THE PHYSIOLOGY OF SEED DORMANCY AND GERMINATION IN *AVENA FATUA* L.

by

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LIST OF ABBREVIATIONS AND NAMES OF CHEMICALS

ABA:	Abscisic acid
BA:	Benzyladenine
Barban:	4-Chloro-butyn-2-yl-1-(chlorophenyl carbamate)
Daminozide:	Succinic acid-2,2 - dimethyl hydrazide
EPTC:	S-ethyl dipropylthiocarbamate
Ethephon:	2-Chloroethyl phosphonic acid
GA_3:	Gibberellic acid
$GA_{4/7}$:	Gibberellin A_4 + gibberellin A_7 (1:1)
G6P:	Glucose-6-phosphate
G6PDH:	Glucose-6-phosphate dehydrogenase
K:	Kinetin
mEu:	Milli enzyme units
PP-pathway:	Pentose phosphate pathway
6PG:	6-Phosphogluconate
6PGDH:	6-Phosphogluconate dehydrogenase
S:	Siemens
SD8339:	Adenine, N-benzyl-9-(tetrahydro-2H-pyran-2-yl)
SHAM:	Salicylic hydroxamic acid

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CHAPTER 1

INTRODUCTION

The study of seed dormancy and germination has for centuries occupied the minds of agronomists, physiologists, brewers, bakers and, more recently, weed scientists. The agronomist requires that the seed that he sows will germinate rapidly and uniformly and produce a vigorous healthy seedling. The physiologist is interested in the understanding of the basic processes involved at the molecular level, and the geneticist in the inheritance of the quiescent character of the seed. Brewers seek a seed that will retain its viability at least until the following crop is harvested but which will also, on imbibition, rapidly set in motion those processes that will convert starch into sugar. The baker is concerned with the baking quality of the seed and, as far as he is concerned, the more dormant the seed the better, as this eliminates the problem of pre-harvest sprouting which is very detrimental to baking quality. The weed scientist seeks to encourage all weed seeds present in the soil to germinate simultaneously so as to enable him to destroy the weed population with one application of herbicide or a single cultivation.

Studies on seed dormancy revolve around three central questions: what causes the induction of dormancy; how is the dormant state maintained in the mature seed and, what factors and conditions are necessary to break dormancy? Despite a wealth of scientific information, these questions have not been satisfactorily answered. The causes of dormancy may be many and varied, such as impermeable seed coat, immature embryos, the presence of inhibitors, light, and a lack of oxygen (Bewley and Black, 1982). It is generally accepted that the mechanism of dormancy in all graminaceous species is similar, if not identical, but the complexity of this mechanism is indicated by the fact that a wide range of chemicals and conditions which are seemingly unrelated to

each other, can relieve dormancy. Factors commonly known to relieve dormancy of graminaceous seeds are stratification of the imbided seed (Friesen and Shebeski, 1961), warm temperature treatment, or after-ripening of the dry seed (Johnson, 1935), scarification of the seed (Hay and Cumming, 1959), increased oxygen tension (Attwood, 1914), gibberellic acid (Green and Helgeson, 1957), respiratory inhibitors such as azide and cyanide (Major and Roberts, 1969a) and anaesthetics such as ethanol (Taylorson and Hendricks, 1979). Any mechanism that will respond to these diverse treatments must indeed be complex and it is not surprising that the final solution of this intricate problem has eluded workers in this field for so long.

Ecologically, dormancy is a ploy whereby the seed avoids growth when conditions are unfavourable and optimises germination on the advent of the optimum growing season. A long dormancy period also ensures that the seed will be able to travel long distances to allow it to colonise new habitats, but a successful weed must possess a degree of variability as regards dormancy and conditions necessary to break it. This allows the seed to come up over a long period of time and there would be rapid natural selection for types which germinated at, or near the most favourable time for optimum reproduction in the new environment. Differential germination within a population can result from genetical differences between individual plants or as the result of the effect of different micro environments under which the seed matures, or as a result of intraplant differences in dormancy level. All three of these factors are operative in wild oat (*Avena fatua*) (Simpson, 1978). This latter type of differential dormancy is especially important in wild oats where the basal floret produces a seed which is relatively non-dormant when compared with the secondary and subsequent seeds which are progressively more dormant (Louw, 1930). The weed has used these subtle differences in dormancy to produce a wide range of naturally adapted ecotypes.

In the cold climates of Canada and northern Europe the seed has been programmed to germinate in the spring. Winter germinating plants would be rapidly destroyed by the severe winter. However, Sawhney and Naylor (1979) found that at Saskatoon, an appreciable number of non-dormant lines still persisted in germinating in the autumn. They, however, found that enough seeds of these non-dormant lines 'escaped' the autumn germination to germinate in the spring and so ensure the survival of the line. In warmer climates, such as the Mediterranean areas where the winter is the optimum growing period, germination is synchronised with the cool damp autumn. Dormancy in these ecotypes can be broken by a brief period of stratification at 5°C (Cairns, 1974).

Avena fatua has traditionally been used to study the mechanism of dormancy in graminaceous species due to the deep and extended nature of the dormancy exhibited and also to its important status as a weed. Over 150 publications have been devoted to some or other aspects of wild oat dormancy, yet much of this work is conflicting and no general concensus has been reached as to the precise nature of the mechanism of dormancy. Many of the inconsistencies are most certainly due to the vast differences between naturalised ecotypes as described above, and few workers have examined a sufficiently wide range of ecotypes.

This study will attempt to further knowledge in the field of seed dormancy and germination physiology using *A. fatua* as the test organism. Botanically the diaspore of *A. fatua* is a caryopsis. Since the term seed is usually used in agriculture to designate the caryopsis this terminology will also be used in this study. The following aspects will be covered:

1. Ecotypical variation as to growth characteristics and germination behaviour of a wide range of *Avena* species will be studied under field and controlled conditions. The results obtained will be related to the conditions

under which the local ecotypes developed to show that *A. fatua* in particular is, of all the *Avena* species, the most adaptable and this could explain its success as a weed.

2. The second aspect to be examined is the effect of plant growth hormones and known artificial germination stimulators on germination of dormant wild oat seed in an attempt to discover if combinations of these substances could be used to break dormancy of seed, either on the mother plant or of seed in the soil. The interaction of kinetin with gibberellin and light, which is well documented in other species, will also be investigated.

3. The effect of sugars on dormancy and gibberellic acid sensitivity of the aleurone layer will form the basis of the third avenue of investigation. An attempt will be made to explain the effect of environment on wild oat dormancy in terms of the carbohydrate availibility to the developing seed during the kernel filling stage.

4. Finally, the finding by Cairns and Craven (1975) that wild oat seed which was fumigated with 'Phostoxin' tablets became non-dormant will be investigated in more detail. This investigation will examine the relative dormancy-breaking properties of the various gases given off by 'Phostoxin' tablets. The mode of action of these gases will be fully studied.

CHAPTER 2

LITERATURE REVIEW

2.1 HISTORY AND ORIGIN

The earliest evidence of wild oats was found in wheat samples from the 12th Egyptian Dynasty (2000 - 1788 B.C.). These wild oats were subsequently identified by Tackholm and Drar (1941) as *Avena sterilis* and *Avena fatua*. It would appear that they occurred as weeds for De Candolle (1890) indicated that there was no evidence of oat production by the ancient Egyptians.

Zade (1918) reported that oats which strongly resembled our present day *A. sativa* were identified in settlements of early bronze age lake dwellers in different parts of Europe. Malzew (1930) thought that these early findings were those of *A. strigosa* sub. spp. *strigosa*. He stated that the perusal of the whole literature on the archaeology of wild oats has convinced us that:

1. *A. strigosa* sub. spp. *strigosa* was found in lake dwellings referred to as the age of bronze.
2. *A. fatua* sub. spp. *fatua* was found in the remains referred to the age following that of bronze, i.e. the age of iron, chiefly its early Hallstatt period.
3. Finally that *A. sativa* was found in great numbers of instances which refer already to the late period of ancient Slavs (900 - 500 B.C.).

Thus, although oats have been found as weeds in ancient cereal samples, their cultivated use is apparently more recent than that of wheat or barley. In this way oats spread mainly concomitantly with barley to the westernmost parts of Europe by about 1500 B.C.

Although the precise origin of wild oats is still a matter of debate, researchers agree that it originated somewhere in South West Asia. Thus Malzew (1930) is of the opinion that *A. fatua* or *A. sterilis*, i.e. all hexaploid oats, have one primitive centre in South West Asia. Vavilov (1926) stated that emmer wheat (*Triticum dicoccum*) with its attending oats, was a widespread crop during the pre-pleistocene climatic optimum. The ensuing deterioration in the climate led to wider cultivation and prominence of oats as a crop due to its better adaptation to the changed conditions than emmer. Natural and artificial selection for certain morphological characteristics of the oat plant led to its spread and success as a crop and/or a weed.

King (1966) reported that floret articulation and its inheritance has been a critical factor in the evolution of oats. Sampson (1954) noted that any mutation in the ancient weed population which favoured non-articulate florets, would have conferred a selective advantage since such grains would have been harvested with the main crop and subsequently been sown in following years. It seems probable that recurrent cycles of selection in this manner led to the cultivation and acceptance of various *Avena* spp. as crops.

Oats were spread in cereal mixtures by the Slavs and Scythians from the centre of origin in South West Asia (Zukovski, 1950). These ancient warlike horsemen must therefore be regarded as one of the primary vectors of wild oat dissemination to Western Europe. Local climatic conditions dictated which of the contaminating wild oat species later became dominant. Afterwards, naturalised ecotypes developed within species which were even better adapted to local conditions. Thurston (1954) for example, cited the presence of *A. ludoviciana* as a serious weed which is confined only to southern England and the warmer parts of Europe. This species germinates in the autumn and is unable to withstand the severe winters at more northerly latitudes.

Avena fatua on the other hand, which germinates in both the autumn and the spring, is found widespread throughout the cooler areas of Europe, Asia and Northern America.

Baum *et al.* (1972) have made an extensive study of wild oat populations which presently occur in the region of origin in the Mediterranean and in South West Asia. They found *A. sterilis* to be the most ubiquitous species adapted to a wide range of conditions. They reported this species to be a vigorous competitor with crops and in Iran, where particularly heavy infestation of the weeds occur, the main crop is often harvested as hay for animal consumption. *Avena ludoviciana* is not mentioned in this study, presumably because Baum *et al.* (1972) regard it as a sub-species of *A. sterilis*. They reported that *A. barbata* occurred throughout the region covered, but particularly in the Mediterranean basin area often growing in saline conditions and in poor sandy soils. *Avena fatua*, however, was found to have penetrated the northernmost parts of the Mediterranean region. Baum *et al.* (1972) regarded this species as of Euro-Siberian or of Western and Central Asiatic extraction rather than of Mediterranean and concluded that *A. fatua* is definitely of continental origin.

2.2 DISPERSAL OF WILD OAT SPECIES

Wild oats has achieved a very deep penetration of world agriculture (Thurston and Phillipson, 1976). These authors state that the enormous development of world trade in seed, food grain and foodstuffs, contributed to this spread and the intensification of cereal farming (especially barley and wheat), greatly aggravated the problem in many parts of the world.

