STUDIES ON THE GERMINATION AND DORMANCY OF *POLYGONUM PERSICARIA* L.

by

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TO MY PARENTS

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Abstract

Studies on the germination and the nature of dormancy of achenes of Polygonum persicaria were carried out.

The release from dormancy was investigated by physical and chemical treatments, environmental factors (temperature and light) and the application of plant hormones.

The imposition of dormancy on non-dormant achenes was examined by prolonged dark incubation at supraoptimal temperature, and also by drying treatments.

The physical (surgical) treatments, such as cutting the tip of achenes or removal of the pericarp, promoted germination of unchilled achenes, while chemical treatments with calcium hypochlorite, and nitrogenous compounds such as nitrates, thiourea, hydroxylamine hydrochloride, and respiratory inhibitors like cyanide and azide, failed to break the dormancy of unchilled achenes. However, unchilled achenes responded positively to treatment with nitrite and hydrogen peroxide, giving an enhanced and significant germination.

Treatment with environmental factors such as temperature and light (white or red) on unchilled achenes illustrated that the population consists of at least four physiological polymorphic components, namely, Dark; Light; Alternating Temperature (dark), and Alternating Temperature plus Light sub-populations.

Stratification treatment resulting in complete dark germination when transferred to higher temperature is more effective at 4°C than 10°C. Germination is enhanced at alternating temperatures in the dark in contrast to constant temperatures. However, a high temperature of 40°C is lethal for germination.

Exogenous application of GA_3, GA_{4+7}, ethylene or nonanoic acid (C9) did not influence the germination of unchilled achenes. In contrast, kinetin

appears to promote germination. Moreover, combined treatments of red light and kinetin with or without GA_3 gave the greatest germination. Results from applying GA_3 during or after stratification did not indicate the involvement of gibberellin during or after chilling. Also, treatment with growth retardants (B995, CCC, AMO 1618, and Ancymidol) argue against gibberellin involvement during or after stratification. The results of applying ABA during stratification or germination indicated that growth processes, i.e. radicle elongation, are inhibited and not germination sensu stricto.

The results of prolonged dark incubation at supra-optimal temperature at 40°C indicated that the effect could be considered as thermoinhibition rather than thermodormancy. However, drying treatment induces secondary dormancy.

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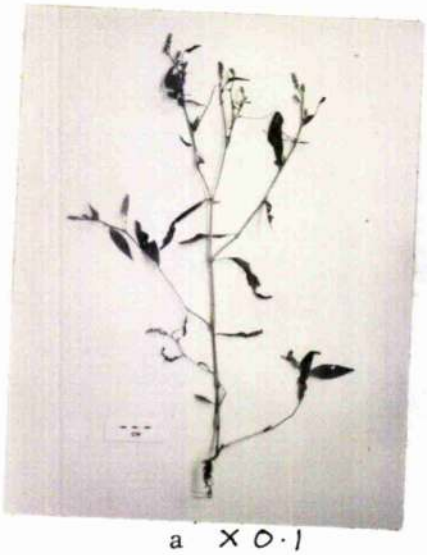


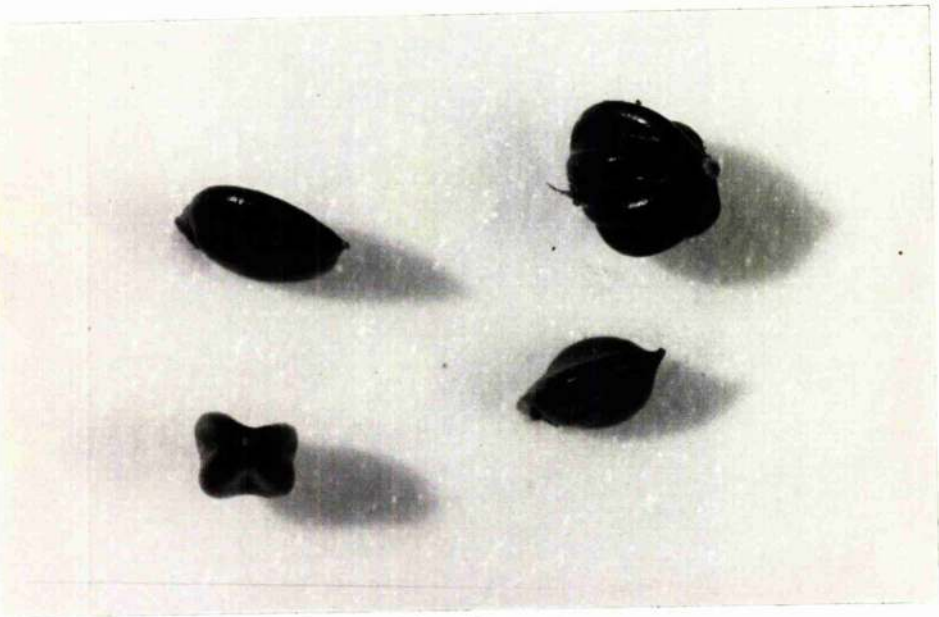
a × 0·1

b × $\frac{2}{3}$

a : A whole plant of Polygonum persicaria.

b : A leaf of P. persicaria with dark patch on the upper surface.

Plate 2



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Different morphological types of achenes of P. persicaria.

CHAPTER 1

GENERAL INTRODUCTION

MORPHOLOGICAL DESCRIPTION

Polygonum persicaria L. is a weed species of temperate zones. It is known by various names ranging from Smartweed in U.S.A., to Redshank, Willowweed and Ladysthumb in Britain (Brenchley, 1920; Clark and Fletcher, 1923; Muenscher, 1955; Percival, 1913; and Salisbury, 1961).

It is a summer, herbaceous annual with erect stems which are somewhat fleshy and glabrous. The height of the plant varies depending on the surrounding plants or crops. In an open field with unshaded sunlight, the average height of the plant could be twenty (20) to thirty (30) cm, whilst amongst potatoes, the height could be as tall as fifty (50) to seventy (70) centimeters.

The leaves are lanceolate, pointed and nearly stalkless, often with a dark purplish patch on the upper surface. It has simple, smooth and alternately arranged leaves. At the base of the leaves, there is a membranous structure called the ochrea, the nature of which may be disputed but it is considered to be modified stipules (see Plate 1 a&b).

The flowering branches carry inflorescences which are short dense erect cylindrical spikes. The flowers have a pink or dark purple perianth, each producing a single seeded fruit. The achenes† consist mainly of two morphological types, trigonal and biconvex in shape, but a third shape "tetragonous" occurs in small number (Hammerton, 1967; Hammerton and Jalloq, 1970). The average weight of the achene is about 2mg and the average size is approximately 2mm in diameter. The colour is shiny black (Plate 2). The number of achenes produced per plant varies greatly, and ranges from 200-800 to 1,200 (Simmonds, 1945-46), though values as high as 6,650 have been reported with an average value of 4,470 achenes (Hammerton and Jalloq, 1970). This production represents a potentially high reproductive

capacity, but the actual reproductive capacity depends also on germination and the purpose of this study was an investigation of germination behaviour.

Morphological descriptions of P. persicaria L. are given in the following references (Bentham, 1865; Butcher, 1961; Forsyth, 1968; Gill and Vear, 1958; Fitter et al., 1974; and Simmonds, 1945-1946).

†Achene is used here to indicate the dispersal unit of P. persicaria and denote the intact fruit or nut. Technically speaking, an achene is a dry indehiscent fruit derived from one carpel, while the gynoecium of P. persicaria has two-three carpels and should be regarded as a nut, but other workers have used the term achene. However, seed is used to denote the dispersal unit of other species mentioned.

HABIT AND HABITAT

Habit appears to be affected by crowding, and the plants more often than not aggregated in the field. This behaviour might confer competitive advantage to the species against neighbouring plants.

Solitary plants are seen in an unshaded, open field, often branched from the base, with the branches spreading and ascending. However, the plant is most commonly found in damp and disturbed environments such as arable land, waste places, roadsides (Muenscher, 1955; Salisbury, 1961). Polygonum persicaria has been classified as a wetland hydrophytic weed (King, 1966) and thrives best in areas with plentiful supplies of water such as water-logged places, marshes, bogs, and streamsides (Brenchley, 1920; Thompson et al., 1977). It is associated with less calcareous soils (Salisbury, 1961); peaty and boggy soils (Brenchley, 1920); damp clay and loamy soils (Staniforth and Cavers, 1979a), and absent from chalky well drained soils (Brenchley, 1920).

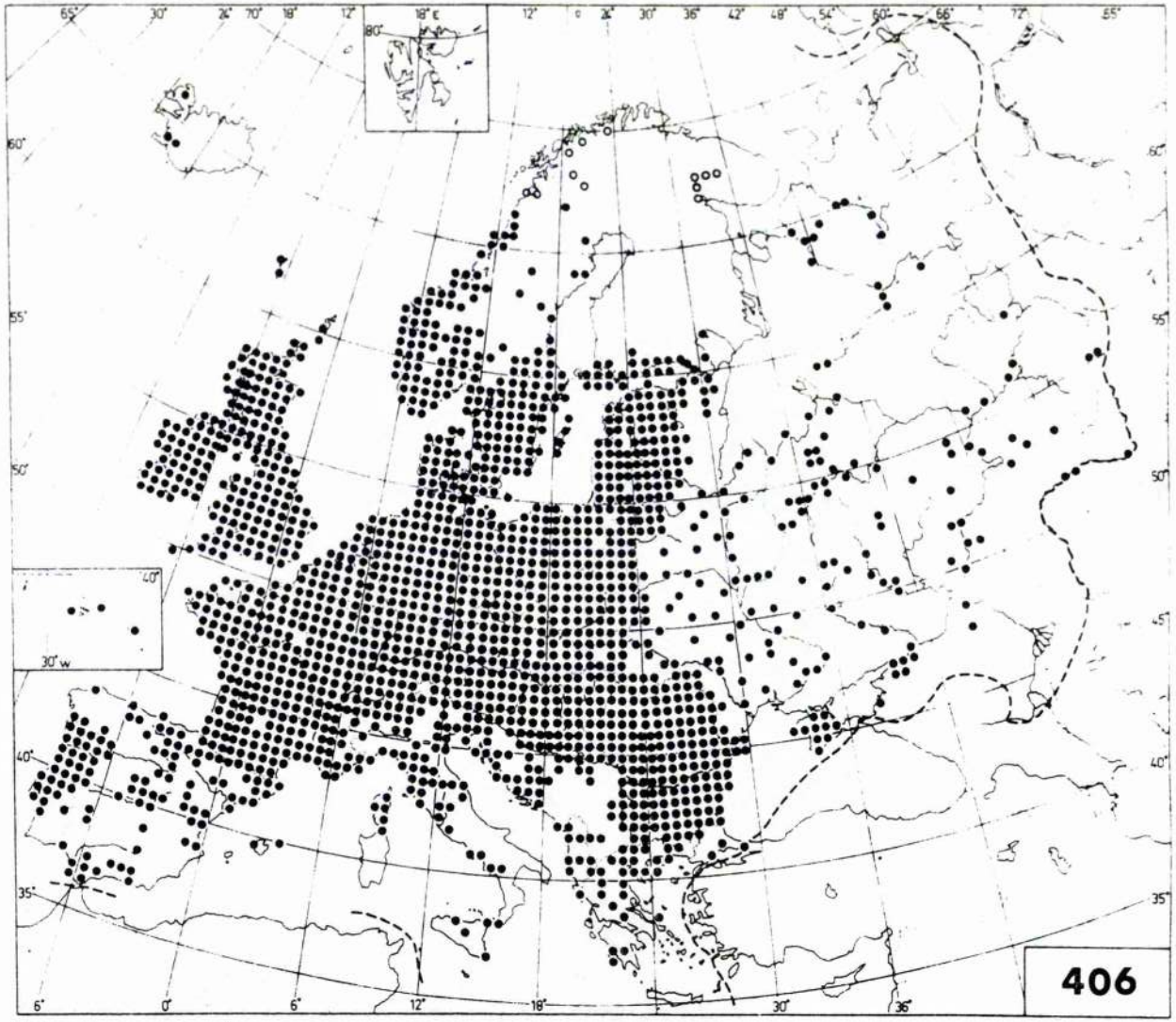
Geographical Distribution Map of

P. persicaria plant.

(Taken from Atlas Florae Europaeae -

(4) Polygonaceae ed. Jaakko and

Juha Suominen 1979)



Polygonum persicaria

GEOGRAPHICAL AND ALTITUDINAL DISTRIBUTION

The geographical distribution of Polygonum persicaria is shown on the opposite page. It is worth noting that P. persicaria is widely distributed throughout Europe. Simmonds (1945-46) noted that the plant also occurs in India, Japan, Australia, North Africa (extending from Egypt to Morocco) and also in North America. This distributional spread would be reflected in the germination behaviour. Thus the achenes are likely to possess multiple mechanisms for breaking of dormancy.

The altitudinal distribution varies widely, growing at 411m in Aberdeen (Scotland); 800m in Graubünden (Europe) to 4260m in Western Tibet (Hooker, 1884; Simmonds 1945-46). The habitat of the parent plant might, as well as the geographical locality, influence the behaviour of achenes. These possibilities should be borne in mind when comparing the results of different workers.

ECONOMIC IMPORTANCE

Polygonum persicaria is commonly found amongst arable plants, e.g. cereals, row crops etc. (Clark and Fletcher, 1923; Gill and Vear, 1958; Staniforth and Cavers, 1979a; and Woodford and Evans, 1963). It has been described as "serious" (Simmonds, 1945-46), "aggressive" (Staniforth and Cavers, 1977), and "worst" (Pitt in Salisbury, 1961) weed of farm land. Witts (1960) observed that P. persicaria was more abundant in arable fields than other Polygonum species because some of its achenes would germinate after herbicide treatment had destroyed the entire seedling population. It is also known to be resistant to selective herbicides, but partial control has been obtained with 2,4-D applied with a wetting agent at the seedling stage (Salisbury, 1961). The achenes have been eaten as food (Schery, 1954). In fact, Potts (1970) noted that they are a better source of nitrogen and calcium than barley grains. The plant has been used as dye (maybe because of its reddish coloration at the early seedling stage) before the advances

made in connection with chemical dyes (Brenchley, 1920). It is economically important because of the potential reduction in yields.

VIABILITY

The following authors have reported about the viability of Polygonum persicaria (see table below).

RETENTION OF VIABILITY	AUTHOR	REMARKS
45 years	Salisbury (1961)	20 years fruits produce seedlings.
30 years	Simmonds (1945-46)	Retained viability for 30 years.
20 years	Toole and Brown (1946)	9 percent seedlings produced. Continuation of Duvel's (1905) work.

Roberts (1972) noted that pronounced dormancy and a long period of potential viability are two attributes essential for the success of any weed species. P. persicaria achenes might be said to possess these attributes.

GERMINATION

Germination may be regarded as the events immediately following imbibition and prior to radicle protrusion (Berrie and Drennan, 1971; Toole et al., 1956). Germination can be divided into a reversible phase I and an irreversible phase II. The events in phase I have been reported not to be affected by hydration-dehydration processes (Berrie and Drennan, 1971) while such treatments, if given in phase II, might kill the seed. Thus, germination in phase I might be regarded as the actual germination (sensu stricto), while phase II is growth. Côme and Thevenot (1982) reported that several workers correlated different stages of germination with separate steps in water uptake and respiratory intensity. Respiratory, glycolysis and Kreb's cycle inhibitors have been used to differentiate germination sensu stricto and growth. When seeds kept in the presence of these inhibitors for a few hours are removed to water, germination is stimulated, suggesting that

germination sensu stricto might be linked to the oxidative pentose phosphate pathway, while growth involves the normal respiratory pathway (Côme and Thevenot, 1982).

The protrusion of the radicle is used to score germination, but many of the biochemical changes seen, e.g. the production of α-amylase are considered as post-germination phenomena (Drennan and Berrie, 1962).

The stimulus triggering "germination proper" may probably lie between the onset of imbibition and cell elongation/division, while the site of control might range from subcellular, e.g. phospholipid membrane, DNA, or could be associated with specific tissues, e.g. the testa or even organs such as the embryonic axis.

DORMANCY

Dormancy has been defined or described by several authors (Amen, 1968; Evenari, 1965; Karssen, 1982; Roberts, 1972; and Vegis, 1964). This has given rise to a catalogue of definitions. It may suffice here to describe it rather than attempt to define it.

Viable seeds when placed in unfavourable (harsh) germination conditions, e.g. where the temperature is unsuitable or oxygen tension limiting, they will not germinate, not because the seeds are dormant but because the condition under which they are incubated will not allow germination. Such a seed may be said to be quiescent and would promptly germinate if returned to conditions permitting the normal physiological activities of the seed. However, if such a seed is kept long enough in the harsh environment, it may not germinate when returned to conditions suitable for germination. In this case, the seed is either dormant or dead. If dormant, it may require special treatments like low temperature incubation for a specific period or red (R) irradiation to potentiate germination and overcome the block imposed by the harsh environment. This is known as induced or secondary dormancy.

Seeds of many temperate species when freshly harvested will not germinate (even though viable) in physiological conditions promoting germination, and may require a special treatment such as red (R) light, temperature shift or low-temperature pretreatment (stratification) to germinate. Again, these seeds are said to possess dormancy but this is primary or innate and not induced. In some other seeds, the barrier to germination is imposed by factors such as burial, the removal of such block promptly allowing germination, such a seed is in a state of imposed or enforced dormancy. Enforced dormancy can be distinguished from induced or innate dormancy by the seeds germinating on being removed from the specific conditions to those which will allow germination. In a sense this is more like quiescence than dormancy. Vegis (1964) has been able to observe different periods of dormancy which he designated as pre, true, and post dormancy. He reported that during predormancy, factors relieving dormancy become less effective and the seed or bud enters a state of deep dormancy. This deep dormancy period passes into a post dormancy period which is characterised by dormancy being broken over a wider range of values of the specific dormancy-breaking factor. This last phase continues until seeds are no longer dormant and germination can occur in non-specific conditions.

The factors imposing, maintaining and relieving dormancy can essentially be separated into a genetic component and the complex interaction of environmental factors.

Genetic Component

The inability of the dormant embryos of Avena fatua to utilize the endosperm under standard germination conditions in contrast to non-dormant species has been attributed to repression of the genome governing elongation growth (Andrew and Simpson, 1969). Naylor and Fedec (1978) reported that in this species some pure lines germinate in the temperature range of 4°C-32°C, while others exhibit strong suppression of germination in the mid portion of

this range. Recently, Upadhyaya et al. (1983) reported in wild oats that sodium azide enhanced alternate respiration in a number of pure lines including Montana 73 by stimulating oxygen uptake, but failed to stimulate germination in Montana 73, unlike the other pure lines. They attributed the differential response to genetic diversity in the control of seed dormancy in Avena fatua. The differences amongst local populations in germination behaviour was attributed partly to genetic adaptation and to agronomic practices. This would indicate that there is genetic plasticity.

It has been shown that in two lines of Grand Rapids lettuce seeds, response to red (R) and far red (FR), the ability to overcome resistance of an external osmotically active medium, and response to abscissic acid (ABA) and gibberellin differ (Globerson et al., 1973).

There is no firm evidence relating to other species but it can be assumed from these two cases that there is likely to be a significant genetic contribution to the expression of dormancy and germination behaviour.

Environmental Factors

Environmental factors play an important role in the dormancy of wild seeds and perhaps for all the wild species investigated so far. While no factor could be said to be most important, light and temperature play a very important role in the regulation of germination and dormancy.

During the course of maturation, the seed would be exposed to variations of temperature, light (quality, quantity and photoperiod) and moisture effect. Any one of these or an interaction amongst them could affect the physiological status of the seed at maturity. The consequence of this would be imposition of dormancy on the seed during maturation. The nature (type) of dormancy would be reflected in the environmental factors operating during maturation. However, dormant seeds are not classified subject to inherent features of the dormant state, but are classified under the factors that break dormancy. Consequently a seed whose dormancy is a property of many factors might be

classified under many headings. The factors imposing dormancy could be seed coverings, temperature, light, or hormonal e.g. ABA. Also dormancy could be relieved by removal of seed coverings, stratification treatment, Red (R) light treatment or hormonal treatment such as gibberellic acid. Chemical treatments e.g. sodium azide, hydroxylamine or sulphuric acid could also relieve dormancy.

These factors will be discussed below.

SEED COVERING EFFECT

Scarification Treatments

The seed coverings which include pericarp, testa and maybe endosperm (but this may act as a storage tissue) may function to regulate germination by offering physical resistance to embryo growth. It may also establish a permeability barrier and might interfere with processes like water uptake during imbibition or regulate gaseous exchange. In such cases the seeds could be regarded as being hard. Seed coverings might impose dormancy by preventing leaching out of the inhibitors from the seed or may even contain inhibitors themselves, and germination is expressed when rainfall leaches out the inhibitors from the seed coverings. Dormancy could be alleviated by treatments that result in opening up or weakening the seed coverings, such as pricking the seed coat, removal of the seed coat, or excision of the embryo. The promotive effects of these physical treatments may be mimicked by weakening of the seed covering with chemicals, e.g. sulphuric acid.

The promotive effects of some physical treatments in breaking dormancy of some species are tabulated below.

Treatments	Species	Author
Incision	Avena fatua	Black, 1959
Scratching, pricking and cutting the seed coat	Betula spp.	Black and Wareing, 1959
Scarification	Striga lutea (witchweed)	Egley, 1972
Moistening and drying, oven-dry, scarification	Eragostis lehmanniana (Lehmann lovegeass)	Hafarkamp et al., 1977
Puncturing, removing of coverings, excision of embryos	Avena fatua	Hart and Berrie, 1966
Puncturing	Avena fatua	Hay, 1962
Hot-water soak, sonication	Barnyardgrass, Redroot pigweed, Quackgrass, Prickly sida, velvet leaf	Holm and Miller, 1972
Sodium hypochlorite (NaOCl)	Polygonum convolvulus and Saponaria vaccaria	Hsiao, 1979
Ethanol	Panicum dichotomiflorum, P. capillare	Taylorson and Hendricks, 1979

Hard Seeds

Seeds which are exposed to standard germination conditions may not germinate, not because they are dormant, but because the seed coat is hard and may be impermeable to water or metabolically active gases such as oxygen and/or carbon dioxide. Seed coats may also offer differential resistance to O_2 and CO_2, so there may arise within the seed a gas mixture which might prevent germination. Hard seeds are found amongst the Leguminosae.

The seed coat has been demonstrated to be impermeable to water in Rhus

ovata (Stone and Juhren, 1951) and dormancy in cotton seed is caused by a blockage of the chalazal cap (Christiansen and Moore, 1959). The barrier to permeation of water in the seed coats of Pisum elatius, P. fulvum and P. humile might be the continuous pectinaceous cap of the palisade coupled with quinones. In contrast, in P. sativum, in which water uptake is not impeded, the cap and quinones are discontinuous, or missing, in the palisade cells (Werker et al., 1979). However, there are many seeds in which dormancy is not regulated or controlled by the coverings preventing the entry of water (Black, 1959; Egley, 1972; and Hadas, 1976).

Seeds of witchweed (Striga lutea) which had been scarified, germinated equally well in air or nitrogen and the block to germination was not due to impermeability to oxygen (Egley, 1972). Lehmann lovegrass (Eragostis lehmanniana) seeds, oven-dried/moistened or scarified, responded well in an atmosphere of 10 and 20 percent oxygen (Haferkamp et al., 1977), and the increased germination may be due to improved seed coat permeability or to a change in the metabolic state of the seed. Oxygen tension above 50 percent could be toxic to Betula species with the pericarp and endosperm slit (Black and Wareing, 1959). The hull of Avena fatua does not prevent oxygen uptake (Black, 1959). Difference in dormancy between lower and upper seeds of cocklebur (Xanthium pennsylvanicum) is not due to restriction of oxygen uptake by seed coverings, but to an inhibitor present in the upper dormant seeds which is leached out in high oxygen tension (Wareing and Foda, 1957).

High concentrations of carbon dioxide, about 30 percent, prevented germination in Betula spp (Black and Wareing, 1959). However, carbon dioxide has also been shown to stimulate germination, about 1-10 percent for basal peanut seed (Toole et al., 1964), 3 percent in Avena fatua (Hart and Berrie, 1966), but concentrations as high as 20 percent inhibited germination. These concentrations do not arise in the soil atmosphere where maximum values recorded do not exceed 4 percent CO_2 or 21 percent O_2 (Karssen, 1980/81b;

Russell, 1966). However, the concentration of CO_2 that could develop within a seed, in the absence of efflux, may be higher than 1 percent but is unlikely to reach the high values used experimentally.

Inhibitory Effect of Seed Coverings

The view that the seed coverings contain inhibitors and these may play a role in the maintenance of dormancy was rejected in Avena fatua (Hay, 1962) and Rhus ovata (Stone and Juhren, 1951). The same conclusion was reached for Striga lutea (Egley, 1972). However, in Betula species, the mechanism involved in relieving dormancy may lie in the interaction between oxygen concentration and any inhibitor present, which can also be removed by prolonged leaching (Black and Wareing, 1959). The ecological significance of the presence of inhibitors is that germination is restricted to favourable environmental conditions like the onset of rainfall which would leach out inhibitors (Mayer and Poljakoff-Mayber, 1982).

TEMPERATURE EFFECT

Vegis (1964) postulated that relatively high temperature and limited supply of oxygen to the embryo imposed by the covering tissue are the causes of dormancy. Koller (1962) observed that increased (Higher) temperature during maturation of Grand Rapid seeds (lettuce) induces higher germination in the seeds in comparison with seeds that matured at lower temperature. He also observed that maturation conditions did not influence the qualitative response but enhanced the quantitative response of the seeds.

Lower temperatures have been reported to produce seeds with deeper dormancy and lower germinability than higher temperatures in Aegilops ovata (Gutterman, 1980/81); in Beta vulgaris (Norstog and Klein, 1972); and in Rosa species (Von Abrams and Hand, 1956). Seeds of Anagallis arvensis showed different degrees of dormancy under different thermoperiods (Grant-Lipp and Ballard, 1963).

Low Temperature

Low temperature pretreatment, referred to as stratification or chilling, is essentially soaking the achenes in water at low temperatures of between 1°C to 10°C for a period of time before subsequent transfer to higher temperature where germination is expressed. It has been reported that germination will not normally occur at this low-temperature pretreatment (Vincent and Roberts, 1979), this being outside the range for normal physiological activities. However, Thevenot et al. (1977) in apple (Pyrus malus) and Webb and Dumbroff (1969) in sugar maple (Acer saccharum) reported that stratification temperature is the same as germination temperature, that is 5°C. Thevenot et al. (1977) further demonstrated that highest percentage germination occurred at 5° C in contrast to seeds which were transferred to higher temperatures of 10°, 15°, 20° and 25°C.

The ecological importance of this phenomenon is that in temperate climates, the achenes lie dormant during the unfavourable cold winter months in the soil (seedlings might die if they germinate), and only germinate in the spring or summer months when environmental conditions suitable for physiological activities prevail. Thus, low temperature pretreatment as well as breaking dormancy, senses the seasons and may act as a time-detecting mechanism. This low-temperature pretreatment phenomenon has been put into agricultural practice by the farmers and has been documented as far back as 1664 by Elvyn and others (Stokes, 1965).

The requirements for low temperature pretreatment are availability of moisture and air, and excessive moisture may reduce access to air and hence reduced germination (Stokes, 1965). Webb and Dumbroff (1969) reported in Acer saccharum that oxygen was not a limiting factor to low temperature pretreatment but they noted that 100 percent oxygen decreased germination. These workers also observed that when seeds are kept imbibed at 5°Cfor 24h, and then stratified in dry vials at 5°C, germination was not prevented, hence only small amounts of water are required for stratification.

Substantial lists of seeds requiring low temperature pretreatment are given by Black (1980/81) and Wareing (1969). Bewley and Black (1982) also noted their optimum temperature and duration required to break dormancy. The stimulatory effect of low-temperature pretreatment is demonstrated by seeds with different types of response which correlates with depth of dormancy.

In embryo-dormant seeds, e.g. apple seeds (Flemion, 1959), the duration of pretreatment might be very long and if the seeds are forced to germinate without the low temperature pretreatment, the resulting seedlings might be dwarf. Normal growth could be resumed if seedlings are stratified. Côme (1980/81) reported in apple embryo that incubation at 15-20°C for 2-3 weeks in a medium in pure nitrogen breaks dormancy. Removal of covering structures might shorten the pretreatment period. Frankland and Wareing (1966) reported in hazel seeds (Corylus avellana) that intact seeds required 12 weeks of chilling, whereas the embryo with the covering removed will germinate after 4 weeks of chilling. Intact seeds of Acer saccharum needed about 60 days of stratification to break dormancy, while removal of pericarp or pricking the seed decreased the stratification period by 43 days and 23 days respectively (Webb and Dumbroff, 1969).

In the case of seeds with coat-imposed dormancy, normal germination can be attained without stratifying the seeds, but stratification widens the germination conditions. The duration of pretreatment need not be too long, e.g. 42 days at 1.5°C breaks dormancy in Rumex crispus (Totterdell and Roberts, 1979). Also Brown and Van Staden (1973) reported 30 days stratification is sufficient to remove coat imposed dormancy in seeds of Leucadendron daphnoides.

Totterdell and Roberts (1979) investigated the stratification effect on Rumex species. They reported that two opposing events occurred during stratification, namely a rapid loss of innate dormancy which is independent of both light and temperature, and a slower induction of secondary dormancy which is temperature-dependent. Short exposures within the temperature range

of 1.5 - 15°C stimulate loss of dormancy, 1.5°C being most effective, but with longer periods, stratification becomes less and less effective and this fall is thought to be due to secondary dormancy, which sets in more rapidly at 10°C than at 1.5°C. In the light, secondary dormancy is apparently deferred, and also the optimum temperature for stratification depends more on the induction of secondary dormancy than on the process leading to loss of dormancy (Totterdell and Roberts, 1979). Capsella bursa-pastoris exhibited secondary dormancy after 7 days of chilling (Roberts and Benjamin, 1979). However, this induction of secondary dormancy by chilling is not universal. Indeed, Vincent and Roberts (1979) failed to induce secondary dormancy in Papaver rhoeas, Spergula arvensis and Chenopodium polyspermum after 24-28 weeks of chilling, rather further increases in germination were observed.

Environmental factors such as nitrate in the soil and light might be expected to influence stratification. Nitrate strongly influences the seeds of Sisymbrium officinale during chilling (Karssen, 1980/81a&b), though it decreased the stimulatory effect of chilling in Capsella bursa-pastoris (Roberts and Benjamin, 1979). Light has no apparent effect upon dormancy breakage at moderate temperature. It was beneficial during chilling in Capsella bursa-pastoris, has positive effect in Chenopodium album after 7 days of chilling, and slightly stimulatory in Poa annua (Roberts and Benjamin, 1979). In species like Papaver rhoeas, stratification was effective if seeds were subsequently illuminated. However, if illumination occurs during pretreatment rather than after in Rumex crispus and Chrysanthemum segetum, germination was stimulated, though in Chenopodium polyspermum and Spergula arvensis germination was the same regardless of when illuminated (Vincent and Roberts, 1979). The duration of chilling which increases the response to light (i.e. few days) does not terminate dormancy in darkness. To achieve this, prolonged periods of chilling, extending over some weeks, must be experienced. The combined effect of stratification, light and nitrate gave the highest germination in most species, and a delay in exposure to light, after stratification in the dark reduces the interaction between stratification and light in removing the

dormancy of S. arvensis, C. polyspermum and R. crispus (Vincent and Roberts, 1979). Stratification, they suggested, interacts with nitrate present during the subsequent period at higher temperature and not during stratification period.

Phytochrome effects have been shown in some species during stratification. Taylorson and Hendricks (1969) reported in Amaranthus retroflexus seeds that subsequent dark germination was prevented if FR irradiation was given between 24-72h of prechilling treatment, and this inhibition was completely reversed by R-light treatment. Also 5 minutes R-light at or before 24h of a 6-day 10°C prechilling period resulted in nearly complete promotion when the seeds were subsequently germinated at 35°C. Okagami and Kawai (1977) also observed that in Dioscorea tokoro and D. tenuipes, R-light irradiation during the incubation after chilling period promoted germination while blue, green and far-red light inhibited the germination of both species. Hence, the resultant germination depends on the nature of the final irradiation and when during prechilling it was applied. It has also been observed that inactivation of Pfr was found to proceed more rapidly at 25° than 20°C, and the failure of imbibition temperature above 20°C to increase dark germination of A. retroflexus seed was attributed to rapid thermal reversion of pre-existent Pfr (Taylorson and Hendricks, 1969).

Stratification requirement is affected by provenance and weather. Fowler and Dwight (1964) investigated the stratification requirement of Pinus strobus (white pine) seeds from 11 different places, and observed that seeds from northern sources required less chilling treatment than seeds from the southern sources. Also, they further correlated average germination of seeds from different provenances to the latitude and mean January temperature of their origins. Indeed, stratification requirement might be an adaptation to climatic factors associated with a particular provenance.

Effect of Low Temperature on embryo growth potential and Metabolism

Low Temperature pretreatment effects on embryo growth potential and metabolism have been well documented in the recent book of Bewley and Black (1982). In fact, a critical look at the listed examples shows that in all cases, embryo growth takes place both at stratification temperatures and non-stratification temperatures. This might suggest that embryo growth is not solely dependent on a low temperature pretreatment which breaks dormancy. Thus the initial embryo expansion cannot be conclusively linked to the dormancy breaking phenomenon. It should also be noted that seeds showing embryo growth potential possess immature embryos, hence embryo dormancy. Bewley and Black noted that in most cases, measurement of respiration is carried out at non-stratifying temperatures and not at low temperature. If the seed is dormant then respiration will be low at the higher temperatures and false conclusions may be made. Comparative control experiments are often not carried out, and comparisons are not made between dormant and non-dormant seeds. A few examples are cited below.

Stokes (1953) reported that during Low Temperature pretreatment, in Heracleum sphondylium, endosperm proteins are broken down to nitrogenous compounds and transferred to the embryo which enlarges from 0.4 percent to 30 percent of the total weight in contrast to 15°C treatment. He also observed that soluble sugars are transferred to the embryo at both 5°C and 15°C and concluded that nitrogen starvation is responsible for cessation of embryo growth at room temperature. Amino acids, organic acids and phosphate gradually increased in peach seeds at both stratification and non-stratification temperatures (Flemion and De Silva, 1960). There is no correlation between breaking of dormancy and peroxidase increase in apple embryo. Peroxidase activity increases in the cold (5°C) and more when embryos are placed at 20°C, though this temperature does not break dormancy (Côme, 1980/81; Thevenot et al., 1977). The growth potential of the embryonic axis of Acer saccharum (elongation of axis) reaches a maximum after 70 days

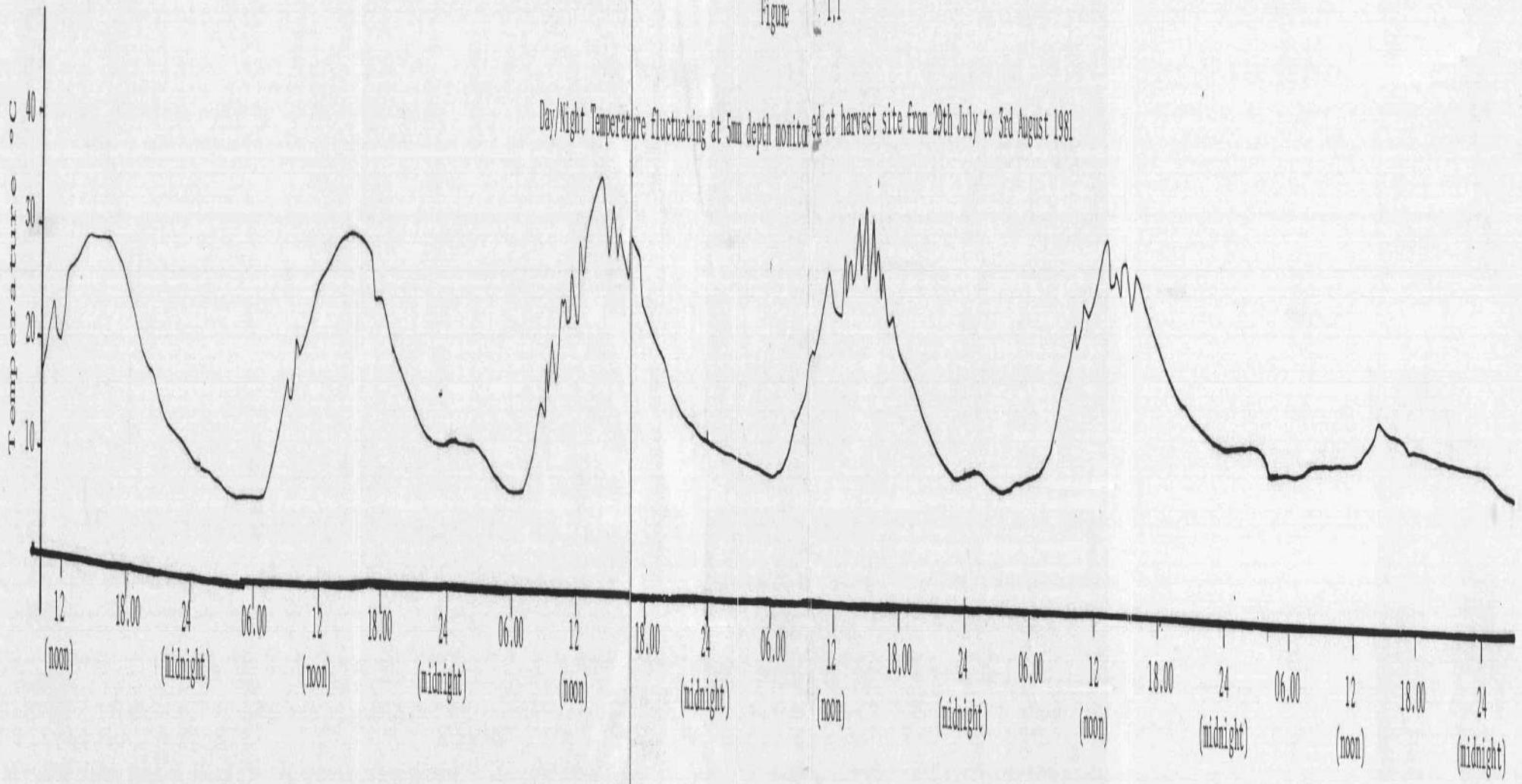
at 5°C with 30 percent germination, although appreciable germination (90%) occurred after 90 days at 5°C. Here again, there is no correlation between the time of maximum axial growth and termination of dormancy. In the above study too, oxygen consumption of the axis remained constant for 5°C treatment in contrast to 20°C treatment where oxygen consumption was greater at the early period of treatment (Simmonds and Dumbroff, 1974). In cherry seeds, the total nitrogen and phosphorus increased in the embryonic axis and are translocated from the cotyledon for 5°C treatment in contrast to 25°C. There was also an increase in respiratory efficiency at 5°C compared to 25°C (Olney and Pollock, 1960). The latter work might suggest that lack of phosphate to the embryonic axis results in reduced RNA synthesis in dormant cherry seeds, thereby maintaining dormancy, but Davies and Pinfield (1979) reported that the capacity of cotyledons and embryonic axis to synthesize RNA at both 5°C or 17°C treatments did not differ, but the embryonic axis capacity for RNA and protein synthesis increased only after an extensive Low Temperature pretreatment period. Bewley and Black (1982) suggested that this increase might be related to the initiation of radicle growth which occurred after this time, rather than to be associated with breaking of dormancy. The latter authors have also reviewed, extensively, the correlation between ATP, RNA, nucleoli, amino acids and phosphate sugars with regard to Low Temperature pretreatment and concluded that these results should be accepted with caution because, as noted earlier, most works lack adequate comparative controls. These phenomenon may not be the actual dormancy-breaking mechanism, but may be secondary events resulting from it, or may even be completely unrelated.

Temperature Shift

The breaking of dormancy could be under the control of temperature shifts, and might be expressed in combination with light. Temperature shifts from low to high temperature have been shown to induce germination in Rumex spp (Totterdell and Roberts, 1981); in Lepidium virginicum (Toole et

Figure 1.1

Day/Night Temperature fluctuating at 5mm depth monitored at harvest site from 28th July to 3rd August 1981



TIME OF DAY

al., 1955). In the case of Lepidium, the shift response was only found in seeds which had been given a saturating dose of R light. The temperature differential between the upper and lower temperature may be critical, as might also be the value of the higher temperature in the shift regime. In fact, day temperatures could be as high as 35°C while night temperatures as low as 10°C (see opposite page, Figure 1.1).

The seeds at the upper surface of the soil would experience this day and night temperature shift in comparison to seeds at depth and might be stimulated to germinate. This is of ecological importance since germination might only occur in summer months when such temperature differential could be attained and a critical high day temperature reached. Also this temperature shift would help the seed to sense its depth in the soil, thus those at deeper depths would not germinate.

Alternating Temperatures

During the summer season, soil temperatures in field conditions are such that the period at the higher is a small fraction (40%) of the 24 h cycle. Light might also be involved because in field circumstances the temperature changes are diurnal and follow the night/day cycle. Totterdell and Roberts (1980), working with seed of Rumex spp., enumerated features of alternating temperatures that should be considered, namely:

(1) Number of cycles,

(2) Amplitude,

(3) The value of the upper/value of the lower temperature,

(4) The time spent at the upper/time spent at the lower temperature within each cycle,

(5) Timing of the cycle with respect to imbibition, and

(6) The rate of cooling/rate of warming.

They observed that the establishment of an 8h (in the high temperature part of the cycle) and a 16h (in the low temperature part of the cycle) is the most stimulatory single regime. The upper temperature must be above 15°C and the lower below 25°C with a minimum amplitude of 5°C and optimum of 15°C.

Thompson (1974) reported that celery (Apium graveolens) seeds displayed restricted germination at constant temperatures, optimal at medium (12°C to 15°C and 22°C to 25°C) and unfavourable below 10°C to above 35°C. Thompson (1969) in Lycopus europaeus reported that high temperature during the light period promotes germination and the temperature differential must exceed 7°C. He also observed that evidence for critical temperature is lacking and that germination occurred irrespective of whether the temperature changes resulted from a rise in the middle (15°C/35°C/15°C) or a fall in the middle (35°C/15°C/35°C).

Thompson et al. (1977) observed that alternating temperature stimulated high germination in species of marshes, bogs, streamsides and other wetland habitats, e.g. Chenopodium rubrum, Polygonum persicaria and Polygonum lapathifolium. They concluded that alternating temperatures provide a mechanism which causes seed to germinate in places favourable for seedling establishment.

High Temperature

Secondary dormancy:- The induction of secondary dormancy in the field by high temperature has been observed on seeds whose primary dormancy has been broken by low-temperature pretreatment of the winter months, e.g. Sisymbrium officinale, and in seeds without primary dormancy, e.g. Senecio vulgaris (Karssen, 1980/81a&b). This secondary dormancy is known as thermodormancy. The effect of dark imbibition at supraoptimal temperatures could be detected if germination is prevented or reduced under the conditions which previously allowed germination. It has been suggested that primary dormancy may either

be embryonal or non-embryonal, while secondary dormancy induced by high temperature in most cases is embryonal in nature, e.g. in lettuce seed (Khan, 1980/81).

The above observation led some authors to suggest that one of the factors inducing secondary dormancy in seeds in the soils might be high temperatures during the summer months. Karssen (1980/81a&b) demonstrated that changes occurring during seasonal cycles are reflected in breaking and imposing dormancy under field conditions.

The induction of secondary dormancy by prolonged dark soaking in water at supraoptimal temperatures has been demonstrated in lettuce seed (Khan, 1980/81), in Chenopodium bonus-henricus (Khan and Karssen, 1980) and in Rumex crispus (Taylorson and Hendricks, 1973). In the above cases, the authors reported that application of exogenous GA_{4+7} or R light irradiation was effective, and even more effective is the combination of kinetin + Ethephon + GA_{4+7} in preventing or breaking secondary dormancy. Khan (1980/81) in lettuce seed observed that while GA_{4+7} was effective in preventing and breaking secondary dormancy, GA_3 was slightly effective, hence there appears to be selectivity of hormonal action. The interplay of exogenous hormones in which GA_{4+7} released, ABA inhibited and this inhibition is overcome by kinetin but not by excess GA_{4+7} in Grand Rapid lettuce seeds after prolonged dark soaking at 25°C was reported by Khan (1980/81). Hence his speculation of primary, preventive and permissive roles of hormones (Khan, 1971) is applicable to both primary and secondary dormancy.

In some cases, the direct effect of prolonged dark soaking at supra-optimal temperatures leads not to thermodormancy but to thermoinhibition, e.g. in lettuce seed (Vidaver and Hsiao, 1975). This could be regarded as deterioration of germination potential of the embryo, and seeds might be killed if kept for a very long time under such dark supraoptimal temperatures. Khan (1980/81) suggested that thermoinhibition could be distinguished from thermodormancy by the action of fusicoccin (FC) or cotylenin (CN) applied at different times to a seed. Whereas, FC and CN are ineffective in overcoming

the thermodormancy imposed by prolonged dark supraoptimal temperatures, thermoinhibition is reversed if these are applied at the beginning of high temperature imbibition. Moreover, FC, kinetin or kinetin + Ethephon + gibberellic acid could effectively prevent thermoinhibition (Braun and Khan, 1976; Khan, 1980/81).

Mechanism:- Evenari (1965) suggested that the mechanism inducing secondary dormancy during imbibition at unfavourable conditions might (1) lead to termination, but the pathway may be blocked, e.g. due to the presence of Pr or absence of Pfr (for photoblastic seeds) and if this block is not lifted by appropriate conditions, the reactions are diverted to pathways which may result in accumulation of deleterious metabolites or respiration switched from one channel to another. (2) that two diametrically opposed processes are initiated, one leading to the build-up of a germination inhibiting substance, and the other leading to full germination. Their relative rate will determine if seeds germinate. If germination is blocked, the inhibiting process prevails.

Secondary dormancy has also been suggested to be an anaerobic process (Bewley and Black, 1982), because anaerobic conditions such as a nitrogen atmosphere allow seeds to escape the effects of high temperature and darkness in Sisymbrium officinale (Karssen 1980/81b). Vidaver and Hsiao (1975), working with lettuce seeds, also concluded that secondary dormancy is an anaerobic process.

Drying:- The effect of high temperature in the soil might act to produce drying in the soil. This can occur in nature in the soil when seeds experience long periods of high temperature under dry conditions which arise due to high levels of solar radiation (insolation). This results in high evapotranspiration and consequently gives drying conditions. Drying in the field may impose or release dormancy. In the laboratory, this process is simulated by keeping dry seeds at elevated temperatures, in the range 35°C -

40°C, and is a form of "after-ripening". It is regarded as any change which occurs in the seeds during storage as a result of which germination is improved (Evenari, 1965). However, in some cases, drying could also impose dormancy, e.g. in lettuce seed (Nutile, 1945). In most cases, dormancy is broken at elevated temperatures [illegible], e.g. in cereals (barley and rice) (Roberts and Smith, 1977), and in embryos of Ambrosia trifida (Davis, 1930). Recently, Bewley and Black (1982) listed seeds whose dormancy can be relieved by high temperature dry storage. It is apparent that most of the seeds whose dormancy can be terminated by high temperature storage have coat-imposed dormancy. The resultant effect of this "after-ripening" process (like low temperature pretreatment) is to widen the conditions under which dormancy can be broken and germination expressed. The rate of the "after-ripening" process has been reported to be accelerated by increased temperature (Roberts and Smith, 1977), but this is again dependent on moisture content (Roberts, 1962). The latter author reported that high moisture content accelerates the loss of viability at high temperatures. In rice seed, carbon dioxide and nitrogen have no effect, but the rate of "after-ripening" is correlated with oxygen tension. However, at very high oxygen tension (100 percent), the high temperature effect is decreased (Roberts, 1962). It appears that an aerobic reaction (s) is involved in the "dry after-ripening process", though the mechanism is still not understood.

Indeed, high temperatures may induce secondary dormancy in imbibed conditions or in dry seeds (in which primary dormancy has been broken) under room temperatures. It can also break primary (innate) dormancy in seeds stored dry under temperatures of 25°C-40°C. Thus, the stimulatory or inhibitory effects of high temperatures depend very much on the state of the seed.

LIGHT

Light might act through its spectral composition, and this effect would be modified by transmission through a canopy of leaves, the seed coverings, seed moisture and the position of the seed, either buried or exposed. Light quality and photoperiod may have a role to play and these parameters will very seasonally.

Involvement of phytochrome during germination has been reported in seeds of Amaranthus retroflexus (Taylorson and Hendricks, 1971). Taylorson (1982) reported that the ratio of R/FR (and accordingly Pfr/P total) reflected changes in natural daylengths, i.e. sunrise-sunset, and screening effect of chlorophyll through the foliage which results in FR enrichment. Hence, the quality of light reaching the embryo during seed maturation and storage (depending on the moisture content) will determine the Pfr/P total ratio in the embryo, and in the case above ensured that most of the P is in the Pr, inactive form.

Gutterman (1980/81) in his review noted that environmental factors influence the phenotypic characteristics of some seeds during maturation. He reported that longer daylengths might impose thicker seed coat coverings in contrast to short-day grown plants, thereby imposing deeper dormancy. Kigel et al. (1977) reported that in Amaranthus retroflexus, seeds grown in short days possess less dormancy and had a higher germination than seeds from parents grown in long days.

However, it is not mandatory that all these environmental factors have to prevail before germination is stimulated or dormancy enforced.

The effect of light is frequently dependent upon temperature. Phytochrome undergoes thermal reactions, e.g. dark reversion, and the ratio of Pfr/P-total coupled with the threshold of Pfr required to stimulate germination in certain conditions might determine if a seed is positively or negatively photoblastic. The positively photoblastic seeds might again be broadly divided into photosensitive and photoperiodic seed.

Light has been shown to influence different photomorphogenetic processes. Black (1969) noted that as early as 1860, Caspary was able to demonstrate a light effect on seed germination. Borthwick et al. (1952), working with lettuce seed (Grand Rapids) were probably the first to demonstrate clearly the reversible photoreaction of phytochrome in seeds.

Extensive treatments of different types of seeds in different physiological states and requiring treatments like light (red, far-red, continuous light or photoperiodism) may be found in the recent books of Bewley and Black (1982), Khan (1982), and Mayer (1980/81). However, a few examples may be cited to show variation which may be relevant to Polygonum persicaria.

Some seeds might require light at higher temperature, e.g. Grand Rapids (Berrie and Taylor, 1981) and in Amaranthus retroflexus (Taylorson and Hendricks, 1969).

Photoperiodic response in the true sense as in flowering has not been unequivocally demonstrated in seeds. In most cases, no critical daylength for breaking dormancy has been shown, and promotion of germination is correlated with increase in the duration of light. Black and Wareing (1955) observed that unstratified seeds of Betula pubescens are stimulated at 20°C and above in both long and short day conditions, and at 15°C or less, long days are promotive in contrast to short days. Vaartaja (1956), while confirming the above results, also reported that in Betula verrucosa, light was not needed for maximum germination at the higher temperature of 25°C and above. However, Fujii (1962) has been able to demonstrate that Eragrostis ferruginea behaved like a short day species in that when the dark period was interrupted with a short period of irradiation, germination was inhibited.

While some seeds could be classified as short or long day seeds, in others photoperiodic expression is dependent entirely on the temperature. In Chenopodium album, Cumming (1963) reported that at 10°C, germination was maximum with a short light period (4h); at 20°C (8h) was most stimulatory in a 24h cycle, while at 30°C, continuous (24h) light gave the maximum germination.

CHEMICAL TREATMENTS

A: Hormones

Effect of Exogenously Applied Hormones:- Dormancy of many intact, unchilled seeds can be alleviated by the application of exogenous hormones. Gibberellic acid (GA_3) applied externally has been demonstrated to break dormancy in seeds that required Low Temperature pretreatment (stratification or chilling) for breaking of dormancy, e.g. in Ruellia humilis (Baskin and Baskin, 1971); in Corylus avellana (Bradbeer and Pinfield, 1967); and in the punctured nuts or seeds of Corylus avellana, and Fagus sylvatica (Frankland and Wareing, 1966). It should be noted that puncturing not only provides passage for rapid entry of GA_3, but also might initiate oxidation-reduction processes, and the latter might lead to dormancy breaking events. However, in some seeds, GA_3 is ineffective both in intact and punctured seeds, e.g. in Acer platanoides (Pinfield et al., 1974); and even when injected into the seed (Webb and Wareing, 1972).

Cytokinin has also been implicated as one of the natural dormancy breaking hormones. While exogenous kinetin stimulated the germination of intact unchilled seeds of Acer pseudoplatanus (Pinfield and Stobart, 1972; Webb and Wareing, 1972), and of seeds of Acer saccharum with the pericarp removed or testa pricked when incubated at Low Temperature (5°C) (Webb and Dumbroff, 1969), it was ineffective in Acer platanoides (Pinfield et al., 1974). Indeed, the dormancy breaking action of kinetin is abnormal and questionable, because in some cases, the cotyledon expanded and ruptured the testa before the growth of the radicles, e.g. in Acer ginnala (Dumbroff and Webb, 1970).

Inhibitors have been suggested to be involved in the control of seed dormancy and germination. Abscisic acid (ABA), because it occurs naturally in seeds, has been invoked to be the major inhibitor imposing and maintaining dormancy. Exogenous ABA inhibited the germination of excised non-dormant embryos of Fraxinus spp (Sondheimer et al., 1968).

It has been argued that exogenously applied hormones failed to inhibit

or stimulate the germination of non-dormant and dormant seeds respectively because they failed to enter the seed.

However, it should be noted that in most of the examples cited above, exogenous hormones were applied to seeds with coverings removed, punctured or even with the hormones injected. Karssen (1976a) reported that much higher levels of ABA in the seeds of Chenopodium album were obtained by exogenous application of the hormone, but these levels do not prevent the breaking of dormancy by low temperature. Uptake of ^{14}C-ABA is not prevented by the covering structure (Karssen, 1976b).

Dormant intact seeds of Fraxinus americana metabolized ^{14}C-ABA both at 5°C and 25°C incubation. While 5°C treatment took 26 days, 25°C incubation took 12 days to metabolize more than 90 percent ^{14}C-ABA, and only the former treatment leads to the breaking of dormancy (Sondheimer et al., 1974).

These observations and others led to the hypothesis that seed dormancy and germination might be regulated by growth inhibitors and promoters. It has been suggested that a balance between promoters and inhibitors, with gibberellin possibly as the major class of promoters and abscisic acid for inhibitors might regulate dormancy (Amen, 1968; Wareing and Saunders, 1971). Khan (1971), working with lettuce seed (Grand Rapids), extended the hypothesis and speculated that gibberellin plays a primary role, inhibitors-preventive and cytokinin a secondary or permissive role in seed dormancy control and regulation.

