy and germination of abscisic acid deficient tomato seeds: studies with the *sitiens* mutant. *Plant Physiology* **99**:952-958.

HARADA J.J. 1997. Seed maturation and control of germination. In: *Cellular and Molecular Biology of Plant Seed Development*. Eds: B.A. Larkins & I.K. Vasil, pp. 545-592. Kluwer Academic Publishers, Netherlands.

HEPBURN H.A., POWELL A.A. & MATTHEWS S. 1984. Problems associated with the routine application of electrical conductivity measurements of individual seeds in the germination testing of peas and soyabeans. *Seed Science and Technology* **12**:403-413.

HEYDECKER W. 1974. Vigour. In: *Viability of Seeds*. Ed: E.H. Roberts, pp. 209-252. Chapman & Hall Ltd, London.

HILHORST H.W.M. 1995. A critical update on seed dormancy. I. Primary dormancy. *Seed Science Research* **5**:61-73.

HILHORST H.W.M. 1997. Primary dormancy in tomato. Further studies with the *sitiens* mutant. In: *Basic and Applied Aspects of Seed Biology*. Eds: R.H. Ellis, M. Black, A.J. Murdoch & T.D. Hong, pp. 191-201. Kluwer Academic Publishers, Dordrecht.

HILHORST H.W.M. 1998. The regulation of secondary dormancy. The membrane hypothesis revisited. *Seed Science Research* **8**:77-90.

HILHORST H.W.M. & KARSSEN C.M. 1992. Seed dormancy and germination: the role of abscisic acid and gibberellins and the importance of hormone mutants. *Plant Growth Regulation* **11**:225-238.

HO C.K., JACOBS G. & DONALD D.G.M. 1995. Effects of sodium hypochlorite, ethanol and culture medium on seed germination of *Paulownia* species. *Seed Science and Technology* **23**:157-163.

HOEKSTRA F.A., WOLKERS W.F., BUITINK J., GOLOVINA E.A., CROWE J.H. & CROWE L.M. 1997. Memebrane stabilization in the dry state. *Comparative Biochemistry and Physiology* **1171**:335-341.

HORN W. 1962. Breeding research on South African Plants: II. Fertility of Proteaceae. *The Journal of South African Botany* **62**:259-268.

HOU J.Q. & ROMO J.T. 1998. Effects of chemical stimulators on germination of winterfat (*Ceratoides lanata* (Pursh) J.T. Howell). *Seed Science and Technology* **26**:9-16.

HUGHES L. & WESTOBY M. 1992. Fate of seeds adapted for dispersal by ants in Australian sclerophyll vegetation. *Ecology* **73**:1285-1299.

HUTCHINSON J. 1973. *The Families of Flowering Plants*. Third Edition. Clarendon Press, Oxford.

ISTA (International Seed Testing Association). 1999. International Rules for Seed Testing. *Seed Science and Technology* **27(S)**:1-333.

JACOBSEN J.V., PRESSMAN E. & PYLIOTIS N.A. 1976. Gibberelin-induced separation of cells in isolated endosperm of celery seed. *Planta* **129**:113-122.

JAMIESON H. Personal communication. National Botanical Institute, Private Bag X7, Claremont, 7735, Republic of South Africa.

JAMIESON H. & BROWN N. 1994. The restio garden at Kirstenbosch. *Veld & Flora* **80**:124-125.

JAMIESON H. & BROWN N. 1995. Restios out of Africa: New ornamentals with potential for growing in Australia. *The Australian Garden Journal* **15**:39-42.

JEFFERY D.J., HOLMES P.M. & REBELO A.G. 1988. Effects of dry heat on seed germination in selected indigenous and alien legume species in South Africa. *South African Journal of Botany* **54**:28-34.

JETT L.W. & WELBAUM G.E. 1996. Changes in broccoli (*Brassica oleracea* L.) seed weight, viability, and vigour during development and following drying and priming. *Seed Science and Technology* **24**:127-137.

JHURREE B., BELLAIRS S.M. & HETHERINGTON S.E. 1998. Germination and dormancy release of seeds of Australian native understorey species used for minesite rehabilitation. *Seed Science and Technology* **26**:587-601.

JOEL D.M., STEFFENS J.C. & MATTHEWS D.E. 1995. Germination of weedy root parasites. In: *Seed Development and Germination.* Eds: J. Kigel & G. Galili, pp. 567-597. Marcel Dekker Inc., New York.