The seeds of most wild species of *Avena* are very variable and when they reach a new country, local conditions will

select the types which are most suited to those local conditions. Henning (1933) cited the use of unrotted manure and the sowing of contaminated seed as the most important vectors of wild oat dissemination in the Cape wheatfields. More recently Thurston (1959) stated that, in spite of efforts of large seed firms in England, contaminated seed remains as the most common source of wild oat distribution.

The severity of the problem may be illustrated by a report by Elliot and Attwood (1970) who found 19% of the grain samples collected in England and Wales, and 16% of those collected in Scotland, to be contaminated by wild oats. Later, Bowler (1973) further demonstrated that 11% of the seed bought from seed merchants and 41% of home grown seed, was contaminated by wild oats. Even straw may serve as a substantial source of wild oat infestation as e.g. Wilson (1970) found 680 000 wild oat seeds in a ton of barley straw from a farm in Oxfordshire. Other methods of wild oat seed dissemination reported by Thurston and Phillipson (1976) are on farm machinery, in sacks, in dung and silage, by wind, by birds and by rodents.

2.3 DISTRIBUTION OF WILD OAT SPECIES IN THE WESTERN CAPE

Louw (1930) identified *A. fatua*, *A. sterilis* and *A. barbata* growing in Cape wheatlands. He commented on the fact that although *A. barbata* appeared to be fairly widespread, it did not occur in large numbers. Whether the *A. barbata* was growing actually in the wheatland or adjacent to them is not clear. Cairns (1974) found four wild oat species in the Western Cape, viz. *A. fatua*, *A. ludoviciana*, *A. sterilis* and *A. barbata*. Of the samples collected in cereal lands about 70% were *A. fatua*, 20% *A. ludoviciana*, and 5% *A. sterilis*, the remaining 5% being fatuoids or other inter-species crosses. No *A. barbata* plants were found in grain lands but this species was abundant along roadsides and other uncultivated but disturbed areas. Henderson and Anderson

(1966) reported that *A. fatua* is frequently found in the southern Cape but was less common in other parts of the country. Thurston and Phillipson (1976) confirmed the identification of the wild oat species present in the Western Cape and commented that the samples from this area revealed affinities with Australian types.

According to Cairns (1974) colour differed widely in the four species present. This was notably so in the case of *A. fatua* and *A. sterilis* whereas *A. ludoviciana* and *A. barbata* were more uniform in colour. *Avena fatua* seeds collected were found to be predominantly dark brown to black but grey, buff and light cream seeds also occurred. Most *A. ludoviciana* seeds collected were grey but a few buff samples were also observed.

Isolated populations, consisting exclusively of *A. sterilis*, were concentrated in the higher rainfall areas around Stellenbosch, but disjuncted communities of this species occurred as far north as Malmesbury. *Avena fatua* by contrast, occurred throughout the region. In the drier area of the Swartland, where the most serious wild oat infestations occur, this species was dominant, which suggest that the local ecotype of *A. fatua* is more drought resistant than the other species (Cairns, 1974).

2.4 DORMANCY AND GERMINATION

Wild oat is today regarded as the most seious weed problem of small grain production throughout the world (Holm *et al.*, 1977). The success of this weed can, to a large extent, be attributed to its extended period of dormancy and associated periodicity of germination under a wide range of climatic conditions (Cairns, 1974). Much work has been done on wild oat dormancy but, despite the wealth of information on the subject, the physiological basis of dormancy is only partially understood (Simpson, 1978).

2.4.1 Variation in dormancy

Thurston (1957) examined the dormancy of a wide range of *A. fatua* seed and found the dormancy to range from 95% to no dormancy, while *A. ludoviciana* dormancy varied from 0-56%. Patterson *et al.* (1976) examined the dormancy of several Australian lines of *A. fatua* and *A. barbata* under a wide range of storage conditions. The germination of *A. fatua* varied from 0-40% but *A. barbata* was found to be much less dormant and germination varied from 50-75%.

In a similar study in South Africa, Cairns (1974) found that local ecotypes of *A. fatua* gave an initial germination of 8% shortly after harvest, which increased to 70% after six months storage at room temperature. *Avena barbata* which was totally dormant at harvest, showed no dormancy at all after six months, while both *A. ludoviciana* and *A. sterilis* showed no germination at harvest and after six months at room temperature, had only germinated 15% and 10% respectively.

The lack of dormancy in *A. barbata* explains why it seldom invades wheatlands where the early germination seedlings would be destroyed by pre-sowing cultivation. In non-cultivated but disturbed areas such as roadsides, the early germinating *A. barbata* dominates, as its rapid germination after the first rains would confer a competitive advantage over the later germinating *A. fatua*. Miller *et al.* (1982) found that 200 accessions of *A. fatua* collected in Minnesota and North Dakota, differed widely in their dormancy characteristics and showed germination figures of between 0 and 92%. These workers also found that seed from the accessions produced in one locality (Langdon) were significantly more dormant than those produced at another (Fargo). They ascribed this difference to the cooler climate prevailing at Fargo.

The position of seed in the wild oat spikelet also affects dormancy. In both *A. fatua* and *A. ludoviciana*, the first

or basal seed is less dormant than the second and third seeds (Louw, 1930; Johnson, 1935; Thurston, 1956; Kommedahl *et al.*, 1958; Morgan and Berrie, 1970; Cairns, 1974 and Peters, 1978). The increase in dormancy with increasing distance from the source of nutrient and growth hormones in the mother plant, has given rise to speculation that the first seed being larger and closer to the source of supply of growth promoting substances, receives a greater share than the second or the third seed (Morgan and Berrie, 1970).

2.4.2 Genetical control of dormancy

Garber and Quisenberry (1923) examined the phenomenon of delayed germination in the 'false wild oat' and concluded that in crosses between *A. sativa* and *A. fatua*, delayed germination is a recessive character and that it is somewhat loosely linked to the fatua-type seed articulation. Imam and Allard (1965) conducted a study on the genetic variability between and within natural populations of wild oats from differing habitats in California and found that the genetic system of wild oats appears to equip this species admirably for the occupation of a complex set of habitats. The occurrence of regular low levels of outcrossing between different co-existing genotypes sets the stage for the recombination that is needed for the development of a diversity of genotypes of ever increasing adaptation to specific environmental niches. At the same time inbreeding ensures that most of the individuals of a population shall be rather highly homozygous, thus providing for the maintenance over a period of generations of genotypes or associations of genotypes that are well adapted to specific micro-niches. In other words, the wild oat species appears to have evolved a remarkably flexible genetic system in which the appropriate compromises between the high recombinational potential of outbreeders and the ability of inbreeders to

hold together desirable complexes of genes.

Large differences in germination behavior were found by Naylor and Jana (1976) in populations collected in the vicinity of Saskatoon in Canada. They found evidence that these differences had a genetic basis and that the cause of this differentiation is genetic adaptation to local environmental conditions. Environmental conditions which could constitute selective pressure in favour of genotypes conferring dormancy, were farming practices such as summer fallowing and the use of herbicides.

Jana *et al.* (1979) conducted breeding experiments with pure lines of *A. fatua* differing characteristically in primary dormancy. Their results indicated that the parental lines differ for at least three genes controlling after-ripening. It was evident that at least two of these genes influence the rate of after-ripening at the different periods after seed maturation. Jana and Naylor (1980) estimated the hereditability of primary seeds in four populations of wild oats by the parent-progeny regression method and found it to be approximately 50% under field conditions.

2.4.3 The influence of environmental factors during seed development on dormancy

2.4.3.1 Temperature

The effect of temperature during seed development on the dormancy of cereals has been extensively studied due to the pre-harvest sprouting problem encountered in many parts of the world where rain is experienced during the harvesting period. Belderok (1961) reported that high temperatures during the mealy-ripe stage of wheat reduced the dormancy of wheat. The same effect was noted for barley by Reiner and Loch (1976) but they also found that low temperatures

between 12 and 16 days after anthesis, reduced post-harvest dormancy. Olsson and Mattsson (1976) examined seed dormancy in wheat under different weather conditions in Sweden. Although generally agreeing with the findings of Belderok (1961), they found that accumulated temperatures during the dough period were not always positively correlated with lack of dormancy. They concluded that other climatic factors may also play a role.

Takahashi (1980) found that there was a difference between short day cereals (rice) and long day cereals (barley) regarding the effect of temperatures during maturation on dormancy. In the former, high temperatures led to more dormant seed whereas in the latter, low temperatures caused enhanced post-harvest dormancy.

Strand (1983) carried out a study on the effects of temperature and rainfall on the seed dormancy of wheat and barley in Norway. He examined the dormancy of the cereals from 1959-1964. In 1959, which was warm, seed dormancy was very low (5%). During 1960-61 intermediate temperatures were experienced and dormancy was significantly higher (35%) than in 1959. During 1963, and particularly in 1964, cool moist conditions were experienced during the ripening period and dormancy was enhanced further (67%).

Sexsmith (1969) found that the seed of two lines of *A. fatua* were more dormant when grown at cool temperatures (18,7°C) than when grown at warmer temperatures (28,4°C).

Sawhney and Naylor (1979) reported that, although differences in dormancy levels between different populations have a genetic basis, genotype expression is significantly influenced by the environment. They showed temperature to be the most important environmental factor which interacts strongly with genotype in the expression of dormancy. They found that low-temperature treatment of dormant *A. fatua*

lines during seed maturation induced long-term seed dormancy. They, however, noted that sensitivity to temperature during seed development was more clearly demonstrable when dormancy was assayed at 4°C than at 20°C. Two of the three dormant lines tested exhibited no sensitivity to different maturation temperatures if they were germinated at 20°C.

Peters (1978) examined the dormancy of grey, brown and yellow lines of *A. fatua* grown at 15°C and 20°C in a growth chamber experiment. The mean germination after two months was 2,8% and 36,7% respectively. The seed was then stored for a further three months at either 15°C or 20°C. The average germination percentage for the wild oat seed matured at 15°C had risen to 11,7% and 47,7% for those matured at 20°C.

2.4.3.2 Moisture

Sexsmith (1969) found that *A. fatua* (var. Intermedia) produced 53% dormant seed when grown in a 'hot' moist environment, but only 6% of the seeds were dormant when produced in a 'hot' dry environment. Peters (1978) found that water stressed *A. fatua* plants produced less dormant seed. Seed matured at 15°C without water stress were 95% dormant, whereas seed from plants given a water stress, were only 53% dormant. He also found that the effect of moisture and temperature was additive rather than synergistic and further that moisture stress during the maturation period was more important than temperature in determining the dormancy of the seed. Sawhney and Naylor (1982) found that in both dormant and non-dormant lines, water-stressed plants exhibited shorter duration of dormancy when compared to the well-watered controls. The magnitude of the effect varied among dormant families.

2.4.3.3 Photoperiod

As recently as 1980 Whatley and Whatley could find no evidence of any effect of photoperiod on the onset of dormancy. However, in an extensive review of the subject, Gutterman (1981) found conclusive evidence that photoperiod did influence dormancy in a wide range of species. Gutterman (1978) showed that the facultative long day desert plant, *Trigonella arabica*, produced much less dormant seed under short days (8 h) than under long days (15 h). Another long day desert plant, *Latuca scariola*, produced seeds under short day conditions which germinated relatively better than those maturing under long days (Gutterman, 1974). In *Portulacca oleracea*, a facultative short day plant, the seeds maturing under short days also germinated more rapidly than those maturing under longer photoperiods (Gutterman, 1974).

