CHAPTER 1

GENERAL INTRODUCTION AND BACKGROUND

1.1 Preamble

Weeds cause considerable losses in crops. Parker and Fryer (1975) estimated the losses due to weeds to be as large as 50% in tropical crops and approximately 11.5% of total potential production worldwide. Furthermore, weeds harbour diseases and disease-causing organisms as well as insect pests, thus contributing to the damage caused by these pests. In many instances, however, losses due to weeds are underestimated because the damage they cause is not as conspicuous as the ravages of insects or diseases.

Weeds are always present in agronomic systems. They are particularly coupled to disturbed soil environments and have developed a number of mechanisms which enable them to survive and perpetuate. In annual species, for example, seeds represent the only link between generations. In contrast, perennials may use vegetative propagules as well as seeds. Dormancy in seeds enables a species to survive conditions unfavourable for growth, preserving the seed for recruitment to the plant population at some later time or in some other place (Chancellor 1982). In some species of grasses, few of the seeds remain viable in the soil for more than one year (Thompson and Grime 1979). Survival of such species may depend on the production of a large number of seeds (Hall et al. 1978). Some other species, mostly dicotyledon, form a persistent seed reserve in the soil (seed bank). Well documented studies indicate that survival of such seeds in the soil may be as long as decades or centuries (Baskin and Baskin 1977a; Kivilaan and Bandurski 1981).
It is not uncommon for agricultural soils to contain in excess of 10,000 seeds/m² (Roberts 1972; H.A. Roberts 1981; Chancellor 1982; Menges 1987). The ability of these seeds to persist in the soil for many years poses a serious problem for agriculture. Conventional herbicidal and cultural procedures control weeds after germination, but normally have little or no effect on non-germinated seeds (Egley 1986; Medd 1987). Thus, only those seeds that germinate will generally be controlled. However, even if 100% weed control is achieved over several years the dormant portion of the seed bank provides a source for future weed infestations requiring repeated control practices (Hurle 1974; Schweizer and Zimdahl 1984a, b).

It would, therefore, appear that one of the best long term prospects in weed management lies in developing techniques that directly regulate the seed bank (Chancellor 1981; Medd and Wilson 1984; Hurtt and Taylorson 1986; Medd 1987; Egley 1990). Seeds may be lost from the seed bank by various means. Although predation and loss of viability can be important at the soil surface, most studies suggest that germination is the primary cause of depletion of buried seeds (Schafer and Chilcote 1970; Taylorson 1970; 1972; Murdoch and Roberts 1982; Zorner et al. 1984a). This makes it worthwhile to consider the possibility of manipulating factors which control seed dormancy and germination. Chancellor (1981) and Taylorson (1985) also suggested that manipulation of seed dormancy might make a powerful weed management tool. However, this concept has not yet led to techniques that can be applied economically on a broad scale in agriculture.

A clear knowledge of the factors involved in the imposition and breaking of dormancy is needed for there to be any future in reducing seed banks and thus the potential for weed reinfestation. The results from such studies should also contribute to a better understanding of the population biology of certain weeds, which, in turn, will allow better planning for the application of control measures.
Because of its persistent seed bank arising from the production of a large number of seeds, the occurrence of seed dormancy and prolonged viability (Lewis 1973; Burnside et al. 1981; Kivilaan and Bandurski 1981) *Amaranthus retroflexus* L. (redroot pigweed) was chosen as the subject of the investigations reported in this thesis.

1.2 *Amaranthus retroflexus* - the weed

*A. retroflexus* is a member of the family *Amaranthaceae* which includes some domesticated grain amaranth species and ornamentals. *A. retroflexus* is a native of North America but it now has a cosmopolitan distribution in both temperate and tropical regions of the world (Weaver and McWilliams 1980).

This weed is a stout, erect summer annual. It has a shallow taproot which is often pinkish or reddish in colour. Stems are erect, growing to a height of up to 2 m and either simple or branched (often profusely) with dense hairs, especially on the upper part. Leaves are alternate, long-stalked, sparsely hairy (on the lower surface), ovate or lanceolate. Leaves and stems have a reddish tinge. The inflorescence, which may be about 5 to 20 cm long and 1.5 cm or more thick, consists of numerous small flowers crowded together into dense, blunt spikes that form a thick, stiff terminal panicle. Small panicles are also produced in leaf axils (Holm et al. 1977).

*A. retroflexus* is considered among the top ranking troublesome weeds in many parts of the world (Holm et al. 1977; Egley 1990) where it continues to persist despite its susceptibility to several herbicides (Hendrick et al. 1974; Ogg and Zimmerman 1976; Schweizer and Zimdahl 1984 a, b). Biotypes of *A. retroflexus* resistant to triazine have been reported from many countries since the first discovery in Austria in 1973. It is now reported as one of the four most prevalent triazine-resistant weeds in France. Resistance to carbamates, linuron and bromacil are also reported (LeBaron 1991). *A. retroflexus* is
a troublesome summer annual weed of cultivated fields, fence lines, waste areas, roadsides, disturbed habitats, and is often a contaminant of grains (Feltner 1970).

*A. retroflexus* reduces the yield of a variety of crops mainly by competition (McLaughlin *et al.* 1976; Senesac and Minotti 1978). Vangessel and Renner (1990) found that as few as one *A. retroflexus* or *Echinochloa crus-galli* L. (barnyard grass) per metre of row reduced marketable *Solanum tuberosum* L. (potato) tuber yield by 19 to 33% when seeded in the row at the time of planting. They also found that in one year, *A. retroflexus* reduced tuber yield more than did *E. crus-galli*. Mixed populations of *A. retroflexus* and *Amaranthus powellii* S. Wats (green pigweed) were found by Hendrick *et al.* (1974) to reduce the yield of *Beta vulgaris* L. (sugarbeet) by as much as 49%.

Apart from the direct effects caused by competition with crops, *A. retroflexus* is also an alternate host for a number of parasites and insect pests and thus contributes to the damage caused by them. For example, it has been found to be an alternate host for the parasite *Orobanche ramosa* L. in *Lycopersicon esculentum* Mill. (tomato) fields (King 1966) and for *Myzus persicae* (Sulzer) (green peach aphids), in *Prunus persica* (L.) Batsch (peach) and *Malus domestica* Borth (apple) orchards (Tamaki 1975).

In some areas, for example, in the Canadian provinces of Quebec and Manitoba, *A. retroflexus* is proclaimed noxious (Weaver and McWilliams 1980). However, in Australia, this weed is not considered noxious (Parsons and Cuthbertson 1992).

One of the factors that makes *A. retroflexus* persist despite its susceptibility to several herbicides is the occurrence of seed dormancy and prolonged seed viability. Studies of seed longevity have been reported for *A. retroflexus*. In the Duvel buried seed experiment in Virginia (USA), seeds of *A. retroflexus* remained viable for 10 years, with percent survival increasing with depth of burial. In Beal’s buried seed experiment in
Michigan (USA), seeds of *A. retroflexus*, buried at a depth of 45 cm, remained viable for 40 years (Kivilaan and Bandurski 1981).

Besides seed dormancy, a further principal cause of persistence of *A. retroflexus* is its prodigious seed production. A single vigorous plant of *A. retroflexus* may produce as many as 100,000 seeds as do other species such as *A. powellii* and *Amaranthus hybridus* L. (smooth pigweed) (Weaver and McWilliams 1980). Hauptli and Jain (1978) reported that pure stands of *A. retroflexus* returned to the soil about 725,000 seeds/m² under conditions of high fertility and water.

The large number of seeds produced and seed longevity appear as two important characteristics that enable a reservoir of seeds to accumulate in the soil. A gradual loss of dormancy of soil-borne seeds may result in sporadic emergence throughout the growing season and the plants that survive to maturity further add to the reservoir of dormant seeds in the soil. Formation of seed banks along these lines ensures survival and makes complete eradication an unrealistic goal.

For these reasons, studies were designed to investigate some of the factors that affect dormancy and germination of *A. retroflexus* seed. In addition, treatments designed to manipulate the seed bank, were studied with an ultimate view to improving management of the weed. As a foundation to these studies a selected review of literature related to seed dormancy was undertaken.

1.3 Seed dormancy and its significance

Seed dormancy results in the failure of a seed to germinate under conditions normally favourable for growth (Amen 1968; Baskin and Baskin 1989). This may be caused by some physiological block(s) within the seed, by morphological features or by
physical or environmental constraints (Bewley and Black 1985). A number of terms are in use to describe a seed's inability to germinate. Innate, induced and enforced dormancy were terms introduced by Harper (1957) to describe, respectively, dormancy which develops in seeds while on the mother plant, dormancy which is induced after shedding and dormancy which is enforced by unsuitable environmental conditions. Innate and induced dormancy are equivalent to primary and secondary dormancy, which are other terms widely used (e.g., Karssen 1982). Figure 1.1 shows that seeds may be shed from the mother plant in a dormant or non-dormant state.

![Diagram of dormancy and germination](image)

**Figure 1.1** Dormancy and germination. Seed characteristics and some of the environmental factors that control dormancy and germination in seeds (after Bewley and Black 1985)

Depending on the prevailing conditions, dormancy may be broken and eventually germination occurs. If germination does not take place secondary dormancy sets in. The use of the term "enforced dormancy" to refer to inhibition of germination due to environmental restraint (Harper 1957, 1977) is questionable. Quiescence would be a more suitable term since the requirements for germination of non-dormant seeds are lacking. Karssen (1982) also questioned the term enforced dormancy. He argued that
inhibition of germination due to unsuitable environmental conditions often leads to the development of secondary dormancy. Hence, he only recognised the terms primary and secondary dormancy.

In many species dormancy persists after maturation and shedding to ensure the temporal dispersal of seeds by preventing immediate or synchronous germination. Cheam (1985) claimed that seed dormancy is perhaps the single most important characteristic in weeds that enables them to survive and persist. According to Pearce (1984), seed dormancy and the ability to germinate throughout the growing season are the main protective mechanisms in numerous weeds which have survived several years of herbicide application during cropping. The importance of dormancy to weed seed survival has also been stressed by Harper (1957) who claimed that it is a major barrier to advances in weed control.

The mechanism(s) or the physiological block(s) to germination have not been fully elucidated, but several models have been proposed to explain the modes of action of dormancy breaking treatments (Roberts and Smith 1977; Bewley and Black 1982; Cohn 1989). Whether or not the physiological mechanisms for primary and secondary dormancy are similar is also not known. There is some evidence from studies of *Avena fatua* L. (wild oat) that both primary and secondary dormancy may have similar mechanisms due to their similar responses to dormancy breaking treatments (Chancellor 1982; Simpson 1983). The patterns of change in dormancy during burial in certain seeds, *e.g.*, *Veronica hederifolia* L. (ivyleaf speedwell) and *Polygonum persicaria* L. (red shank) (Karssen 1980/81) and in *Ambrosia artemisiifolia* L. (annual ragweed) (Baskin and Baskin 1980) clearly show that secondary dormancy is broken in the same period of the year as primary dormancy. In this case, it may be assumed that both types of dormancy are broken by the same environmental factors.
However, contrasting results have been obtained from other species. Although it has been shown that low temperature brings about the loss of both primary and secondary dormancy in a number of species, e.g., *Rumex crispus* L. (curly dock) (Totterdell and Roberts 1979), this treatment was ineffective in breaking secondary dormancy of *Chenopodium bonus-henricus* L. (perennial goosefoot) (Khan and Karssen 1980; Karssen 1980/81). These results suggest a different nature of primary and secondary dormancy in the latter species. There is some other evidence to suggest that more than one state of dormancy may be present in a seedlot, each of which may require a different dormancy breaking treatment. At least two states of dormancy were found to exist in *A. fatua* (Adkins and Simpson 1988). Although both states of dormancy were overcome similarly by gibberellic acid and ethanol, differences in the response to other treatments were observed. For example, while one form of dormancy was readily broken by short periods of after-ripening and was insensitive to nitrite and azide, the other state of dormancy was persistent, but sensitive to nitrite and azide.

1.3.1 Changes in dormancy

Primary dormancy may persist for a variable length of time after the seed has been shed. It is later lost, but if conditions are not suitable, germination will not occur. During the period when the seed does not germinate, secondary dormancy may develop (Fig. 1.1). Secondary dormancy can be repeatedly broken and induced during many successive years (Karssen 1982; Fig. 1.2). Annual cycles in which dormancy is seasonally broken and induced are well documented in some annual plants of either temperate or tropical origin (Taylorson 1970, 1972; Baskin and Baskin 1985, 1989; Garwood 1989; Bouwmeester 1990).

At times of no dormancy, there is no direct block, so germination is likely to proceed unless environmental factors such as moisture and temperature are unsuitable
(Roberts and Potter 1980; Karssen 1982; Bouwmeester 1990). Exposure to light (Taylorson 1970, 1972), fluctuations of temperature (Baskin and Baskin 1985), or a combination of factors may be required by some seeds for germination to occur (E.H. Roberts 1981; Baskin and Baskin 1985; Roberts et al. 1987).

It has been shown that dormancy cycling depends on seasonal fluctuations in soil temperature (Bouwmeester 1990). These seasonal responses may help to explain why seedlings emerge sporadically after cultivation as well as the periodicity of emergence which is characteristic of many species (Stoller and Wax 1973; Roberts and Neilson 1980, 1981). Periodicity of germination, complicates weed control practices since weeds may germinate after a mechanical cultivation has been completed or after a herbicide has degraded.

![Diagram of dormancy stages](image)

**Figure 1.2** Changes in dormancy: 1, factors breaking dormancy; 2, factors coinciding with conditions required for germination; 3, factors inducing dormancy; 4, factors differing from conditions required for germination and/or factors inhibiting germination (adapted from Karssen 1982).
1.4 Dormancy control

In nature, factors such as light, temperature and aging may convert a seed from the dormant to the non-dormant state. Regulation of seed dormancy is controlled by the genetic makeup of seeds and environmental factors which operate interactively (Chancellor 1982; Bewley and Black 1985; Adkins et al. 1987; Fig. 1.1).

1.4.1 Light in dormancy regulation

Light is one of the principal factors shown to control dormancy in seeds. In a large number of species, dormancy is terminated when the hydrated seed is illuminated with white light (Bewley and Black 1982). In most species, germination is promoted by short exposures to white light with relatively low energy and inhibited by long exposures (Gorski and Gorska 1979). For example, Kadman-Zahavi (1960) found that a short red or white illumination given at different intervals after sowing greatly stimulated germination of *A. retroflexus* at 37°C while continuous white light had an inhibitory effect. Some species require long exposure to light for breaking dormancy, *e.g.*, *Plantago major* L. (great plantain) (Pons 1991), while others, such as *Bidens pilosa* L. (hairy beggar ticks) germinate irrespective of the illumination regime (Reddy and Singh 1992). Hence, the actual response of seeds to light varies greatly between species and seedlots, and may further depend on pre-treatment conditions during any germination test. Furthermore, these responses may vary depending upon different spectral compositions (Bewley and Black 1982).

The stimulating effect of light on seed germination of wild species has been known for a long time. Since the experiments of Borthwick *et al.* (1952) it has generally been agreed that red/far-red reversibility of a response is the action of the pigment, phytochrome (Toole 1973; Bewley and Black 1982; Frankland and Taylorson 1983;
Taylorson 1987). In darkness, this pigment is in an inactive form with an absorption maximum at 660 nm. It can absorb red light and is, therefore, designated \( P_R \). \( P_R \) is quickly converted into the active \( P_{fr} \) form upon irradiation with red light. \( P_{fr} \) has an absorption maximum at 730 nm and absorbs far red irradiation, hence its name. Upon far red light irradiation \( P_{fr} \) is converted back to \( P_R \). Much less is known, however, about how phytochrome mediates further steps in the regulation of dormancy.

The known light sensitivity of many seeds (Toole 1973) has led to speculation that light might be important in inducing germination of many weed species in cultivated fields.

1.4.2 Temperature in dormancy regulation

Temperature affects three separate physiological processes in seeds as was recognised by Roberts (1988): (i) together with moisture content, temperature determines the rate of deterioration in seeds; (ii) it affects the rate of dormancy loss in dry seeds and in the patterns of dormancy change in moist seeds; and (iii) it determines the rate of germination in non-dormant seeds.

1.4.2.1 Effect of temperature on dry seeds

At maturity a large proportion of weed seeds are dormant and will fail to germinate under conditions that later are satisfactory for germination and seedling growth. In some species, the germination of all seeds may occur shortly after dispersal, whereas, in others, dormancy will be retained in a significant proportion of the population for several years (Koller 1972). This behaviour is attributable to complex physio-chemical changes within the seed known as "after-ripening" (Steinbauer and
After-ripening, which takes place only in seeds with low water content, is therefore common to seeds in storage as well as to those in the field during exposure to long dry periods. It has been found that after-ripened seeds slowly become partially or completely independent of such factors as, light, pre-chilling or alternating temperatures, initially needed after harvest, and gain the ability to germinate under conditions which were previously unfavourable (Bewley and Black 1982). For example, in *Dactyliis glomerata* L. (cocksfoot, orchard grass) the proportion of individuals which require light and/or alternating temperatures to trigger germination declines during dry storage (Probert *et al.* 1985). In some seeds, after-ripening may be necessary before a phytochrome response can be shown, and in light sensitive species, prolonged after-ripening may lead to loss of light dependence (Taylorson 1982).

Dormancy may not be completely lost during after-ripening. In *R. crispus*, for example, dormancy was only reduced by about 50% after five years of after-ripening. The remainder of the seeds showed greater responsiveness to factors such as alternating temperatures and light (Cavers 1974). Hence Bewley and Black (1982) described the after-ripening effect as "graded".

Temperature plays a major role in determining the rate of after-ripening although other factors such as water and oxygen may also be involved (Bewley and Black 1982). E.H. Roberts (1962), working with *Oryza sativa* L. (rice) seeds demonstrated a negative linear relationship between the logarithm of the period of dry storage required for 50% germination (mean dormancy period) and temperature. Subsequent experiments revealed that the rate of after-ripening is constant within a species (Roberts 1965).

In species adapted to areas of the world which experience rainfall throughout the year and in soil types with high water retention, seeds may not experience dry periods, hence, the ecological significance of changes in dormancy behaviour observed during dry storage may be difficult to interpret. However, for species adapted to areas experiencing
dry seasons, physiological changes during dry storage probably reflect a naturally occurring mechanism that governs the timing of germination in the field. The relevance of after-ripening or maintenance of viability in dry-stored seeds may also be related to the storage, movement, and planting of crop seed contaminated with weed seeds that could result in dissemination of weeds.

1.4.2.2 Effect of temperature on imbibed dormant seeds

1.4.2.2.1 Temperature fluctuations

In many species, seeds require alternating temperatures or fluctuations to achieve a high percentage germination. Of 85 species selected from 15 families, Steinbauer and Grigsby (1957) found that more than 80% showed higher germination at alternating temperatures compared with constant temperatures. Roberts and Totterdell (1981) have clearly demonstrated the effect of temperature change on dormancy in seeds of *R. crispus* and *Rumex obtusifolius* L. (broadleaf dock). At constant temperatures, seeds from most collections of *Rumex* species failed to germinate unless irradiated. However, high germination in darkness was observed when seeds experienced alternations between 20°C and 30 or 35°C. It was also noted that these species responded almost as well to one or two 2-hour shifts to 35°C from 20°C as they did to several alternations, suggesting that temperature shifts could have the same basic action as alternating temperature.

The sensitivity of seeds to alternating temperatures may be influenced by other environmental factors such as light (Roberts and Benjamin 1979; Totterdell and Roberts 1980; Probert et al. 1986). In *Rumex* species for example, an interdependence between light and the effects of high temperature shifts have been shown (Takaki et al. 1981, 1985). Other factors that tend to increase the sensitivity of seeds to alternating
temperatures are nitrate (Probert et al. 1987), pre-chilling (Probert et al. 1989) and dry storage (Thompson and Grime 1983).