JOHRI B.M., AMBEGAOKAR K.B. & SRIVASTAVA P.S. 1992. *Comparative Embryology of Angiosperms.* Volume 2. Springer-Verlag, Berlin.

JONES S.K., GOSLING P.G. & ELLIS R.H. 1997. Dormancy in Sitka spruce seeds. In: *Basic and Applied Aspects of Seed Biology.* Eds: R.H. Ellis, M. Black, A.J. Murdoch & T.D. Hong, pp. 235-244. Kluwer Academic Publishers, Dordrecht.

KAMRA S.K. & SIMAK M. 1965. Physiological and genetical effects on seed of soft x-rays used for radiography. *Botaniska Notiser* **118**:254-264.

KEELEY J.E. 1987. Role of fire in seed germination of woody taxa in California chaparral. *Ecology* **68**:434-443.

KEELEY J.E. 1991. Seed germination and life history syndromes in the California chaparral. *The Botanical Review* **57**:81-116.

KEELEY J.E. 1992. A Californian's view of fynbos. In: *The ecology of fynbos: nutrients, fire and diversity.* Ed: R.M. Cowling, pp. 372-388. Oxford University Press, Cape Town.

KEELEY J.E. 1994. Seed-germination patterns in fire-prone Mediterranean-climate regions. In: *Ecology and biogeography of Mediterranean ecosystems in Chile, California and Australia.* Eds: M.T.K. Arroyo, P.H. Zedler, and M.D. Fox, pp. 239-273. Springer-Verlag, New York.

KEELEY J.E. & BOND W.J. 1997. Convergent seed germination in South African fynbos and Californian chaparral. *Plant Ecology* **133**:153-167.

KEELEY J.E. & FOTHERINGHAM C.J. 1997. Trace gas emissions and smoke-induced seed germination. *Science* **276**:1248-1250.

KEELEY J.E. & FOTHERINGHAM C.J. 1998a. Mechanism of smoke-induced seed germination in a post-fire chaparral annual. *Journal of Ecology* **86**:27-36.

KEELEY J.E. & FOTHERINGHAM C.J. 1998b. Smoke-induced seed germination in California chaparral. *Ecology* **79**:2320-2336.

KEELEY J.E., MORTON B.A., PEDROSA A. & TROTTER P. 1985. Role of allelopathy, heat and charred wood in the germination of chaparral herbs and suffrutescents. *Journal of Ecology* **73**:445-458.

KELLY K.M. & VAN STADEN J. 1985. Scarification and seed structure of *Aspalathis linearis. Journal of Plant Physiology* **121**:37-45.

KEPCZYNSKI J. & KEPCZYNSKA E. 1997. Ethylene in seed dormancy and germination. *Physiologia Plantarum* **101**:720-726.

KERMODE A.R. 1995. Regulatory mechanisms in the transition from seed development to germination: interactions between the embryo and the seed environment. In: *Seed Development and Germination.* Eds: J. Kigel & G. Galili, pp. 273-332. Marcel Dekker Inc., New York.

KHAN A.A. 1997. Quantification of seed dormancy: physiological and molecular considerations. *HortScience* **32**:609-614.

KHAN M.A. & UNGAR I.A. 1998. Seed germination and dormancy of *Polygonum aviculare* L. as influenced by salinity, temperature, and gibberellic acid. *Seed Science and Technology* **26**:107-117.

KILIAN D. & COWLING R.M. 1992. Comparative seed biology and co-existence of two fynbos shrub species. *Journal of Vegetation Science* **3**:637-646.

KIRCHER P. 1986. Untersuchungen zur Blueten- und Infloreszenzmorphologie, Embryologie und Systematik der Restionaceen im Vergleich mit Gramineen und verwandte Familien. *Dissertationes Botanicae* **94**:1-219.

KOCHANKOV V.G., GRZESIK M., CHOJNOWSKI M. & NOWAK J. 1998. Effect of temperature, growth regulators, and other chemicals on *Echinacea purpurea* (L.) Moench seed germination and seedling survival. *Seed Science and Technology* **26**:547-554.

KOLLER D. 1964. The survival value of germination-regulating mechanisms in the field. *Herbage Extracts* **34**:1-7.

KRUGER F.J. 1979. South African heathlands. In: *Heathlands of the World. A. Descriptive Studies.* Ed: R.L. Specht, pp. 19-80. Elsevier, Amsterdam.