In most of the above-mentioned cases the daylength experienced during the final stage of maturation determined the dormancy of the seed. In the case of tomato, the daylength experienced by detached ripe fruits influenced germination (Gutterman, 1978). Germination of seed in the fruit kept under short days was 38%, while no germination occurred in fruits kept under long days.

Wurzburger and Koller (1976) found that in the monocotyledon, *Aegilops Kotschyi*, which produces two caryopses per spikelet, the basal dormant caryopsis was subject to environmental influences to which the parent plant was exposed during its development. Basal seeds were more dormant when produced by plants grown at low temperatures or in 16 h daylength, than seeds produced from plants grown at higher temperatures or 8 h daylength. The upper seed was unaffected by environmental conditions experienced by the mother plant, and germinated readily.

2.4.4 The influence of environmental factors on after-ripening, dormancy and germination

2.4.4.1 Temperature

Simpson (1978) described after-ripening as the loss of dormancy caused by exposure of the seed to varying environmental conditions over time. Quail and Carter (1968) demonstrated that after-ripening in storage was faster with increasing temperatures up to 30°C together with increasing relative humidities over the range 16-100%. Quail and Carter (1968) showed that the optimum temperature for the after-ripening of *A. ludoviciana* was between 25° and 30°C. Peters (1978) found that the average germination of seeds stored at 15° and 20°C for three months was 22% and 37% respectively.

Naylor and Fedec (1978) found large differences in optimum after-ripening temperatures between dormant and non-dormant families of *A. fatua*. They found that dormant strains after-ripened very slowly in the middle temperature range (16-24°C), but after-ripened more quickly at high (32°C) and especially at low (4°C) temperatures. It must, however, be pointed out that this experiment was carried out on imbibed caryopses whereas previous work had been done on dry seed. It is, however, interesting to note that dormant families are identified on the basis of their germination performance in the middle temperature range.

Sawhney and Naylor (1979) found that there was a strong correlation between maturation temperature and germination temperature. The dormant line (Montana 73) matured at 28°C, gave 100% germination after 49 days at 4°C, but if the maturation temperature was 20°C, the seed produced was still 90% dormant after 133 days at 4°C. Another dormant line (AN 51) matured at 20°C, achieved 100% germination after

258 days at 4°C, but those kept at 20°C had only achieved 5% germination over the same period.

A wide range of optimum germination temperatures has been reported for wild oat seed collected in different parts of the world. Friesen and Shebeski (1961) found that the optimum germination temperature of *A. fatua* samples collected in Canada was 21°C, while Quail and Carter (1968) reported an optimum germination temperature of 10°C and 15°C respectively for *A. ludoviciana* and *A. fatua* collected in Australia. Whalley and Burfitt (1972) however, recorded an optimum germination temperature of 20°C for Australian *A. fatua*. Koch (1968), working in Germany, showed that under laboratory conditions the germination of *A. fatua* at 20°C amounted to 10% of the seeds germinating at 15°C. At temperatures above 15°C there was a steady decline in germination up to 35°C where germination ceased.

Cairns (1974) showed that the optimum germination temperature of *A. fatua* collected in the Western Cape depended on the state of after-ripening. The optimum germination temperature of dormant seed was 5°C, whereas a non-dormant selection showed a broad temperature optimum, but with significantly less germination at 5°C than at 25°C. When the dormant seeds were either treated with gibberellic acid or pricked, the optimum germination temperature moved up to 10°C and even at 15°C more seeds germinated than at 5°C.

The above literature illustrates the fallacy of an optimum germination temperature for wild oats. The environment under which the seed matured, the genetic makeup of the seed, the conditions of the after-ripening period and the duration thereof are all factors that will influence the temperature at which the highest percentage of the seeds will germinate.

2.4.4.2 Light and photoperiod

The germination of many seeds is sensitive to white light; some seeds requiring light for germination and others being prevented from germinating by light treatment (Smith, 1972). Johnson (1935) reported 'inconclusive' results on the effects of both sunlight and artificial light on the germination of *A. fatua*. He found that in newly-ripened seeds, light promoted the germination of both hulled and de-hulled seed by 15%. He also found that light was detrimental to the germination of well after-ripened seeds. Cumming and Hay (1958) investigated the effect of light quality on germination of *A. fatua*. Infra-red, blue and white light inhibited germination in that order of magnitude, but he found minimal inhibition with red light. They also concluded that under natural conditions, daylight inhibits the germination of partially dormant wild oat seed on the soil surface.

Similar findings were reported by Hsiao and Simpson (1971), who found that the inhibitory effect of light was more pronounced in the case of newly-harvested than in older seeds.

Hsiao and Simpson (1971) also established that in low volumes of water, germination was inhibited by white light and that in high volumes, germination was promoted when compared to seeds germinating in darkness. Whittington *et al.* (1971) found that light promotes the germination of *A. fatua* at both low (5°C) and high (18°C) temperatures, whereas in *A. ludoviciana* light is inhibitory only at the higher temperature.

Photoperiod can also affect the germination of *A. fatua* seed. Thurston (1964) reported 53% germination for seed held under an 8 h photoperiod, but only 18% germinated under 16 h days. The inhibitory effect of the longer photoperiod persisted even after the seeds had been de-hulled and pricked. Light has also been implicated in the after-ripening of *A. ludoviciana*

and Quail and Carter (1969) found that white light exerted a significant effect by shortening the after-ripening period needed for maximum germination. Hsiao and Simpson (1971) found that white light significantly reduced the promotive effect of gibberellic acid in *A. fatua*. Simpson (1978) commented that this effect is a further indication of the significance of light effects on metabolism, since gibberellic acid has been shown to influence enzyme reactions in both the embryo and endosperm.

Recently Hilton (1982) showed that the germination of *Bromus sterilis* was inhibited by red light and stimulated by far-red light. A single pulse of red light for 10 min inhibited germination but this could be reversed by a subsequent 10 min period of far-red light. Whether this is an isolated case or whether this phenomenon is more general, remains to be established.

Hilton and Bitterli (1983) found that in U.K. *A. fatua* ecotypes, light was stimulatory to seed germination. They ascribe this effect to the cropping system employed there, whereby recently-shed dormant seed is interred during the autumn cultivation. The seed is then stimulated to germinate in spring when the semi-dormant seed is brought to the surface or receives a light flash during preparation and sowing of the spring crop.

2.4.4.3 Mineral elements

The role of mineral salts, particularly potassium nitrate, in fulfilling requirements for dormant seed was reported by Crocker and Barton (1953) to be a well known phenomenon. The effect of nitrogenous fertilizers on germination of *A. fatua* in the field was examined by Sexsmith and Pittman (1963) who found that early applications of nitrogenous

fertilizers caused increased germination of *A. fatua* seed in the soil. They suggested that the apparent breaking of dormancy by nitrates may offer an alternative method for combatting wild oats.

This hypothesis was in fact tested by Sexsmith and Russel (1963) but no reduction in wild oat stand due to early season application of nitrogenous fertilizers, followed by cultivation, was noted. On the contrary, their panicle counts in the field indicated a cumulative increase in wild oat infestations with seasonal applications of nitrogenous fertilizers, thus nullifying their previous hypothesis.

Similar findings were reported by Watkins (1971), who found that a significantly greater number of wild oat seeds germinated prior to sowing on fertilized lands than in adjacent unfertilized areas. He also reported that annual dressings of nitrogen led to greatly increased numbers of wild oat seeds in the soil. He found a fivefold increase in seed numbers of wild oat seeds in unfertilized areas but an eightfold increase in fertilized plots. Under more controlled conditions, application of nitrate (3 000 p.p.m.) resulted in a 16% germination of three month old wild oat seeds compared to no germination in the controls. Cairns (1974) found that nitrate (5 000 p.p.m.) significantly increased the germination of *A. fatua* and *A. sterilis* but had no effect on either *A. ludoviciana* or *A. barbata*.

2.4.4.4 Water

While water is essential for the normal germination of wild oat seeds, variation in the volume of water in which seeds are imbibed, has significant effects on seed dormancy (Simpson, 1978). Experiments by Hsiao and Simpson (1971) showed that the behaviour of wild oat seed to different wavelengths of light could be influenced by the quantity

of water the seeds were imbibed in. Small and large volumes of water restricted germination when compared to the medium range of volumes. Light interacted with low volumes of water to inhibit germination in the light compared to darkness, but large volumes of water together with white light, promoted germination compared to darkness. Cumming and Hay (1958) found that if non-dormant seed was immersed in water they became secondary dormant. Simpson (1978) commented that the explanation given for this phenomenon was that respiration became limited due to the low diffusion of oxygen into the non-agitated water.

Embryos immersed for 24 hrs in deoxygenated water saturated with nitrogen, did not germinate but remained viable (Simmonds and Simpson, 1971). They found that embryos leached in the same manner in oxygenated water germinated during the treatment. Naylor and Simpson (1961) found that by leaching excised dormant embryos with large volumes of water, led to enhanced gibberellin-promoted germination. The authors attributed this effect to the leaching out of a gibberellin inhibitor.

Secondary dormancy can also be induced by interrupting germination by drying (Johnson, 1935). Kommedahl (1958) however, commented that this effect could only be obtained if the hulls were present. Simpson (1978) reported an experiment where dry, dehulled, non-dormant *A. fatua* caryopses were placed in a petri dish saturated with water vapour. The seeds germinated readily and he deduced that the variability in germination, associated with the variation in the volume of water available for germination, is not due to lack of water uptake by the embryo. Rather, he concluded that these and other reported observations suggest an interaction of water volume with hulls, and possibly the pericarp and testa, which in turn modifies the effects of light and movement of gases, metabolites, and growth regulators that are essential for normal germination. There is however,

by no means unanimity on this question and recently McIntyre and Hsaio (1983), have reported a direct relationship between water uptake by the embryo and germinability.

2.4.4.5 Gases

Attwood (1914) first commented on the fact that oxygen level could influence germination of *A. fatua*. He found that by pricking the seed, germination of dormant caryopses were stimulated. He attributed this stimulation to the improvement of oxygen diffusion to the embryo. He also found a marked increase in germination with an increase in oxygen concentration. Louw (1930) could find no effect of increased oxygen concentration on the germination of *A. fatua* but his methods were somewhat primitive and it is doubtful if any valid conclusions can be drawn from this result.

Baker and Leighty (1958) showed that in less dormant strains of *A. fatua*, germination was stimulated with increasing oxygen concentration but, in the most dormant strain tested, increasing the oxygen concentration had no stimulatory effect on germination. However, if this strain was de-hulled and pricked, 41% germinated. Whether pricking of the seed releases dormancy by increasing oxygen available to the embryo is open to some doubt as Hay (1962), found that pricking the seeds through a layer of lanolin also promoted germination.

Bibbey (1948) could find no effect of increased CO_2 concentration on the germination of *A. fatua*. However, Hart and Berrie (1968), found that in the absence of CO_2, white light inhibited germination. With CO_2 at 20%, light showed no interaction, but germination of the intact seeds was severely inhibited.

Simpson (1978) summarised the effect of CO_2 and O_2 by saying that both gases are essential for germination and affect

germination by affecting the metabolism of the embryo in particular. Structures such as the hull and seed coat can restrict gas exchange but they are not the sole determinants of dormancy associated with the gas composition of the atmosphere. The level of metabolic activity of the embryo can also determine both oxygen and carbon dioxide requirement of the embryo. The rate and type of metabolism are a function of the degree of dormancy present in the embryo.