1.4.2.2.2 Low temperature effect (pre-chilling)

Pre-chilling involves holding imbibed seeds at low temperatures for a period of time before subjecting them to a higher temperature for germination (Bewley and Black 1985). Breaking of dormancy in seeds of many species is enhanced by pre-chilling (Stokes 1965; Lewak and Rudnicki 1977; Nikolaeva 1977). The effects of pre-chilling on dormancy breaking is particularly common in woody species (Bewley and Black 1982). The optimum temperature for breaking dormancy in most species is generally close to 5°C. However, species differ as to the most effective range. In a detailed study of *R. obtusifolius* and *R. crispus*, Totterdell and Roberts (1979) reported that dormancy breaking was possible from 1.5°C to 15°C. Totterdell and Roberts (1979), and VanderWoude and Toole (1980) have reported that lowering the temperature of imbibition to about 5 to 10°C in light sensitive species often results in increased dark germination at a subsequent higher favourable temperature. Thus, pre-chilling may reduce the requirement for light in those seeds that require light to break dormancy. In some light requiring seeds, pre-chilling causes an enhanced response to light. For example, in *Capsella bursa-pastoris* (L.) Medik (shepherd's purse) and *Chenopodium polyspermum* L. (many-seeded goosefoot) light has no apparent action upon dormancy breakage at constant moderate temperatures unless the seeds have been chilled previously (Popay and Roberts 1970; Vincent and Roberts 1979). Several other studies have shown that, in certain species, pre-chilling reduces the requirement for other environmental factors needed for breaking dormancy (Roberts and Benjamin 1979; Vincent and Roberts 1979).

The effectiveness of pre-chilling in breaking dormancy is dependent upon the temperature and duration of the low temperature experience. Nikolaeva (1977) and
VanderWoude and Toole (1980) observed that several hours or a few days of low temperature incubation promoted the germination of non-deeply-dormant seeds. According to Taylorson and Hendricks (1969), effective pre-chilling temperatures for *A. retroflexus* lie below 20°C, and that pre-chilling at 10°C for 144 hours can induce high levels of germination in darkness. However, even within a single population, genetic differences in the depth of dormancy between individual seeds may result in differential effects of pre-chilling. Hence, the pre-chilling treatments required may not be specific.

Although the effects of pre-chilling are widespread, there is evidence that low temperature can also result in the induction of dormancy. An extended period of incubation at low temperatures has been shown to lead to induction of secondary dormancy (Willemsen 1975; Bouwmeester 1990). The actual mechanism by which pre-chilling breaks dormancy is unclear. However, membrane phospholipid phase changes at critical temperatures have been implicated (Bewley and Black 1982).

1.4.2.3 Temperature and germination of non-dormant seeds

Once dormancy is broken, temperature plays an important role in promoting seed germination. Depending upon the species, seed germination is possible only within well defined temperature limits (Bewley and Black 1982, 1985). The optimum temperature for germination varies with seed age, the physiological state of seeds and species. For example, the extremes of optimum constant temperature for germination, as noted from the experiments of Steinbauer and Grigsby (1957) range from 5°C for *A. artemisiaefolia* to 35°C for *A. retroflexus*. Furthermore, the optimum temperature for any particular species may vary depending on seed treatment prior to germination. For example, seeds which have been after-ripened gain the ability to germinate at less favourable conditions. Hence, the minimum temperature required for germination by such seeds may be lowered. Seeds that do not germinate due to unfavourable temperature are often
induced into secondary dormancy (Karssen 1982). Although all seeds of a species may germinate over a wide range of temperatures, the time needed to achieve maximum germination percentage to be reached varies with the temperature (Roberts 1988). Temperature plays a major role in determining the time of weed seed germination in the field (Karssen 1982; Bouwmeester 1990).

1.4.3 The role of chemical stimulants

A number of chemicals have been shown to stimulate germination when applied to dormant seeds (Bewley and Black 1982). It is beyond the scope of this study to discuss all such chemicals. Attention is focused on some chemicals that show potential for use in the field.

The reduction of the dormant weed seed populations through chemical stimulation of germination has been considered by a number of workers (Egley and Dale 1970; Eplee 1975; Fawcett and Slife 1975, 1978; Egley 1980, 1990). Many of these studies were done under controlled laboratory, growth chamber or greenhouse conditions. The most notable example of the use of chemicals to increase germination of buried seeds is the use of ethylene on Striga lutea L. (= S. asiatica) (witchweed) (Egley and Dale 1970). In the United States, ethylene has become of great importance in the control of S. lutea by stimulating germination and reducing the population of dormant seeds in the soil (Eplee 1975; Bebawi and Eplee 1986). Ethylene may be supplied either directly as the gas or by means of an ethylene generating, water soluble chemical such as ethephon (Saini et al. 1986). Mayer and Poljakoff-Mayber (1982) have shown the effectiveness of ethephon in stimulating germination of dormant weed seeds under laboratory conditions. Under certain soil conditions, ethephon has been known to generate ethylene (Babiker and Hamdoun 1983; Goudey et al. 1987a). However, the use of ethephon to break dormancy in the field has been limited (Egley 1990).
Although ethylene has been known for some time to stimulate germination in seeds, its mechanism of action remains to be determined. However, roles of ethylene in respiration and in alteration of protein body membranes have been proposed. Ross (1984) gives a detailed review.

Nitrate has long been known to stimulate germination. Steinbauer and Grigsby (1957) studied the germination of 85 species of weed plants in 15 families and half showed more germination in the presence of nitrate. In *Chenopodium album* L. (fat hen, common lambs quarters) (Saini *et al.* 1985a) and *Sisymbrium officinale* (L) Scop. (hedge mustard), a positive interaction between endogenous nitrate content and germination in water was demonstrated (Bouwmeester 1990; Hilhorst and Karssen 1990).

Attempts to stimulate weed seed germination in the field with nitrates have given variable results. Watkins (1966) investigated the effects of three forms of fertilizers - urea, ammonium sulphate and calcium ammonium nitrate on the emergence of *Avena ludoviciana* L. (wild oat) in Queensland (Australia). He found no differences among the fertilizers, but all stimulated germination. H.A. Roberts (1962) also found some indications that increased application of nitrogen fertilizer led to a greater loss of soil weed seed populations, presumably the result of increased germination. Similarly, application of ammonium nitrate in the late spring stimulated emergence of *Abutilon theophrasti* Medic. (velvet leaf) seedlings in the field (Hurtt and Taylorson 1986). The germination of *S. officinale* seeds was also increased by application of nitrate in the field (Karssen 1980/81).

Contrasting results were obtained by Fawcett and Slife (1978), Schimpf and Palmblad (1980), and Egley (1990) who found no evidence that nitrate stimulated
germination of various weed seeds in the field. One explanation for such variability in responses is that other soil factors may affect the sensitivity of seeds to nitrate.

The effects of various tests on dormancy and germination by using nitrate, nitrite and hydroxylamine has prompted formulation of hypotheses concerning dormancy mechanisms of nitrate (Hendricks and Taylorson 1974; Roberts and Smith 1977). However, there has been no general agreement as to their mechanism of action.

Many other chemicals have been tested for dormancy breaking activity. Low concentrations of butylate (S-ethyl di-isobutylthiocarbamate), EPTC (S-ethyl dipropyl thiocarbamate), vernolate (S-propyl dipropylthiocarbamate) and diallate [S-(2,3-dichloroallyl) di-isopropylthiocarbamate] all members of the thiocarbamate family of herbicides were shown to increase *A. theophrasti* density in the field, and diallate has been found effective on *Datura stramonium* L. (jimson weed) and *A. retroflexus* (Fawcett and Slife 1975). Tri-allate [S-(2,3,3-trichloroallyl) di-isopropylthiocarbamate] is an improved formulation of diallate that does not volatilise easily. However, its effect on dormancy breaking has yet to be demonstrated.

The above investigations reveal that it may be quite feasible to reduce weed seed populations in the soil by use of chemical stimulants. However, it is also necessary to investigate the effects of other factors, especially under field conditions, that may interfere with the response of seeds to these stimuli.

1.4.4 Interacting factors

The interactions on the control of seed dormancy between temperature and light sensitivity, for example, are many and varied (Toole *et al.* 1955; Steinbauer and Grigsby 1957; Toole 1973; Takaki *et al.* 1981). Breaking of dormancy in almost all seeds is subject to complex interactions between the temperature and light conditions. For
example, a seed may be light requiring at one temperature but not at another (Bewley and Black 1982).

The degree of inhibition by continuous light may also depend on temperature. *Amaranthus* species, for example, are photo-inhibited at lower temperatures, but not at higher temperatures and the reverse is true for some other species (Bewley and Black 1982). There are examples in which high temperatures may impose a light requirement for the termination of dormancy. In *Chenopodium botrys* L. (Jerusalem oak goosefoot) light is obligatory at all temperatures but the duration needed varies with temperature (Cumming 1963). At 10°C, a single short photoperiod is sufficient to break dormancy; longer photoperiods are required at intermediate temperatures. As the temperature increases to 30°C frequent short photoperiods are required.

In several light requiring species, pre-chilling or low temperature treatment as well as alternating temperatures break dormancy in darkness. In some other cases the joint action of temperature fluctuation and light is required. Hence, in some species, the light requirement in dormancy and germination is not absolute. Seasonal changes in temperature result in seasonal changes in dormancy status in seeds of many species. These changes may also cause changes in the light requirement for dormancy breaking, e.g., in *A. artemisiafolia* (Baskin and Baskin 1980) and *R. obtusifolius* (Van Assche and Vanlenderghhe 1989). Karssen (1982) drew attention to the interaction between light and temperature as being involved in the seasonal control of dormancy.

Interactions between the chemical stimulants and other factors such as light and temperature may be required for any effective response. Interactions between nitrate and light have been demonstrated in a large number of species (Vincent and Roberts 1977, 1979; Roberts and Benjamin 1979; Williams 1983; Probert *et al.* 1987, Singh and Amritphale 1992). Light, nitrate or alternating temperatures, given individually to *R. crispus* seeds resulted in no germination. Yet, 68 and 100% germination was obtained
when light and alternating temperatures were applied together or when all three factors were applied, respectively. In seeds of *S. officinale*, the effectiveness of nitrate depended on the amount of $P_{fr}$ (Hilhorst and Karssen 1988, 1990). Nitrate has also been shown to change the light response of seeds appreciably.

In the same way as temperature fluctuation, nitrate can replace a requirement for light, *e.g.*, in *Plantago lanceolata* L. (common plantain) (Pons 1989) and *Sinapis arvensis* L. (wild mustard, charlock) (Goudey et al. 1987b). In some other instances nitrate must act together with light to break dormancy, *e.g.* in *S. officinale* (Hilhorst et al. 1986). The dormancy breaking mechanism is further complicated if more than two interacting factors are involved. In a number of weed species studied by Vincent and Roberts (1977), for example, factors such as pre-chilling, nitrate, temperature fluctuation and light in various combinations showed additive effects or positive interaction. Under field conditions, it is common for seeds to be exposed to several interacting factors. The response to these factors may be viewed as mechanisms which seeds use for regulating germination to a time and place best suited to ensure subsequent seedling development.

### 1.4.5 Dormancy regulation during seed development

In the previous sections on dormancy control, discussion was based on the influence of external factors. In many plant species it has been found that the level of primary dormancy is dependent to some degree, on the environmental conditions under which the mother plant develops. Day length during seed development on the mother plant, for example, affects subsequent seed germination behaviour in *C. album* (Karssen 1970), and *Portulaca oleracea* L. (common purslane) (Gutterman 1974). When seeds of these plants were matured under short days (8 hours) from flower bud formation, the germination was higher in comparison with seeds matured under long days (16 to 18 hours).
Variation in the parental photoperiod environment during seed maturation as well as the condition of the mother plant during flower induction has been shown to cause qualitative changes in the germination behaviour of seeds of *A. retroflexus*, but appears not to affect seed viability, seed weight or seed coat thickness (Kigel *et al.* 1977, 1979). Even within an inflorescence on one plant, there may be differences in dormancy arising from differences in day length during ripening (Gutterman 1982).

### 1.4.6 Other factors

The other environmental factors that are claimed to influence the changes in dormancy and germination are oxygen and carbon dioxide concentration and moisture (Schonbeck and Egley 1980a,b; 1981a). While there have been suggestions that reduced oxygen (Symons *et al.* 1987) and increased carbon dioxide affect dormancy in seeds, there has been little evidence of the effect of carbon dioxide levels in soil on changes in dormancy (Karssen 1980/81). During the course of one year Karssen (1980/81) did not observe great fluctuations in oxygen and carbon dioxide concentrations. Furthermore, the soil atmosphere within the zone of most weed seed germination (0 to 3 cm) is seldom oxygen deficient (Taylorson 1982). Hence, the influence of this factor on dormancy under soil conditions has not been clearly demonstrated. In the laboratory, carbon dioxide concentrations of 0 to 4.5% did not significantly influence germination in *A. retroflexus* (Schonbeck and Egley 1980a). Schonbeck and Egley (1981a, 1989), however, showed that removal of carbon dioxide from air reduced germination as compared to that obtained with normal air.

Although factors such as light and temperature are important in controlling dormancy and germination, the germination of all seeds is ultimately determined by the availability of water. Schonbeck and Egley (1980a) demonstrated that germination of *A. retroflexus* increased with increasing water potential.
1.5 Aims of the project

It is quite clear that a number of factors are involved in releasing seeds from dormancy. Although individual factors are more often investigated under laboratory conditions, it is evident that under field conditions several interacting factors are likely to be involved. This complicates our understanding of how dormancy is broken. However, various investigations from different workers have revealed that it is possible to manipulate some of the factors that control dormancy and germination.

The aim of the work reported in this thesis, therefore, was to examine hypotheses regarding dormancy breaking and to investigate how this information could be used to reduce weed seed populations in the soil by being able to manipulate their dormancy. Before the ultimate aim of manipulating dormancy in the field is achieved it is first necessary to understand the factors involved. Hence, the initial studies outlined in Chapters 2 and 3 were designed to investigate the effects of light and temperature on dormancy and germination of freshly harvested seeds of *A. retroflexus* as well as after dry storage. The work that followed (Chapter 4) involved examining changes in dormancy as well as the fate of seeds under natural conditions in the field. Having knowledge of the influence of some of the factors in dormancy breaking, it was necessary in the concluding work (Chapter 5) to evaluate the effects of chemical stimulants. All the results are finally integrated and discussed in Chapter 6.
CHAPTER 2

EFFECTS OF LIGHT AND TEMPERATURE ON BREAKING
DORMANCY AND GERMINATION

2.1 Introduction

The sporadic emergence of weed seedlings from soil containing dormant seeds is a formidable obstacle to efficient and lasting weed control. Weed control could be improved if reliable methods were available to break dormancy and induce synchronous germination thereby reducing seed populations in the soil (Saini et al. 1985b). Before such manipulations are possible, it is first necessary to understand the effects of various factors in breaking seed dormancy.

Several environmental factors such as light, temperature and moisture are known to control seed dormancy, and thus to promote or inhibit weed seed germination (Egley and Duke 1985; Taylorson 1987). In particular, the importance of light and temperature in regulating dormancy and germination has been commonly demonstrated (Steinbauer and Grigsby 1957; Toole 1973; Takaki et al. 1981). However, the requirement for these factors varies considerably between species (Singh and Achhireddy 1984; Jain and Singh 1989; Mekki and Leroux 1991; Reddy and Singh 1992).

Temperature plays an important role in dormancy breakage as well as in germination (Roberts 1988). It has been shown that in imbibed seeds low temperatures break dormancy while high temperatures induce it. In some species
(Totterdell and Roberts 1979). Although low temperatures may break dormancy, high temperatures are more often required for germination. Induction of secondary dormancy may occur if germination temperatures are unfavourable. In A. retroflexus, different workers have reported different germination temperatures. Steinbauer and Grigsby (1957) reported that 35°C was the optimum temperature. Weaver and McWilliams (1980) showed that A. retroflexus required a maximum of 30 to 40°C, while Bewley and Black (1985) noted that A. retroflexus germinated best at temperatures above 25°C. Being a summer annual, it is highly likely that temperature is implicated in regulating dormancy, but this has not been clearly demonstrated.

Many seeds germinate as well in darkness as in light such as Cirsium arvense (L.) Scop. (Canada thistle) (Wilson 1979), Morrenia odorata (H. and A.) Lindl. (milkweed) (Singh and Achhireddy 1984) and B. pilosa (Reddy and Singh 1992), while some others germinate poorly or not at all when deprived of light, such as, Scoparia dulcis L. (goatweed) (Jain and Singh 1989) and Matricaria maritima L. (false chamomile) (Mekki and Leroux 1991). In cultivated fields, sporadic emergence, after cultivation, has sometimes been attributed to exposure of seeds to light (Wesson and Wareing 1969a). However, the effects of light may either be promotive or inhibitory depending upon time of exposure or germination temperatures. For example, in B. pilosa light had a stimulatory effect on germination at 25/20 to 35/30°C day/night temperatures but was inhibitory on germination at 15/10°C (Reddy and Singh 1992).

In A. retroflexus seeds, short exposures to white light were found to be promotive while continuous illumination inhibited germination at 37°C (Kadman-Zahavi 1960). The light quantity needed for full stimulation was short illuminations of three seconds with 1000 foot candles (approximately 96 Wm⁻²) of white light. It was noted that the time required for complete germination of seeds which had received a short light stimulus depended upon incubation temperatures, for example,
complete germination was achieved 20 hours after illumination at 37°C, and 40 hours at 20°C. The effect of light on dormancy and germination of *A. retroflexus* seeds has also been tested by Schonbeck and Egley (1980a). However, only constant temperatures were used. Different seedlots were incubated in continuous light or in darkness at either 30 or 35°C for seven days. Seed of all lots showed a similar degree of light responsiveness and germinated to significantly higher percentages in the light than in the dark. Since it has been demonstrated that light effects depend on germination temperatures it is worthwhile to conduct tests at various temperatures.

Clearly, light and temperature do not operate singly under field conditions. They interact to produce an effective response, and as Karssen (1982) has reported, these interactions are involved in seasonal control of dormancy breakage. Thus, although some responses to light for *A. retroflexus* are known for constant temperatures, changes in dormancy under alternating or shifting temperature regimes have yet to be described.

The aim of this study was, therefore, to test the hypothesis that light and temperature control the breakage of dormancy and germination of *A. retroflexus* seeds. The effects of prolonged continuous illumination were examined on freshly harvested seeds incubated at different temperatures. During some period of the year weed seeds in the field may be exposed to cold temperatures. Thus, one experiment also examines the effect of pre-chilling temperatures on the breakage of dormancy and germination. The interactions between these two factors (light and temperature), and others in the termination of dormancy were examined in subsequent studies.
2.2 General materials and methods

2.2.1 Seed source and preparation

*A. retroflexus* seeds used in all experiments were from two seed sources collected in two consecutive years (May 1991 and May 1992) from plants grown in the horticultural fields of the University of Western Sydney, Hawkesbury, Richmond, New South Wales, Australia. The precise locations where the 1991 and 1992 plants were grown were slightly different due to cropping requirements of the land. The 1991 plants were located at 150° 44' 37"E and 33° 36' 42"S, while the 1992 plants were at 150° 44' 46"E and 33° 36' 36"S. The 1992 plants were generated from some of the seeds harvested in 1991.

For each collection, inflorescences were hand harvested and then air dried in a greenhouse for one week. The inflorescences were manually threshed by gentle rubbing between palms. After passing through a 1 mm sieve (Endecotts Ltd., London, England), entire black seeds were manually selected for use in all studies since it is known that both brown and damaged seeds have very low viability (Frost and Cavers 1975). Seed stocks were then stored at room temperature until assigned to treatments, or as otherwise stated.

2.2.2 Germination conditions

All laboratory germination experiments were performed in 9 cm diameter glass petri dishes lined with two layers of Whatman number 2 filter paper (Whatman International Ltd., Maidstone, England). The dishes were moistened with 5 ml of distilled water, unless otherwise specified, wrapped with Parafilm (American National Can, Greenwich, CT., U.S.A) to prevent moisture loss and then placed in an
environmental chamber programmed for the appropriate temperature and light regime. Additional water was applied at intervals as required.

Light was supplied by three 30 Watt cool white fluorescent lamps in programmable germination incubators (Thermoline Scientific Equipment Pty., Ltd., Smithfield, Australia) with internal dimensions of 111 x 50 x 36 cm, or in controlled temperature rooms. Total darkness treatments were achieved by wrapping Petri dishes with two layers of domestic grade aluminium foil.

Protrusion of the radicle was the criterion used to record germination success. Once counted, the germinated seeds were removed from the dish. For those treatments in continuous darkness, scoring was performed in a dark room under low intensity filtered green light.