KRUGER F.J. & BIGALKE R.C. 1984. Fire in fynbos. In: *Ecological Effects of Fire in South African Ecosystems.* Eds: P. de V. Booysen & N.M. Tainton, pp. 67-114. Springer-Verlag, Berlin.

KRUPKO S. 1962. Embryological and cytological investigations in *Hypodiscus aristatus* Nees (Restionaceae). *The South African Journal of Botany* **28**:21-44.

LAKON G. 1942. Topographischer Nachweis der Keimfähigkeit der Getreidefrüchte durch Tetrazoliumsalze. *Bericht über den Deutschen Botanischen Gesellschaft* **60**:299-305.

LAKON G. 1949. The topographical tetrazolium method for determining the germination capacity of seeds. *Plant Physiology* **24**:389-394.

LAMONT B.B., LE MAITRE D.C., COWLING R.M. & ENRIGHT N.J. 1991. Canopy seed storage in woody plants. *Botanical Review* **57**:277-317.

LE MAITRE D.C. 1988a. Effects of season of burn on the regeneration of two Proteaceae with soil-stored seed. *South African Journal of Botany* **54**:575-580.

LE MAITRE D.C. 1988b. The effects of parent density and season of burn on the regeneration of *Leucadendron laureolum* (Proteaceae) in the Kogelberg. *South African Journal of Botany* **54**:581-584.

LE MAITRE D.C. & MIDGLEY J.J. 1992. Plant reproductive ecology. In: *The ecology of fynbos: nutrients, fire and diversity.* Ed: R.M. Cowling, pp. 135-174. Oxford University Press, Cape Town.

LEOPOLD A.C., GLENISTER R. & COHN M.A. 1988. Relationship between water content and afterripening in red rice. *Physiologia Plantarum* **74**:659-662.

LEVYNS M.R. 1929. The problem of the rhenoster bush. *South African Journal of Science* **26**:166-169.

LINDER H.P. 1984. A phylogenetic classification of the genera of the African Restionaceae. *Bothalia* **15**:11-76.

LINDER H.P. 1985. Conspectus of the African species of Restionaceae. *Bothalia* **15**:387-503.

LINDER H.P. 1987. The evolutionary history of the Poales / Restionales – a hypothesis. *Kew Bulletin* **42**:297-318.

LINDER H.P. 1990. The thatching reed of Albertinia. *Veld and Flora* **76**:86-89.

LINDER H.P. 1991. A review of the Southern African Restionaceae. *Contributions from the Bolus Herbarium* **13**:209-264.

LINDER H.P. Personal communication. Department of Botany, University of Cape Town, Private Bag, Rondebosch, 7701, Republic of South Africa.

LONGER D.E. & DEGAGO Y. 1996. Field weathering potential of normal and hardseeded soybean genotypes. *Seed Science and Technology* **24**:273-280.

LUBKE R. 1996. Thicket biome. In: *Vegetation of South Africa, Lesotho and Swaziland.* Eds: A.B. Low & A.G. Rebelo, pp. 14-18. Department of Environmental Affairs & Tourism, Pretoria.

LUSH W.M., GROVES R.H. & KAYE P.E. 1981. Presowing hydration-dehydration treatments in relation to seed germination and early seedling growth of wheat and ryegrass. *Australian Journal of Plant Physiology* **8**:409-425.

LUSH W.M., KAYE P.E. & GROVES R.H. 1984. Germination of *Clematis microphylla* seeds following weathering and other treatments. *Australian Journal of Botany* **32**:121-129.

MACKAY W.A., DAVIS T.D. & SANKHLA D. 1995. Influence of scarification and temperature treatments on seed germination of *Lupinus havardii. Seed Science and Technology* **23**:815-821.

MAJER J.D. 1982. Ant-plant interactions in the Darling Botanical District of Western Australia. In: *Ant-Plant Interactions in Australia.* Ed: R.C. Buckley, pp. 45-61. Dr W Junk Publishers, The Hague.

MANNING J.C. & VAN STADEN J. 1987. The role of the lens in seed imbibition and seedling vigour of *Sesbania punicea* (Cav.) Benth. (Leguminosae: Papilionoideae). *Annals of Botany* **59**:705-713.