Dormancy in many weed seeds is broken by ethylene and the possibility that ethylene can be used to break dormancy in *A. fatua* has been investigated by several workers. Chancellor *et al.* (1971) found that Ethephon did not stimulate the germination of a dormant batch of *A. fatua* but Adkins and Ross (1981) reported a small but significant increase in germination of *A. fatua* which had been after-ripened for 12 months with increasing ethylene concentration. The inability of ethylene to stimulate the dormancy of freshly harvested seed and the fact that ethylene evolution was not correlated to loss of dormancy, would seem to indicate that this gas does not play an important role in the loss of dormancy in *A. fatua*.

Cairns (1974) reported that the mixture of gases (PH_3, NH_3 and CO_2) given off by 'Phostoxin' (Degesch) tablets, dramatically stimulated the germination of dormant *A. fatua*.

2.4.5 Influence of chemicals on dormancy

2.4.5.1 Gibberellic acid

The effect of gibberellic acid (GA_3) on the breaking of dormancy in *A. fatua* is well documented (Cairns, 1974). Green and Helgerson (1957) first reported that GA_3 could break the dormancy of freshly harvested *A. fatua* seed. He found the

optimum concentration of GA_3 to be 50 ppm. The response to exogenous GA_3 decreased with the length of the after-ripening period. Black and Naylor (1959) grew exised panicles in a range of GA_3 concentrations and found the seed to be non-dormant when compared to the water controls.

Naylor and Simpson (1961) reported an interaction between GA_3 and sucrose. Highly dormant *A. fatua* seeds would germinate when given a low (0,5 ppm) dose of GA_3 provided that sugar was also given. In the absence of sugar, 50 ppm GA_3 was needed to elicit the same response. They concluded that the high level of GA_3 is needed for both the production and utilization of sugar, whereas the low dose is sufficient to allow the dormant embryo to utilize the exogenously applied sugar.

Simpson (1965) found that naked (without pericarp, testa or endosperm) dormant embryos of *A. fatua* required an exogenous source of sugar, amino acids and GA_3 in order to germinate. The non-dormant embryos did not require GA_3 because they produce endogenously a gibberellin-like factor. This factor is absent in freshly harvested embryos but increases in amount with the length of the after-ripening period.

How gibberellin stimulates germination is not at this stage clear. The fact that gibberellin stimulates the production of α-amylase has led several researchers to believe that the stimulation of germination is via amylase synthesis (Chen and Chang, 1972) but as they showed that the stimulation of α-amylase by gibberellin is a post germination phenomenon, this would seem unlikely. Drennan and Berrie (1962) also eliminated the lack of α-amylase synthesis as a possible cause of dormancy. An interesting finding was made by Peters (1978) who found that the α-amylase content of *A. fatua* seeds which had been subject to drought stress during their maturation, was significantly higher than in seeds from well-watered plants. The drought-stressed seed was

also very much less dormant than the well-watered controls.

Until recently it has been assumed that the main source of amylase in the germinating cereal seed was the aleurone layer, but Gibbons (1980, 1983) working on barley, has shown that the scutellum is far more important as a site of amylase synthesis during the early stages of germination. It is interesting to note that the effect of gibberellin on α-amylase synthesis by the scutellum has not been examined in any depth although Simpson and Naylor (1962) could find no effect of GA_3 on the amylase synthesised by embryos with attached scutellum.

2.4.5.2 Other plant growth regulators

At this stage plant growth regulators, apart from gibberellin, do not seem to play an important role in the regulation of wild oat seed dormancy (Roberts, 1963a). Andrews and Burrows (1972) demonstrated that abscisic acid (ABA) can inhibit germination of excised embryos of dormoats (*A. fatua* x *A. sativa*) and that this inhibition can be reversed by GA_3. This work does not however, provide conclusive evidence that ABA is implicated in the natural regulation of germination and dormancy. In fact, Berrie *et al.* (1979) could find no correlation between ABA content in *A. fatua* and *A. sativa* and the level of dormancy. Braun and Khan (1975) also could not find any relationship between ABA levels in germinating and dormant lettuce seeds.

Bewley and Black (1982), in their review of dormancy, are of the opinion that there is little direct evidence to link ABA content to the state of dormancy in the seed. This view is echoed by Walton (1981) in another review article who states that there are few definite statements that can be made concerning a role for ABA in the germinative process.

The influence of cytokinins on wild oat germination has not been fully investigated but Sharma *et al.* (1976) found that both kinetin and benzyladenine significantly stimulated the germination of partially dormant *A. fatua*.

2.4.5.3 Respiratory inhibitors

Common inhibitors of cytochrome oxidase (such as potassium cyanide, sodium azide and carbon monoxide) were found by Roberts, (1964a; 1964b) to stimulate the breaking of seed dormancy in several species. He found that none of the 17 other inhibitors tested, had any stimulatory effect. Later, Major and Roberts (1969a) found that other respiratory inhibitors (such as sodium fluoride, iodoacetate, malonate and monofluoroacetate) were also able to produce significant increases in germination in dormant seed.

Roberts (1969) proposed that the oxidative processes involved in the breaking of dormancy are linked to the pentose phosphate pathway (PP - pathway). Any treatment which inhibits conventional respiration, would favour the PP-pathway. Under the relatively anaerobic conditions prevailing in seeds, the elimination of cytochrome oxidase, which has a very high affinity for oxygen, would drastically reduce the competitive ability of the glycolitic pathway in favour of the PP-pathway.

Kovacs and Simpson (1976) found that the levels of extractable glucose-6-phosphate dehydrogenase (G6PDH), a key enzyme in the PP-pathway, rose with increasing time after inhibition of non-dormant *A. fatua* seed but declined in dormant grains. Glycolitic activity was similar in both dormant and non-dormant seeds. These authors concluded therefore, that the ability to maintain and/or increase the initial levels of PP-pathway enzymes following imbibition is essential for germination.

Uphadyaya *et al.* (1981) however, found that the levels of G6PDH and 6-phosphogluconate dehydrogenase (6PGDH) were similar in both genetically dormant and non-dormant lines of *A. fatua*. Embryos and endosperms of both dehydrogenases exhibited high activity in both dormant and non-dormant lines. Activity of the two enzymes remained constant during the first 48 h of incubation in water in the dormant line, but in the non-dormant line there was a sharp increase after 24 h incubation - well after the onset of germination. These workers also found that the level of G6PDH and 6PGDH activity in dormant seeds treated with GA_3 increased only after germination had taken place. Adkins and Ross (1981) also, could find no obvious connection between dormancy breaking and increased activity of enzymes of the pentose phosphate pathway in *A. fatua*.

However, De Jimenez and Quiroz (1983) working on maize, found that G6PDH activity in the axis, is related to susceptibility to pre-harvest sprouting. It was found that if the ratio of enzyme activity in the scutellum to that in the axis was lower than 3, the cultivar was susceptible to pre-harvest sprouting. These authors concluded that an active pentose phosphate pathway is directly related to pre-harvest sprouting in maize.

Hendricks and Taylorson (1975) ascribe the dormancy breaking properties of thiourea, hydroxylamine and nitrite to an inhibition of H_2O_2 decomposition by catalase. This inhibition spares H_2O_2 for peroxidase action. Peroxidase, together with pyridine-nucleotide-quinone-oxidoreductase, can couple H_2O_2 to NADPH re-oxidation which is essential for an active pentose phosphate cycle. This hypothesis is supported by recent work by Noll (1983) who found that there was a direct association between peroxidase activity and grain dormancy in severeal Canadian wheat cultivars. He found that the higher the peroxidase activity, the less dormant the seed. This held true between and within cultivars for samples of

varying degrees of dormancy. However, Esashi *et al.*(1979), working with cocklebur (*Xanthium pensylvanicum*) could not find any evidence that the H_2O_2 sparing action by the inhibition of catalase and the concomitant stimulation of the pentose phosphate cycle, played any part in the release from secondary dormancy by substances such as azide, cyanide and hydroxylamine. They also found that the degree of catalase inhibition by these inhibitors did not reflect their effectiveness in promoting germination. Instead, Esashi *et al.* (1979) proposed that respiratory inhibitors which stimulate germination, activated a cyanide insensitive alternative respiratory pathway. They furthermore found that inhibitors of this alternative pathway such as benzohydroxamic acid and salicylhydroxamic acid, induced secondary dormancy in non-dormant lower seeds.

Esashi *et al.* (1979) developed their hypothesis further by establishing that both the conventional and alternative respiratory pathway must be operative to induce germination. When azide and cyanide were applied as inhibitors of the cytochrome respiratory path, only a moderate stimulation of germination was obtained, but when applied together with alternative respiratory pathway inhibitors such as benzohydroxamic acid, a marked stimulation in germination resulted. Esashi *et al.* (1979) could however, not stimulate primary dormant upper seeds to germinate with convential respiratory inhibitors such as azide and cyanide. They offered no explanation for this finding but reiterate their view that increased participation of the pentose phosphate cycle is not involved in the release of dormancy in cocklebur seeds.

Uphadhyaya *et al.* (1982) found that sodium azide could break dormancy in most dormant oat lines, but not in Montana 73. Chlormequat, an inhibitor of GA synthesis, completely prevented the azide effect, suggesting that stimulation of germination by azide requires gibberellin biosynthesis. Salicylhydroxamic acid (3 mmol dm^{-3}) completely inhibited the

germination promoted by azide. At this concentration SHAM did not prevent the germination of either genetically non-dormant seeds or after-ripened seeds of a dormant line. This finding indicates that a SHAM-sensitive process, presumably alternative respiration, is necessary for the stimulation of germination in the presence of azide, but not in the germination of genetically non-dormant, GA-treated or after-ripened seeds.

2.4.5.4 Herbicides

Certain herbicides applied to wild oats can affect the dormancy and viability of seeds of surviving plants (Peters, 1978). However, information on the effect of wild oat herbicides on dormancy is meagre. Barban is one of the oldest wild oat herbicides and has been used widely for more than two decades yet, until recently, nothing was known of its effect on seed dormancy of surviving plants.

Peters (1978) tested the effects of sub-lethal dosages of barban, chlorfenprop-methyl and benzoylprop-ethyl applied to *A. fatua* plants at the 5½-6 leaf stage, on the dormancy of seeds produced. Barban produced seeds which were slightly more dormant than the controls but the other two herbicides stimulated germination. In subsequent work, Peters (1982) showed that benzoylprop-ethyl significantly stimulated the germination of seeds from sprayed plants. Analogues of benzoylprop-methyl such as flamprop-isopropyl also stimulated germination of seeds from sprayed plants significantly but other herbicides such as S-ethyl dipropylthiocarbamate (EPTC) and diclofop-methyl only slightly stimulated germination. Peters (1978) also treated seed with benzoyl-methyl and its analogues, but germination of the seed was not significantly affected.

2.4.5.5 Miscellaneous treatments

Hsiao (1979) found that maximum germination of dormant *A. fatua* could be obtained by treating the seeds for two hours with 6% sodium hypochlorite, one hour in water and then incubating the seed in GA_3. He found that the hypochlorite-treatment mimics the effect of piercing and led to greater penetration of GA_3. Lal and Reed (1980) treated wild oat seed with microwave energy at a frequency of 2450 MHz. Germination was inhibited and the extent of inhibition was affected by exposure time, microwave energy level and moisture content of the seed. Dormancy of the seed was not significantly affected.