Involvement of Hormones during Stratification:- The action of Low Temperature pretreatment in terminating dormancy might involve the disappearance of inhibitors, especially ABA, and/or the appearance of promoters, notably gibberellin(s) and cytokinins. The level of endogenous ABA has been reported to drop during the Low Temperature pretreatment period which breaks dormancy in Pyrus malus (Barthe und Balard, 1978); in Fagus sylvatica (El-Antably, 1976); in Acer saccharum (Enu-Kwesi and Dumbroff, 1978); in apple (Rudnicki, 1969); and in dormant Fraxinus americana, the level was as low as that

found in non-dormant Fraxinus ornus seeds (Sondheimer et al., 1968). In most of the experiments described above, the disappearance of ABA under non-stratification temperatures was not tested. ABA declined after 3 weeks of Low-Temperature pretreatment in apple, even though appreciable germination was only observed after 12 weeks exposure to Low Temperature. A similar pattern is seen in Acer platanoides (Pinfield and Davies, 1978). While the above works tend to implicate ABA in seed dormancy control, other studies have shown that ABA amounts could also drop in seeds kept under non-Low Temperature pretreatment period that does not break dormancy, e.g. in Corylus avellana (William et al., 1973); in Pyrus malus (apple) (Balboa-Zavala and Dennis, 1977); and in peach (Prunus persica (Bonamy and Dennis, 1977). In fact, Dennis et al. (1978) went further to demonstrate that some species like pear (Pyrus communis) seeds had low levels of ABA, yet required very long Low-Temperature pretreatment periods, implying that the stratification period is not correlated with endogenous ABA content. Thus, the above examples demonstrated that Low-Temperature pretreatment events are not solely correlated with a drop in ABA content, and hence the question whether the drop in ABA content is a prerequisite for breaking of dormancy is still unanswered. Berrie et al. (1979) noted that dormancy in Avena fatua was better correlated with volatile fatty acid levels than ABA levels. This is reasonable when one considers that the plant cell membranes consist of lipids and lipoproteins, and the state of these lipids might determine the induction and breaking of dormancy.

Growth promoters such as gibberellin and cytokinin might also play a role in terminating the dormancy of seeds requiring stratification. Villiers and Wareing (1965) observed in Fraxinus excelsior that Low-Temperature pre-treatment does not reduce the inhibitor content of the embryos but increases the content of germination promoter which enables the embryo to overcome the effects of the inhibitors. Gibberellin and cytokinin-like substances have been suggested to increase during the process of Low-Temperature pretreatment in Fagus sylvatica, even though controlled experiments were not carried out

(El-Antably, 1976). Extracts of seeds of Acer platanoides kept at 5°C had a higher gibberellin-like activity compared to seeds kept at 17°C. However, the cytokinin activity remained low, and no consistent differences could be detected between the 5°C and 17°C treatments (Pinfield and Davies, 1978). Taylor and Wareing (1979a,b) reported that in Pseudotsuga menziesii, gibberellin-like substances increase during stratification and were 7 times higher after 7 weeks, but declined subsequently, while cytokinin-like activity did not change. Also the latter workers noted that in Pinus lambertiana, a 20-fold increase in gibberellin-like activity after 8 weeks of Low Temperature pretreatment was detected, unlike non-stratified seeds. Cytokinin-like activity also increased, but by 200-fold, after 10 weeks. Both gibberellin and cytokinin-like activities declined rapidly after reaching the maximum values. In Leucadendron daphnoides, cytokinin is found in both stratified and non-stratified seeds (Brown and Van Staden, 1973). They suggested that stratification might bring about an interconversion of bound cytokinin to free cytokinin rather than a de novo synthesis as occurred in Protea compacta where stratified seeds resulted in a simultaneous increase in butanol-soluble cytokinins. Stratification of Acer saccharum resulted in high levels of cytokinin-like activity after 20 days while appreciable germination was recorded only after 50 days of stratification (similar to ABA in apple - as noted earlier), with subsequent decrease in cytokinin-like activity when the stratification period was extended.

Indeed, in most of the work noted above, the transient increase in gibberellin or cytokinin-like substances during Low Temperature pretreatment is difficult to correlate with termination of dormancy. That is, these hormones increased and decreased before any appreciable germination is observed. It may be that only the transient increase is required for the termination of dormancy, and sensitivity rather than the continuous presence of these hormones throughout the Low Temperature pretreatment period might be important (Bewley and Black, 1982). Wareing (1982) suggested that the transient increase in gibberellin-like substances might be released from bound

forms, thereby "priming" the pathway for gibberellin biosynthesis in the tissue of the embryo.

In hazel nut (Corylus avellana), little or no increase in gibberellin-like activity was detected during the Low Temperature pretreatment period (5°C) which breaks dormancy, while a substantial increase occurred when seeds were transferred to higher temperatures suitable for germination (Bradbeer et al., 1978). Thus, Low Temperature pretreatment increases the capacity of the embryo to synthesise gibberellin when seeds are transferred to higher temperature. Also exogenous gibberellic acid (GA_3) can stimulate the germination of non-stratified dormant hazel seeds (Arias et al., 1976).

The dormant embryos of Fraxinus excelsior can be stimulated to germinate by application of exogenous gibberellic acid (GA_3); nonetheless, the stunting or dwarfism of seedlings grown from non-stratified embryos is not removed by exogenous GA_3, in contrast to stratified embryos. It implies that the mode of action of GA_3 and stratification might differ (Villiers and Wareing, 1960).

The role of external application of inhibitors and promoters, and their involvement during the Low Temperature pretreatment period, have been extensively documented and reviewed by authors like Bewley and Black (1982); Black (1980/81); Khan (1980/81, 1982); Taylorson and Hendricks (1977); Trewavas (1982); Walton (1980/81) and Wareing (1982). These authors question if reduction in abscisic acid (ABA), increase in gibberellin or cytokinin is necessary or sufficient enough to break dormancy. Trewavas suggested that it is the sensitivity of the receptor site which might change with development, rather than the concentration of growth hormones.

B: Inorganic Chemicals

Potassium nitrate (KNO_3) has been demonstrated to break dormancy in several wood seeds (Steinbauer and Grigsby, 1957a). Some other nitrogen-containing compounds, e.g. nitrites, cyanides (KCN), azides (KN_3), hydroxylamine (NH_2OH) and thiourea ($CS(NH_2)_2$) stimulated germination in seeds of

pigweed (Amaranthus albus) and lettuce seed (Lactuca sativa) (Hendricks and Taylorson, 1972, 1974, 1975). These chemicals have also been reported to promote germination in barley and rice (Roberts, 1969, 1973), while in Phalaris arundinacea, the stimulation of germination by cyanide, azide, nitrate and hydrogen peroxide occurred only in light (Junttila and Nilsen, 1980). It has been speculated that these chemicals function by irreversible inhibition of catalase activity, thus sparing hydrogen peroxide, which is oxidised by peroxidase action and hence promoting pentose phosphate (PP) pathway by making available NADP (Hendricks and Taylorson, 1972, 1974, 1975). They further suggested that nitrate functions by being reduced to nitrite or hydroxylamine (not ammonium ions) and that the hydroxylamine ions formed might be coupled to a respiratory chain which promotes germination through cyanide-resistant respiratory activity (alternate path).

Esashi et al. (1979) observed that stimulation of germination and inhibition of catalase in non-dormant upper seeds of cocklebur (Xanthium pennsylvanicum) depended very much on imbibition. In unimbibed (dry) seeds nitrates, nitrites, hydroxylamine, cyanide, azide inhibited catalase activity, and while other chemicals also stimulated germination, nitrate and nitrites did not. However, in seeds soaked for 7 days, catalase inhibition by these chemicals, except sodium azide, was not observed, although seeds were still responsive to germination. They rejected the hypothesis that these chemicals promoted germination by inhibiting catalase action on the following grounds. That potassium nitrate, and nitrite inhibited catalase activity but were ineffective in stimulating germination.

Secondly, thiourea and hydroxylamine did not inhibit catalase action in seeds presoaked for 7 days, and lastly that catalase activity is much higher in the lower cocklebur seeds which have a higher germination potential than in the upper ones.

Roberts (1969, 1973) postulated that dormancy breaking is associated with the operation of the pentose phosphate (PP) pathway, and in dormant seeds this is restricted. He also speculated that any treatment that increases oxygen

tension like removal or puncturing of seed coverings leads to germination by allowing the PP to operate. A decrease of the oxygen requirement of the cytochrome oxidase pathway by inhibitors of cytochrome oxidase (KCN, NaN_3, NH_2OH and CO) would be expected to break dormancy and stimulate germination through the PP pathway. Junttila and Nilsen (1980) noted that respiratory intensity is not correlated with germination in Phalaris arundinacea.

An alternate path (sensitive to hydroxamic acids but insensitive to cyanides) may be involved in regulation of seed dormancy in cocklebur seeds (Esashi et al., 1979, 1981), in wild oats (Upadhyaya et al., 1982). The alternate path has been suggested to be predominant during the early phase of respiration followed by the cyanide-sensitive pathway, e.g. in barley (Roberts, 1969, 1973), in cocklebur seeds (Esashi et al., 1981). However, Burguillo and Nicolés (1977) reported that in chick pea, initial respiration is through the cyanide-sensitive pathway and, after 12h, a shift to alternate respiration occurs. In Avena fatua, Upadhyaya et al. (1981) reported that seed dormancy cannot be attributed to different levels of dehydrogenase for the PP and cyanide-sensitive pathway, and GA_3 did not increase dehydrogenase levels in embryo prior to the onset of dormancy in dormant line. Also, Upadhyaya et al. (1982) observed that SHAM (an inhibitor of alternate path) is necessary for the stimulation of germination in the presence of azide but not in the germination of genetically non-dormant, gibberellic acid-treated dormant or after-ripened seeds.

The stimulatory effect of these chemicals is not universal. Khan (1980/81) observed that cyanide has little or no effect in removing the primary dormancy in celery (Apium graveolens), Chenopodium bonus-henricus, and Chenopodium album, or even scarified seeds of Rumex crispus.

Plate 3

Photograph of seed cleaner and blower used to prepare achenes for experimental use.

CHAPTER 2

GENERAL MATERIALS AND METHODS

HARVESTING AND PREPARATION OF ACHENES

The achenes of Polygonum persicaria were harvested by hand during the period of September/October as the fruit ripened.† The plants were removed from the field, with or without the roots attached, and left either in the laboratory or glasshouse to dry for about one or two weeks. Fruits were removed from the dried stalks by gentle hand rubbing. The mature achenes were separated from the detritus and immature or dead achenes using an air-draught method. This process is carried out in dry conditions which ensured that the original characters of the achenes harvested from the field are maintained. The seed blower or cleaner (plate 3) consists of a cylindrical jar with a series of equally spaced holes at the bottom and top, and "spiral steps" at the sides. It is fitted into a hollow head connected to a blower with regulation of air flow. The material is evenly spread on the lower grid, and the top of the cylinder is covered by hand when the air blower is switched on. The air flow up the cylinder is modulated by removing and replacing the hand to generate a "fountain" effect which carries the light materials to the greatest height along the cylinder. When it falls back the debris is held in the pockets made by the vanes. The detritus is thrown away and the procedure repeated several times until all the detritus has been removed and the mature achenes collected. It is subjected to further separation and cleaning by sieving in small quantities to make sure that nearly all the detritus, immature and dead achenes have been removed. The achenes are then stored dry in a glass jar in a deep-freeze at -20°C, and removed as required for future experiments.

† Achenes were harvested at the Garscube Research Laboratories' Gardens of the Botany Department, University of Glasgow.

VIABILITY TESTS

These tests were carried out by two different test methods, namely:

1) Tetrazolium Test Method: The tetrazolium test method, as developed by Lakon (1949), was used. This test does not test for actual germination but tests for dehydrogenase activity. It is a simple method, and results are obtained within 24h. Cottrell (1947, 1948) reported that the results obtained by this test correlated well with actual germination. Dehydrogenases reduce the colourless tetrazolium salt to a red-coloured substance known as formazan in active living tissue in contrast to unstained dead tissues. The test is carried out in darkness because the farmazan is bleached by light. The method used is described below.

Achenes were soaked in water for 24h in the dark at 20°C, split longitudinally through the embryo and soaked in 1 percent solution of 2,3,5-Triphenyltetrazolium chloride (TTC), then left for 2h in the dark. The embryonic region was then examined for accumulation of formazan, by noting the amount of red colour.

2) Direct Germination Test by Tip Removal from Achenes: The tips of dry achenes were cut off using a scalpel, and germination tested in distilled water at 20°C dark. Germination count was recorded after 72 hours.

LIGHT SOURCES

White light or Red (R) and Far red (FR) were given as described below.

White Light:- White light in the incubator was obtained from 5 fluorescent tubes (Stella MCF/U8W, Warm White, made in Holland), with each tube measuring 26.6cm in length by 1.4cm in width. The tubes were attached vertically, and lining the inside door of the incubator, giving an irradiation of 220μWcm^{-2}. The lower irradiance used i.e. 3 tubes instead of 5 gave an irradiance of 130μWcm^{-2}

Red (R) and Far red (FR) Irradiation:- Red and Far red irradiation was carried out using a light source based on a standard slide projector. The lamp was 150W 24V tungsten halogen and the light emitted was filtered through interference filters (narrow and wide band) manufactured by Barr and Stroud, Anniesland, Glasgow, with secondary and unwanted transmissions blocked by optical filters. The radiation emitted by this irradiator was passed through a 20cm tube filled with water, which acted as a light guide and infra-red filter. The R light transmission peaked at 660 nm and peak width at half height was 10 nm, while in FR light at 730 nm and the peak width at half height was 8 nm. Radiation incident on the achenes was measured by both a compensated thermopile (Kipp and Zonen, Helft, Holland) and a 40x optometer (United Detector Technology Inc., Santa Monica, California). The R gave an irradiation of 660 nmol $m^{-2}s^{-1}$ while FR gave an irradiation of 12 μmol $m^{-2}s^{-1}$. In order to ensure uniformity of dose, and to compensate for possible variations of flux density, the petri dishes were rotated on a turntable at $16^{2}/_{3}$ r.p.m. (Berrie and Taylor, 1981).

SOLUTIONS:

The solutions were made up as described below. The chemicals used were "ANALAR" where possible, otherwise G.L.R. were used without further purification. The required weight was dissolved in a small amount of distilled water, using heat if necessary, and then made to volume by adding distilled water. Standard laboratory glassware was used. The solutions were then stored in a refrigerator and subsequently removed for use.

LOW TEMPERATURE PRETREATMENT (CHILLING OR STRATIFICATION)

Low-Temperature treatment was carried out by soaking achenes in distilled water in 9cm glass petri dishes lined with one layer of No.3 or No.181 Whatman filter papers. The amount of water added was adequate to allow a slight run-off of water in the dish throughout the experimental period. Drying

of water did not occur throughout the incubation period at the incubation temperature. The dishes were covered and kept at the required low temperature (stated in the results) for the treatment period in days or weeks and subsequently removed for experiments.

EXPERIMENTAL CONDITIONS:

The experiments were either carried out with 50 achenes per 5cm glass petri dishes on 4.5cm Whatman No. 3 filter paper or 100 achenes per 9cm glass petri dishes on 9cm Whatman grade 181 filter paper. The amount of distilled water or solution added depended very much on the experiments in question (stated in the results), and of course on the size of petri dish used.

The R and FR irradiation, and any other manipulation during the course of all the experiments, were conducted under a green safe-light previously shown to be photomorphogenetically inactive in lettuce seed germination. Also for white light illumination, the controls (i.e. dark treatments) and for all other treatments, the dishes were enclosed in cylindrical light tight cans or covered with double aluminium foil to exclude light. For light treatments, dishes were exposed to light only when needed. Treatments were either replicated twice or thrice (stated in the results). Germination was also recorded under a green safe-light in the dark room.

The dishes were incubated at the required temperature ±1°C, in incubators. The incubators were checked daily to ensure that the required temperature was maintained. Germination was recorded after 10-21 days (indicated in the results), but varied with the type of experiment. Germination is considered to have taken place when the radicle protrudes to about 2mm.

STATISTICS:

The results involving one treatment, but with many factors involved, e.g. varying during course of imbibition at 40°C in hours, were analysed by one factor analysis of variance. And for comparison of a similar treatment, but

involving two different treatments like light vs dark, two factor analysis of variance was used. When such treatments involved temperature, light, duration at 35°C, three factor analysis of variance was used. Before the analyses, the mean value (percentage) of each replicate was transformed into angular values and used for the calculations (Fisher and Yates, 1963).

For comparison between two treatments with only one factor involved, a contingency chi squared (χ^2) test was used. The χ^2 value was determined taking account of the small number of corrections.

The significance of the calculated F or χ^2 values was determined at 5, 1 and 0.1 percent level (Fisher and Yates, 1963).

CHAPTER 3

THE EFFECT OF PHYSICAL AND CHEMICAL TREATMENTS ON GERMINATION

INTRODUCTION

Physical Treatments

The achenes of Polygonum persicaria have a black shiny pericarp. The weight of one achene averages approximately 2.70 mg of which about 1.025 mg is pericarp (40 percent of the fresh weight). The role of the pericarp in maintaining dormancy and controlling germination cannot be neglected in this species.

In Polygonum species, it has been reported that the pericarp is a barrier to germination (Dalci, 1975; Ransom, 1935). Treatments such as pricking the achenes and exposure to sulphuric acid or acetone enhanced the germination of P. persicaria (Timson, 1965). However, Staniforth and Cavers (1979b) stated that in species of Polygonum, including P. persicaria, scarification either by sulphuric acid or abrasion with sandpaper has no effect on the germination of unchilled intact achenes. Justice (1941) reported that scarified achenes did not germinate, but soaking in concentrated sulphuric acid allowed chilled achenes to germinate though unchilled achenes were unaffected by acid scarification. Although some species responded to scarification and hot water soaking, Holm and Miller (1972) noted that P. pennsylvanicum did not germinate when so treated.

These scarification treatments do not occur naturally, but they may be linked to some effects of natural environments. Soil abrasion might act on the seed coverings in the same way as removal of the tip of the achenes or pricking. Degeneration of the coats (pericarp) could be effected by bacteria and fungi in the soil; also the achenes are eaten and dispersed by animals, e.g. cotton tail rabbits (Staniforth and Cavers, 1977) and birds. It seems likely that the nature of the pericarp might be altered due to the "mechanical" action of the bird's crop and/or the enzymatic activities in the gut. This

could remove any physical constraint that the pericarp might impose and germination might be enhanced when achenes are excreted in an environment conducive to germination. The effect of sulphuric acid scarification might be compared with the action of micro-organisms in the gut or soil.

Chemical Treatments

The effects of chemicals like Potassium nitrate (KNO_3), Potassium nitrite (KNO_2), Hydroxylamine hydrochloride (NH_3ClOH), Thiourea ($CS(NH_2)_2$), Sodium azide (NaN_3), Sodium cyanide (NaCN), and Hydrogen peroxide (H_2O_2) on the regulation of dormancy and germination of achenes have not been extensively studied. Attempts were made to see if these chemicals break the dormancy of intact unchilled achenes. The possible mechanisms of actions of these chemicals have been discussed in the General Introduction (page 29).

Water Absorption

Justice (1941) observed that in Polygonum species, the pericarp was not a barrier to water entry, nonetheless it might reduce the rate of water uptake. The absorption of water was also determined here.

Gaseous Uptake

Ransom (1935) reported in Polygonaceae that the rate of respiration in the achenes with pericarp removed is higher than in intact achenes. He postulated that there was a greater oxygen availability. Storage in certain oxygen and carbon dioxide atmospheres enhanced germination in other Polygonum species, with the exception of P. persicaria. The effects of different O_2 and CO_2 atmospheres were investigated for P. persicaria.

MATERIALS AND METHODS

Achenes

The achenes used for the experiments were harvested, cleaned and stored as described on page 32.

Surgical Treatments

The pericarp was carefully removed by hand using a razor blade, leaving the seed coat, endosperm and the embryo intact (the possibility of wounding the seed during the course of pericarp removal was not ruled out). Each germination treatment was confined to one replicate of 50 seeds (achenes with the pericarp removed, because of the problems associated with dissection). Germination tests were carried out with the seeds; seeds incubated with pericarp at the ratio of 1 seed to 2 mg pericarp at 20° or 35°C in the dark.

Germination tests were also carried out with achenes whose tips had been chipped off or punctured. Also viability test was examined by the tetrazolium chloride test method.

Water Absorption

Water absorption of the intact achenes, and achenes that had their tips cut off, was determined as described below. Any dirt on the surface of the achenes was initially removed with tissue paper. The initial fresh weights of batches of 50 achenes were determined. The achenes were then placed in a 5cm glass petri dish lined with 4.25 cm Whatman No. 3 filter paper. The oven dry weights of one batch of each treatment were determined at 80°C after periods of 24h, 48h and 72h to give a constant weight. 5ml of distilled water was added to other fresh batches and incubated individually for 1, 4, 8, 16, 24, 36 and 48h at 20°C in the dark. After the desired time at 20°C, dishes were removed and water adhering on the surface of the achenes was gently blotted off with tissue paper and the weight subsequently determined. The

experiments were carried out under laboratory conditions.

Percentage moisture content was calculated as

Fresh weight = X_1 g

Oven dry weight = X_2 g

$\therefore$ Percentage moisture content = $\frac{X_1 - X_2}{X_1} \times 100 = A.$

The water uptake after a specific period of imbibition was determined as shown below.

Initial fresh weight = X_3 g

Weight after a specified period of imbibition at 20°C in the dark = X_4 g

Calculated dry weight = $X_3 \times \frac{100 - \text{Initial moisture percentage}}{100}$

$= X_3D$

$\therefore$ Percentage water content = $\frac{X_4 - X_3D}{X_4} \times 100$

Chemical Treatments

Achenes were soaked in the dark at 20°C for 24h in either 5ml of distilled water or in different concentrations of chemicals (indicated in the results) in a 9cm glass petri dish lined with one 9cm grade 181 Whatman filter paper. Subsequently, the achenes soaked in water were germinated in chemical treatments, and those soaked in chemical treatments were germinated in distilled water. Achenes were also treated with chemicals and germinated at 35°C in the dark immediately without prior imbibition at 20°C in the dark.

After each treatment, achenes were dispensed into 5cm glass petri dishes on 4.25 cm Whatman No. 3 filter paper moistened with 2ml of distilled water or appropriate solution with 50 achenes per petri dish (treatments replicated thrice) or in a 9cm glass petri dish on 9cm grade 181 filter paper moistened with 10ml of distilled water or appropriate solution with 100 achenes per petri dish and treatments replicated twice. All manipulations were carried out under the green safe-light and germination took place at 35°C in the dark.

A germination count was recorded after 14 days.

Effect of Gaseous Environment

The effect of different gaseous atmospheres was determined. Gas mixtures were prepared as described below.
Four replicates each of 50 achenes per 5cm glass petri dish (uncovered) on 4.25cm Whatman No. 3 filter paper and moistened with 2ml of distilled water were placed in the tray of the desiccator. 10ml of distilled water were placed in the bottom of the desiccator to saturate the atmosphere and prevent drying out of the achenes in the petri dishes. The desiccator was then joined to a glass manifold with rubber tubing which was finally fitted to a mercury manometer. The glass manifold was connected variously to cylinders of N_2, O_2 and CO_2 was obtained from subliming dry ice. The manifold was also connected to a vacuum pump.

The desired gas mixtures were obtained by allowing entry of each component of the mixtures to the evacuated system according to the manometer reading. The whole system was purged twice with the mixtures to ensure that the final recipe was as stated. The desired quantity of gas mixtures was introduced as described by Hart and Berrie (1966).

In all cases, the experiment was set up under laboratory conditions and germination took place at 30° and 35°C in the dark.

RESULTS

Viability Tests

The tetrazolium chloride test was carried out with 100 achenes as described on page 33. The embryonic regions of all the achenes tested coloured red due to farmazan formation, indicating positive dehydrogenase activity.

The results from the tetrazolium chloride test, which gave positive dehydrogenase activity, and the germination of achenes with tips cut off

(Table 3.1) which gave about 80 percent germination suggested that the sample of achenes used in the subsequent experiments had a high proportion, about 80 percent, which were viable.

Germination of Unchilled Intact (Whole) and Punctured Achenes

Germination of intact (whole), unchilled achenes occurred only at 35°C in the dark and significantly improved (Table 3.2) in the light. When the tips of those achenes which did not germinate under the conditions tested in Table 3.2 were cut off, germination was expressed at 20°C in darkness, except for the achenes that had been held at 40°C in the dark. This indicates that failure of the achenes to germinate is due to a dormant state. Germination of unchilled and intact (whole) and punctured achenes is only expressed within the narrow range of (35°C), nonetheless germination at this temperature is not substantial. At 40°C, the temperature is injurious to achenes, and they lose viability. This temperature might be considered lethal for the germination of this species.

Removal of Pericarp

It has been shown that cutting the tips of achenes off (viability test), and puncturing enhanced germination significantly over that of intact achenes (Tables 3.1 and 3.2). This might suggest that any treatment that might result in the removal of the pericarp would stimulate germination. Indeed, removal of the pericarp (Table 3.3), stimulated germination both at 20°C and 35°C in the dark. Germination at 35°C was significantly greater over the 20°C treatment. The percentage germination of seeds (i.e. without pericarp) and seeds germinated in pericarp at 20°C or 35°C was not significantly different, but was significant over that of intact achenes (χ^2 [Chi-squared) test).

The above results at least suggest that seeds (without the pericarp) are not dormant and that dormancy is a property of the pericarp. The maintenance of this dormancy could be to restrain embryo expansion, prevent water uptake or

Table 3.1. The Percentage Germination of achenes of P. persicaria L. in which the tip was surgically removed.

Germination Temperature (in the dark)	Percentage Germination Replicates†			Mean	Total	Comment
20°C	54	60	56	56.66		3 days exposure
	24	20	26	23.33		Transfer to 35°C for another 2 days
					80	Total percentage germination
35°	82	88	82	84		3 days exposure
	0	2	6	2.66		Further 2 days exposure
					86.66	Total percentage germination

† 50 achenes in 2ml distilled water

Table 3.2. The Percentage Germination of unchilled whole or punctured achenes of P. persicaria.

		Germination scored after 14 days									
		Dark Germination					Light Germination				
Germination Temperature	Treatment	Percentage Germination Replicates†				Mean	Percentage Germination Replicates				Mean
35°C	Whole (Intact)	36	30	42	23	32.7	50	56	52	75	58.25
35°C	Punctured	70	65	65	64	66	69	62	69	65	66.25

† 50 achenes in 4.25 cm petri dish in 2ml of distilled water.

Whole (Intact) and Punctured achenes did not germinate or germinated poorly at 10°, 15°, 20°, and 25°C both in the dark and in the light. Also, germination failed to occur at 40°C in the dark.

Statistical Treatment of Results:-

Source	χ^2 (Chi-squared	p	Level of Significance
Dark Treatments: Intact vs Punctured	74.4317	$p < 0.001$	***
Light Treatments: Intact vs Punctured	5.1184	$0.05>p>0.01$	*
Intact : Dark vs light	41.5807	$p < 0.001$	***
Punctured : Dark vs light	0	$p > 0.05$	N.S.

KEY TO LEVEL OF SIGNIFICANCE

N.S. indicates Not Significant

* " Significant at 1 percent level

** " Significant at 1 - 0.1 percent level

*** " Significant at less than 0.1 percent level

Table 3.3. The Effect of Pericarp Removal on the Germination of achenes of P. persicaria. Germination recorded after 72h.

Treatment	Percentage Germination 20°C (dark)	35°C (dark)
Intact achenes	0	32
Seeds (without pericarp)	38	76
†Seeds + Pericarp (in the ratio of 1 seed to 2mg pericarp)	40	70

50 achenes or seeds per treatment.

Analysis of results : Contingency χ^2 (Chi-squared).

Source	χ^2	p	Level of Significance
20°C Germination: Seeds vs Seeds+Pericarp	0.0210172	$p > 0.05$	N.S.
35°C Germination: Intact vs Seeds	37.2182	$p < 0.001$	***
" : Intact vs Seeds+Pericarp	27.391	$p < 0.001$	***
" : Seeds vs Seeds+Pericarp	0.634196	$p > 0.05$	N.S.
Seeds : 20°C vs 35°C	27.9274	$p < 0.001$	***
Seeds + Pericarp : 20°C vs 35°C	16.9899	$p < 0.001$	***

† In this case, the seed (achenes without the pericarp) were incubated in the presence of pericarp which were added separately to the petri dish in the amount indicated.

gaseous exchange. In this regard, the effect of water uptake was investigated.

Water Uptake

The water uptake at 20°C in the dark for intact achenes and those with tips cut off (Figure 3.1) was essentially similar up to 8h exposure time. However, at the 16h exposure period, the uptake of water by the achenes with tips cut off increases. This increase in water uptake is due not only to water absorbed, but also to radicle protrusion in contrast to intact achenes. The percentage water uptake after 8h, prior to radicle protrusion, is about 37 percent in achenes that had their tips cut off; this water uptake is attained by intact achenes between 16 to 24h exposure time. Thus, these results indicate that the pericarp does not prevent or even retard water absorption of intact, unchilled achenes.

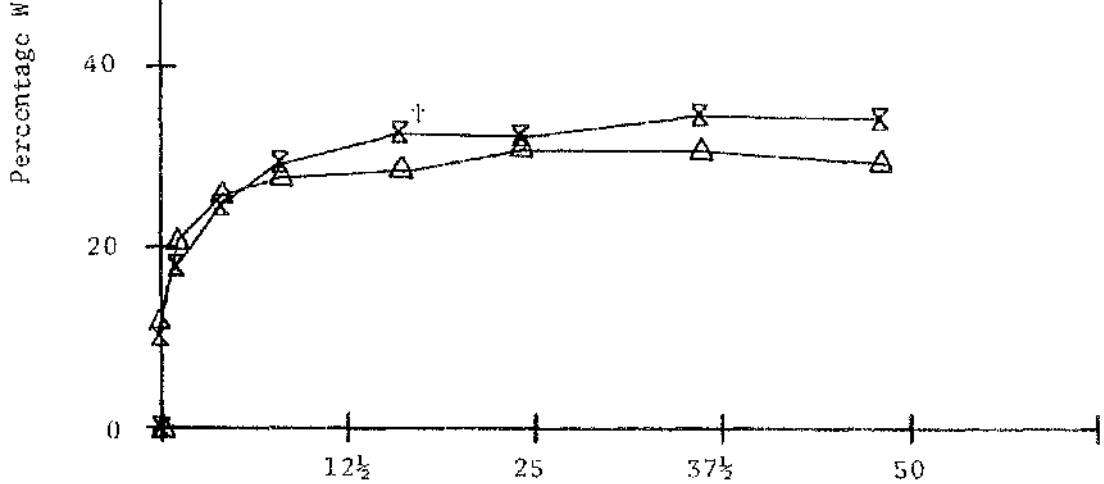
Chemical Treatments

The suggestion that chemical treatments which allow degradation of the pericarp, hence reducing the mechanical force imposed on the expanding embryo, was examined. The effects of chemical treatments which may also promote germination through physiological means, as has been speculated by Hendricks and Taylorson (1972) or Roberts (1973) (refer General Introduction page 29), was also investigated in P. persicaria. The design of the experiments was such that achenes were either kept in water at 20°C in the dark for 24h and then exposed to the chemicals and germinated at 35°C in the dark, or exposed to the chemicals at 20°C (dark) for 24h, transferred to water and germinated at 35°C in the dark. This would allow the separation of the effects of chemicals on germination sensu stricto and on growth.

The achenes were also exposed continuously to the chemicals and germinated at 35°C in the dark.

Figure 3.1

The Percentage Water Uptake at 20°C darkness



Δ : Intact Achenes

$\overline{\underline{X}}$: Achenes with tips cut off

† : 16h exposure time, first radicle protrusion observed for achenes with tips cut off.

Calcium hypochlorite ($Ca(ClO)_2$)

When achenes were treated with $Ca(ClO)_2$ after 24h exposure to water or exposed to $Ca(ClO)_2$ for 24h before subsequent germination in water, the total percentage germinations for 0, 1, and 10 mM treatments were similar and not significantly different (Tables 3.4A and B). Also treatments in which achenes were germinated immediately in $Ca(ClO)_2$ did not improve germination (Table 3.4C). It is evident that $Ca(ClO)_2$ does not improve the germination of intact unchilled achenes, and any chemical scarification action it may possess is not effective on P. persicaria achenes.

Hydrogen Peroxide (H_2O_2)

Treatments in which achenes were germinated in H_2O_2 after being kept in water for 24h (Table 3.5A) stimulated significant germination at concentrations of 5 & 10 volume H_2O_2, while higher concentrations of 20 and above inhibited complete germination.

When the achenes were exposed to H_2O_2 for 24h, and subsequently germinated in water, percentage germination increased progressively with increase in the H_2O_2 concentrations (Table 3.5B).

The germination of achenes without prior imbibition at 20°C in the dark is shown in Table 3.5C. Germination was enhanced at 5 & 10 volume concentrations, and with subsequent increase to 15 Volume; germination decreased when compared to water (control) treatment. With further increase to 20 volume, germination was completely inhibited.

From the foregoing observations, H_2O_2 could both promote or inhibit the germination of unchilled achenes depending on the application and the concentrations. The mechanism of action might be purely due to chemical scarification of the pericarp because it is a strong oxidising agent. Alternatively, it may be physiological, as has been speculated by Hendricks and Taylorson (1972) or Roberts (1973). The former authors suggested that H_2O_2 acts through being spared by inhibition of catalase action by the action of nitrite, hydro-

Table 3.4. The mean percentage germination of unchilled achenes of P. persicaria treated with calcium hypochlorite ($Ca(ClO)_2$. Germination conditions - 35°C darkness. Counted after 14 days.

Treatment	Percentage Germination Concentration of $Ca(ClO)_2$ (mM) 0	1	10
A. Achenes kept in water at 20 C for 24h and then transferred to $Ca(ClO)_2$ at 35°C	†32.33	34	40.66
B. Achenes exposed to $Ca(ClO)_2$ at 20°C for 24h and then transferred to water at 35°C.	32.33	32.33	41.33
C. Exposed continuously to $Ca(ClO)_2$ at 35°C.	39.66	49.66	35.66

† Replicated thrice with 50 achenes per replicate in 2ml of H_2O or soln.

Analysis of Results : One factor variance:-

A. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	2	25.3916	12.6958	1.75357	N.S.
Concentrations (Treatments)	2	41.8779	20.939	2.89213	N.S.
Error	4	28.96	7.23999		
Total	8	96.2295			

B. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	2	6.7207	3.36035	0.450865	N.S.
Concentrations (Treatments)	2	57.5996	28.7998	3.86412	N.S.
Error	4	29.8125	7.45313		
Total	8	94.1328			

C. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	2	14.7471	7.37354	0.73811	N.S.
Concentrations (Treatments)	2	105.467	52.7334	5.27875	N.S.
Error	4	39.959	9.98975		
Total	8	160.173			

Table 3.5. The mean percentage germination of unchilled achenes of *P. persicaria* treated with Hydrogen Peroxide (H_2O_2). Germination conditions - 35°C darkness. Counted after 14 days.

Treatment	Percentage Germination: Concentration of H_2O_2 (volume) 0	5	10	15	20	50
A. Achenes kept in water at 20°C for 24h, then transferred to H_2O_2 at 35°C	†35.5	57.5	66.5	34.5	0	0
B. Achenes exposed to H_2O_2 at 20°C (dark) for 24h, then transferred to water at 35°C	35.5	28.5	48.5	46.0	69.0	82.5
C. Exposed continuously to H_2O_2 at 35°C	33.5	67.0	70.5	20.5	0	0

† Mean of 2 replicates with 100 achenes per replicate in 10ml of H_2O or soln.

Observations for Treatments A and C.

0-10 volumes : Seedlings appear normal.

15 volumes : Seedlings malformed and stunted.

20 and 50 volumes : Pericarp turned yellowish in colour, tips split open and swollen.

Analysis of Results:- One factor variance.

0 vol to 15 volumes treatments

A. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	1	2.87598	2.87598	0.191674	N.S.
Concentrations	3	525.308	175.103	11.67	*
Error	3	45.0137	15.0046		
Total	7	573.197			

0 vol to 50 volumes treatments

A. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	1	1.91797	1.91797	0.208603	N.S.
Concentrations	5	5711.47	1142.29	124.239	***
Error	5	45.9717	9.19434		
Total	11	5759.36			

Table 3.5 contd.

Analysis of Results:-

0 - 50 volumes

B. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	1	3.96875	3.96875	1.6908	N.S.
Concentrations	5	1533.26	306.652	130.642	***
Error	5	11.7363	2.34727		
Total	11	1548.96			

0 - 15 volumes

C. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	1	9.03027	9.03027	1.19682	N.S.
Concentrations	3	1319.82	439.941	58.307	**
Error	3	22.6357	7.54525		
Total	7	1351.49			

0 - 50 volumes

C. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	1	6.02051	6.02051	1.17379	N.S.
Concentrations	5	6391.96	1278.39	249.243	***
Error	5	25.6455	5.1291		
Total	11	6423.62			

xylamine and thiourea, while the latter author speculated that these chemicals act by being hydrogen acceptors. However, H_2O_2 may also stimulate germination by increasing oxygen tension or promoting oxidation of any inhibitory substance that the pericarp might contain.

The effects of chemicals like KNO_3, KNO_2, $NH_3Cl.OH$ and $CS(NH_2)_2$ on the germination of P. persicaria achenes were investigated in the light of the above speculations.

Potassium nitrate (KNO_3)

Treatments in which achenes were initially exposed in water before germinating in KNO_3 did not differ much from the water (control) treatment; however, it was significant at 1 percent level (Table 3.6A). 100 mM treatment inhibited complete germination of the achenes.

When achenes were imbibed in 1, 10 or 100 mM KNO_3 and subsequently germinated in water, percentage germinations were comparable to that of water (control) treatment (Table 3.6B).

Germinations of unimbibed achenes in 1 or 10 mM concentrations did not increase substantially over the control treatment, and treatments were not significant when the 100 mM treatment is not taken into consideration (Table 3.6C).

Potassium nitrite (KNO_2)

Potassium nitrite enhanced significant germination when given after the achenes had been soaked in water and subsequently germinated in 1 or 10 mM concentrations. When the concentration was increased to 100 mM, germination was completely prevented (Table 3.7A).

There was a progressive stimulation and significant germination when achenes were exposed to KNO_2 for 24h, and germinated in water (Table 3.7B).

In the unimbibed treatments, significant germination was stimulated, although with an increase in KNO_2 concentration to 100 mM, germination was

Table 3.6. The mean percentage germination of unchilled achenes of *P. persicaria* treated with Potassium nitrate (KNO_3). Germination conditions 35°C (darkness). Counted after 14 days.

Treatment	Percentage Germination Concentration of KNO_3 (mM) 0	1	10	100
A. Achenes kept in water at 20°C (dark) for 24h, then transferred to KNO_3 at 35°C.	†32.33	44	41	0
B. Achenes exposed to KNO_3 at 20°C (dark) for 24h, then transferred to water at 35°C.	32.33	31	32	44
C. Exposed continuously to KNO_3 at 35°C.	39.66	49.66	47.66	0

† Mean of 3 replicates with 50 achenes per replicate.

Analysis of Results - One factor variance:-

0 to 10 mM

A. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	2	61.457	30.7285	15.489	*
Concentrations	2	77.8838	38.9419	19.6291	**
Error	4	7.93555	1.98389		
Total	8	147.276			

0 to 100 mM

B. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	2	32.9121	16.4561	1.01544	N.S.
Concentrations	3	119.789	39.9297	2.4639	N.S.
Error	6	97.2354	16.2059		
Total	11	249.937			

Table 3.6 contd.

Analysis of Results:-

0 to 10 mM

C. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	2	0.804688	0.402344	0.020142	N.S.
Concentrations	2	56.6982	28.3491	1.41921	N.S.
Error	4	79.9014	19.9753		
Total	8	137.404			

0 to 100 mM

C. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	2	0.603516	0.301758	0.0226029	N.S.
Concentrations	3	4118.64	1372.88	102.834	***
Error	6	80.1025	13.3504		
Total	11	4199.34			

Table 3.7. The mean percentage germination of unchilled achenes of *P. persicaria* treated with Potassium nitrite (KNO_2). Germination conditions - 35°C (darkness). Counted after 14 days.

Treatment	Percentage Germination Conc. of KNO_2 (mM) 0	1	10	100
A. Achenes kept in water at 20°C (dark) for 24h, then transferred to KNO_2 at 35°C.	†32.33	56.66	65	0
B. Achenes exposed to KNO_2 at 20°C (dark) for 24h, then transferred to water at 35°C.	32.33	38.66	52.66	70.33
C. Exposed continuously to KNO_2 at 35°C.	39.66	60.66	70.33	0

† Mean of 3 replicates with 50 achenes per replicate.

Analysis of Results : One factor variance.

0 to 10 mM

A. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	2	18.8008	9.40039	3.46384	N.S.
Concentrations	2	590.457	295.229	108.785	***
Error	4	10.8555	2.71387		
Total	8	620.113			

0 to 100 mM

B. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	2	0.707031	0.353516	0.0232898	N.S.
Concentrations	3	892.471	297.49	19.5983	**
Error	6	91.0762	15.1794		
Total	11	984.254			

0 to 10 mM

C. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	2	16.3248	8.16211	1.62354	N.S.
Concentrations	2	507.645	253.822	50.4883	**
Error	4	20.1094	5.02734		
Total	8	544.078			

prevented.

Hydroxylamine hydrochloride ($NH_3Cl.OH$)

Treatments (0 and 1 mM) in which achenes were germinated in $NH_3Cl.OH$ after 24h imbibition in water were not significantly different, and with increase in concentrations to 10 and 100 mM, germination was prevented (Table 3.8A).

When achenes were treated in $NH_3Cl.OH$ for 24h before germinating in water, the percentage germination increased slightly at 10 and 100 mM concentrations, but these were not significant (Table 3.8B).

Germination of achenes exposed continuously to $NH_3Cl.OH$ at 35°C decreased slightly at 10 and 100 mM concentrations, though this decrease is not substantial and also not significant (Table 3.8C).

Thiourea ($CS(NH_2)_2$)

Treatments in which germination was carried out in $CS(NH_2)_2$ after the achenes had been kept in water for 24h did not significantly enhance germination (Table 3.9A).

Germination was essentially similar when achenes, after 24h imbibition in $CS(NH_2)_2$, were germinated in water (Table 3.9B).

The treatments in which unimbibed achenes were germinated in $CS(NH_2)_2$ did not promote significant germination (Table 3.9C).

Sodium cyanide (NaCN)

Treatments that will increase the oxygen to Pentose Phosphate pathway have been speculated to break dormancy, e.g. treatment with inhibitors of cytochrome oxidase, e.g. NaCN or NaN_3 (Roberts, 1973). The effects (if any) on the unchilled achenes of P. persicaria were tested.

Treatments in which achenes were exposed to water before being germinated in NaCN (Table 3.10A) failed to stimulate germination. Germination amongst

Table 3.8. The mean percentage germination of unchilled achenes of P. persicaria treated with hydroxylamine hydrochloride ($NH_3Cl.OH$). Germination conditions - 35°C darkness. Counted after 14 days.

Treatment	Percentage Germination Conc. of $NH_3Cl.OH$ (mM) 0	1	10	100
A. Achenes kept in water at 20°C (dark), then transferred to $NH_3Cl.OH$ at 35°C.	†35.5	49	0	0
B. Achenes exposed to $NH_3Cl.OH$ at 20°C (dark), then transferred to H_2O at 35°C.	35.5	34	50	49
C. Exposed continuously to $NH_3Cl.OH$ at 35°C.	33.5	36.5	30	24.5

† Mean of 2 replicates with 100 achenes per replicate.

Observations: 10 and 100 mM removed the colour of the pericarp which turned brownish in colour, germination appears visible but radicle elongation inhibited. Some achenes had their tips split open, and those that germinated had stunted seedlings.

Analysis of Results : One factor variance.

0 and 1 mM

A. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	1	2.25098	2.25098	1.00109	N.S.
Concentrations	1	60.8413	60.8413	27.0582	N.S.
Error	1	2.24854	2.24854		
Total	3	65.3408			

0 to 100 mM

A. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	1	1.12549	1.12549	1.00072	N.S.
Concentrations	3	3341.34	1113.78	990.314	***
Error	3	3.37402	1.12467		
Total	7	3345.84			

Table 3.8 contd.

Analysis of Results:

B.

Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	1	13.2588	13.2588	1.29919	N.S.
Concentrations	3	151.973	50.6576	4.9638	N.S.
Error	3	30.6162	10.2054		
Total	7	195.848			

C.

Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	1	36.9746	36.9746	4.47735	N.S.
Concentrations	3	66.127	22.0423	2.66916	N.S.
Error	3	24.7744	8.25814		
Total	7	127.876			

Table 3.9. The mean percentage germination of unchilled achenes of *P. persicaria* treated with Thiourea ($CS(NH_2)_2$.
Germination conditions - 35°C darkness. Counted after 14 days.

Treatment	Percentage Germination Conc. of $CS(NH_2)_2$ (mM) 0	1	10	100
A. Achenes kept in water at 20°C (dark) for 24h, then transferred to $CS(NH_2)_2$ at 35°C.	†35.5	37.5	40.2	43.5
B. Achenes exposed to $CS(NH_2)_2$ at 20°C (dark) for 24h, then transferred to water at 35°C.	35.5	29.5	34.5	41.5
C. Achenes exposed continuously to $CS(NH_2)_2$ at 35°C.	33.5	29.5	21.8	42.5

† Mean of 2 replicates with 100 achenes per replicate.

Observations: 100 mM concentration turned achenes brownish in colour, and prevented radicle elongation.

Analysis of Results : One factor variance.

0 to 100 mM

A. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	1	0.178711	0.178711	0.0607167	N.S.
Concentrations	3	24.8105	8.27018	2.80978	N.S.
Error	3	8.83008	2.94336		
Total	7	33.8193			

0 to 100 mM

B. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	1	0.977539	0.977539	0.137161	N.S.
Concentrations	3	52.2773	17.4258	2.44505	N.S.
Error	3	21.3809	7.12695		
Total	7	74.6357			

0 to 100 mM

C. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	1	12.748	12.748	0.485784	N.S.
Concentrations	3	178.11	59.3701	2.26239	N.S.
Error	3	78.7266	26.2422		
Total	7	269.585			

Table 3.10. The mean percentage germination of unchilled achenes of *P. persicaria* treated with sodium cyanide (NaCN). Germination conditions - 35°C (darkness). Counted after 14 days.

Treatment	Percentage Germination: Concentration of NaCN (mM) 0	0.1	0.5	1.0	3.0	5.0	10
A. Achenes kept in water at 20°C (dark) for 24h, then transferred to NaCN.	†35.5	31.5	28.0	35.0	25.5	28.5	29.0
B. Achenes exposed to NaCN at 20°C (dark) for 24h, then transferred to water at 35°C.	35.5	15.0	12.5	18.5	19.0	24.0	15.5
C. Exposed continuously to NaCN at 35°C.	33.5	33.5	33.0	40.5	31.0	39.5	42.0

† Mean of 2 replicates with 100 achenes per replicate.

Analysis of Results : One factor variance.

0 to 10 mM

A. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	1	26.8818	26.8818	1.35595	N.S.
Concentrations	6	69.3467	11.5578	0.582989	N.S.
Error	6	118.95	19.825		
Total	13	215.179			

0 to 10 mM

B. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	1	18.9756	18.9756	5.36546	N.S.
Concentrations	6	341.657	56.9429	16.1009	**
Error	6	21.2197	3.53662		
Total	13	381.853			

0 to 10 mM

C. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	1	3.80859	3.80859	0.468206	N.S.
Concentrations	6	81.791	13.6318	1.67582	N.S.
Error	6	48.8066	8.13444		
Total	13	134.406			

treatments was essentially similar and not significant.

When the achenes were kept in NaCN for 24h and subsequently germinated in water, germination was not stimulated, rather it decreased. The significant effect (Table 3.10B) was due to decreased germination.

Germination without prior exposure to water at 20°C (dark) was not enhanced. Percentage germination was similar and not significant (Table 3.10C).

Sodium Azide (NaN_3)

When the achenes were kept in water before germinating in NaN_3 (Table 3.11A), percentage germination between the water and 0.1 mM treatments was similar. With further increase in NaN_3 concentrations to 0.5 mM, germination was greatly reduced, and was completely prevented with increase in NaN_3 concentrations to 1 mM.

However, treatments in which achenes were germinated in water after being kept in NaN_3 for 24h did allow germination which was significantly greater at the 5 percent level.

At high concentrations of 1 to 10 mM, germination was not prevented (Table 3.11B) in contrast to achenes that were kept in water and germinated in NaN_3.

Germination of achenes exposed continuously to NaN_3 was not promoted within the range of 0.1 to 0.5 mM. With increase in concentration to 1.0 mM, germination was completely inhibited. The NaN_3 treatments of 0 to 0.5 mM did not promote significant germination (Table 3.11C).

Effect of Gaseous Environment

In an attempt to determine the effects of gaseous environments, achenes were germinated directly in varying composition of O_2 or CO_2 and germinated at 35°C or 30°C in the dark (Figure 3.2).

The results demonstrate that 25 to 100 percentage oxygen environment did

Table 3.11. The mean percentage germination of unchilled achenes of *P. persicaria* treated with sodium azide (NaN_3).
Germination conditions - 35°C (darkness). Counted after 14 days.

Treatment	Concentration of NaN_3 (mM) 0	0.1	0.5	1.0	3.0	5.0	10.0
A. Achenes kept in water at 20°C (dark) for 24h, then transferred to NaN_3 at 35°C.	†35.5	30.5	5.0	0	0	0	0
B. Achenes exposed to NaN_3 at 20°C (dark) for 24h, then transferred to water at 35°C.	35.5	21.5	24	31.5	41	46.5	35
C. Achenes exposed continuously to NaN_3 at 35°C.	33.5	36.5	45.0	0	0	0	0

† Mean of 2 replicates with 100 achenes per replicate.

Observations:

A and C : Achenes germinated in 0.5 to 1 mM had their tips split open, germination appears visible, but radicle protrusion prevented.

B : Achenes exposed to 100 mM had their tips split open.

Analysis of Results : One factor variance

0 to 0.5 mM

A. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	1	4.00146	4.00146	0.07872	N.S.
Concentrations	2	671.644	335.822	6.6065	N.S.
Error	2	101.663	50.8315		
Total	5	777.308			

Table 3.11 contd.

Analysis of Results:

0 to 10 mM

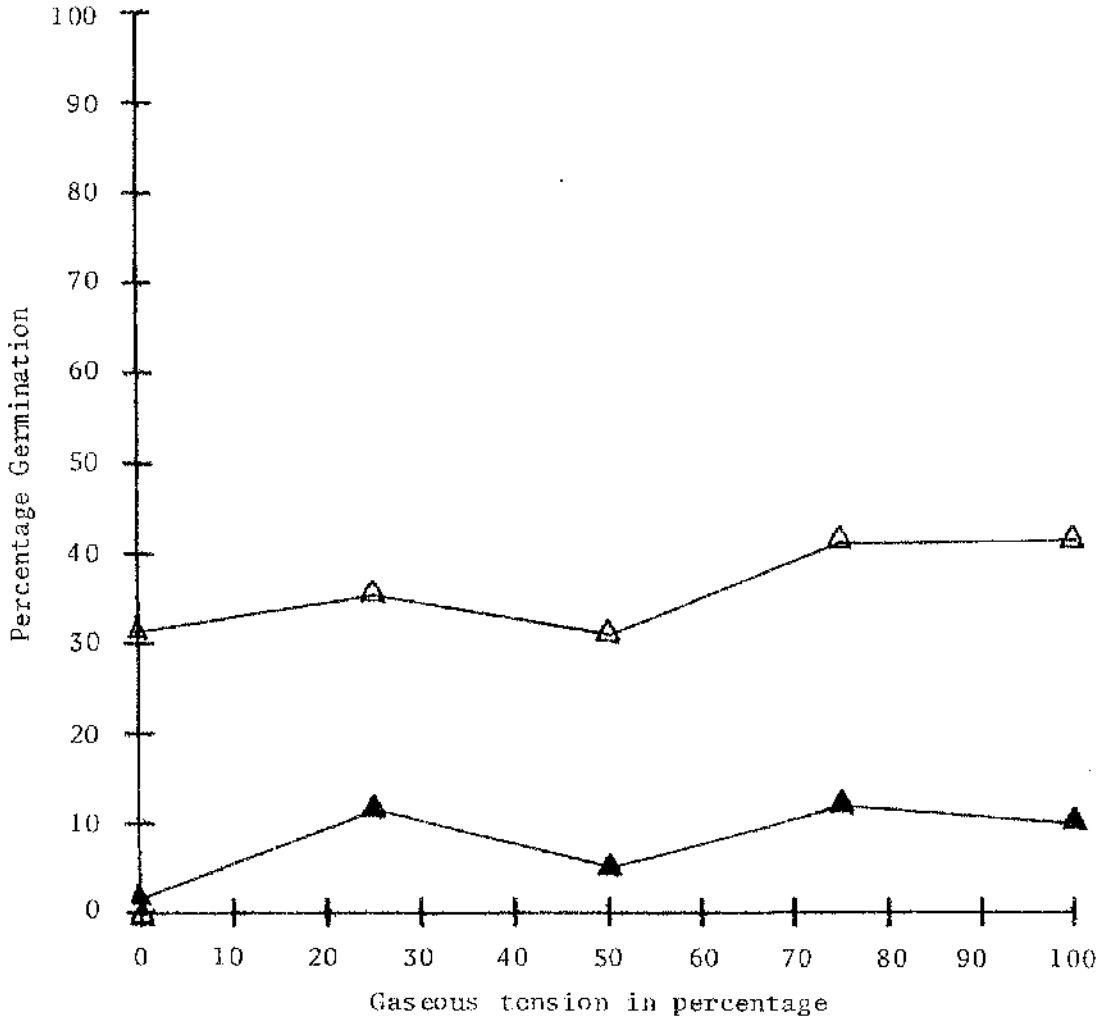
B. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	1	1.93164	1.93164	0.155826	N.S.
Concentrations	6	361.867	60.3112	4.86531	*
Error	6	74.377	12.3962		
Total	13	438.176			

0 to 0.5 mM

C. Source	Degree of Freedom	Sum of Square	Mean Square	F	Level of Significance
Replicates	1	3.52637	3.52637	0.771416	N.S.
Concentrations	2	49.0859	24.543	5.36894	N.S.
Error	2	9.14258	4.57129		
Total	5	61.7549			

Figure 3.2

Effect of different O_2 composition on the germination of achenes at 30°C and 35°C in the dark. Germination count recorded after 14 days.



△ Germination at 35°C (dark)

▲ " at 30°C (dark)

Achenes did not germinate in 1, 2, and 4 percent CO_2 environment and also in 100 percent N_2 environment.

not influence germination.

Also, an anaerobic environment of 1, 2, or 4 percent carbon dioxide or 100 percent nitrogen did not promote germination (Figure 3.2).

DISCUSSION

The tests for viability, i.e. the 100 percent dehydrogenase activity observed coupled with high germination recorded when the tips of the achenes were cut off, indicate that the achenes have a high potential germination capacity.

Germination of intact, unchilled achenes was only expressed slightly at 35°C in the dark and significantly enhanced in the light. However, percentage germination was not comparable to the viability estimated. These observations agree with Vegis's (1963) classification of Polygonum persicaria achenes as "Narrowed range for germination at high temperature"; and Lauer's (1953) who recognised the ability of P. persicaria to germinate at high temperatures of 35°C - 37°C.

The above results tend to suggest that the pericarp might be one of the factors controlling dormancy. This prevention of germination might be considered as possibly being

(1) A mechanical restrain on embryo growth,

(2) Imposing dormancy by the presence of inhibitors, and

(3) Prevention of water or gaseous uptake.

It is then suggested that removal of the pericarp by reducing the force imposed on the embryo by the pericarp would promote germination. Puncturing of the achenes stimulated significant germination in contrast to intact achenes at 35°C only. Treatments with pericarp removed stimulated greater germination at 35°C than 20°C in the dark. That germination of intact unchilled achenes was expressed when the tips of achenes were cut off or when pericarp was carefully removed, and not when punctured at 20°C in the dark, might suggest that achenes' metabolism at 20°C was not enhanced

sufficiently to overcome the constraint imposed by the presence of pericarp in contrast to 35°C temperature. That seeds (without pericarp) germinated when incubated in their pericarp might rule out the possibility of water soluble inhibitors preventing germination even if present.