2.2.3 Treatment of data

The data for germination were expressed as percentages of the numbers of seeds sown. Statistical analyses were conducted on arcsin transformed data by using analysis of variance routines in Genstat 5 (Payne et al. 1987). Arcsin transformation is regarded as an appropriate transformation for percentages arising from set numbers, as is the case for seeds set to germinate. However, since the distribution is discontinuous at zero and 100%, 100/4n was substituted for zero germination and 100 - 100/4n substituted for 100% germination, where n = initial number of seeds per replicate. In all cases a plot of residual values for raw and transformed data was examined to ensure that the transformation resulted in uniform distribution. Where analyses of variance indicated units had high residuals (p.311 Payne et al. 1987), these were individually examined and those units with values more than twice, or less than half the mean of the remaining treatment units were deleted and treated as
missing values when reanalysed. Unless stated, results throughout this thesis are presented as transformed data. Where appropriate, means of individual treatments were compared using a Least Significant Difference (Steele and Torrie 1960). Differences were declared significant if their probability of arising by chance was less than 0.05, unless otherwise specified.

2.3 Materials and methods

2.3.1 Experiment 1 - Effect of light and temperature on dormancy and germination

Seeds used in this experiment were collected in 1991 as described in Section 2.2.1. At the time of commencing this experiment the seeds had been stored dry at room temperature for one week. A completely randomised experimental design was used to test the germination response of *A. retroflexus* seeds to eight temperature regimes under continuous light or dark conditions. Seeds were germinated in Petri dishes (Section 2.2.2) with treatments consisting of four replicates of 150 seeds.

Treatments consisted of four constant temperatures of 12, 20, 35, and 40°C. Under these temperatures germination was assessed after six days and subsequently at four day intervals for four weeks. The temperature shift treatments consisted of incubation at one constant temperature for the first 14 days followed by incubation at a second constant temperature for a further 14 days. These temperatures were 15 then 20°C; 20 then 30°C; 12 then 35°C, and 20 then 35°C. These are denoted as 15+20°C, etc. Germination was assessed 14 days after the start of incubation at the first temperature. Subsequent counts, after transfer to the second temperature, were at four day intervals. Incubation at 12, 15 or 20°C either at the same or shifted temperature was in controlled temperature rooms. Final percent germination was
determined after 28 days from the start of incubation (the sum of total germination at the first temperature and that at the second temperature).

2.3.2 Experiment 2 - Effect of pre-chilling temperature and duration on dormancy and germination

Seeds used in this experiment were from the seed collection made in 1992 (Section 2.2.1). At the commencement of this experiment the seeds had been stored dry at 0°C for two months. A factorial experiment consisting of three pre-chilling temperatures (0, 12 and 20°C), five pre-chilling durations (1, 3, 6, 9 and 12 days), two illumination conditions (continuous light or darkness) and two germination temperatures (35 and 20/30°C) was conducted. The alternating temperatures were set for 12 hours per day at each temperature. Each treatment consisted of four replicates of 50 seeds. The seeds to be pre-chilled were placed on moistened filter papers in Petri dishes, and dark treatments wrapped with aluminium foil as in the germination test (Section 2.2.2). The dishes were then kept in temperature controlled rooms under the appropriate pre-chilling temperature. Seeds which were not pre-chilled were used as a control. Germinated seeds were counted after seven days of incubation.

2.4 Results

2.4.1 Light and temperature

Light significantly interacted with temperature (p<0.05) (Appendix Table 1) to stimulate the germination of fresh seed of *A. retroflexus*. Under constant temperatures light increased germination only at 35°C and had no effect at temperature shifts of 20+30°C or 20+35°C. At 12+35°C, light significantly
increased germination. The effects due to incubation temperatures were also found to be significant (p<0.001). Seeds of *A. retroflexus* did not germinate at 12°C or at a temperature shift of 15 followed by 20°C. Minimal germination occurred at 20°C (5%) and at 35°C in the light (16%) or at a temperature shift of 20 followed by 30°C (7%). At 40°C, a slight but significant increase in germination in light (19%) was observed. Germination was significantly increased when seeds experienced temperature shifts of 12 followed by 35°C (65%) and 20 followed by 35°C (66%) over that observed at constant temperatures (Fig. 2.1).

![Graph showing the effect of light and temperature on dormancy and germination of *A. retroflexus* seed. Seeds were incubated at constant or shifted temperatures either in continuous light or in the dark. Total germination at constant temperatures was recorded after 28 days. In the temperature shift treatment, seeds were incubated at the first temperature for 14 days and then transferred to the second temperature for another 14 days, denoted e.g., 12+15°C. Total germination at these temperatures was the sum of germination at the first and the second temperature. The vertical line indicates the least significant difference (LSD) at p = 0.05.](image)

**Figure 2.1** The effect of light and temperature on dormancy and germination of *A. retroflexus* seed. Seeds were incubated at constant or shifted temperatures either in continuous light or in the dark. Total germination at constant temperatures was recorded after 28 days. In the temperature shift treatment, seeds were incubated at the first temperature for 14 days and then transferred to the second temperature for another 14 days, denoted e.g., 12+15°C. Total germination at these temperatures was the sum of germination at the first and the second temperature. The vertical line indicates the least significant difference (LSD) at p = 0.05.
The rate of germination was considerably slower under constant temperatures, and more so in the dark than in the light (data not presented). Maximal germination occurred under temperature shifts. Flushes of germination were observed with the majority of seeds germinating within 14 days of incubation. At temperature shifts, germination was complete within 5 days after transfer of seeds from the first to the second temperature.

2.4.2 Pre-chilling

Breaking of dormancy and the stimulation of germination was significantly \( p < 0.001 \) affected by the interaction of pre-chilling temperature, duration of pre-chilling, incubation temperature and illumination condition (Appendix Table 2). Pre-chilling at 0°C for one day resulted in significantly higher germination percentage in the dark than in light when incubated at 35°C (Fig. 2.2a). Seeds pre-chilled for three and nine days at 0°C germinated to significantly higher percentages in the light than in darkness. When incubated at 20/30°C (Fig. 2.2b) germination of seeds pre-chilled for one, three and six days was higher in the dark. Generally, germination of seeds pre-chilled at 0°C was lower than that of non-pre-chilled ones.

Unlike pre-chilling at 0°C, seeds pre-chilled at 12°C germinated to significantly \( p < 0.001 \) higher percentages than the non-pre-chilled ones at 35°C. However, lower germination occurred in the light in one day pre-chilled seeds as compared with germination in the light of control seeds (Fig. 2.2c). Incubation in the light resulted in a significant increase in germination with increasing pre-chilling duration. Similarly, germination in the dark increased with increasing duration of pre-chilling except for seeds pre-chilled for six days (Fig. 2.2c). At 20/30°C incubation generally resulted in a lower or unchanged percentage germination both in the light and in darkness than that of control seeds except for germination in the light of seeds pre-chilled for nine days (Fig. 2.2d).
Pre-chilling at 20°C resulted in significantly (p<0.001) higher germination than that of the control at 35°C in the light. In the dark only those seeds pre-chilled for nine or 12 days germinated to significantly higher percentages than did control seeds (Fig. 2.2e). Incubation at 35°C in light resulted in an increase in germination with increasing pre-chilling duration. In the dark, a significant decline in germination occurred in seeds pre-chilled for three days. Germination increased thereafter to a maximum in those seeds pre-chilled for nine days.

Lower percent germination occurred both in the light and in the dark in seeds pre-chilled at 20°C and incubated at 20/30°C. Germination in the light was significantly higher than that in the dark only in seeds pre-chilled for six, nine or 12 days (Fig. 2.2f).

In general, seeds pre-chilled at 12°C germinated to higher percentages than did control seeds or those pre-chilled at either 0 or 20°C. Seeds pre-chilled at any of the three temperatures had a higher germination percentage when incubated at 35°C both in the light or dark than when incubated at 20/30°C (Fig. 2.2a, c, e versus b, d, f, respectively).

2.5 Discussion

2.5.1 Light and temperature

A high temperature requirement for germination of *A. retroflexus* has been reported by Kadman-Zahavi (1960), McWilliams *et al.* (1968), and Weaver and McWilliams (1980). In the present study, more seeds germinated at 35 and 40°C (16 and 19%) than at 20°C (5%). However, the germination was quite low, contrasting
Figure 2.2 The effect of pre-chilling on the germination of *A. retroflexus* seeds incubated at a constant temperature of 35°C or at an alternating temperature (20/30°C) in the light or in darkness. Germination was tested at a regular interval up to 12 days of pre-chilling at: (i) 0°C and incubated at (a) 35°C, (b) 20/30°C; (ii) 12°C and incubated at (c) 35°C, (d) 20/30°C; (iii) 20°C and incubated at (e) 35°C, (f) 20/30°C. Germination was recorded seven days after incubation. The vertical lines indicate the LSD at p = 0.05.
with the results of Schonbeck and Egley (1980a) who recorded 42 to 98% germination of *A. retroflexus* at 35°C after 5 days of incubation. Results from Kadman-Zahavi (1960) showed that seeds of *A. retroflexus* germinated to 70 to 90% at 37°C, depending on light intensity, 10 days after sowing.

Freshly harvested seeds were used in this experiment, and it has been reported that at maturity, many seeds of *A. retroflexus* are dormant (Baskin and Baskin 1977b). This may have accounted for the low total germination. Seeds used by Kadman-Zahavi (1960) had been stored at room temperature for an unknown period of time before use, and those used by Schonbeck and Egley (1980a) had been stored for a period of up to 8 years. Since changes in dormancy of *A. retroflexus* are known to occur in storage, after-ripening may have taken place, thus accounting for higher germination in those studies (Schonbeck and Egley 1980a).

*A. retroflexus* seeds germinated to significantly higher percentages at temperature shifts than at constant temperatures. An appreciable number of seeds germinated in the dark as well at these temperatures (Fig. 2.1). It is well documented that alternating temperature regimes or temperature shifts often break dormancy while constant temperature regimes have little or no effect (Morinaga 1926; Taylorson 1969; Koller 1972; Hegarty 1973; Totterdell & Roberts 1980). Koller and Negbi (1959) reported that alternating temperature or temperature shifts seem to eliminate any differences between the processes carried out in constant light and those carried out in constant darkness. They argued that when the temperature is alternating, a level of germination is achieved which is unaffected by constant light or constant darkness.

However, it is not clear by what mechanism the alternating temperature-light interaction acts in the breaking of dormancy. One possibility is that the changes in temperature are effective in connection with the action and preservation of
phytochrome (Bewley and Black 1982). These authors explained that the rate of dark reversion of phytochrome could be reduced by low temperatures, while its activity might be enhanced by higher temperatures. No clear evidence for or against this interpretation has been found.

Stimulation of germination by shifts from low to high temperatures bear some relation to what might be expected under field conditions. During late autumn and winter, seeds are exposed to cool temperatures which apparently break dormancy. These non-dormant seeds germinate best under higher temperatures, explaining why *A. retroflexus* do not germinate until late spring and early summer.

The results on the breakage of dormancy by light in seeds of *A. retroflexus* are in agreement with those of Kadman-Zahavi (1960), and Schonbeck and Egley (1980a). Germination would therefore be promoted by habitat disturbance such as ploughing that brings seeds to the soil surface. However, continuous light used in this experiment produced a less dramatic response; a result also noted by Schonbeck and Egley (1980a). Kadman-Zahavi (1960), and Taylorson and Hendricks (1971) found that a brief exposure to red light caused dormant *A. retroflexus* seeds to germinate to nearly 100%. Actual inhibition by continuous high intensity illumination has been reported by Kadman-Zahavi (1960). He found that under continuous illuminations there were two germination periods: a number of seeds germinated during the first day. This was followed by little additional germination between the first and second day. A second period of high germination followed (third and subsequent days). During this period germination rose to a peak at about 150 foot candles (approximately 14 Wm⁻²) and declined thereafter with increasing light intensity, suggesting that light had opposing effects - one stimulating, the other inhibiting germination.
The inhibitory effects of continuous illumination as suggested by Gorski and Gorska (1979) may be through the so called high-energy reaction that causes conformational change in phytochrome or through a continual displacement of phytochrome from its site of action.

The results obtained in this study may therefore reflect a combination of both stimulatory and inhibitory light effects such as might be experienced by a seed lying on the surface of the soil.

2.5.2 Pre-chilling

Dormancy of summer annuals is often broken by subjecting imbibed seeds to low temperatures (Baskin and Baskin 1977b; Karssen 1982). The effectiveness of pre-chilling on breaking dormancy is dependent on the duration of the low temperature treatment. Taylorson and Hendricks (1969) showed that dormancy of *A. retroflexus* is effectively broken by temperatures below 20°C, and they found that high levels of germination occurred in darkness after seeds were pre-chilled at 10°C for 144 hours.

In the present study, dormancy was broken more effectively in seeds pre-chilled at 12°C than in those pre-chilled at either 0 or 20°C. In fact, induction of dormancy occurred in those seeds that were pre-chilled at 0°C. Both breaking and induction of dormancy was quite evident, especially when seeds were incubated in the dark, and at 20/30°C. Totterdell and Roberts (1979) hypothesised that the loss of dormancy at low temperatures, of seeds of *R. obtusifolius* and *R. crispus* was a result of two processes: loss in primary dormancy; and induction of secondary dormancy. They reported that temperatures just above zero caused the most effective dormancy breakage, because the rate of induction of secondary dormancy was lowest at these
temperatures. As pre-chilling temperatures increase, the loss in dormancy becomes less and less pronounced. The lack of germination at 20°C and above in the case of *A. retroflexus*, as reported by Taylorson and Hendricks (1969), can be attributed to the occurrence of secondary dormancy.

The loss in dormancy in pre-chilled seeds was also influenced directly by germination temperatures. When seeds were incubated at 20/30°C induction of dormancy was apparent in seeds pre-chilled at any temperature. According to Taylorson (1982), most seeds influenced by pre-chilling require a high temperature for germination. Therefore, although dormancy may be broken by low temperatures, germination itself generally awaits higher temperatures.

The action of pre-chilling in the breaking of dormancy is not well understood. However, VanderWoude and Toole (1980), from their study of pre-chilling in *Lactuca sativa* L. (lettuce) seeds, proposed that a membrane transition may be involved. They reported that a change in membrane order and lipid composition reflected in a decrease in viscosity, may be responsible for the pre-chilling phenomena. More recently, Karssen *et al.* (1989) have shown that pre-chilling results in an increase in the seed’s responsiveness to endogenous gibberellins which are involved in breaking dormancy. They explained that changes in membrane structure and composition during chilling might increase the availability of gibberellin receptors.

It has been found, in this chapter, that light and temperature affect dormancy and germination of freshly harvested *A. retroflexus* seeds. However, not all the seeds may be exposed to these factors at the same time and, therefore, germination will await a later period. Changes in dormancy may occur while seeds await favourable conditions for breaking dormancy and inducing germination. In the next chapter, changes in dormancy in dry stored seeds of *A. retroflexus* were investigated.
CHAPTER 3

EFFECTS OF STORAGE TEMPERATURE AND DURATION ON DORMANCY AND GERMINATION

3.1 Introduction

Many of the seeds of *A. retroflexus*, like many other species, are dormant when harvested. When stored dry, physiological changes take place over time within seeds which result in the loss of dormancy. This process has been termed "after-ripening" (Bewley and Black 1982). Since weed seeds may be dispersed within the soil profile as well as to other locations, or they may be harvested together with crops, they may experience various storage conditions which affect dormancy.

The best known effects of after-ripening have been noted in cereals such as *Hordeum vulgare* L. (barley), *O. sativa* and *Triticum aestivum* L. (wheat), and other grasses such as *A. fatua* whose seeds lose dormancy during storage (Roberts and Smith 1977). Grass weed species as well as domesticated grains commonly exhibit dormancy manifested by the ability of the seeds to germinate over a limited temperature range (Taylorson and Brown 1977). This kind of dormancy is not only broken by a few days of pre-chilling, but is also lost by dry storage over a few months with the result that seeds germinate over a wider range of temperatures.

The effects of the temperature and time relationship on after-ripening have been examined in detail for only a few species, notably *O. sativa* and *H. vulgare* (E.H. Roberts 1962, 1965; Roberts and Smith 1977). It was demonstrated that higher temperatures during dry storage hastened loss of dormancy. Other workers have
shown that the duration and conditions of storage altered the germination responses of a number of species (Cavers 1974; Taylorson and Brown 1977).

This study was designed to investigate how dormancy changes during storage of *A. retroflexus* seeds. The effects of storage temperatures, duration of storage and incubation temperatures on dormancy and germination of *A. retroflexus* seeds were investigated. The objectives of this experiment were twofold: (i) to investigate changes in dormancy of seeds in dry storage for different periods under different temperatures; and (ii) to determine how these stored seeds respond to different germination temperatures.

3.2 Materials and methods

3.2.1 Long term storage

Small samples of *A. retroflexus* seeds were placed in air tight plastic containers and stored in temperature controlled rooms. A factorial design with five constant storage temperatures (0, 12, 20, 25 and 36°C), four storage durations (1, 3, 6 and 12 months), and six incubation temperature regimes was used. During the first 14 days seeds were incubated at 20, 25 or 35°C. Those seeds which had not germinated after 14 days incubation were later transferred to or held at 35°C for the next 14 days, hence, designated as 20+35, 25+35 and 35+35°C. Four replicates, each of 50 seeds from the 1991 seedlot (Section 2.2.1) were used for each treatment. Seeds were incubated under continuous light.
3.2.2 Short term storage

Similarly, seeds for this experiment were kept in air tight plastic containers and stored in temperature controlled rooms. A factorial design with five constant storage temperatures (0, 12, 20, 25, and 36°C), 13 storage durations (zero to 12 weeks) and six incubation temperatures (20, 25, 35, 12+35, 20+35 and 25+35°C) were used. Seeds were either incubated at constant temperatures (20, 25, 35°C) for a total period of 28 days or at 12, 20 and 25°C for 14 days prior to transferring them to 35°C for the next 14 days, (designated, e.g., 12+35°C). Germination was recorded after the first 14 days of incubation and from day 14 to day 28 of incubation. Total germination, 28 days from the start of incubation, was the sum of germination after the first 14 days and that from day 14 to 28 of incubation. Seeds were incubated under continuous light. Three replicates, each of 50 seeds from the 1992 seedlot (Section 2.2.1) were used for each treatment.

3.2.3 Treatment of data

The data for percent germination were arcsin transformed (Section 2.2.3). Separate analyses were conducted (Section 2.2.3) for individual storage durations. Residual variances from each storage duration were analysed using Bartlett's test of homogeneity (Bartlett 1937). Where variances were found to be homogeneous, a combined analysis of variance was undertaken to test for the interaction of treatments over time. All analyses were separated into: (i) germination after the first 14 days incubation; (ii) germination during the second period (from day 14 to 28); and (iii) total germination from the start of incubation.
3.3 Results

3.3.1 Long term storage

Results from the Bartlett’s tests indicated non-homogeneous variance for 0 to 14 days ($\chi^2$ on 3 d.f. = 23.1 ($p < 0.001$)), homogeneous variance for 14 to 28 days ($\chi^2$ on 3 d.f. = 1.2 ($p > 0.05$)) and homogeneous variance for total germination ($\chi^2$ on 3 d.f. = 1.0 ($p > 0.05$)). Hence results for separate analyses are presented for 0 to 14 days and results for combined analyses are given for 14 to 28 days and for total germination.

After the first 14 days incubation of seeds which had been stored at different temperatures, the effects of storage and incubation temperatures depended upon the duration of storage. Storage temperatures had significant ($p < 0.001$) effects in seeds stored at all durations (Appendix Tables 3 to 6). When stored at any temperature seeds after-ripened to some degree. The rate and degree of after-ripening increased with increasing storage temperature. Regardless of incubation temperature or duration of storage, seeds stored at 36°C germinated to higher percentages than those stored at lower temperatures (Fig. 3.1a to c). Significant ($p < 0.001$) effects due to incubation temperature were noted in seeds stored at all durations except those stored for six months. Seeds generally germinated to higher percentages with increasing incubation temperature. Only those seeds stored for 12 months showed some interactions between storage and incubation temperature (Appendix Table 6). At lower storage temperature, germination increased significantly ($p < 0.001$) with increasing incubation temperature. As storage temperature increased, seeds germinated equally well at lower temperature.
Figure 3.1 Germination after the first 14 days incubation of *A. retroflexus* seeds stored between 1 and 12 months. Seeds were stored at different temperatures, and after a period of 1, 3, 6 and 12 months four replicates each of 50 seeds were taken from each storage temperature and tested for germination in the light at (a) 20°C; 25°C; (c) 35°C. The vertical lines indicate the LSD at $p = 0.05$. (Analyses not combined).
Significant interactions (p<0.001) between storage temperatures, incubation temperatures and duration of storage were found after seeds were transferred from 20 or 25°C to 35°C, or held at 35°C for the next 14 days of incubation (Appendix Table 7). Incubation of seeds at 20+35°C gave the highest germination (Fig.3.2a) while those held at 35°C (Fig. 3.2c) germinated the least.