MATTHEWS S. & BRADNOCK W.T. 1967. The detection of seed samples of wrinkle-seeded peas (*Pisum sativum* L.) of potentially low planting value. *Proceedings of the International Seed Testing Association* **32**:553-563.

MAYNARD M.L. & GATES D.H. 1963. Effects of wetting and drying on germination of crested wheatgrass seed. *Journal of Range Management* **16**:119-121.

MCDONALD M.B., SULLIVAN J. & LAUER M.J. 1994. The pathway of water uptake in maize seeds. *Seed Science and Technology* **22**:79-90.

MENEY K.A. & DIXON K.W. 1988. Phenology, reproductive biology and seed development in four rush and sedge species from western Australia. *Australian Journal of Botany* **36**:711-726.

MEYER H.J. & VAN STADEN J. 1986. Inorganic nutrient requirements of *in vitro* cultured *Manihot esculenta* explants. *South African Journal of Botany* **52**:472-480.

MILEWSKI A.V. & BOND W.J. 1982. Convergence of myrmecochory in mediterranean Australia and South Africa. In: *Ant-Plant Interactions in Australia*. Ed: R.C. Buckley, pp. 89-98. Dr W Junk Publishers, The Hague.

MILEWSKI A.V. & ESTERHUYSEN E. 1977. Habitat of Restionaceae endemic to western Cape coastal flats. *Journal of South African Botany* **43**:233-241.

MILLER R.W. & DONAHUE R.L. 1990. *Soils: An Introduction to Plant Growth*. Sixth Edition. Prentice Hall, Englewood Cliffs, New Jersey.

MIYAJIMA D. 1996. Germination of *Zinnia* seed with and without pericarp. *Seed Science and Technology* **24**:465-473.

MŁODZIANOWSKI F. 1964. The structure and the later stages of development in the embryo sac of *Thamnochortus fruticosus* Berg (Restionaceae). *Bulletin de la Societe des Amis* **IV**:3-11.

MOHAMED-YASSEEN Y., BARRINGER S.A., SPLITTSTOESSER W.E. & COSTANZA S. 1994. The role of seed coats in seed viability. *The Botanical Review* **60**:426-439.

MOORE P.D. 1996. Fire damage soils our forests. *Nature* **384**:312-313.

MOORE R.P. 1969. Tetrazolium insights into leaky embryos and bloated seeds. *Seed Technologist News* **40**:5-6.

MOORE R.P. 1970. Tetrazolium for diagnosing causes for disturbances in seed quality. *Zeitschrift Landwirtschaftliche Forschung* **24**:104-109.

MORPETH D.R., HALL A.M. & CULLUM F.J. 1997. The involvement of microbes and enzymes in the pretreatment of woody seeds to overcome dormancy. In: *Basic and Applied Aspects of Seed Biology*. Eds: R.H. Ellis, M. Black, A.J. Murdoch & T.D. Hong, pp. 261-277. Kluwer Academic Publishers, Dordrecht.

MORRISON D.A., McCLAY K., PORTER C. & RISH S. 1998. The role of the lens in controlling heat-induced breakdown of testa-imposed dormancy in native Australian legumes. *Annals of Botany* **82**:35-40.

MURASHIGE T. & SKOOG F. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiologia Plantarum* **15**:473-497.

MUSIL C.F. 1991. Seed bank dynamics in sand plain lowland fynbos. *South African Journal of Botany* **57**:131-142.

MUSIL C.F. & DE WITT D.M. 1990. Post-fire regeneration in a sand plain lowland fynbos community. *South African Joumal of Botany* **56**:167-184.

MUSIL C.F. & DE WITT, D.M. 1991. Heat-stimulated germination in two Restionaceae species. *South African Joumal of Botany* **57**:175-176.

MUSTART P.J. & COWLING R.M. 1991. Seed germination of four serotinous Agulhas Plain Proteaceae. *South African Journal of Botany* **57**:310-313.

NAN Z.B., HANSON J. & YESHI W.M. 1998. Effects of sulphuric acid and hot water treatments on seedborne fungi and germination of *Stylosanthes hamata, S, guianensis* and *S. scabra. Seed Science and Technology* **26**:33-43.

NAREDO M.E.B., JULIANO A.B., LU B.R., DE GUZMAN F. & JACKSON M.T. 1998. Responses to seed dormancy-breaking treatments in rice species (*Oryza* L.). *Seed Science and Technology* **26**:675-689.