The pericarp does not appear to prevent water uptake because water uptake of the intact achenes was comparable to the water uptake achenes in which the tip had been cut off, bearing in mind that radicle protrusion was observed at the 16h of imbibition. Thus, the stimulation of germination by puncturing cannot be considered to result from water uptake.

The observations of Hammerton (1967), Justice (1941), Ransom (1935), and Staniforth and Cavers (1979b) that scarification treatments such as pericarp removal, abrasion with sandpaper, did not promote germination, are in contrast to the results reported here, which indicated that dormancy might be a function of the pericarp, and in agreement with Timson (1965) and Dalci (1975). The innate dormancy is not embryonic in nature but may be associated with the presence of the pericarp.

If dormancy is solely a property of the pericarp, then chemical treatments that possess scarification effects should stimulate germination. However, treatments with $Ca(ClO)_2$ either before or after 20°C dark imbibition had no effect. Even when achenes were germinated in $Ca(ClO)_2$ without prior imbibition, germination was not affected.

The results of treatment in H_2O_2 showed that H_2O_2 is able to break the dormancy of P. persicaria because significant stimulation of germination was obtained with this treatment. However, the effect depended on the time of application (either exposed continuously or for a short period), and of course depended on the concentrations. High concentrations (15 volume H_2O_2 and above) are inhibitory if achenes are kept continuously in the presence of H_2O_2, but when exposed to H_2O_2 for 24h, germination was greatly enhanced even at high concentrations. The mechanism of H_2O_2 action might be purely due to chemical scarification, because it is a strong oxidising agent. Indeed, concentrations from 15 volume removed the colour from the pericarp, which

turned yellowish. Tips of achenes opened up and swelled while the achenes that germinated had malformed and stunted seedlings, especially when germination was allowed to take place in the presence of H_2O_2. The possibility that H_2O_2 might cause oxidation of inhibitory substances present in the pericarp seems unlikely because cutting a small tip off the achenes, or even puncturing, results in high germination. Apparently, even if inhibitors are present, they do not appear to play a role in the germination and dormancy of P. persicaria.

The view that H_2O_2 may also function to promote germination through physiological means as speculated by Hendricks and Taylorson (1972) and Roberts (1973), was also investigated indirectly.

When the achenes were treated with KNO_3 germination was not substantially promoted, and 100 mM treatment inhibited complete germination. However, treatment with KNO_2 promoted significant germination irrespective of the time of application in contrast to KNO_3. The mechanism by which nitrite promoted germination is not understood if Hendricks and Taylorson's (1972) hypothesis that promotion is through catalase inhibition or acting as an electron acceptor, hence promoting Pentose Phosphate pathway activity (Roberts, 1973), are to be considered. The observations that treatments with $NH_3Cl.OH$ or $CS(NH_2)_2$ failed to stimulate germination irrespective of the application time, argue against the above hypotheses. Germination in the presence of these chemicals, especially at high concentrations (10 or 100 mM) of $NH_3Cl.OH$ or $CS(NH_2)_2$, removed the colour of the pericarp which turned brownish; achenes had their tips split open while seedlings were stunted and malformed. These observations tend to support the view that these chemicals may function through their scarification action, but are inhibitory to growth processes rather than germination sensu stricto. However, the promotive effect of KNO_2 cannot be explained through a scarification effect. Germination of achenes treated with different concentrations of NaCN after imbibition in water at 20°C in the dark, or germinated immediately in NaCN, were essentially similar and not significantly different. Moreover, treatments in which achenes were exposed

to NaCN for 24h at 20°C in the dark prior to germination decreased percentage germination significantly. Treatments in which achenes were kept in different concentrations of NaN_3 were similar although the increase when compared to the control, was significant at the 5 percent level. However, germination in the presence of NaN_3 (0 to 0.5 mM concentrations) either before or without prior imbibition in water at 20°C in the dark for 24h, gave comparable germination. There was no significance amongst treatments. With an increase in NaN_3 concentration from 1 mM to 10 mM, germination was completely inhibited. The mechanism of promotion of germination ascribed to NaCN and NaN_3, i.e. inhibition of cytochrome oxidase, thus promoting the Pentose Phosphate pathway or Alternate path in dormant seeds, might not be universal because the germination of unchilled achenes of P. persicaria was not stimulated by either NaCN or NaN_3. Indeed, treatments with NaN_3 split open the tip of the achenes, with inhibition of radicle extension, which might suggest inhibition of growth rather than any involvement in germination sensu stricto.

The view that dormancy might be maintained by the pericarp preventing the entry of metabolically active gases like O_2 and CO_2 could not be accepted. The percentage germination of achenes germinated in different oxygen or carbon dioxide environments was comparable to the air treatment. This indicates that even though the pericarp might reduce gaseous exchange, this is not the primary cause of dormancy. One can infer from the above results that even if H_2O_2 acts by providing oxygen, stimulation of germination was not as a result of it. Ransom (1935) reported that the rate of respiration in the seeds of Polygonaceae with fruit coat removed is higher than those with coats intact, and suggested greater oxygen availability. Storage in certain oxygen and carbon dioxide concentrations, while promoting the germination of other Polygonum species, has no effect on P. persicaria (Justice, 1941). Nevertheless, the possibility that the pericarp might retard the diffusion of gases to the embryo still exists.

As reported in the General Introduction, page 2 , P. persicaria flourishes best in areas with plentiful supply of water, mostly in waterlogged peat and

mud soils. Similar habitats have also been observed during the course of this study. Such poor habitats are likely to be deficient in oxygen, and this could imply that oxygen tension may not be a factor in controlling dormancy.

CHAPTER 4

ROLE OF TEMPERATURE AND LIGHT ON THE GERMINATION OF UNCHILLED ACHENES

INTRODUCTION

It has been reported that achenes of P. persicaria will not germinate and fail to respond to light when kept at constant temperatures (Bayer and Bucholtz, 1957; Justice, 1941; and Staniforth and Cavers, 1979b).

Even when exposed to alternating temperature regimes of 25°C/10°C, 30°C/15°C, 35°C/20°C with 14h light at the higher temperature and 10h dark at the lower, germination of achenes was not induced (Staniforth and Cavers, 1979b). Also Bayer and Bucholtz (1957) observed no marked benefit in germination from alternating temperatures. However, Thompson, Grime and Mason (1977) noted that P. persicaria responded to alternating temperature with a temperature differential of about 8°C. Vincent and Roberts (1977) reported positive interaction between alternating temperature of 25°C (8h)/ 1°C (16h) or 1°C (8h)/25°C (16h) in a 24h diurnal cycle with light or nitrate. They observed that germination was greater when the three factors were combined, and even greatest when chilling treatment was included. They concluded that one factor cannot be regarded as substituting for any of the other factors because positive interactions were demonstrated between pairs of stimulatory factors. These factors, i.e. alternating temperature, light and nitrate, may not have precisely the same physiological effect.

The effect of light in stimulating the germination of buried seeds, including that of Polygonum persicaria (Wesson and Wareing, 1969), cannot be underestimated. Also, other environmental factors associated with soil like alternating temperature and nitrate interact with light to promote the germination of buried seeds (Vincent and Roberts, 1977). As well as these factors which have been considered, soil oxygen tension may also be involved in the expression of germination of weed species like P. persicaria when achenes are exposed after burial.

In the Vincent and Roberts (1977) report noted above, one component of

temperature regime used was 1°C. This temperature might also act as a chilling temperature rather than solely as an alternating temperature. Indeed, Vincent and Roberts (1979) reported that 14 or 28 days 1°C pretreatment promoted germination and either period had a similar effect in Polygonum persicaria. Of course, it should be expected that the cumulative effect of 20 such 25°C (8h)/1°C (16h) cycles would perhaps substitute for continuous stratification, then followed by transfer to higher temperature. Also, the effect of light was not quantified. Light was given when the incubators were opened at the beginning and at the end of the day, during the transfer from one cycle to another.

In the experiments carried out here, the effects of different environmental factors on unchilled achenes were investigated in terms of constant and alternating temperatures; photoperiod; and red (R) and far red (FR) exposures.

MATERIALS AND METHODS

The achenes used for the experiments were collected, cleaned and stored as described in the General Materials and Methods.

The various treatments such as red light irradiation, fluorescent light illumination, preparation of achenes for incubation, and germination counts were carried out under a green safe-light in the dark room as described on pages 33 and 34.

Temperature shifts and alternating temperature treatments were carried out by having the initial imbibition at the lower temperature (unless otherwise stated) for the set time, then transferring to the upper temperature in a 24h diurnal cycle. For example, temperature cycling of 20°C (16h)/35°C (8h) indicates that after dark imbibition at the lower temperature of 20°C, achenes were transferred to 35°C for a duration of 8h in the dark, then transferred back to 20°C for another 16h, and that is regarded as one cycle. If red (R) light or far red (FR) were used, the red light (R) or far red (FR) symbol is

indicated on the short hand representation of the cycle where such treatments are given. Also the number of cycles employed, the length of the component parts of each cycle and values of the temperature employed during any given cycle are indicated in the presentation of the results, e.g. 20°C (16h) R/ 35°C (8h) dark.

Temperature cycling was carried out by hand transfer from one incubator to another. Maintaining a constant temperature and subjecting achenes to temperature shift or alternating temperature by hand transfer ensures that the correct temperatures are maintained throughout the course of the experiments. However, the disadvantage is that the desired temperature required may not be attained at the higher temperature part of the cycle if very short temperature shifts or shocks are employed before transfer back to the lower temperature. Hence, the time spent and the value of the temperature attained mainly at the higher temperature part of the cycle may be considerably less than the stated treatment itself.

RESULTS

Constant Temperature

Germination of unchilled achenes of P. persicaria at constant temperatures only occurred at high temperature of 35°C (Table 4.1). Germination in the light at this temperature was significantly higher ($p < 0.001$). However, the total percentage germination under continuous light treatment was less than 50 percent (Table 4.1).

Temperature Shifts

Achenes germinated at 20°C (dark) after being subjected to temperature shifts to 35°C for 1h to 4h behaved as though they were kept at constant 20°C, i.e. did not germinate (Table 4.2). When the temperature shift to high temperature was extended to 8h and the temperature regime of 20°C (16h) D/

35°C (8h) D repeated for 3 cycles, there was slight germination, increasing with the number of cycles (Table 4.3, nos. 5,7 and 10). If Red (R) was incorporated into the temperature shifts, then germination was further enhanced (Table 4.3, nos. 6,8 and 11). Percentage germination was improved if the achenes were irradiated with red light (R) before transfer to constant 35°C (dark) (Table 4.3, nos. 4) in contrast to non-irradiated achenes. While one such shift with irradiation gave some increase in germination, two shifts were stimulatory and three shifts with R gave greatest stimulation and these are significantly different from each other (Table 4.3, nos. 6,8 and 13; and Table 4.3 χ^2 test, nos. 5, 2 and 7).

It seems that a single temperature shift does not completely break dormancy, but germination is slightly promoted when the length of the shift at high temperature was increased to 8h compared to 1, 2, or 4h. Though substantial germination was only observed when the 8h shift is coupled with R light exposure and repeated three times. Nevertheless, dormancy was not completely broken, only 58 percent germination under the most favourable conditions employed viz. 3 cycles of 20°C (16h) R/35°C (8h) D in a 24h diurnal cycle (Table 4.3, no.13). These results do indicate that rather than a short temperature shift, temperature alternations involving longer periods at the upper temperature part of the cycle and many cycles might be promotive.

Alternating Temperatures

The results from exposing achenes to a series of different temperature regimes showed that unchilled achenes demonstrated a requirement for an alternating temperature (Figure 4.1, a and b). It is worth emphasising that alternating temperatures, except where the value of the upper temperature is 35°C (Figure 4.1, a&b; nos. 4,8,11&13) failed to stimulate germination both in 8h or 16h regimes in a 24h diurnal cycle. It could be argued that amplitude by itself is not the factor controlling germination because even when the difference between the lower and upper temperatures is 20°C, e.g. 5°C/25°C regime, germination was very poor (less than 10 percent) either in

Table 4.1. The percentage germination of unchilled achenes at constant temperatures either in continuous light or darkness. Germination scored after 14 days.

Germination Temperature in °C	Percentage Germination: Dark Replicates†		Dark Mean	Light Replicates		Light Mean
15	0	0	0	0	0	0
20	0	2	1	0	2	1
25	0	0	0	1	1	1
35	18	23	20.5	49	44	46.5

† 100 achenes in 10ml of distilled water.

Analysis of Results - Contingency χ^2 (Chi-squared):

Source	χ^2	P	Significant level
35°C : Dark vs light	15.1723	$p < 0.001$	***

Table 4.2. The mean percentage germination of unchilled achenes initially exposed to water for different periods at 20°C (dark), and subjected to a temperature shift at 35°C for 1,2 or 4h, then transferred back to 20°C in the dark for germination. Counted after 14 days.

Imbibition Time at 20°C (dark) in h	Percentage Germination: 1h exposure at 35°C (D)* Replicates†			Mean	2h exposure at 35°C (D) Replicates			Mean	4h exposure at 35°C (D) Replicates			Mean
4	0	0	0	0	0	0	0	0	3	0	2	1.66
16	0	2	0	0.66	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0	3	1	1.33
24	0	1	0	0.66	0	0	0	0	3	1	0	1.33
48	0	0	1	0.66	0	0	0	0	0	0	0	0
72	0	0	0	0	0	0	0	0	0	0	0	0

* D = Darkness

† 50 achenes per replicate, in 2ml of distilled water.

Table 4.3. The mean percentage germination of unchilled achenes initially kept in water for 16h at 20°C in the dark, then subjected to 1, 2 or 3 cycles of 20°C (16h) D/35 C (8h) D† with or without 30 minutes R given at the transition from 20°C to 35°C. Germination took place at 20°C in the dark, and count scored after 14 days.

Serial No.	Conditions/Treatment Temperature °C	30 minutes R irradiation	Percentage Replicates			Germination Mean
1	Constant 20 (D)	-	0	0	0	0
2	" "	1 x R	0	0	0	0
3	" 35 (D)	-	40	34	26	33.3
4	" "	1 x R	46	44	32	40.7
5	1 cycle 20(16h)D/35(8h)D	-	0	4	8	4
6	" "	1 x R	6	16	4	8.4
7	2 cycles 20(16h)D/35(8h)D	-	4	12	10	8.6
8	" "	1 x R	48	44	24	38.6
9	" "	2 x R	30	46	32	36.0
10	3 cycles 20(16h)D/35(8h)D	-	10	10	28	16
11	" "	1 x R	38	48	46	44
12	" "	2 x R	44	49	48	47
13	" "	3 x R	66	56	52	58

† D = Darkness
R = Red light

Table 4.3.

Analysis of Results : Contingency χ^2 (Chi-squared) test

Serial No.	Source	χ^2	p	Significant level
1	35°C D : R vs No. R	3.15316	$p > 0.05$	N.S.
	Increasing no. of cycles without R			
2	1 cycle vs 2 cycles	4.74808	$0.05>p>0.01$	*
3	1 cycle vs 3 cycles	22.6852	$p < 0.001$	***
4	2 cycles vs 3 cycles	6.79787	$p < 0.01$	**
	Maintaining constant cycles, while increasing the no. of R			
5	1 cycle (No.R) vs 1 cycle (1xR)	4.74808	$0.05>p>0.01$	*
6	2 cycles (No.R) vs 2 cycles (1xR)	73.0765	$p < 0.001$	***
7	3 cycles (No.R) vs 3 cycles (1xR)	54.6746	$p < 0.001$	***
8	2 cycles (1xR) vs 2 cycles (2xR)	0.349069	$p > 0.05$	N.S.
9	3 cycles (1xR) vs 3 cycles (2xR)	0.430151	$p > 0.05$	N.R.
10	3 cycles (2xR) vs 3 cycles (2xR)	6.84378	$p < 0.01$	**
	Effect of same no. of R, while increasing the no. of cycles			
11	1 cycle (1xR) vs 2 cycles (1xR)	73.0765	$p < 0.001$	***
12	2 cycles (1xR) vs 3 cycles (1xR)	1.54646	$p > 0.05$	N.S.
13	2 cycles (2xR) vs 3 cycles (2xR)	7.02983	$p < 0.01$	**

the dark or light (Figure 4.1 a&b; no.3). The subsequent experiments involved only regimes where the value of the upper temperature is at 35°C.

The alternating temperature regimes of 15°C/35°C with an amplitude of 20°C gave the highest germination (Figure 4.2 a&b; no.3) and not 5 C/35°C (Figure 4.2 a&b; no.1) with an amplitude of 30°.

The value of the upper temperature is critical and has to be at 35°C before any substantial germination can occur (Figures 4.1 a&b; and 4.2 a&b) for the regimes tested. It might be argued that 35°C is the most essential and singularly important value compared to other values, and independent of the value at the lower temperature part of the cycle. The analyses of variance for Figure 4.1 (page 88) demonstrate that the high value of F value (149.619) compared to F value (14.5182) of Figure 4.2 (page 90) for temperature regimes indicates the degree of importance of exposure to 35°C since the contribution to F in Figure 4.1 is due to the substantial difference between germination at a high of 35°C and a high which is not 35°C.

As the value of the lower temperature part of the cycle increases from 5°C to 15°C, percentage germination also increases (Figure 4.2 a&b), and this is more pronounced in the dark treatments compared to the light treatments. However, germination was observed to decrease when the value of the lower temperature increased to 20°C (Figure 4.2 a&B; no.4). Treatments having 15°C at the lower temperature part of the cycle are most stimulatory and gave the highest germination.

The duration of the period spent at the upper temperature is certainly promotive. An increase in the period spent at the upper temperature from 8h to 16h increases germination correspondingly in both dark and light (Figure 4.2 a&b).

Germination was enhanced in both the 8h and 16h light treatments, but the 16h light treatments gave the greater response. From the values for F_1, it would seem that light is of greater importance than temperature in promoting germination of achenes (Figure 4.2 Analysis of Variance, page 90).

Percentage germination for the light treatments was better than the

controls, i.e. constant light or dark; 8h or 16h photoperiod at 35°C constant (Figure 4.2 a&b).

Rate of Warming/Cooling

It was observed that the time taken to attain 35°C at the upper temperature part of the cycle was about 20 minutes in the light and about 39 minutes in the dark, while the time taken to attain 20°C at the lower temperature part of the cycle was about 37 minutes. From these observations, it seems likely that the rate of warming or cooling might not be a critical factor when the period spent at the upper temperature part of the cycle is 8h or 16h in a 24h diurnal cycle. However, if short period, e.g. 1h, is spent at the upper temperature part of the cycle, then the rate of warming could be critical and the period spent at the upper temperature would be considerably less than 1h.

Effect of Increase in the Duration of Illumination

The results of increasing the daylengths at constant 35°C (Figure 4.3,a) from 4h to 20h (i.e. so-called short to long days) showed that increasing daylengths did not enhance total percentage germination considerably. There is no significant difference between 8h and 16h illumination (Figure 4.3 χ^2 test, no.1, page 92a). However, there is a slight increase in germination from 0h to 16h illumination and thereafter, percentage germination was fairly constant (Figure 4.3, a). With increasing daylength at the upper temperature of 15°C/35°C in a 24h diurnal cycle, maximum germination was attained at 16h and it did not differ from 20 illumination (Figure 4.3,c), but was considerably higher than 8h illumination. Here, Chi-squared (χ^2) test between 8h and 16h demonstrated a significant difference (Figure 4.3, χ^2 test, no.2, page 92a). An analysis of variance carried out between the daylength treatments at constant 35°C and an alternating temperature (Figure 4.3, a&c, page 92a) demonstrated a significant difference with an F value of 33.4619. Also statistical interaction with an F value of 47.1001 occurred between daylengths and alternating temperature. This might also indicate a physiological inter-

action for a certain proportion of achenes in the population. The effect of light treatment is significant over dark treatment (Figure 4.3, b&c, page 92a). From the foregoing account, light undoubtedly stimulated the germination of achenes, and the duration of illumination seems to be a significant factor.

Effect of Reduced Light Intensity

The effect of reduced light intensity was monitored (Figure 4.4, a,b,c,d &e). Percentage germination was similar between the 40 and 100 percent light intensity. Although, at continuous light treatment, germination in 100 per cent intensity was significantly higher at the 5 percent level, over 40 per cent light intensity (Figure 4.4, χ^2 test, no.2), these results cannot be taken as being very substantial.

Effect of Imbibition on Light Response

The responsiveness of the achenes to light from the time of onset of imbibition at 15°C (dark) increases slightly from 0h to 48h imbibition under all germination test conditions viz. 35°C constant, 8hD/16hL at 35°C and 15°C (8h)/35°C (16h) (Figure 4.5, a,b,c&d). But when illumination was not given until after 48h from the onset of imbibition at 15°C, light response decreased considerably. Indeed, the achenes should not be kept imbibed at 15°C for long if they are to respond to light. The responsiveness of achenes to temperature at constant 35°C dark or 15°C (8h)/35°C (16h) (Figure 4.5, a&d) in the dark after the onset of different imbibition time at 15°C follows a similar pattern shown by the response of achenes to light (Figure 4.5, b&e). For all the treatments, the illuminated achenes gave higher percentages of germination than that of dark treatments (Figure 4.5).

Effect of Light given at the Lower Temperature part of the cycle

In the experiments described so far, light was given at 35°C. The effect of light at the lower temperature part of the cycle was also examined. This experiment might be considered artificial in relation to what actually operates in nature (i.e. in the field), because light is normally associated with high temperatures, i.e. during the day. In this case, achenes were initially imbibed at the higher temperature (35°C) part of the cycle from 2h to 36h and transferred to the lower part of the cycle (i.e. 20°C) for 8h duration and transferred back to 35°C for 16h. The results (Figure 4.6, a&b) showed that after the onset of imbibition at 35°C, irrespective of the imbibition time, achenes germinated well when transferred to the lower temperature of 20°C in the light. It seems that response to light is not associated with any particular temperature phase, as long as achenes received light immediately either after or before 35°C temperature.

Effect of Temperature Rhythm

The concept that germination might be modified by temperature rhythms, and that the character of the first treatment (i.e. temperature) given to an imbibing achene might determine the final response was tested. The results (Figure 4.7) demonstrate that starting the cycles from either low or high temperature was of no consequence providing the optimum conditions for germination were given viz. light, and 16h period at 35°C. Both the duration of imbibition and the cyclic temperature regimes were not significantly different, and once again, analysis of variance showed light to be a significant factor (Figure 4.7, page 98). The control treatments at 35°C continuous light or dark, or at 16h light/8h dark at 35°C germinated less than the alternating temperature treatments exposed to light (Figure 4.7).

From the foregoing experiments, there is no doubt that light and alternating temperature play a significant role in promoting the germination of unchilled achenes. The possible involvement of phytochrome in this

response was then examined.

PHYTOCHROME INVOLVEMENT

Effect of Imbibition on Red (R) Light irradiation at 20°C and 35°C (dark)

Achenes which were irradiated with 30 minutes red at various times from the onset of imbibition germinated better than the non-irradiated achenes (Figure 4.8). At 20°C, this effect is not observable (Figure 4.8, a), but at 35°C the effect is clear with red light treatment doubling the percentage germination (Figure 4.8, b). After 4h of imbibition, germination increased, and the maximum response attained after 16h imbibition, and then the response decreasing slightly to 48h imbibition treatment (Figure 4.8, b). However, imbibition time was not significant in the response of achenes to Red (R) treatment (Figure 4.8, 1 factor Variance, page 100). If after irradiation the achenes were kept at 20°C, they did not germinate (Figure 4.8, a). Germination was only observed when achenes were transferred to 35°C in the dark. This implies that the effect of R is conditional on the achenes being germinated at 35°C. Also, it is worth noting that this irradiation does not permit complete germination since the percentage germination did not exceed 40 percent whilst the maximal possible germination of this batch was about 80 percent.

Effect of Different Duration of Red Exposures at 35°C (dark)

That the response might be a function of duration of irradiation was tested by exposing achenes to red light for 5,10,20,30 or 60 minutes (Figure 4.9). These exposures result in almost equal germination. The duration of R exposure was not a significant factor in manifestation of the red effect (Figure 4.9, 2 Factor Variance, page 102). The increase in germination compared to the control was about 20 percent. These results might suggest that about 20 percent of the achenes are under phytochrome control. However, this speculation awaits further confirmation by R/FR irradiation.

Effect of R, FR, R + FR and R + FR + R

An experiment employing red and far-red exposures was carried out to test phytochrome involvement. The results confirmed previous findings of R enhancement of germination (Figure 4.10, a&b); and they also show that FR reverses R enhancement, but FR has no inhibitory effect on achenes not previously irradiated, that is dark germinating achenes. Achenes given the sequence of R-FR-R behaved in a classic phytochrome pattern (Figure 4.10, a&b). The promotive effect of R is significant over the control or FR treatment (Figure 4.10 a, nos. 1,3&5; 4.10 b, nos. 6,8&10). This implies that under the conditions used for germination, namely 35°C dark germination, approximately 20 percent are not under the influence of phytochrome control, while another sub-population, about 20 percent are, and with the rest of the population about 60 percent are not responding to the given treatments, although the viability test (Table 3.1, page 43) would indicate about 80 percent viability. This fraction of the population that did not germinate was looked at under an alternating temperature regime.

Effect of Increased Frequency of R Irradiation

It has been shown that as long as exposure time to R was between 10 to 30 minutes, a promotive effect was observed. Nevertheless, the view that germination could be increased further by subsequent reinforcement with R after every 24h at 35°C was examined. The results (Figure 4.11) illustrate that after the first R irradiation, further series of irradiation did not increase the total percentage germination significantly (Figure 4.11, 2 Factor Variance, page 106). This shows that one 30 minute R exposure was effective and might indicate that this light control phenomenon is not photoperiodic in nature. Also, the view that at constant 35°C, only about 20 percent of the achenes respond to R is acceptable and suggests that to effect a complete germination, the achenes should be germinated in conditions other than constant 35°C.

As seen in the results from previous experiments in which achenes were exposed to an alternating temperature with the light treatment given at the upper temperature part of the cycle, total percentage germination was substantial. From Figure 4.11 it can be seen that reinforcement of R light treatments failed to increase germination. Also, dark alternating temperature failed to induce germination above 30-35 percent. It could be argued that a proportion of the population is responsive to alternating temperature coupled with light. In this proportion, the expression of germination is only possible when both are given simultaneously or in combination. Thus, interaction between alternating temperature and light is the key word here.

In this regard, achenes were subjected to 1,3,6 or 9 cycles of alternating temperatures, with a corresponding number of R treatments. The results (Figure 4.12) demonstrated that while 1 or 3 cycles of only alternating temperatures, or even 6, promoted slight germination, their corresponding treatments with R exposure enhanced germination more, and were significantly different (Figure 4.12 χ^2 test, page 108). When the alternating temperature treatment is combined with R exposures, germination was stimulated but this effect reached a maximum after 6 cycles (Figure 4.12). The fact that emerges here is that alternating temperature with R irradiated at every transfer to 35°C stimulated germination more than when R treatment was given to achenes held at 35°C.

This experiment was taken further and the effect of R, FR irradiations were investigated before, after, and before and after every transfer to the 35°C part of the cycle. It was carried out to test if the germination of the proportion that responds to alternating temperature and light simultaneously also respond to FR irradiation. The results (Figure 4.13) demonstrate that while germination is enhanced by R, it is also suppressed by FR. Germination of achenes subjected to an alternating temperature in combination with R irradiation was promoted regardless of the point of R irradiation. The total percentage germination of these R treatments was considerably higher than the constant 35°C dark germination with or without R irradiation, or the alternating

temperature without R irradiation (Figure 4.13, page 110); and also these results are significantly different from R treatments (Figure 4.13, pages 111 & 112). Moreover, the total percentage germination of those achenes subjected to an alternating temperature coupled with R were comparable to the previous treatments where alternating temperatures are coupled to fluorescent white light.

The corresponding FR treatments gave lower germination in contrast to R treatments (Figure 4.13). Here again, the point of irradiation was of no consequence, and germination is suppressed in response to FR irradiation. The percentage germination here is comparable to those of 35°C constant dark germination with or without FR irradiation, and the results were not significantly different (Figure 4.13, 1 Factor Variance b&d).

The results of the alternating temperature in combination with R treatments were significantly different from the respective FR treatments (Figure 4.13, 1 Factor Variance C). The difference in percentage germination between these treatments was about 40-50 percent. These results perhaps suggest the existence of a sub-population that is responsive only to alternating temperature coupled with light.

From these experiments in which both light and alternating temperature were employed, the following possibilities might apply. It is generally observed that about 20 percent of a proportion of the population are dark germinating, and would germinate even when irradiated with FR. Continuous illumination, at least 16h light/8h dark photoperiod or R irradiation, in most cases enhanced the total percentage germination to about 40 percent, that is a 20 percent increase over dark germinations.

That this light enhanced germination is under phytochrome control is confirmed by the reversal of red induced germination by far-red. Thus, it might be argued that there is present in the population a second light-requiring fraction which constitutes about 20 percent of the population. The statistical analysis of these findings suggests that the combined effects of constant high temperature and light are additive, rather than synergistic

or antagonistic.

When achenes are germinated under alternating temperature regimes in darkness, there is an increase in the total percentage germination to 35-40 percent, i.e. 20 percent more than under constant temperature of 35°C. This might indicate that there is another fraction, also approximately 20 percent of the population, which responds to alternating temperature and not to light. But the germination of achenes under the dark conditions did not exceed the continuous light treatments.

These unchilled achenes which do not respond to any of the individual specific conditions which have been discussed and are known to be viable, might be induced by a combination of these, that is, an alternating temperature and light. Thus, it is postulated that there exists a fourth group in the population of unchilled achenes responding only to a combination of alternating temperature coupled with light. The difference in germination between the alternating temperature treatments either illuminated, or given R light with their respective dark treatments, is approximately 30-40 percent. Hence, this group might constitute a particular component of the total population.

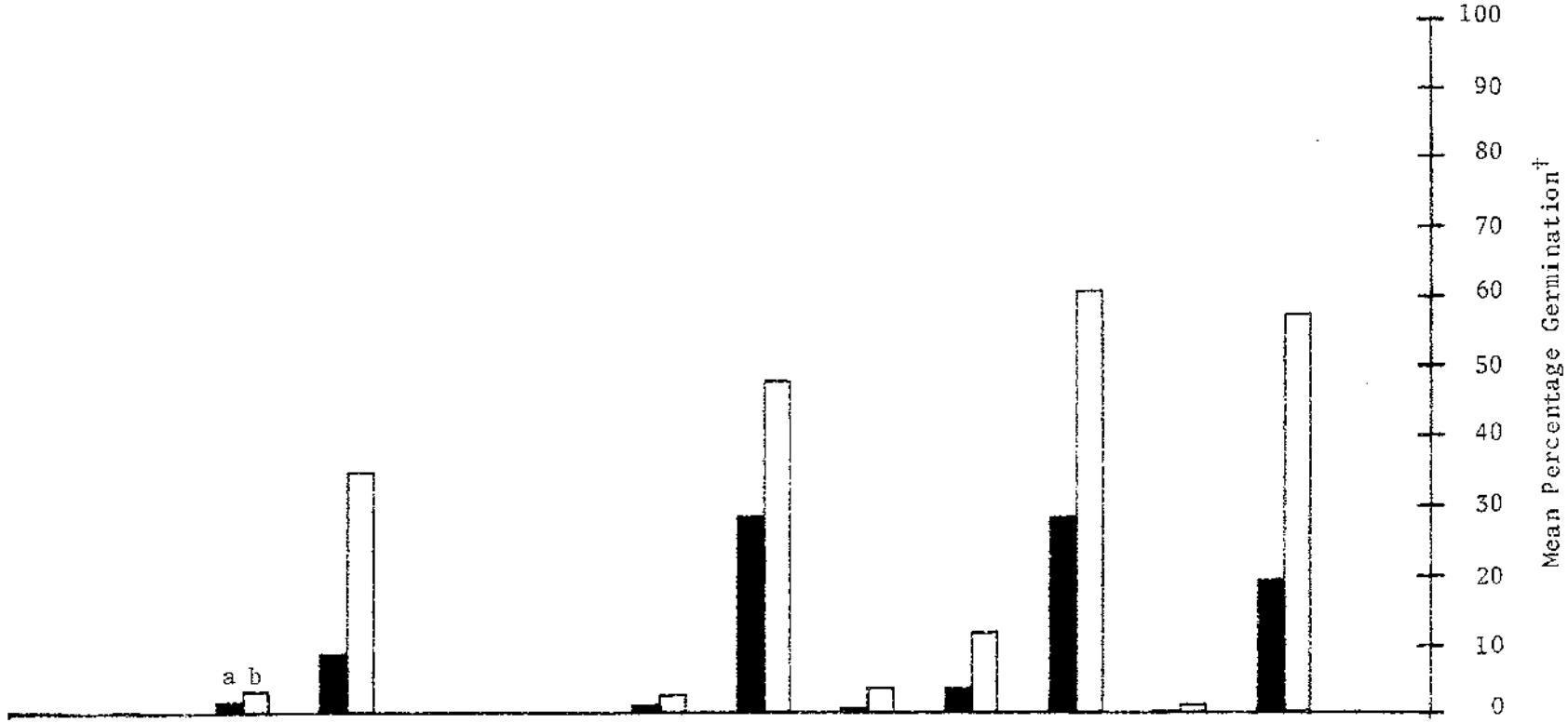
In contrast to the situation regarding constant temperature and light exposure where the effects are simply additive, it seems that when unchilled achenes are exposed to alternating temperature and light at the same time, there is a positive interaction (Figure 4.3 a&c, Analysis of Results, page 92a. An overall summary could be presented by considering the population to consist of sub-populations, namely, the dark germination, the light-requiring, the alternating temperature (dark) germination and the alternating temperature plus li germination of achenes. However, there is approximately another 25-30 percent of the sub-population that does not germinate and cannot be classified under any of the groups mentioned above. The germination of this latter group might be promoted by other environmental factors, e.g. stratification treatment.

A diagrammatic representation of the different sub-populations is shown in Table 4.

Table 4.0. Postulated sub-population existing in the population of *P. persicaria* achenes harvested 1981. The total percentage germination is given in the "combination" boxes and the marginal differences indicate the proportions that respond to light or alternating temperatures. This assumes that there is neither synergism nor antagonism in treatments.

Treatments	Constant 35°C	Alternating Temperature	Fraction requiring Alternating Temperature
Dark	Sub-population I 20 percent	40 percent	Sub-population III 20 percent
Light	40 percent	80 percent	40 percent
Light-requiring (under phytochrome control)	Sub-population II 20 percent	40 percent	Sub-population IV 20 percent

FIGURE 4.1[a]



Lower Temperature	5°	5°	5°	5°	10°	10°	10°	10°	15°	15°	15°	20°	20°
Upper Temperature	15°	20°	25°	35°	15°	20°	25°	35°	20°	25°	35°	25°	35°
Nos.	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)

ALTERNATING TEMPERATURES

a. 16h D at the lower/8hD at the Upper Temp.

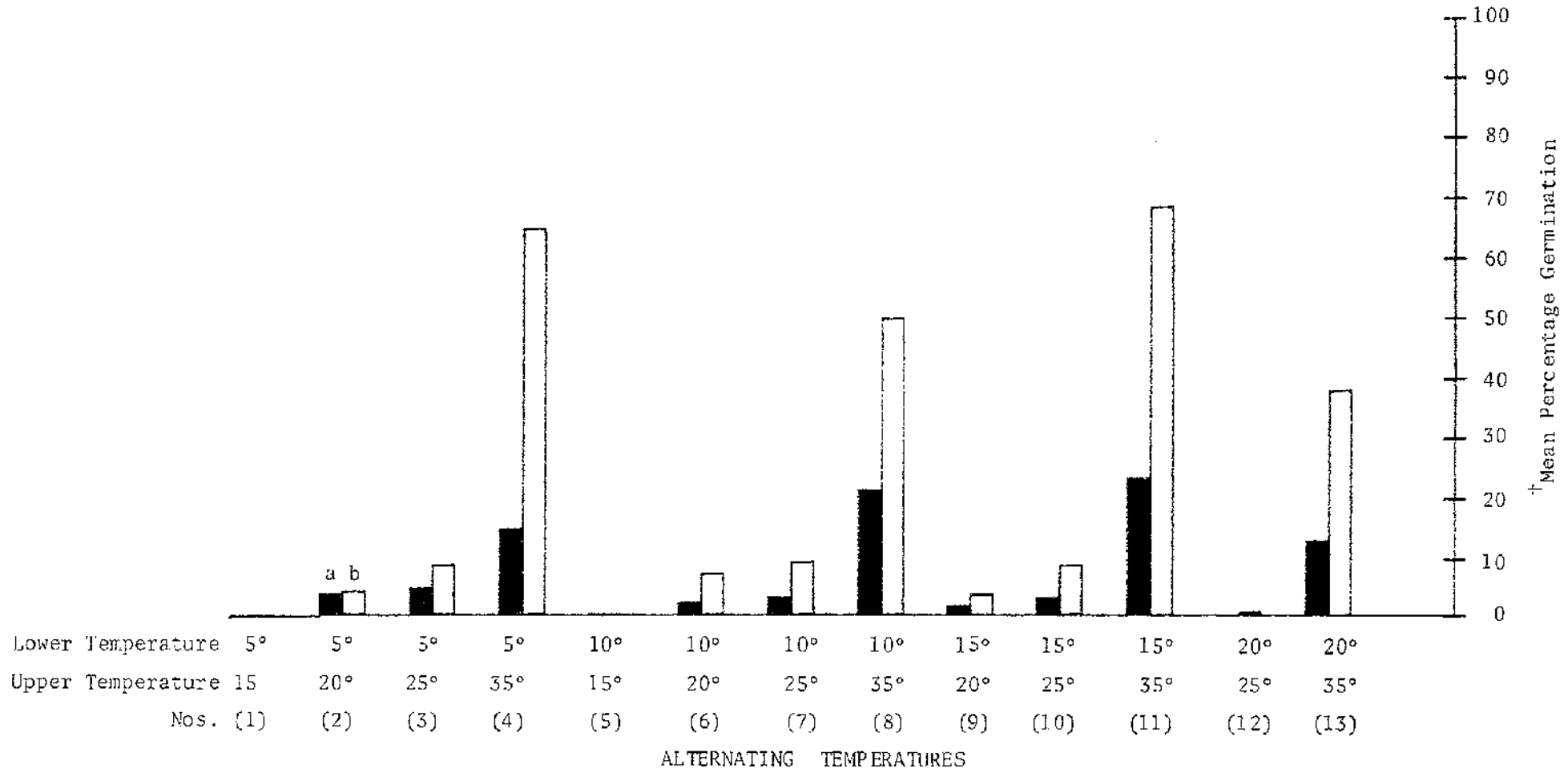
b. 16h D at the lower/8h L at the Upper Temp.

Temp. = Temperature.

The mean percentage germination of unchilled achenes subjected to Different Alternating Temperature Regimes in a 24h diurnal cycle.

†Germination scored after 14 cycles.
Treatment replicated twice with 100 achenes per replicate in H_2O.

FIGURE 4.1 b



a. 8h D at the Lower/16h D at the Upper Temp.
b. 8h D at the Lower/16h L at the Upper Temp.

D = Darkness
L = Light
Temp. = Temperature

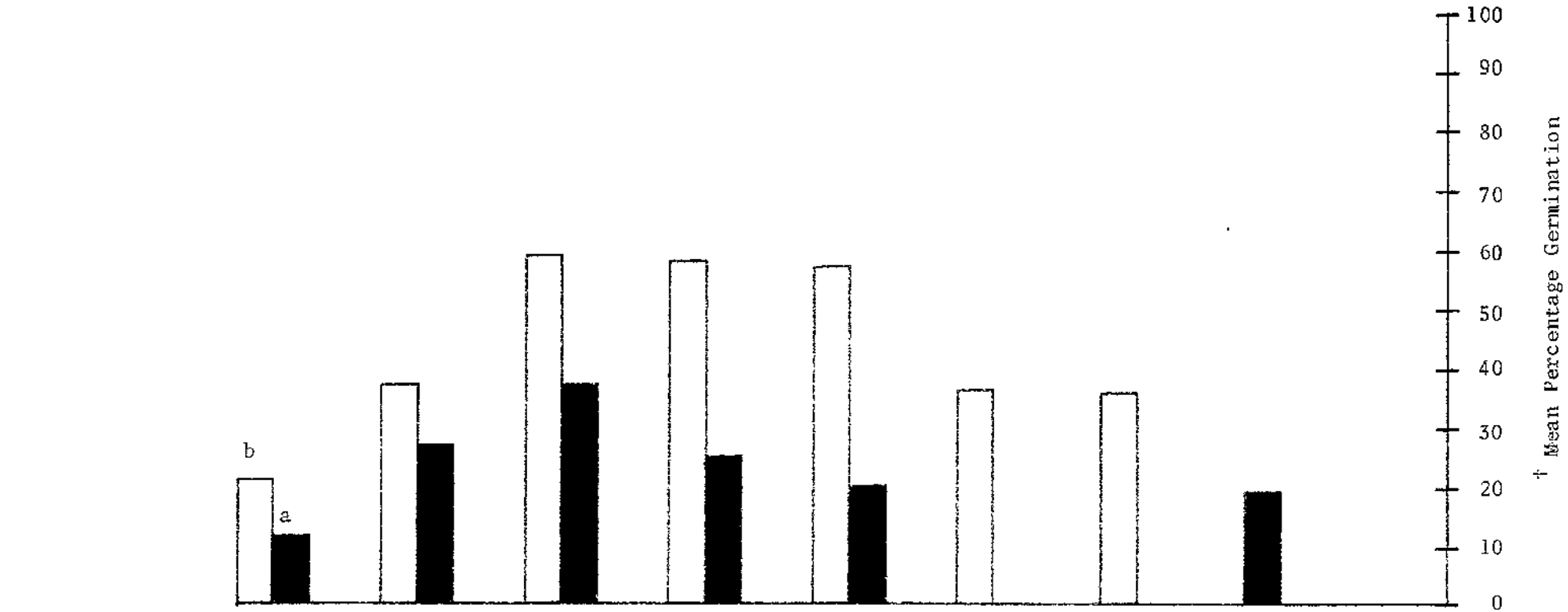
The mean percentage germination of unchilled achenes subjected to Different Alternating Temperature Regimes in a 24h diurnal cycle.
Germination scored after 14 cycles.
†Treatment replicated twice with 100 achenes per replicate in 10ml of H_2O.

Figure 4.1, a & b.

Analysis of Results:- 3 Factor Variance

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	1	39.0059	39.0059	3.2964	N.S.
Temperature Regimes (A)	12	21245	177042	149.619	***
Short (8h) vs Long (16h) Duration (B)	1	174.449	174.449	14.7428	***
Dark vs Light (C)	1	2072.82	2072.82	175.176	***
A x B	12	1043.46	86.9554	7.3486	**
B x C	1	35.9082	35.9082	3.0346	N.S.
A x C	12	187982	156.652	13.2387	***
A x B x C	12	158.678	13.2231	1.1175	N.S.
Error	51	603.475	11.8328		
Total	103	27252.6			

FIGURE 4.2 a



a. 16hD at Lower Temp/8hD at Upper temp.
b. 16hD at Lower Temp/8hL at Upper temp.

Temp. = Temperature

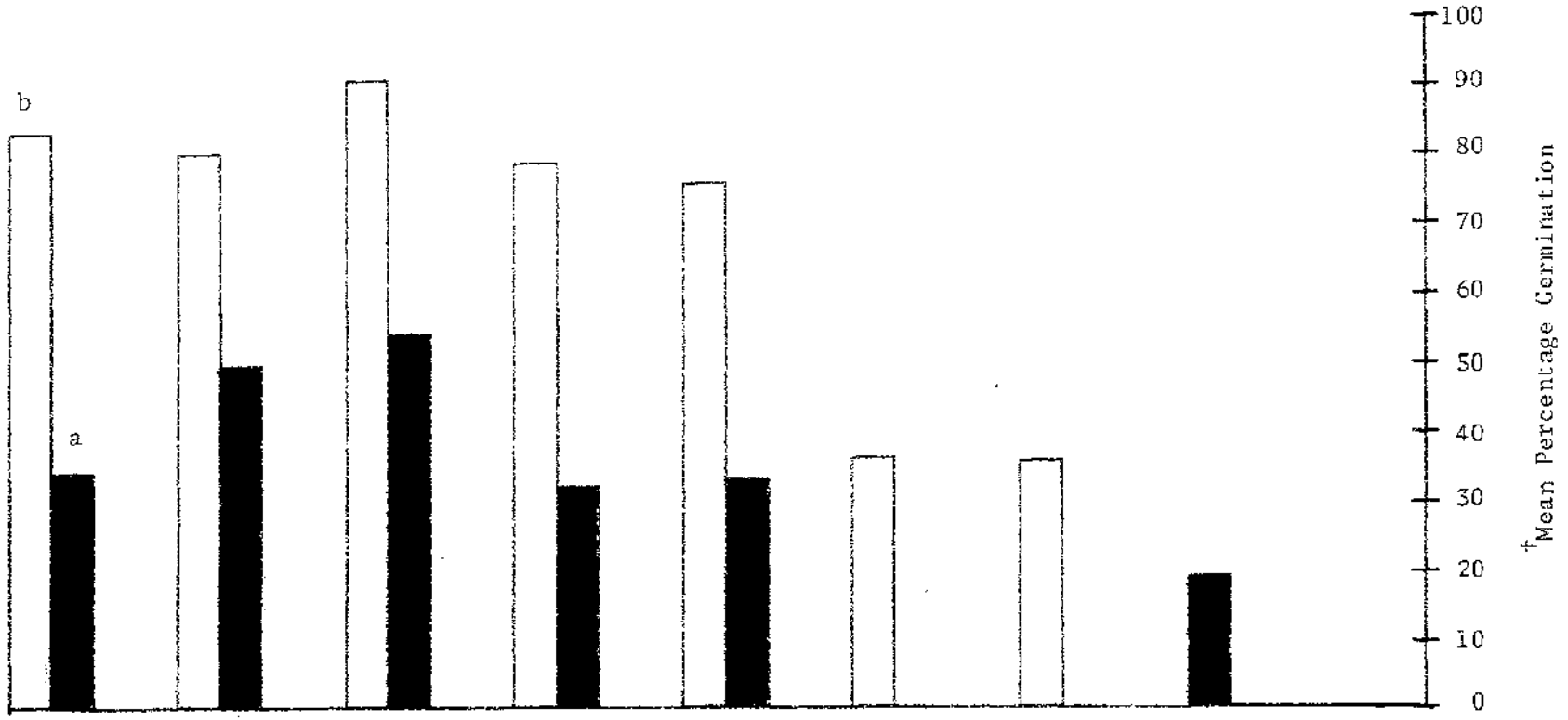
The mean percentage germination of unchilled achenes subjected to Different Alternating Temperature Regimes in a 24h diurnal cycle.
†Germination scored after 14 cycles.
Treatment replicated twice with 100 achenes per replicate in 10ml of distilled water.

Figure 4.2.

Analysis of Results:- 3 Factor Variance

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	1	22.0859	22.0859	1.4444	N.S.
Temperature Regime (A)	4	887.977	221.994	14.5182	***
Short (8h) vs Long (16h) Duration (B)	1	2773.93	2773.93	181.413	***
Dark vs Light	1	3688.36	3688.36	241.216	***
A x B	4	429.68	107.42	7.02518	*
B x C	1	328.883	328.883	21.5087	***
A x C	4	157.188	39.2969	2.56998	N.S.
A x B x C	4	144.031	36.0078	2.35488	N.S.
Error	19	290.523	15.2907		
Total	39	8722.66			

FIGURE 4.2 b

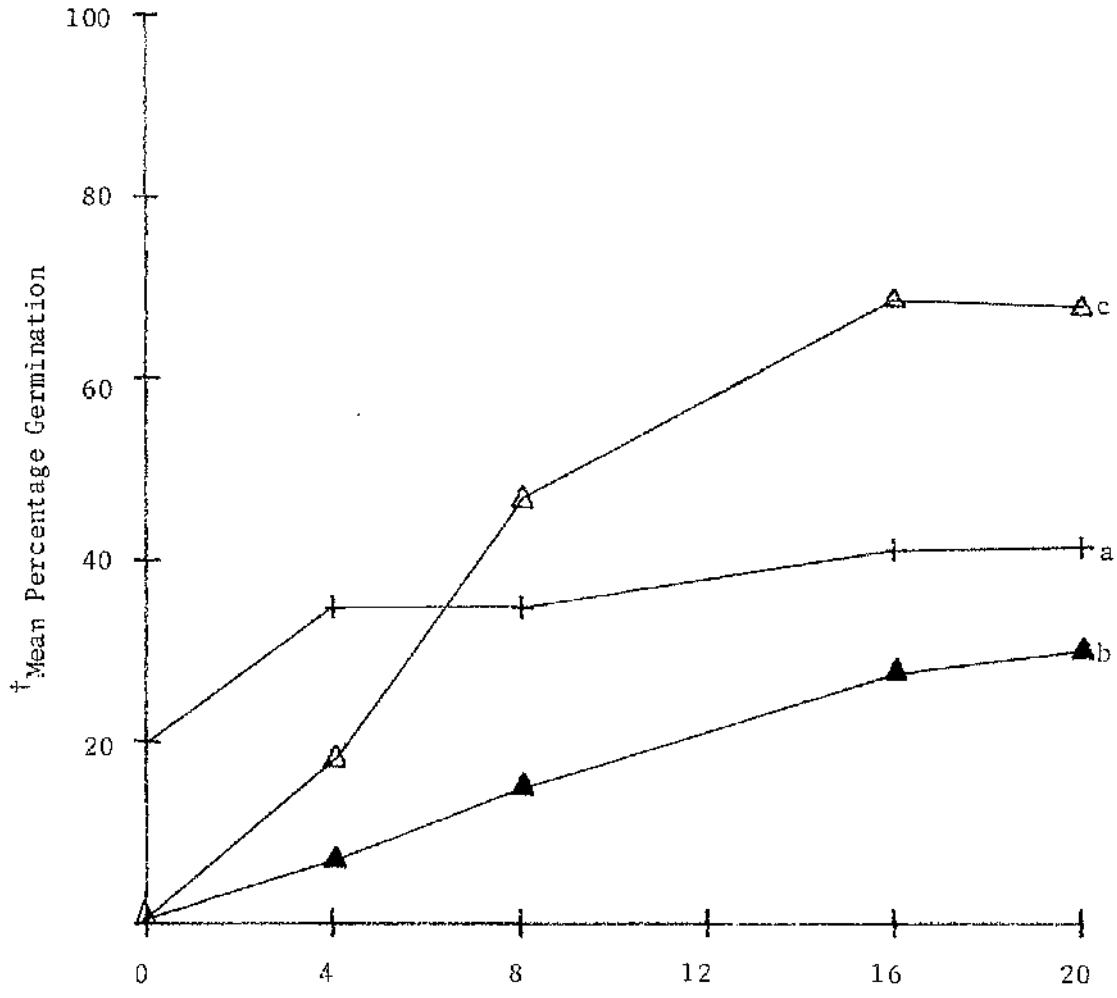


a. 8hD at Lower/16hD at Upper Temp.
b. 8hD at Lower/16hL at Upper Temp.

D = Darkness
L = Light
h = Hours
Temp. = Temperature

The mean percentage germination of unchilled achenes subjected to Different Alternating Temperature Regimes in a 24h diurnal cycle.
Germination scored after 14 cycles.
†Treatment replicated twice, with 100 achenes per replicate.

FIGURE 4.3



Effect of different period of illumination at 35 °C (upper temperature part of the cycle) in a 24h diurnal alternating temperature of 15°C/35°C with corresponding time at the lower temperature part of the cycle. Counted after 14 cycles.

▲ Dark Treatment - 15°C/35°C.

Δ Light Treatment - 15°C/35°C.

* Continuous at 35°C.

† Each treatment was replicated thrice, with 100 achenes per replicate in 10ml of distilled water.

Achenes were soaked in water for 16h at 15°C (dark) before subsequent Alternating Temperature treatments.

Figure 4.3.

Analysis of Results:- χ^2 (Chi-squared) Test.

Serial No.	Source	χ^2	p	Significant level
1	35°C: 8h Light vs 16h Light	2.29595	$p > 0.05$	N.S.
2	15°C/35°C: 8h Light vs 16h Light	28.9067	$p < 0.001$	***

2 Factor Variance

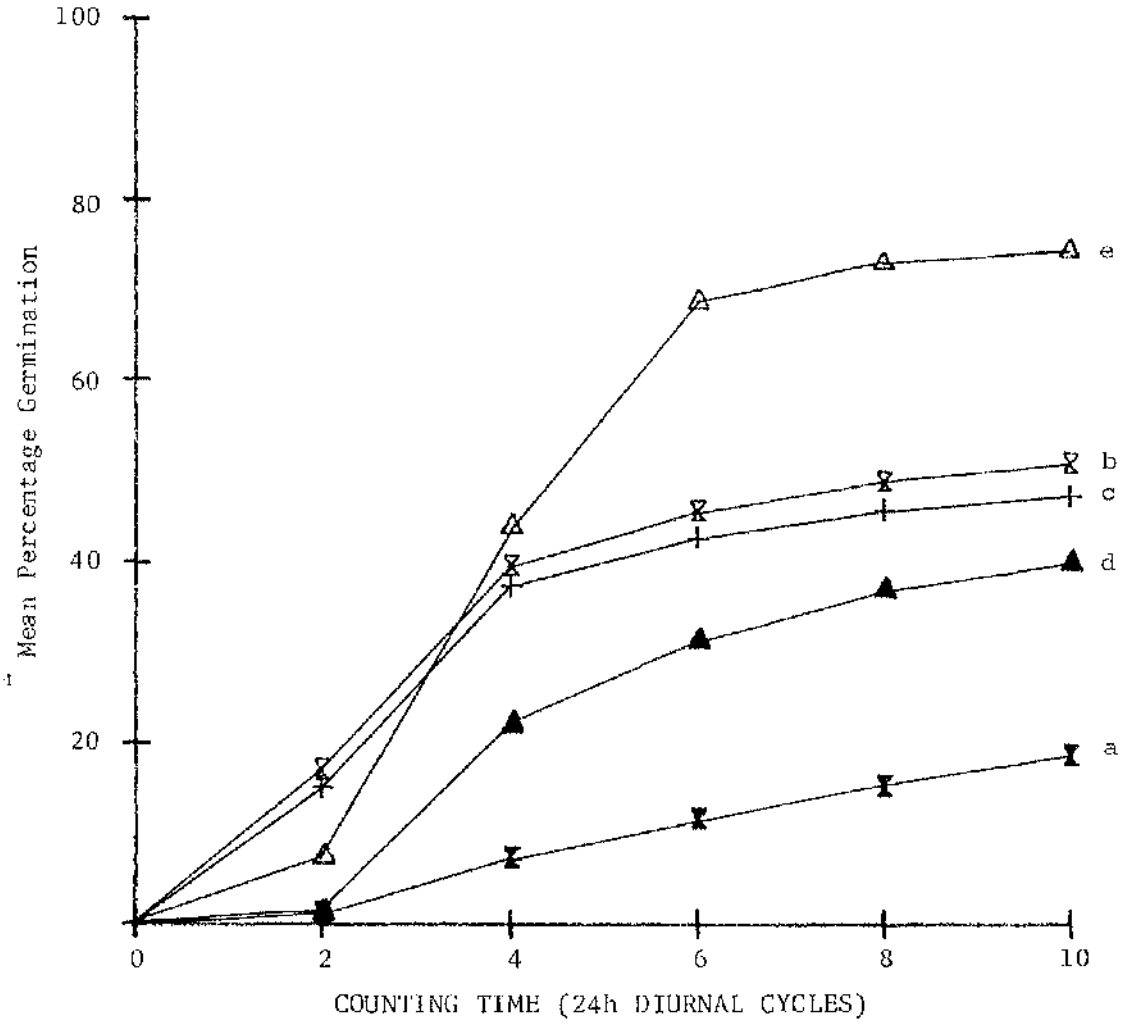
4.3, a&c

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	2	1.3125	0.65625	0.127073	N.S.
Daylength (A)	3	518.426	172.809	33.4619	***
Constant 35°C vs 15°C/35°C (B)	1	962.676	962.676	186.408	***
A x B	3	729.723	243.241	47.1001	***
Error	14	72.3008	5.16434		
Total	23	2284.44			

4.3, b&c

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	2	1.82227	0.911133	0.14267	N.S.
Light vs Dark (A)	1	2204.17	2204.17	345.141	***
Daylengths (B)	3	2308.26	769.419	120.48	***
A x B	3	184.734	61.5781	9.64222	**
Error	14	89.4082	6.3863		
Total	23	4788.39			

FIGURE 4.4



Effect of reduced light intensity (40%) on the germination of unchilled achenes at different temperatures.
†Each treatment replicated thrice with 100 achenes per replicate.