The effect of duration of storage during this second germination period depended on both storage and incubation temperature. When incubated at 20+35°C (Fig. 3.2a) germination of seeds stored at 0 and 12°C increased significantly (p<0.01) with increasing duration of storage. Storage at 20, 25 and 36°C resulted in increasing germination with duration of storage up to three months. Thereafter, duration did not affect germination (Fig. 3.2a). Incubation at 25+35°C and 35+35°C resulted in a significant decline in germination in seeds stored for 12 months.

Storage temperature, storage duration and their interaction, as well as the interaction between storage duration and incubation temperature were found to significantly (p<0.001) affect total germination (Appendix Table 8). Germination increased with increasing storage temperature and duration of storage. However, germination of seeds stored for a period of six or 12 months did not differ from each other, regardless of the incubation and storage temperatures (Fig. 3.3a to c). Incubation temperature did not affect the total germination of A. retroflexus seeds.

3.3.2 Short term storage

Results from the Bartlett’s tests indicated homogeneous variance for 0 to 14 days ($\chi^2$ on 14 d.f. = 12.43 (p>0.05)), non-homogeneous variance for 14 to 28 days ($\chi^2$ on 14 d.f. = 55.72 (p<0.001)) and non-homogeneous variance for total germination ($\chi^2$ on 14 d.f. = 46.39 (p<0.001)).
Figure 3.2 Germination from day 14 to 28 of incubation of *A. retroflexus* seeds stored between 1 and 12 months. Seeds were stored at different temperatures, and after a period of 1, 3, 6 and 12 months they were tested for germination in the light. This germination period was after the seeds had been transferred to the second temperature, i.e., (a) from 20 to 35°C; (b) from 25 to 35°C; (c) seeds held at 35°C for the next 14 days. The vertical lines indicate the LSD (p=0.05). (Combined analyses).
Figure 3.3 Total germination 28 days from the start of incubation of *A. retroflexus* seeds stored between 1 and 12 months. (a) germination after 24 days at 20°C + 14 days after transfer to 35°C; (b) germination after 14 days at 25°C + 14 days after transfer to 35°C; (c) germination after the first 14 days at 35°C + the second 14 days at the same temperature. The vertical lines indicate the LSD (p=0.05). (Combined analyses).
Hence results of a combined analysis are presented for 0 to 14 days and results of separate analyses are given for 14 to 28 days and for total germination.

After the first 14 days incubation, significant ($p<0.001$) interaction occurred between storage temperature, incubation temperature and the duration of storage (Appendix Table 9). Storage temperature significantly affected dormancy and germination of *A. retroflexus* seed, indicating that seeds stored at all temperatures after-ripened to some degree. Germination increased with an increase in storage temperature. Seeds stored at 36°C had the highest germination and those stored at 0°C the lowest, regardless of germination temperature or duration (Fig. 3.4).

Significant effects due to incubation temperature occurred during the first 14 days of incubation. Germination was highest at 35°C and declined as temperature decreased, being the least in the 12+35°C treatment (effectively 12°C for this period) (Fig. 3.4a to f). Germination of seeds in the 20+35°C and 25+35°C treatments (effectively 20 and 25°C, respectively, for this period) was similar to those designated to the constant temperatures of 20 and 25°C (Fig. 3.4a and b compared with 3.4e and f), respectively.

When incubated at 20°C, few seeds stored at 0 and 12°C germinated, even after 12 weeks of storage (Fig. 3.4a). Significantly ($p<0.001$) more seeds germinated after being stored at 20 or 25°C for 10 or 6 weeks, respectively. Seeds stored at 36°C showed significantly higher germination as early as the third week of storage. At the 35°C incubation temperature, more germination occurred after short periods of storage (Fig. 3.4c). In general, germination increased when seeds were subjected to longer periods of storage. Increased storage duration, as well, promoted germination at lower incubation temperatures.
Figure 3.4 Germination after the first 14 days incubation of *A. retroflexus* seeds stored for up to 12 weeks. Seeds were stored at different temperatures and durations. Every week, three replicates each of 50 seeds were taken from each storage temperature and tested for germination in the light at (a) 20°C; (b) 25°C; (c) 35°C; (d) 12+35°C; (e) 20+35°C and (f) 25+35°C. 12+35°C, for example, denotes temperature shift of 14 days at 12°C followed by 14 days at 35°C. The vertical lines indicate the LSD (p=0.05) for the combined analyses.
A separate analysis was done for the second germination period (from day 14 to 28). This was the period after the seeds had been transferred to the second incubation temperature for the temperature shift treatments. All treatments (storage temperature, incubation temperature and their interaction) still showed significant effects (Appendix Table 10). [It should be noted that the appendix table shown herein is for seeds stored for one week. Since the variances were non-homogenous according to Bartlett’s test (Section 3.2.3) 13 such tables, one for each storage duration, should be presented. However, only one has been shown as an example, and the rest could be presented on request]. During this period, seeds stored at higher temperatures again mostly resulted in the highest germination. Germination percentage increased with increasing duration of storage up to the seventh week. Thereafter, there was a decrease, showing that with longer storage time seeds take a shorter period of time to germinate. In this period, seeds incubated at temperature shifts (Fig. 3.5d to f) had significantly higher germination percentages than those incubated at constant temperatures (Fig. 3.5a to c), although germination declined as the incubation temperature imposed during the initial period increased (Fig. 3.5d to f).

The above trends were also evident for total germination (i.e., after 28 days incubation) (Fig. 3.6). Significant interactions (p<0.001) between storage temperatures and incubation temperatures were found (Appendix Table 11). [This is again the analysis of variance table for seeds stored for one week.] Germination increased with increasing storage temperature as well as duration. The highest germination was observed when seeds experienced temperature shifts. At 12±3°C, even those seeds stored at 0°C showed some appreciable germination (Fig. 3.6d). With longer storage duration more seeds stored at lower temperatures were able to germinate.
Figure 3.5 Germination from day 14 to 28 of incubation of *A. retroflexus* seeds stored and tested at a weekly interval for 12 weeks. Seeds were stored at different temperatures and durations. Germination data was recorded after the second period at (a) 20°C; (b) 25°C (c) 35°C; (d) 12+35°C; (e) 20+35°C; (f) 25+35°C. At this period (Figure 3.5d-f) seeds hab been transferred to the second temperature in temperature shift germination treatments. The vertical lines indicate LSD (p=0.05) for each storage duration. (Analyses not combined).
Figure 3.6 Total germination after 28 days from the start of incubation of *A. retroflexus* seeds stored and tested at a weekly interval for 12 weeks. This was the sum of germination after 14 days (Fig. 3.4) and that after the second germination period from da 14 to 28 (Fig. 3.5) at (a) 20°C; (b) 25°C (c) 35°C. Total germination of seeds subjected to temperature shift was the sum of germination at the first temperature for 14 days and that after transfer to the second temperature, *i.e.* (d) 12+35°C; (e) 20+35°C and (f) 25+35°C. The vertical lines indicate the LSD (p=0.05) for each storage duration. (Analyses not combined).
3.4 Discussion

Storage temperature and duration of storage significantly affected dormancy of *A. retroflexus* seeds and germination occurred at a wider range of temperatures once dormancy had been broken. Percentage germination increased with increasing storage temperature of from 0 to 36°C (Fig. 3.1 to 3.6). These results agree with the observations that the dormancy breaking process is temperature and time dependent as reported for after-ripening in cereals and grass weeds (Roberts 1965; Paterson *et al.* 1976; Taylorson and Brown 1977).

Heat pre-treatment, even as little as a few days, is sufficient to break seed dormancy in some species (Rees 1961; Graves *et al.* 1975), whereas low temperatures retard or greatly prevent the after-ripening process. For example, storage of *Euphorbia heterophylla* L. (wild poinsettia) seed at 36°C resulted in nearly 100% germination in darkness at 25°C while samples from the same seedlot stored at 5°C for 12 weeks failed to germinate (Bannon *et al.* 1978). *R. crispus* seeds stored for five years at 2 to 4°C resulted in no appreciable change in dormancy (Cavers 1974); on the other hand, *R. obtusifolius* was affected by storage for nine months at 1.5°C (Totterdell and Roberts 1979).

The present results are similar to those presented by Schonbeck and Egley (1980a), who found that seeds of *A. retroflexus* were less dormant after two months storage at 24 to 28°C, while at 0 to 5°C after-ripening proceeded much more slowly as might be expected based on the observations outlined above for other species. After six months at 0 to 5°C, however, a slight but consistent increase in germination was recorded.

In general, loss of dormancy by *A. retroflexus* seeds followed the pattern described by Cavers (1974) for *R. crispus*. He found that most seeds would
germinate under favourable temperatures, and under less favourable temperature regimes germination increased with increasing periods of dry storage. Fresh seeds of *A. retroflexus* and *A. powellii*, only germinated at temperatures above 35°C. With increasing periods of dry storage, more seeds germinated at progressively lower temperatures (Frost 1971). Similarly, Derksen (1993) found that dry storage for 15 months increased the response of *Arabidopsis thaliana* L. (mouse ear cress) seeds to applied gibberellins in darkness. Whereas the response to a single red light irradiation was absent in freshly harvested seeds, dry stored seeds were highly responsive.

A secondary effect of the after-ripening process is its ability to bring seeds from a relatively deep dormant to successively less dormant states as the duration of treatment increases (Taylorson and Brown 1977). Data from both the long term and short term storage experiments indicate that storage at 36°C for an appropriate period of time, increases the range of environmental conditions over which *A. retroflexus* would germinate. That is, dry, warm conditions seemingly modified the seed for rapid germination under low temperatures. Under low storage temperatures, a longer period of time was required to break dormancy.

Although 36°C was an appropriate temperature for breaking dormancy of stored seeds, the duration required for an effective response varied with the seedlot. For example, germination of the 1992 seedlot (short term storage experiment) stored at 36°C and incubated at 35°C was almost complete by the third month of storage. On the other hand, even after 12 months storage the 1991 seedlot germinated to only 65%. It is therefore difficult to generalize by suggesting a single after-ripening treatment be employed for *A. retroflexus* seed. Considerable seedlot to seedlot variation in relative dormancy is commonly encountered within a given weed species, and arises chiefly from the response of the genotype to its environment during maturation of the seed (Kigel et al. 1977; Taylorson 1977 and Fenner 1991). In *A. retroflexus*, changes in the parental photoperiodic environment caused quantitative
changes in the germination of the resulting seeds by affecting their responsiveness to promotive treatments such as (a) pre-chilling or short illumination, and (b) the rate at which primary dormancy was lost in storage (Kigel et al. 1977).

These experiments showed that both the source of seeds and the conditions under which they were stored have a profound effects on the germination response of *A. retroflexus* seeds.

The mechanisms involved in breaking dormancy due to the effects of after-ripening are not fully understood. One possibility proposed by Wareing and Saunders (1971) is the loss of an inhibitor that initially prevents the seed from responding to light or other stimuli. Other experiments by Karssen et al. (1983) on *A. thaliana* and by Groot and Karssen (1992) on *Lycopersicon esculentum* Mill. (tomato) seeds clearly indicated that abscisic acid (ABA) may play a role in the induction of dormancy. Groot and Karssen (1992) demonstrated that the level of dormancy of wild-type seeds of *L. esculentum* decreased during several weeks of dry storage. When these seeds were incubated immediately after harvest, only a few germinated. Eight weeks of dry storage at 7°C induced germination. It was shown that the level of ABA was reduced considerably after long periods of dry storage; probably accounting for loss in dormancy. In the same study, Groot and Karssen (1992) found that ABA-deficient *L. esculentum* seeds lacked dormancy and germinated directly after harvest. They concluded that endogenous ABA plays an important role in the induction of dormancy during seed development. However, it is unknown whether or not such loss in endogenous ABA occurs in *A. retroflexus* seeds during after-ripening.

In this chapter and the previous one, it has been shown that under controlled environmental conditions, dormancy and germination of *A. retroflexus* seeds is affected by light and temperature treatments imposed under laboratory conditions.
The fate of seeds and changes in dormancy under more natural storage conditions in the field are reported in the next chapter.
CHAPTER 4

CHANGES IN DORMANCY AND VIABILITY AS AFFECTED BY DEPTH AND DURATION OF BURIAL

4.1 Introduction

Buried weed seeds are a perpetual concern to agriculturalists. These seeds are distributed within the surface layers of soil principally by tillage implements. They can eventually germinate and the resulting plants compete with crops for water, nutrients and light. Information on seed viability and longevity in the soil is thus important since potential weed problems exist as long as weed seeds remain viable. Various studies on buried seeds have been conducted to determine their longevity in the soil. The best known studies were the classical burial trials initiated by Beal (Darlington and Steinbaur 1961) in 1897 in Michigan (USA) and by Duvel in 1902 in Virginia (USA) (Toole 1946). In Beal’s study, for example, some weed species such as Verbascum blattaria L. (moth mullein) and Verbascum thapsus L. (great mullein) were still viable after 100 years (Kivilaan and Bandurski 1981). More recently, 50-year studies have been started in Stoneville, USA (Egley and Chandler 1983) and in sub-arctic Alaska (USA) (Conn 1990). Such studies indicate the potential longevity of seeds and, in the absence of further seed inputs, foreshadow the longevity of seed banks.

Seed longevity in the soil may be influenced by several factors. In general, greater seed longevity is favoured by deep burial (Taylorson 1970; Stoller and Wax 1974; Zorner et al. 1984b). Seeds disseminated in the soil environment are exposed
to a number of variables such as light, temperature, moisture and the gaseous environment which often interact to varying degrees. Depending upon the location of the seeds in the soil profile, exposure to these variables differs considerably and thus, may contribute to differences in viability, dormancy breaking and germination. Seasonal changes in these variables may likewise affect dormancy and germination of buried seeds.

Some of the first direct evidence for changes in germination responses of buried seeds came from studies by Schafer and Chilcote (1970), and Taylorson (1970). They buried seeds in soil outdoors, exhumed and tested samples for germination at regular intervals. These authors showed that buried seeds go through dormancy/non-dormancy cycles. Since 1970, the existence of dormancy/non-dormancy cycles in buried seeds of annual plants has been more widely documented and there have been attempts to explain the mechanism(s) causing cyclical dormancy in seeds (Baskin and Baskin 1977b; 1980; 1981a; b; Karssen 1982; Bouwmeester 1990).

In the previous two chapters, it was shown that under controlled conditions factors such as light and temperature play important roles in the control of dormancy and germination. The studies described in this chapter were undertaken to investigate the effects of depth and duration of burial on dormancy and germination of *A. retroflexus* seeds under seasonal fluctuations of temperature and light intensity with the objective of determining changes in relative dormancy and longevity during one year of study.
4.2 Materials and methods

4.2.1 Experimental design

Seeds of *A. retroflexus* were buried at four depths in soil within pots which were in turn buried in the field and recovered for testing after being buried for 1, 3, 6, 9 and 12 months. The pots were arranged in a split plot design with four replications. Pots containing seeds buried at like depths were grouped to form the main plots. Sub plots consisted of the five replicate pots corresponding to the five durations of burial. These were arranged randomly within each main plot.

4.2.2 Seed preparation and burial in the field

Seeds which had been harvested in the autumn of 1991 and stored at room temperature for a period of 6 months were used for the experiment. Fifty seeds were enclosed separately in 10 by 10 cm bags made from multifilament printing screen material (Edman Wilson & Co. Pty. Ltd. Artarmon, Australia). The mesh was small enough to retain the seeds yet still allow the passage of water, gases and microorganisms. The bags were presumed to be non-inhibitory to germination since Egley and Chandler (1978) demonstrated that direct contact of seeds with polyethylene screen during incubation in petri dishes did not affect germination.

On 30th November 1991, single screen bags containing the seeds were placed in individual pots, either on the soil surface or buried at depths of 2.5, 5 and 10 cm in steam sterilised soil. The pots had eight holes (1.5 by 1.5 cm) at the lateral side near the base. The purpose of sterilising the soil was to prevent growth from any contaminating seeds, thus eliminating the possibility of roots entangling the mesh
bags, making sampling difficult. The pots were then buried in a ploughed field within the horticultural grounds of the university at 105° 44' 46"E and 33° 36' 36"S.

4.2.3 Seed retrieval and germination test

The seed bags were retrieved from the soil after being buried for 1, 3, 6, 9 and 12 months. At each sampling time four pots for each depth of burial (one from each block) were removed, taking care not to disturb neighbouring pots. The recovered bags were transferred to the laboratory and washed under running tap water. They were then opened and the number of seeds which had deteriorated or germinated was recorded. Intact, non-germinated seeds from each bag were transferred to 9 cm diameter glass petri dishes for germination tests. Seeds were incubated at 12°C for seven days in the dark and then transferred to 35°C for 14 days in the light after which the number of germinated seeds was recorded. Non-germinated seeds were treated with 100 μl/L Ethrel® (480 g/L ethephon) (May and Baker Rural Pty., Ltd., NSW, Australia) and seeds incubated for another seven days at 35°C in light. The number of germinated seeds was again recorded. Seeds that did not germinate after treatment with ethephon were considered non-viable.

The total number of viable seeds consisted of the sum of the seeds that had germinated in the field, plus the seeds germinated in the laboratory before and after treatment with ethephon.

Air temperature and that at 10 cm depth over the one year period of this study was obtained from the meteorological station at the university approximately one km from the field site.
4.2.4 Data treatment

Data from the four replicates for each recovery date and depth of burial were analysed after arcsin transformation (Section 2.2.3). In order to examine the trends in germination, orthogonal components were used to separate linear and quadratic responses of depth of burial and duration of burial main effects and of the depth by duration interaction. Separate analyses were performed for: (a) germination in the field; (b) germination in the laboratory; (c) germination after treatment with ethephon; (d) germination of non-physiologically dormant seeds (i.e., a+b above) and (e) total germination (viability) (i.e., a+b+c above).

4.3 Results

4.3.1 In situ germination

Significant effects (p<0.001) due to depth of burial were found in in situ germination of A. retroflexus seeds (Appendix Table 12). Germination increased with decreasing depth of burial regardless of the duration of burial. Seeds on the soil surface germinated to significantly higher percentages than those buried at depth and seeds buried at 10 cm gave the least germination (Fig. 4.1). These responses had both significant linear (p<0.001) and quadratic (p<0.001) components, indicating a diminishing trend with increasing depth.

The effect of duration of burial on in situ germination was significant at p<0.05 (Table 4.1). Seeds buried for a period of six months germinated to significantly higher percentages (p = 0.05), giving rise to a significant (p<0.001) quadratic effect (Appendix Table 12). There were also some significant components
of the depth of burial by duration of burial interaction because there was little or no germination in situ at the 5 and 10 cm depths until the sixth month of burial.

![Graph showing germination percentage over time with different burial depths.](image)

**Figure 4.1** *In situ* germination of buried *A. retroflexus* seeds. Seeds in mesh bags were placed in pots at various depths and buried in the field. Four pots representing four replicates of each depth of burial were retrieved at intervals over 12 months. The number of seeds that had germinated before retrieval were determined. The vertical line indicates the LSD at $p=0.05$

4.3.2 Germination in the laboratory

Significant differences ($p<0.001$) due to depth and duration of burial were observed in laboratory germination results (Appendix Table 13). Unlike germination in situ, germination of seeds in the laboratory increased directly with depth of burial (Fig. 4.2). This was true for all burial durations. However, after 1 and 3 months burial, no differences in germination were observed between seeds buried at 5 and those buried at 10 cm; and after 12 months, no differences in germination were found between seeds on the soil surface and those buried at 2.5 and 5 cm.
Table 4.1 Mean of in situ germination of *A. retroflexus* seed after varying durations of burial. Values followed by the same letter are not significantly different using an LSD (p=0.05) = 2.4.