Norris and Wilkinson (1980) studied the effect of centrifugation and hydrostatic pressure on the germination of *A. sativa*. They found that centrifugation of dry seed had little effect on subsequent germination and seedling growth, but the centrifugation of imbibed seeds led to an inhibition of germination and stunted seedlings. Since the seed used was non-dormant, it is not clear what effect these treatments would have on the behaviour of dormant seeds.

2.4.6 The role of inhibitors

Green and Helgerson (1957) were the first to report that substances inhibitory to germination, could be extracted out of the hulls. However, Baker and Leighty (1958) indicated that there was no inhibitor present in hulls that could inhibit germination of non-dormant seed. Black (1959) found inhibitors in both the hulls and caryopses of *A. fatua* and suggested that the hulls may retard the leaching out of these inhibitors and thus inhibit germination. He also found more inhibitor in moist seed held under nitrogen than in moist seed held under oxygen.

Methanolic extracts of dormant and non-dormant seeds were

found by Hay (1962) to contain inhibitors of seedling growth but not of germination. However, he found that an aqueous solution of hulls caused a reversible inhibition of germination. This inhibition could be achieved without injuring the embryos and could be reversed by GA_3. Because the inhibitor could stop germination in the presence of sucrose, the author concluded that dormancy can be due to a block at a point other than the conversion of starch to sugar.

Naylor and Simpson (1961) found evidence of a water-soluble inhibitor in the embryo of *A. fatua*. By leaching dormant embryos, they increased the capacity of the embryos to respond to a given dose of GA_3 to substitute for exogenous sucrose. They concluded that the control of germination during the period of after-ripening is through changes in the inhibitor content rather than in endogenous gibberellin.

A later study of Chen *et al.* (1981) found that aqueous extracts of dormant wild oat had a weak inhibitory effect on lettuce seed germination. Both paper and thin-layer chromatographic analyses of the ether soluble acidic fraction showed the presence of phenolics and short chain fatty acids. They concluded that although wild oat hulls contain a variety of phenolic inhibitors, these substances play no role in the regulation of wild oat seed germination.

Berrie *et al.* (1975) examined the function and occurrence of short chained fatty acids in plants and found that nonanoic acid at 10^{-3} mol dm^{-3} was most effective in preventing the germination of non-dormant *A. sativa* and *A. fatua*. Stewart and Berrie (1979) found that short chain fatty acids were also inhibitory to lettuce seed germination. Once again nonanoic acid was the most effective inhibitor. They also found that the inhibitory effect of the fatty acids increased with increase in temperature.

Berrie *et al.* (1979) compared the fatty acid contents of

non-dormant *A. sativa* and dormant *A. fatua* during after-ripening. The relationship between dormancy and nonanoic acid was good. *Avena fatua* still contained 30,7 mg kg^{-1} nonanoic acid 133 days after harvest; the figure for *A. sativa* at this stage of after-ripening was 0,28g kg^{-1}. The results of Berrie *et al.* (1979) would be more impressive if germination figures at the different stages after harvest, when the fatty acid levels were monitored, had been included.

2.4.7 Secondary dormancy

The basic difference between primary and secondary dormancy concerns the time of onset of dormancy (Bewley and Black, 1982). In the former, dormancy arises in the developing and maturing seed; in the latter, dormancy sets in the already mature seed. These authors could not recognise any fundamental biochemical or physiological distinction between the two. Any factor which inhibits germination can also induce secondary dormancy.

Dormancy in *A. fatua* can be induced by soaking the seeds in water that has been boiled to remove most of the dissolved oxygen (Hay and Cumming, 1959). Thurston (1960) found that secondary dormancy could be induced in *A. ludoviciana* by exposing the imbibed seeds to a temperature of 24,5°C. The dormancy thus induced could be removed by stratification between 4-7°C. Friesen and Shebeski (1961) exposed non-dormant *A. fatua* seed to 4,4°C before incubation at 21,1°C. This treatment also induced dormancy. Bacthaler (1957) induced dormancy in *A. fatua* at high temperatures (25-30°C). It is thus evident that dormancy can be induced in wild oats under a wide variety of conditions, as previously described (section 4.4.4.1) the optimum germination temperature can vary widely, so it is not surprising that the optimum temperature for the induction of dormancy varies also.

Relatively little work has been done on the mechanism of secondary dormancy (Chancellor, 1976). Hay (1962) found that secondary dormancy was not due to the build up of inhibitors in the hulls of seeds. Andrews and Burrows (1972) found that in dormoats (*A. fatua* x *A. sativa*) low temperatures could break primary but not secondary dormancy. However, Hay and Cumming (1959) reported that primary and secondary dormancy were similar, in that potassium nitrate, hydrogen peroxide, gibberellic acid, hull removal and puncturing of the seed coat relieve both the primary and secondary types of dormancy.

Simmons and Simpson (1972) suggested that the induction of secondary dormancy could be explained either by depletion of adenosine triphosphate which would stimulate Krebs cycle activity and so inactivate the pentose phosphate cycle, or it could be caused by a build up of inhibitors. Bewley and Black (1982), in their review of secondary dormancy, concluded that 'we do not understand secondary dormancy'.

2.4.8 Periodicity of germination

Thurston (1951) found that cultural practices and depth of burial affected periodicity of germination. Cultivation increased the total number of wild oat seedlings by over 20% over a 6-12 month period. Chancellor (1976) reported that in England, *A. fatua* germinates mainly in spring and to a lesser extent in autumn. Whittington *et al.* (1971) found that *A. fatua* would not germinate at low temperatures (5°C) unless the husk was removed. Quail and Carter (1968), working in Australia, found that lower temperatures stimulated the germination of both *A. fatua* and *A. ludoviciana* but the latter species germinated better at the lower temperatures.

Peters (1978) speculated that the reason for the emergence

of *A. fatua* in both spring and autumn was that the high temperatures in summer and the low temperatures in winter are both able to break dormancy. Cairns (1974) found that *A. fatua* germinated only in the autumn in the Western Cape. Any seedlings emerging in spring, at the beginning of the long dry Mediterranean summer, would have no chance of survival and therefore selection against spring germinating types would be intense.

Avena fatua seems to be the only wild *Avena* spp. (with the possible exception of *Avena strigosa*) which has been able to adapt to spring rather than autumn germination. *Avena ludoviciana*, *A. sterilis* and *A. barbata* germinate almost exclusively in the autumn and are thus not able to survive in the more extreme climates of Northern Europe and Canada (Cairns 1974).

CHAPTER 3

CHARACTERIZATION OF *AVENA* ECOTYPES FROM DIFFERENT HABITATS

3.1 INTRODUCTION

In the many studies on wild oat dormancy few, if any, have compared dormancy of ecotypes that have become naturalised in different parts of the world. Some workers, such as Imam and Allard (1965) working in California, Patterson (1976) in West Australia and Miller *et al.* (1982) in Minnesota, have compared the dormancy of different selections from sites within a climatic zone. These sites may have differed in terms of rainfall, temperature, soil type and agricultural practices but did not differ as to the optimum growing season or in photoperiod. Mediterranean ecotypes for example germinate in the autumn, and the long dry summer would eliminate any types which germinated in the spring. Similarly, types which germinate in the autumn in severe continental climates, would not be able to survive the long, cold winter.

Any comprehensive study on the mechanism of wild oat dormancy should take into consideration the many different ecotypes of the weed which have become naturalised in the different parts of the world as well as the strategies they have adopted to synchronise germination with the advent of the optimum growing season. Photoperiodic response of naturalised populations is related to latitude of origin (Thurston, 1957), but whether this response is also related to dormancy, has not been investigated.

In this study germination behaviour, seed viability and growth habit of British wild oat ecotypes were compared to South African ecotypes. British *A. fatua* germinates mainly in the spring as it is not hardy enough to survive the winter but there is always a small flush of autumn-germinating

plants. British *A. ludoviciana* is however, much hardier and more resistant to cold, and consequently, germinates mainly in the autumn. All South African ecotypes germinate in the autumn. Spring germinating types would not survive, due to the long dry summer experienced in the Western Cape which has a typically Mediterranean type climate.

3.2 MATERIAL AND METHODS

3.2.1 The course of germination of some *Avena* species under U.K. conditions

Seed from South Africa and U.K. ecotypes of the various *Avena* species were harvested in the Western Cape during July and at Oxford in the U.K. during August respectively. The seed was stored at room temperature until sowing in 2 cm diameter pots placed outside at Oxford on 20 October, 1975. It was decided not to water the pots but to rely on natural rainfall, but due to the extremely dry weather conditions (the winter of 1975-76 was the driest in 250 years) watering was commenced on 29 November 1975 and continued, when necessary, throughout the duration of the trial. Germination was recorded weekly and germinating seedlings were removed taking care not to disturb the soil.

3.2.2 Effect of white light on the germination of three *A. fatua* ecotypes

Germination of *A. fatua* from Oxford, Montana and Rondebosch was carried out in a growth chamber at 16°/10°C day/night temperature. The seed was germinated in petri dishes as described in 3.2.3. Seed was exposed to the light by placing the petri dishes in transparent plastic bags. Darkness was provided by enclosing the petri dishes in light proof black plastic bags sealed with insulation tape. It was assumed that the heat exchange between the bags and the atmosphere within the incubator was not impaired. The light

source consisted of both fluorescent and incandescent lamps, delivering an intensity of 0,86 $Jm^{-2}s^{-1}$. The photoperiod was 12 h. This light source was used to deliver light of a similar composition to that of sunlight.

3.2.3 Variation in growth habit of various ecotypes of five *Avena* species

This trial consisted of two plantings, a winter planting, sown on 17 December, and a summer planting, sown on 17 June and was carried out in the open in Oxford, England. The winter plantings included the following ecotypes: Western Cape *A. fatua*, *A. ludoviciana*, *A. sterilis* and *A. barbata*, Oxfordshire *A. fatua* and *A. ludoviciana* and *A. fatua* from Montana, U.S.A. The summer plantings included the above ecotypes as well as *A. byzantina*, *A. fatua* and *A. sterilis* from Kenya, *A. byzantina* from the Orange Free State and *A. fatua* from Zimbabwe. The latitude of origin of the above ecotypes were: Oxfordshire 52°N, Montana 47°N, Western Cape 33°S, O.F.S. 28°S, Zimbabwe 18°S and Kenya 0°. These ecotypes were obtained from agricultural research workers in the various countries and were, as far as possible, lines derived from naturalised populations encountered as weeds in one or more crops.

Prior to planting the seeds were all dehusked and pricked to break dormancy. They were set to germinate at 15°C in the dark in 9 cm petri dishes lined with 3 Whatman No.1 filter papers and moistened with 6 cm^3 water. Uniform seedlings of the various ecotypes were planted out in 21 cm diameter pots outside and given supplementary watering when necessary.

3.2.4 Growth habit and dormancy of various *Avena* ecotypes grown under controlled environment

Dormancy of the different ecotypes used in the above experiments could not be compared due to the different times of

maturation and the consequent effect of the environment at the time of ripening on the level of dormancy (Sexsmith, 1969; Peters 1978; Sawhney and Naylor, 1979). Consequently, the various ecotypes were grown under controlled environment at two different temperatures.