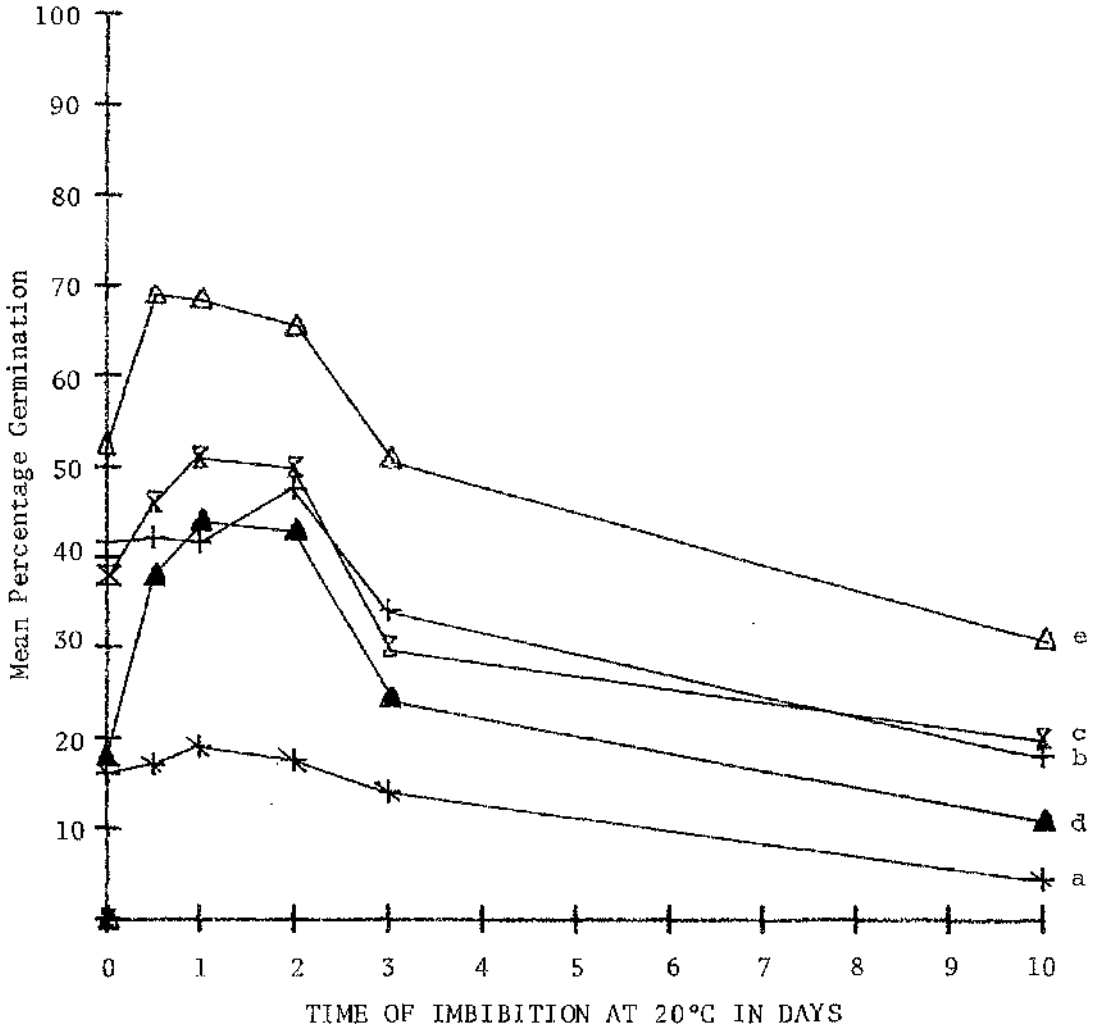
a : Continuous Dark at 35°C
b : Continuous Light at 35°C
c : 35°C-8hD/16hL
d : 15°C(8h)D/35°C(16h)D
e : 15°C(8h)D/35°C(16h)L
D = Darkness
L = Light
h = Hours

Figure 4.4.

Analysis of Results : Comparison between 40 and 100 percent illumination.

Number	Source	χ^2	p	Significant Level
1	35°C : 8h Dark/16h Light	2.18981	$p > 0.05$	N.S.
2	Continuous Light Illumination at 35°C	6.45516	$0.05 > p > 0.01$	*
3	15°C(8h) Dark/35°C(16h) Light	2.10876	$p > 0.05$	N.S.

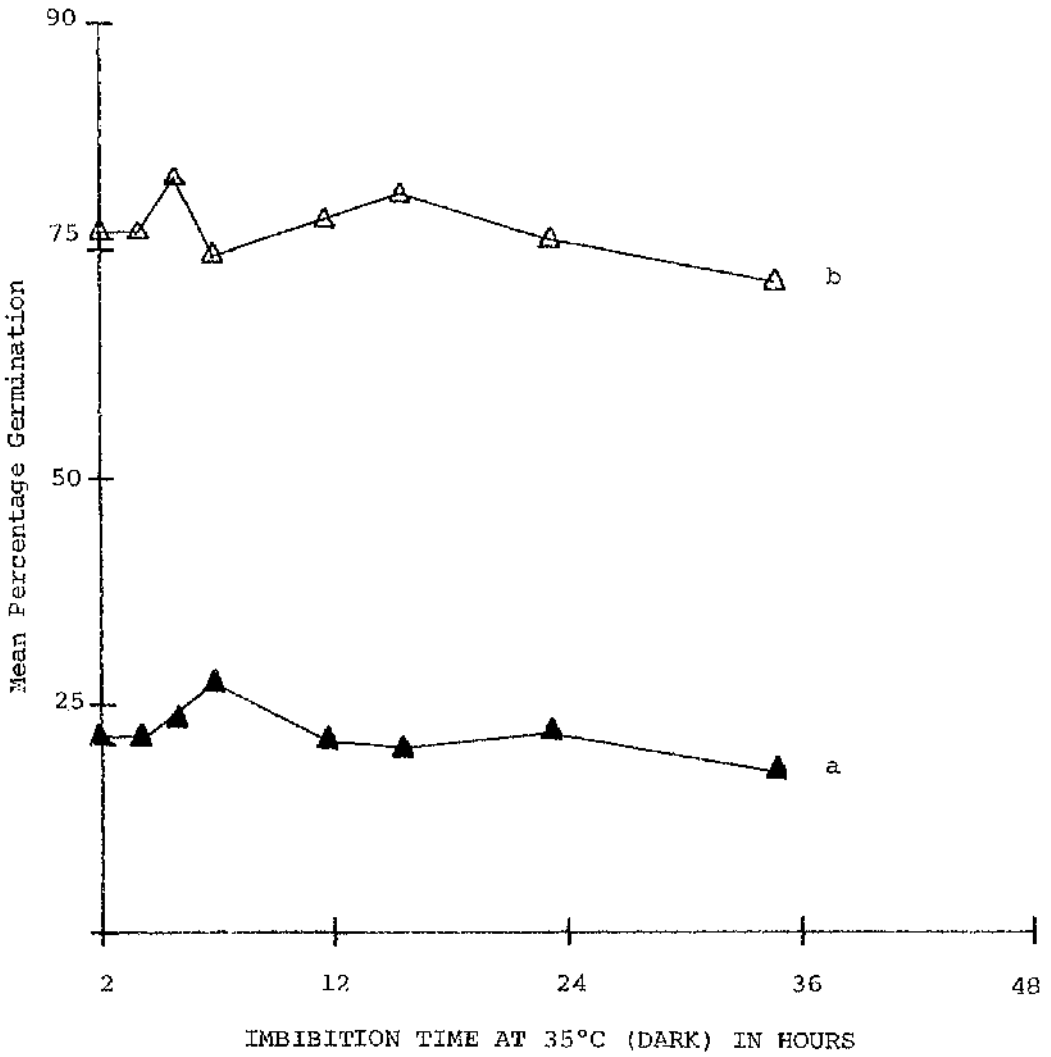
FIGURE 4.5



Effect of light response to imbibition on the germination of unchilled achenes at alternating and constant temperatures.
Germination scored after 14 cycles.

a : Continuous 35°C Dark
b : Continuous 35°C Light
c : 35°C-8h Dark/16h Light
d : 15°C(8h)D/35 C(16h)D
e : 15°C(8h)D/35C(16h)L

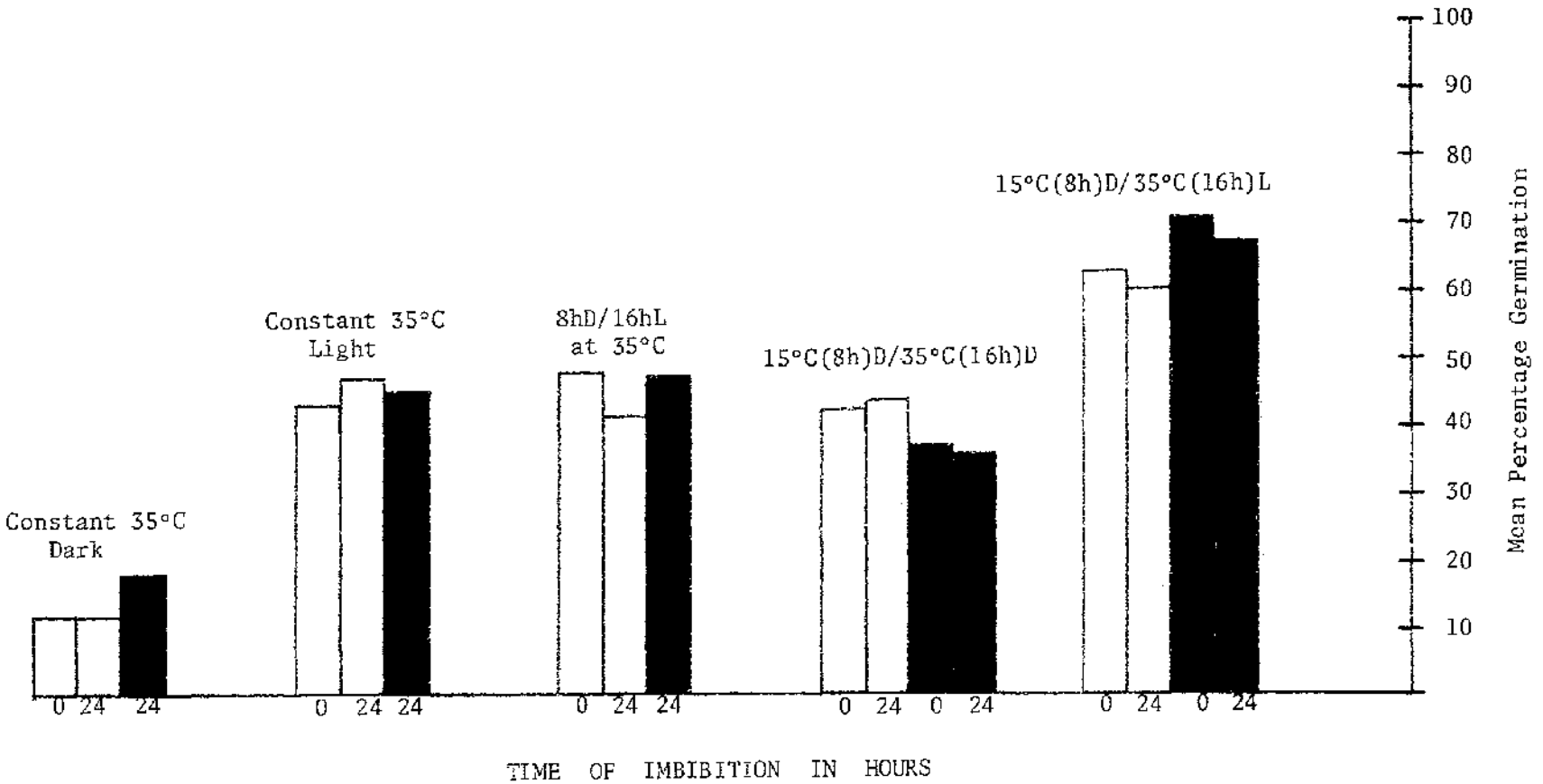
FIGURE 4.6



Response of unchilled achenes to alternating temperatures of 35°C/20°C with light illuminated at 20°C for 8h in a 24h diurnal cycle. Counted after 14 cycles.

a: 35°C(16h)D/20°C(8h)D
b. 35°C(16h)D/20°C(8h)L

FIGURE 4.7



Effect of 24h diurnal cyclic temperature rhythm on the germination of unchilled achenes at different temperatures.

□ High temperature to Low temperature

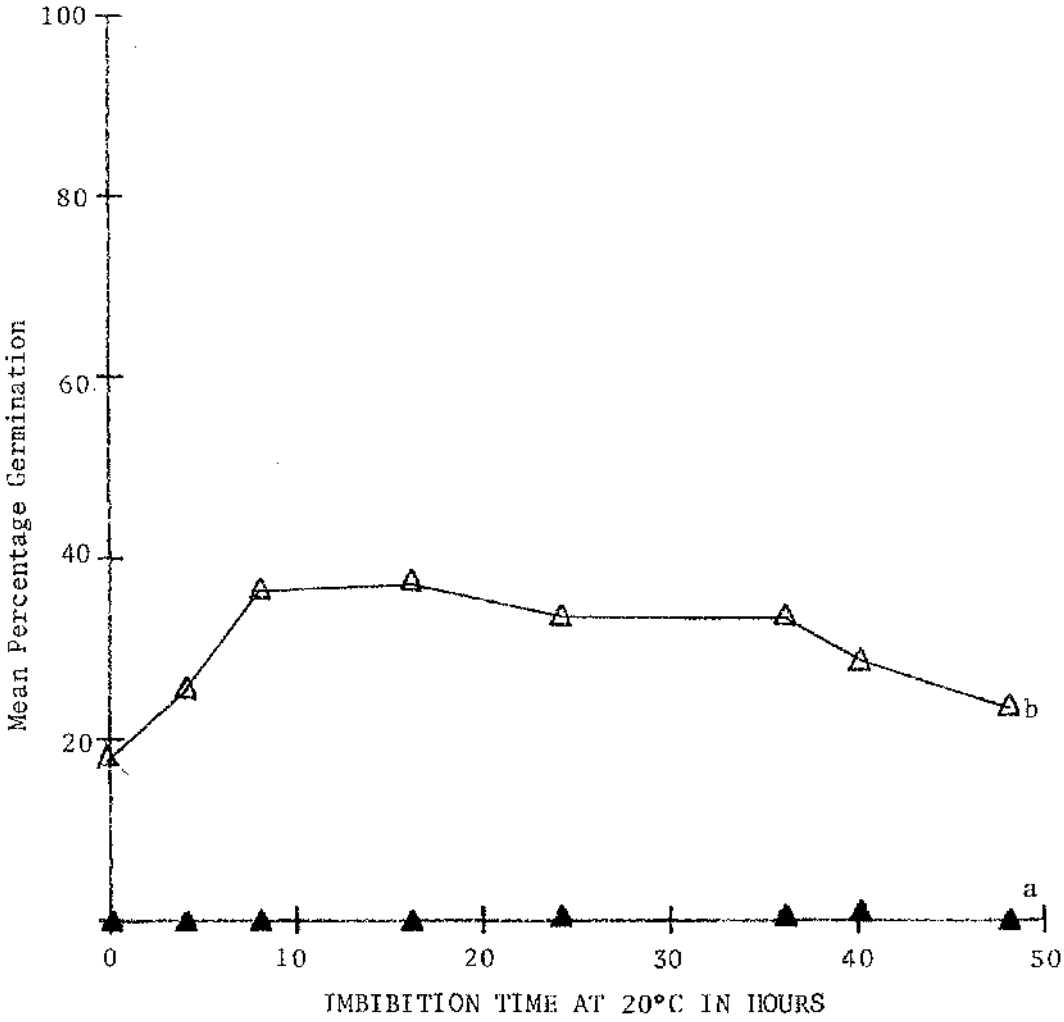
■ Low temperature to High temperature

Figure 4.7.

Analysis of Results : 3 Factor Variance

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	2	8.69141	4.3457	0.273356	N.S.
Duration of Imbibition(A)	1	7.23828	7.23828	0.455307	N.S.
Temperature Cyclic Rhythm (B)	1	0.410156	0.410156	0.025799	N.S.
Dark vs Light (C)	1	1326.08	1326.08	83.4128	***
A x B	1	2.55469	2.55469	0.160696	N.S.
B x C	1	103.355	103.355	6.50133	*
A x C	1	4.71484	4.71484	0.29657	N.S.
A x B x C	1	0.132813	0.132813	0.00835	N.S.
Error	14	222.566	15.8976		
Total	23	1675.74			

FIGURE 4.8



Germination response of achenes imbibed at 20°C (dark) and irradiated with 30 minutes Red light (R) and subsequently germinated at 35°C or 20°C dark. Counted after 14 days.

a : 20°C Dark

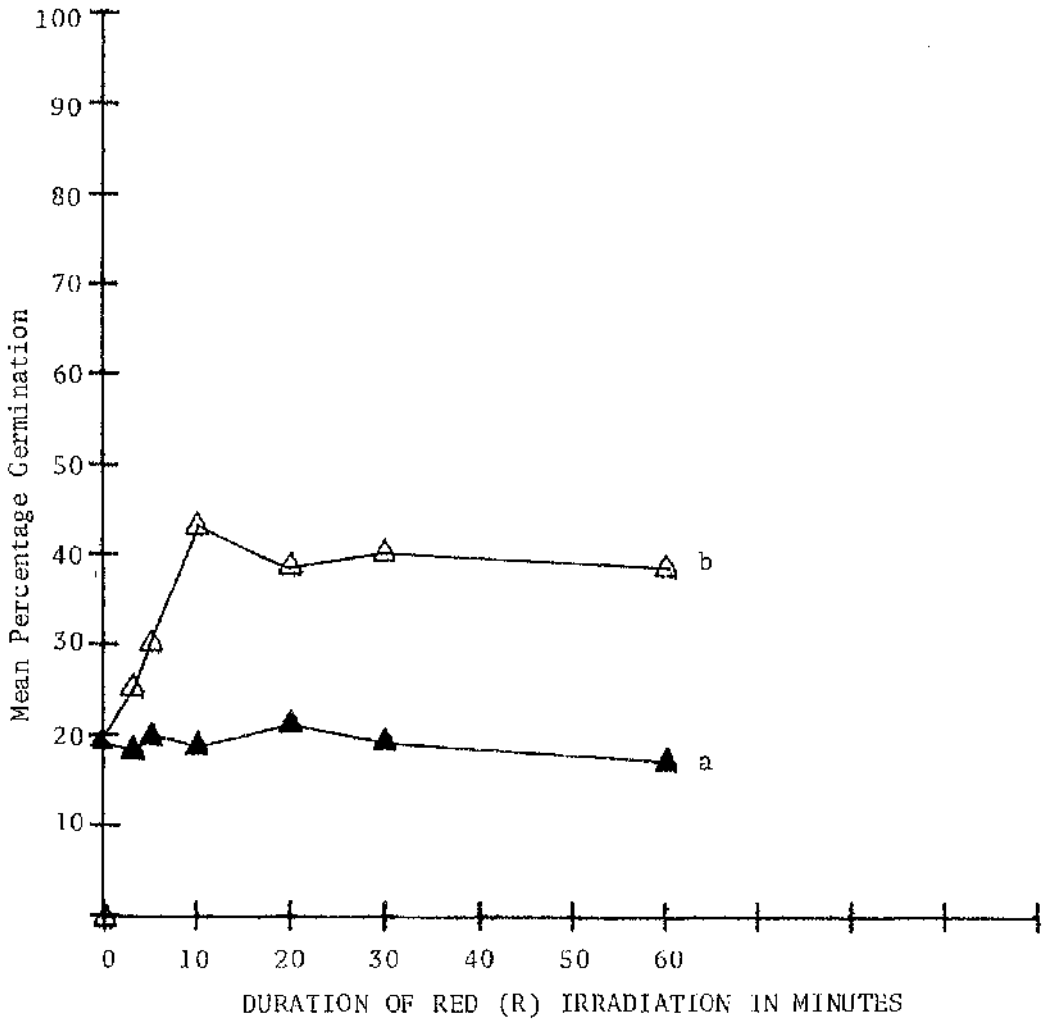
b : 35°C Dark

Figure 4.8.

Analysis of Results : 1 Factor Variance.

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	1	7.71875	7.71875	0.623984	N.S.
Imbibition Time at 20°C in Hours	6	141.368	23.5614	1.0947	N.S.
Error	6	74.2207	12.3701		
Total	13	223.308			

FIGURE 4.9



The percentage germination response of unchilled achenes irradiated with different duration of R light and germinated at 35°C in the dark.
Germination count scored after 14 days.
Achenes were initially kept at 20°C in the dark for 16h prior to R exposure. Each treatment was replicated twice with 100 achenes per treatment.

a : Non-irradiated achenes

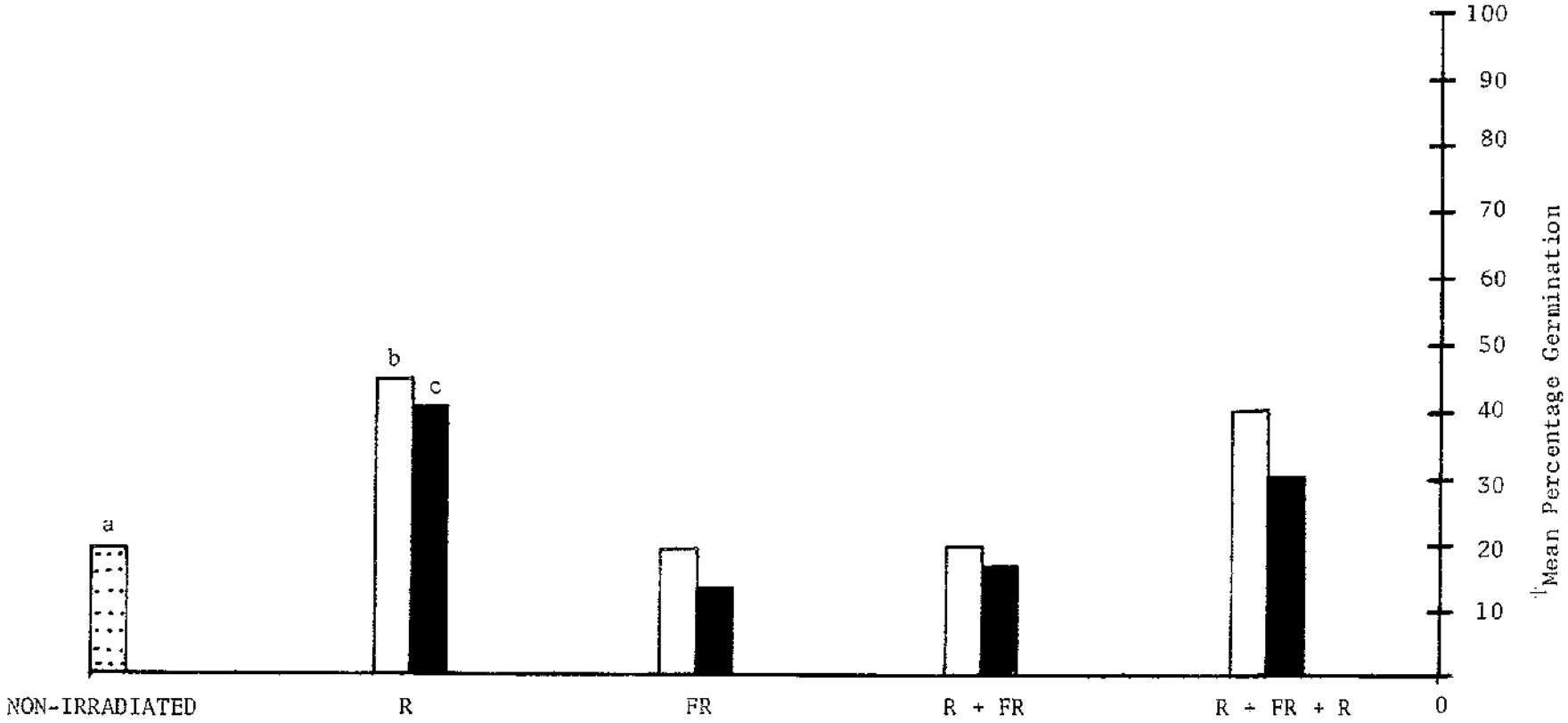
b : R Exposure

Figure 4.9.

Analysis of Results : 2 Factor Variance

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	1	3.76172	3.76172	1.37966	N.S.
Light vs Dark (A)	1	71.949	713.949	261.85	***
Duration of R Exposure(B)	5	95.3379	19.0676	6.99327	N.S.
A x B	5	87.3262	17.4652	6.40559	N.S.
Error	11	29.9922	2.72656		
Total	23	930.367			

FIGURE 4.10



Germination Response of Unchilled Achenes to 10 minutes Irradiation of R; FR; R+FR and R+FR+R. Germination at 35°C (dark). Germination scored after 14 days.

a : Non-irradiated

b : 10 minutes Irradiation

c : 30 minutes Irradiation

Irradiation was given after initial 16h imbibition at 20°C (dark).

† Each treatment was replicated twice with 100 achenes per replicate in 10ml of distilled water.

Figure 4.10.

Analysis of Results : χ^2 (Chi-squared) Test.

10 minutes Irradiation

Serial Number	4.10b Source	χ^2	p	Significant Level
1	Control vs R	28.605	$p < 0.001$	***
2	Control vs FR	0.0159261	$p > 0.05$	N.S.
3	R vs FR	28.605	$p < 0.001$	***
4	FR vs R+FR	0	$p > 0.05$	N.S.
5	R+FR vs R+FR+R	18.9579	$p < 0.001$	***

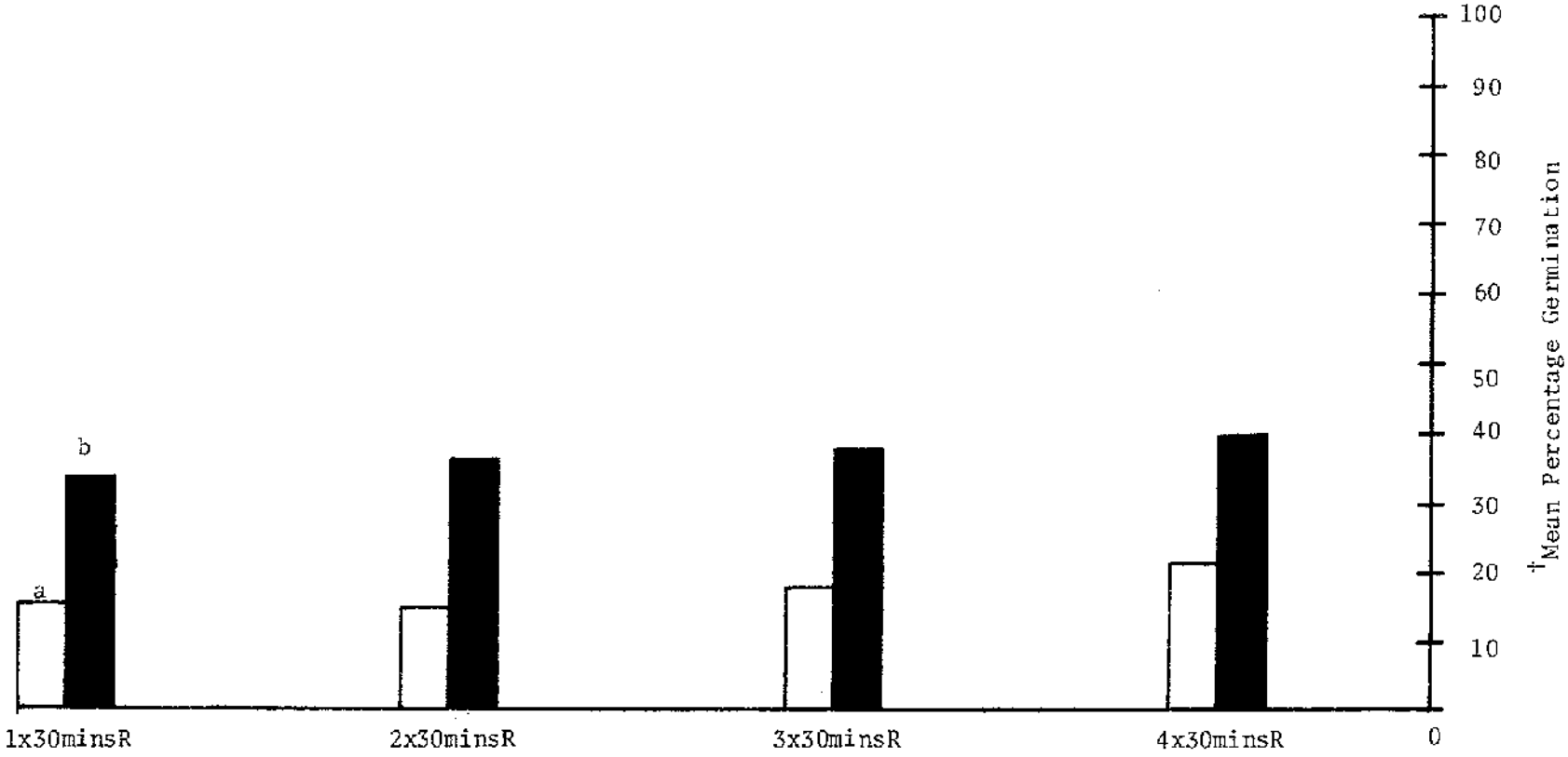
30 minutes Irradiation

	4.10c Source	χ^2	p	Significant Level
6	Control vs R	22.0119	$p < 0.001$	***
7	Control vs FR	1.83697	$p > 0.05$	N.S.
8	R vs FR	36.7729	$p < 0.001$	***
9	FR vs R+FR	0.696359	$p > 0.05$	N.S.
10	R+FR vs R+FR+R	9.33219	$p < 0.01$	**

10 minutes vs 30 minutes Irradiation

	Source	χ^2	p	Significant Level
11	R Treatments	0.499796	$p > 0.05$	N.S.
12	FR Treatments	2.19561	$p > 0.05$	N.S.

FIGURE 4.11



Effect of different frequencies of 30 minutes Red light treatment on the germination of unchilled achenes at 35°C Dark after 16h imbibition at 20°C. Germination counted after 14 days.

a : Non-irradiation

b : Red Light Treatment

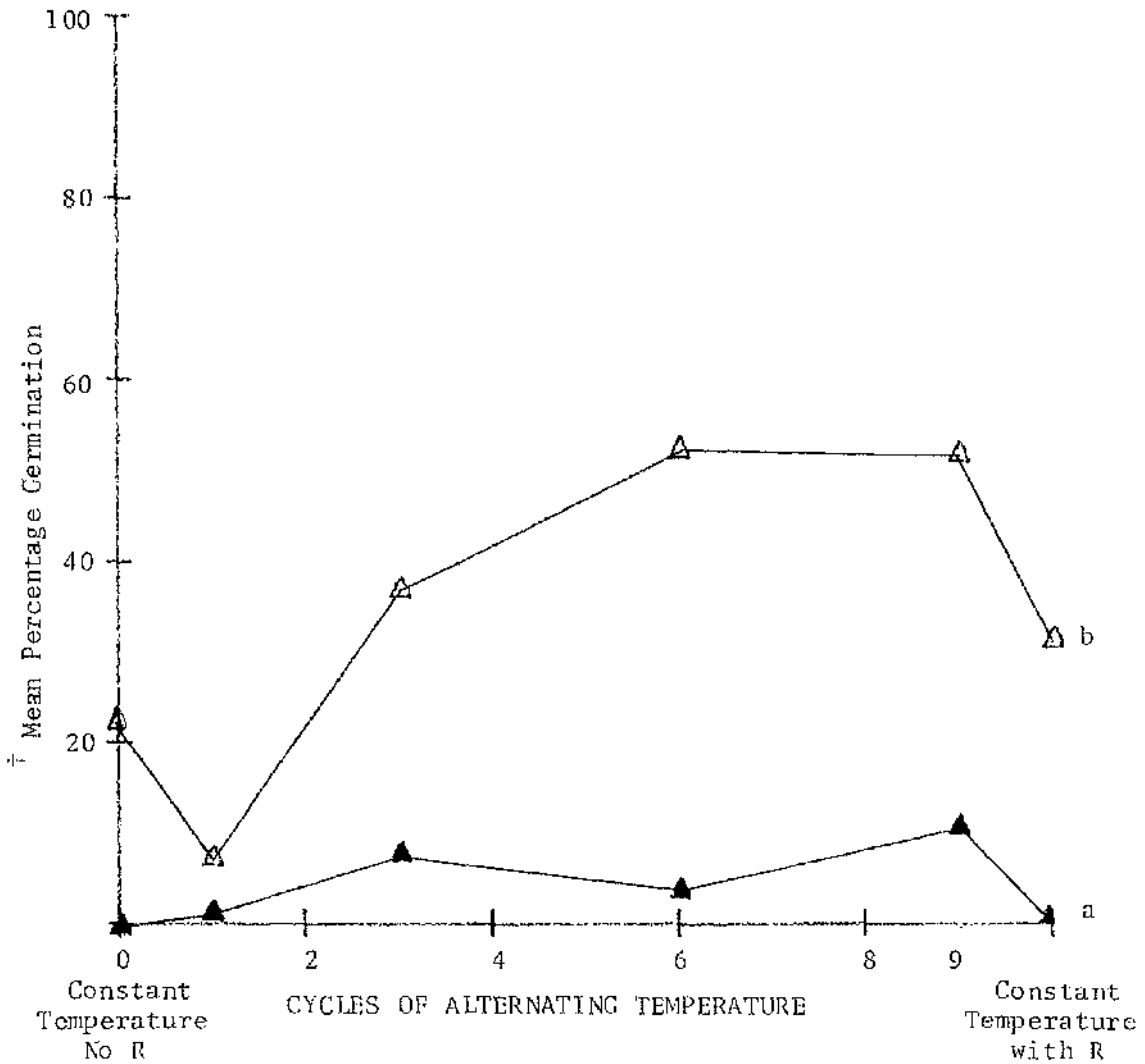
†Each treatment was replicated twice with 100 achenes per replicate in 10ml of distilled water.

Figure 4.11.

Analysis of Results : 2 Factor Variance.

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	1	12.4229	12.4229	1.00726	N.S.
Dark vs Light (A)	1	664.351	664.351	53.8665	***
R Frequency (B)	3	39.4863	13.1621	1.0672	N.S.
A x B	3	3.98047	1.32682	0.107581	
Error	7	86.333	12.3333		
Total	15	806.573			

FIGURE 4.12



Interaction of alternation of temperature with 10 minutes R irradiation. 1 cycle represents 20°C (8h) R/35 C (16h) D.

†Achenes were subjected to Alternating Temperature after an initial 16h at 20°C (dark) imbibition. After Alternating Temperature treatment, dishes were left at 20°C for 14 days and germination count recorded.

a : Non-irradiated

b : Red light treatment

Figure 4.12.

Analysis of Results : χ^2 (Chi-squared) Test.

Source		χ^2	p	Significant level
35°C constant	R vs No R	4.13384	0.05>p>0.01	*
1 cycle of 20°C(8h)R/ 35°C(16h)D	R vs No R	7.03893	p < 0.01	**
3 " "	R vs No R	46.5807	p < 0.001	***
6 " "	R vs No R	113.669	p < 0.001	***
9 " "	R vs No R	76.0167	p < 0.001	***
1 cycle vs 3 cycles	Non-Irradiated	7.95691	p < 0.01	**
3 cycles vs 6 cycles	"	2.17199	p > 0.05	N.S.
6 cycles vs 9 cycles	"	6.09009	0.05>p>0.01	*
1 cycle vs 3 cycles	Irradiated	48.6145	p < 0.001	***
3 cycles vs 6 cycles	"	9.10034	p < 0.01	**
6 cycles vs 9 cycles	"	0	p > 0.05	N.S.

Figure 4.12.

Analysis of Results : 2 Factor Variance.

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant Level
Replicates	1	10.0781	10.0781	1.31998	N.S.
R vs No R (A)	1	2118.3	2118.3	277.444	***
Increasing No. of Cycle (B)	3	1051.61	350.536	45.9115	***
A x B	3	360.818	120.273	15.7527	**
Error	7	53.4453	7.63505		
Total	15	3594.25			

1 Factor Variance

Replicates	1	6.66101	6.66101	0.746634	N.S.
Number of Cycles (No R)	3	173.533	57.8445	6.4838	*
Error	3	26.7642	8.92139		
Total	7	206.959			

1 Factor Variance

Replicates	1	3.64355	3.64355	0.413163	N.S.
Number of Cycles (with R)	3	1238.89	412.965	46.8284	***
Error	3	26.4561	8.81868		
Total	7	1268.99			

FIGURE 4.13

a : 35°C Dark

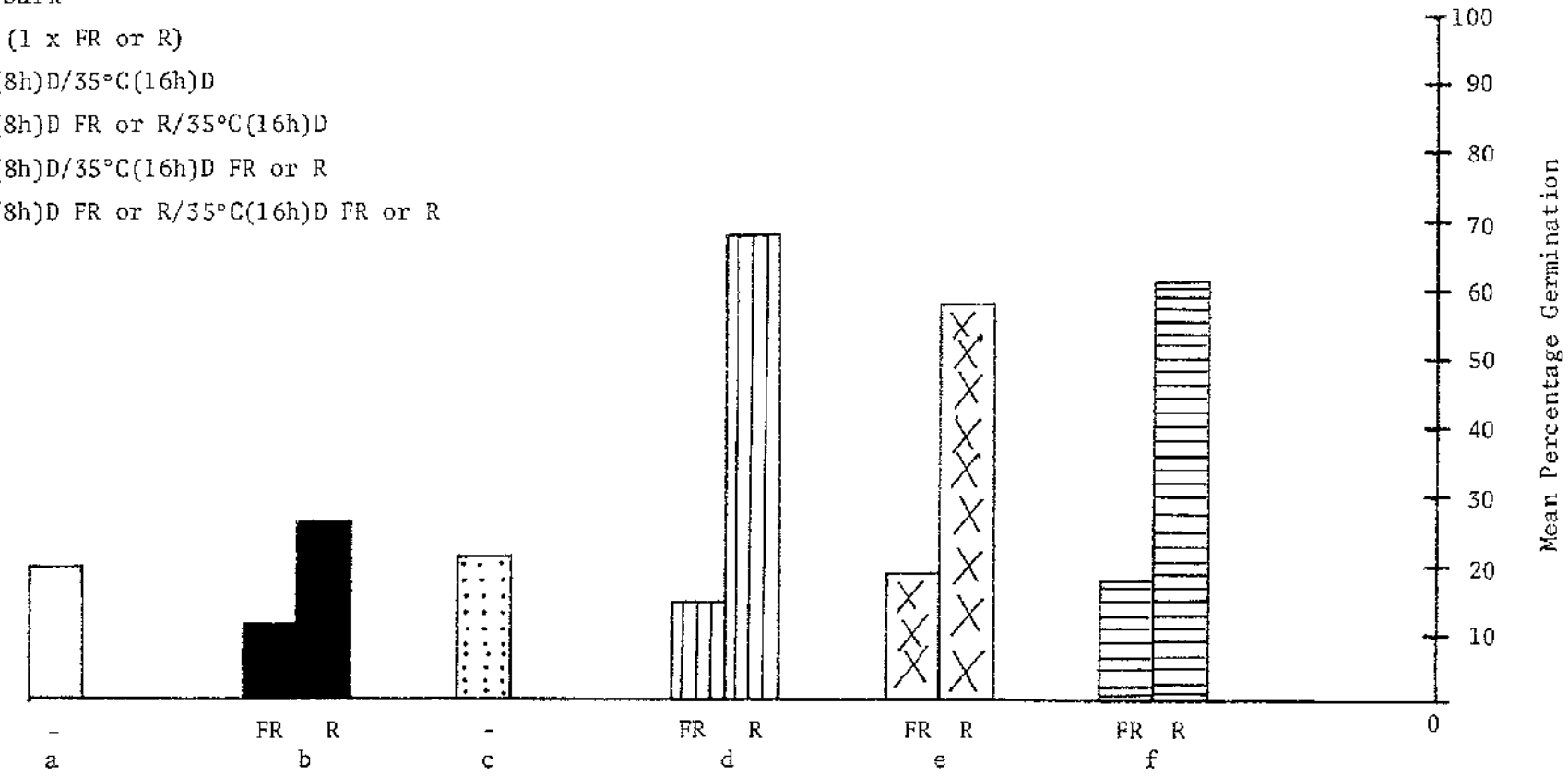
b : 35°C (1 x FR or R)

c : 20°C(8h)D/35°C(16h)D

d : 20°C(8h)D FR or R/35°C(16h)D

e : 20°C(8h)D/35°C(16h)D FR or R

f : 20°C(8h)D FR or R/35°C(16h)D FR or R



Interaction of alternating temperatures with 10 minutes R or FR irradiation given at different points during transfer to lower or upper temperatures in a 24h diurnal cycle. 20°C(8h)/35 C(16h) 1 cycle.

Achenes were initially soaked for 16h at 20°C (dark) before irradiation and given (4xR or FR + Alt. Temp.) + 3 cycles of Alt. Temp. Dishes were then kept for a further 7 days at 20°C and germination scored.

Figure 4.13.

Analysis of Results : 1 Factor Variance.

Point of Red (R) Irradiation

a Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant Level
Replicates	2	0.238281	0.119141	0.005048	N.S.
Point of R. irradiation	2	53.3398	26.6699	1.13008	N.S.
Error	4	94.4004	23.6001		
Total	8	147.979			

Point of Far Red (FR) Irradiation

b					
Replicates	2	7.80322	3.90161	0.433801	N.S.
Point of FR irradiation	2	18.729	9.3645	1.04119	N.S.
Error	4	35.9761	8.99402		
Total	8	62.5083			

Effect of R during Alternating Temperature

c					
Replicates	2	10.1836	5.0918	0.262926	N.S.
R during Alt. Temp.	3	1452.1	484.033	24.9941	***
Error	6	116.195	19.3659		
Total	11	1578.48			

Effect of FR during Alternating Temperature

d					
Replicates	2	9.52881	4.7644	0.433192	N.S.
FR during Alt. Temp.	3	38.8262	12.9421	1.17672	N.S.
Error	6	65.9902	10.9984		
Total	11	114.345			

Figure 4.13.

Analysis of Results : 2 Factor Variance.

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	2	2.99609	1.49805	0.110622	N.S.
R vs FR (A)	1	3508.43	3508.43	259.078	***
Point of Irradiation (B)	2	4.45117	2.22559	0.164347	N.S.
A x B	2	67.6191	33.8096	2.49665	N.S.
Error	10	135.42	13.542		
Total	17	37.1891			

CHAPTER 5

EFFECTS OF PRETREATMENT AT LOW TEMPERATURE

INTRODUCTION

The requirement of Polygonum persicaria for low temperature pretreatment has been investigated by several workers, namely Bayer (1958, Hammerton (1967), Justice (1941), Ransom (1935), Staniforth and Cavers (1979b), and Timson (1965). P. persicaria has been reported to possess embryo dormancy which can only be broken by low temperature pretreatment (Staniforth and Cavers, 1979b).

In Polygonaceae, the most stimulatory medium for stratification is a jar of water and the least is moist paper towelling (Justice, 1941).

The optimum period and temperature range for low temperature incubation varies from 1 week at 2°C (Karssen, 1980/81b); 4-6 weeks at 2°C (Bayer and Buchholtz, 1957); and 15 weeks at 4°C (Staniforth and Cavers, 1979b). Furthermore, Justice (1941) reported that the period at low temperature is reduced if achenes were initially immersed in concentrated sulphuric acid for 15 minutes which was confirmed by Steinbauer and Grigsby (1957b).

Removal of pericarp has been observed to enhance and shorten the low temperature pretreatment period (Justice, 1941; Ransom, 1935). Perhaps it provides ready passage for gaseous diffusion. Stratification in certain oxygen and carbon dioxide concentrations stimulated germination in contrast to the control in other Polygonaceae, but the effect on P. persicaria was not tested (Justice, 1941).

Nitrate during stratification slightly increased germination and shortened the pretreatment period (Bayer and Buchholtz, 1957). It also promoted the removal of the primary light requirement and helped prevent the development of secondary dormancy on extended exposure to low temperature (Karssen, 1980/81b; Vincent and Roberts, 1979).

In contrast to P. persicaria, P. scandens and P. virginianum, which are

restricted to rather dry conditions, do not respond to low temperature pretreatment at 2-4°C (Justice, 1941).

In the light of above discussion and Totterdell and Roberts' (1979) observations in Rumex species, stratification requirements of P. persicaria were investigated in detail.

EXPERIMENTS AND RESULTS

LOW TEMPERATURE PRETREATMENT

Low temperature pretreatment (chilling or stratification) has been reported to break dormancy in P. persicaria (see page 113). The effects of different low temperatures on the dormancy breaking of P. persicaria were examined.

Achenes were placed in distilled water and incubated at temperatures of 4°C, 10°C, 15°C, 20°C and 25°C for periods of 2, 4, 8, 12, 16 and 24 weeks in the dark as described in the General Materials and Methods. At these intervals, two replicates each of 100 achenes were counted from each of the "pools" and dispensed into 9cm glass petri dishes in 5ml of distilled water. The above procedure was carried out under darkroom conditions as described on page 34 Achenes from each of the low temperature incubations were then germinated at 4°C, 10°C, 15°C, 20°C, 25°C, 35°C and 40°C or subjected to an alternating temperature of 15°C (8h) D/35°C (16h) D. Germination took place in the dark and was recorded after 14 days for the constant temperatures and 10 days for the alternating temperatures.

This experiment was carried out to determine the following, namely:-

(1) The optimum temperature and period of exposure for breaking dormancy and to test if dormancy breaking is temperature dependent.

(2) The optimum conditions for germination after stratification.

(3) To investigate if induction of secondary dormancy occurs, and if it does, is it temperature dependent?

Viability tests were also determined at each transfer, and also after

every germination test to determine if achenes had lost viability during the course of the treatments. This test was carried out as described on page 33 No.2.

The effects of stratification are presented in Figure 5.1. The results illustrate that chilling is only effective at 4°C and 10°C; however, the loss of dormancy is faster at the lower temperature. Higher temperatures are ineffective. The low germination percentages observed after prolonged exposure of achenes to 15°C, 20°C and 25°C (c. 16 percent) could be due to death of achenes, but this is not likely to be the cause since tests for viability indicated that at least 70 percent of achenes responded positively to tip removal. Perhaps there was loss of some viability at the highest temperature, but this does not account for the lack of germination. It is concluded that dormancy breaking can only occur at temperatures of 10°C and less. These results agree with those of Justice (1941), Staniforth and Cavers (1979b) and Vincent and Roberts (1979).

Achenes gave the highest percentage germination when subjected to an alternating temperature after low temperature treatment. After two weeks of stratification at 4°C, germination was almost complete (90 percent). After the same period of exposure at 10°C, germination was enhanced (63.5 percent) but not comparable with 4°C pretreatment, maximum germination (93.33 percent) not occurring until after 24 weeks exposure to 10°C. At 15°C, 20°C, or 25°C pretreatment, any achenes that germinated under the optimum condition were considered to represent that component of the population that does not require stratification and germination did not exceed 38.5 percent.

When the achenes were subjected to germination temperatures after stratification at 4°C or 10°C, there was a progressive loss of dormancy when transferred to constant germination temperatures of 15°C, 20°C, 25°C and 35°C. The expression of substantial germination at these temperatures was observed early after 4°C stratification (4 weeks duration gave about 55-68 percent) in contrast to 10°C treatment (4 weeks duration gave about 2-47 percent) where considerable germination occurred at the later stages of stratification (about 40-70 percent).

Germination did not occur when achenes were transferred to lower temperatures after stratification, e.g. achenes did not germinate at 4°C after 10°C stratification even after 16 weeks exposure. This is of ecological importance because it then means that achenes would not germinate even though dormancy was broken in winter months (4°C keeps the achenes in a quiescent state). Germination, however, awaits a favourable temperature during the spring or summer months. These results agree with the observation of Totterdell and Roberts (1979) in Rumex spp that germination was observable only when there is a transfer to a higher temperature after stratification.

When achenes were transferred to 40°C, there was no germination after stratification at 4°C or 10°C. A viability test on these was negative, all appearing dead. This indicates that at this temperature embryos were killed, hence 40 C might be considered supraoptimal or lethal for germination.

One feature of interest is the ability of achenes to commence germination at stratification temperatures. After 24 weeks of low temperature pretreatment, germination was 18 and 25 percent at 4°C and 10°C respectively. It is possible that if achenes were left for a longer time, considerable germination could be obtained. The germination of achenes at these low temperatures is not at variance with the earlier observations that considerable germination is obtained only on transfer to the higher temperatures. Firstly total percentage germination was low, and secondly the exposure period, i.e. 24 weeks, is far in excess of winter months (normally 12-14 weeks). Also, even if considerable germination occurs, the temperature might be too low to allow the growth and development of the seedlings.

STRATIFICATION AND IRRADIATION

Effect of Red (R) or Far Red (FR) Irradiation after stratification

Stratification pretreatment, and R or FR irradiations were carried out as described in the General Materials and Methods. The effects of R or FR were investigated to determine the response of achenes to light irradiation after

low temperature pretreatment.

The results of Chapter 4 demonstrate that about 20 percent of the unchilled, dormant achenes are under phytochrome control. The results of stratification (Figure 5.1) indicate progressive loss of dormancy with stratification period at constant temperatures. It was speculated that the unchilled achenes responding to R/FR treatment might lose responsiveness as the stratification period increased, or alternatively stratification might enhance or induce response to irradiation.

The following experiments were then conducted to test for possible phytochrome involvement.

Achenes were stratified at 4°C for 0.5, 1, 1½, 2, 3, 6, 9, 12, 15 and 20 weeks. At each test interval, achenes were removed from the "pool" and irradiated with R or FR for 5, 10, 30 or 60 minutes duration. Immediately after irradiation, achenes were kept at 20°C in the dark for subsequent expression of germination. This germination temperature was used because germination was not completely expressed in darkness, and also from the previous results (Chapter 4), unchilled achenes do not respond to R irradiation at 20 C constant temperature.

Treatment consisted of 100 achenes in 9cm glass petri dishes as described on page 35, and were replicated twice. Germination was determined after 14 days. The results of 5, 10, 30, or 60 minutes irradiations were not significantly different between the treatments, hence only 30 minutes results are presented (Figure 5.2).

When stratified achenes were germinated at 20°C and given a light treatment, the progressive loss of dormancy is still noted (Figure 5.2). There is a consistent increase in the total percentage germination for the R irradiated over that of non-irradiated achenes. This increase is about 10 percent and is less than the increase promoted by red in unchilled achenes (20 percent, Chapter 4). This might indicate that a smaller proportion of the population is stimulated by R irradiation. It might be suggested that as stratification

progresses, achenes become less responsive to R treatment; however, as long as complete germination was not attained an R influence was still manifest.

The FR irradiated achenes gave a percentage germination similar to that of non-irradiated achenes during the first 6 weeks of stratification (Figure 5.2). However, after this period, germination was comparable to that of R irradiated achenes. The analyses of variance demonstrate that the effect of R irradiation for 0.5 to 6 weeks period of stratification is significantly different at 0.1 percent over that of control (non-irradiated), or FR treatments (Figure 5.2, Analysis of Results). However, after 12 weeks of stratification, there is no significant difference between R and FR, while the difference between R and control treatments decreased to 5 percent level (Figure 5.2, Analysis of Results). The FR irradiation is not significantly different over that of the control treatment for 0.5 to 6 weeks stratification period, but it was for 12 to 20 weeks at 5 percent level (Figure 5.2, Analysis of Results). It could be argued that as stratification effects develop, there is a gradual loss of response to R or FR treatments, and dark germination increases until the effect of R is completely lost. There is no interaction between stratification period and R irradiation, which might suggest that the effects of stratification and R are additive, but only when each is not fully saturated.

Effect of R, FR or R+FR

It has been shown that R stimulated the germination of achenes previously exposed to low temperature. It was then necessary to determine if this promotive effect is under the phytochrome control. Achenes were irradiated with 30 minutes each of R, FR, or R+FR after a specific period at 4 C and germinated at both 20°C and 35°C in the dark.

The results (Figure 5.3) demonstrate that the enhancement effect of R treatment is reversed by FR after 8 weeks of stratification. The total percentage germination amongst non-irradiated (i.e. control), FR or R+FR did

not differ significantly (Figure 5.3, χ^2 test). However, these treatments in most cases are significantly different from the R irradiated achenes after 8 weeks of chilling (Figure 5.3, χ^2 test).

Irradiation of chilled achenes exposed to alternating temperature

The effective point at which R or FR might be expected to enhance or inhibit the germination of achenes exposed to cyclical temperature regimes was examined.

Achenes were placed in distilled water at 4°C for 8 weeks, and 100 achenes (replicated thrice) dispensed into 9cm glass petri dishes in 5ml of distilled water. The dishes were subjected to 5 cycles of an alternating temperature of 20°C (16h) D/35°C (8h) D in a 24h diurnal cycle. R or FR was given at different points of the alternating temperature (before, after or before and after) and then the irradiated achenes transferred to 35°C (upper temperature part of the cycle). After the cyclic treatments, the dishes were kept at the lower temperature of 20°C (dark) to allow the expression of germination. Germination count was recorded after 7 days.

The promotive or inhibitory effects of R or FR respectively were further confirmed by results in Figure 5.4, both where germination is at 20°C or 35°C. These effects were investigated further at the near optimal condition of alternating temperatures (i.e. with 8h at the upper temperature instead of 16h). The results indicated that germination at alternating temperature only (i.e. with no irradiation) stimulated considerable germination (Figure 5.4) and was significantly different from the germination at constant temperatures, both non-irradiated and irradiated (Figure 5.4, χ^2 (Chi-squared) test. When the alternating temperature is coupled with R treatments, almost complete germination was obtained (Figure 5.4), and was significantly different from the non-irradiated treatment (Figure 5.4, 1 Factor Variance). However, if FR irradiation was given, total percentage germination decreased and was comparable to that of control (non-irradiated achenes) (Figure 5.4). Both the enhancement or

inhibitory effect of R or FR respectively is manifested in the alternating temperature treatment. The point of R or FR irradiation during the cycling period is of no consequence, and high percentage germination was still obtained if R or FR is given before or after transfer to 35°C temperature (Figure 5.4, 2 Factor Variance).