<table>
<thead>
<tr>
<th>Duration of burial (months)</th>
<th>% germination (arcsin transformed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11.7b</td>
</tr>
<tr>
<td>3</td>
<td>13.0ab</td>
</tr>
<tr>
<td>6</td>
<td>15.3a</td>
</tr>
<tr>
<td>9</td>
<td>14.0ab</td>
</tr>
<tr>
<td>12</td>
<td>12.5b</td>
</tr>
</tbody>
</table>

Figure 4.2 Germination in the laboratory of *A. retroflexus* seeds retrieved from the field. Seeds in mesh bags were placed in plastic pots at various depths and buried in the field. Four pots representing four replicates for each depth of burial were retrieved at regular intervals over 12 months. Intact non-germinated seeds were subjected to germination test at a temperature shift of 12°C in the dark for seven days followed by 35°C in the light for 14 days. The vertical line indicates the LSD at p=0.05.
Seeds of *A. retroflexus* experienced changes in dormancy and germination during a one year burial duration. Burial for three months resulted in a significant decline in germination. This was followed by an increase in germination to a maximum at nine months burial. Germination declined again by 12 months burial.

4.3.3 *Germination of ethephon treated seeds*

Following treatment with ethephon, differences in germination due to depth of burial were found to be significant (*p* < 0.05) (Appendix Table 14). The highest germination occurred for seeds on the soil surface (Fig. 4.3). A significant linear component (*p* < 0.001) was found in germination at different depths. Germination of seeds at 10 cm was significantly lower than those on the soil surface or buried at 2.5 or 5 cm. At these three shallower depths no differences in germination were observed. Significant differences due to duration of burial were also evident. The least germination was in seeds buried for 9 months, and the highest after 3 months burial (Fig. 4.3). As with the time trends in laboratory germinated seeds, these fluctuations in germination due to the duration of burial resulted in significant deviations from the linear and quadratic model.

Comparison between these results and the previous ones (Section 4.3.2) show that seeds that did not germinate well without treatment (*e.g.*, those at shallow depths), germinated to significantly higher percentages after treatment with ethephon. Most of the seeds buried more deeply, on the other hand, germinated before treatment.
4.3.4 Non-physiologically dormant seeds

The non-physiologically dormant seeds included those seeds that germinated *in situ* plus those that germinated in the laboratory before treatment with ethephon. Statistical analysis showed that there were significant differences ($p < 0.001$) due to depth of burial as well as to duration of burial, but no interaction occurred between the two (Appendix Table 15).

![Figure 4.3](image.png)

*Figure 4.3* Germination of retrieved *A. retroflexus* seeds after treatment with ethephon. Seeds that did not germinate when subjected to germination test (Fig. 4.2) were treated with 100μL/L Ethrel$^\text{®}$ and incubated at 35°C in the light for a period of seven days. The vertical line indicates the LSD at $p=0.05$. 
Germination increased with increasing depth of burial with a significant (p<0.001) linear component (Fig. 4.4). Over the burial duration, seeds experienced changes in germination (Section 4.2.2). Seeds buried for three months showed a decline in germination. This was followed by increases up to a maximum in seeds buried for nine months. Germination decreased thereafter. Again, these fluctuating responses resulted in significant (p<0.001) deviation from the linear and quadratic model.

![Graph showing germination percentage vs. burial duration](image)

**Figure 4.4** Germination of non-physiologically dormant seeds of *A. retroflexus*. Seeds in mesh bags were placed in pots at various depths and buried in the field. Four pots representing four replicates of each depth of burial were retrieved at intervals over 12 months and tested for germination in the laboratory. Non-physiologically dormant seeds represented the sum of seeds that germinated *in situ* and those that germinated in the laboratory before treatment with Ethrel. The vertical line indicates LSD at p=0.05.
4.3.5 Total germination (Viability)

Significant differences (p<0.001) due to depth and duration of burial were found, but the interaction between these two factors was not significant. Viability declined with time, but was consistently higher for deeply buried seeds with a significant linear component (p<0.001) (Fig. 4.5; Appendix Table 16). Differences in viability at differing burial depths varied with time. After one month burial, for example, only those seeds buried at 10 cm maintained the highest viability. Thereafter, there was no difference in loss in viability between seeds placed on the soil surface and those buried at 2.5 cm and between burial at 5 cm and that at 10 cm.

![Graph showing total germination of A. retroflexus seeds after burial.](image)

**Figure 4.5** Total germination of *A. retroflexus* seeds after burial. Seeds in mesh bags were placed in pots at various depths and buried in the field. Four pots representing four replicates of each depth of burial were retrieved at interval and tested for germination in the laboratory. Total germination represents the sum of seeds that germinated *in situ* and those that germinated in the laboratory before and after treatment with Ethrel. The vertical line indicates LSD at p=0.05.
There was, however, considerable variability in the loss of viability over time indicated by the significant deviations from the linear and quadratic model (Appendix Table 16).

4.4 Discussion

4.4.1 In situ germination

Seeds placed at the surface gave the highest in situ germination (Fig. 4.1). Wesson and Wareing (1967) proposed that seed germination in soil was relative to the requirement for light. Exposure to light seems to be of major importance in the stimulation of germination at the surface (Roberts 1972; Wesson and Wareing 1969a,b). Seeds on the surface also experience wide daily fluctuations in temperature which may be responsible for after-ripening or pre-chilling, and hence, breaking of dormancy which allows germination. According to Wesson and Wareing (1967), seeds that do not germinate in the field probably exhibit a type of dormancy that is broken by factors other than temperature and light. They argued that such seeds express enforced dormancy in the burial site to be able to survive for a longer period.

At deeper depths, in situ germination was quite low. The reason for inhibition of germination at these depths may be two fold. The soil may either: (i) lack one of the stimulatory factors required for germination, or (ii) contain certain inhibitory factors. It is evident that both light and the amplitude of the diurnal temperature fluctuations diminish with increasing depth. In the present study only small differences were found between mean monthly air and soil temperatures at 10 cm depth, particularly during the declining phase. Therefore, factors other than temperature were probably responsible for the observed differences in in situ germination. One factor could be light since Woolley and Stoller (1978) found, for
example, that less than 1% of incident light penetrates further than 2.2 mm in clay, loam or sand. Harper (1957) suggested either decreased oxygen or increased carbon dioxide concentration as the effective factor inhibiting germination at deeper depths. However, Karssen (1980/81) was unable to confirm these suggestions. Other factors involved in the inhibition may be the volatile components in the soil atmosphere as indicated in studies of different species (Wesson and Wareing 1969b; Holm 1972).

4.4.2 Germination in the laboratory

Seeds buried at depth germinated to significantly higher percentages in the laboratory (without ethephon) than those placed on the surface or shallowly buried (Fig. 4.2). This suggests that germination in the field of deeply buried seeds was not possible due to secondary dormancy which was broken when favourable germination conditions were provided in the laboratory. At shallow depths, seeds that did not germinate in situ acquired a type of dormancy that could not be broken by laboratory conditions similar to those provided to deeply buried seeds. These seeds did not germinate readily without the dormancy breaking treatment.

4.4.3 Seed viability

Total viability of seeds buried in the soil decreased with time (Fig. 4.5), and depth of placement markedly influenced its loss. Shallowly placed seeds lost viability more quickly than did deeply buried seeds (Fig. 4.5). These results are in agreement with those of other workers, such as Taylorson (1970), Stoller and Wax (1974), Cheam (1987), Conn and Farris (1987). Seeds of Achillea millefolium L. (common yarrow), which are positively photoblastic lost viability more slowly when buried (Kannangara and Field 1985). Taylorson (1970) found that very few E. crus-galli
seeds survived one year in the soil at the 2.5 cm depth, but many survived at 15 cm depth. One reason for the rapid decline in viability at shallower depths is that seeds near or at the soil surface are exposed to the most variable and extreme environmental conditions which could promote metabolic failure (Taylorson 1970; Stoller and Wax 1974).

Contrary to these results Egley and Chandler (1978) found no differences in the loss of *A. retroflexus* seed viability due to depth of burial. Burnside *et al.* (1981), on the other hand, exhumed and germinated seeds of 12 economic weed species buried beneath 23 cm of soil and found that germination in only *A. retroflexus* and *Panicum dichotomiflorum* Michx (fall panicum) did not drop significantly over the 10-year burial period. By using a hyperbolic function to determine the duration of time required for a minimum level of germination to be approached, these authors predicted that *A. retroflexus* seed germination would reach 1% after 155 years of burial.

### 4.4.4 Dormancy changes

Baskin and Baskin (1977b) and Karssen (1982) found that the degree of dormancy of some weed seeds in the soil may vary with season. During the one year burial period used in the present study, fluctuations in germination of *A. retroflexus* seeds were evident. Seeds germinated to higher percentages when retrieved after a cold period of the year and germination declined as warmer periods were encountered. This was only true for non-physiologically dormant seeds (*i.e.*, the sum of the seeds that germinated *in situ* and those that germinated in the laboratory without being induced chemically) (Fig. 4.6a). Hence, it represents a fraction of seeds that responded to environmental conditions, in this case, temperature changes. This decline in germination during warm periods showed that seeds became secondarily
dormant under these conditions. *A. retroflexus* seeds are known to become secondarily dormant when high temperatures persist (Schonbeck and Egley 1981b). The reverse response was observed in seeds treated with Ethrel® (Fig. 4.6b).

**Figure 4.6** Germination of *A. retroflexus* seeds in relation to seasonal fluctuations in temperature during the burial experiment: (a) non-physiologically dormant seeds; (b) seeds treated with ethrel in the laboratory after retrieval from the field. The vertical lines indicate the LSD for germination at $p=0.05$. 

Seeds retrieved during warm periods responded better to treatments with ethephon (Fig. 4.6b). This was the dormant portion of the seeds that did not germinate in situ or in the laboratory, and hence required another form of treatment. These results agree with the typical dormancy cycles reported for summer annual species (Baskin and Baskin 1977; Karssen 1982). In such species, seeds become non-dormant during winter and germinate in spring or early summer, given favourable conditions. Seeds that fail to germinate re-enter dormancy in summer and may become non-dormant the following winter.

From information on changes in dormancy status, it thus appears possible to predict when exhumed seeds of A. retroflexus will germinate. This is important in the development of strategies for improved weed control methods.

Under controlled environmental conditions, light and temperature were found to control dormancy and germination of A. retroflexus seeds (Chapters 2 and 3). In this chapter it was demonstrated that dormancy was also affected by natural environmental conditions in the field. However, only a small proportion of seeds respond to these factors, especially under field conditions. In the next chapter, the results of investigations of germination stimulants on dormancy and germination of A. retroflexus seeds are reported.
CHAPTER 5

THE EFFECT OF CHEMICAL GERMINATION STIMULANTS ON
DORMANCY AND GERMINATION

5.1 Introduction

The problem of weed reinfestation from persistent seed banks has led to efforts to try to develop field methods for breaking seed dormancy, with the ultimate aim of reducing seed populations in the soil. For species which produce a persistent seed bank, weed control could be improved if reliable methods became available to induce synchronous germination of dormant seeds in the field (Saini et al. 1985b). Although seed dormancy can be broken by natural conditions such as light and temperature, a large number of seeds do not respond to these conditions and require some other form of treatment to induce germination.

One approach to reducing the survival advantage provided by seed dormancy, is to artificially break dormancy thereby allowing germination to occur and then kill seedlings with conventional weed control practices (Egley 1980; Chancellor 1981). Provided reseeding is prevented, the population of viable long-lived dormant seeds would thus be reduced. The long-term result of this strategy would be an overall reduction in the number of weed control treatments necessary to manage the weed population.

With a view to developing methods to purge seed banks, Hurtt and Taylorson (1986) suggested that chemicals which break dormancy could be applied to soil. The successful use of ethylene in stimulating germination of S. lutea (Bebawi and Eplee
1986) has indicated the feasibility of reducing weed seed populations in the soil in other weed species. Ethylene has been found to stimulate germination of *A. retroflexus* both in the laboratory and under growth chamber conditions (Egley 1980; Schonbeck and Egley 1980a, b). The use of ethephon, an ethylene producing compound, could thus be exploited for stimulation of germination in *A. retroflexus*. Nitrate (Fawcett and Slife 1978; Schimpf and Palmblad 1980; Schonbeck and Egley 1989), and diallate (Fawcett and Slife 1975) are other chemical agents known to stimulate germination of *A. retroflexus*. The effect on dormancy breaking and hence germination stimulation by tri-allate, a related compound to diallate, is not known.

This chapter describes experiments designed to evaluate the effects of ethephon, nitrate and tri-allate on the germination of *A. retroflexus* seed under laboratory conditions and, ethephon and nitrate, under field conditions in the context of managing seed banks.

5.2 Materials and methods

5.2.1 Laboratory studies

Two factorial experiments were designed to test the roles of ethephon and nitrate on seeds of *A. retroflexus* in petri dishes. The first consisted of nine rates of Ethrel® (0, 0.0001, 0.001, 0.01, 0.1, 1, 10, 100, and 1000 μL/L derived from 480 g/L ethephon), four incubation temperatures (20 or 35°C constant, and 20/30 or 11/24°C alternating for 12/12 hrs) and two illumination regimes of darkness or continuous light. The second experiment involved six rates of potassium nitrate (0, 0.002, 0.01, 0.05, 0.1, and 0.5%) four incubation temperatures (20 or 35°C constant, and 20/30 or 20/35°C alternating for 12/12 hrs) and two illumination regimes of darkness or continuous light.
Lots of 50 seeds each from the 1992 seedlot (Section 2.2.1) were placed in petri dishes and moistened with 5 ml of the appropriate germination medium. All the other procedures for germination tests were as described in section 2.2.2. Each treatment was replicated four times and germination was scored after seven days of incubation.

5.2.1.1 Data analyses

Analysis of variance was conducted on percentage germination after arcsin transformation (Section 2.2.3). Ethephon and nitrate experiments were analysed separately and the effects due to incubation temperatures, concentrations of each chemical, illumination and their interactions were determined.

5.2.2 Pot experiment

The effects of nitrate, ethephon and tri-allate applied to 14 cm diameter pots containing seeds of *A. retroflexus* was tested in a randomised block design having five replications. Three rates of each of the three chemicals (Table 5.1) were applied to pots containing sterilised soil in which envelopes (Section 4.2.2) containing 50 seeds from the 1991 seed lot (Section 2.2.1) were buried 2.5 cm below the surface. In addition there were three untreated control pots for each replicate. Pots were initially watered from above until the soil was saturated and then sub-irrigated to maintain soil moisture at field capacity. This was achieved by applying water to trays in which individual pots had been seated. Pots were randomly arranged on a bench in a shade house within blocks containing like chemical treatments.

After eight weeks, the seeds were retrieved, washed free of soil in the laboratory, bags were opened and seeds prepared for germination tests (Section
2.2.2). The seeds were incubated at 12°C in the dark for a period of seven days. They were then transferred to 35°C in the light and germination was counted after 14 days incubation at 35°C.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Rate number 1 (kg/ha)</th>
<th>Rate number 2 (kg/ha)</th>
<th>Rate number 3 (kg/ha)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate</td>
<td>25.0</td>
<td>34.0</td>
<td>100</td>
</tr>
<tr>
<td>Ethephon</td>
<td>5.5</td>
<td>11.0</td>
<td>22</td>
</tr>
<tr>
<td>Tri-allate</td>
<td>0.3</td>
<td>0.6</td>
<td>1</td>
</tr>
</tbody>
</table>

5.2.2.1 Data analyses

Percent germination data were arcsin transformed before analyses of variance were carried out (Section 2.2.3). Because there were three untreated control pots in each replicate, the untreated control was first contrasted with the mean of all other treatment combinations. The other treatment combinations were then split into their factorial components of chemical type and rate of chemical for analysis.

5.2.3 Field studies

Identical experiments were conducted on horticultural fields on adjacent sites in the University at 150° 44' 46"E and 33° 36' 36"S during the summers of 1991/92 and 1992/93 to test the response of A. retroflexus seeds to the application of nitrate and ethephon. Each experiment was laid out as a randomised block design with three
replications. The sites were first disc ploughed then rotary tilled with tractor mounted implements to produce a fine textured seedbed. Plots were 1.0 by 1.0 m into which 3,000 seeds were hand sown and covered by lightly raking by hand.

Nitrate (in the form of ammonium nitrate) and ethephon (in the form of ethrel) at the rates of 34 and 11 kg/ha respectively were applied by hand as a one litre solution as soon as the first seedlings were observed to emerge. Water was applied to control plots. The plots were occasionally irrigated during the first three weeks, to maintain soil moisture at field capacity.

Treatment effects were measured in terms of weed emergence. This was done in one randomly located quadrat (0.25 m²) within each plot. The first count was made 28 days after treatment and subsequent counts at two week intervals. There were four counts in total.

The initial experiment was conducted using seed from the 1991 seedlot (Section 2.2.1) which was sown on October 29th 1991 and treatments were applied on November 4th 1991. In the 1992 experiment seeds from the 1992 seedlot (Section 2.2.1) were sown on December 14th 1992 and treatments were applied on December 19th 1992. Similar to the 1991 experiment, the first weed count was made 28 days after treatment and subsequent counts at two week intervals.

5.2.3.1 Data analyses

Data from the 1991 and 1992 experiments were analyzed separately. In each experiment the number of emerged seedlings/m² for each treatment were based on the means of the three replicates or blocks. Before an analysis of variance was conducted percent emergence data were transformed into arcsin scale (Section 2.2.3).
Orthogonal components were used to separate linear and quadratic responses to seedling emergence over time and its interaction with the type of chemical applied.

5.3 Results

5.3.1 Laboratory studies

5.3.1.1 Effects of ethephon

Ethephon stimulated germination of *A. retroflexus* with a significant \( p < 0.05 \) interaction between ethephon concentration, incubation temperature and illumination (Appendix Table 17). The degree to which germination increased was markedly affected by ethephon concentration and incubation temperature but to a much lesser extent by illumination (Fig. 5.1). At concentrations up to 1 μL/L the highest germination occurred when seeds were incubated at 35°C followed by incubation at 20/30°C. At these temperatures there was no further stimulation of germination above that recorded for 10 μL/L ethephon. Germination was lower at 11/24°C and 20°C and at these temperatures there was little stimulation of germination at concentrations below 1 μL/L. Germination was significantly higher at 11/24°C than at 20°C as opposed to the results at lower concentrations.

Although illumination had a significant interacting effect with concentration and incubation temperature on germination, there was no consistent response. Incubation at 20°C resulted in seeds germinating to significantly higher percentages in the light than in the dark, only at concentrations of 10 μL/L and above. Incubation at 35°C, on the other hand, resulted in higher germination percentages in the light than in the dark at some lower concentrations up to 0.01 μL/L. Above these concentrations, no differences in germination in the light and in the dark were
observed (Fig. 5.1a). When seeds were incubated at 11/24°C no differences in germination were found between light and dark illumination. Similarly, incubation at 20/30°C resulted in no significant differences between light and dark germination except at a concentration of 1µL/L (Fig. 5.1b).

In general, seeds treated with 1000 µL/L germinated to the highest percentage. However, be noted that at this concentration radicle protrusion occurred but development was arrested, and the shoot did not develop at all. Treatment with 10 and 100 µL/L ethrel resulted in development of shoots with hooked hypocotyls. This was especially noticeable in those seeds incubated at high temperature (35°C).

5.3.1.2 Effects of nitrate

*A. retroflexus* seed germination was significantly (*p* < 0.001) affected by the interaction between nitrate concentration, incubation temperature and illumination (Appendix Table 18). The effects of the different concentrations on germination depended upon incubation temperature. At 20°C, the response to nitrate was only evident at concentrations of 0.1 and 0.5%. Germination at lower concentrations did not differ from the control. When incubated at 20/30°C, a significant response was noted at the lowest concentration of 0.002%, but this did not differ with response to other concentrations of up to 0.1%. The highest germination percentage was still obtained at a concentration of 0.5%. The responses at 35°C and at 20/35°C were slightly different. At these temperatures, germination increased with an increase in concentration up to an optimum of 0.1%. Thereafter, germination declined significantly (Fig. 5.2a and 5.2b).
Figure 5.1 Germination response of *A. retroflexus* seeds to different concentrations of ethephon. Four replicates each of 50 seeds were incubated either in the light or in the dark for seven days at constant temperatures of (a) 20°C and 35°C; or at alternating temperatures of (b) 11/24°C or 20/30°C. The vertical lines indicate the LSD (p = 0.05).
Figure 5.2 Germination response of *A. retroflexus* seeds to different concentrations of nitrate. Four replicates each of 50 seeds were incubated either in the light or in the dark for seven days at constant temperatures (a) 20ºC and 35ºC; or at alternating temperatures of (b) 20/30ºC and 20/35ºC. The vertical lines indicate the LSD (p=0.05).
Illumination had a significant interacting effect with concentration and incubation temperatures on germination. However, there was no consistent response. Regardless of concentration, no significant differences were found between germination in the dark and that in the light when seeds were incubated at 20°C. Incubation at 35°C resulted in significantly higher germination in the light than in the dark at all concentrations except 0.01% (Fig. 5.2a). At 20/30°C control seeds and those treated with 0.05 and 0.1% potassium nitrate germinated to significantly higher percentages in the dark than in the light.