Wild oat seed of the various *Avena* ecotypes were germinated on filter paper in petri dishes kept at 15°C in the dark. Uniform seedlings were then planted out in 17 cm diameter pots in two growth chambers held at 18°/20°C day/night temperatures. A 12h-photoperiod was provided throughout the experiment. The number of days taken by the different ecotypes to flower was recorded. Ripe seed was harvested daily by hand by gently tugging the awns. The seed was then stored at 4°C in the dark until germination tests were carried out.

Three replicates of 25 seeds per treatment were transferred to 9 cm petri dishes lined with three layers of Whatman No.1 filter paper. Six cm^3 water were applied to each dish and the seeds were incubated at either 5° or 15°C in the dark. Germination was recorded daily for 21 days (no further germination occurred after this period) and germinated seeds were removed after each count. Ungerminated seeds were tested for viability by dehusking, pricking and treatment with 1 mmol dm^{-3} GA_3 solution for a further 14 days. Seeds which had not germinated by this time were visibly putrid and non-viable (Peters, 1978).

3.2.5 Statistical procedures

Unless otherwise stated, all experiments consisted of at least three replications. Analysis of variance was carried out on a Univac 1110 computer using the BMD 08V programme. The least significant difference test (LSD) was used to determine significant differences between treatment means.

3.3 RESULTS AND DISCUSSION

3.3.1 Time course of germination of some *Avena* species under U.K. conditions

Germination of the different species is illustrated in Fig. 3.1 a,b,c and d. Germination was recorded until the end of August when the experiment was terminated. Since no germination in any of the ecotypes occurred between May and August, this period is not represented on the graphs.

The germination pattern of the two *A. fatua* ecotypes (Fig. 3.1a and b) conforms with the germination pattern likely to result in successful establishment in their natural environments. *Avena fatua* from the Western Cape germinated only in the autumn as it does in its natural habitat. Spring germinating types would have long since been removed by natural selection due to the inability of these plants to survive the long dry Mediterranean summers. The U.K. *A. fatua* shows the typical bimodel germination pattern characteristic of this ecotype. Peters (1978) found that the autumn flush of germination resulted from relatively non-dormant basal seeds. Subsequent germinations depended on the depth of dormancy of smaller second seed. It is surprising that *A. fatua* (U.K.) germinated more in the autumn than the spring, but this may have been due to the extremely mild conditions experienced during that year.

Avena ludoviciana (Western Cape) (Fig. 3.1c and d) germinated more rapidly than its UK counterpart (Fig. 3.1c and d). Both ecotypes however, germinated exclusively in the autumn, with the exception of a single seed of the UK ecotype which germinated in the spring. This species is more hardy than *A. fatua* and is able to survive a moderately severe winter (Chancellor, 1976). *Avena ludoviciana* is thus confined to the temperate parts of Southern Europe but is not very

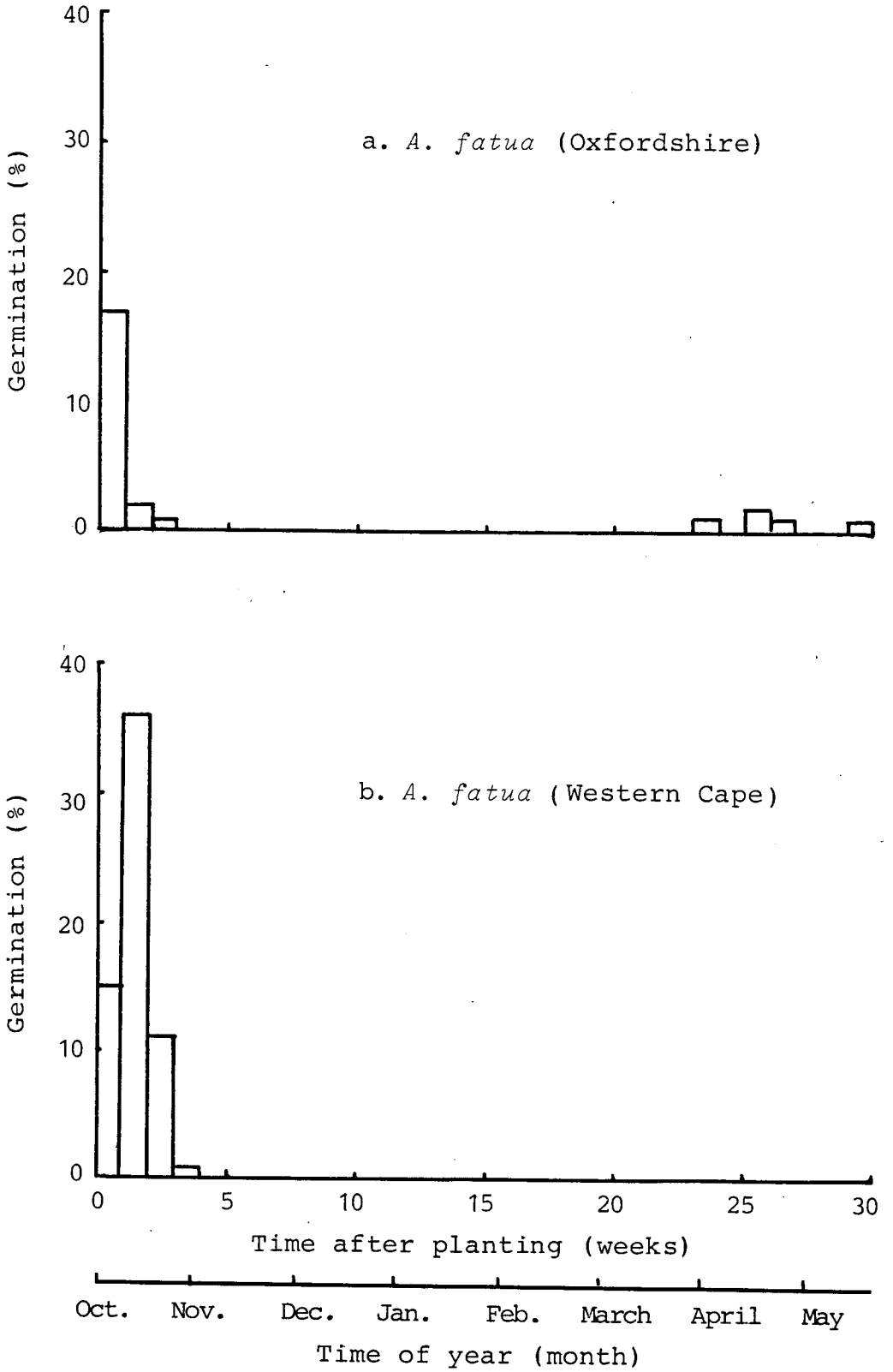

Fig. 3.1 a & b Time course of germination of U.K. and S.A. ecotypes of *A. fatua* under U.K. field conditions.

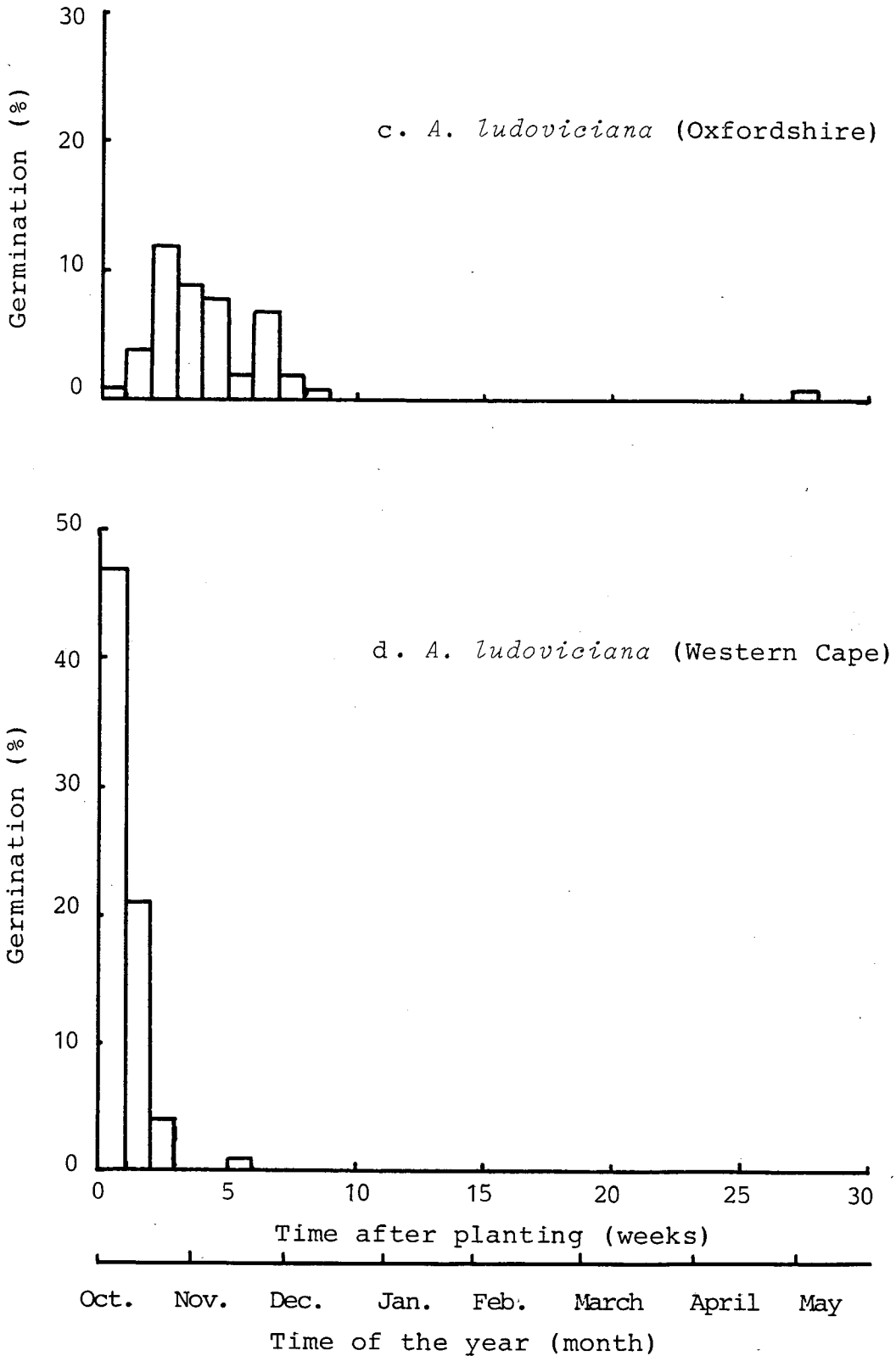

Fig. 3.1 c & d Time course of germination of U.K. and S.A. ecotypes of *A. ludoviciana*, grown under U.K. field conditions.