The above observations contrast with the work of Mancinelli et al. (1966) in tomato seeds, where they found that far-red irradiation induces phytochrome requirement in previously dark germinating tomato seeds.

Effect of stratification in an atmosphere enriched in carbon dioxide or nitrogen

The effects of carbon dioxide and nitrogen during stratification at 4°C were determined as described below.

Three hundred achenes were placed inside a 50ml Pyrex ampoule along with cut pieces of 4.25cm Whatman No. 3 filter paper to absorb and hold the 3ml of distilled water added to it. The ampoule was then joined to a glass manifold with rubber tubing which was fitted to a mercury manometer. The glass manifold was connected to sources of N_2, CO_2 and to a vacuum pump by rubber tubing, and gas flow regulated by stopcocks.

The gas mixtures were introduced by allowing entry of each component of the mixtures to the evacuated system according to the manometer readings. The whole system was purged twice with the mixture to ensure that the final recipe was as stated. The desired quantity of gas mixtures introduced was as described by Hart and Berrie (1966).

After introducing the gas mixtures, the stopcocks were closed. The ampoule was then sealed and disconnected, covered with foil and incubated at 4°C for 8 weeks. The atmospheres used were 1, and 4 percent CO_2 in N_2, and 100 percent N_2. This procedure was adopted to ensure that the gas mixture introduced at the start of the experiment was maintained throughout the low temperature pretreatment period.

After the low temperature pretreatment period, the ampoules were broken

and the achenes dispersed as batches of 100 into 9cm petri dishes in 5ml of distilled water as described in the General Materials and Methods.

Treatments were replicated thrice, and germination took place at 20 C in the dark. Radicle protrusion was recorded after 14 days.

It has earlier been noted (Stokes, 1965) that access to air is a prerequisite for stratification to take place. To test this, P. persicaria achenes were stratified in atmospheres enriched in carbon dioxide or nitrogen or kept in air. The results (Figure 5.5) demonstrate that percentage germination of achenes stratified in air did not significantly differ from those stratified in 4 percent CO_2/96 percent N_2 (Figure 5.5, χ^2 test, no. 1). At 1 percent CO_2/99 percent N_2, total percentage germination was higher compared to air stratified achenes and highest germination occurred in 100 percent nitrogen (Figure 5.5). The χ^2 (Chi-squared) test value between air treatment and 4 percent CO_2/96 percent N_2; 1 percent CO_2/99 percent N_2 or 100 percent N_2 increases with an increase in nitrogen concentrations (Figure 5.5, χ^2 test, nos. 1,2,3). This might suggest that nitrogen atmospheres enhance stratification process. And again, the χ^2 value for 100 percent N_2 and 4 percent CO_2/96 percent N_2 is greater than that for 100 percent N_2 and 1 percent CO_2/99 percent N_2 (Figure 5.5, χ^2 test, nos. 5,4), suggesting a promotive effect of higher nitrogen concentration or inhibitory effect of greater CO_2 concentrations. However, there is no significant difference between the air (control) treatment and 4 percent CO_2 treatment (Figure 5.5, χ^2 test, no. 1), an indication that even this high concentration (i.e. 4 percent CO_2) did not retard the stratification process. It could be argued from these results that while an anaerobic atmosphere in nitrogen enhances stratification process, high CO_2 concentrations might not affect it. Whether the mechanism involved in stratification is an aerobic or anaerobic is not known. These observations agree with those of Webb and Dumbroff (1969) that oxygen was not a limiting factor influencing stratification in Acer saccharum.

Germination Response from different harvests to stratification at 4 °C

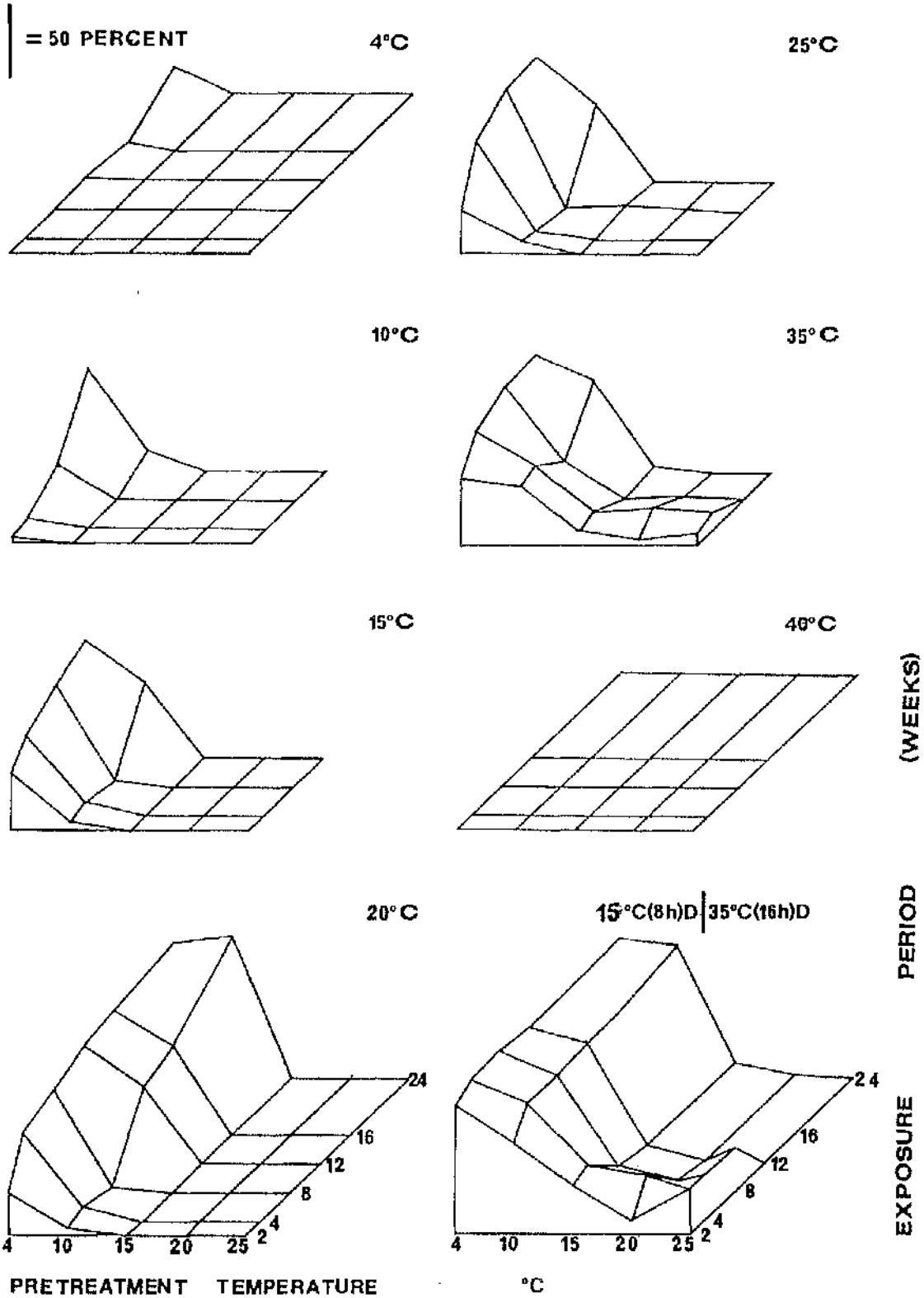
The effects of stratification at 4 C were compared for 1981 and 1982 harvested achenes at germination temperatures of 15 C, 20 C, 25 C or 35 C in the dark after different stratification periods of 2, 4, 6, 8, 10 or 12 weeks.

The degree of dormancy could be influenced by environmental factors operating during the maturation of the achenes (as reported for other species in the General Introduction). This could be reflected in the stratification process because achenes possessing deep dormancy might take a longer period of stratification to achieve the same percentage germination as less dormant achenes. The results of stratification at 4 C (Figure 5.6) demonstrate that the degree of dormancy differs between the achenes harvested in 1981 and those of 1982. The achenes of 1982 harvest are less dormant, giving a higher percentage germination than those harvested in 1981 when given an equal period of stratification at 4 C.

The differences between the harvests are quantitative not qualitative, dormancy being an innate character of the achene at maturity, but the depth of dormancy varies according to the conditions during maturation.

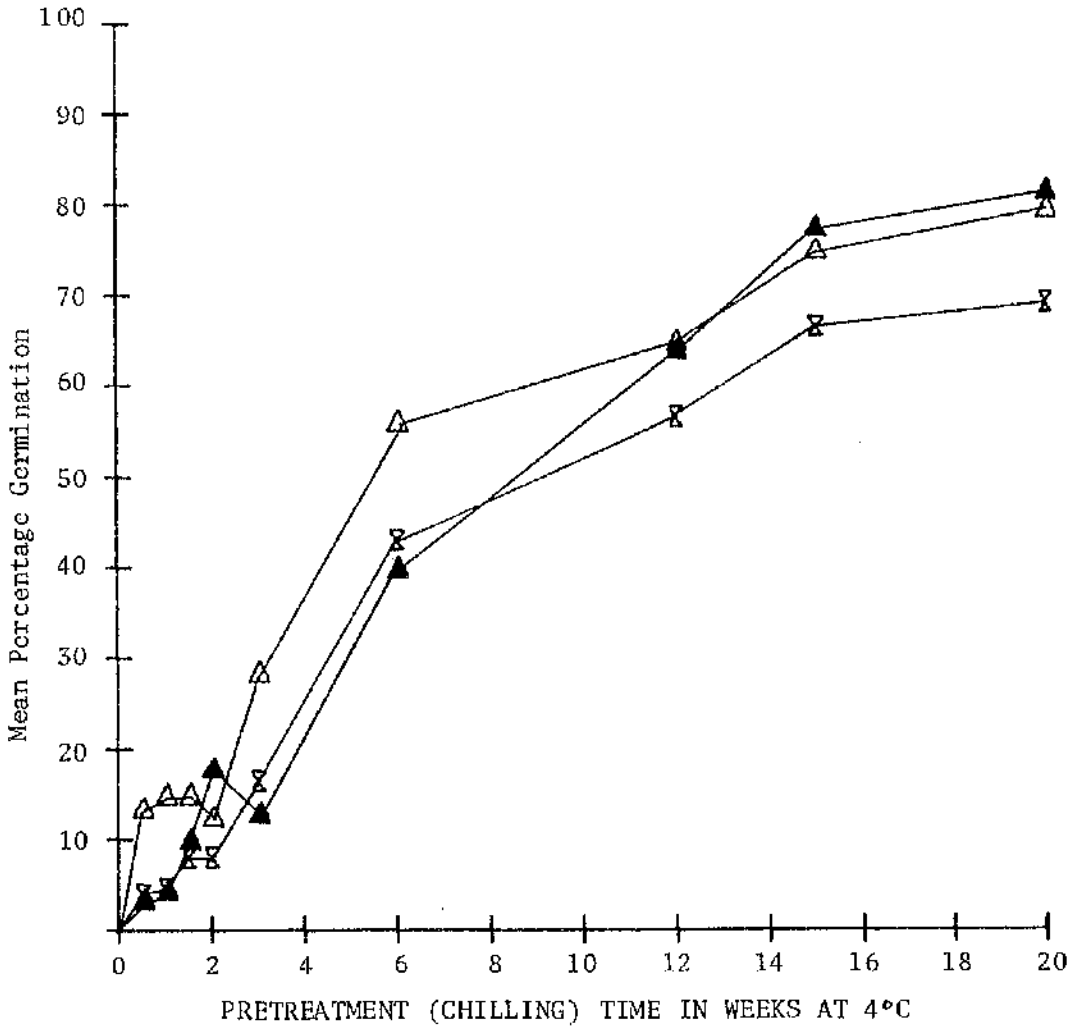
The climatological data for the months of June to October and of 1981 and 1982 are presented in Table 5.1. The variations in air temperature, rainfall, precipitation and hours of bright sunshine between 1981 and 1982 during the maturation of the achenes might impose a different degrees of dormancy, which are reflected in their response to stratification.

FIGURE 5.1



The mean percentage germination of achenes exposed to Low Temperature pretreatments for different periods in weeks and germinated at different temperatures.

FIGURE 5.2



Effect of 30 minutes R or FR irradiation after stratification on the germination of achenes at 20°C (dark).

Δ 30 minutes R treatment

▲ 30 " FR treatment

$\overline{X}$ 0 " non-irradiated treatment

Figure 5.2.

Analyses of Results : 2 Factor Variance

R vs FR Treatment

0.5 to 6 weeks stratification period

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant Level
Replicates	1	3.4502	3.4502	0.673104	N.S.
Stratification Period (A)	5	2064.68	412.937	80.5605	***
R vs FR (B)	1	290.506	290.506	56.6752	***
A x B	5	187.617	37.5234	7.3205	N.S.
Error	11	56.3838	5.1258		
Total	23	2602.64			

12 to 20 weeks stratification period

Replicates	1	0.15625	0.15625	0.0526732	N.S.
Stratification Period (A)	2	227.434	113.717	38.3349	***
R vs FR (B)	1	2.42969	2.42969	0.819068	N.S.
A x B	2	2.96904	1.48047	0.499078	N.S.
Error	5	14.832	2.96641		
Total	11	247.813			

Figure 5.2.

Analyses of Results contd.

R vs Control (Non-irradiated) Treatment

0.5 to 6 weeks stratification period

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	1	2.40332	2.40332	0.643888	N.S.
Stratification Period (A)	5	2383.62	476.725	127.722	***
R vs Control (No R) (B)	1	369.73	369.73	99.0568	***
A x B	5	27.0586	5.41172	1.44989	N.S.
Error	11	41.0576	3.73251		
Total	23	2823.87			

12 to 20 weeks stratification period

Replicates	1	2.16406	2.16406	0.194427	N.S.
Stratification Period (A)	2	150.742	75.3711	6.7716	*
R vs Control (No R) (B)	1	98.0391	98.0391	8.80817	*
A x B	2	2.00781	1.00391	0.0901944	N.S.
Error	5	55.6523	11.1305		
Total	11	308.605			

Figure 5.2.

Analyses of Results contd.

FR vs Control (Non-irradiated) Treatment

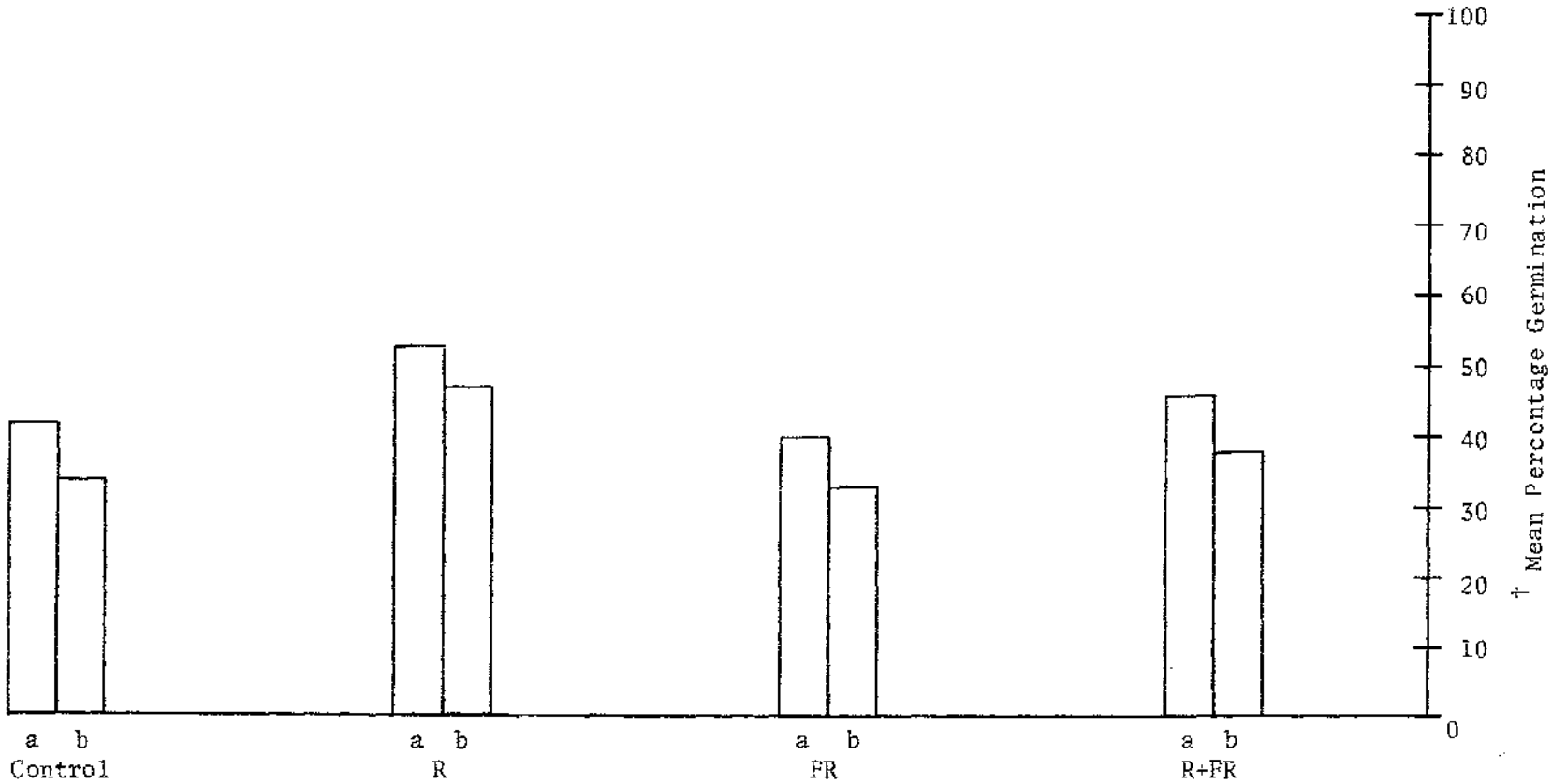
0.5 to 6 weeks stratification period

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	1	0.000976563	0.000976563	0.000192884	N.S.
Stratification Period (A)	5	2222.2	444.44	87.738	***
FR vs Control (No R) (B)	1	4.7666	4.7666	0.941468	N.S.
A x B	5	88.1729	17.6346	3.48307	N.S.
Error	11	55.6924	5.06294		
Total	23	2370.83			

12 to 20 weeks stratification

Replicates	1	0.0234375	0.0234375	0.0016662	N.S.
Stratification Period (A)	2	193.387	96.6934	6.87406	*
FR vs Control (No R) (B)	1	131.344	131.344	9.33741	*
A x B	2	7.87891	3.93945	0.280061	N.S.
Error	5	70.332	14.0664		
Total	11	402.965			

FIGURE 5.3



Germination Response of 8 weeks chilled achenes (4°C) to 30 minutes R, FR, and R+FR, and germinated at 20°C or 35°C in the dark. Germination scored after 14 days.
†Treatments were replicated thrice with 100 achenes per replicate.

a : 20°C Germination

b : 35°C "

Figure 5.3.

Analysis of Results : χ^2 (Chi-squared) Test

GERMINATION AT 20°C DARK

Source		χ^2	p	Significant level
Control (No Irradiation)	vs R	6.84378	$p < 0.01$	**
"	vs FR	0.110103	$p > 0.05$	N.S.
"	vs R+FR	0.973247	$p > 0.05$	N.S.
R	vs FR	9.16741	$p < 0.01$	**
R	vs R+FR	2.40677	$p > 0.05$	N.S.

GERMINATION AT 35°C DARK

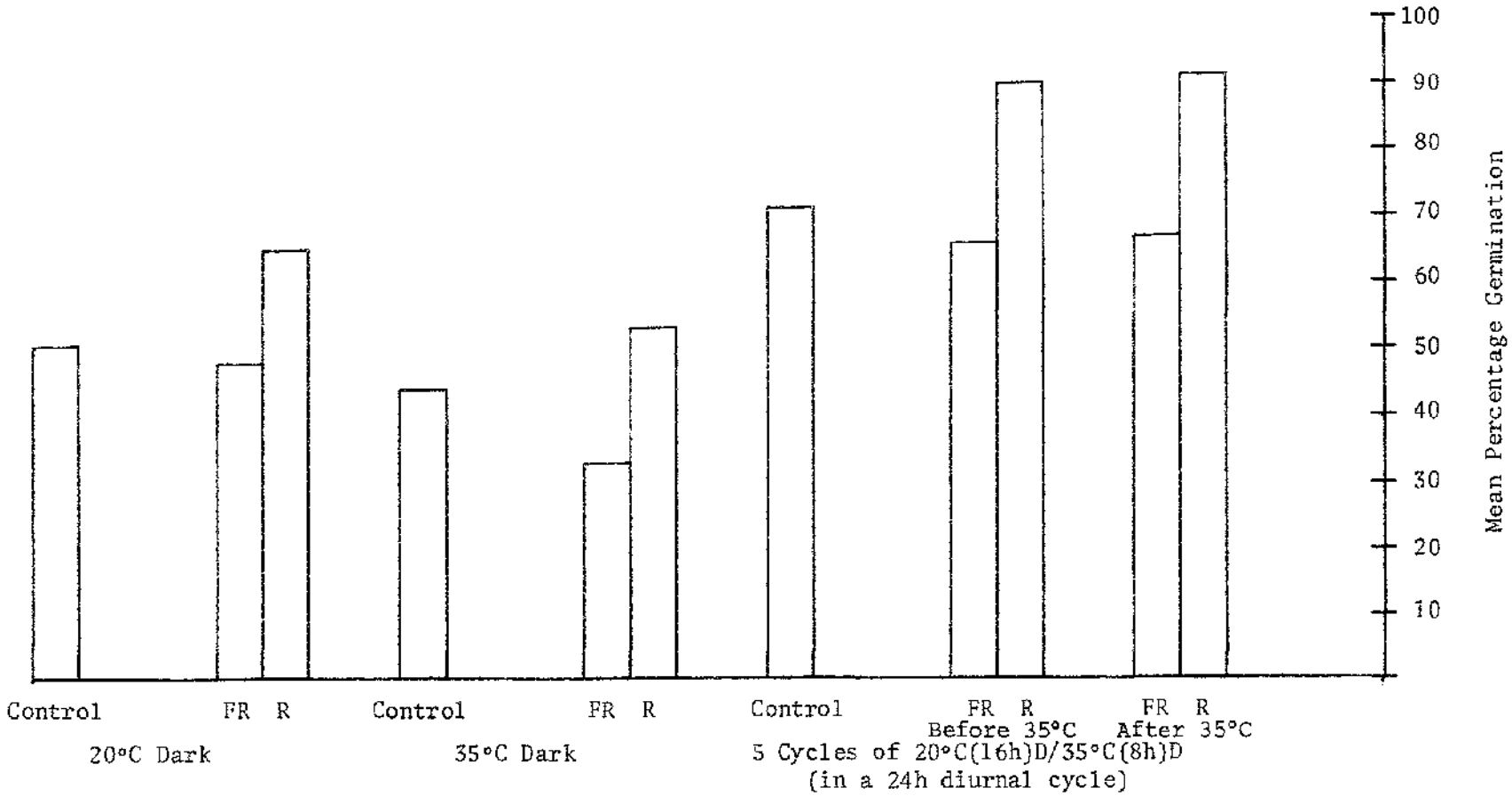
Control (No Irradiation)	vs R	10.014	$p < 0.01$	**
"	vs FR	0.0075	$p > 0.05$	N.S.
"	vs R+FR	1.04595	$p > 0.05$	N.S.
R	vs FR	11.1267	$p < 0.001$	***
R	vs R+FR	4.62007	$0.05>p>0.01$	*

2 Factor Variance

COMPARISON BETWEEN 20°C AND 35°C TREATMENTS

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	2	2.23438	1.11719	0.234687	N.S.
Light Irradiations (A)	3	274.625	91.5417	19.2301	***
20°C vs 35°C (B)	1	40.0469	40.0469	8.41264	*
A x B	3	23.7734	7.92448	1.66469	N.S.
Error	14	66.6445	4.76032		
Total	23	407.324			

FIGURE 5.4



Germination Response of 8 weeks pretreated (chilled) achenes at 4°C to 30 minutes R or FR Irradiation at different temperatures.

Figure 5.4.

Analyses of Results : χ^2 (Chi-squared) Test

Source	χ^2	p	Significant level
Constant 20°C : R vs Non-irradiated (No R)	12.6505	$p < 0.001$	***
" : FR vs "	0.326725	$p > 0.05$	N.S.
" : R vs FR	17.6539	$p < 0.001$	***
Constant 35°C : R vs Non-irradiated (No R)	4.86346	$0.05>p>0.01$	*
" : FR vs "	6.75368	$p < 0.01$	**
" : R vs FR	23.6267	$p < 0.001$	***
No R : 20°C(16h)D/35°C(8h)D vs 20°C	26.0684	$p < 0.001$	***
R : " vs "	2.22593	$p > 0.05$	N.S.
FR : " vs "	32.9725	$p < 0.001$	***
No R : 20°C(16h)D/35°C(8h)D vs 35°C	44.6732	$p < 0.001$	***
R : " vs "	19.9399	$p < 0.001$	***
FR : " vs "	85.3125	$p < 0.001$	***

1 Factor Variance

Comparison amongst Alternating Temperature Treatments

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	2	49.7305	24.8652	1.7808	N.S.
R vs Non-irradiated (No R)	2	448.395	224.197	16.0567	*
Error	4	55.8516	13.9629		
Total	8	553.977			

Figure 5.4.

Analyses of Results contd.

1 Factor Variance

Comparison amongst Alternating Temperature treatments

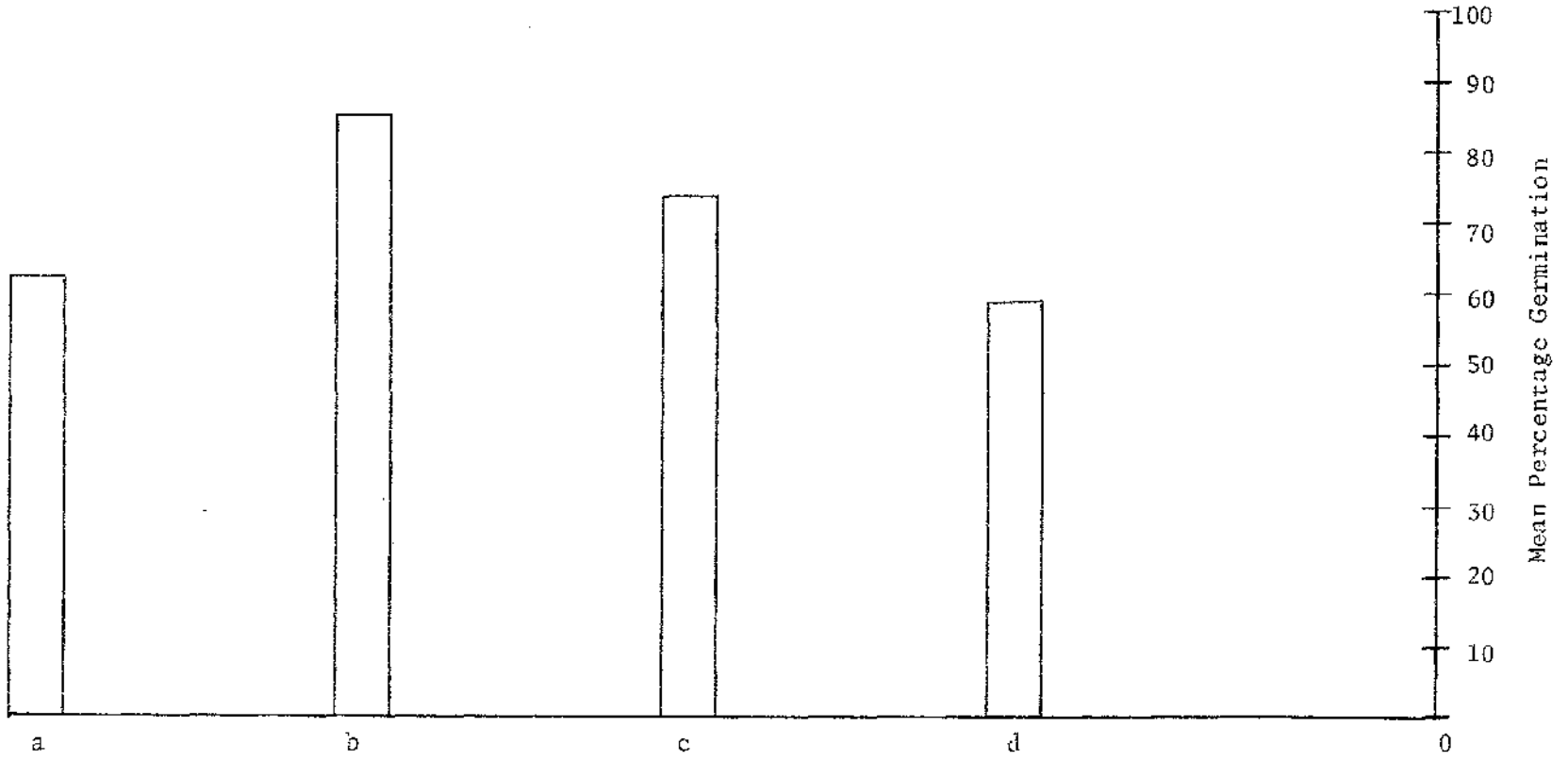
Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	2	9.71289	4.85645	0.916091	N.S.
FR vs Non-irradiated (No R)	2	25.5566	12.7783	2.41043	N.S.
Error	4	21.2051	5.30127		
Total	8	56.4746			

2 Factor Variance

Alternating Temperature (R) vs Alternating Temperature (FR)

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	2	25.4258	12.7129	0.981207	N.S.
R vs FR (A)	1	995.535	995.535	76.8375	***
Point of Irradiation (B) (Before or After)	1	6.3125	6.3125	0.487212	N.S.
A x B	1	0.0898438	0.08984380	0.0069343	N.S.
Error	6	77.7383	12.9564		
Total	11	1105.1			

FIGURE 5.5



Effect of stratification at 4°C for 8 weeks in gas mixtures of carbon dioxide and nitrogen. Germinated at 20°C dark.

a : Air (control)

b : 100 percent N_2

c : 1 percent CO_2/96 percent N_2

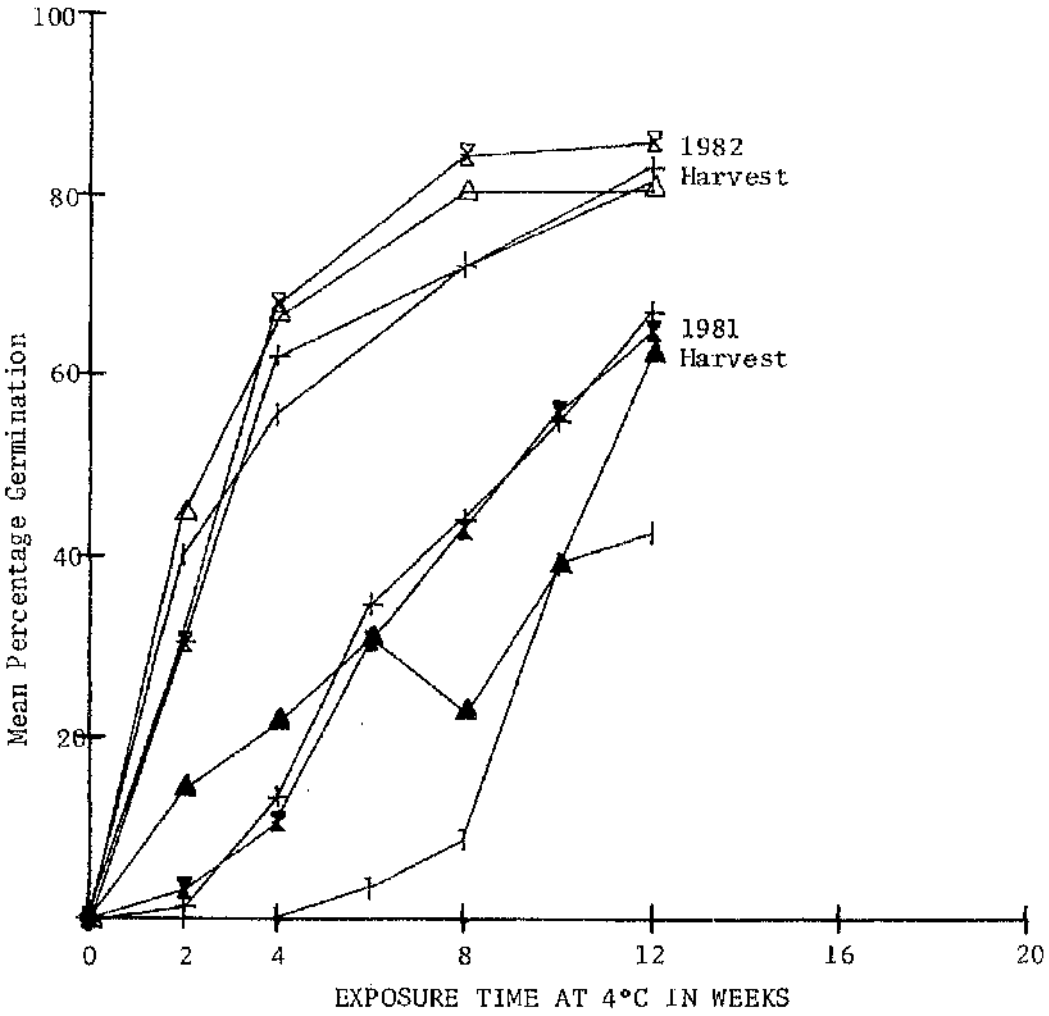
d : 4 percent CO_2/99 percent N_2

Figure 5.5.

Analysis of Results : χ^2 (Chi-squared) Test

Serial Number	Source	χ^2	p	Significant level
1	Air (control) vs 4 percent CO_2	0.567466	$p > 0.05$	N.S.
2	" vs 1 percent CO_2	8.38768	$p < 0.01$	**
3	" vs 100 percent N_2	40.2233	$p < 0.001$	***
4	100 percent N_2 vs 1 percent CO_2	11.9671	$p < 0.001$	***
5	" vs 4 percent CO_2	50.859	$p < 0.001$	***
6	1 percent CO_2 vs 4 percent CO_2	13.8675	$p < 0.001$	***

FIGURE 5.6



Germination Response of Different Harvest to 4°C at different temperatures. Counted after 14 days.

1 : 15°C

\+ : 20°C

X : 25°C

Δ : 35°C

Figure 5.6.

Analysis of Results : 1 Factor Variance

1981 vs 1982 Harvest

15 C Germination

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	1	10.3984	10.3984	1.10696	N.S.
Exposure Time (A)	3	6030.87	2010.29	214.004	***
1981 vs 1982 (B)	1	2320.83	2320.83	247.062	***
A x B	3	496.111	165.37	17.6044	**
Error	7	65.7559	9.39369		
Total	15	8923.97			

20 C Germination

Replicates	1	1.88281	1.88281	0.282627	N.S.
Exposure Time (A)	3	2496.91	832.303	124.936	***
1981 vs 1982 (B)	1	2832.89	2832.89	425.242	***
A x B	3	300.84	100.28	15.0529	**
Error	7	46.6328	6.66183		
Total	15	5679.16			

25 C Germination

Replicates	1	7.29297	7.2927	0.680075	N.S.
Exposure Time (A)	3	2666.45	888.815	82.8827	***
1981 vs 1982 (B)	1	3317.76	3317.76	309.384	***
A x B	3	369.391	123.13	11.482	**
Error	7	75.0664	10.7238		
Total	15	6435.96			

Figure 5.6.

Analysis of Results : 1 Factor Variance contd.

35 C Germination

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant Level
Replicates	1	20.6992	20.6992	3.77422	N.S.
Exposure Time (A)	3	2303.18	767.727	139.984	***
1981 vs 1982 (B)	1	1069.29	1069.29	194.969	***
A x B	3	241.574	80.5248	14.6826	***
Error	7	38.3906	5.48438		
Total	15	3673.13			

Table 5.1. CLIMATOLOGICAL DATA (SUMMARY) AVERAGES OF PRECIPITATION, DURATION OF BRIGHT SUNSHINE, AND AIR TEMPERATURE AT GLASGOW AIRPORT.

1981

	AIR TEMPERATURE IN DEGREES °C							RAINFALL			WEATHER (NUMBER OF DAYS)		BRIGHT SUNSHINE
	Means of		Daily Mean	Highest Max.		Lowest Min.		Total Fall	Most in a day		Precipitation		Daily Mean
	Max(A)	Min(B)	Mean of A+B	Max.	Date	Min.	Date	(mm)	(mm)	Date	0-2mm or more	I.0mm or more	(h)
JUNE	16.6	9.3	12.9	21.7	21	3.4	29	71	13	5	17	9	5.08
JULY	17.8	10.7	14.3	21.0	11	4.2	24	91	21	11	18	13	4.76
AUGUST	19.2	10.5	14.9	24.1	27	3.2	23	14	5	3	11	3	5.49
SEPT	16.9	9.3	13.1	21.2	3	2.5	6	227	39	26	22	16	3.69
OCTOBER	10.0	2.8	6.4	14.1	9	-4.6	14	147	29	1	26	21	3.39

1982

JUNE	17.6	9.7	13.7	29.6	5	3.0	13	68	16	15	14	11	4.83
JULY	21.1	10.9	16.0	27.6	20	7.0	2	13	3	14	9	6	6.49
AUGUST	18.2	11.0	14.6	27.3	3	3.1	28	97	18	17	23	17	4.29
SEPT	15.8	8.5	12.1	22.5	17	-1.0	30	144	25	4	19	18	3.66
OCTOBER	13.2	6.2	9.7	16.8	3	-0.4	23	137	27	30	23	-	2.12

CHAPTER 6

EFFECT OF HIGH (SUPRAOPTIMAL) TEMPERATURE

INTRODUCTION

SECONDARY DORMANCY

Thermodormancy

In the field, buried achenes are subjected to annual cycles of temperature ranging from stimulatory low during winter months (low- "stratification") which breaks dormancy to unfavourable high summer temperatures (at which achenes will not germinate) which might induce secondary dormancy. This seasonal cycle results possibly in changes in the dormancy characteristics of the population of buried achenes. Burial has been reported to induce secondary dormancy in the achenes (Staniforth and Cavers, 1979b). Achenes buried in the soil were in a state of primary dormancy which was removed in autumn and winter months and secondary dormancy imposed in late spring and early summer months (Karssen, 1980/81b; Roberts and Neilson, 1980). These authors suggested that high temperatures were responsible for the induction of this secondary dormancy. Thus, P. persicaria shows a seasonal cyclic pattern of non-dormancy and dormancy which reflect changing temperatures. Karssen (1980/81b) observed that the secondary dormancy was not imposed by lack of oxygen or high carbon dioxide level at the depth of 10cm tested. Ransom (1935), however, suggested that lack of O_2 induces secondary dormancy. Burial also induced light sensitivity in previously non-light sensitive achenes, and germination occurred only in the light (Wesson and Wareing, 1969). In the laboratory, the induction of secondary dormancy by high temperature has been noted (Bayer and Buchholtz, 1957; Ransom, 1935).

Room Temperature Drying after Stratification

Drying at room temperature has been reported to impose secondary dormancy on non-dormant achenes, and this could be broken by stratification

(Bayer and Buchholtz, 1957; Staniforth and Cavers, 1979b). Justice's (1941) results demonstrated that only a small proportion of Polygonum persicaria exhibited secondary dormancy and that this species is least affected, compared to other Polygonum species, after 50 days of drying at room temperature. The germination of the other Polygonum species after induction of secondary dormancy ranged from 0-20 percent while controls gave from 65-98 percent. However, P. persicaria gave 71 percent after drying and the control gave 90 percent.

AFTER-RIPENING

Dry Storage at High Temperature

The effect of high temperature dry storage on dormant achenes has been reported by Justice (1941), who observed that temperatures of 20°, 25°, 30°, 35° and 40°C did not break dormancy of achenes even after 6 months. Also, he noted that air-dry storage at room temperature (1-36 months) for achenes with pericarp removed, scarified, intact and even with naked seeds did not result in germination.

In most of the works cited above, the effects of high temperature both on imbibed and non-imbibed achenes have not been carried out in detail. Also the effects of light and growth regulators, e.g. gibberellic acid (GA_3), and kinetin, in affecting secondary dormancy induced by high temperature in the imbibed state, have not been tested. In this study, the view that Red light (R) and growth regulators like kinetin and GA_3 might prevent or break "thermodormancy", was investigated. Also the effect of drying at room temperature (20°C in the dark) was investigated in achenes with primary dormancy broken by stratification treatments. The effects of high temperature of 35°C in terminating the primary dormancy of achenes in dry storage was also tested.

MATERIALS AND METHODS

Achenes

The achenes used were harvested, cleaned and stored as described in the General Materials and Methods.

Stratification Treatment

The treatment was carried out as described on page 34.

Light Treatment

Illumination in the growth cabinet was provided by fluorescent tubes. Red light (R) irradiation was given by means of the irradiator (page 34). All manipulations and germination counts were conducted under greensafe light conditions.

High Temperature Treatment on Stratified Achenes - ("thermodormancy")

Achenes stratified at 4°C were counted into 9cm glass petri dishes and either incubated in distilled water or growth regulators (GA_3), kinetin (K), or GA_3+K. Dishes were kept in continuous dark at 40 C for specified periods. Growth regulators were applied either during or after the 40°C treatment period. In some experiments, achenes soaked in water at 40°C in the dark were irradiated daily with Red (R) light either during or after the period at 40°C dark incubation.

Achenes exposed to 40°C were germinated in 9cm glass petri dishes on one 9cm Whatman grade 181 filter paper and moistened with 5ml or 10ml (except where indicated) of distilled water or appropriate solutions.

To determine the ability of growth regulators to prevent or break the "secondary dormancy" induced by high temperatures, achenes were incubated in solutions of growth regulators either before or after the high temperature treatment and germinated at the appropriate conditions.

Also the effect of Red (R) to prevent or break the "secondary dormancy" was equally tested by irradiating achenes daily with R light during the period

at 40°C treatment or after.

The effect of a second period of chilling in terminating the "secondary dormancy" induced by high temperature was examined. After the desired period at 40°C in the dark, treated achenes were transferred back to 4°C for a given duration and then germinated at desired conditions.

Room Temperature Drying after Stratification Treatment

After stratification at 4°C or 10°C for 12 or 24 weeks respectively, water adhering on the surface of achenes was removed by blotting with filter paper. The achenes were stored in 9cm glass petri dishes covered with aluminium foil and kept dry in the room (under the greensafe light) at 20°C. After a specific interval (indicated in the results), achenes were removed, transferred back to 4°C for 4 weeks before germinating at the desired conditions. In some experiments, achenes were incubated in GA_3 or kinetin after the drying treatment.

Dry Storage at 35°C (dark) on Dormant Achenes - (After-ripening)

The effects of air-dry storage at 35°C on achenes were investigated. Fresh achenes were stored air-dry at 35°C in the dark. After specific intervals (indicated in the results), achenes were removed and germination tested under different temperatures and conditions.

Germination Conditions

After the desired treatments, achenes were germinated at 20°C in the dark, in continuous 35°C in the dark or light, or subjected to an alternating temperature of 20°C/35°C with 8h or 16h spent at the upper temperature part of the cycle either in the light or dark in a 24h diurnal cycle.

Germination (radicle emergence) was scored after 10, 14 or 21 days (indicated in the results). Each treatment was replicated twice or thrice, and each replicate contained 100 achenes per replicate in 10ml of distilled water or appropriate solutions.

RESULTS

SECONDARY DORMANCY

Effect of 40°C Temperature on non-dormant achenes ("Thermodormancy")

Stratification has been demonstrated to remove the primary dormancy and promote dark germination at 20°C, a temperature at which unchilled achenes will not germinate (refer to Chapter 5). The achenes used in the investigation of secondary dormancy were those whose primary dormancy had been broken by chilling, that is non-dormant achenes.

When achenes were chilled for 9 weeks in water and kept at 40°C in the dark for various time intervals, there was a decrease in germination as indicated by the decreased percentage germination at 20°C in the dark (Table 6.1, no. 1). Germination was reduced to 25 percent after 72h (3 days), and with longer imbibition, achenes failed to germinate. They were probably killed as viability tests were negative. The effect of imbibing achenes at 40°C (dark) with time is significant (Table 6.1, no. 1).

When the achenes were irradiated with 30 minutes R light, there was a gradual decrease in percentage germination, and a marked drop was observed after 48h at 40°C (Table 6.1, no. 4b). In contrast to non-irradiated treatment, a sharp decrease in percentage germination was observed after 12h (Table 6.1, no. 1). There is no difference with regard to the time at which R is given, that is either during or after 40°C treatment, because the F values were almost similar (Table 6.1, nos. 4a&b). The F values of R treatments were slightly lower than the F value of non-irradiated treatment (Table 6.1, nos. 1 and 4a&b).

The effects of GA_3 applied to chilled achenes during or after the treatment at 40°C are shown in Table 6.1, nos. 2a&b). The results indicated a gradual decrease in percentage germination with a sharp decrease after 48h at 40°C incubation for GA_3 treatments (Table 6.1, nos. 2a&b). The effect of the high temperature is slightly moderated by applied GA_3 and this is indicated by their F values (Table 6.1, nos. 2a&b), which are less than the water (control) treatment (Table 6.1, no. 1). Also it seems that GA_3 is more

effective when applied during the supraoptimal temperature than after it.

The results obtained with kinetin are essentially similar to those from GA_3 treatments whether when applied during or after the 40°C treatment (Table 6.1, nos. 3a&b). The high F values calculated when kinetin was applied after 40°C (Table 6.1, no. 3b), could be due to the high promotive effect of kinetin after 24h and the low percentage germination after 72h, due to inhibitory effect of 40°C. An overall examination of the results indicates that kinetin treatment applied either during or after 40°C exposure enhanced percentage germination over the water (control) treatment (Table 6.1, nos. 3a&b).

The same trends of results were demonstrated when achenes were further chilled for 12 weeks, exposed to 40°C either in GA_3 or kinetin (Table 6.2). There is an enhanced percentage germination when compared to 9 weeks stratification (Table 6.1), this is due to increased duration of stratification. The F value for kinetin treatment (Table 6.2, no. 3) is the lowest for all the treatments. And this is confirmed by another set of results (Table 6.3a, no. 3), where the F value is the smallest. This might suggest that kinetin might be moderating the high temperature inhibition more effectively than GA_3. The effect of supraoptimal temperature induced by 72h dark incubation was not relieved by a second chilling treatment of 4 weeks even when germinated at an alternating temperature of 15°C (8h) D/35 C (16h) L. This germination condition normally results in complete germination without the supraoptimal temperature treatment (Table 6.3b, no. 1).

Effect of Drying at 20°C (dark) on non-dormant achenes (chilled)

When achenes stratified for 12 weeks were kept dry in the dark at 20°C for 1 to 10 days, and then transferred to 20°C (darkness) for germination, there was a gradual and significant decrease in germination in contrast to undried stratified achenes (Table 6.4, no. 1). In this situation, secondary dormancy can be said to be imposed because a second chilling treatment (after the drying treatment) relieves the imposed secondary dormancy (Table 6.4, no.4).

There is a significant difference between the treated achenes and those that were further chilled after the drying treatment (Table 6.4, 2 Factor Variance). This is a true test of imposition of secondary dormancy.

The secondary dormancy imposed by drying can be effectively removed by subjecting the achenes to alternating temperature both in the dark and in the light (Table 6.4, nos. 2 & 3). The promotive effects of these alternating temperatures are significantly different from the treated achenes at 20°C in the dark (Table 6.4, 2 Factor Variance).

When the achenes were stratified for 24 weeks, and kept dry for 20 days, secondary dormancy was induced (Table 6.5, no. 1). Here also, the control treatments were significantly different from the treated achenes (Table 6.5, nos. 1,2,3,5 & 6). Alternating temperatures both in the light or dark abolished the effect of drying, thus secondary dormancy is broken. Application of kinetin does not enhance the germination and was not significant over that of the treated achenes, but GA_3 was slightly promotive (Table 6.5, nos. 4 and 3).

AFTER RIPENING

Effect of dry storage at 35°C (dark) on unchilled (dormant) achenes

When the unchilled achenes were stored dry at a temperature of 35°C in the dark, the results (Figure 6.6, a&b) indicated a progressive loss of dormancy. Germination was enhanced when the achenes were subsequently germinated at 35°C (dark) or in an alternating temperature of 15 C(8h)D/ 25°C(16h)L. In contrast, storage at -20°C does not result in substantial progressive loss of dormancy. The resultant effect of high temperature dry storage is similar to the effect of low temperature pretreatment (stratification), that is the widening of temperature range at which germination is expressed. However, the mechanisms are probably different. The results presented here do not agree with those of Justice (1941) who reported that high temperature dry storage failed to break dormancy.

DISCUSSION

The effect of supraoptimal temperature at 40°C on chilled achenes of P. persicaria is not an indication of thermodormancy as seen in Grand Rapids (Mesa 659) lettuce seeds (Khan, 1980/81) or in Chenopodium bonus-henricus (Khan and Karssen, 1980), but rather might be considered as thermoinhibition (Vidaver and Hsiao, 1975). The results presented here do not agree with those of Ransom (1935) and Bayer and Buchholtz (1957).

In P. persicaria, R irradiation does not abolish the effect of supraoptimal temperature but rather diminishes or moderates its influence up to 36h exposure at 40°C, unlike in Chenopodium bonus-henricus where R prevented the establishment of the secondary dormancy (Khan and Karssen, 1980).

The growth regulator kinetin has been reported to be more effective than gibberellin or light in preventing the establishment of secondary dormancy while the combination of kinetin + ethephon + GA_{4+7} gave the best results (Khan, 1980/81; Khan and Karssen, 1980). In the case of P. persicaria, the results indicate that kinetin ameliorates the influence of the high temperature (40°C) to a greater extent when compared to GA_3 or light. Khan (1980/81), working with lettuce seeds, suggested that prolonged dark incubation in water at supraoptimal temperature causes changes enhancing the rapid penetration of kinetin. It might be suggested that the action of kinetin prevents stress and removes adverse cellular changes more effectively than GA_3 or light.

It appears that the effect of supraoptimal temperature (40°C) is on the embryo, because chilling treatment which previously breaks the primary dormancy is unable to reverse this thermoinhibition effect. Khan (1982), working with Grand Rapids lettuce seeds, showed decrease in the embryo growth rate or growth potential during prolonged dark soaking in water at 25°C.

Table 6.1. The mean percentage germination of 9 weeks chilled achenes at 4°C, then exposed to 40°C for different times in hours and germinated at 20°C in the dark. GA_3, kinetin or 30 minutes R were given either during or after 40°C treatment. Germination was scored after 14 days.

	Treatments						
	1	2 a	2 b	3 a	3 b	4 a	4 b
Time at 40°C (dark) in hours	Exposed to water	1mM GA_3 During 40°C	1mM GA_3 After 40°C	0.1mM Kinetin During 40°C	0.1mM Kinetin After 40°C	30 minutes R During 40°C	30 minutes R After 40°C
0h	†60	62.5	55.5	55.5	62.5	77.5	77.5
6h	65	62	69.5	67.5	67.5	71	74.5
12h	53	62.5	70.5	64	65	68.5	77.5
24h	40	60.0	57.5	71	71.5	61.0	61.0
36h	48.5	60.0	52	56	48.5	54.0	60
48h	43.0	59.5	48.5	59.5	36	47.5	55
72h	25	40.5	27.5	40.5	30	35	25
Constant 40°C	0	0	0	0	0	0	0

† Mean of two replicates, with 100 achenes per replicate.

Analysis of Results : 1 Factor Variance (Summary)

F value	15.2948	3.2036	7.6905	4.89599	20.0161	13.4142	12.1358
Significant level	**	N.S.	*	*	**	**	**

Table 6.1.

Analyses of Results : 1 Factor Variance.

Water Treatment

1. Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	1	0.0175781	0.0175781	0.00214	N.S.
Duration at 40°C	6	752.791	125.465	15.2948	**
Error	6	49.2188	8.20313		
Total	13	802.027			

1mM GA_3 Treatment

2a.					
Replicates	1	3.71094	3.71094	0.286951	N.S.
Duration at 40°C	6	248.586	41.431	3.20369	N.S.
Error	6	77.5938	12.9323		
Total	13	329.891			
2b.					
Replicates	1	3.79688	3.79688	0.19365	N.S.
Duration at 43°C	6	904.709	150.785	7.69058	*
Error	6	117.639	19.6064		
Total	13	1026.14			

0.1mM Kinetin Treatment

3a.					
Replicates	1	11.5234	11.5234	0.808478	N.S.
Duration at 40°C	6	418.703	69.7839	4.89599	*
Error	6	85.5195	14.2533		
Total	13	515.746			
3b.					
Replicates	1	43.584	43.584	4.69148	N.S.
Duration at 40°C	6	1115.7	185.951	20.0161	**
Error	6	55.7402	9.29004		
Total	13	1215.03			

Table 6.1.

Analyses of Results contd. : 1 Factor Variance.

30 minutes R Treatment

4a. Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	1	0.6875	0.6875	0.05879	N.S.
Duration at 40°C	6	941.195	156.866	13.4142	**
Error	6	70.1641	11.694		
Total	13	1012.05			

4b.					
Replicates	1	7.01172	7.01172	0.332448	N.S.
Duration at 40°C	6	1535.75	255.958	12.1358	**
Error	6	126.547	21.0911		
Total	13	1669.31			

Table 6.2. The mean percentage germination of 12 weeks chilled achenes at 4°C, then exposed to 40°C (dark) for different times in hours in GA_3, kinetin or irradiated with R light and germinated in distilled water at 20°C in the dark.
Germination scored after 14 days.

Exposure Time at 40°C (dark) in hours	Treatments 1 0 (Water)	2 1mM GA_3	3 0.1mM Kinetin	4 30 minutes R
0	†75.5	79.5	74	83.5
48	48.5	83	62.5	47
60	40	72.5	57.5	47.5
72	37.5	45.0	42.5	38

† Mean of two replicates, with 100 achenes per replicate.

Analysis of Results : 1 Factor Variance (Summary)

F value	58.6007	53.3627	21.5038	43.7582
Significant Level	**	**	*	**

Table 6.2.

Analyses of Results : 1 Factor Variance.

Water Treatment

6.2(1) Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	1	8.61133	8.6133	2.3452	N.S.
Duration at 40°C	3	645.523	215.174	58.6007	**
Error	3	11.0156	3.67188		
Total	7	665.15			

1mM GA_3 Treatment

6.2(2)					
Replicates	1	0.09375	0.09375	0.0224055	N.S.
Duration at 40°C	3	669.848	223.283	53.3627	**
Error	3	12.848	4.18424		
Total	7	682.494			

0.1mM Kinetin Treatment

6.2(3)					
Replicates	1	2.64453	2.64453	0.474921	N.S.
Duration at 40°C	3	359.223	119.741	21.5038	*
Error	3	16.7051	5.56836		
Total	7	378.572			

30 minutes R light Treatment

6.2(4)					
Replicates	1	4.80273	4.80273	0.68585	N.S.
Duration at 40°C	3	919.264	306.421	43.7582	**
Error	3	21.0078	7.0026		
Total	7	945.074			

Table 6.3a. The mean percentage germination of 12 weeks chilled achenes at 4°C, then exposed to 40 C for different times in hours in GA_3, kinetin or GA_3 + kinetin, and germinated in distilled water at 20°C in the dark. Germination was recorded after 14 days.