NAVEH Z. 1974. Effect of fire in the Mediterranean region. In: *Fire and Ecosystems*. Eds: T.T. Kozlowski & C.E. Ahlgren, pp. 401-434. Academic Press, New York.

NEGI A.K. & TODARIA N.P. 1995. Pre-treatment methods to improve germination in *Terminalia tomentosa* wight & arn. *Seed Science and Technology* **23**:245-248.

NIKOLAEVA M.G. 1977. Factors controlling the seed dormancy pattern. In: *The Physiology and Biochemistry of Seed Dormancy and Germination*. Ed: A.A. Kahn, pp. 51-74. North-Holland, Amsterdam/New York.

NOWAG A., PINNOW H. & SPETHMANN W. 1997. Controlled stratification of *Prunus avium* L. seeds. In: *Basic and Applied Aspects of Seed Biology*. Eds: R.H. Ellis, M. Black, A.J. Murdoch & T.D. Hong, pp. 335-338. Kluwer Academic Publishers, Dordrecht.

OBROUCHEVA N.V. 1997. Hydration up to threshold levels as the triggering agent of the processes preparing germination in quiescent seeds. In: *Basic and Applied Aspects of Seed Biology*. Eds: R.H. Ellis, M. Black, A.J. Murdoch & T.D. Hong, pp. 555-562. Kluwer Academic Publishers, Dordrecht.

PAMMENTER N.W. & BERJAK P. 1999. A review of recalcitrant seed physiology in relation to desiccation-tolerance mechanisms. *Seed Science Research* **9**:13-37.

PAPIÓ C. & TRABAUD L. 1991. Comparative study of the aerial structure of five shrubs of Mediterranean shrublands. *Forest Science* **37**:146-159.

PARKER V.T. & KELLY V.R. 1989. Seed banks in California chaparral and other Mediterranean climate shrublands. In: *Ecology of Soil Seed Banks*. Eds: M.A. Leck, V.T. Parker & R.L. Simpson, pp. 231-255. Academic Press Inc, San Diego, California.

PICKER M. Personal communication. Department of Zoology, University of Cape Town, Private Bag, Rondebosch, 7701, Republic of South Africa.

PIERCE S.M. & MOLL E.J. 1994. Germination ecology of six shrubs in fire-prone fynbos. *Vegetatio* **110**:25-41.

PIOTTO B. 1995. Influence of scarification and prechilling on the germination of seeds of *Pistacia lentiscus*. *Seed Science and Technology* **23**:659-663.

POULSEN K.M. 1995. Application of the IDS-method to *Pinus caribaea* seed. *Seed Science and Technology* **23**:269-275.

POULSEN K.M. 1996. Prolonged cold, moist pretreatment of conifer seeds at controlled moisture content. *Seed Science and Technology* **24**:75-87.

POWELL A.D., DULSON J. & BEWLEY J.D. 1984. Changes in germination and respiratory potential of embryos of dormant Grand Rapids lettuce seeds during long-term imbibed storage, and related changes in the endosperm. *Planta* **162**:40-45.

PRASAD P. & NAUTIYAL A.R. 1996. Physiology of germination in *Bauhinia*: involvement of seed coat inhibition of germination in *B. racemosa* Lam. seeds. *Seed Science and Technology* **24**:305-308.

PRIESTLEY D.A. 1986. *Seed Ageing: Implications for Seed Storage and Persistence in the Soil*. Cornell University Press, Ithaca.

QUICK W.A. & HSIAO A.I. 1984. Changes in inorganic phosphate and seed germinability during afterripening of wild oats. *Canadian Journal of Botany* **62**:2469-2471.

RAZDAN M.K. 1993. *An Introduction to Plant Tissue Culture*. Intercept Limited, Andover, Hampshire, U.K.

REBELO A.G. 1996. Fynbos biome. In: *Vegetation of South Africa, Lesotho and Swaziland*. Eds: A.B. Low & A.G. Rebelo, pp. 62-74. Department of Environmental Affairs & Tourism, Pretoria.

REHMAN S., HARRIS P.J.C. & BOURNE W.F. 1998. The effect of hardening on the salinity tolerance of *Acacia* seeds. *Seed Science and Technology* **26**:743-754.