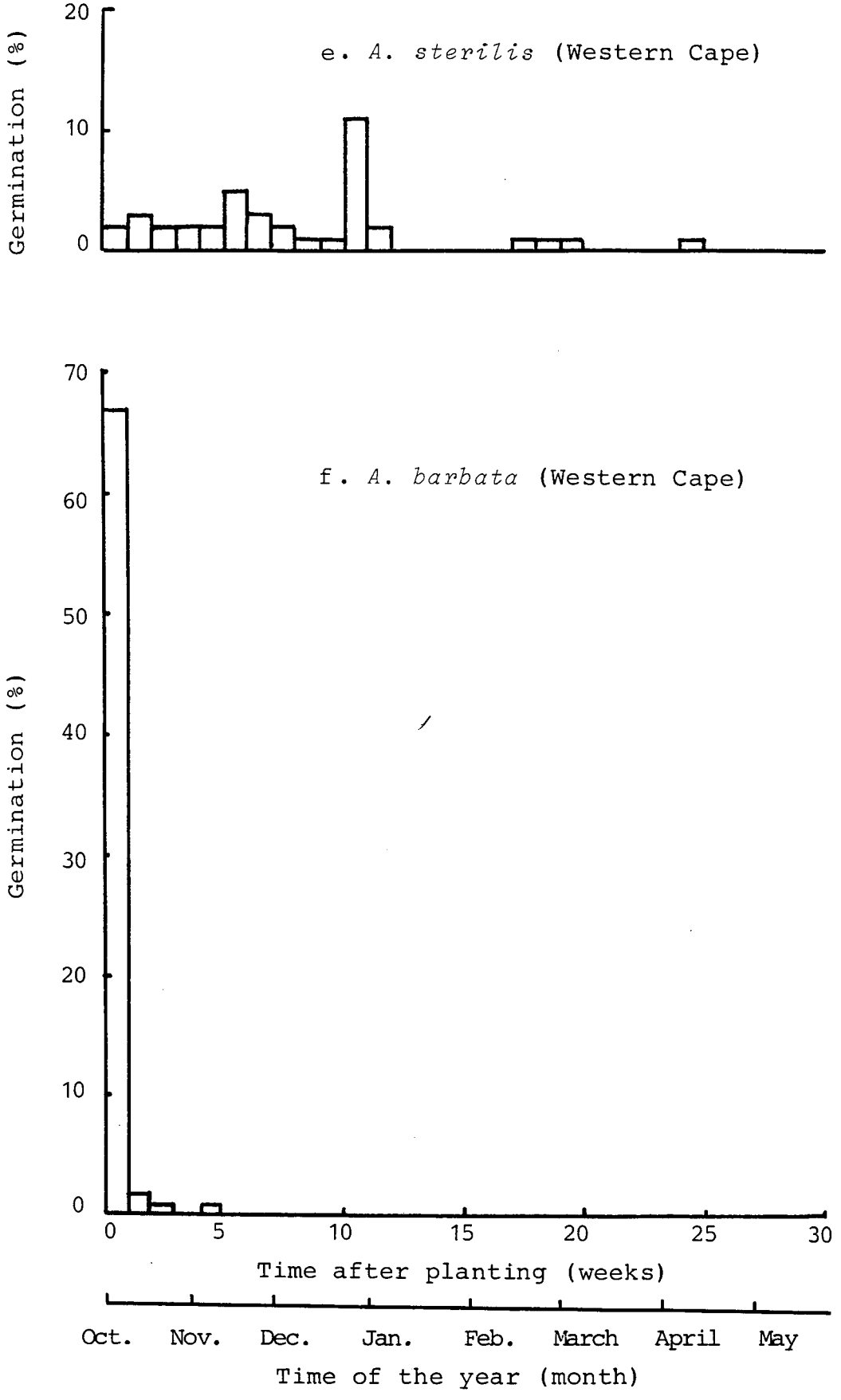

Fig. 3.1 e & f Time course of germination of S.A. ecotypes of *A. sterilis* and *A. barbata* grown under U.K. field conditions.

common in the Mediterranean Basin of the Middle East where *A. sterilis* is more common (Baum *et al.*, 1976). The same can be said of the Western Cape where *A. ludoviciana* is very rare only being recorded for the first time by Cairns in 1974.

Avena sterilis showed no definite flush of germination and germinated sporadically over the first three months. Again, the mild weather may have influenced this species but this germination pattern could also have been caused by the large number of seeds per floret (up to five) with widely differing dormancy (Cairns, 1974). The ubiquity of this species in the Middle East, growing under a wide range of conditions, can be explained by the differential dormancy of the seeds produced by a single spikelet (Baum *et al.*, 1972).

Avena barbata was the least dormant and germinated rapidly with 67% of the seeds emerging after the first week. The lack of dormancy of this species has been cited as a reason why this species is hardly ever considered to be a weed problem (Cairns, 1974).

It is interesting to note that in both *A. fatua* and *A. ludoviciana* the U.K. ecotypes were more dormant than their South African counterparts. Whether this is a result of environmental conditions during maturation or whether under genetic control (or both) will be examined later (see section 3.3.4). However, in the light of the findings of Patterson *et al.* (1976), who found a correlation between rainfall and the dormancy of *A. fatua* and *A. barbata* (the wetter the location the more dormant the naturalised population) it would not be surprising if Western Cape ecotypes are genetically less dormant than those of the U.K.

3.3.2 Effect of white light on the germination of three *A. fatua* ecotypes

The effect of white light on the germination of *A. fatua*

(Montana, Oxford and Rondebosch) is presented in Fig. 3.2. White light stimulated the germination of the Oxford ecotype but inhibited germination in both the Montana and Rondebosch ecotypes.

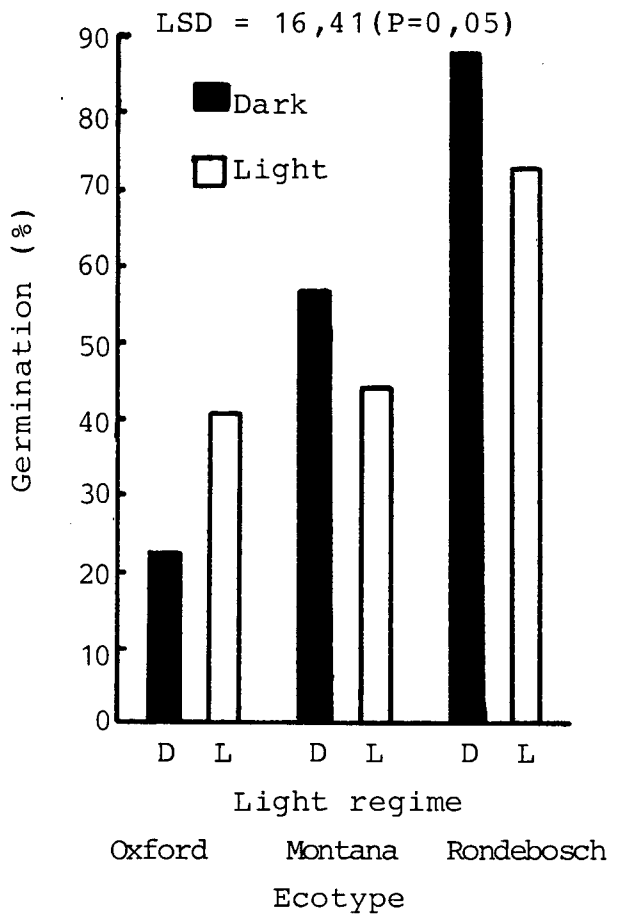

Fig. 3.2 Effect of white light on the germination of three *A. fatua* ecotypes

The inhibitory effect of light on the germination of *A. fatua* has been demonstrated by several workers (Hay and Cumming, 1959; Hart and Berrie, 1968; Hsiao and Simpson, 1971 and Cairns, 1974). However, recent work by Adkins (1981) and Hilton and Bitterli (1983) working with Oxfordshire *A. fatua*,

found that light promoted the germination of 23 partially dormant lines.

Hilton and Bitterli (1983) postulated that U.K. *A. fatua* ecotypes were peculiar in their reaction to light and that this trait developed as a result of the cropping system employed in that part of the world. Freshly shed seed is prevented from germinating immediately after being shed by innate dormancy and is incorporated into the soil shortly afterwards with the autumn cultivations. Cultivation of the land in the spring, when the crop is sown, brings some of the partially after-ripened seed into contact with light which results in a flush of germination. Under this system it would be an advantage for the seed to respond positively to a light stimulus during or after the spring cultivation. These authors develop their hypothesis further by explaining that the negative effect of light on the germination of Canadian ecotypes is due to the fact that the seed (also shed in autumn), is not incorporated with autumn cultivations but remains on the surface throughout the winter. Under these conditions it would be a distinct advantage if light inhibited germination until such time that the seeds were incorporated into the soil with pre-sowing cultivations in the spring.

On the basis of the above hypothesis it could be expected that ecotypes naturalised in a Mediterranean-type climate such as is experienced in the Western Cape, would also produce seed which showed a negative germination response to light. Here, the cropping system is different to that in Canada in that the growing season is in the winter. Similar to the Canadian situation however, the shed seed lies on the soil for the whole of the long dry summer. It would be fatal to this seed to germinate during an unseasonal summer shower.

The results of the present experiment are consistent with

the above hypothesis postulated by Hilton and Bitterli (1983) and illustrates the extremely adaptive nature of *A. fatua* in regulating its germination to correspond to the season where the seedling would have the maximum chance of survival and seed production. These results can also help to explain the many inconsistancies in the reported work on the effect of light on *A. fatua* germination and expose the error of basing any theory on the effect of climatic variables on growth and germination of this weed on a few local ecotypes.

3.3.3 Variation in growth of various ecotypes of five *Avena* species

A photograph taken of the winter plantings 24 weeks after planting (June 3) illustrates that all the Western Cape lines had headed, whereas the Oxford and Montana ones had not (Plate 1).

The time taken to 50% heading, anthesis and seed fall of the seven ecotypes is shown in Table 3.1, where it is evident that latitude of origin determines the photoperiodic response.

Differences, however, could be seen between the various species e.g. the panicles of English and Cape *A. fatua* emerged slightly after those of the respective *A. ludoviciana* ecotypes but that anthesis occurred first in the former species. The sensitivity of photoperiodic adaptation in *A. fatua* is evident from the fact that only 4° separated the latitude of origin of the Oxfordshire and Montana populations yet, the former ecotype flowered 11 days later. The possibility that the vernalization requirement might have influenced the photoperiodic response was not investigated but it would seem more than likely that, if anything, the Montana wild oats would have the greater vernalization requirement. Subsequent work (results not shown) conducted in glasshouses

and growth chambers, showed that vernalization had little or no effect on the photoperiodic response of *A. fatua*. This, however, was not the case with *A. ludoviciana* which, at least in the Oxford ecotype, has a strong obligate vernalization requirement.

Plate 1. Developmental stage of seven *Avena* ecotypes in June under U.K. field conditions. They were all planted in mid December.

1. = *A. fatua* U.K.
2. = *A. fatua* S.A.
3. = *A. ludoviciana* U.K.
4. = *A. ludoviciana* S.A.
5. = *A. fatua* Canada.
6. = *A. barbata* S.A.
7. = *A. sterilis* S.A.

Table 3.1 Time taken by seven *Avena* ecotypes to attain certain defined morphological stages under winter conditions in the U.K.

Ecotype	Origin		Days to: *		
	Area	Latitude	Panicle Emergence[1]	Anthesis[2]	Seed fall[3]
A. barbata	W. Cape	33° S	155	167	186
A. fatua	W. Cape	33° S	168	175	192
A. ludoviciana	W. Cape	33° S	167	178	195
A. sterilis	W. Cape	33° S	167	174	198
A. fatua	Montana	45° N	183	193	202
A. fatua	Oxford	52° N	193	204	207
A. ludoviciana	Oxford	52° N	188	205	213

* Means of 4 observations

1. 50% Emergence of panicle from the flag leaf sheath.
2. 50% Appearance of anthers.
3. 50% Seed shed from plants.