Time at 40°C (dark) in h	Treatments 1 Water	2 1mM GA_3	3 0.1mM Kinetin	4 1mM GA_3 + 0.1mM Kinetin
0	†83	90	80	95
16	81	81.5	79.5	84.5
30	80.5	85.5	82	82
54	67.5	72	77	72
72	54.5	65	79	64.5

† Mean of two replicates, with 100 achenes per replicate.

Analysis of Results : 1 Factor Variance (Summary)

F values	19.1524	46.9735	0.549284	31.8946
Significant level	**	**	N.S.	**

Table 6.3b. The mean percentage germination of 12 weeks chilled achenes at 4°C, then exposed to 40°C (dark) for different time intervals in hours and given a second period of stratification at 4°C and subsequently subjected to 10 cycles of alternating temperatures of 15°C(8h)/35°C(16h) in a 24h diurnal period.

	Treatments		
	1	2	3
Exposure Time at 40°C (dark) in hours	4 weeks at 4°C after 40°C (dark) → 15°C(8h)D/35°C(16h)D	15°C(8h)D/35°C(16h)D after 40°C exposure	15°C(8h)D/35°C(16h)L after 40°C exposure
0	†97	95	98
16	95	86	89.5
30	88.5	85	86.5
54	85	73.5	85.5
72	34.5	52.5	56.5

† Mean of two replicates, with 100 achenes per replicate

D = Darkness

L = Light

Analysis of Results : 1 Factor Variance (Summary)

F values	50.9447	36.5765	37.34
Significant level	**	**	**

Table 6.3a.

Analyses of Results : 1 Factor Variance.

Water Treatment

6.3a(1) Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	1	0.335938	0.335938	0.0540626	N.S.
Duration at 40°C	4	476.043	119.011	19.1524	**
Error	4	24.8555	6.21387		
Total	9	501.234			

1mM GA_3 Treatment

6.3a(2)					
Replicates	1	3.97266	3.97266	1.79761	N.S.
Duration at 40°C	4	415.238	103.81	46.9735	**
Error	4	8.83984	2.20996		
Total	9	428.051			

0.1mM Kinetin Treatment

6.3a(3)					
Replicates	1	14.6367	14.6367	2.43787	N.S.
Duration at 40°C	4	13.1914	3.29785	0.549284	N.S.
Error	4	24.0156	6.00391		
Total	9	51.8438			

1mM GA_3 + 0.1mM Kinetin Treatment

6.3(4)					
Replicates	1	2.30469	2.30469	0.44884	N.S.
Duration at 40°C	4	655.086	163.771	31.8946	
Error	4	20.5391	5.13477		
Total	9	677.93			

Table 6.3b.

Analyses of Results : 1 Factor Variance.

6.3b(1) Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	1	40.3945	40.3945	3.16408	N.S.
Duration at 40°C	4	2601.56	650.391	50.9447	**
Error	4	51.0664	12.7666		
Total	9	2693.02			
6.3b(2)					
Replicates	1	0.402344	0.402344	0.05484	N.S.
Duration at 40°C	4	1073.29	268.323	36.5765	**
Error	4	29.3438	7.33594		
Total	9	1103.04			
6.3b(3)					
Replicates	1	22.2031	22.2031	2.69161	N.S.
Duration at 40°C	4	1232.07	308.019	37.34	**
Error	4	32.9961	8.24902		
Total	9	1287.27			

Table 6.4. The mean percentage germination of 12 weeks chilled achenes at 4°C and then kept dry at Room Temperature (20°C in the dark) and subsequently germinated at different temperatures.

Drying Period at Room Temp. (20°C dark)	Germination Temperature/Conditions 1 20°C (dark)	2 15°C(8h)D/35°C(16h)D	3 15°C(8h)D/35°C(16h)L	4 4°C for 4 weeks 20°C (dark)
0 Day	†83	94.5	97.5	86
1 "	58	94	94	82
3 "	62	95	95	82
10 "	42.5	84.5	84.5	80

† Mean of two replicates with 100 achenes per replicate.

D = Darkness

L = Light

Analysis of Results : 1 Factor Variance (Summary)

F value	21.2035	2.57636	2.85841	0.284132
Significant level	*	N.S.	N.S.	N.S.

Table 6.4.

Analyses of Results : 1 Factor Variance

20°C (dark) Treatment

Source (1)	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	1	2.09375	2.09375	0.207363	N.S.
Drying Period at 20°C (dark)	3	642.275	214.092	21.2035	*
Error	3	30.291	10.097		
Total	7	674.66			

15°C(8h)D/35°C(16h)D Treatment

Source (2)					
Replicates	1	42.3203	42.3203	2.10246	N.S.
Drying Period at 20°C (dark)	3	155.578	51.8594	2.57636	N.S.
Error	3	60.3867	20.1289		
Total	7	258.283			

15°C(8h)D/35°C(16h)L Treatment

Source (3)					
Replicates	1	20.1523	20.1523	0.783765	N.S.
Drying Period at 20°C (dark)	3	220.488	73.4961	2.85841	N.S.
Error	3	77.1367	25.7122		
Total	7	317.777			

4°C for 4 weeks → 20°C (dark)

Source (4)					
Replicates	1	4.5	4.5	0.09224	N.S.
Drying Period at 20°C (dark)	3	41.582	13.8607	0.28413	N.S.
Error	3	146.348	48.7826		
Total	7	192.43			

Table 6.4.

Analyses of Results : 2 Factor Variance

20°C D vs 15°C(8h)D/35°C(16h)D

Source	Degree of freedom	Sum of Square	Mean Square	F	Significant level
Replicates	1	13.1289	13.1289	0.748767	N.S.
Drying Period at 20°C (A)	3	620.266	206.755	11.7916	**
20°C D vs 15°C(8h)D/ 35°C(16h)D (B)	1	2031.75	2031.75	115.875	***
A x B	3	178.445	59.4818	3.39236	N.S.
Error	7	122.738	17.534		
Total	15	2966.33			

20°C D vs 15°C(8h)D/35°C(16h)L

Replicates	1	4.60938	4.60938	0.25799	N.S.
Drying Period at 20°C (A)	3	768.852	256.284	14.344	**
20°C D vs 15°C(8h)D/ 35°C(16h)D (B)	1	2227.84	2227.84	124.697	***
A x B	3	93.9141	31.3047	1.75219	N.S.
Error	7	125.063	17.8661		
Total	15	3220.28			

20°C D vs 4 weeks at 4°C → 20°C D

Replicates	1	6.38672	6.38672	0.2528	N.S.
Drying Period at 20°C (A)	3	447.906	149.302	5.90956	*
20°C D vs 4 weeks at 4°C → 20°C D	1	877.66	877.66	34.7389	***
A x B	3	235.949	78.6498	3.11305	N.S.
Error	7	176.852	25.2645		
Total	15	1744.75			

Table 6.5. The percentage germination of achenes stratified at 10°C (dark) for 24 weeks and kept dry at 20°C (dark) for 20 days. Germinated in various temperature conditions.

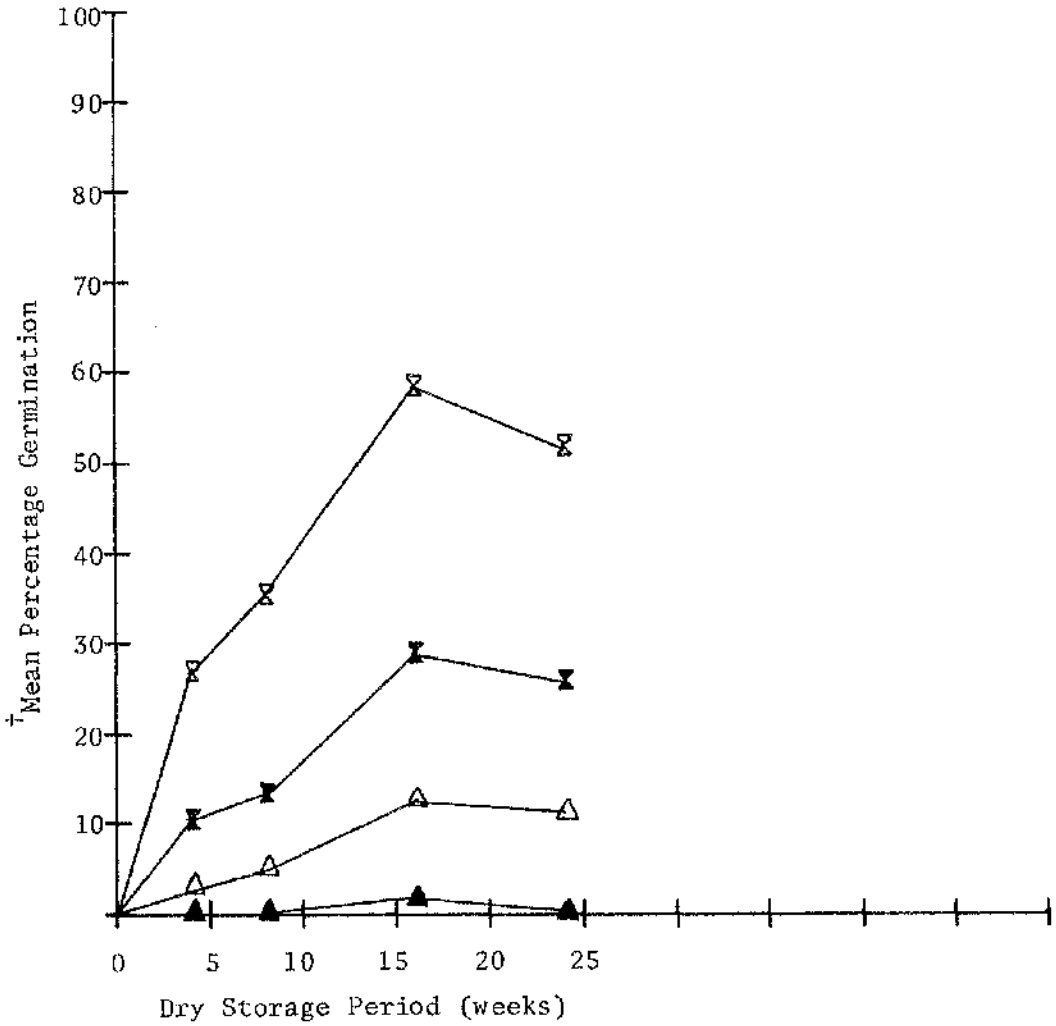
Serial No.	Germination Temperature	Drying at 20°C (dark)	Percentage Germination Replicates			Mean
1	20°C dark	+	†62	62	62	62
2	0.1mM Kinetin at 20°C dark	+	75	61	66	67.33
3	1mM GA_3 at 20°C dark	+	77	73	72	74
4	15°C(8h)D/35°C(16h)D	+	96	94	88	92.66
5	15°C(8h)D/35°C(16h)L	+	96	84	92	90.66
6	20°C dark	-	98	100	98	98.66
7	15°C(8h)/35°C(16h)D	-	96	92	92	93.33

† 100 achenes per replicate

Analysis of Results : χ^2 (Chi-squared) Test

Serial Number	Source	χ^2	p	Significant level
1	1 vs 6	125.336	$p < 0.001$	***
2	1 vs 7	83.1049	$p < 0.001$	***
3	1 vs 3	9.38266	$p < 0.01$	**
4	1 vs 2	1.64122	$p > 0.05$	N.S.
5	1 vs 4	78.7367	$p < 0.001$	***
6	1 vs 5	66.6554	$p < 0.001$	***

FIGURE 6.1a



The mean percentage germination of achenes stored Dry at -20°C (dark) or 35°C (dark) and germinated at 15°C(8h)/25°C(16h). Germination scored after 14 diurnal cycles.

-20°C (dark) Storage ▲ Dark Germination

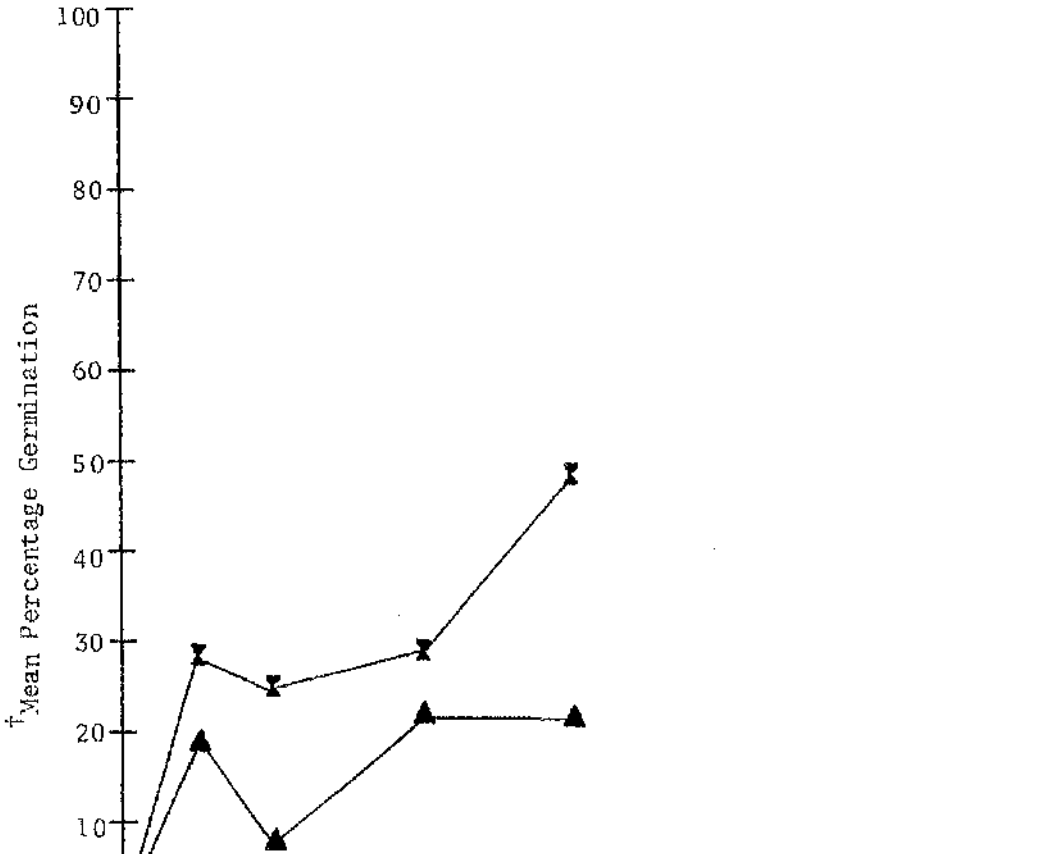
" △ Light "

35°C (dark) Storage ⧗ Dark Germination

" X̲ Light "

†Mean of two replicates with 100 achenes per replicate.

FIGURE 6.1b



The mean percentage germination of achenes stored Dry at -20°C (dark) or 35°C (dark) and germinated at 35°C dark.
Germination recorded after 14 days.

-20°C (Dark) Storage ▲ Dark Germination

35°C (Dark) Storage ⧗ Dark Germination

†Mean of two replicates, with 100 achenes per replicate.

Table 6.6a.

Analysis of Results : 2 Factor Variance

-20°C vs 35°C Storage

Germination at 15°C(8h)D/25°C(16h)D

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	1	7.15625	7.15625	0.93201	N.S.
Storage Period in weeks (A)	3	228.998	76.3325	9.94143	**
-20°C vs 35°C Storage (B)	1	1890.08	1890.08	246.161	***
A x B	3	88.2959	29.432	3.83317	N.S.
Error	7	53.7476	7.67822		
Total	15	2268.27			

Germination at 15°C(8h)D/25°C(16h)L

Replicates	1	1.81934	1.81934	0.4526	N.S.
Storage Period in weeks (A)	3	551.451	183.817	45.728	***
-20°C vs 35°C Storage (B)	1	2455.21	2455.21	610.78	***
A x B	3	37.2217	12.4072	3.0865	N.S.
Error	7	28.1387	4.01981		
Total	15	3073.84			

Table 6.6b.

Germination at 35°C Dark

Replicates	1	9.76563	9.76563	1.44586	N.S.
Storage Period in weeks (A)	3	329.665	109.888	16.2697	**
-20°C vs 35°C Storage (B)	1	429.529	429.529	63.5945	***
A x B	3	100.16	33.3867	4.94312	*
Error	7	47.2793	6.75419		
Total	15	916.399			

CHAPTER 7

TREATMENT WITH HORMONES AND GROWTH RETARDANTS

INTRODUCTION

The effects of exogenously applied hormones and the involvement of these hormones during stratification have been discussed for other species in the General Introduction.

In Polygonum persicaria, the effects of exogenous hormones were tested both for stratified and non-stratified achenes, and under promotive and non-promotive conditions. While application of exogenous hormones does not indicate the involvement of hormones, it might indicate the presence in the achenes of receptor sites for these hormones which may change in sensitivity.

With regard to the involvement of endogenous ABA, Karssen (1982) correlated percentage germination with endogenous levels of ABA during stratification at 2°C in P. persicaria. He observed that the endogenous content of ABA decreased very rapidly from about 180 ng/g dry wt. to approximately 50 ng/g dry wt. after 2 weeks, while germination was approximately 15 percent for 2°C stratification (dormancy breaking temperature). The endogenous content of ABA of achenes kept at 25°C (non-dormancy breaking temperature) treatment decreased even more than that of those given a 2 C treatment, from approximately 180 ng/g dry wt. to approximately 25 ng/g dry wt. The maximum percentage germination was approximately 85 percent, after 9 weeks at 2°C pretreatment period, with endogenous ABA content at 25 ng/g dry wt. From these observations, it is clear that endogenous ABA decreased faster at 25°C than at 2°C and though a substantial decrease in the level of endogenous ABA was observed at 2 weeks of stratification, termination of dormancy occurred at 9 weeks of stratification. Thus, even though decrease in endogenous ABA takes place during stratification, nevertheless its involvement in the regulation of germination and dormancy of Polygonum persicaria is in doubt or questionable.

These observations have been described in detail because so far, it is

the first, and well documented report about the role of endogenous ABA in dormancy control of P. persicaria.

The effects of exogenous abscisic acid (ABA) were tested during and after the stratification period to see if ABA inhibits the stimulatory effect of low temperature pretreatment. Also the effects of exogenous application of gibberellic acid (GA_3) and kinetin were investigated.

MATERIALS AND METHODS

The effects of hormones and growth retardants were investigated under different germination conditions.

White light, Red (R) and Far Red (FR) irradiation, and stratification treatments were carried out as described in the General Materials and Methods.

Solutions of abscisic acid (ABA), gibberellic acid (GA_3), gibberellin 4+7 (GA_{4+7}), kinetin (KIN), and the growth retardants (B995, CCC, AMO 1618, Ancymidol) were made as described on page 34. Solutions of 0.1 and 1 ppm of ethylene (ETH) using Ethrel were made in distilled water.

Hormones and Unchilled Achenes: Unchilled achenes were incubated in solutions of GA_3 (1mM), KIN (0.1 mM); ETH (0.1 1ppm); GA_{4+7} (1mM); GA_3 + KIN ; and GA_{4+7} + KIN in 9cm glass petri dishes. The dishes were kept at 15°C or 20°C for 16h or 24h (indicated in the results, to imbibe in distilled water or appropriate solution(s)). The dishes were then transferred to 20°C or 35°C either in the dark or light. R was given after imbibition at the lower temperature of 20°C or 15°C in the dark before being transferred to the desired temperatures for germination.

Fatty Acids and Germination: Nonanoic acid (C9) was applied in the manner described by Berrie et al. (1975). Concentrations of 0.1mM and 1mM were made up in a solution of redistilled methanol and applied to Whatman No.3 filter paper lining the base of glass petri dishes. The volume and concentration

of the solution used was appropriate to give, on evaporation of the methanol, an amount that would result in the desired nominal molar concentration when a standard volume of redistilled water was added to the dish. 1 ml of test solution was added to 4.5cm diameter glass petri dishes, and on evaporation, 1.5 ml of distilled water was added to 50 achenes per petri dish. Treatments were replicated thrice and incubated at the lower temperature (indicated in the results) for 16h. Germination was at 25°C or 35°C either in the dark or light, or in alternating temperatures of 15/25°C or 15/35°C with 16h spent at the upper temperature part of the cycle in a 24h diurnal cycle. Also light was given at the upper temperature part of the cycle.

Hormones and Stratification: Achenes were stratified in distilled water or in 0.01, 0.1 and 1mM GA_3 for 2,4,6,12,15 and 20 weeks at 4°C. Achenes stratified in GA_3 were dispensed into glass petri dishes, moistened with distilled water, while those stratified in distilled water were incubated in 0.01, 0.1 and 1mM GA_3, after the desired stratification period. Red (R) or Far Red (FR) light was given and germination took place at 20°C in the dark.

Growth Retardants and Stratification Treatment: Achenes were stratified in CCC (6.33, 0.633 and 0.0633 mM); B995 (6.25, 0.625 and 0.0625 mM); AMO 1618 (2.79, 0.279, 0.0279 mM); and Ancymidol (0.01 and 0.001 mM) or in distilled water at 4°C for 4,9 and 12 weeks. After the desired pretreatment period, achenes that were stratified in growth retardants were dispensed into petri dishes and incubated in distilled water. While those stratified in distilled water were incubated in the different concentrations of growth retardants. The dishes were subsequently germinated at 20°C in the dark.

ABA and Stratification Treatment: Achenes were stratified at 4°C for 24 weeks in distilled water, dispensed into glass petri dishes and incubated in 1mM ABA for 24 or 48h at 20°C in the dark. After this incubation period, achenes were

transferred to distilled water, and either irradiated with 10 minutes R light or not, and incubated back at 20°C in the dark. Also chilled achenes were germinated in the presence of ABA at 20°C in the dark.

Achenes were also chilled at 4°C for 8 weeks in 1mM ABA, transferred to distilled water and incubated in GA_3, KIN or irradiated with R light and germinated at 20°C in the dark.

Germination Conditions: Achenes, either unchilled or chilled (100 in number), were dispensed into 9cm glass petri dishes lined with one Whatman grade 181 filter paper moistened with 10ml (non-chilled achenes) or 5ml (chilled achenes) of distilled water or appropriate solution(s). Treatments were replicated twice or thrice (indicated in the results) and enclosed in cylindrical cans or covered with a double layer of aluminium foil and germinated under desired conditions. For light treatments, dishes were exposed only during the required light period. All procedures were carried out in the dark room, as described in the General Materials and Methods. Germination was scored after various times depending on the experiments (indicated in the results).

RESULTS

The Effects of Exogenously applied Hormones

Unchilled Achenes

The effects of GA_{4+7}, GA_3, kinetin or kinetin combined with GA_{4+7} or GA_3 are shown in Table 7.1. The results demonstrate that neither GA_3 nor GA_{4+7} has any significant effect on the germination of unchilled achenes at 35°C in the dark (Table 7.1a, nos. 1 & 2).

The treatment in which kinetin was applied alone stimulated germination and is significant over the water (control) treatment (Table 7.1d, no.3). When kinetin was given in combination with GA_3 or GA_{4+7}, germination increased slightly but cannot be considered to be substantial over that of kinetin treatment alone (Table 7.1).

Germination in the light enhanced germination (Table 7.1) and these were significantly different over their corresponding dark treatments (Table 7.1c). The trend of light germinations was essentially similar to dark germinations.

The results do not indicate any one factor substituting for another; indeed, highest germination is obtained when GA_3 or GA_{4+7} is combined with kinetin and germinated in the presence of light (Table 7.1). Perhaps this promotive effect could be attributed primarily to the effects of kinetin and light, the GA_3 or GA_{4+7} influence being slight or negligible.

The above results were confirmed in the second set of this experiment in which hormone treated achenes were irradiated with 30 minutes Red (R) light i.e. that GA_3 did not stimulate germination (Table 7.2, no.1), kinetin enhanced significant germination (Table 7.2, no.2), while the combination of GA_3 and kinetin gave results comparable to germination in only kinetin treatment (Table 7.2, no.3). When R irradiation was given the germination was promoted and was significantly greater than non-irradiated achenes, or GA_3 treatment (Table 7.2, nos. 4 & 5). This indicates that while R promoted germination slightly, GA_3 did not. However, the treatment with kinetin alone enhanced germination over that of R light treatment (Table 7.2, no.6). The combination of R + GA_3 treatment enhanced germination over that of GA_3 and not R treatments (Table 7.2); this enhancement of germination is attributed to R irradiation. The R + kinetin treatment gave germination which was better than either alone. The combination of the three factors, i.e. R, GA_3 and kinetin, gave the highest germination (Table 7.2), which was significantly greater than GA_3 + kinetin or R + GA_3 treatments (Table 7.2, nos. 11 & 12), but was not when compared with R + kinetin treatment (Table 7.2, no.13). It might be argued that GA_3 or kinetin failed to stimulate complete germination because entry is prevented by the pericarp.

This appears not to be the case because it can be seen that puncturing, which may be assumed to aid entry, does not result in the hormone treated achenes germinating to a greater extent than intact achenes (Table 7.3).

Indeed, achenes that were punctured and incubated at 20°C either in the dark or light failed to germinate or germinated poorly (Table 7.3A). When achenes were germinated at 35°C in the dark (Table 7.3B), germination for Intact + Water (H_2O) treatment is similar to that of Intact + 1mM GA_3 (Table 7.3, χ^2 test no.1), while Intact + 0.1mM kinetin treatment gave germination significantly greater over the water or GA_3 treatment (Table 7.3, χ^2 test nos. 2&3). This again indicates the enhancement effect of kinetin in contrast to GA_3. The germination of punctured achenes in water, GA_3 or kinetin was essentially similar (Table 7.3B, nos. 2,4&6). These observations suggest that failure to promote germination is not due to lack of entry of these applied hormones. Puncturing and not the hormones stimulated germination. Germination in light treated intact achenes was higher than in correponding dark treatments (Table 7.3B), indicating a stimulatory effect of light. The Punctured + Water (H_2O) for dark and light treatments were similar (Table 7.3, no.1), suggesting that puncturing might substitute for the stimulatory effect of light. The Punctured + GA_3 or kinetin light treatments cannot be compared to their corresponding dark treatments because they were affected by fungus during the course of the experiments (Table 7.3, nos.4&6), hence the germination results recorded should be treated with a degree of caution.

Ethylene has been demonstrated to stimulate germination in witchweed seed (Egley, 1972); peanut seed (Ketring and Morgan, 1970; Toole et al., 1964). The effects of ethylene were examined in many species by Taylorson (1979). He observed promotion of germination in some species, inhibition in some, while in species like Polygonum convolvulus and P. pennsylvanicum and in many others, ethylene has no effect. The effect on P. persicaria (Figure 7.1) demonstrates that promotion of germination is dependent upon the germination conditions, i.e. temperature and light. It is not affected by applied ethylene (ethrel). The breaking of dormancy for unchilled achenes follows the general trend earlier demonstrated in Chapter 4.

Fatty acids, especially nonanoic acid, have been implicated in the

germination and dormancy of some species (Berrie et al., 1975). The test carried out with applied nonanoic acid demonstrates that germination is dependent on temperature and light conditions, but not affected by nonanoic acid (Figure 7.2).

Chilled Achenes

When the achenes were stratified in the presence of GA_3 and subsequently germinated in water, the results (Table 7.4A or Figure 7.3A) demonstrate that the treatment enhanced germination. The duration of stratificiation at 4°C is significant, and so also is the light treatment given during germination (Table 7.4, Analysis of Variance). The results also show that there is statistical interaction between exposure time and GA_3 treatments (Table 7.4, Analysis of Variance). However, it appears that the interaction observed was mainly due to the early period of the stratification (2-6 weeks) and with further exposure time, germination between water and GA_3 treatments was essentially similar (Table 7.4A or Figure 7.3A). This might indicate that the requirement for gibberellin, if any, is seen only during the early period of stratification. This is indirect evidence that GA may be synthesized, or the achene becomes more responsive to the endogenous GA as stratification proceeds.

Treatment in which achenes were stratified in GA_3 and irradiated with R or FR light (Table 7.4B&C or Figure 7.3B,C&D) demonstrates an interaction between the GA_3 concentrations and light treatments (Table 7.4, Analysis of Variance). This interaction might be due entirely to the behaviour of those achenes which show an enhanced germination over those treated with either GA_3 or GA_3 + FR, indicating a stimulatory effect of R. The germinations in GA_3 and GA_3 + FR were essentially similar (Table 7.4, A&C or Figure 7.3B,C,D), suggesting that FR has no effect, either inhibitory nor stimulatory, when achenes were stratified in GA_3. However, the water + R treatment gave germination comparable to water + FR treatment (Table 7.4B&C or Figure 7.3B,

C&D). This also confirms earlier observations (Chapter 5) that responsiveness to FR is lost as stratification progresses. It appears from these results that while R promotion is still evident with GA_3, FR is not. In the absence of results from an experiment in which R irradiation is followed by FR, it cannot be claimed that phytochrome is involved in the system. In any case the amount of enhancement is small and unlike that of photoblastic lettuce seeds.

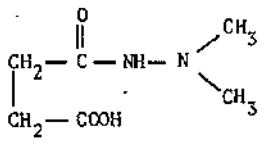
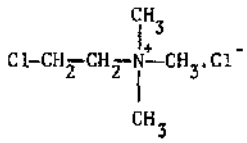
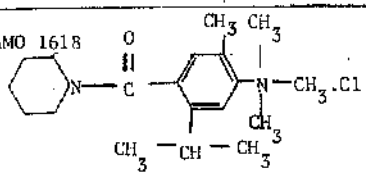
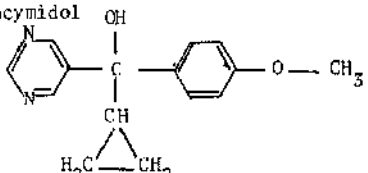
Treatments in which GA_3 was applied after stratification (Table 7.5 or Figure 7.4) showed a similar pattern of germination, i.e. the difference between the GA_3 and the water treatment (control) gradually decreases as the stratification period increases.

When achenes were germinated in GA_3 after stratification, percentage germination was significantly greater than those chilled in GA_3 and germinated in water (Tables 7.4A & 7.5, Figures 7.3A & 7.4). Perhaps it might be suggested that the effect of applied GA_3 during stratification is not to substitute for de novo synthesis of gibberellin during stratification, but rather the effect is a carry-over of the hormone, and the effect is expressed when the achenes are transferred to a higher temperature to enable germination to be expressed.

The Effects of Exogenously Applied Growth Retardants during Stratification

In an attempt to elucidate further the possible role of gibberellin during stratification, the achenes were chilled in different concentrations of growth retardants which have been reported to block synthesis of gibberellin. These growth retardants have been used as tools for the study of GA synthesis in the seeds. If gibberellin is synthesized either during stratification or after transfer to a higher temperature, then application of an exogenous growth retardant during or after stratification should inhibit this synthesis and thus prevent germination.

The inhibitors chosen were B995, CCC, AMO 1618 and Ancymidol. These are

Inhibitor	Structure	Reference	Source	Step Inhibited	Remarks
B 995		Dennis et al. (1965)	Cell Free Extract of Immature fruit of Marah (Echinocystis) macrocarpa Greene endosperm (wild cucumber) seed.	Did not inhibit (-)-kaurene formation	Appears to be somewhat inhibitory at higher concentrations
	N-dimethylamino succinamic acid	Frost & West (1977)	"	Did not inhibit cyclization of GGPP to CPP(A step) or CPP to ent-kaurene (B step)	At any level test
		Ninnemann et al. (1964)	Cultures of Fusarium moniliforme Sheld (Gibberella fujikuroi SAW. WR) (Fungus)	No decrease in GA production was observed.	B955 was not metabolised
	Cathey (1964)				
CCC		Dennis et al. (1965)	Marah macrocarpa Greene (cell free extract)	Did not significantly inhibit ent-kaurene synthesis at 10 or 100 µg/ml.	
	(2-chloroethyl) trimethylammonium chloride	Ninnemann et al. (1964)	Cultures of Fusarium moniliforme Sheld (Fungus)	GA production nullified.	
		Barnes et al. (1969)	Fusarium moniliforme Sheld	Inhibited GA_3 biosynthesis at cyclization step of GGPP to ent-kaurene	
	Cathey (1964)	Frost and West (1977)	Immature fruit of Marah macrocarpus Greene endosperm (cell free extract	Inhibited cyclization of GGPP to CPP. CPP to ent-kaurene not affected.	
AMO 1618		Dennis et al. (1965)	Cell free extract of endosperm of nucellus of Marah (Echinocystis) macrocarpa Greene (wild cucumber) seed	Inhibited cyclization of GGPP to ent-kaurene	
	4-Hydroxyl-5-isopropyl-2-methyl phenyl trimethyl ammonium chloride, 1-piperidine carboxylate.	Frost and West (1977)	"	Cyclization of GGPP to CPP inhibited (A step)	
	Cathey (1964)	Barnes et al. (1969)	Fusarium moniliforme Sheld (Gibberella fujikuroi SAW. WR)	Cyclization of GGPP to ent-kaurene	
Ancymidol		Coolbaugh et al. (1978)	Immature fruit of Marah macrocarpus Greene (endosperm cell free extract)	Inhibits oxidations of kaurene, kaurenol and kaurenal but not kaurenoic acid	
	α-cyclo-propyl-α[p-methoxyphenyl]-5-pyrimidinemethenol alcohol	"	Cultures of Fungus Fusarium moniliforme Sheld	Did not inhibit the oxidations of kaurene, kaurenoic acid or production of GA_3 in vitro or in vivo	Active at low concentrations

Despite the inhibition of gibberellin biosynthesis reported above, some of these growth retardants can also increase the endogenous level of gibberellin, e.g. 1 mg/CCC stimulated GA levels 150-fold while 1 mg/AMO 1618 increased the quantity of GA; however higher concentrations of AMO 1618 (200 mg/l) reduced the amount of GA unlike CCC in peas (Reid and Crozier, 1970a&b). Halevy and Shilo (1969) observed that CCC increased the content of the gibberellins in gladiolus plants.

The results of experiments in which growth retardants are used to reduce GA biosynthesis therefore need to be interpreted with some caution.

thought to inhibit at different positions as indicated in the chart on the opposite page.

When achenes were stratified in water and germinated in different concentrations of B995, CCC, AMO 1618 or Ancymidol at 20°C in the dark, germination was not prevented (Table 7.6A,B,C&D or Figure 7.5A,B,C&D). Indeed, treatments with growth retardants were comparable to water treatments, and this might suggest that even if gibberellin substance is synthesized, it might not affect germination.

Treatments in which achenes were stratified in growth retardants had percentage germinations slightly less than the water stratified achenes (Table 7.7A,B,C&D or Figure 7.5A,B,C&D). It does not seem that the slight decrease in germination due to the growth retardants might be the result of inhibition of de novo synthesis of gibberellin during stratification.

Treatments with B995 or Ancymidol seem to be more effective in decreasing the percentage germinations of achenes (Tables 7.6 & 7.7, 2 Factor Analysis of Variance, pages 196 & 198). In all cases, achenes incubated in growth retardants after chilling gave better percentage germination than those chilled in growth retardants (Tables 7.6 & 7.7 or Figures 7.5A,B,C&D).

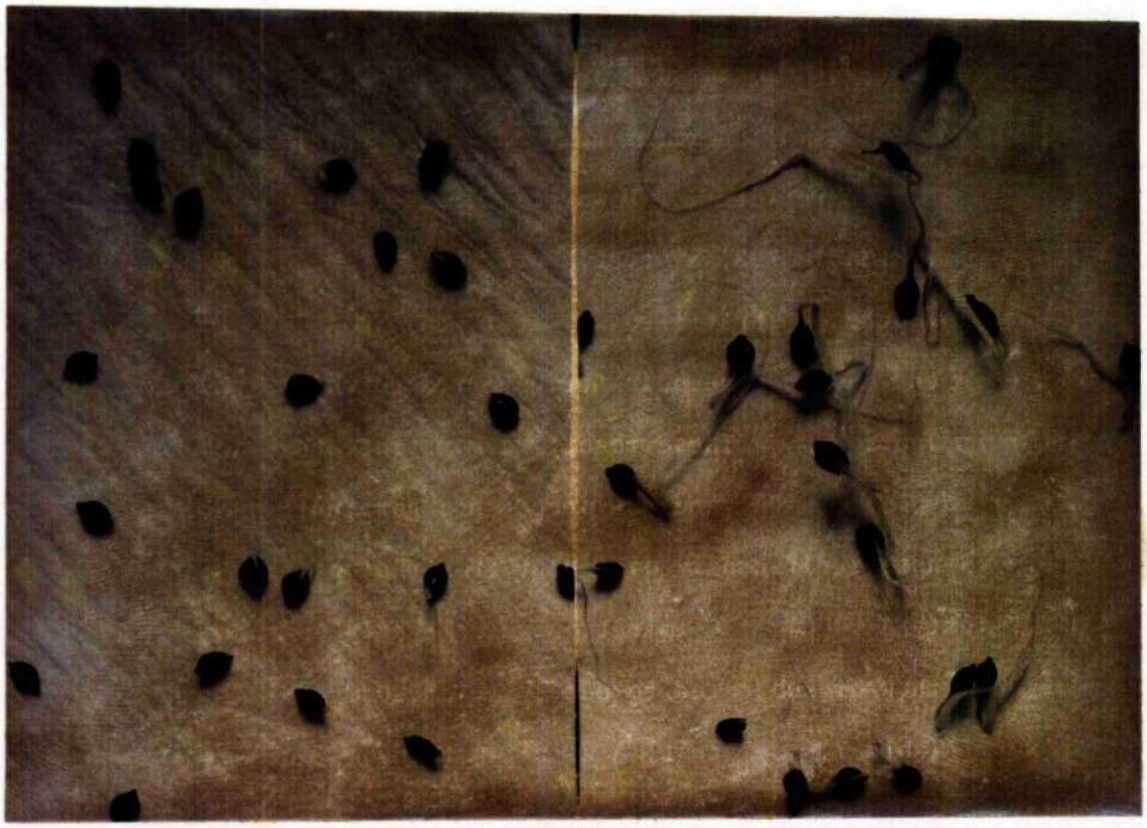
Treatment with 1 or 0.1mM Ancymidol retarded the length of the radicles. Radicles were thicker and shorter than the water treatment in contrast to 0.01 or 0.001mM.

The overall results appear to indicate that the involvement of gibberellin either during or after stratification is questionable.

Abscisic Acid (ABA)

The involvement of endogenous ABA during stratification in P. persicaria has been discussed on page 163. It has been speculated in other species that endogenous ABA declines during cold treatment and consequently dormancy is broken. It is then argued that application of exogenous ABA either during or after stratification should prevent germination.

Plate 4



a × 2·5 b

a : Achenes germinated at 20°C (dark) in the presence of 1mM ABA after stratification.

b : Achenes exposed to 1mM ABA for 24h and transferred to water at 20°C in the dark after stratification.

When achenes were stratified in ABA and then germinated in water at 20°C in the dark (Table 7.8) germination was not prevented, and it was comparable to water stratified achenes. Stratification in ABA was not significantly different to stratification in water (Table 7.8, χ^2 test, A vs B). Even when achenes were germinated in GA_3 or kinetin after stratification in ABA (Table 7.8, χ^2 test, B vs C; B vs D), percentage germination was not significantly greater than germination in water. However, R irradiation did enhance germination slightly (Table 7.8, χ^2 test, B vs E).

The results of achenes stratified in water and exposed to 1mM ABA for 24h or 48h at 20°C in the dark before being transferred to water at 20°C in the dark are shown in Table 7.9. These results demonstrate that achenes germinated on removal from ABA, and the percentage germination of achenes kept for 24h in ABA is not significantly different from that of the control (H_2O) (Table 7.9, χ^2 test, A vs C). Germination was slightly enhanced if achenes were irradiated with R or subjected to one cycle of 20°C (8h)D/35 C(16h)D after 48h ABA exposure (Table 7.9, χ^2 test, A vs E & A vs G). Achenes exposed continuously under the influence of ABA germinated (Table 7.9B), although radicle elongation was prevented (Plate 4). It appears that exogenous application of ABA does not prevent germination, rather it affects radicle elongation. These observations agree with those of Karssen (1976a) in Chenopodium album.

DISCUSSION

It has been clearly demonstrated that exogenous application of GA_3 or GA_{4+7} is ineffective in terminating the primary dormancy of unchilled achenes. The argument that these hormones might not enter into the achenes is unlikely because incubating punctured achenes in exogenous GA_3 or GA_{4+7} failed to stimulate germination. Although the possibility that GA_3 or GA_{4+7} is inactive in P. persicaria is not ruled out, or alternatively the hormones did not reach the receptor sites. In contrast to GA_3, kinetin enhanced the germination of

unchilled intact achenes. The enhancement effect observed when GA_3 or GA_{4+7} is combined with kinetin appears to be due to the effect of kinetin, hence interaction between GA_3 or GA_{4+7} with kinetin seems unlikely. However, Okusanya and Unger (1983) in Spergularia marina, observed interaction between GA_3 with kinetin, and noted that without kinetin, no germination occurred within the first 2 weeks of incubation.

The percentage germination of unchilled achenes incubated in GA_3 and irradiated with R is not significantly different from that of R irradiation alone (Table 7.2, χ^2 test, no.8), indicating that additive or synergistic interaction does not occur. It appears that the promotive effect observed could be attributed to the effect of R light, and this argues against the speculation that R light might act to promote the synthesis of gibberellin or substitute for GA, at least in P. persicaria.

When R is given in the presence of exogenous kinetin, germination increases and is better than either given alone. It seems plausible to suggest that R does not cause the formation of cytokinin. In fact, the combination of R or white light, GA_3 and kinetin gave the highest stimulation of germination. These observations fail to fit into Khan's (1971) hypothesis that GA and cytokinin perform primary and secondary roles respectively. An overall view of the results suggests that cytokinin might be more relevant in breaking dormancy in P. persicaria. It is apparent that exogenous application of GA_3 or GA_{4+7} does not stimulate germination while kinetin does not completely break dormancy. However, in Kalanchöe blossfeldiana seeds, Dedonder et al. (1983) observed that a high concentration ($2x10^{-3}M$) of exogenous GA_3 induces FR promotion of germination in contrast to water incubated seeds; and during the period of highest light sensitivity, one single 5 minutes FR irradiation induces more germination than one 2 minutes R irradiation. This indicates that the effects of exogenous gibberellin coupled with R or FR are complex and differ within species, thus ascribing a universal mechanism of action for different species could be misleading.

The possibility that low temperature increases the amount of endogenous

gibberellin was also not evident in P. persicaria. It was suggested that if chilling acts to promote an increase in the endogenous gibberellin, then application of exogenous gibberellin during stratification should act to reduce stratification period, i.e. substituting for de novo synthesis of endogenous gibberellins. This suggestion assumes that after a prolonged period of stratification, achenes not stratified in gibberellin should have produced considerable endogenous gibberellin and consequently the germination should be comparable to achenes that were stratified in gibberellin. The results show consistency of promotion of germination by GA_3 over that of water stratified achenes. Even after 15 weeks of stratification in GA_3, total percentage germination was not above 70 percent.

Williams et al. (1974), working with Hazel seeds, speculated that chilling treatment which breaks dormancy activates the GA-producing mechanism, the subsequent synthesis of GA occurring at the higher germination temperature. Application of GA_3 after chilling in water on transfer to 20°C promoted germination, but not adequate enough to allow the holding of the above view.

Growth retardants applied either during or after chilling did not prevent germination, even at the highest concentrations used, though germination percentage can be reduced by these substances. The percentage germination for achenes germinated in different growth retardants after chilling lay between 63 to 70 percent, and for those chilled in growth retardants, it was between 33-54 percent, while the control gave 67 percent after 12 weeks of chilling. These results do not support the hypothesis that chilling terminates dormancy by acting to cause an increase in the level of promotive hormones (GA) or to increase the capacity for their production at elevated temperature. However, it is still possible that endogenous-free gibberellin content increases by hydrolysis of the conjugate forms, because these forms are not susceptible to the action of growth retardants, although in after-ripened wild oats (Avena fatua L.), conjugate forms are not an important source of biologically active GAs during germination (Metzger, 1983).

Cathey (1964) has observed that growth retardants apparently do not prevent germination but were only operative when growth was started by light or gibberellin. AMO 1618 was reported to retard the germination of light-promoted seeds of Lepidium virginicum, although it did not influence the dormancy of non-promoted seeds (Cathey and Stuart, 1961). However, Wittwer and Tolbert (1960) observed that CCC inhibited the percentage germination of lettuce seed and also antagonised the stimulation of germination by both red light and gibberellin. Metzger (1983) reported inhibition of GA biosynthesis with CCC in non-dormant (after-ripened) wild oats (Avena fatua L.) seeds without subsequent prevention of germination. He went further to demonstrate that wild oat plants fed with CCC produced viable seeds which lacked a detectable GA-like substance, but after-ripened at a slower rate than the untreated. Berrie and Robertson (1973) noted statistical interaction between exogenously applied GA_3 and B995 in lettuce seeds. They argued that the interaction cannot be interpreted as meaning that competition for the site of action of GA_3 exists, since such interaction was also observed for kinetin.

The actions of growth retardants have also been demonstrated in other parts of the plants. Cathey and Stuart (1961) enumerated the effects of AMO 1618 and CCC on growth responses of several species of plants in terms of reduction of internode length and production of dark green leaves, and also stimulation of plant height. Application of CCC in gladiolus plants resulted in stimulation of stem length, an increase in number of flowers per spike, and a slight advancement of anthesis date (Halevy and Shilo, 1970).

An overall picture might suggest that even though growth retardants inhibit GA biosynthesis, the involvement of gibberellin apparently appears not to be a necessary prerequisite for successful germination, at least in P. persicaria.

With regard to ABA, our results demonstrate that radicle elongation and not germination is affected by exogenously applied ABA. The results of Karsson (1982) noted in page 163 support the view that the fall in ABA levels during stratification is not primarily responsible for terminating dormancy in

P. persicaria.

Consequently, it seems plausible to suggest that dormancy release in P. persicaria is not correlated with any changes in growth promoters or inhibitors.

Table 7.1. The effects of exogenously applied hormones on the germination of unchilled achenes at 35°C either in the dark or light. Germination scored after 14 days.

	Percentage Germination							
	Dark Germination				Light Germination			
Treatments	†Replicates			Mean	Replicates			Mean
Water (Control)	23	19	21	21	55	53	46	51.33
1mM GA_3	24	19	24	22.33	47	56	55	52.66
1mM GA_{4+7}	23	19	16	19.33	55	51	59	55
0.1mM Kinetin	40	45	38	41	70	71	65	68.66
1mM GA_3 + 0.1mM Kinetin	54	50	54	52.66	72	74	71	72.33
1mM GA_{4+7} + 0.1mM Kinetin	53	51	44	49.33	91	69	77	79

† 100 achenes per replicate

Analysis of Results : χ^2 (Chi-squared) test

7.1a.

Dark Germination

Serial No.	Source	χ^2	p	Significant level
1	Water vs 1mM GA_3	0.0883798	p > 0.05	N.S.
2	Water vs 1mM GA_{4+7}	0.165634	p > 0.05	N.S.
3	Water vs 0.1mM Kinetin	27.1233	p < 0.001	***
4	0.1mM Kinetin vs 1mM GA_3 + 0.1mM Kinetin	7.73771	p < 0.01	**
5	0.1mM Kinetin vs 1mM GA_{4+7} + 0.1mM Kinetin	3.87622	0.05>p>0.01	*

Table 7.1.

7.1b

Light Germinations

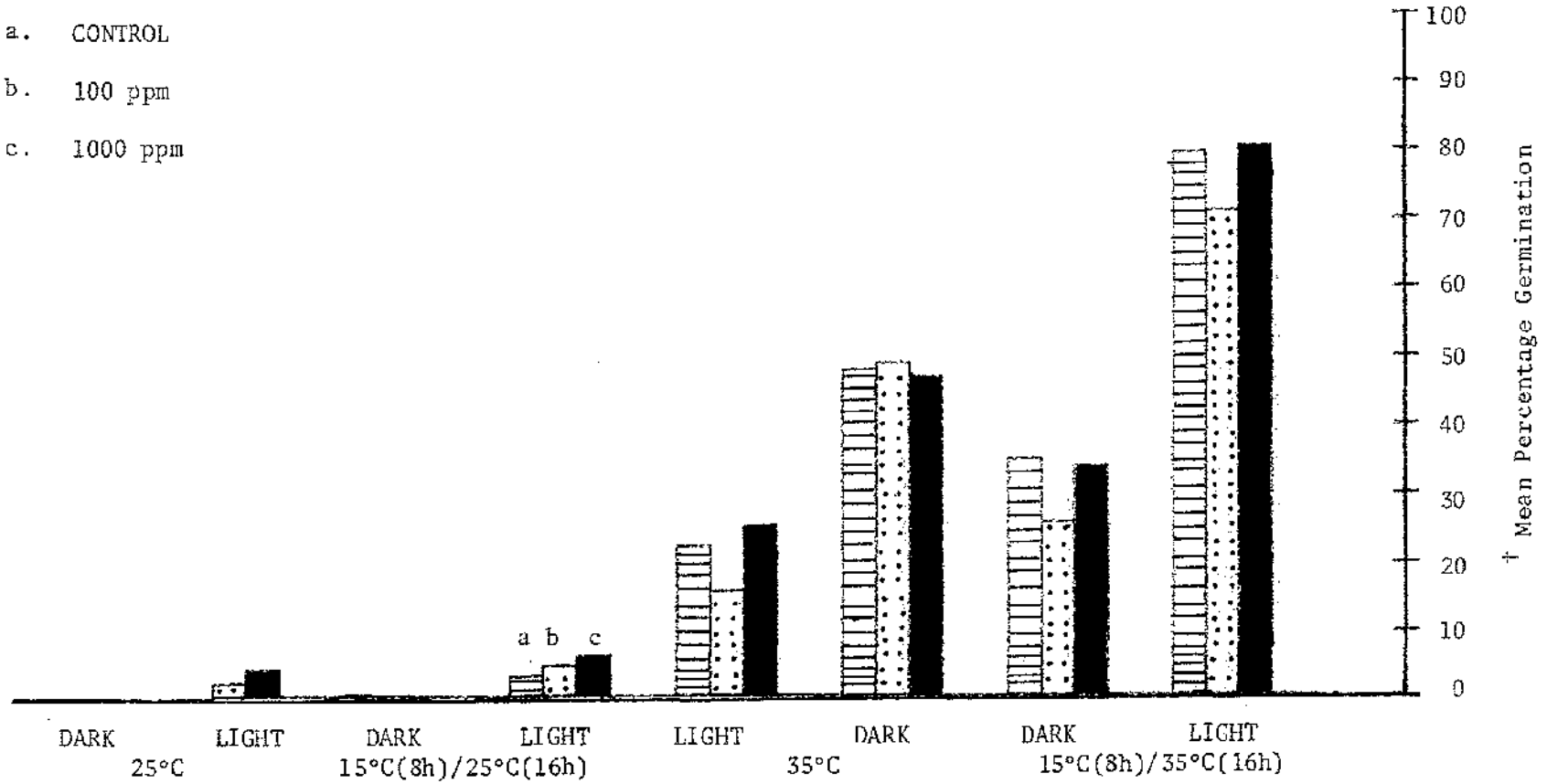
Source	χ^2	p	Significant level
Water vs 1mM GA_3	0.0600962	$p > 0.05$	N.S.
Water vs 1mM GA_{4+7}	0.669352	$p > 0.05$	N.S.
Water vs 0.1mM Kinetin	18.0625	$p < 0.001$	***
0.1mM Kinetin vs 1mM GA_3 + 0.1mM Kinetin	0.801379	$p > 0.05$	N.S.
0.1mM Kinetin vs 1mM GA_{4+7} + 0.1mM Kinetin	7.76409	$p < 0.01$	**

7.1c

Dark vs Light Germination

Source	χ^2	p	Significant level
Water	58.476	$p < 0.001$	***
1mM GA_3	57.6	$p < 0.001$	***
1mM GA_{4+7}	80.1894	$p < 0.001$	***
0.1mM Kinetin	45.2495	$p < 0.001$	***
1mM GA_3 + 0.1mM Kinetin	23.9218	$p < 0.001$	***
1mM GA_{4+7} + 0.1mM Kinetin	56.1329	$p < 0.001$	***

FIGURE 7.1



The mean percentage germination of unchilled achenes treated with exogenous ethylene (Ethrel) and germinated at different conditions. Germination recorded after 14 days.

Light at the Upper Temperature part of the cycle.

†Each treatment is replicated twice, with 100 achenes per replicate.

Table 7.2. The mean percentage germination of unchilled achenes treated with exogenously applied hormones or irradiated with Red (R) light and germinated at 35°C in the dark. Germination scored after 14 days.

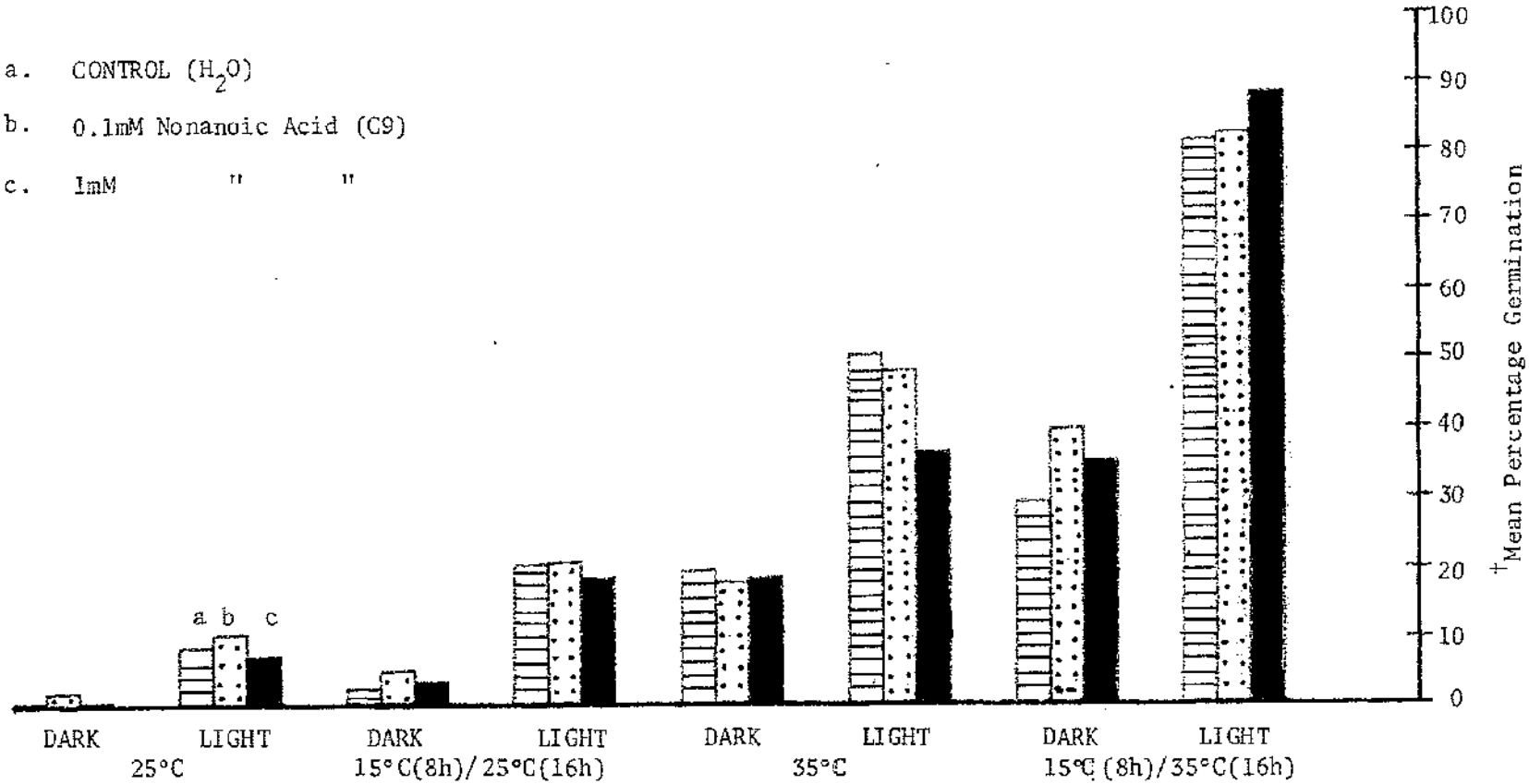
Treatment	Percentage Germination Replicates			Mean
Water (Control)	14	16	18	16
1mM GA_3	9	12	17	12.66
0.1mM Kinetin	49	47	41	45.66
1mM GA_3 + 0.1mM Kinetin	34	41	43	39.33
30 minutes Red light (R)	34	28	31	31
30 minutes R + 1mM GA_3	44	35	34	37.66
30 minutes R + 0.1mM Kinetin	61	54	54	56.33
30 minutes R + 1mM GA_3 + 0.1mM Kinetin	60	56	63	59.66

Each replicate consists of 100 achenes

Analysis of Results : χ^2 (Chi-squared) test

Serial No.	Source	χ^2	p	Significant level
1	Water vs GA_3	1.09945	$p > 0.05$	N.S.
2	Water vs Kinetin	60.5197	$p < 0.001$	***
3	Kinetin vs GA_3 + Kinetin	2.20972	$p > 0.05$	N.S.
4	Water vs R	17.9484	$p < 0.001$	***
5	R vs GA_3	28.477	$p < 0.001$	***
6	R vs Kinetin	13.0364	$p < 0.001$	***
7	GA_3 vs R + GA_3	48.4609	$p < 0.001$	***
8	R vs R + GA_3	2.66867	$p > 0.05$	N.S.
9	R vs R + Kinetin	38.1115	$p < 0.001$	***
10	Kinetin vs R + Kinetin	6.40923	$0.05>p>0.01$	*
11	GA_3 + Kinetin vs R + GA_3 + Kinetin	24.0024	$p < 0.001$	***
12	R + GA_3 vs R + GA_3 + Kinetin	28.1867	$p < 0.001$	***
13	R + Kinetin vs R + GA_3 + Kinetin	0.554187	$p > 0.05$	N.S.