REN C. & KERMODE A.R. 1999. Analyses to determine the role of the megagametophyte and other seed tissues in dormancy maintenance of yellow cedar (*Chamaecyparis nootkatensis*) seeds: morphological, cellular and physiological changes following moist chilling and during germination. *Journal of Experimental Botany* **50**:1403-1419.

REYNOLDS E.S. 1963. The use of lead citrate at high pH as an electron opaque stain for electron microscopy. *Journal of Cell Biology* **17**:208-212.

RICE B. & WESTOBY M. 1986. Evidence against the hypothesis that ant-dispersed seeds reach nutrient-enriched microsites. *Ecology* **67**:1270-1274.

RIZZINI C.T. 1973. Dormancy in seeds of *Anona crassiflora* Mart. *Journal of Experimental Botany* **24**:117-123.

ROBERTS E.H. 1973. Predicting the storage life of seeds. *Seed Science and Technology* **1**:499-514.

ROBERTS E.H. 1974. Storage environment and the control of viability. In: *Viability of Seeds*. Ed: E.H. Roberts, pp. 14-58. Chapman and Hall Ltd, London.

ROTH-BEJERANO N., SEDEE N.J.A., VAN DER MEULEN R.M. & WANG M. 1999. The role of abscisic acid in germination of light-sensitive and light-insensitive lettuce seeds. *Seed Science Research* **9**:129-134.

ROWELL D.L. 1994. *Soil Science: Methods and Applications*. Longman Scientific & Technical. Harlow, Essex, England.

ROY J. & SONIÉ L. 1992. Germination and population dynamics of *Cistus* species in relation to fire. *Journal of Applied Ecology* **29**:647-655.

SACHETI U. & AL-RAWAHY S.H. 1998. The effects of various pretreatments on the germination of important leguminous shrub-tree species of the Sultanate of Oman. *Seed Science and Technology* **26**:691-699.

SIDDIQUE M.A. & GOODWIN P.B. 1985. Conductivity measurements on single seeds to predict the germinability of French beans. *Seed Science and Technology* **13**:643-652.

SIMON E.W. 1974. Phospholipids and plant membrane permeability. *New Phytologist* **73**:377-420.

SKORDILIS A. & THANOS C.A. 1995. Seed stratification and germination strategy in the Mediterranean pines *Pinus brutia* and *P. halepensis*. *Seed Science Research* **5**:151-160.

SLINGSBY P. & BOND W. 1981. Ants - friends of the fynbos. *Veld and Flora* **67**:39-45.

SLINGSBY P. & BOND W.J. 1985. The influence of ants on the dispersal distance and seedling recruitment of *Leucospermum conocarpodendron* (L.) Buek (Proteaceae). *South African Journal of Botany* **51**:30-34.

SMALL J.G.C., ROBBERTSE P.J., GROBBELAAR N. & BADENHORST C.M. 1982. The effect of time of application and sterilization method of gibberellic acid, and temperature on the seed germination of *Erica junonia*, and endangered species. *South African Journal of Botany* **1**:139-141.

SPURR A.R. 1969. A low viscosity epoxy resin embedding medium for electron microscopy. *Journal of Ultrastructural Research* **26**:31-43.

STABELL E., UPADHYAYA M.K. & ELLIS B.E. 1996. Development of seed coat-imposed dormancy during seed maturation in *Cynoglossum officinale*. *Physiologia Plantarum* **97**:28-34.

STAHL M. & STEINER A.M. 1998. Mass relations and moisture equilibria between the embryo with scutellum and the endosperm part of non-sprouted and sprouted cereal seeds. *Seed Science and Technology* **26**:835-838.

STATISTICA. 1999. Version 5.5 for Windows StatSoft, Inc., Tulsa, Oklahoma.

STOCK W.D. & LEWIS O.A.M. 1986. Soil nitrogen and the role of fire as a mineralizing agent in a South African coastal fynbos ecosystem. *Journal of Ecology* **74**:317-328.

SUNILKUMAR K.K. & SUDHAKARA K. 1998. Effect of temperature, media and fungicide on the storage behaviour of *Hopea parviflora* seeds. *Seed Science and Technology* **26**:781-797.

SUTCLIFFE M.A. & WHITEHEAD C.S. 1995. Role of ethylene and short-chain saturated fatty acids in the smoke-stimulated germination of *Cyclopia* seed. *Journal of Plant Physiology* **145**:271-276.