Higher germination in the light than in the dark was obtained when seeds were treated with 0.5% potassium nitrate. Seeds incubated at 20/35°C, germinated to significantly higher percentages in the light than in the dark at all concentrations except 0.5% where no differences in germination were found (Fig. 5.2b).

5.3.2 Pot experiment

The application of chemicals significantly stimulated total germination of *A. retroflexus* \(p<0.001\) (Appendix Table 19). Both nitrate and ethephon significantly increased germination, whereas tri-allate had no effect (Table 5.2).

On average, all three rates of chemical applied stimulated germination over that of the untreated control (Table 5.3). It was observed that rates 1 and 2 significantly \(p<0.001\) increased germination but there was no further increase in germination with the highest application rate.
Table 5.2 Germination stimulation of *A. retroflexus* seeds by different chemicals. Values followed by the same letter are not significantly different at LSD (p=0.05) = 4.8.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>% germination (arcsin transformed) (mean of 5 replicates and 3 rates for each chemical)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>40.3c</td>
</tr>
<tr>
<td>Nitrate</td>
<td>56.1b</td>
</tr>
<tr>
<td>Ethephon</td>
<td>62.5a</td>
</tr>
<tr>
<td>Tri-allate</td>
<td>42.9c</td>
</tr>
</tbody>
</table>

Table 5.3 Germination stimulation of *A. retroflexus* seeds by different rates of chemicals used. Values followed by the same letter are no significantly different at LSD (p=0.05) = 4.8.

<table>
<thead>
<tr>
<th>Rate number</th>
<th>% germination (arcsin transformed) (mean of rates from the three chemicals)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>40.3c</td>
</tr>
<tr>
<td>1</td>
<td>48.6b</td>
</tr>
<tr>
<td>2</td>
<td>57.9a</td>
</tr>
<tr>
<td>3</td>
<td>55.0a</td>
</tr>
</tbody>
</table>

5.3.3 Field studies

In the 1991 experiment the emergence of *A. retroflexus* seedlings was stimulated by the application of ethephon and nitrate over time, giving a significant
(p<0.001) treatment by time interaction (Appendix Table 20). At the first and the second seedling counts, stimulation of emergence by ethephon and nitrate did not differ significantly from each other, but stimulation by both treatments was significantly (p<0.05) higher than that of the control (Fig. 5.3a). At the third and fourth counts, the three treatments differed significantly from each other with the highest emergence observed in ethephon treated plots. Over the period of seedling counts both linear and quadratic effects were significant (p<0.001), indicating a curvilinear response for each treatment, but the linear slope of the response differed between treatments (p<0.001).

In the 1992 experiment, treatments, time of seedling count and their interactions were found to significantly (p<0.001) affect *A. retroflexus* seedling emergence (Appendix Table 21). Similar to the 1991 experiment, more seeds emerged in treated plots than in the control. Differences in emergence among the three treatments were observed from the first to the last seedling count (Fig. 5.3b). The highest emergence again occurred in the ethephon treated plots. Seedling emergence increased over time with both significant (p<0.001) linear and quadratic effects indicating a curvilinear response for each treatment, but the curvature of the response differed between treatments (p<0.05).

5.4 Discussion

Ethephon and nitrate treatments applied in petri dishes in the laboratory, in pots and in the field significantly stimulated germination of *A. retroflexus* over that of untreated seeds.
Figure 5.3 Field emergence of *A. retroflexus* seeds in control and plots treated with either nitrate (34 kg/ha) or ethephon (11 kg/ha): (a) 1991 experiment (b) 1992 experiment.
Ethephon has been found to be an effective dormancy breaking compound for seeds of several species that respond to ethylene (Egley and Duke 1985). Goudey et al. (1987a) showed that ethephon is decomposed within the seeds as well as in the soil and hence acts via ethylene.

5.4.1 Laboratory studies

In the laboratory, germination of *A. retroflexus* seeds increased with ethephon concentration under all experimental conditions, but the response was significantly modified by temperature and light (Fig. 5.1). Schonbeck and Egley (1980a,b; 1988) obtained similar results with ethylene showing a log-linear dose response for *A. retroflexus*. Schonbeck and Egley (1980b) found that ethylene response thresholds decreased slightly with increasing temperature, and they suggested that this trend might facilitate ethylene stimulation of soil-borne seeds when the ground is warm. Similar positive response to treatments with ethylene, either as a gas or in the form of ethylene producing compounds, in other weed species have been observed (Saini et al. 1986; Mekki and Leroux 1991).

Light was ineffective in stimulating germination of *A. retroflexus* seeds at low temperatures (20°C and 11/24°C). Seed germination of some species has been found to be actually inhibited by light at low temperatures, and Vidaver (1977) suggested that this mechanism prevents germination when cool day time temperatures foretell a possible night time frost. Taylorson (1979) reported that germination in species stimulated by ethylene was also promoted by light, but light was not always necessary in this present study, for the response to ethephon. For example, at high temperatures and high concentrations germination in the dark was as good as in the light.

Germination of *A. retroflexus* increased with increasing ethephon concentration. At the highest concentration (1000 μl/L), however, some
developmental processes were arrested. Inhibition of germination by ethylene is uncommon, but has been noted previously in a number of species. Taylorson (1979) reported that the inhibition of *Potentilla norvegica* L. (rough cinquefoil) seeds was abrupt and sensitive at less than 1 ppm ethylene, and germination of *Solanum carolinense* L. (horse nettle) seeds was also inhibited. Apart from the effect on germination, ethylene treatment has also been known to produce other effects. Laboratory and field investigations by Babiker and Hamdoun (1983) revealed that ethephon at 0.5 to 6 kg/ha gave appreciable germination of *Striga hermonthica* (Del) Benth (witchweed), but reduced the number of aerial shoots. Egley and Dale (1970) also found that *S. lutea* seeds germinated with ethylene at 1 μL/L or greater had twisted or curled radicles by the end of a four day period.

Based on the laboratory response it is possible that the application of a high concentration of ethephon in the field would lead to seed germination but render emergence of some *A. retroflexus* seedlings impossible, and hence reduce the need for post emergence weed control measures. The effectiveness of this will, however, depend on factors such as pH that influence the decomposition of ethephon (Biddle *et al.* 1976; Babiker and Hamdoun 1983; Goudey *et al.* 1987a). The soil may also act as a sink for ethylene possibly by binding the gas (Schonbeck and Egley 1981b), thereby, annulling the effect of high ethylene concentrations.

Although it has been known for some time that ethylene stimulates germination in seeds, its mechanism of action is still not known. However, various roles for ethylene in respiration and in the alteration of protein body membranes have been proposed. Ross (1984) gives a detailed review.

The response of *A. retroflexus* seeds to treatments with nitrate was similar to that of ethephon treatment (Fig. 5.2). Germination increased with increasing concentration and this was favoured by the presence of light and high temperature,
particularly the alternating temperatures. Germination of many weed seeds has been shown to be enhanced by nitrate, especially when used in sequence or combination with light and temperature (Henson 1970; Vincent and Roberts 1977; Williams 1983; Probert et al. 1987; Goudey et al. 1988). Results reported herein showed that at high temperatures seeds germinated to significantly higher percentages in the light than in the dark. In seeds of *S. officinale* it was shown that the effectiveness of nitrate in stimulating germination depended on the level of $P_{fr}$ (Hilhorst and Karssen 1988; 1990).

Seeds germinated appreciably well in the dark, and at low temperatures, germination was better in the dark. Contrasting results were obtained by Schonbeck and Egley (1980a) who found no evidence of stimulation in *A. retroflexus* seeds in darkness by nitrate concentrations of between 0 and 0.2%. Roberts and Lockett (1975) explained that germination response to nitrate may depend on the environmental history of the seed particularly during seed development, probably accounting for the contrasting results.

At high temperatures potassium nitrate concentration of 0.1% was the optimum concentration with an inhibitory effect at higher concentrations. An inhibitory effect of nitrate on germination has also been reported by Henson (1970) in *C. album*. He found that nitrate in excess of 250 ppm only promoted germination in the light. However, high concentrations with light retarded radicle extension, slowed germination and depressed the final germination percentage. Goudey et al. (1988) postulated that in fertilized arable soils nitrate concentrations may rise to inhibitory levels. It is not known whether this inhibition is a toxic effect, specific inhibition or an osmotic effect.

A number of hypotheses have been proposed to account for the action of nitrate in seed germination. One of these, proposed by Hilton and Thomas (1986)
involves stimulation of oxygen uptake. Application of nitrate to seeds of several species resulted in increased uptake of oxygen by these seeds. This was ascribed to breakage of dormancy. Hilhorst and Karssen (1990), critically evaluating several experiments, concluded that the increased oxygen uptake was the result and not the cause of germination. Thus, the effect of nitrate was on germination which led to increased oxygen uptake.

More recently it has been hypothesised that nitrate acts as a co-factor of phytochrome (Hilhorst and Karssen 1988; Hilhorst 1990a,b). The merit of this hypothesis over the two discussed above is in the fact that it integrates the effects of light, temperature and nitrate on germination and dormancy breakage. The actual mechanism of nitrate action in the induction of germination, however, is still obscure.

5.4.2 Pot experiment

Seeds were stimulated to germinate by nitrate and ethephon irrespective of the dose rate. Rate one and two increased germination significantly over that of the control. A further increase in the rate resulted in no increase in germination (Table 5.2, 5.3). Critical factors in the soil such as soil types, moisture, inhibitors and soil atmospheres may affect stimulation of germination by these chemicals.

Diallate has been found to be effective on germination stimulation of *A. retroflexus* and *Datura stramonium* L. (*jimson weed*) (Fawcett and Slife 1975). There has been no report on germination stimulation by tri-allate, a related chemical. However, only the second rate (0.6 kg/ha) increased germination significantly over that of the control. Fawcett and Slife (1975) observed that many seedlings in the field were killed by treatment rates above 0.6 kg/ha, but it is not clear whether these high
rates also have detrimental effects on seeds themselves, thus affecting germination, and whether tri-allate had similar effects.

5.4.3 Field studies

Stimulation of germination by ethephon and nitrate was also observed in the field (Fig. 5.3). Similar results on stimulation of germination in *A. retroflexus* by ethephon in the field were obtained by Egley (1990). Germination stimulation by ethephon in the field or in the soil in growth chambers has also been observed in other species. In controlled environment studies, application of 100 mg/L solution of ethephon resulted in a significant promotion of germination of *C. album* in the soil (Saini et al. 1986). Field investigations by Babiker and Hamdoun (1983) indicated that ethephon at 0.5 to 6 kg/ha gave appreciable germination of *S. hermonthica*. Similarly, application of ethephon, with kinetin and gibberellins prevented the development of secondary dormancy of *A. artemisifolia* seeds in the field (Samimy and Khan 1983). Only 20% of the treated seeds remained viable compared to 89% of the control seeds. This reduction in viable seeds was probably due to enhanced germination in the soil prior to recovery of the seeds.

Varying results have been observed from treatment with nitrate in the field. Application of 34 kg/ha in the form of ammonium nitrate stimulated germination of *A. retroflexus* resulting in 62% emergence. This result is of the same order as that recorded by Hurtt and Taylorson (1986) who found stimulation of *A. retroflexus* by nitrate treatment in the field. More seeds in the potassium nitrate treated plots than in the control germinated *in situ* before recovery and Egley (1989) attributed this to the stimulative effect of nitrate. In contrast, Fawcett and Slife (1978), Schimpf and Palmblad (1980), and Egley (1990) found no indication of stimulation by nitrate treatment in the field. It therefore appears that the stimulating effect of nitrate in the
field probably is affected by soil and climatic conditions. Fawcett and Slife (1978) suggested that nitrate treatment did not stimulate germination because the natural soil fertility provided enough nitrate to saturate any mechanism affecting seed germination.

It has clearly been demonstrated in these studies that stimulation of germination in the field by nitrate and ethephon is possible. The effectiveness of these stimuli, however, will depend on other factors that interact with them. In the laboratory study, factors such as temperature and light were shown to affect the sensitivity of seeds to the stimuli and, in the field, other factors may also play an important role.
CHAPTER 6

GENERAL DISCUSSION

Being a summer annual species, seeds of *A. retroflexus* constitute the vital link between generations. To provide this link, it is evident that the species has adopted dormancy strategies that enable its seeds to persist. Consequently, seed dormancy in weeds such as *A. retroflexus* leads to the formation of seed banks and hence to perpetual weed problems and the continual need for control. Manipulation to promote germination is one plausible strategy for depleting seed reserves in the soil. The studies undertaken in this project were thus conceived to provide an understanding of the factors that regulate dormancy and germination with a view to developing improved management practices.

6.1 Effects of light and temperature on seed dormancy

The germination studies in Chapters 2 and 3 revealed that light and temperature affect dormancy of *A. retroflexus* seeds. In general, *A. retroflexus* seeds germinated to higher percentages in the light, but it was shown that the requirement for light was obviated when seeds experienced temperature shifts, (i.e., a shift from low to high temperatures) or after pre-chilling (Fig. 2.1). The substitution of temperature shifts for light has been reported in other work (Probert and Smith 1986) which indicated that temperature shifts somehow interfere with phytochrome action.
When seeds are buried in the soil it would appear from studies herein that seeds germinate well so long as the changes in temperature are met. In nature the effect of light on buried seeds is limited since it does not penetrate to great depths (Woolley and Stoller 1978; Tester and Morris 1987) and hence is likely only to affect those seeds at or near the soil surface. Thus only those seeds brought to the soil surface, such as might occur through cultivation or soil disturbance, might have a requirement for light fulfilled. By preventing germination, the light mechanism plays an important role in the preservation of seeds. This role is especially important in small seeded species such as *A. retroflexus*, whereby establishment from depth cannot be sustained by their meagre nutrient reserves.

The observed requirement for a temperature shift or alternating temperature in *A. retroflexus* also represents an important adaptation which ensures that germination occurs at or close to the soil surface. Soil temperature measurements in studies conducted by Thompson *et al.* (1977); and Van Assche and Vanlerberghe (1989) indicate that temperature fluctuations are greatest on or close to the surface of bare soil and in breaks in the vegetation canopy.

6.2 Effects of storage environment on seed dormancy and longevity

After maturity, weed seeds may be dispersed to various locations and so experience various storage conditions. They may lie on the soil surface for an extended period of time, other seeds may be buried, and others may be harvested with crop seeds. Consequently, both the conditions under which seeds are stored, and the duration of storage are likely to bring about changes in dormancy. In the experiments reported in Chapter 3 the effect of storage temperatures and duration were investigated. It was observed that dormancy is more likely to be broken by high temperatures and prolonged storage duration. Given these conditions, seeds were likely also to germinate at lower temperatures (Figs. 3.1 to 3.6). Thus under field
conditions, seeds may experience prolonged dry conditions and this would modify them to be able to germinate at less favourable conditions.

Seeds in arable land are often distributed to different depths in the soil as a consequence of cultivation (Cousens and Moss 1990). Because spatial distribution affects seeds, the fate of *A. retroflexus* seeds buried at different depths was investigated over a period of one year. Although it was argued in Section 6.1 that conditions for germination might occur near the soil surface, this was not borne out in the burial experiment. Only a small percentage of seeds on the soil surface germinated *in situ* (Fig. 4.1). Most of the seeds that did not germinate in the field were induced into dormancy as was shown by their inability to readily germinate in the laboratory (Fig. 4.2). This is indicative of a further survival mechanism for this species which ensures that not all seeds germinate under conditions that are prone to rapid drying. *In situ* germination of seeds at deeper depths was not favoured. In contrast to seeds placed at the surface, more deeply buried seeds germinated readily under laboratory conditions showing that they either had low dormancy or no dormancy at all.

It is logical that, in the soil, germination of non-dormant seeds awaits the availability of suitable conditions such as moisture and temperature (Roberts and Potter 1980; Karssen 1982; Bouwmeester 1990), light (Taylorson 1970), temperature fluctuations (Baskin and Baskin 1985), or a combination of factors (Roberts *et al.* 1987). From the results of studies presented herein, it is evident that dormancy in deeply buried seeds is broken but germination will not occur unless favourable conditions are encountered. This could be one explanation why seedlings emerge immediately after cultivation (Roberts and Ricketts 1979; Roberts and Potter 1980).

Irrespective of the depth of burial, seeds underwent changes in dormancy throughout the year of burial. Seeds retrieved after a cold period of the year were
less dormant than those retrieved after a warm period (Fig. 4.4). These results concur with the laboratory observations whereby pre-chilling resulted in high percent germination. This behaviour conforms with the general notion that dormancy is broken at a time preceding apparently favourable periods for germination and seedling emergence in the field. This pattern, in which dormancy is broken and induced during the course of a year for buried seeds of some summer and winter annual plants in both temperate and tropical soil environments has been well documented (Taylorson 1970, 1972; Baskin and Baskin 1985, 1989; Garwood 1989; Bouwmeester 1990). These authors demonstrated that annual dormancy patterns coincide closely with seasonal changes in temperature.

At all depths of burial, seeds lost viability over time, but this loss was greater for those on the soil surface (Fig. 4.5). Seeds on the soil surface would, thus, not form persistent seed banks, since most of them would be depleted through in situ germination or loss in viability. In most cases, however, *A. retroflexus* seeds would readily be buried because of their small size. Ease of burial due to small seed size has been documented by Thompson (1987) for a range of species.

Both laboratory and burial experiments revealed that environmental factors play an important role in the regulation of dormancy and germination of *A. retroflexus* seeds. At any particular time, however, only a small fraction of seeds may respond to these factors. The residual fraction remains dormant and forms a persistent seed bank. A challenge therefore remains to devise methods to manipulate dormancy of seeds unaffected by seasonal environmental factors.
6.3 Effects of chemical stimulants on dormancy

Dormancy of those seeds that do not respond positively to natural environmental factors may be manipulated chemically. Results reported in Chapter 5 showed that nitrate and ethephon were effective in stimulating germination of *A. retroflexus* seeds under controlled environmental conditions as well as in the field (Figs. 5.1 to 5.3). The stimulative effects of these chemicals depended on other factors as was demonstrated in the laboratory studies. A combination of stimuli (such as, light, high temperature and ethephon or nitrate) had a synergistic effect when compared to the application of a single stimulus. Vincent and Roberts (1977, 1979) stressed that light, nitrate, and fluctuating temperature, for example, could strongly influence weed seed germination in the field. Since factors such as light and fluctuating temperatures may only be experienced at or near the soil surface, it would appear that only those seeds lying at these sites would be most affected by the chemical stimulants.

The requirement for high temperature for effective stimulation of germination by the chemicals indicates that the stimulants should be applied in the field during periods of hot weather. For summer annuals such as *A. retroflexus* and *C. album* this is the period when most of the seeds are dormant (Baskin and Baskin 1977). A more logical suggestion given by Egley (1986) was that a stimulant should be applied when the maximum number of seeds are non-dormant hence late winter would be an appropriate time for summer annuals. During this season, however, the temperatures would be too low for germination and therefore the effects of applied chemicals would more likely be sub-maximal.

Based on laboratory observations reported herein, various factors should also be taken into account when evaluating the effectiveness of chemical stimulants particularly under field conditions. Varying results from treatments of nitrates in the
field have been reported by different workers (Fawcett and Slife 1978; Schimpf and Palmblad 1980; Hurtt and Taylorson 1986; Egley 1990). The erratic results may have been attributed to the different environmental conditions under which the treatments were conducted. In the laboratory, for example, it was shown that factors such as pH and moisture content influence the decomposition of ethephon (Goudey et al. 1987a).