FIGURE 7.2



The mean percentage germination of unchilled achenes treated with exogenous nonanoic acid (C9) and germinated under different conditions. Germination scored after 14 days.

+Light at the Upper Temperature part of the cycle.
+Each treatment is replicated thrice, with 100 achenes per replicate.

Table 7.3. The percentage germination of achenes punctured and germinated in exogenously applied GA_3 or Kinetin and germinated at 20°C or 35°C in the dark or light. Germination counted after 14 days.

A. Percentage Germination at 20°C in the dark

No.	Treatment	Dark Germination †Replicates				Mean	Light Germination Replicates				Mean
1	(Whole) Intact + Water (H_2O)	0	0	0	0	0	0	0	0	0	0
2	Punctured + Water	0	0	0	0	0	0	0	0	0	0
3	Intact + 1mM GA_3	0	0	0	0	0	0	0	0	0	0
4	Punctured + 1mM GA_3	4	4	10	8	6.5	6	6	10	8	7.5
5	Intact + 0.1mM Kinetin	0	0	0	0	0	8	6	12	10	9
6	Punctured + 0.1mM Kinetin	0	0	0	2	0.5	10	8	8	8	8.5

B. Percentage Germination at 35°C in the dark

No.	Treatment	Dark Germination †Replicates				Mean	Light Germination Replicates				Mean
1	(Whole) Intact + Water (H_2O)	36	30	42	23	32.75	50	56	52	75	58.25
2	Punctured + Water (H_2O)	70	65	65	64	66	69	62	69	65	66.25
3	Intact + 1mM GA_3	37	33	46	37	38.25	70	71	60	-	67
4	Punctured + 1mM GA_3	70	75	67	69	70.25	*48	46	34	-	42.66
5	Intact + 0.1mM Kinetin	58	69	60	81	67	73	77	85	-	78
6	Punctured + 0.1mM Kinetin	71	60	72	78	70.25	*36	46	41	-	41

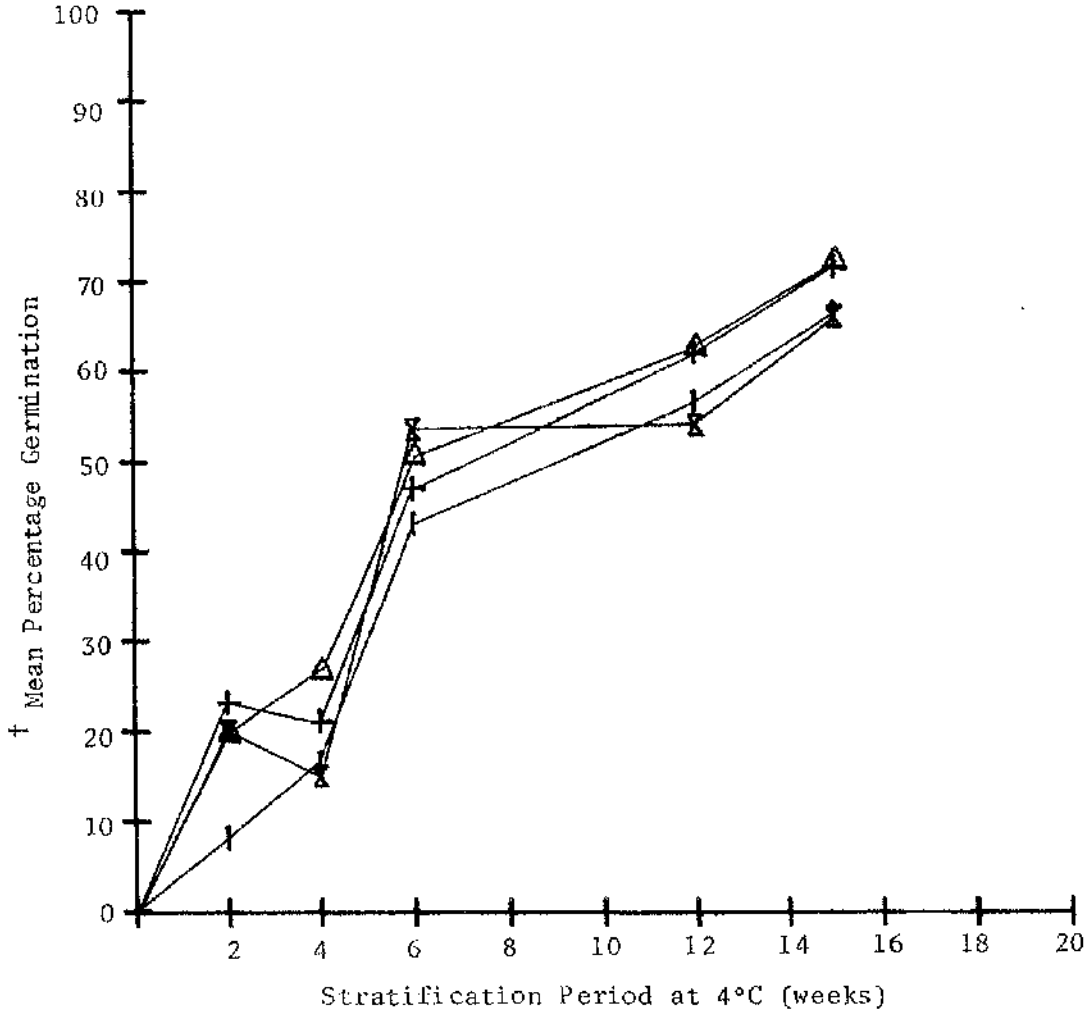
† Those replicated 4 times consist of 50 achenes per replicate in 2ml of distilled water or appropriate solution, while those replicated 3 times consist of 100 achenes per replicate in 10ml of distilled water or solution.

* Affected by fungus during the course of experiment.

Analysis of Results : χ^2 (Chi-squared) Test. Germination at 35°C (dark)

Serial No.	Source	χ^2	p	Significant level
1	Intact Achenes:Water vs GA_3	2.40747	$p > 0.05$	N.S.
2	Intact Achenes:Water vs Kinetin	92.4806	$p < 0.001$	***
3	Intact Achenes:GA_3 vs Kinetin	65.1596	$p < 0.001$	***
4	Punctured Achenes:Water vs GA_3	1.47365	$p > 0.05$	N.S.
5	Punctured Achenes:Water vs Kinetin	1.47365	$p > 0.05$	N.S.
6	Punctured Achenes:GA_3 vs Kinetin	0.00598104	$p > 0.05$	N.S.

FIGURE 7.3A



The mean percentage germination of achenes stratified in GA_3, transferred to water and germinated at 20°C in the dark. Germination scored after 14 days.

†Mean of two replicates, with 100 achenes per replicate.

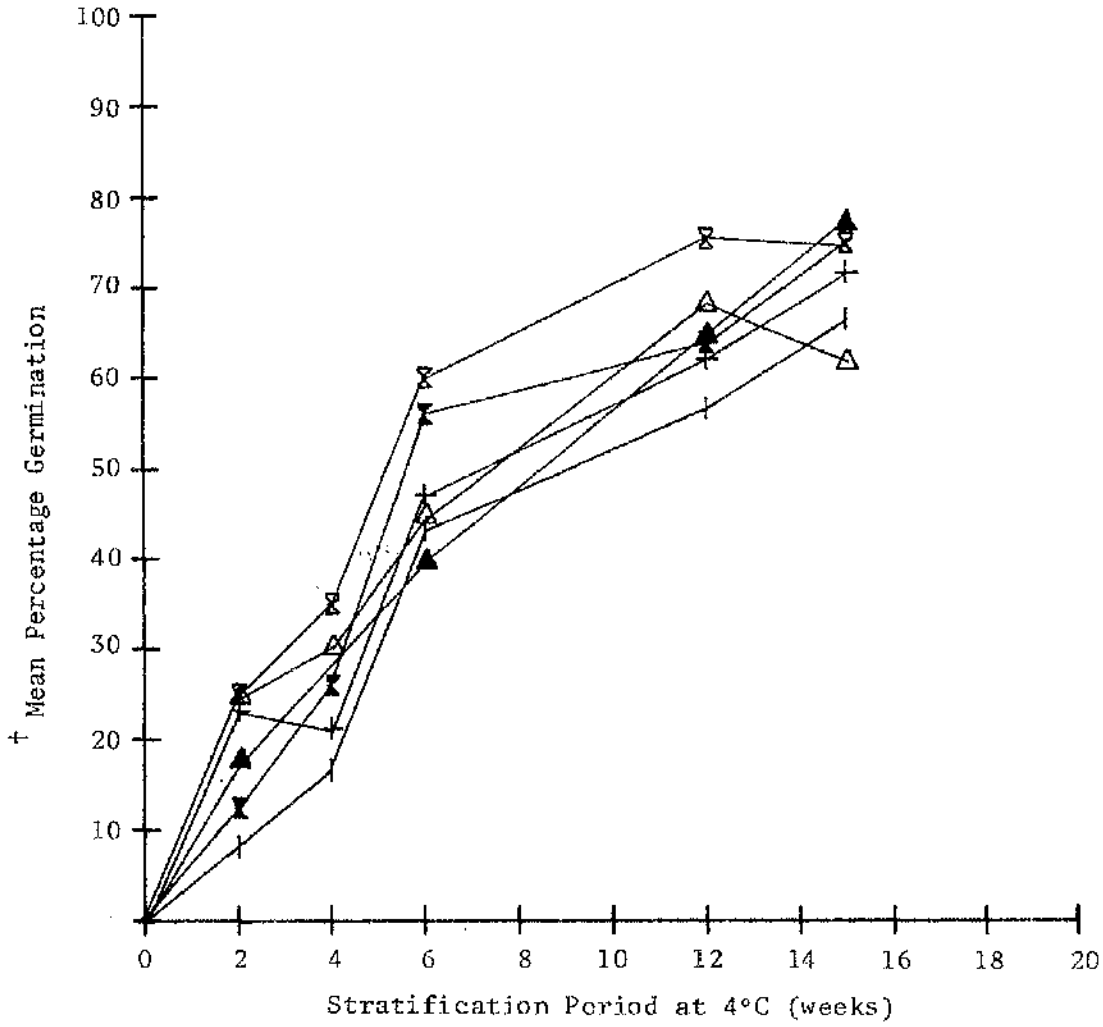
Δ : 1mM GA_3

$\overline{X}$: 0.1mM GA_3

\+ : 0.01 mM GA_3

I : NO GA_3

FIGURE 7.3B



The mean percentage germination of achenes stratified in GA_3, transferred to water and irradiated with 30 minutes R or FR. Germinated at 20°C in the dark. Germination recorded after 14 days.

†Mean of two replicates, with 100 achenes per replicate.

1 : Water (NO GA_3)

\+ : 0.01mM GA_3

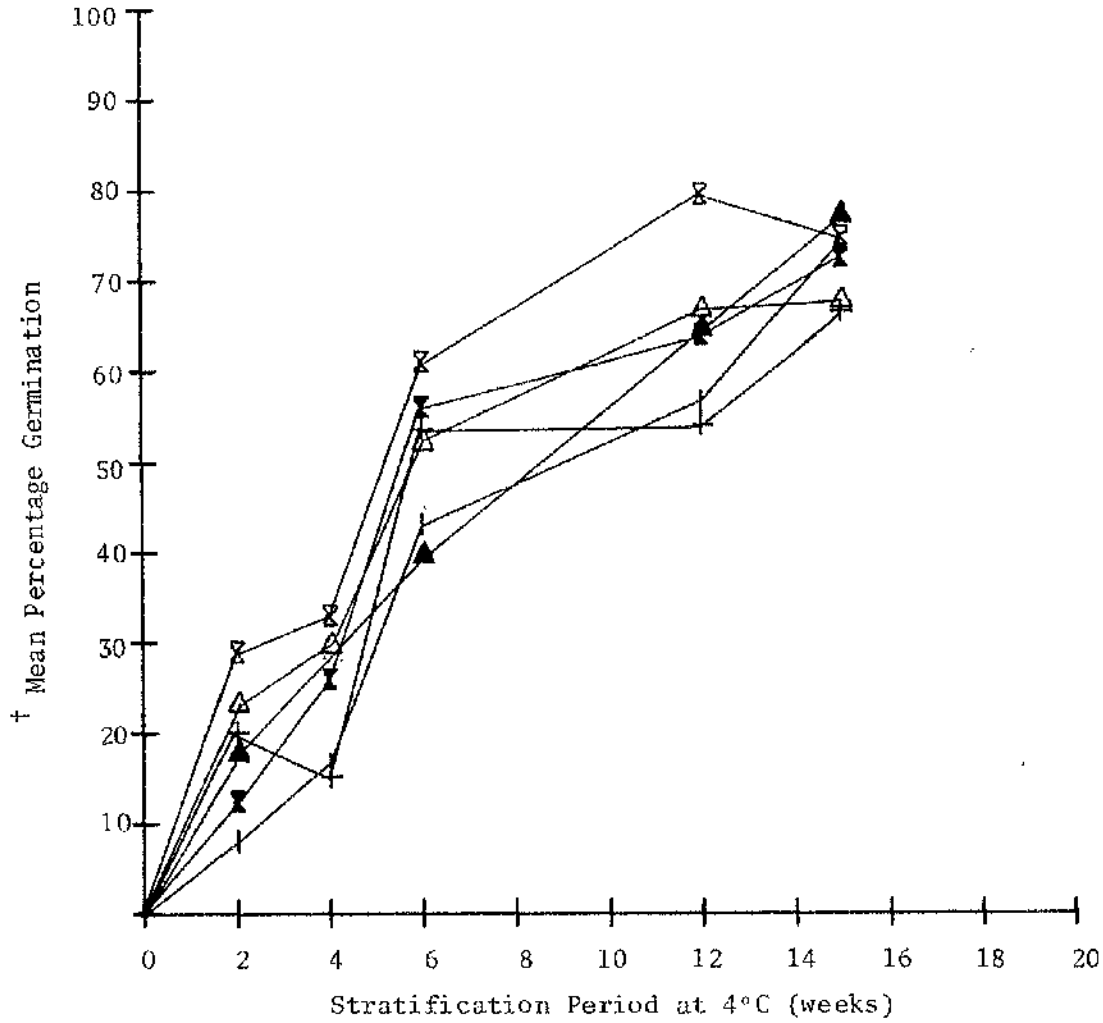
Δ : 0.01mM GA_3 + FR

▲ : Water (0 GA_3) + FR

$\overline{X}$: 0.01mM GA_3 + R

⧗ : Water (0 GA_3) + R

FIGURE 7.3C



The mean percentage germination of achenes stratified in GA_3, transferred to water and irradiated with 30 minutes R or FR. Germinated at 20°C in the dark. Germination recorded after 14 days.
†Mean of two replicates, with 100 achenes per replicate.

1 : Water (NO GA_3)

\+ : 0.1mM GA_3

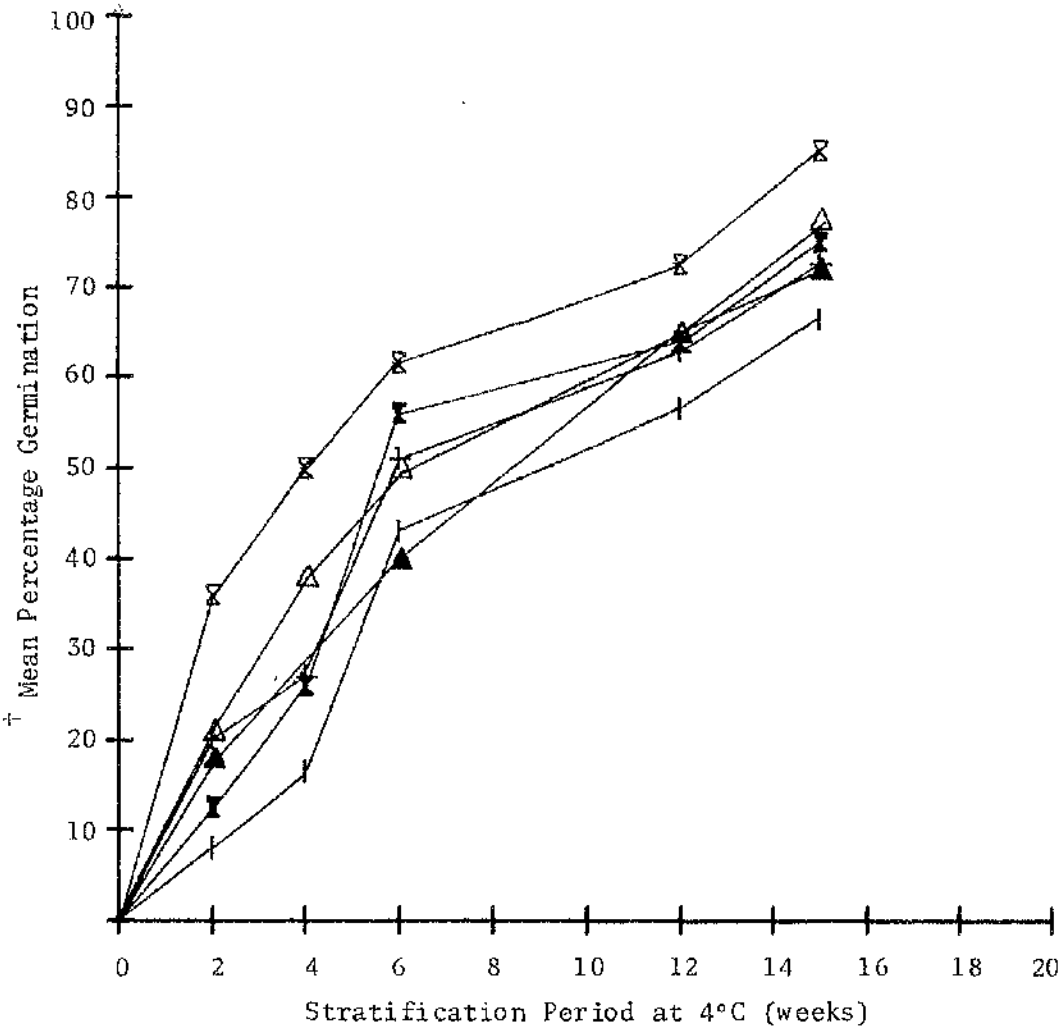
Δ : 0.1mM GA_3 + FR

▲ : Water (0 GA_3) + FR

$\overline{X}$: 0.1mM GA_3 + R

⧗ : Water (0 GA_3) + R

FIGURE 7.3D



The mean percentage germination of achenes stratified in GA_3, transferred to water and irradiated with 30 minutes R or FR. Germinated at 20°C in the dark. Germination recorded after 14 days.
†Mean of two replicates, with 100 achenes per replicate.

1 : Water (NO GA_3)

\+ : 1mM GA_3

Δ : 1mM GA_3 + FR

▲ : Water (0 GA_3) + FR

X̄ : 1mM GA_3 + R

⧗ : Water (0 GA_3) + R

Table 7.4. The mean percentage germination of achenes stratified at 4°C in GA_3 and germinated in water at 20°C in the dark. Germination scored after 14 days.

A. No Irradiation

Exposure Period at 4°C in weeks	Percentage Germinations: Concentrations of GA_3			
	Water (0)	0.01mM	0.1mM	1mM
2	†8	23	20	20
4	16.5	21	15	27
6	43	47	53.5	51
12	56.5	62	54	63
15	66.5	71.5	66	72.5

B. 30 minutes R Irradiation after Stratification

Exposure Period at 4°C in weeks	Percentage Germination: Concentrations of GA_3			
	Water	0.01mM	0.1mM	1mM
2	†12.5	25	29	36
4	26	35	33	50
6	56	60	61	61.5
12	64	75.5	79.5	72.5
15	75	75	72.5	85

C. 30 minutes FR Irradiation after Stratification

Exposure Period at 4°C in weeks	Percentage Germination: Concentrations of GA_3			
	Water	0.01mM	0.1mM	1mM
2	†18	25	23.5	21
4	30	30.5	30	38
6	40	45	52.5	50
12	65	68.5	67	65
15	77.5	62	68	72

†Mean of two replicates, with 100 achenes per replicate.

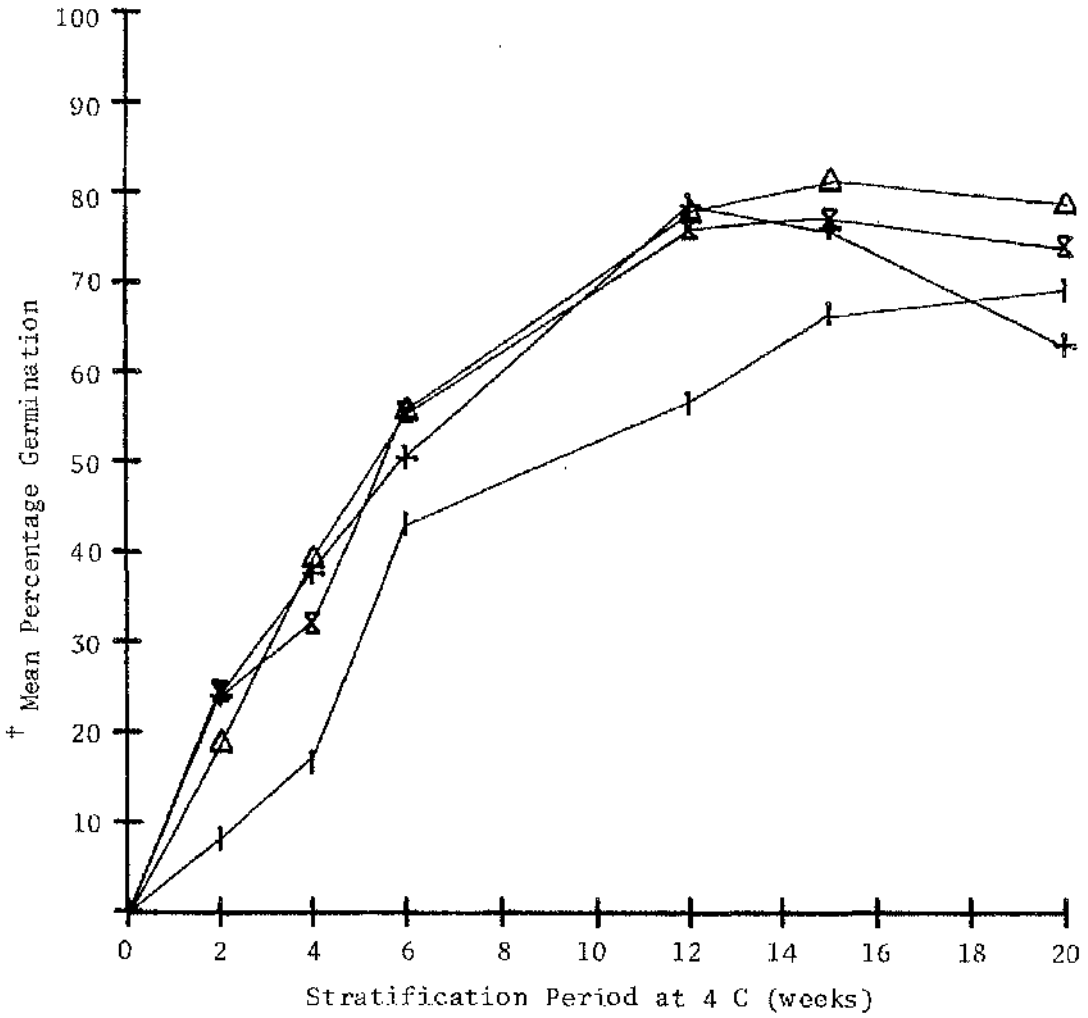
Table 7.4.

Analysis of Results : 3 Factor Variance

A, B & C.

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	1	1.01563	1.01563	0.20046	N.S.
Exposure Time at 4°C in weeks (A)	4	17298.3	4324.57	853.566	***
GA_3 Concentrations (B)	3	481.656	160.552	31.6891	***
Light Treatments (C)	2	1046.59	523.297	103.286	***
A x B	12	424.828	35.4023	6.98757	***
B x C	6	178.313	29.7188	5.86577	***
A x C	8	245.453	30.6816	6.05582	***
A x B x C	24	231.625	9.65104	1.90488	*
Error	59	298.922	5.06647		
Total	119	20206.7			

FIGURE 7.4



The mean percentage germination of achenes stratified at 4°C in water and germinated in GA_3 at 20°C in the dark. Germination scored after 14 days. †Mean replicated twice, with 100 achenes per replicate.

Δ : 1mM GA_3

X : 0.1mM GA_3

\+ : 0.01mM GA_3

1 : NO GA_3

Table 7.5. The mean percentage germination of achenes stratified at 4°C in water and germinated in GA_3 at 20°C in the dark. Germination recorded after 14 days.

Exposure Period at 4°C in weeks	Percentage Germination Concentrations of GA_3			
	Water (0)	0.01mM	0.1mM	1mM
2	†8	24	24.5	19.0
4	16.5	37.5	32.0	39.5
6	43	50.5	55.5	56.0
12	56.5	78.5	76.0	78.0
15	66.5	76	77.0	81.5
20	69	63	74.0	79.0

†Mean of two replicates, with 100 achenes per replicate.

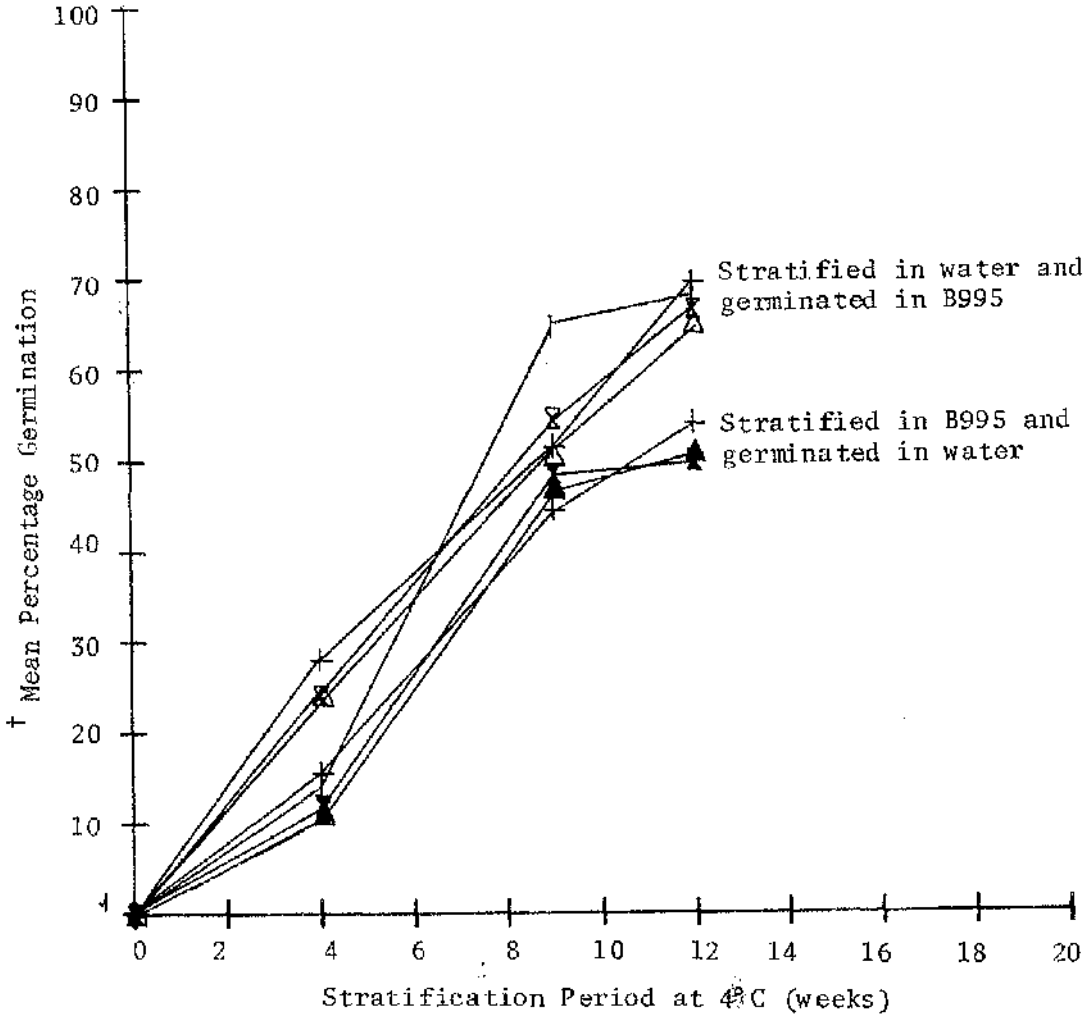
Analysis of Results : 2 Factor Variance

Source (7.5)	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	1	6.82031	6.82031	1.33424	N.S.
Exposure Period at 4°C in weeks (A)	5	769.438	153.888	30.1046	***
GA_3 Concentrations (B)	3	8683.23	2894.41	566.226	***
A x B	15	777.391	51.826	10.1386	***
Error	23	117.57	5.11175		
Total	47	10354.4			

Comparison between stratification in water (H_2O) and stratification in GA_3 at 4°C. Tables 7.5 vs 7.4A

Replicates	1	1.1875	1.1875	0.273568	N.S.
Exposure Period at 4°C in weeks (A)	4	478.539	119.635	27.5606	***
GA_3 Concentrations (B)	5	8777.3	1775.46	404.411	***
A x B	20	1399.31	69.9656	16.1182	***
Error	29	125.883	4.34679		
Total	59	10782.2			

FIGURE 7.5A



The mean percentage germination of achenes stratified at 4°C in distilled water and germinated in B995 or stratified in B995 and germinated in water at 20°C in the dark.
Germination count recorded after 14 days.
†Mean of two replicates, with 100 achenes per replicate.

B995 Concentrations:

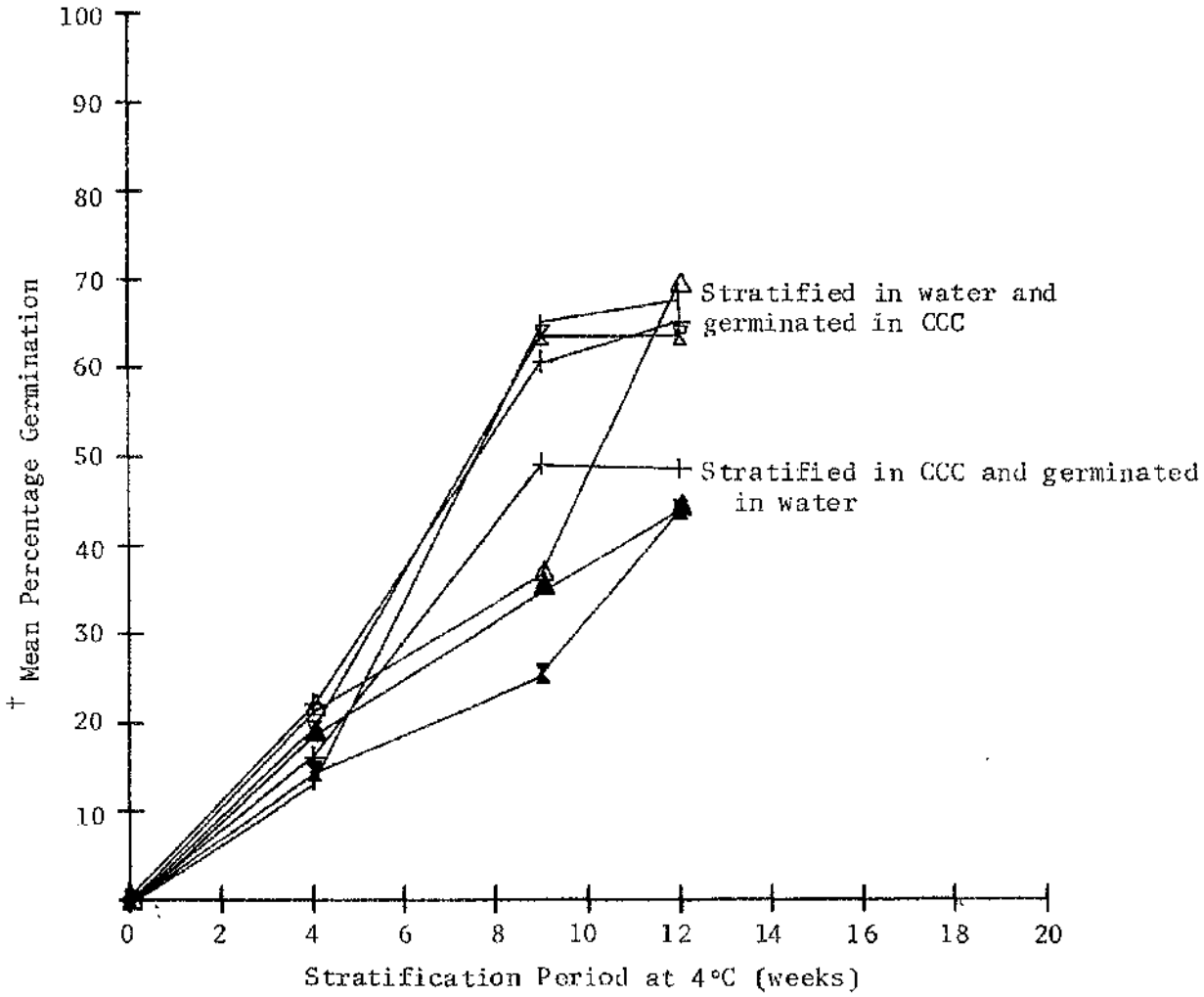
1 Water (Control)

\+ 0.0625mM

X 0.625mM

Δ 6.25mM

FIGURE 7.5B

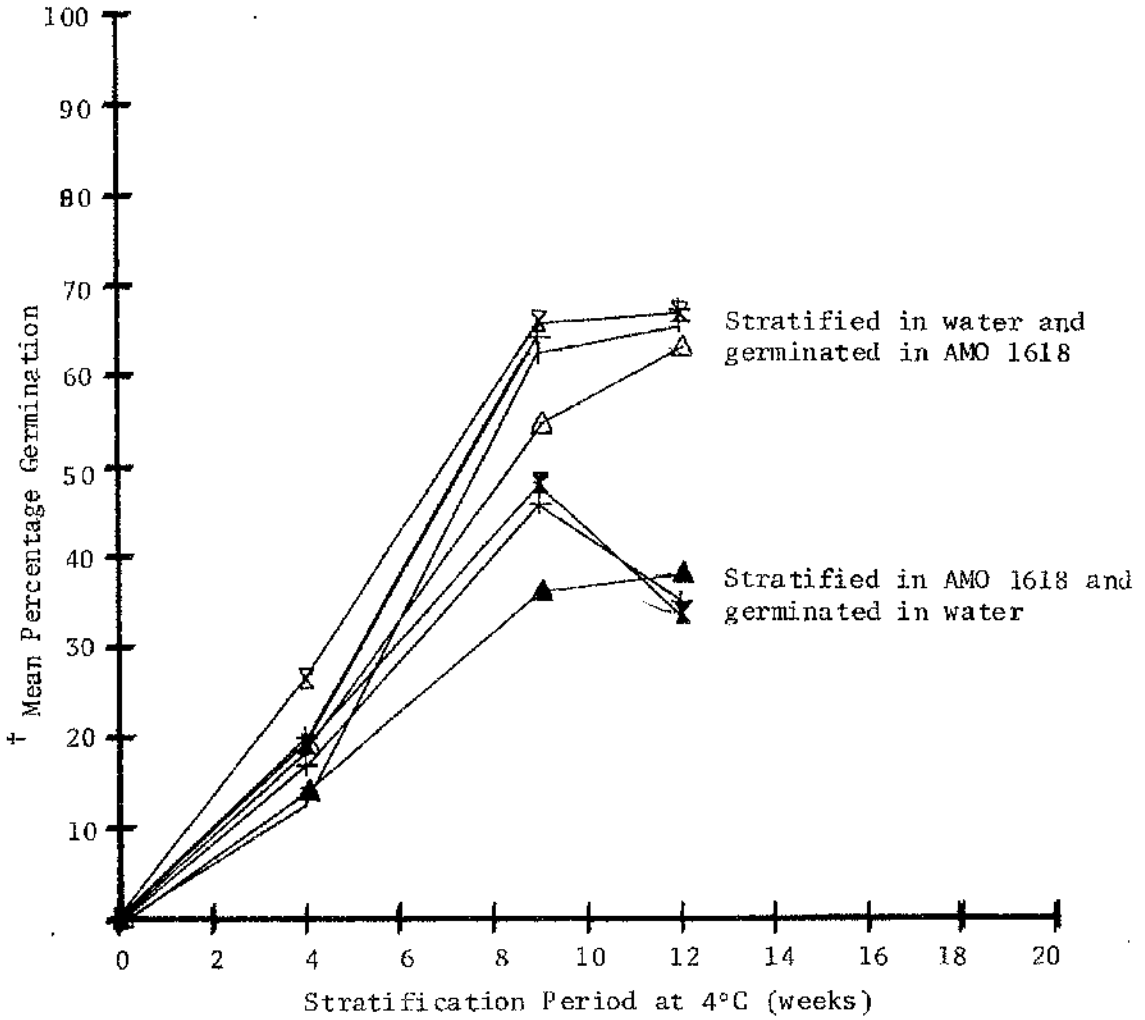


The mean percentage germination of achenes stratified at 4°C in distilled water and germinated in CCC or stratified in CCC and germinated in water at 20°C in the dark.
Germination count recorded after 14 days.
†Mean of two replicates, with 100 achenes per replicate.

CCC Concentrations:

1	Water
+	0.0633mM
X	0.633mM
Δ	6.33mM

FIGURE 7.5C



The mean percentage germination of achenes stratified at 4°C in distilled water and germinated in AMO1618 or stratified in AMO1618 and germinated in water at 20°C in the dark.
Germination scored after 14 days.
+Mean of two replicates, with 100 achenes per replicate.

AMO1618 Concentrations:

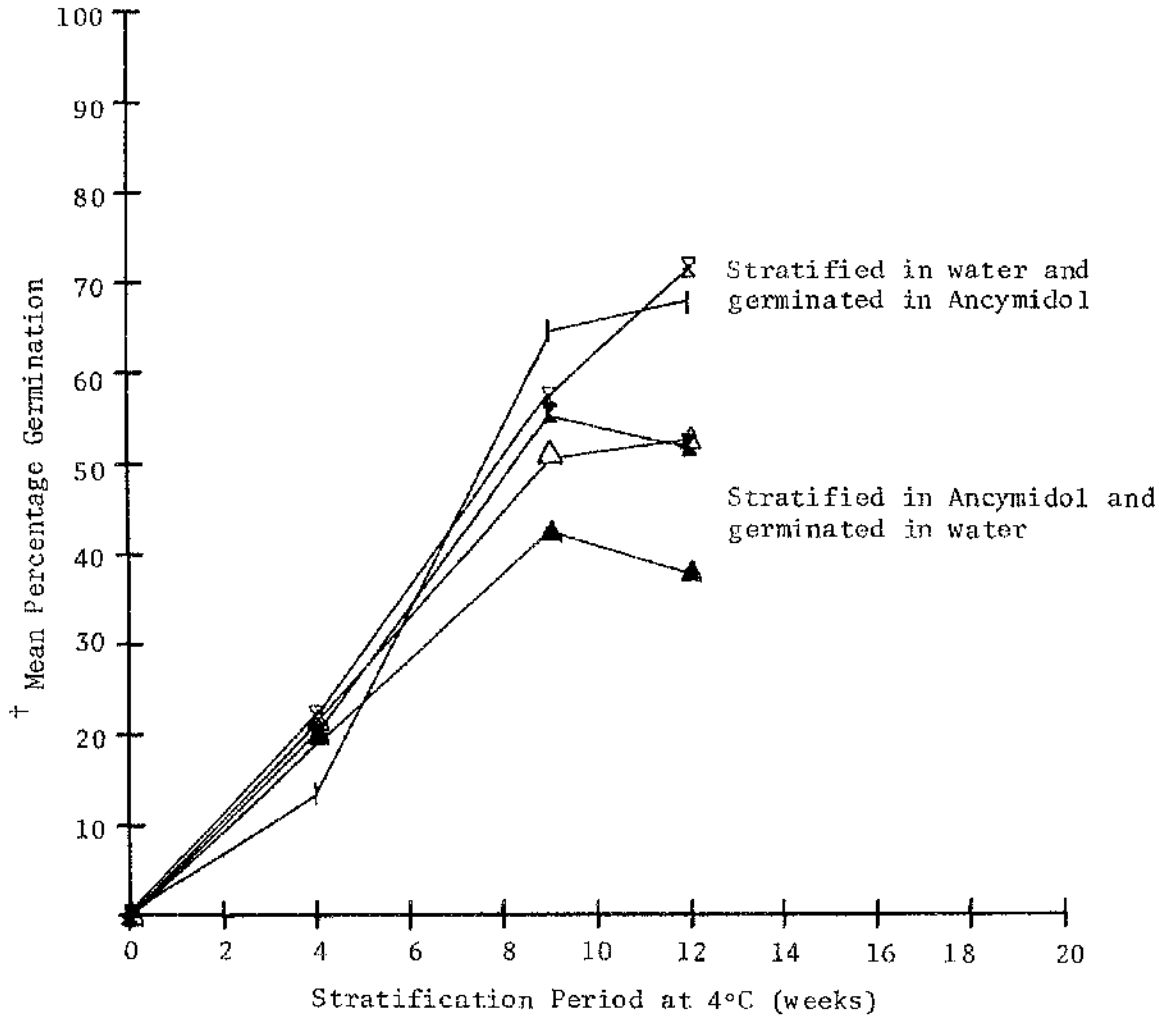
1 Water (Control)

+ 0.0279mM

X 0.279mM

Δ 2.79mM

FIGURE 7.5D



The mean percentage germination of achenes stratified at 4°C in distilled water and germinated in Ancymidol or stratified in Ancymidol and germinated in water at 20°C in the dark.

†Mean of two replicates, with 100 achenes per replicate.

Ancymidol Concentrations:

1 Water (Control)

$\overline{X}$ 0.001mM

Δ 0.01mM

Table 7.6. The mean percentage germination of achenes stratified at 4°C in water, transferred to growth retardants and germinated at 20°C in the dark. Germination count recorded after 14 days.

A.

Exposure Period at 4°C in weeks	Percentage Germination Concentrations of B995			
	†Water (0)	0.0625mM	0.625mM	6.25mM
4	13.5	28	24	24
9	64.5	44.5	54.5	50.5
12	67.5	69.5	66.5	65

B.

Exposure Period at 4°C in weeks	Percentage Germination Concentrations of CCC			
	†Water (0)	0.0633mM	0.633mM	6.33mM
4	13.5	22	19	22
9	64.5	60.5	63.5	37
12	67.5	65	63.5	69.5

C.

Exposure Period at 4°C in weeks	Percentage Germination Concentrations of AMO1618			
	†Water (0)	0.0279mM	0.279mM	2.79mM
4	13.5	20	26.5	19
9	64.5	62.5	66	54.5
12	67.5	66	67	63

D.

Exposure Period at 4°C in weeks	Percentage Germination Concentrations of Ancymidol		
	†Water (0)	0.001mM	0.01mM
4	13.5	22	21.5
9	64.5	57.5	51
12	67.5	71.5	52.5

† Mean of two replicates, with 100 achenes per replicate.

Table 7.6.

Analysis of Results : 2 Factor Variance.

A. Stratification in Water and Germination in B995

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant Level
Replicates	1	7.375	7.375	2.73335	N.S.
Exposure Period at 4 C (wks)	2	261.684	130.842	3.8369	*
B995 Concentrations	3	2531.63	843.875	24.7466	***
Error	17	579.7107	34.1006		
Total	23	3380.39			

B. Stratification in Water and Germination in CCC

Replicates	1	6.10938	6.10938	0.678017	N.S.
Exposure Period at 4 C (wks)	2	269.207	134.604	2.1711	N.S.
CCC Concentrations	3	2892.65	964.218	15.5528	***
Error	17	1053.9372	61.9963		
Total	23	4221.91			

C. Stratification in Water and Germination in AMO 1618

Replicates	1	0.144531	0.144531	0.019604	N.S.
Exposure Period at 4 C (wks)	2	337.375	168.688	3.1834	N.S.
AMO1618 Concentrations	3	2883.19	961.063	18.1369	***
Error	17	900.8167	52.9892		
Total	23	4121.52			

D. Stratification in Water and Germination in Ancymidol

Replicates	1	23.8086	23.8086	4.45053	N.S.
Exposure Period at 4 C (wks)	2	82.0859	41.043	2.2842	N.S.
Ancymidol Concentrations	2	2659.85	1329.92	74.0172	***
Error	12	215.6129	17.9677		
Total	17	2981.36			

Table 7.7. The mean percentage germination of achenes stratified at 4°C in growth retardants and germinated in water (H_2O) at 20°C in the dark. Germination counted after 14 days.

A.

Exposure Period at 4°C in weeks	Percentage Germination Concentrations of B995			
	†Water (0)	0.0625mM	0.625mM	6.25mM
4	13.5	15.5	12	11
9	64.5	51.5	48.5	47
12	67.5	54	50	51

B.

Exposure Period at 4°C in weeks	Percentage Germination Concentrations of CCC			
	†Water (0)	0.0633mM	0.633mM	6.33mM
4	13.5	16	14.5	19
9	64.5	49	25.5	35.5
12	67.5	48.5	44	44.5

C.

Exposure Period at 4°C in weeks	Percentage Germination Concentrations of AMO1618			
	†Water (0)	0.0279mM	0.279mM	2.79mM
4	13.5	17	19	14
9	64.5	46	48	36
12	67.5	35	33.5	38

D.

Exposure Period at 4°C in weeks	Percentage Germination Concentrations of Ancymidol		
	†Water (0)	0.001mM	0.01mM
4	13.5	20	20
9	64.5	55.5	42.5
12	67.5	52	38

† Mean of two replicates, with 100 achenes per replicate.

Table 7.7.

Analysis of Results : 2 Factor Variance

A. Stratification in B995 and Germination in Water

Source	Degree of Freedom	Sum of Square	Mean Square	F	Significant level
Replicates	1	9.13281	9.13281	1.70555	N.S.
Exposure Period at 4°C (wks)	2	371.82	185.91	2.72	N.S.
B995 Concentrations	3	2528.39	842.796	12.34	***
Error	17	1160.2223	68.2483		
Total	23	4069.56			

B. Stratification in CCC and Germination in Water

Replicates	1	0.181641	0.181641	0.024418	N.S.
Exposure Period at 4°C (wks)	2	449.346	224.673	3.4068	N.S.
CCC Concentrations	3	1674.39	558.131	8.46	**
Error	17	1121.1062	65.9474		
Total	23	3245.02			

C. Stratification in AMO1618 and Germination in Water

Replicates	1	0.636719	0.636719	0.125175	N.S.
Exposure Period at 4°C (wks)	2	381.016	190.508	3.03	N.S.
AMO1618 Concentrations	3	1469.08	489.693	7.788	**
Error	17	1068.8031	62.8707		
Total	23	2919.53			

D. Stratification in Ancymidol and Germination in Water

Replicates	1	7.74023	7.74023	1.60048	N.S.
Exposure Period at 4°C (wks)	2	211.082	105.541	3.95	*
Ancymidol Concentrations	2	1951.94	975.97	36.58	***
Error	12	320.1605	26.68		
Total	17	2490.92			

Table 7.8. The mean percentage germination of achenes stratified at 4°C in 1mM ABA for 8 weeks and germinated at 20°C in the dark. Germination scored after 14 days.

Serial No.	Treatments	†Percentage Replicates		Germination Mean
A	Stratification in Water/Germination in Water	77	65	71
B	Stratification in ABA/Germination in Water	67	67	67
C	Stratification in ABA/Germination in 1mM GA_3	71	70	70.5
D	Stratification in ABA/Germination in 0.1mM Kinetin	71	71	71
E	Stratification in ABA/Germination in Water + 10 mins. R	75	86	80.5
F	Stratification in ABA/Germination in 1mM ABA	58	60	59

†Replicate consists of 100 achenes per petri dish.

F Treatment : Growth of radicles retarded

Analysis of Results : χ^2 (Chi-squared) Test

Source	χ^2	p	Significant level
A vs B	0.572698	$p > 0.05$	N.S.
B vs C	0.418909	$p > 0.05$	N.S.
B vs D	0.57269	$p > 0.05$	N.S.
B vs E	8.72963	$p < 0.01$	**
B vs F	2.41313	$p > 0.05$	N.S.

Table 7.9. The mean percentage germination of achenes stratified at 4°C for 24 weeks in water and exposed to 1mM ABA for different durations, then transferred to water and germinated at 20°C in the dark. Germination counted after 14 days.

Serial No.	Treatments	†Percentage Replicates		Germination Mean
A	Germinated continuously in Water	98	100	99
B	Germinated continuously in ABA	68	56	62
C	24h in ABA → Transferred to Water	100	92	96
D	48h in ABA → Transferred to Water	80	85	82.5
E	24h in ABA → Transferred to Water + 10mins R	88	85	94
F	48h in ABA → Transferred to Water + 10mins R	100	84	92

†Replicate consists of 100 achenes per petri dish

D = Dark Germination

Analysis of Results : χ^2 (Chi-squared) Test

Source	χ^2	p	Significant level
A vs B	84.8702	$p < 0.001$	***
A vs C	2.5641	$p > 0.05$	N.S.
A vs D	30.4966	$p < 0.001$	***
A vs E	5.99556	$0.05 > p > 0.01$	*
A vs F	9.8313	$p < 0.01$	**

CHAPTER 8

GENERAL DISCUSSION

Unchilled Achenes : Physical and Chemical Treatments

Intact unchilled achenes of Polygonum persicaria possess an innate dormancy which appears not to be due to the immaturity of the embryo but could be associated with the pericarp. The germination of intact unchilled achenes is around 20 percent (depending on the harvest) at 35°C in the dark and is improved in continuous light almost doubling to about 40 percent. However viability, tested by tetrazolium treatment, indicates a potential germination of 80 percent or even more. "Surgical" treatments such as removal of an achene tip, puncturing or removal of pericarp, result in substantial germination even at 20°C, at which intact achenes do not germinate. It appears likely that one of the ways (if not the major one) through which the pericarp maintains dormancy is by acting to restrain the growth of the embryo through mechanical means. Nonetheless selective permeability to water or gases is not ruled out.

The results obtained with certain chemicals shown previously to enhance germination in other species do not support Hendricks and Taylorson's (1972) or Roberts' (1973) hypotheses. The stimulation of germination observed by treatment with H_2O_2 argues more for a scarification effect. The view that the pericarp contains inhibitor does not appear likely, although the results obtained cannot allow conclusive argument against it.

Unchilled Achenes : Temperature and Light Effects

The results presented in Chapter 4 demonstrate that approximately 20 percent of the population can germinate at 35°C in the darkness. Another sub-population consisting of approximately a further 20 percent can germinate only when irradiated with R or exposed to white light. This fraction was shown to be under phytochrome control because the R promotion of germination of these

achenes was reversed by FR irradiation. Part of the remainder of the original population, amounting to about 40 percent of the total could equally be divided further into two components, one responding to alternating temperature in darkness, while the other responded to alternating temperature coupled with R or white light at the upper temperature part of the cycle. If a sample were subjected to optimum germination conditions of 15°C or 20°C dark for 8h/35°C light for 16h, the potential germination expected from viability test was realised.

From the foregoing observations, the existence of at least 4 physiological polymorphic types is postulated within the population, namely, sub-populations of

(1) Dark component germinating at 35°C in darkness,

(2) Light requiring component germinating at 35 C in continuous light,

(3) Alternating Temperature dark component germinating at 15°C or 20°C (8h) Darkness/35°C (16h) Darkness, and

(4) Alternating Temperature + (plus) light requiring component of 15°C or 20°C (8h) Darkness/35°C (16h) Light.

An hypothesis regarding the role of phytochrome in the germination of Polygonum persicaria must take account of the sub-populations which have been recognised. The following speculations were taken into consideration in trying to explain the mechanism of germination through phytochrome action.

(1) A legacy of Pfr at maturity (Taylorson, 1982).

(2) Pfr retained in dry seeds (Berrie et al., 1974; Hsiao and Vidaver, 1971).

(3) Germination only possible at 35°C and with sufficient Pfr for a given time of imbibition (Taylorson and Hendricks, 1971).

(4) Pfr destroyed more rapidly at 35°C than 20°C (Taylorson and Hendricks, 1969).

In the case of the dark germination, it is suggested that there is sufficient Pfr present at maturity to allow germination. That phytochrome can persist in seeds of lettuce (Lactuca sativa L. (Grand Rapids) that have undergone hydration-dehydration phenomenon was reported by Berrie et al.

(1974), and Hsiao and Vidaver (1971). This might imply that Pfr was fixed in this sub-population during seed maturation and subsequent dehydration.