SWIFT A.J. 1994. *Phylogeny of Willdenowia (Restionaceae): Implications for Speciation Mechanisms and Evolution of Regeneration Strategies in the Fynbos*. BSc Honours Thesis, University of Cape Town.

TAKAYANAGI K. & MURAKAMI K. 1968. Rapid germinability test with exudates from seed. *Nature* **218**:493-494.

TAYLOR A.G., LEE S.S., BERESNIEWICZ M.M. & PAINE D.H. 1995. Amino acid leakage from aged vegetable seeds. *Seed Science and Technology* **23**:113-122.

TAYLOR H.C. 1978. Capensis. In: *Biogeography and Ecology of Southern Africa*. Ed. M.J.A. Werger, pp. 171-229. Dr W Junk Publishers, The Hague.

TEKRONY D.M. & EGLI D.B. 1997. Accumulation of seed vigour during development and maturation. In: *Basic and Applied Aspects of Seed Biology*. Eds: R.H. Ellis, M. Black, A.J. Murdoch & T.D. Hong, pp. 369-384. Kluwer Academic Publishers, Dordrecht.

THANOS C.A. & GEORGHIOU K. 1988. Ecophysiology of fire-stimulated seed germination in *Cistus incanus* ssp. *creticus* (L.) Heywood and *C. salvifolius* L. *Plant, Cell and Environment* **11**:841-849.

THANOS C.A. & RUNDEL P.W. 1995. Fire-followers in chaparral: nitrogenous compounds trigger seed germination. *Journal of Ecology* **83**:207-216.

THANOS C.A., KADIS C.C. & SKAROU F. 1995. Ecophysiology of germination in the aromatic plants thyme, savory and oregano (Labiatae). *Seed Science Research* **5**:161-170.

THAPLIYAL R.C. & CONNOR K.F. 1997. Effects of accelerated ageing on viability, leachate exudation, and fatty acid content of *Dalbergia sissoo* Roxb. seeds. *Seed Science and Technology* **25**:311-319.

THAPLIYAL R.C. & NAITHANI K.C. 1996. Inhibition of germination in *Nyctanthes arbor-tristis* (Oleaceae) by pericarp. *Seed Science and Technology* **24**:67-73.

THOMSEN K.A. 1997. The effect of harvest time and drying on dormancy and storability in beechnuts. In: *Basic and Applied Aspects of Seed Biology*. Eds: R.H. Ellis, M. Black, A.J. Murdoch & T.D. Hong, pp. 45-51. Kluwer Academic Publishers, Dordrecht.

TILLMAN-SUTELA E. 1997. Effect of incubation temperature on the variation of imbibition in northern pine (*Pinus sylvestris* L.) seeds. *Seed Science and Technology* **25**:101-113.

TOOROP P.E., VAN AELST A.C. & HILHORST H.W.M. 1998. Endosperm cap weakening and endo-ß-mannanase activity during priming of tomato (*Lycopersicon esculentum* cv. Moneymaker) seeds are initiated upon crossing a threshold water potential. *Seed Science Research* **8**:483-491.

TOYOMASU T., YAMANE H., MUROFUSHI N. & INOUE Y. 1994. Effects of exogenously applied gibberellin and red light on the endogenous levels of abscisic acid in photoblastic lettuce seeds. *Plant Cell Physiology* **35**:127-129.

TRYKA S., NIKOLENKO V.F. & ALEXEEVA D.I. 1997. The influence of internal mechanical cracks on ultraweak luminescence from wheat seeds and seedlings. *Seed Science and Technology* **25**:381-389.

TYAGI C.S. 1992. Evaluating viability and vigour in soybean seed with automatic seed analyzer. *Seed Science and Technology* **20**:687-694.

UPRETI J. & DHAR U. 1997. Study on seed germination of a leguminous liana – *Bauhinia vahlii* Wight & Arnott. *Seed Science and Technology* **25**:187-194.

VAN DER VENTER H.A. & ESTERHUIZEN A.D. 1988. The effect of factors associated with fire on seed germination of *Erica sessiliflora* and *E. hebecalyx* (Ericaceae). *South African Journal of Botany* **54**:301-304.

VAN RENSBURG S. 1995. *The Effect of Smoke Treatment on the Germination of Four Species of Mesembryanthemum: Some Preliminary Observations*. BSc Honours Thesis, University of Cape Town.

VAN STADEN J. 1966. Studies on the germination of seed of Proteaceae. *The Journal of South African Botany* **32**:291-298.