6.4 Concluding remarks

Studies reported in this thesis revealed that dormancy in *A. retroflexus* is broken, not just by a single factor (such as light) but, by exposure to a combination of treatments such as pre-chilling, alternating temperatures, after-ripening or applied chemical stimulants. It is reasonable to suppose that such interactions may commonly occur in nature. As discussed earlier, it is more likely that environments favourable for germination would occur at or near the soil surface. However, under field conditions, many seeds are buried and hence, are less likely to respond. Even under favourable conditions, not all seeds will germinate at the same time. Seeds from different plants or even from different parts of an inflorescence on the same plant, may have variable dormancy status which most likely results from the environmental conditions experienced by the mother plant during seed production (Kigel 1977, 1979; Adkins and Simpson 1988; Fenner 1991) (Section 1.4.5). Variability in the quality and dormancy status of seeds is presumably a further adaptation that favours survival.

Weed management by manipulation of weed seed dormancy in the soil, therefore, cannot be managed by a single method. An integrated approach that utilises ecological, mechanical and chemical methods may be advocated. Non-inverting tillage that brings buried seeds to the soil surface may be useful for both breaking dormancy and, exposing seeds to favourable germination conditions may be
used. This should be done during optimum conditions for germination to avoid induction into dormancy. Deep inverting tillage on the other hand should be avoided since it may delay germination by burying seeds, prolonging their viability and adding to seed persistence problems. Cousens and Moss (1990) reported that burial by cultivation may either induce or enforce dormancy. Conversely, in seeds with no dormancy and which cannot undergo secondary dormancy, burial may be one form of control since seeds would germinate \textit{in situ} but fail to emerge. Chemicals that stimulate germination could then be usefully applied to further reduce seed banks. Furthermore, if reseeding is prevented, the persistent weed seed population may be reduced by such stimulation of germination.
REFERENCES


Cheam, A.H. (1987). Longevity of *Bromus diandrus* Roth. seed in soil at three sites in Western Australia. *Plant Protection Quarterly* 2, 137-139.


Roberts, E.H. and Benjamin, S.K. (1979). The interactions of light, nitrate and alternating temperature on the germination of *Chenopodium album*, *Capsella*
bursa-pastoris and Poa annua before and after chilling. *Seed Science and Technology* 7, 379-392.


APPENDIX 1

This appendix contains the analysis of variance for all the experiments undertaken in this study.

Table 1 ANOVA table for the effect of light and temperature on dormancy and germination of *A. retroflexus* seed (Fig. 2.1).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>m.s.</th>
<th>v.r</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>incubation temperature</td>
<td>5</td>
<td>5730.55</td>
<td>215.40</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>light</td>
<td>1</td>
<td>284.02</td>
<td>10.68</td>
<td>0.002</td>
</tr>
<tr>
<td>inc temp. light</td>
<td>5</td>
<td>71.30</td>
<td>2.68</td>
<td>0.037</td>
</tr>
<tr>
<td>Residual</td>
<td>36</td>
<td>26.60</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD (p=0.05) = 7.4
C.V. = 19%

\(^1\) incubation temperature
Table 2 ANOVA table for the effect of pre-chilling temperature, duration and incubation temperature on dormancy and germination of *A. retroflexus* seed (Fig. 2.2).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>m.s</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>inctemp¹</td>
<td>1</td>
<td>35814.29</td>
<td>2662.34</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>light</td>
<td>1</td>
<td>9525.75</td>
<td>708.12</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>chilltemp.²</td>
<td>2</td>
<td>13431.40</td>
<td>998.45</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>dura³</td>
<td>4</td>
<td>1537.20</td>
<td>114.27</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>store⁴</td>
<td>1</td>
<td>135.22</td>
<td>0.05</td>
<td>0.002</td>
</tr>
<tr>
<td>inctemp . light</td>
<td>1</td>
<td>3617.82</td>
<td>268.94</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>inctemp. store⁵</td>
<td>1</td>
<td>2012.32</td>
<td>149.59</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>light.store⁶</td>
<td>1</td>
<td>1.71</td>
<td>0.13</td>
<td>0.722</td>
</tr>
<tr>
<td>inctemp.light.store</td>
<td>1</td>
<td>75.45</td>
<td>5.61</td>
<td>0.019</td>
</tr>
<tr>
<td>inctemp.dura</td>
<td>4</td>
<td>327.74</td>
<td>24.36</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>light.dura</td>
<td>4</td>
<td>594.15</td>
<td>44.17</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>inctemp.temp.</td>
<td>2</td>
<td>3785.77</td>
<td>281.42</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>light.chilltemp</td>
<td>2</td>
<td>2938.44</td>
<td>218.44</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>store.dura.chilltemp</td>
<td>8</td>
<td>502.96</td>
<td>7.39</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>inctemp.light.store.dura</td>
<td>4</td>
<td>109.69</td>
<td>8.15</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>inctemp.light.store.chilltemp</td>
<td>2</td>
<td>552.22</td>
<td>41.05</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>inctemp.store.dura.chilltemp</td>
<td>8</td>
<td>111.38</td>
<td>8.28</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>light.store.dura.chilltemp</td>
<td>8</td>
<td>193.00</td>
<td>14.35</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>inctemp.light.store.dura.chilltemp 8</td>
<td>114.29</td>
<td>8.50</td>
<td>&lt; .001</td>
<td></td>
</tr>
</tbody>
</table>

Residual 192 13.45
Total 255

LSD (p=0.05) = 5.1
C.V.. = 11.9%

¹ incubation temperature
² pre-chilling temperature
³ pre-chilling duration
⁴ non pre-chilled (control) versus mean of pre-chilled treatments
⁵ interaction of incubation temperatures with pre-chilled and control
⁶ interaction of illumination with pre-chilled and control
Table 3 ANOVA table for germination after 14 days incubation of seeds stored for one month (Fig. 3.1).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>m.s</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>incubation temperature</td>
<td>2</td>
<td>837.60</td>
<td>51.87</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>storage temperature</td>
<td>4</td>
<td>136.02</td>
<td>8.42</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>inctemp(^1), storetemp(^2)</td>
<td>8</td>
<td>8.65</td>
<td>0.54</td>
<td>0.823</td>
</tr>
<tr>
<td>Residual</td>
<td>45</td>
<td>16.15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD (p=0.05) = 8.1
C.V = 31.4%

---

Table 4 ANOVA table for germination after 14 days incubation of seeds stored for three months(Fig. 3.1).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>m.s</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>incubation temperature</td>
<td>2</td>
<td>160.34</td>
<td>37.94</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>storage temperature</td>
<td>4</td>
<td>430.01</td>
<td>101.75</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>inctemp(^1), storetemp(^2)</td>
<td>8</td>
<td>2.80</td>
<td>0.66</td>
<td>0.721</td>
</tr>
<tr>
<td>Residual</td>
<td>43</td>
<td>4.23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>57</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD (p=0.05) = 4.2
C.V = 8.7%

\(^1\)incubation temperature

\(^2\)storage temperature
**Table 5** ANOVA table for germination after 14 days incubation of seeds stored for six months (Fig. 3.1).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>m.s</th>
<th>v.r</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>incubation temperature</td>
<td>2</td>
<td>46.81</td>
<td>3.04</td>
<td>0.058</td>
</tr>
<tr>
<td>storage temperature</td>
<td>4</td>
<td>951.34</td>
<td>61.76</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>inctemp(^1).storetemp(^2)</td>
<td>8</td>
<td>6.25</td>
<td>0.41</td>
<td>0.911</td>
</tr>
<tr>
<td>Residual</td>
<td>43</td>
<td>15.40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>57</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD (p=0.05) = 8

C.V. = 12.4%

---

**Table 6** ANOVA table for germination after 14 days incubation of seeds stored for 12 months (Fig. 3.1).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>m.s</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>incubation temperature</td>
<td>2</td>
<td>2224.93</td>
<td>125.97</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>storage temperature</td>
<td>4</td>
<td>618.38</td>
<td>35.01</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>inctemp(^1).storetemp(^2)</td>
<td>8</td>
<td>83.33</td>
<td>4.72</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Residual</td>
<td>45</td>
<td>17.66</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>59</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD (p=0.05) = 8.6

C.V. = 8.9%

\(^1\)incubation temperature

\(^2\)storage temperature
Table 7 ANOVA table for germination from day 14 to 28 of incubation of seeds stored 1 to 12 months at different temperatures (Fig. 3.2).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>m.s</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>storetemp$^1$</td>
<td>4</td>
<td>161.20</td>
<td>4.18</td>
<td>0.005</td>
</tr>
<tr>
<td>inctemp$^2$</td>
<td>2</td>
<td>2648.02</td>
<td>99.83</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>dura$^3$</td>
<td>3</td>
<td>4906.45</td>
<td>127.31</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>storetemp.dura</td>
<td>12</td>
<td>41.26</td>
<td>1.07</td>
<td>0.400</td>
</tr>
<tr>
<td>storetemp.inctemp</td>
<td>8</td>
<td>19.03</td>
<td>0.72</td>
<td>0.676</td>
</tr>
<tr>
<td>dura.inctemp</td>
<td>6</td>
<td>1103.44</td>
<td>41.60</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>storetemp.dura.inctemp</td>
<td>24</td>
<td>53.26</td>
<td>2.01</td>
<td>0.008</td>
</tr>
<tr>
<td>Residual</td>
<td>107</td>
<td>26.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>226</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD (p=0.05) = 7.2
C.V. = 17.9%

$^1$storage temperature

$^2$incubation temperature

$^3$storage duration
Table 8 ANOVA table for total germination 28 days from start of incubation of seeds stored 1 to 12 months at different temperatures (Fig. 3.3).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>m.s</th>
<th>v.r</th>
<th>F.pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>storetemp(^1)</td>
<td>4</td>
<td>2251.10</td>
<td>71.31</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>inctemp(^2)</td>
<td>2</td>
<td>7.54</td>
<td>0.37</td>
<td>0.690</td>
</tr>
<tr>
<td>dura(^3)</td>
<td>3</td>
<td>12209.62</td>
<td>386.78</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>storetemp.dura</td>
<td>12</td>
<td>105.52</td>
<td>3.34</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>storetemp.inctemp</td>
<td>8</td>
<td>14.07</td>
<td>0.70</td>
<td>0.694</td>
</tr>
<tr>
<td>dura.inctemp</td>
<td>6</td>
<td>210.26</td>
<td>10.40</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>storetemp.dura.inctemp</td>
<td>24</td>
<td>31.11</td>
<td>1.54</td>
<td>0.071</td>
</tr>
<tr>
<td>Residual</td>
<td>107</td>
<td>20.21</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>226</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD (p=0.05) = 6.2

C.V. = 9.7%

\(^1\)storage temperature

\(^2\)incubation temperature

\(^3\)storage duration
Table 9 ANOVA table for germination after 14 days incubation of seed stored for 1 to 12 weeks and tested at a weekly interval (Fig. 3.4).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>m.s</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>storetemp&lt;sup&gt;1&lt;/sup&gt;</td>
<td>4</td>
<td>25804.04</td>
<td>1560.80</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>inctemp&lt;sup&gt;2&lt;/sup&gt;</td>
<td>5</td>
<td>36170.51</td>
<td>2187.84</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>dura&lt;sup&gt;3&lt;/sup&gt;</td>
<td>11</td>
<td>6176.81</td>
<td>373.62</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>storetemp.dura</td>
<td>44</td>
<td>403.37</td>
<td>24.40</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>storetemp.inctemp</td>
<td>20</td>
<td>1622.09</td>
<td>98.11</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>dura.inctemp</td>
<td>55</td>
<td>424.26</td>
<td>25.66</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>storetemp.dura.inctemp</td>
<td>20</td>
<td>53.26</td>
<td>3.22</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Residual</td>
<td>705</td>
<td>16.53</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1064</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD (p=0.05) = 6.5
C.V. = 18.4

<sup>1</sup>storage temperature

<sup>2</sup>incubation temperature

<sup>3</sup>storage duration
<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>m.s</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>storetemp(^1)</td>
<td>4</td>
<td>861.08</td>
<td>39.11</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>inctemp(^2)</td>
<td>5</td>
<td>3033.99</td>
<td>137.81</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>storetemp.inctemp</td>
<td>20</td>
<td>84.16</td>
<td>3.82</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Residual</td>
<td>57</td>
<td>22.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD (p=0.05) = 9.7
C.V. = 20.4%

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>m.s</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>storetemp(^1)</td>
<td>4</td>
<td>1306.33</td>
<td>44.95</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>inctemp(^2)</td>
<td>5</td>
<td>3660.61</td>
<td>125.97</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>storetemp.inctemp</td>
<td>20</td>
<td>94.39</td>
<td>3.25</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Residual</td>
<td>57</td>
<td>29.06</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD (p=0.05) = 10.4
C.V. = 20.7%

\(^1\)storage temperature
\(^2\)incubation temperature
Table 12 ANOVA table for germination *in situ* of seeds buried in the field at different depths for up to 12 months (Fig. 4.1).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>m.s</th>
<th>v.r</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>depth(^1)</td>
<td>3</td>
<td>1389.83</td>
<td>63.87</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>lin(^2)</td>
<td>1</td>
<td>3378.05</td>
<td>155.23</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>quad(^3)</td>
<td>1</td>
<td>756.91</td>
<td>34.78</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>deviations</td>
<td>1</td>
<td>34.52</td>
<td>1.59</td>
<td>0.240</td>
</tr>
<tr>
<td>Residual</td>
<td>9</td>
<td>21.76</td>
<td>1.96</td>
<td></td>
</tr>
<tr>
<td>duration(^4)</td>
<td>4</td>
<td>1.05</td>
<td>2.79</td>
<td>0.036</td>
</tr>
<tr>
<td>lin</td>
<td>1</td>
<td>7.50</td>
<td>0.68</td>
<td>0.415</td>
</tr>
<tr>
<td>quad</td>
<td>1</td>
<td>106.33</td>
<td>9.57</td>
<td>0.003</td>
</tr>
<tr>
<td>deviations</td>
<td>2</td>
<td>5.18</td>
<td>0.47</td>
<td>0.630</td>
</tr>
<tr>
<td>depth.duration</td>
<td>12</td>
<td>20.25</td>
<td>1.82</td>
<td>0.071</td>
</tr>
<tr>
<td>lin.lin</td>
<td>1</td>
<td>108.06</td>
<td>9.73</td>
<td>0.003</td>
</tr>
<tr>
<td>quad.lin</td>
<td>1</td>
<td>45.97</td>
<td>4.14</td>
<td>0.047</td>
</tr>
<tr>
<td>deviations(^5)</td>
<td>10</td>
<td>8.90</td>
<td>0.80</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>48</td>
<td>11.11</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD (p=0.05) = 4.7

C.V. = 25%

\(^1\) depth of burial

\(^2\) linear effect

\(^3\) quadratic effect

\(^4\) duration of burial

\(^5\) where the interactions between the orthogonal components were non significant they were lumped into the deviation term.
Table 13 ANOVA table for germination in the laboratory of retrieved seeds after burial for 1,3,6,9 and 12 months at different depths (Fig. 4.2).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>m.s</th>
<th>v.r</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>depth(^1)</td>
<td>3</td>
<td>3084.51</td>
<td>77.10</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>lin(^2)</td>
<td>1</td>
<td>8687.41</td>
<td>217.16</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>quad(^3)</td>
<td>1</td>
<td>538.71</td>
<td>13.47</td>
<td>0.005</td>
</tr>
<tr>
<td>deviations</td>
<td>1</td>
<td>27.41</td>
<td>0.69</td>
<td>0.429</td>
</tr>
<tr>
<td>Residual</td>
<td>9</td>
<td>40.00</td>
<td>0.40</td>
<td></td>
</tr>
<tr>
<td>duration(^4)</td>
<td>4</td>
<td>875.71</td>
<td>30.70</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>lin</td>
<td>1</td>
<td>1217.02</td>
<td>42.67</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>quad</td>
<td>1</td>
<td>246.99</td>
<td>8.66</td>
<td>0.005</td>
</tr>
<tr>
<td>deviations(^5)</td>
<td>2</td>
<td>1019.41</td>
<td>35.74</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>depth.duration</td>
<td>12</td>
<td>15.41</td>
<td>0.54</td>
<td>0.877</td>
</tr>
<tr>
<td>deviations(^5)</td>
<td>12</td>
<td>15.41</td>
<td>0.54</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>48</td>
<td>28.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD (p = 0.05) = 7.6
C.V. = 11.9%

\(^1\) depth of burial
\(^2\) linear effect
\(^3\) quadratic effect
\(^4\) duration of burial
\(^5\) where the interactions between the orthogonal components were non significant they were lumped into the deviation term.
Table 14 ANOVA table for germination of retrieved seeds when treated with ethephon in the laboratory (Fig. 4.3).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>m.s</th>
<th>v.r.</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>depth(^1)</td>
<td>3</td>
<td>303.58</td>
<td>5.28</td>
<td>0.023</td>
</tr>
<tr>
<td>lin(^2)</td>
<td>1</td>
<td>899.04</td>
<td>15.63</td>
<td>0.003</td>
</tr>
<tr>
<td>quad(^3)</td>
<td>1</td>
<td>11.62</td>
<td>0.20</td>
<td>0.664</td>
</tr>
<tr>
<td>deviations</td>
<td>1</td>
<td>0.09</td>
<td>0.00</td>
<td>0.970</td>
</tr>
<tr>
<td>Residual</td>
<td>9</td>
<td>57.51</td>
<td>1.86</td>
<td></td>
</tr>
<tr>
<td>duration(^4)</td>
<td>4</td>
<td>2041.72</td>
<td>65.94</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>lin</td>
<td>1</td>
<td>4984.69</td>
<td>161.00</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>quad</td>
<td>1</td>
<td>1059.08</td>
<td>34.21</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>deviations</td>
<td>2</td>
<td>1061.56</td>
<td>34.29</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>depth.duration</td>
<td>12</td>
<td>37.53</td>
<td>1.21</td>
<td>0.303</td>
</tr>
<tr>
<td>deviations(^5)</td>
<td>12</td>
<td>37.53</td>
<td>1.21</td>
<td>0.303</td>
</tr>
<tr>
<td>Residual</td>
<td>48</td>
<td>30.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD (p=0.05) = 7.9

C.V. = 18.1%

\(^1\) depth of burial
\(^2\) linear effect
\(^3\) quadratic effect
\(^4\) duration of burial
\(^5\) where the interactions between the orthogonal components were non significant they were lumped into the deviation term.
Table 15 ANOVA table for germination of non-physiologically dormant seeds (sum of germination in situ and germination in the laboratory before treatment with ethephon) (Fig. 4.4).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>m.s</th>
<th>v.r</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>depth(^1)</td>
<td>3</td>
<td>1297.16</td>
<td>49.99</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>lin(^2)</td>
<td>1</td>
<td>3837.30</td>
<td>147.89</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>quad(^3)</td>
<td>1</td>
<td>24.04</td>
<td>0.93</td>
<td>0.361</td>
</tr>
<tr>
<td>deviations</td>
<td>1</td>
<td>30.14</td>
<td>1.16</td>
<td>0.309</td>
</tr>
<tr>
<td>Residual</td>
<td>9</td>
<td>25.95</td>
<td>1.10</td>
<td></td>
</tr>
<tr>
<td>duration(^4)</td>
<td>4</td>
<td>800.65</td>
<td>33.83</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>lin</td>
<td>1</td>
<td>1031.23</td>
<td>43.57</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>quad</td>
<td>1</td>
<td>414.07</td>
<td>17.50</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>deviations</td>
<td>2</td>
<td>878.65</td>
<td>37.13</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>depth.duration</td>
<td>12</td>
<td>16.28</td>
<td>0.69</td>
<td>0.755</td>
</tr>
<tr>
<td>deviations(^5)</td>
<td>2</td>
<td>16.28</td>
<td>0.69</td>
<td>0.755</td>
</tr>
<tr>
<td>Residual</td>
<td>48</td>
<td>23.64</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD (p=0.05) = 6.9
C.V.. = 9.9%

\(^1\)depth of burial
\(^2\)linear effect
\(^3\)quadratic effect
\(^4\)duration of burial
\(^5\)where the interactions between the orthogonal components were non significant they were lumped into the deviation term.
Table 16 ANOVA table for total germination (viability) of seeds after burial for a period of up to 12 months (Fig. 4.5).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>m.s</th>
<th>v.r</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>depth$^1$</td>
<td>3</td>
<td>774.65</td>
<td>40.68</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>lin$^2$</td>
<td>1</td>
<td>2241.39</td>
<td>117.70</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>quad$^3$</td>
<td>1</td>
<td>45.44</td>
<td>2.39</td>
<td>0.157</td>
</tr>
<tr>
<td>deviations</td>
<td>1</td>
<td>37.10</td>
<td>1.95</td>
<td>0.196</td>
</tr>
<tr>
<td>Residual</td>
<td>9</td>
<td>19.04</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>duration$^4$</td>
<td>4</td>
<td>571.74</td>
<td>27.53</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>lin</td>
<td>1</td>
<td>2014.16</td>
<td>97.16</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>quad</td>
<td>1</td>
<td>10.66</td>
<td>10.66</td>
<td>0.002</td>
</tr>
<tr>
<td>deviations</td>
<td>2</td>
<td>25.88</td>
<td>1.25</td>
<td>0.296</td>
</tr>
<tr>
<td>depth.duration</td>
<td>12</td>
<td>28.16</td>
<td>1.36</td>
<td>0.219</td>
</tr>
<tr>
<td>lin.lin</td>
<td>1</td>
<td>237.83</td>
<td>11.47</td>
<td>0.001</td>
</tr>
<tr>
<td>deviations$^5$</td>
<td>11</td>
<td>9.10</td>
<td>0.44</td>
<td></td>
</tr>
<tr>
<td>Residual</td>
<td>48</td>
<td>20.73</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>79</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD (p=0.05) = 6.5.