The sub-population which germinates at 35°C but requires light can be considered to have sufficient phytochrome to give enough Pfr on irradiation to permit germination or if there is insufficient P_{tot} to allow de novo synthesis of phytochrome. Taylorson and Hendricks (1971) in Amaranthus retroflexus reported that synthesis of Pfr occurs at 35°C and not at 20°C. Alternatively, it could be argued that full realisation of germination potential was not achieved at 35°C in the light because rapid thermal reversion of Pfr to Pr occurs and nullifies the action even of high levels of Pfr produced in the illuminated achenes before their effect could be expressed as has been suggested by Cumming (1963) in Chenopodium botrys. There is a possibility that supraoptimal amounts of Pfr are formed in continuous light leading to a reduction in germination; this was speculated by Cumming (1963). Whether this is applicable to P. persicaria is not known because germination in continuous light resulted only in about 40 percent germination.

The final sub-population which requires alternating temperature + light, probably requires that there be de novo synthesis of phytochrome at 35°C in the light. However, after a prolonged period at 35°C, thermal reversion of Pfr to Pr occurs and exceeds Pfr formation. Consequently transferring achenes to lower temperatures reduces or delays the conversion of Pfr to Pr. With subsequent transfer to 35°C in the light, a steady state of Pfr is again established, thus promoting germination. Indeed, inactivation of Pfr was found to proceed about 4 times more rapidly at 25°C than at 20°C in Amaranthus retroflexus seeds (Taylorson and Hendricks, 1969).

An alternative hypothesis that is centred on the membrane as controlling elements in the germination could be advanced. That the membrane might be involved in P. persicaria is suggested by the need for a high temperature in the germination of unchilled achenes. In explaining the mechanism of action, these assumptions were taken into consideration, namely:-

(1) That promotion of germination/gradual loss of membrane integrity occurs

at high temperature (Hendricks and Taylorson, 1976).

(2) That the reorganization of membrane integrity takes place at the lower temperature part of the cycle (Hendricks and Taylorson, 1978).

(3) That de novo synthesis of Pfr occurs in the light (Taylorson and Hendricks, 1971).

(4) That thermal inactivation of Pfr to Pr is faster at a higher temperature (Taylorson and Hendricks, 1969).

In the dark germinating sub-population, germination occurs before the membranes' integrity is lost.

In the case of the light-requiring component, Pfr interacts with the membrane (or primary site of action χ- Duke et al., 1977) before the loss of membrane integrity or before Pfr was inactivated to Pr at the high temperature of 35°C.

In the third population, the results of germination of optimum alternating temperature in the dark did not exceed 50 percent; however, when R or white light was incorporated at the upper temperature, germination was as high as about 80 percent. That this effectiveness of alternating temperature coupled with light or R might indicate that Pfr interacts with membrane. Hendricks and Taylorson (1978) in Amaranthus retroflexus seeds correlated membrane transition with increase in amino-acid leakage and suggested physiological responses that correlate with Pfr action at the lower temperature. They speculated that lowering the temperature increased organization of membranes and this enhances Pfr action in contrast to 40°C, where membranes are poorly organised. With regard to P. persicaria, it might be suggested that the diurnal lower temperature exposure allows reorganization of membrane integrity which has been altered by a prolonged period at high temperature. This affords an interaction site for Pfr which when produced interacts immediately with its primary site of Action X at 35°C in the light. However this should occur before the loss of membrane integrity or thermal reversion of Pfr to Pr at 35°C. Thus the single process of membrane permeation is both expected to promote and inhibit germination as

temperatures are changed or permeation continues, and these could lead to secondary events such as alteration of membrane pores, or change in activity of membrane-bound enzymes.

The third component of the population (i.e. Alternating Temperature dark sub-population) could not be distinctively or clearly recognised because when achenes were subjected to an alternating temperature + FR (Figure 4.13), the percentage germination was similar to the sub-population II(i.e. Light germinating at constant 35°C). It might be argued that this component of the population is the same as the component that requires continuous light at 35°C, because both had almost similar percentage germination. If this is the situation, then the population might be thought of as comprising 3 clearly defined sub-populations.

The mechanism of germination of the whole population based on phytochrome as largely the controlling factor has one deficiency. That is, a legacy of Pfr at maturity with regard to the component germinating at 35°C in darkness. If germination of this component is due to the already present Pfr at maturity, then irradiating with FR should result in reduced germination. However, this is not the case because this sub-population always germinates even when irradiated with FR and incubated at 35°C in the dark. The mechanism of germination might not be dependent on Pfr.

Other theories in respect of the mechanism of alternating temperatures have been advanced. Cohen (1958) has proposed an explanation based on the physical changes of state with maximum temperature reached in the higher temperature of the two temperature phases. This change, he suggested, might involve the conversion of an inactive enzyme precursor to an active state or result in alterations in membrane permeability which permit interaction between previously separated compounds. This argues for the existence of a particular critical temperature to effect the required changes. This is partly applicable to P. persicaria, because of the need for a critical temperature of 35°C. However it did not take into account the need for light. Toole et al. (1955) suggested that the high temperature part of the cycle

increases respiration, hence creates respiratory substrates or compounds (e.g. substrates supplying energy; hydrogen-transferring intermediates and intermediates for synthesis). These substrates, while unfavourable for germination at the high temperature, might be favourable at the reaction rates involved at the lower temperature. The observation that longer duration (16h) at the upper temperature gave better germination than short duration (8h) might argue against this theory, at least in P. persicaria. Also, the above argument could be advanced against Thompson's (1969) suggestion that it would seem likely that the need for relatively long periods at low temperature depends on the rate of completion of necessary metabolic processes during that time in Lycopus europaeus L. Lang (1965) has suggested that the effect of temperature changes does not consist in the removal of specific blocks to germination, but rather in an increase of the general physiological activity level of the seed which enables the latter to germinate more rapidly and overcome a germination block if such is present.

The physiological polymorphism exhibited by unchilled achenes of P. persicaria is of great survival value, because the differences of individuals to respond to different factors is of ecological advantage in different geographical locations. It ensures a continuing seed bank of achenes because not all would be expected to germinate in response to a particular dormancy breaking stimulus.

Stratification

The results of Chapter 5 indicated that 4°C and 10°C are stratifying temperatures. Dormancy release is faster at 4°C when compared to 10°C pre-treatment, consequently 4°C could be considered a more effective stratification temperature. Higher temperatures of 15°C, 20°C or 25°C did not break dormancy, and so are considered as non-stratification temperatures.

When germinated under an alternating temperature of 15°C(8h)/35°C(16h) in the dark germination was advanced. (About 90 percent germination was

obtained after 2 weeks of stratification at 4°C.) However, 40°C (dark) completely prevented germination, and can be considered inhibitory.

The conditions which break dormancy and permit germination can be related to events in the natural environment, viz. the soil. Hence, it is of ecological importance. The soil temperatures of the winter months might be within the temperature range of 1°C-10°C, which results in the loss of dormancy without subsequent germination, which of course awaits fluctuating temperatures of the spring or summer months. Indeed, after only two weeks of stratification almost complete germination could result if achenes are subjected to alternating temperatures. If the seed has to provide a viable seedling when conditions are suitable for seedling growth, it should remain quiescent. Stratification for 24 weeks can occur without detriment to subsequent germination. This duration far exceeds normal winter periods.

The pericarp plays an important role during the winter months. It enables the non-dormant (dormancy broken by low temperature of the winter months) viable dispersal unit to survive conditions unfavourable to germination. This can be achieved by the pericarp (coat) reducing the physiological contact between embryo and the environment to a minimum. Lack of coat protection results in ready germinability and loss of viability.

It has also been demonstrated that an anaerobic atmosphere does not retard the stratification process, rather stratification in the presence of nitrogen is more effective. The ecological importance is that the nature of the habitat in which achenes are found, that is, marshy, peaty or boggy places, as reported in the General Introduction, might lack adequate aeration. Anaerobiosis is not a state that interferes with loss of dormancy during the winter months.

Achenes of different harvests have been shown to possess different degrees of dormancy by their response to stratification treatment. Thus, the results presented in this study differ slightly in total percentage germination under specific germination conditions, because achenes of different harvests were used, even though collected at the same site. Nevertheless, the pattern of response to specific stimuli is essentially the same. Hence, germination

response of different harvests differs quantitatively and not qualitatively.

The mechanism of low temperature action (effect) is not understood. The results of stratification treatments demonstrate that the requirement for Pfr decreases with an increase in the stratification time. And dark germination occurs at 20°C, a temperature which did not promote germination of unchilled achenes either in the dark or light. It could be then argued that chilling acts to remove the Pfr requirement of unchilled achenes. Also the R promotive effect decreases with an increase in stratification period. It is clear that stratification abolishes any light response. It might be speculated that if the mechanism of action ascribed to Pfr (interaction with membrane) is indeed the case, then one can infer that stratification treatment might be affecting the membrane. Bewley and Black (1982) have suggested that stratification might act on the enzyme system or affect the structural organization of the membrane; however, these suggestions are yet to be proven experimentally. In some species, chilling has been reported to cause changes in the content of certain metabolites and of some biochemical processes, as has been noted on page 16, although it is difficult to relate these changes specifically to the primary release from dormancy.

Stratification acts to decrease the heterogeneity of the individuals in the population and makes them reponsive to a single treatment.

Secondary Dormancy and After-ripening

It has been shown in Chapter 6 that a supraoptimal temperature of 40°C does not impose secondary dormancy, rather causes cellular stress leading to deterioration of the achenes, the effect being on the embryo. The harmful effect can be ameliorated more by kinetin treatment in contrast to GA_3 or light. However, drying is considered to impose true secondary dormancy, and this can be broken by a second period of chilling or alternating temperature and not by kinetin or GA_3. Drying might be less likely to affect the membranes and embryo in contrast to 40°C. It can be argued that kinetin or GA_3 are not

involved in the process(es) relieving secondary dormancy. The mechanism by which secondary dormancy is imposed by drying is not known.

Dry storage at a temperature of 35°C could also relieve primary dormancy, by a mechanism not understood.

The promotive effects of high temperature depend on the nature of the achenes (i.e. either dormant or non-dormant) and their state, that is whether imbibed or dry.

Though soil temperatures in the summer months might reach high values, there is little likelihood that the achenes would be killed or secondary dormancy imposed. Ecologically, this would provide for summer germination, but if the achenes dry out, then secondary dormancy is imposed, and in this ecological circumstance non-dormant achenes (i.e. those chilled by winter months) would be returned to the seed bank, and germinate when favourable conditions prevail or the achenes are subjected to a second chilling treatment.

Hormone treatments

The results obtained in Chapter 7 with whole (intact) or punctured unchilled achenes of P. persicaria when GA_3 or GA_{4+7} was applied do not indicate any regulatory role for these hormones in dormancy breaking of unchilled achenes. The argument that R initiates the synthesis of gibberellin is not applicable to unchilled achenes of P. persicaria because R + GA_3 treatment gave higher germination than GA_3 but not R treatment. However, kinetin treatment promoted significant germination over non-treated achenes. But again, de novo synthesis of cytokinin by R is not supported by the results obtained when both are given together, rather additive promotion of germination appears to be the case. Germination was greatest when R, GA_3 and kinetin were given together. The above observations argue against substitution or replacement of one treatment by another treatment, and consequently do not favour common regulatory mechanism, at least in P. periscaria. Bewley, Negbi and Black (1968) demonstrated that the effect of Pfr was evident within

5 minutes, which almost precludes the possibility of the mode of action of light being the initiation of the synthesis of GA, since the de novo production of GA would require a longer time for expression. Also, they found that GA and light acted synergistically. The more recent work by Berrie and Taylor (1981) where light-induced and GA-induced populations were analysed numerically led them to the same conclusion. That the absolute amount of GA_1 was the factor preventing germination was disputed by Metzger (1983). He observed that both unimbibed dormant and non-dormant seed of wild oats (Avena fatua L.) seeds contained similar amounts of GA_1, although the level declined faster in dormant than non-dormant seeds during imbibition. He suggested that greater GA biosynthesis might be held responsible for the slower rate of decline in GA_1 levels in imbibing non-dormant seeds, and speculated that germination might be regulated by the rate of GA turnover. He concluded that increased GA biosynthesis in imbibing non-dormant seeds might be a secondary rather than a primary event associated with germination.

The treatments in which achenes were stratified in GA_3 and irradiated with R light, enhanced germination to a greater extent than when either was given alone. This does not favour the hypothesis that R might act to promote GA synthesis. Indeed, the stimulatory effect of applied GA_3 is not universal. In Dioscorea tokoro, it inhibited germination in the dark or when given in combination with R light (Okagami and Kawai, 1977). Also GA_3, while promoting the germination of some seeds that require chilling, failed to overcome dwarfism of unchilled seedlings (Villiers and Wareing, 1960).

The observations of applying growth retardants either during stratification or after might suggest that stratification did not act to promote de novo synthesis of GA.

The results of applied ABA, and Karssen's (1982) observations on the involvement of endogenous ABA during stratification, did argue against ABA being responsible for the maintenance of primary dormancy, although radicle elongation could be inhibited after the germination process has started in

P. persicaria.

The hypothesis that promotive conditions allowed the production of gibberellin, so initiating germination, cannot be sustained by these experiments. Whilst it is generally accepted that the growth retardants act by preventing the synthesis of gibberellin, there are instances where GA synthesis is not clearly inhibited. Regardless of the role these growth retardants play in gibberellin metabolism, it is accepted that they may influence the gibberellin "balance sheet".

The relative lack of response of Polygonum persicaria to exogenous gibberellin and the equivocal results obtained with the growth retardants, would strengthen the conception that gibberellin is not involved in the germination of these achenes. Substantiating evidence could only be obtained by undertaking an analysis for endogenous gibberellin.

CONCLUSION

It could be suggested that where isolated embryos, or seeds without the pericarp, germinate, dormancy in this case might be determined by the seed coat. This, however, is not always correct, because the dormant seed could germinate if subjected to the action of light, alternating temperature, stratification or exogenous hormones, as indeed is the case with P. persicaria. These dormancy breaking treatments, in some cases e.g. light, have been suggested to affect the embryo or membranes.

Thus, while we must consider that the pericarp offers some resistance to germination, it is the inability of the embryo to overcome this that characterises the phenomenon of dormancy. When the embryo is in a state of sufficient activity, established by specific environmental conditions, this dormancy is broken, the resistance overcome and germination expressed.

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APPENDIX

CHAPTER 3

The mean percentage germination of unchilled achenes of P. persicaria treated with chemicals.

Table 3.4. Calcium hypochlorite ($Ca(ClO)_2$)

Concentrations	Treatments A: Imbibed in water at 20 C for 24h and then germinated in chemical				Treatments B: Imbibed in chemical at 20 C for 24h and then germinated in water				Treatments C: Germinated dry in chemical			
0mM(H_2O)	31	38	28	32.33	31	38	28	32.33	40	46	33	39.66
1mM	27	38	37	34	29	31	37	32.33	43	54	52	49.66
10mM	38	40	44	40.66	40	40	44	41.33	35	34	38	35.66

Table 3.5 Hydrogen Peroxide (H_2O_2)

Concentrations	A			B			C		
0 volume	33	38	35.5	33	38	35.5	35	32	33.5
5 volumes	58	57	57.5	28	29	28.5	65	69	67
10 volumes	65	68	66.5	49	48	48.5	76	65	70.5
15 volumes	42	27	34.5	42	50	46	22	19	20.5
20 volumes	0	0	0	69	69	69	0	0	0
50 volumes	0	0	0	83	82	82.5	0	0	0

Table 3.6 Potassium nitrate (KNO_3)

Concentrations	A			B			C		
0mM(H_2O)	31	38	28	31	38	28	40	46	33
		32.33			32.33			39.66	
1mM	39	50	43	29	29	35	43	53	53
		44			31			49.66	
10mM	40	48	35	37	22	37	52	40	51
		41			32			47.66	
100mM	0	0	0	49	35	48	0	0	0
		0			44			0	

Table 3.7 Potassium nitrite (KNO_2)

Concentrations	A			B			C		
0mM(H_2O)	31	38	28	31	38	28	40	46	33
		32.33			32.33			39.66	
1mM	57	57	56	43	40	33	64	59	59
		56.66			38.66			60.66	
10mM	62	69	64	53	50	55	72	70	69
		65			52.66			70.33	
100mM	0	0	0	68	64	79	0	0	0
		0			70.33			0	

Table 3.8 Hydroxylamine hydrochloride ($NH_2OH.HCl$)

Concentrations	A		B		C	
0mM(H_2O)	33	38	33	38	35	32
	35.5		35.5		33.5	
1mM	49	49	27	41	40	33
	49		34		36.5	
10mM	0	0	52	48	31	29
	0		50		30	
100mM	0	0	48	50	32	17
	0		49		24.5	

Table 3.9 Thiourea ($CS(NH_2)_2$)

Concentrations	A	B	C
0mM(H_2O)	33 38	33 38	35 32
	35.5	35.5	33.5
1mM	39 36	26 33	25 34
	37.5	29.5	29.5
10mM	39 42	35 34	25 18
	40.2	34.5	21.8
100mM	45 42	45 38	33 52
	43.5	41.5	42.5

Table 3.10 Sodium Cyanide (NaCN)

Concentrations	A	B	C
0mM(H_2O)	33 38	33 38	35 32
	35.5	35.5	33.5
0.1mM	36 27	14 16	36 31
	31.5	15	33.5
0.5mM	30 26	10 15	35 31
	28	12.5	33
1mM	32 38	14 23	34 47
	35	18.5	40.5
3mM	17 34	20 18	31 31
	25.5	19	31
5mM	29 28	23 25	43 36
	28.5	24	39.5
10mM	21 37	15 16	45 39
	29	15.5	42

Table 3.11 Sodium azide (NaN_3)

Concentrations	A		B		C	
0mM(H_2O)	33	38	33	38	35	32
	35.5		35.5		33.5	
0.1mM	41	20	16	27	33	40
	30.5		21.5		36.5	
0.5mM	3	7	27	21	43	47
	5		24		45	
1mM	0	0	32	31	0	0
	0		31.5		0	
3mM	0	0	41	41	0	0
	0		41		0	
5mM	0	0	50	43	0	0
	0		46.5		0	
10mM	0	0	41	29	0	0
	0		35		0	

Figure 3.1 The Percentage Water Uptake at 20°C in the dark.

Exposure Time in h	Treatments: Intact Achenes	Achenes with tips cut off
0 (Moisture content)	11.538	10.69
1	25.892	22.269
4	32.51	30.667
8	34.994	36.809
16	36.09	40.978
24	39.202	40.598
36	38.682	43.462
48	37.105	42.918

Figure 3.2 Effect of different oxygen compositions on the germination of achenes.

Germination at 30°C dark

Atmospheric Pressure 74cm of Hg

Percentage of Gaseous Composition	Gaseous Composition in cm			Percentage Germination Replicates				
	Hg	N_2	O_2	1	2	3	4	Mean
100 percent O_2	0	-	74	8	8	8	18	10.5
75 " "	0	18.5	55.5	10	16	10	14	12.5
50 " "	0	37	37	8	2	8	4	5.5
25 " "	0	55	18.5	8	20	12	8	12
Air (Control)	-	-	-	0	0	4	4	2

Germination at 35°C dark

100 percent O_2	0	--	74.5	52	52	30	34	42
75 " "	0	18.6	55.9	44	44	40	40	42
50 " "	0	37.3	37.3	34	30	28	34	31.5
25 " "	0	55.9	18.6	32	38	44	30	36
Air (Control)	-	-	-	32	28	36	30	31.5

Effect of different carbon dioxide compositions on the germination of achenes.

Germination at 30°C in the dark

Atmospheric Pressure 73.5 cm of Hg

Percentage of Gaseous Composition	Gaseous Composition in cm			Percentage Germination Replicates				
	Hg	N_2	CO_2	1	2	3	4	Mean
100 percent N_2	0	73.5	0	0	0	0	0	0
4 " CO_2	0	70.5	2.9	0	0	0	0	0
2 " CO_2	0	72	1.5	0	0	0	0	0
1 " CO_2	0	72.8	0.7	0	0	0	0	0
Air (Control)	-	-	-	2	2	0	0	1

CHAPTER 4

Figure 4.1 The mean percentage germination of unchilled achenes subjected to different alternating regimes in a 24h diurnal cycle. Counted after 14 cycles.

Serial Number	Alternating Temperatures in a 24h diurnal cycle	Percentage Germination[a.] 8h at the Upper Temperature: Light at the Upper Temp. Replicates			Dark at the Upper Temp. Replicates			[b.] 16h at the Upper Temperature: Light at the Upper Temp. Replicates			Dark at the Upper Temp. Replicates		
(1)	5°C/15°C	1	0	0.5	0	1	0.5	0	0	0	0	0	0
(2)	5°C/20°C	0	0	0	0	1	0.5	5	4	4.5	4	4	4
(3)	5°C/25°C	4	3	3.5	0	4	2	10	8	9	2	8	5
(4)	5°C/35°C	30	40	35	11	7	9	66	64	65	17	13	15
(5)	10°C/15°C	0	0	0	0	0	0	0	0	0	0	0	0
(6)	10°C/20°C	0	0	0	0	0	0	7	8	7.5	5	0	2.5
(7)	10°C/25°C	0	6	3	0	3	1.5	8	11	9.5	6	1	3.5
(8)	10°C/35°C	48	48	48	21	36	28.5	43	57	50	19	24	21.5
(9)	15°C/20°C	5	3	4	1	1	1	5	3	4	1	3	2
(10)	15°C/25°C	12	12	12	2	6	4	10	8	9	3	2	3.5
(11)	15°C/35°C	54	67	61	28	29	28.5	70	67	68.5	21	26	23.5
(12)	20°C/25°C	1	2	1.5	0	1	0.5	0	2	1	0	0	0
(13)	20°C/35°C	54	61	57.5	18	21	19.5	41	35	38	14	12	13

Figure 4.2 The mean percentage germination of unchilled achenes subjected to different alternating temperature regimes in a 24h diurnal cycle. Counted after 18 cycles.

Serial Number	Alternating Temperatures in a 24h diurnal cycle	Percentage Germination Treatment 4.2a. 8h at the Upper Temperature Light at the Upper Temp. Replicates			Dark at the Upper Temp. Replicates			Treatment 4.2b. 16h at the Upper Temperature Light at the Upper Temp. Replicates			Dark at the Upper Temp. Replicates		
(1)	5°C/35°C	17	24	20.5	11	13	12	85	82	83.5	35	33	34
(2)	10°C/35°C	27	48	37.5	25	30	27.5	84	77	80.5	54	46	50
(3)	15°C/35°C	66	53	59.5	43	32	37.5	91	91	91	54	55	54.5
(4)	20°C/35°C	52	65	58.5	22	29	25.5	79	79	79	29	36	32.5
(5)	25°C/35°C	53	62	57.5	20	21	20.5	67	85	76	39	38	33.5

The mean percentage germination of unchilled achenes kept at constant temperature of 35°C. Counted after 18 cycles.

35°C Continuous Dark			8h Light/16h Dark at 35°C			16h Light/8h Dark at 35°C			35°C Continuous Light		
29	10	19.5	36	37	36.5	42	31	36.5	39	41	40

Figure 4.3 Effect of different period of illumination at 35°C (Upper temperature part of the cycle) in a 24h diurnal alternating temperature. Counted after 14 cycles.

Percentage Germination

Light Period at 35°C	Germination Conditions: 15°C / 35°C — Light given at 35°C Replicates			Mean	Continuous darkness Replicates			Mean	Germination Conditions: 35°C constant Replicates			Mean
0h	-	-	-	-	-	-	-	-	25	17	17	19.66
4h	20	19	16	18.33	10	4	8	7.33	33	41	30	34.66
8h	48	49	44	47	15	12	19	15.33	32	33	39	34.66
16h	69	65	73	69	27	29	27	28	40	44	39	41
20h	64	68	71	68	28	36	27	30.33	44	39	41	41.33
24h	-	-	-	-	-	-	-	-	36	41	44	40.33

Figure 4.4 Effect of reduced light intensity (40%) on the germination of unchilled achenes at different temperatures. Counted after 10 cycles

Percentage Germination

Germination Temperature/Conditions	40% illumination Replicates			Mean	100% illumination Replicates			Mean
35°C Constant dark	14	26	16	18.66	25	17	17	18.33
16h Light/8h Dark at continuous 35°C	47	42	53	47.33	40	44	39	41
35°C Continuous Light	51	52	50	51	36	41	44	40.33
15°C(8h) dark/35°C(16h) dark	38	41	42	40.33	27	29	27	28.00
15°C(8h) dark/35°C(16h) Light	78	76	70	74.66	69	65	73	69

Figure 4.5 Effect of light response to imbibition on the germination of unchilled achenes at different Temperatures/Conditions. Counted after 10 cycles.

Germination Temperature/Condition	Imbibition Time at 15 C (dark) in Days					
	0	0.5	1	2	3	10
35°C (dark) Constant	13 19 16.0	16 18 17	19 19 19	18 17 17.5	13 15 14	7 2 4.2
35°C (light) Constant	33 50 41.5	41 43 42	37 46 41.5	47 48 47.5	30 38 34	22 14 18
16h(light)/8h(dark) at 35°C	35 41 38	46 46 46	52 50 51	51 50 50.5	28 32 30	19 21 20
15°C(8h)Dark/35°C(16h) Dark	12 21 16.5	38 38 38	46 42 44	48 38 43	20 29 24.5	10 12 11
15°C(8h)Dark/35°C(16h) Light	57 45 51	70 68 69	71 66 68.5	63 68 65.5	50 52 51	30 32 31

Figure 4.6 Response of unchilled achenes to an alternating temperature of 35°C/20°C with light illuminated at 20°C for 8h in a 24h diurnal cycle. Counted after 14 cycles.

Imbibition Time at 35°C (dark) in hours	Percentage Germination					
	Dark Treatments			Light Treatments		
	Replicates		Mean	Replicates		Mean
2	19	25 21	21.66	79	75 78	77.33
4	21	20 30	23.66	84	86 80	83.33
6	34	27 22	27.66	78	72 74	74.66
12	21	15 28	21.33	78	79 79	78.66
16	18	22 21	20.33	86	81 77	81.33
24	26	21 20	22.33	79	72 78	76.33
36	23	16 15	18	77	71 67	71.66

Figure 4.7 Effect of 24h diurnal cyclic temperature rhythm on the germination of unchilled achenes at different temperatures. Counted after 14 cycles.

15°C (8h) / 35°C (16h)

Imbibition Time at 15°C dark in hours	Pattern of Exposure from start of imbibition	Percentage Germination Dark at 35°C Replicates			Mean	Light at 35°C Replicates			Mean
0	LT - HT - LT	39	38	35	37.33	73	70	70	71
0	HT - LT - HT	42	40	46	42.66	66	65	58	63
24	LT - HT - LT	23	45	40	36	73	62	66	67
24	HT - LT - HT	52	43	36	44	53	68	60	60.33

35°C Constant Temperature

Imbibition Time at 15°C dark in hours	Pattern of Exposure from start of imbibition	Percentage Germination Dark treatment Replicates			Mean	Light treatment Replicates			Mean
0	HT - LT - HT	12	14	10	12	45	45	40	40.33
24	LT - HT - LT	18	16	21	18.33	45	46	45	45.33
24	HT - LT - HT	12	14	10	12	34	58	50	47.33

16h (Light) / 8h (Dark) at Constant 35°C

Imbibition Time at 15°C dark in hours	Pattern of Exposure from start of imbibition	Percentage Germination 16h Light / 8h Dark at 35°C Replicates			Mean
0	HT - LT - HT	46	50	48	48
24	LT - HT - LT	47	49	47	47.66
24	HT - LT - HT	40	41	44	41.66

L = Light Treatment

D = Dark Treatment

LT - HT - LT = Cycling started from low temperature to high and then to low in a 24h diurnal cycle.

HT - LT - HT = Cycling started from high temperature to low and then to high in a 24h diurnal cycle.

Figure 4.8 Germination Response of achenes imbibed at 20°C (dark) and irradiated with 30 minutes Red (light) R and subsequently germinated at 35°C and 20°C dark.

Imbibition Time at 20°C dark	30 minutes Red (R) light irradiation	Percentage Germination 20°C dark Germination Replicates		Mean	35°C dark Germination Replicates		Mean
Control	-	0	0	0	17	17	17
4h	R	0	0	0	32	19	25.5
8h	"	0	0	0	40	33	36.5
16h	"	0	0	0	40	35	37.5
24h	"	1	0	0.5	36	31	33.5
36h	"	1	0	0.5	34	33	33.5
40h	"	0	2	1	23	34	28.5
48h	"	0	0	0	22	25	23.5

Figure 4.9 The percentage germination response of unchilled achenes irradiated with different duration of R light and germinated at 35°C in the dark.

Duration of Exposure to R in minutes	Percentage Germination Non-Irradiated Replicates		Mean	30 minutes R irradiation Replicates		Mean
0	17	22	19.5	17	22	19.5
3	18	19	18.5	28	23	25.5
5	20	20	20	30	31	30.5
10	17	21	19.0	40	47	43.5
20	20	23	21.5	38	40	39
30	18	21	19.5	40	41	40.5
60	20	15	17.5	37	41	39

Figure 4.10a&b. Germination of unchilled achenes to 10 minutes irradiation of R, FR, R+FR, and R+FR+R at 20°C and 35°C in the dark.

	Percentage Germination					
	10 minutes irradiation			30 minutes irradiation		
Type of Irradiation	Replicates		Mean	Replicates		Mean
Control	18	21	19.5	15	23	19
R	40	50	45	39	43	41
FR	22	17	19.5	13	14	13.5
R+FR	22	18	20	14	20	17
R+FR+R	35	46	40.5	31	30	30.5

Figure 4.11 Effect of different frequencies of 30 minutes Red light treatment on the germination of unchilled achenes at 35°C (dark) after 16h imbibition at 20°C (dark).

	Percentage Germination					
	Non-irradiated			Irradiated		
30 minutes R irradiation = 1R	Replicates		Mean	Replicates		Mean
1 x 30 minutes R	12	19	15.5	41	27	34
2 x 30 minutes R	15	15	15	37	36	36.5
3 x 30 minutes R	17	19	18	37	39	38
4 x 30 minutes R	25	18	21.5	47	33	40

Figure 4.12 Interaction of alternating temperature with 10 minutes R irradiation, and germinated at an alternating temperature of 20°C (8h) R / 35°C (16h) : 1 cycle.

Serial Number	Treatment Temperature °C/ Conditions	10 minutes Red (R) light irradiation	Percentage Germination Replicates		Mean
1	20°C Continuous	-	0	0	0
2	20°C "	1 x R	0	0	0
3	35°C "	-	22	22	22
4	35°C "	1 x R	32	31	31.5
5	1 Cycle (20°C(8h)R/ 35°C(16h)	-	1	2	1.5
6	1 Cycle "	1 x R	6	9	7.5
7	3 Cycles "	-	9	7	8
8	3 " "	3 x R	41	33	37
9	6 " "	-	4	4	4
10	6 " "	6 x R	57	48	52.5
11	9 " "	-	15	7	11
12	9 " "	9 x R	51	53	52

Figure 4.13 Interaction of alternating temperature with 10 minutes R or FR irradiation given at different points during transfer to lower or upper temperature in a 24h diurnal cycle - 20°C (8h)/35°C (16h).

Germination Temperatures/ Conditions	Point and frequency of R or FR irradiation	Percentage Germination Replicates			Mean
35°C dark	-	18	23	18	19.66
" "	10 minutes R	27	22	30	26.33
" "	" " FR	7	13	14	11.33
20°C(8h)/35°C(16h)	-	18	18	28	21.33
"	4 x 10 minutes R; R before every 35°C	76	62	66	68
"	4 x 10 minutes R; R after every 35°C	49	61	65	58
"	4 x 10 minutes R; R before & after every 35°C	62	64	58	61.33
"	4 x 10 minutes FR; FR before every 35°C	13	13	18	14.66
"	4 x 10 minutes FR; FR after every 35°C	17	21	19	19
"	4 x 10 minutes FR; FR before & after every 35°C	18	23	13	18

CHAPTER 5

Figure 5.1 The mean percentage germination of achenes exposed to Different Temperature Pretreatment for different periods in weeks and germinated at different temperatures.

Pretreatment at 4°C Dark

Germination Temperatures

Pretreatment Time at 4°C	4°C	10°C	15°C	20°C	25°C	35°C	40°C	15°C(8h) Dark/35°C (16h)Dark	Viability Test
2 weeks	0 0 0	5 3 4	40 40 40	31 30 30.5	33 28 30.5	44 46 45	0 0 0	88 92 90	100
4 weeks	0 0 0	6 6 6	56 55 55.5	64 60 62	70 66 68	66 68 67	0 0 0	97 100 98.5	100
8 weeks	0 0 0	26 21 23.5	71 74 72.5	77 65 72	87 82 84.5	78 83 80.5	0 0 0	100 95 97.5	100
12 weeks	2 2 2	72 70 71	81 82 81.5	80 86 83	90 82 86	80 82 81	0 0 0	93 96 94.5	95
16 weeks	3 8 3.5	-	-	87 85 86	-	-	-	-	-
24 weeks	18 18 18	-	-	94 94 94	-	-	-	98 96 97	100

Pretreatment at 10°C Dark

Germination Temperatures

Pretreatment Time at 10°C dark	4°C	10°C	15°C	20°C	25°C	35°C	40°C	15°C(8h) Dark/35°C (16h)Dark	Viability Test
2 weeks	0 0 0	0 0 0	8 2 5	3 7 5	6 12 9	36 46 41	0 0 0	64 63 63.5	100
4 weeks	0 0 0	0 0 0	2 2 2	11 9 10	5 8 6.5	46 51 46.5	0 0 0	81 83 82	75
8 weeks	0 0 0	0 0 0	2 1 1.5	3 4 3.5	0 2 1	24 31 27.5	0 0 0	80 85 82.5	100
12 weeks	0 0 0	15 14 14.5	47 52 49.5	54 55 54.5	51 55 53	68 60 64	0 0 0	82 87 84.5	100
16 weeks	0 0 0	17 16 16.5	30 53 41.5	65 58 61.5	58 63 60.3	67 74 70.5	0 0 0	86 82 84	100
24 weeks	-	26 24 25	-	98/100/98 98.6	-	-	0 0 0	96/92/92 93.33	95

Figure 5.1 contd.

Pretreatment at 15°C Dark

Germination Temperatures

Pretreatment Time at 15°C dark	4°C	10°C	15°C	20°C	25°C	35°C	40°C	15°C(8h) Dark/35°C (16h)Dark	Viability Test
2 weeks	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	9 12 10.5	0 0 0	37 31 34	100
4 weeks	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	12 16 14	0 0 0	41 36 38.5	90
8 weeks	0 0 0	0 0 0	0 0 0	0 0 0	2 4 4	1 2 1.5	0 0 0	26 11 18.5	85
12 weeks	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	4 3 3.5	0 0 0	14 9 11.5	50
24 weeks	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	16 7 11.5	0 0 0	10 11 10.5	75

Pretreatment at 20°C Dark

Germination Temperatures

Pretreatment Time at 20°C dark	4°C	10°C	15°C	20°C	25°C	35°C	40°C	15°C(8h) Dark/35°C (16h)Dark	Viability Test
2 weeks	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	4 5 4.5	0 0 0	10 11 10.5	100
4 weeks	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	20 12 16	0 0 0	28 35 31.5	75
8 weeks	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	5 2 3.5	0 0 0	11 6 8.5	80
12 weeks	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	1 0 0.5	60
24 weeks	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	4 2 3	0 0 0	0 2 1	70

Figure 5.1 contd

Pretreatment at 25°C Dark

Pretreatment Time at 25°C dark	Germination Temperatures 4°C	10°C	15°C	20°C	25°C	35°C	40°C	15°C(8h) Dark/35°C (16h)Dark	Viability Test
2 weeks	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	8 7 7.5	0 0 0	29 36 32.5	100
4 weeks	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	13 10 11.5	0 0 0	32 34 33	75
8 weeks	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	3 3 3	0 0 0	31 31 31	100
12 weeks	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	80
24 weeks	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	1 2 1.5	0 0 0	1 0 0.5	63

Figure 5.2 Effect of different amounts of R or FR irradiation after stratification on the germination of achenes at 20 C in the dark.

Pretreatment Time at 4°C dark	Type of Irradiation 5 min R	10 min R	30 min R	60 min R	5 min FR	10 min FR	30 min FR	60 min FR	Control
0.5 weeks	8 5 6.5	7 8 7.5	13 14 13.5	11 5 8	4 3 3.5	5 5 5	4 3 3.5	4 3 3.5	4 4 4
1 week	6 8 7	10 14 12	13 17 15	14 11 12.5	4 3 3.5	4 5 4.5	5 4 8.5	3 5 4	4 5 4.5
1½ weeks	17 22 19.5	15 17 16	17 13 .15	20 29 24.5	8 10 9	8 10 9	9 11 10	8 7 7.5	10 6 8
2 weeks	17 19 18	16 18 17	10 15 12.5	15 13 14	5 6 5.5	7 8 7.5	14 22 18	13 15 14	6 10 8
3 weeks	18 21 19.5	18 27 22.5	28 29 28.5	29 24 26.5	16 16 16	22 15 18.5	16 10 13	20 26 23	17 16 16.5
6 weeks	44 57 50.5	53 50 51.5	54 58 56	55 60 57.5	49 50 49.5	42 50 46	39 41 40	52 53 52.3	44 42 43
12 weeks	-	-	68 60 64	-	-	-	68 60 64	-	63 50 56.5
15 weeks	-	-	74 76 75	-	-	-	76 79 77.5	-	63 70 66.5
20 weeks	-	-	79 80 79.5	-	-	-	82 81 81.5	-	64 74 69

Figure 5.3 Germination response of 8 weeks pretreated (chilled) achenes at 4°C to 30 minutes R, FR, or R+FR and germinated at 20°C or 35°C in the dark.

Germination Temperatures	Treatments											
	Non-irradiated			30 minutes R			30 minutes FR			30 minutes R+FR		
20°C (dark)	43	41 42%	42	55	50 53%	54	39	35 40%	47	44	47 46%	48
35°C (dark)	35	36 34%	30	49	51 47%	40	33	33 33%	33	40	35 38%	38

Figure 5.4 Germination response of 8 weeks pretreated (chilled) achenes at 4°C to 30 minutes R or FR irradiation at different temperatures.

Germination Temperature/ Conditions	Percentage Germination			
	Irradiation Treatment	Replicates		Mean
Constant 20°C D	None	44	50 58	50.66
" "	R only	66	68 62	65.33
" "	FR only	45	49 50	48
" 35°C D	None	34	44 54	44
" 35°C D	R only	40	60 60	53.33
" 35°C D	FR only	26	34 40	33.33
5 Cycles of 20°C (16h)D/35°C(8h)D	None	66	74 74	71.33
" "	5 x R only before transfer to 35°C	92	84 94	90
" "	5 x R only after transfer to 35°C	94	86 94	91.33
" "	5 x FR only before transfer to 35°C	62	68 64	66
" "	5 x FR only after transfer to 35°C	70	68 64	67

Figure 5.5 Effect of Stratification at 4°C for 8 weeks in gas mixtures of carbon dioxide and nitrogen. Counted after 14 days.

Stratification atmospheres at 4°C	Percentage Germination at 20°C dark Replicates			Mean
Air (Control)	60	68	60	62.66
100 percent N_2	84	81	92	85.66
1 percent CO_2/99 percent N_2	80	76	66	74
4 percent CO_2/96 percent N_2	58	60	60	59.33

Figure 5.6 Germination response of different harvest (1981/1982) to 4°C and germinated at different temperatures.

1981 HARVEST

Stratification Period at 4°C (wks)	Germination Temperatures 15°C			Mean	20°C			Mean	25°C			Mean	35°C			Mean
2 weeks	0	0	0	0	1	0	1	1.33	1	2	2	3.33	6	7	9	14.6
4 weeks	0	0	0	0	3	9	8	13.33	5	4	7	10.6	8	14	11	22
8 weeks	3	2	8	8.66	23	22	21	44	20	16	19	43	11	17	7	23
12 weeks	17	24	23	42.6	34	31	36	67	38	26	34	65	33	25	36	62.66

1982 HARVEST

Stratification Period at 4°C (wks)	15°C		Mean	20°C		Mean	25°C		Mean	35°C		Mean
2 weeks	40	40	40	31	30	30.5	33	28	30.5	44	46	45
4 weeks	56	55	55.5	64	60	62	70	66	68	66	68	67
8 weeks	71	74	72.5	77	65	72	87	82	84.5	78	83	80.5
12 weeks	81	82	81.5	80	86	83	90	82	86	80	82	81

CHAPTER 6

Table 6.1 The mean percentage germination of 9 weeks chilled achenes at 4°C, then exposed to 40°C for different times in hours and germinated at 20°C in the dark.

a.

Time at 40°C in hours	Percentage Germination Treatment Applied During 40°C Incubation											
	0 (H_2O)			1mM GA_3			0.1mM Kinetin			30 minutes R		
	Replicates		Mean	Replicates		Mean	Replicates		Mean	Replicates		Mean
0	57	63	60	57	68	62.5	50	60	55.5	70	85	77.5
6	70	60	65	60	64	62	74	61	67.5	70	72	71
12	57	49	53	67	58	62.5	67	61	64	72	65	68.5
24	40	40	40	54	66	60	72	70	71	60	62	61
36	45	52	48.5	57	63	60	56	56	56	55	53	54
48	40	46	43	63	56	59.5	67	52	59.5	50	45	47.5
72	25	25	25	43	38	40.5	38	43	40.5	36	34	35
40°C Constant	0	0	0	0	0	0	0	0	0	0	0	0

b.

	Percentage Germination Treatment Applied After 40°C Incubation											
0	57	63	60	50	60	55.5	57	68	62.5	70	85	77.5
6	70	60	65	67	72	69.5	67	68	67.5	70	79	74.5
12	57	49	53	77	64	70.5	62	68	65	85	70	77.5
24	40	40	40	58	57	57.5	63	80	71.5	61	61	61
36	45	52	48.5	54	50	52	47	50	48.5	60	60	60
48	40	46	43	50	47	48.5	33	39	36	54	56	55
72	25	25	25	19	36	27.5	32	28	30	22	28	25
40°C Constant	0	0	0	0	0	0	0	0	0	0	0	0

Table 6.2 The mean percentage germination of 12 weeks chilled achenes at 4°C, then exposed to 40°C (dark) for different times in hours in GA_3, kinetin or irradiated with Red light and germinated in distilled water at 20°C in the dark.

Exposure Time at 40°C in hours	Percentage Germination Treatment Applied During 40°C Incubation											
	0 (H_2O) Replicates		Mean	1mM GA_3 Replicates		Mean	0.1mM Kinetin Replicates		Mean	30 minutes R Replicates		Mean
0	71	80	75.5	81	78	79.5	70	78	74	80	87	83.5
48	47	50	48.5	85	81	83	61	64	62.5	47	47	47
60	40	40	40	70	75	72.5	60	55	57.5	50	45	47.5
72	37	38	37.5	44	46	45	42	43	42.5	34	42	38

Table 6.3 The mean percentage germination of 12 weeks chilled achenes at 4°C, then exposed to 40°C for different times in hours in GA_3, kinetin or GA_3+ kinetin and germinated in distilled water at 20°C (dark).

a.

Exposure Time at 40°C in hours	Percentage Germination Treatment Applied During 40°C Incubation											
	0 (H_2O) Replicates		Mean	1mM GA_3 Replicates		Mean	0.1mM Kinetin Replicates		Mean	1mM GA_3+0.1mM Kinetin Replicates		Mean
0	80	86	83	89	91	90	79	81	80	96	94	95
16	80	82	81	84	79	81.5	85	74	79.5	83	86	84.5
30	81	80	80.5	86	85	85.5	84	80	82	83	81	82
54	71	64	67.5	74	70	72	78	76	77	71	73	72
72	57	52	54.5	66	64	65	80	78	79	60	69	64.5

Table 6.3 The mean percentage germination of 12 weeks chilled achenes at 4°C, then exposed to 40°C (dark) for different time intervals in hours and given a second period of stratification at 4°C and subsequently subjected to 10 cycles of alternating temperatures of 15°C (8h)/35 C (16h) in a 24h diurnal cycle.

b.

Exposure Time at 40°C in hours	Percentage Germination Conditions								
	15°C(8h)D/35°C(16h)D			15°C(8h)D/35°C(16h)L			4 wks at 4°C→15°C(8h)D/35°C(16h)D		
	Replicates		Mean	Replicates		Mean	Replicates		Mean
0	93	97	95	96	100	98	94	100	97
16	88	84	86	90	89	89.5	94	96	95
30	85	85	85	85	88	86.5	89	88	88.5
54	77	70	73.5	85	86	85.5	84	86	85
72	52	53	52.5	54	59	56.5	31	38	34.5

Table 6.4 The mean percentage germination of 12 weeks chilled achenes at 4°C and then kept dry at room temperature (20°C in the dark) and subsequently germinated at different temperatures.

Exposure Period Kept dry at 20°C in dark	Percentage Germination Conditions											
	20°C Dark			15°C(8h)D/35°C(16h)D			15°C(8h)D/35°C(16h)L			4°C for 4 wks → 20°C Dark		
	Replicates		Mean	Replicates		Mean	Replicates		Mean	Replicates		Mean
0 day (0 h)	80	86	83	93	96	94.5	98	97	97.5	87	85	86
1 day (24 h)	62	54	58	90	98	94	90	98	94	90	74	82
3 days (72 h)	66	58	62	92	98	95	92	98	95	90	86	82
10 days (240 h)	42	43	42.5	87	82	84.5	87	82	84.5	72	88	80

Table 6.5 The percentage germination of achenes stratified at 10°C (dark) for 24 weeks and kept dry at 20°C (dark) for 20 days.

Serial Number	Germination Temperature/Condition	Percentage Germination Exposure Period kept dry at 20°C	Replicates			Mean
1	20°C Dark	20 days	62	62	62	62
2	20°C Dark in 0.1mM Kinetin	"	75	61	66	67.33
3	20°C Dark in 1mM GA_3	"	77	73	72	74
4	15°C(8h)Dark/35°C(16h)Dark	"	96	94	88	92.66
5	15°C(8h)Dark/35°C(16h)Light	"	96	84	92	90.66
6	20°C Dark	-	98	100	98	98.6
7	15°C(8h)Dark/35°C(16h)Dark	-	96	92	92	93.33

Figure 6.1 The mean percentage germination of achenes stored dry at -20°C (dark) or 35°C (dark) and germinated at 15°C(8h)/25°C(16h) in a 24h diurnal cycle.

Percentage Germination

Storage Period in wks	a: 15°C (8h) / 25°C (16h)				b: 35°C Constant Dark			
	-20°C Storage		35°C Storage		-20°C Storage		35°C Storage	
	Dark Replicates	Light Replicates	Dark Replicates	Dark Replicates	Dark Replicates		Dark Replicates	
4	1 0 (0.5)	5 2 (3.5)	13 8 (10.5)	28 26 (27)	19 19 (19)		30 27 (28.5)	
8	0 1 (0.5)	6 5 (5.5)	13 14 (13.5)	33 38 (35.5)	5 11 (8)		28 22 (25)	
16	2 2 (2)	11 15 (13)	30 28 (29)	58 59 (58.5)	21 23 (22)		25 33 (29)	
24	1 0 (0.5)	13 10 (11.5)	26 26 (26)	54 50 (52)	18 25 (21.5)		48 49 (48.5)	

CHAPTER 7

Table 7.4 or Figure 7.3 The mean percentage germination of achenes stratified at 4°C in GA_3 and germinated in water at 20°C in the dark. Counted after 14 days.

A.

Percentage Germination

Stratification at 4°C in GA_3 and Germination in Water

Period at 4°C in wks	0.01mM GA_3 Replicates		Mean	0.1mM GA_3 Replicates		Mean	1mM GA_3 Replicates		Mean	0 (H_2O) Replicates		Mean
2	22	24	23	21	19	20	21	19	20	6	10	8
4	19	23	21	16	14	15	31	23	27	15	18	16.5
6	41	53	47	53	54	53.3	54	48	51	44	42	43
12	60	64	62	54	54	54	64	62	63	63	50	56.5
15	75	68	71.5	70	63	66.0	74	71	72.5	63	70	66.5

B.

Stratification at 4°C in GA_3 + 30 minutes R → Germinated in Water

2	27	23	25	29	29	29	34	38	36	10	15	12.5
4	35	35	35	32	34	33	46	54	50	27	25	26
6	65	55	60	57	65	61	63	60	61.5	54	58	56
12	81	70	75.5	79	80	79.5	72	73	72.5	68	60	64
15	74	76	75	67	78	72.5	86	84	85	74	76	75

C.

Stratification at 4°C in GA_3 + 30 minutes FR → Germinated in Water

2	25	25	25	24	23	23.5	18	24	21	14	22	18
4	31	30	30.5	28	32	30	36	40	38	29	31	30
6	47	43	45	53	52	52.5	53	47	50	39	41	40
12	67	70	68.5	66	68	67	67	63	65	65	65	65
15	62	62	62	68	68	68	72	72	72	76	79	77.5

Table 7.5 or Figure 7.4 — The mean percentage germination of achenes stratified at 4°C in water and germinated in GA_3 at 20°C in the dark. Counted after 14 days.

Period at 4°C in wks	Percentage Germination: Stratification at 4°C in Water and Germinated in GA_3											
	0.01mM GA_3			0.1mM GA_3			1mM GA_3			0 (Water)		
	Replicates		Mean	Replicates		Mean	Replicates		Mean	Replicates		Mean
2	20	28	24	25	24	24.5	19	19	19	6	10	8
4	33	42	37.5	31	33	32	37	42	39.5	15	18	16.5
6	47	54	50.5	56	55	55.5	55	57	56	44	42	43
12	79	78	78.5	78	74	76	75	80	77.5	63	50	56.5
15	75	77	76	77	77	77	80	83	81.5	63	70	66.5
20	63	63	63	71	77	74	80	78	79	64	74	69

Table 7.6 or Figure 7.5 — The mean percentage germination of achenes stratified at 4°C in Water, transferred to growth retardants and germinated at 20°C in the dark. Counted after 14 days.

Period at 4°C in wks	Percentage Germination: Concentrations of B995											
	0.0625mM			0.625mM			6.25mM			0 (Water)		
	Replicates		Mean	Replicates		Mean	Replicates		Mean	Replicates		Mean
4	24	32	28	23	25	24	22	26	24	15	12	13.5
9	43	46	44.5	53	56	54.5	51	50	50.5	66	63	64.5
12	68	71	69.5	67	66	66.5	65	65	65	64	71	67.5

Period at 4°C in wks	Percentage Germination: Concentrations of CCC											
	0.0633mM			0.633mM			6.33mM			0 (Water)		
	Replicates		Mean	Replicates		Mean	Replicates		Mean	Replicates		Mean
4	27	17	22	17	21	19	19	25	22	15	12	13.5
9	65	56	60.5	61	66	63.5	39	35	37	66	63	64.5
12	65	65	65	63	64	63.5	76	63	69.5	64	71	67.5

Table 7.6 or Figure 7.5 contd.

Percentage Germination

Concentrations of AMO 1618

Period at 4°C in wks	0.0279mM			0.279mM			2.79mM			0 (Water)		
	Replicates		Mean	Replicates		Mean	Replicates		Mean	Replicates		Mean
4	19	21	20	28	25	26.5	13	25	19	15	12	13.5
9	63	62	62.5	68	64	66	57	52	54.5	66	63	64.5
12	64	68	66	69	65	67	67	59	63	64	71	67.5

Percentage Germination

Concentrations of Ancymidol

Period at 4°C in wks	0.001mM			0.01mM			0 (Water)		
	Replicates		Mean	Replicates		Mean	Replicates		Mean
4	20	24	22	21	22	21.5	15	12	13.5
9	53	62	57.5	47	55	51	66	63	64.5
12	67	77	71.5	52	53	52.5	64	71	67.5

Table 7.7 or Figure 7.5 The mean percentage germination of achenes stratified at 4°C in growth retardants and germinated in water at 20°C in the dark.

Percentage Germination

Concentrations of B995

Period at 4°C in wks	0.0625mM			0.625mM			6.25mM			0 (Water)		
	Replicates		Mean	Replicates		Mean	Replicates		Mean	Replicates		Mean
4	18	13	15.5	13	11	12	12	10	11	15	12	13.5
9	50	53	51.5	50	47	48.5	53	41	47	66	63	64.5
12	58	50	54	48	52	50	50	52	51	64	71	67.5

Percentage Germination

Concentrations of CCC

Period at 4°C in wks	0.0633mM			0.633mM			6.33mM			0 (Water)		
	Replicates		Mean	Replicates		Mean	Replicates		Mean	Replicates		Mean
4	16	16	16	16	13	14.5	18	20	19	15	12	13.5
9	56	42	49	26	25	25.5	32	39	35.5	66	63	64.5
12	46	57	48.5	45	43	44	46	43	44.5	64	71	67.5

Table 7.7
or
Figure 7.5 contd.

Percentage Germination

Concentrations of AMO 1618

Period at 4°C in wks	0.0279mM Replicates		Mean	0.279mM Replicates		Mean	2.79mM Replicates		Mean	0 (Water) Replicates		Mean
4	15	19	17	19	19	19	16	12	14	15	12	13.5
9	47	45	46	46	50	48	32	40	36	66	63	64.5
12	37	33	35	30	37	33.5	41	35	38	64	71	67.5

Percentage Germination

Concentrations of Ancymidol

Period at 4°C in wks	0.001mM Replicates		Mean	0.01mM Replicates		Mean	0 (Water) Replicates		Mean
4	22	18	20	23	17	20	15	12	13.5
9	55	56	55.5	47	38	42.5	66	63	64.5
12	50	54	52	40	36	38	64	71	67.5

Figure 7.1 The mean percentage germination of unchilled achenes treated with exogenous ethylene (Ethrel) and germinated at different conditions. Counted after 14 days.

Percentage Germination

Ethrel concentrations in ppm	25°C Constant Light				15°C(8h)/25°C(16h) Light at 25°C Light				35°C Constant Light				15°C(8h)/35°C(16h) Light at 35°C Light			
	25°C Constant Dark				15°C(8h)/25°C(16h) Light at 25°C Dark				35°C Constant Dark				15°C(8h)/35°C(16h) Light at 35°C Dark			
0	0	0	0	0	5	2	1	0	48	48	22	23	76	83	33	37
	0		0		3.5		0.5		48		22.5		79.5		35	
100 (0.1ml/ 1000ml)	3	2	0	0	6	4	0	0	51	47	17	15	68	74	23	29
	3.5		0		5		0		49		16		71		26	
1000 (1ml/ 1000ml)	6	3	0	0	5	8	0	0	52	42	22	29	85	76	38	30
	4.5		0		6.5		0		47		25.5		80.5		34	

Figure 7.2 The mean percentage germination of unchilled achenes treated with exogenous Nonanoic Acid (C9) and germinated under different conditions. Counted after 14 days.

Concentration of Nonanoic Acid (C9) in mM	Percentage Germination							
	25°C		15°C(8h)/25°C(16h)		35°C		15°C(8h)/35°C(16h)	
	Constant		Light at 25°C		Constant		Light at 35°C	
	Light	Dark	Light	Dark	Light	Dark	Light	Dark
0 (H_2O)	7 9 10	0 1 1	19 27 16	4 0 4	48 48 56	18 22 19	85 77 83	28 28 32
	8.66	0.66	20.66	2.66	50.65	19.66	81.66	29.33
0.1mM	14 8 10	7 0 0	23 17 23	5 4 7	60 39 46	13 23 18	82 84 82	44 35 41
	10.66	2.33	21	5.33	48.33	18	82.66	40
1mM	11 7 4	0 2 0	22 11 23	7 4 0	35 33 42	18 24 14	94 - 83	33 37 36
	7.33	0.66	18.66	3.66	36.66	18.66	88.5	35.33