VAN STADEN J. 1978. Seed viability in *Protea neriifolia*. I. The effects of time of harvesting on seed viability. *Agroplantae* **10**:65-67.

VAN STADEN J. & BROWN N.A.C. 1973a. The effect of oxygen on endogenous cytokinin levels and germination of *Leucadendron daphnoides* seed. *Physiologia Plantarum* **29**:108-111.

VAN STADEN J. & BROWN N.A.C. 1973b. The role of the covering structures in the germination of seed of *Leucospermum cordifolium* (Proteaceae). *Australian Journal of Botany* **21**:189-192.

VAN STADEN J. & BROWN N.A.C. 1977. Studies on the germination of South African Proteaceae – a review. *Seed Science and Technology* **5**:633-643.

VAN WILGEN B.W. 1981. Some effects of fire frequency on fynbos plant community composition and structure at Jonkershoek, Stellenbosch. *South African Forestry Journal* **118**:42-55.

VAN WILGEN B.W. & VIVIERS M. 1985. The effect of season of fire on serotinous Proteaceae in the western Cape and the implications for fynbos management. *South African Forestry Journal* **133**:49-53.

VERMA V.D. & RAM H.H. 1987. Genetics of electrical conductivity in soybean. *Seed Science and Technology* **15**:125-134.

VERTUCCI C.W. & FARRANT J.M. 1995. Acquisition and loss of desiccation tolerance. In: *Seed Development and Germination*. Eds: J. Kigel & G. Galili, pp. 237-271. Marcel Dekker Inc., New York.

VILLAGRA P.E. 1995. Temperature effects on germination of *Prosopis argentina* and *P. alpataco* (Fabaceae, Mimusoideae). *Seed Science and Technology* **23**:639-646.

VINCENT E.M. & CAVERS P.B. 1978. The effects of wetting and drying on the subsequent germination of *Rumex crispus*. *Canadian Journal of Botany* **56**:2207-2217.

VLEESHOUWERS L.M., BOUWMEESTER H.J. & KARSSEN C.M. 1995. Redefining seed dormancy: an attempt to integrate physiology and ecology. *Journal of Ecology* **83**:1031-1037.

VOZZO J.A. 1988. Seed radiography. *Materials Evaluation* **46**:1450-1455.

WALCK J.L., BASKIN J.M. & BASKIN C.C. 1997a. A comparative study of the seed germination biology of a narrow endemic and two geographically-widespread species of *Solidago* (Asteraceae). 5. Effect of dry storage on afterripening and survivorship. *Seed Science Research* **7**:311-318.

WALCK J.L., BASKIN J.M. & BASKIN C.C. 1997b. A comparative study of the seed germination biology of a narrow endemic and two geographically-widespread species of *Solidago* (Asteraceae). 3. Photoecology of germination. *Seed Science Research* **7**:293-301.

WANG M. 1997. The role of abscisic acid in the regulation of barley grain germination. *Seed Science and Technology* **25**:67-74.

WANG M., VAN DER MEULEN R., VISSER K., VAN SCHAIK H-P., VAN DUIJN B. & DE BOER H. 1998. Effects of dormancy-breaking chemicals on ABA levels in barley grain embryos. *Seed Science Research* **8**:129-137.

WELBAUM G.E., BRADFORD K.J., YIM K-O., BOOTH D.T. & OLUOCH M.O. 1998. Biophysical, physiological and biochemical processes regulating seed germination. *Seed Science Research* **8**:161-172.

WESTOBY M., HUGHES L. & RICE B.L. 1991. Seed dispersal by ants; comparing infertile with fertile soils. In: *Ant-plant interactions*. Eds: C.R. Huxley & D.F. Cutler, pp. 434-447. Oxford University Press, Oxford.

WESTOBY M., RICE B., SHELLEY J.M., HAIG D. & KOHEN J.L. 1982. Plants' use of ants for dispersal at West Head, New South Wales. In: *Ant-Plant Interactions in Australia*. Ed: R.C. Buckley, pp. 89-98. Dr W Junk Publishers, The Hague.

WICKLOW D.T. 1977. Germination response in *Emmenanthe penduliflora* (Hydrophyllaceae). *Ecology* **58**:201-205.

ZAR J.H. 1996. *Biostatistical Analysis*. Third Edition. Prentice-Hall, Inc., Upper Saddle River, New Jersey.