C.V. = 6.7%

$^1$depth of burial

$^2$linear effect

$^3$quadratic effect

$^4$duration of burial

$^5$where the interactions between the orthogonal components were non significant they were lumped into the deviation term.
Table 17 ANOVA table for germination response of seeds to different concentrations of ethephon in the laboratory at different temperatures (Fig. 5.1).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>m.s</th>
<th>v.r</th>
<th>F pr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>inctemp(^1)</td>
<td>3</td>
<td>37206.42</td>
<td>2374.17</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>conc(^2)</td>
<td>8</td>
<td>15500.99</td>
<td>989.13</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>illum(^3)</td>
<td>1</td>
<td>79.49</td>
<td>5.07</td>
<td>0.025</td>
</tr>
<tr>
<td>inctemp.conc</td>
<td>24</td>
<td>891.87</td>
<td>56.91</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>inctemp.illum</td>
<td>3</td>
<td>89.62</td>
<td>5.72</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>conc.illum</td>
<td>8</td>
<td>9.86</td>
<td>0.63</td>
<td>0.753</td>
</tr>
<tr>
<td>inctemp.conc.illum</td>
<td>24</td>
<td>25.12</td>
<td>1.60</td>
<td>0.042</td>
</tr>
<tr>
<td>Residual</td>
<td>216</td>
<td>15.67</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>287</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD (p=0.05) = 5.5

C.V. = 9.1%

\(^1\)incubation temperature

\(^2\)concentration

\(^3\)illumination
Table 18 ANOVA table for germination response of seeds to different concentrations of nitrate in the laboratory at different temperatures (Fig. 5.2).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f.</th>
<th>m.s</th>
<th>v.r</th>
<th>F p r</th>
</tr>
</thead>
<tbody>
<tr>
<td>inctemp&lt;sup&gt;1&lt;/sup&gt;</td>
<td>3</td>
<td>24433.10</td>
<td>1048.64</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>conc&lt;sup&gt;2&lt;/sup&gt;</td>
<td>5</td>
<td>1462.26</td>
<td>62.76</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>illum&lt;sup&gt;3&lt;/sup&gt;</td>
<td>1</td>
<td>1029.96</td>
<td>44.20</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>inctemp.conc</td>
<td>15</td>
<td>183.49</td>
<td>7.88</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>inctemp.illum</td>
<td>3</td>
<td>1210.40</td>
<td>51.95</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>conc.illum</td>
<td>5</td>
<td>33.82</td>
<td>1.45</td>
<td>0.209</td>
</tr>
<tr>
<td>inctemp.conc.illum</td>
<td>15</td>
<td>87.73</td>
<td>3.77</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Residual</td>
<td>143</td>
<td>23.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>190</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD (p=0.05) = 6.7
C.V. = 13.2%

<sup>1</sup>incubation temperature
<sup>2</sup>concentration
<sup>3</sup>illumination
Table 19 ANOVA table for germination of seeds treated with different concentrations of nitrate, ethephon and tri-allate in pots (Table 5.2 and 5.3).

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>d.f</th>
<th>m.s</th>
<th>v.r</th>
<th>F pr</th>
</tr>
</thead>
<tbody>
<tr>
<td>control(^1)</td>
<td>1</td>
<td>2064.71</td>
<td>47.59</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>control.rates(^2)</td>
<td>2</td>
<td>335.86</td>
<td>7.74</td>
<td>0.001</td>
</tr>
<tr>
<td>chem(^3)</td>
<td>2</td>
<td>1512.10</td>
<td>34.86</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>control.rates.chem</td>
<td>4</td>
<td>44.27</td>
<td>1.02</td>
<td>0.406</td>
</tr>
<tr>
<td>Residual</td>
<td>48</td>
<td>43.38</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>57</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

LSD (p=0.05) = 8.4 when comparing rates from each chemical.
C.V. = 4.8 when comparing rates from each chemical with control.

\(^1\)compares non-treated (control) with treated seeds irrespective of the chemical used or rate

\(^2\)Compares non-treated (control) with different rates irrespective of the chemicals applied

\(^3\)different chemicals applied
### Table 20 ANOVA table for seedling emergence in the field measured over time in control and

<table>
<thead>
<tr>
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LSD (p=0.05) = 6.8

C.V. = 10.2%

\(^1\)treatment

\(^2\)time of weed count

\(^3\)linear effect

\(^4\)quadratic effect
Table 21 ANOVA table for seedling emergence in the field measured over time in control and plots treated with nitrate or ethephon during the 1992 experiment (Fig. 5.3b).

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LSD (p=0.05) = 3.1
C.V. = 4.1%

\(^1\)treatment
\(^2\)time of weed count
\(^3\)linear effect
\(^4\)quadratic effect
AMARANTHUS RETROFLEXUS SEED DORMANCY AND
GERMINATION RESPONSES TO ENVIRONMENTAL FACTORS
AND CHEMICAL STIMULANTS

by

Elizabeth Nabwile Omami

B. Ag.Sc., University of Manitoba, Canada

A thesis submitted in fulfilment of the requirements for the degree of Master of Science (Honours) at the University of Western Sydney, Hawkesbury, School of Horticulture.
Richmond, N.S.W., 2753
AUSTRALIA

July, 1993
PLEASE NOTE

The greatest amount of care has been taken while scanning this thesis,

and the best possible result has been obtained.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>SUMMARY</strong></td>
<td>v</td>
</tr>
<tr>
<td></td>
<td><strong>DECLARATION</strong></td>
<td>viii</td>
</tr>
<tr>
<td></td>
<td><strong>ACKNOWLEDGEMENTS</strong></td>
<td>ix</td>
</tr>
<tr>
<td></td>
<td><strong>LIST OF FIGURES</strong></td>
<td>xi</td>
</tr>
<tr>
<td></td>
<td><strong>LIST OF TABLES</strong></td>
<td>xii</td>
</tr>
<tr>
<td>1.1</td>
<td>Preamble</td>
<td>1</td>
</tr>
<tr>
<td>1.2</td>
<td><em>Amaranthus retroflexus</em> - the weed</td>
<td>3</td>
</tr>
<tr>
<td>1.3</td>
<td>Seed dormancy and its significance</td>
<td>5</td>
</tr>
<tr>
<td>1.3.1</td>
<td>Changes in dormancy</td>
<td>8</td>
</tr>
<tr>
<td>1.4</td>
<td>Dormancy control</td>
<td>9</td>
</tr>
<tr>
<td>1.4.1</td>
<td>Light in dormancy regulation</td>
<td>10</td>
</tr>
<tr>
<td>1.4.2</td>
<td>Temperature in dormancy regulation</td>
<td>11</td>
</tr>
<tr>
<td>1.4.3</td>
<td>The role of chemical stimulants</td>
<td>16</td>
</tr>
<tr>
<td>1.4.4</td>
<td>Interacting factors</td>
<td>18</td>
</tr>
<tr>
<td>1.4.5</td>
<td>Dormancy regulation during seed development</td>
<td>20</td>
</tr>
<tr>
<td>1.4.6</td>
<td>Other factors</td>
<td>21</td>
</tr>
<tr>
<td>1.5</td>
<td>Aims of the project</td>
<td>22</td>
</tr>
</tbody>
</table>

**CHAPTER 2. EFFECTS OF LIGHT AND TEMPERATURE ON DORMANCY AND GERMINATION**

<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Introduction</td>
<td>23</td>
</tr>
<tr>
<td>2.2</td>
<td>General materials and methods</td>
<td>26</td>
</tr>
<tr>
<td>2.2.1</td>
<td>Seed source and preparation</td>
<td>26</td>
</tr>
<tr>
<td>2.2.2</td>
<td>Germination conditions</td>
<td>26</td>
</tr>
<tr>
<td>2.2.3</td>
<td>Treatment of data</td>
<td>27</td>
</tr>
<tr>
<td>2.3</td>
<td>Materials and methods</td>
<td>28</td>
</tr>
<tr>
<td>2.3.1</td>
<td>Effects of light and temperature</td>
<td>28</td>
</tr>
<tr>
<td>2.3.2</td>
<td>Effects of pre-chilling</td>
<td>29</td>
</tr>
<tr>
<td>2.4</td>
<td>Results</td>
<td>29</td>
</tr>
</tbody>
</table>
CHAPTER 3. EFFECTS OF STORAGE TEMPERATURE AND DURATION ON DORMANCY AND GERMINATION

3.1 Introduction

3.2 Materials and methods
   3.2.1 Long term storage
   3.2.2 Short term storage
   3.2.3 Treatment of data

3.3 Results
   3.3.1 Long term storage
   3.3.2 Short term storage

3.4 Discussion

CHAPTER 4. CHANGES IN DORMANCY AND VIABILITY AS AFFECTED BY DEPTH AND DURATION OF BURIAL

4.1 Introduction

4.2 Materials and methods
   4.2.1 Experimental design
   4.2.2 Seed preparation and burial in the field
   4.2.3 Seed retrieval and germination test
   4.2.4 Data treatment

4.3 Results
   4.3.1 In situ germination
   4.3.2 Germination in the laboratory
   4.3.3 Germination of ethephon treated seed
   4.3.4 Germination of non-physiologically dormant seed
   4.3.5 Total germination (Viability)

4.4 Discussion
   4.4.1 In situ germination
   4.4.2 Germination in the laboratory
   4.4.3 Seed viability
   4.4.4 Dormancy changes
CHAPTER 5. EFFECT OF CHEMICAL STIMULANTS ON DORMANCY AND GERMINATION OF A. RETROFLEXUS SEED

5.1 Introduction 71

5.2 Materials and methods 72
   5.2.1 Laboratory studies 72
   5.2.2 Pot experiment 73
   5.2.3 Field studies 74

5.3 Results 76
   5.3.1 Laboratory study 76
   5.3.2 Pot experiment 80

5.4 Discussion 82
   5.4.1 Laboratory studies 84
   5.4.2 Pot experiment 87
   5.4.3 Field studies 88

CHAPTER 6. GENERAL DISCUSSION

6.1 Effects of light and temperature on seed dormancy 90
6.2 Effects of storage environment on seed dormancy and longevity 91
6.3 Effect of chemical stimulants on dormancy 94
6.4 Concluding remarks 95

REFERENCES CITED 97

APPENDIX 110
SUMMARY

A large number of weed seeds in the soil persist because of seed dormancy. This poses a continuing weed control problem. For any control measure to be effective in the long term it must greatly reduce the weed seed population in the soil. Depletion of the seed bank through the manipulation of seed dormancy has been suggested as one of the ultimate goals in weed control. An understanding of factors that regulate dormancy and germination are essential before such an approach can prove effective. This study was designed to investigate the effects of some of the factors which control dormancy and germination in Amaranthus retroflexus seeds. Treatments were also designed to evaluate the influence of known chemical stimulants in stimulating germination.

Germination studies were conducted at different temperatures, and either in continuous white light or in the dark. Incubation temperatures significantly affected germination of A. retroflexus seeds. Seeds germinated to 5, 16 and 19% at constant temperatures of 20, 35 and 40°C in the light, respectively. A significant increase in germination to 65 and 66% was observed when seeds experienced a temperature shift treatment of 12°C for 14 days followed by 35°C for the next 14 days, or 20°C followed by 35°C, respectively. Although light significantly interacted with temperature, its effect on germination varied with the incubation temperature. Seeds germinated to significantly higher percentages in the light than in the dark at a constant temperature of 35°C and at temperature shift of 12°C followed by 35°C. No differences occurred in germination due to light at other temperatures.

Seeds were also subjected to different pre-chilling treatments at 0, 12 and 20°C for up to 12 days. At a regular interval they were tested for germination at different temperatures (35°C or at an alternating temperature regime of 20/30°C) either in the
light or in darkness. Seeds germinated to significantly higher percentages at 35°C than at 20/30°C, and generally higher in the light than in the dark. Pre-chilling at 12°C produced the most effective response. Pre-chilling at 0°C and incubation at 20/30°C induced dormancy in seeds.

In an attempt to determine the changes in dormancy during dry storage, two lots of *A. retroflexus* seeds, harvested in two consecutive years (May 1991 and 1992), were stored dry at different temperatures between 0 and 36°C. At regular intervals, germination tests were conducted at different temperatures in the light. In both seed lots, the loss in dormancy increased significantly with an increase in storage temperature and duration. The time required for maximum germination, however, varied depending upon the seedlot. The 1992 seedlot was less dormant, and total germination at 35°C of seeds stored at 36°C was 68% after four weeks storage as compared to only 29% for the 1991 seedlot. Seeds germinated to significantly higher percentages at high temperatures. However, storage at high temperatures and for prolonged duration resulted in seeds gaining the ability to germinate at lower temperatures.

Changes in dormancy in seeds under field conditions were also examined. Seeds were buried at different depths (0 to 10 cm) and for different duration (1 to 12 months) to determine changes in viability and dormancy. Both surface-sown and buried seeds lost viability with time but this loss was greater in surface and shallowly buried seeds. Depletion of the seed bank of surface-sown seeds was mainly through *in situ* germination, and those seeds that did not germinate were induced into dormancy. *In situ* germination of deeply buried seeds was very low, however, seeds germinated readily after retrieval. Changes in dormancy occurred during the one-year burial period of this study. Dormancy was broken during cold periods of the year and induced as warmer periods progressed.
The effects of chemical stimulants on dormancy and germination of *A. retroflexus* were investigated under controlled environment conditions as well as in the field. In laboratory tests, the response of seeds to ethephon concentrations of between 0.0001 and 1000 µL/L and to nitrate concentrations of between 0.002 and 0.5% were assessed at different temperatures either in continuous white light or in the dark. Under all experimental conditions, germination increased significantly with concentration. Stimulation of germination was greater at higher temperatures, but a greater response was observed at lower temperatures. The response to light varied depending upon temperature.

In pot trials each rate of nitrate (25, 34, 100 kg/ha) and ethephon (5.5, 11, 22 kg/ha) significantly stimulated germination over that of the control but there were no differences in stimulation among the rates. Only one rate of tri-allate (0.6 kg/ha) was effective. Results from the field experiments repeated in two consecutive years showed that ethephon (11 kg/ha) and nitrate (34 kg/ha) treatments significantly stimulated emergence over that of the control.
DECLARATION

I hereby declare that this work has not been submitted for a higher degree to any other university or institution

Elizabeth N. Omami
ACKNOWLEDGEMENTS

Many thanks to my sponsors, AIDAB (Australian International Development Assistance Bureau), without whom this study would not have been undertaken. The helpful discussions and support from my supervisors Dr A.M. Haigh and Dr R.W. Medd during the research and through the preparation of this thesis are acknowledged. I am grateful for the friendly and supportive environment created by the staff of the School of Horticulture and elsewhere in the University.

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I would also like to acknowledge the assistance of my colleagues, particularly to kukhu Nasambu Khaemba and Genevieve Stewart for assisting me at one moment in counting seeds and in proof reading the final manuscript; to Magda Hribar and Sumardi for their fruitful help with the word processing program on the computer; to Jenny Jobling for her very productive tutorial on Coplot; to Silke Ullrich for providing me with bits and pieces of very essential information and in proof reading a draft of the final document; to kuka Francis Maloba for his care and help during the typing of the manuscript, orio koo, and. to all those who assisted me in one way or another, sincere thanks.
Last, but not least, sincere thanks to my family for their encouragement and for patiently waiting for me to complete my studies. Above all, I am very grateful to Almighty God who made all these things possible.
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Dormancy and germination.</td>
<td>6</td>
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<tr>
<td>1.2</td>
<td>Changes in dormancy.</td>
<td>9</td>
</tr>
<tr>
<td>2.1</td>
<td>The effect of light and temperature on dormancy and germination.</td>
<td>30</td>
</tr>
<tr>
<td>2.2</td>
<td>The effect of pre-chilling on the germination of <em>A. retroflexus</em> seeds incubated at a constant temperature of 35°C or at an alternating temperature (20/30°C) in the light or in darkness.</td>
<td>33</td>
</tr>
<tr>
<td>3.1</td>
<td>Germination after the first 14 days incubation of <em>A. retroflexus</em> seeds stored between 1 and 12 months.</td>
<td>42</td>
</tr>
<tr>
<td>3.2</td>
<td>Germination from day 14 to 28 of incubation of <em>A. retroflexus</em> seeds stored between 1 and 12 months.</td>
<td>44</td>
</tr>
<tr>
<td>3.3</td>
<td>Total germination 28 days from the start of incubation of <em>A. retroflexus</em> seeds stored between 1 and 12 months.</td>
<td>45</td>
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<tr>
<td>3.4</td>
<td>Germination after the first 14 days of incubation of <em>A. retroflexus</em> seeds stored for up to 12 weeks.</td>
<td>47</td>
</tr>
<tr>
<td>3.5</td>
<td>Germination from day 14 to 28 of incubation of <em>A. retroflexus</em> seeds stored and tested at a weekly interval for 12 weeks.</td>
<td>49</td>
</tr>
<tr>
<td>3.6</td>
<td>Total germination after 28 days of incubation of <em>A. retroflexus</em> seeds stored and tested at a weekly interval for 12 weeks.</td>
<td>50</td>
</tr>
<tr>
<td>4.1</td>
<td><em>In situ</em> germination of buried <em>A. retroflexus</em> seeds.</td>
<td>60</td>
</tr>
<tr>
<td>4.2</td>
<td>Germination in the laboratory of <em>A. retroflexus</em> seeds retrieved from the field.</td>
<td>61</td>
</tr>
<tr>
<td>4.3</td>
<td>Germination of retrieved <em>A. retroflexus</em> seeds after treatment with ethephon.</td>
<td>63</td>
</tr>
<tr>
<td>4.4</td>
<td>Germination of non-physiologically dormant seeds of <em>A. retroflexus</em>.</td>
<td>64</td>
</tr>
<tr>
<td>4.5</td>
<td>Total germination of <em>A. retroflexus</em> seeds after burial.</td>
<td>65</td>
</tr>
<tr>
<td>4.6</td>
<td>Germination of <em>A. retroflexus</em> seeds in relation to seasonal fluctuations in temperature during the burial experiment.</td>
<td>69</td>
</tr>
<tr>
<td>5.1</td>
<td>Germination response of <em>A. retroflexus</em> seed to different concentrations of ethephon.</td>
<td>78</td>
</tr>
<tr>
<td>5.2</td>
<td>Germination response of <em>A. retroflexus</em> seed to different concentrations of nitrate.</td>
<td>79</td>
</tr>
<tr>
<td>5.3</td>
<td>Field emergence of <em>A. retroflexus</em> seedlings in control and plots treated with either nitrate or ethephon.</td>
<td>83</td>
</tr>
</tbody>
</table>
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1</td>
<td>Mean of <em>in situ</em> germination of <em>A. retroflexus</em> seed after varying durations of burial.</td>
<td>61</td>
</tr>
<tr>
<td>5.1</td>
<td>Rates of nitrate, ethephon and tri-allate applied in pots.</td>
<td>74</td>
</tr>
<tr>
<td>5.2</td>
<td>Germination stimulation of <em>A. retroflexus</em> seed by different chemicals.</td>
<td>81</td>
</tr>
<tr>
<td>5.3</td>
<td>Germination stimulation of <em>A. retroflexus</em> by different rates of chemicals used.</td>
<td>81</td>
</tr>
</tbody>
</table>