Table 3.1 illustrates the relevant data for the summer plantings. *Avena fatua* from Kenya and Zimbabwe headed and flowered very similarly and the Western Cape ecotype of this species was not far behind. One would have expected a greater degree of photoperiodic variation in these ecotypes, but it must be borne in mind that these three ecotypes were probably derived from the initial infestations at the Cape which was the first centre of cultivation of small cereals in this area. Also, the excessively long 18 h-daylength for these cultivars would tend to mask photoperiodic differences between these ecotypes. That there were differences in photoperiodic behaviour in these ecotypes, is borne out by the results of a growth chamber trial with a photoperiod of 12 hrs (see section 3.3.3).

Predictably, the Northern ecotypes from high latitudes took

appreciably longer for floral induction. *Avena sterilis* and *A. byzantina* from Kenya flowered within a week of *A. fatua* from that latitude showing that these ecotypes have little if any vernalization requirement. The same however, cannot be said of *A. barbata* and *A. sterilis* from the Western Cape and *A. byzantina* from the Orange Free State. These ecotypes took far longer to flower than their latitude of origin would indicate. These three ecotypes occur exclusively as winter weeds in their respective habitats but so do *A. fatua* and *A. ludoviciana* from the Western Cape which do not have a vernalization requirement.

Avena ludoviciana from Oxford did not head at all, showing that this ecotype had an obligate vernalization requirement. This explains why this species germinates more or less exclusively in the autumn in England. It will obviously also be confined to areas where the winter is mild enough for it to be able to survive - hence the failure of this species to colonise Northern Europe or Northern America.

The fact that none of the *A. fatua* ecotypes had even a facultative vernalization requirement is interesting, and may indicate why this species is by far the most successfull of the *Avena* species in colonizing the different parts of the world. It would be interesting to find out how long a population of *A. fatua* would take to become photoperiodically adapted to an environment with a longer or shorter photoperiod than that experienced at the habitat where the naturalised population evolved.

3.3.4 Growth habit and dormancy of various *Avena* ecotypes grown under controlled environmental conditions

The number of days to anthesis of 12 *Avena* ecotypes is given in Table 3.3. With the exception of *A. fatua* from Montana, all ecotypes flowered earlier with a day/night temperature of 26°/20°C, compared to 18°/12°C.

Table 3.2 Time taken by 12 *Avena* ecotypes to attain certain defined morphological stages under U.K. summer conditions

Ecotype	Origin		Days to:		
	Area	Latitude	Panicle Emergence[1]	Anthesis[2]	Seed fall[3]
A. byzantina	Kenya	0°	34	40	62
A. fatua	Kenya	0°	38	45	64
A. sterilis	Kenya	0°	37	47	70
A. fatua	Zimbabwe	10° S	37	44	70
A. byzantina	O.F.S.	28° S	63	67	89
A. barbata	W. Cape	33° S	104	106	123
A. fatua	W. Cape	33° S	38	47	64
A. ludoviciana	W. Cape	33° S	44	49	77
A. sterilis	W. Cape	33° S	63	80	96
A. fatua	Montana	45° N	64	75	87
A. fatua	Oxford	52° N	63	73	92
A. ludoviciana *	Oxford	52° N	-	-	-

* did not head

1. 50% Emergence of panicle from the flag leaf sheath.
2. 50% Appearance of anthers.
3. 50% Seed shed from plants.

The behaviour of the various ecotypes was similar to that seen in the summer planting in the U.K. The East African ecotypes and Western Cape *A. fatua* flowered about the same time. Western Cape *A. ludoviciana* however, took longer to flower, particularly at 26°/20°C, indicating that this species too, has a slight vernalization requirement but that it can be overcome by long days (U.K. summer plantings). *Avena barbata* and *A. sterilis* from the Western Cape flowered more or less at the same time as they did in the U.K., indicating that they are less photoperiodically sensitive - at

least without being vernalized. The Northern ecotypes of *A. fatua* also performed much the same as they did in the U.K. summer plantings. As expected, *A. ludoviciana* from Oxford again did not head as a result of its obligate vernalization requirement.

Table 3.3 Time taken by 12 *Avena* ecotypes to reach anthesis at 18°/12°C and 26°/20°C, day/night temperature under a 12 h photoperiod

Ecotype	Origin		Days to anthesis:[1]	
	Area	Latitude	Growth temperature 18°/12°C	26°/20°C
A. byzantina	Kenya	0°	59	46
A. fatua	Kenya	0°	56	42
A. sterilis	Kenya	0°	59	53
A. fatua	Zimbabwe	18° S	56	42
A. byzantina	O.F.S.	28° S	77	68
A. barbata	W. Cape	33° S	91	78
A. fatua	W. Cape	33° S	56	43
A. ludoviciana	W. Cape	33° S	79	65
A. sterilis	W. Cape	33° S	81	59
A. fatua	Montana	47° N	68	73
A. fatua	Oxford	52° N	84	73
*A. ludoviciana**	Oxford	52° N	-	-

* did not head

1. 50% Appearance of anthers.

Germination of seed harvested from the above ecotypes was carried out at 5°C and 15°C and is illustrated in Table 3.4. Overall, maturation temperature had no significant effect on germination at either incubation temperatures. This result must, at first, seem surprising as several workers have found that the higher the maturation temperature of wild oat,

the lower the level of dormancy (Sexsmith, 1969; Peters, 1978; Naylor and Fedic, 1978; Sawhney and Naylor, 1979). However, none of these studies examines the behaviour of ecotypes which have evolved at latitudes less than 40° from the equator.

If the ecotype originating from latitudes higher than 30° north or south are compared (Western Cape, British and North American ecotypes) the mean germination for those grown at the higher temperature was 26,5%, whereas the corresponding figure for those grown at the lower temperature was only 12,5%. It must therefore, be assumed that the reaction of tropical and sub-tropical ecotypes, as regards effect of maturation temperature on dormancy, is essentially different from ecotypes originating at higher latitudes.

All ecotypes germinated significantly better at lower germination temperature, again with the exception of Kenyan *A. fatua* and *A. sterilis* grown at 18°/12°C and also the latter ecotype grown at 26°/20°C.

The Zimbabwean *A. fatua* was very dormant but surprisingly less so at the lower maturation temperature. The result obtained with O.F.S. *A. byzantina* was even more unusual as no germination took place in the seeds matured at the higher temperature irrespective of incubation temperature. All the seeds from these matured at 18°/12°C and incubated at 5°C germinated whereas 44% germinated at 15°C. These figures seem to indicate that this ecotype has a strong stratification requirement if matured at warm temperatures. In its native habitat it experiences fairly cold winters and usually germinates during the winter or early spring. However, this species is known for its irregular germination pattern and it is most probable that this obligate stratification requirement is lost with after-ripening. This hypothesis could not be tested due to a lack of seed but a further experiment conducted in a glasshouse at

Stellenbosch in the summer-yielded seed of this ecotype which germinated in excess of 80% at both incubation temperatures.

Table 3.4 Germination of 11 *Avena* ecotypes grown at 18°/12°C and 26°/20°C day/night temperature and incubated at 5°C and 15°C.

Ecotype	Origin		Germination (%)			
			Growth temperature			
			18°/12°C		26°/20°C	
	Area	Latitude	Incubation temperature			
			5°C	15°C	5°C	15°C
A. byzantina	Kenya	0°C	100	29	93	28
A. fatua	Kenya	0°C	53	87	85	81
A. sterilis	Kenya	0°C	49	57	56	71
A. fatua	Zimbabwe	18° S	9	7	1	1
A. byzantina	O.F.S.	28° S	100	44	0	0
A. barbata	W. Cape	33° S	0	0	0	0
A. fatua	W. Cape	33° S	37	1	77	3
A. ludoviciana	W. Cape	33° S	43	3	52	3
A. sterilis	W. Cape	33° S	21	3	39	4
A. fatua	Montana	47° N	1	0	9	5
A. fatua	Oxford	52° N	16	0	69	4
	$\bar{x}$		39,0	21,0	63,7	18,2
			$\bar{x}$ = 30,0		$\bar{x}$ = 51,0	

LSD = 2,38 (P = 0,05)

Avena barbata did not germinate at all in any treatment. This result is not unexpected as Cairns (1974) found that the Western Cape ecotype of this species was very dormant at harvest and that no germination took place for three

months after harvest. However, dormancy was of short duration and 100% germination was achieved after six months of after-ripening.

All the Western Cape ecotypes germinated much better at the lower incubation temperature. This response to mild stratification is typical of species naturalised in a Mediterranean region. The Montana *A. fatua* was very dormant and gave a very similar mean germination to that obtained with Zimbabwean *A. fatua*. However, the Montana ecotype produced less dormant seeds when grown at the higher temperature. The *A. fatua* from Oxford also showed a strong to mild stratification, particularly those grown at 26°/20°C.

Imam and Allard (1965) working on variability between and within natural populations from different habitats in California, found that geographical variation can be attributed to the selective effects of local environment and that it is adaptive in nature. The nature of the differentiation from site to site and region to region in wild oats, suggests that the variations observed have developed in response to and are maintained by natural selection. These authors also commented on the genetic system of wild oats and suggested that it is admirably suited to occupy a wide range of different habitats. This is brought about by the occurrence of regular low levels of outcrossing (1-10%) between different co-existing genotypes which sets the stage for the recombination that is needed for the development of a diversity of genotypes of ever increasing adaptation to specific environmental niches. At the same time inbreeding provides that most individuals in the population shall be rather highly homozygous, thus providing over a number of generations, genotypes or associations of genotypes which are best able to exploit that particular habitat. Imam and Allard (1965) postulated that the flexibility of the genetic system of wild oats has contributed to the success of this species in occupying many and varied complex habitats.

The success of the various ecotypes of wild oat used in the experiments would seem to bear out the above findings. The rate at which an ecotype can adapt to a new environment would seem to be fairly rapid as wheat has not been grown in Kenya and Zimbabwe for more than 25-35 years at the most, yet, the wild oat ecotypes encountered there, differ significantly from the parental ecotypes from higher latitudes. The considerable environmental component in the expression of dormancy would also confer an adaptive advantage on wild oats. Jana and Naylor (1980) reported that in the Prairie wild oat populations, the environmental component of the expression of dormancy is about 50%. Even so, the mortality of a population of wild oats suddenly transferred to a very different environment to that to which it is adapted, can be heavy during the first few generations. Field plantings at Stellenbosch of some of the foreign ecotypes have resulted in more than 60% mortality within one generation (results not shown).

On the basis of the results of these experiments and due to the world wide importance of *A. fatua* it was decided to carry out further work on the mechanism of dormancy on this species only. The Montana, Western Cape and Kenyan ecotypes represent a wide range of dormancy and were consequently used for most of the further experiments. The Zimbabwean ecotype was used for a few experiments but later rejected due to the poor seed production under glasshouse conditions. This ecotype was also something of an anomaly as all the other tropical and sub-tropical ecotypes were largely non-dormant and it is possible that this ecotype is a fairly recent introduction to this area.

The Montana ecotype is a dark brown inbred population obtained from Prof. Simpson of the University of Saskatchewan, Saskatoon in Canada. The Oxford ecotype was obtained from Dr. N.C.B. Peters, of the A.R.C. Weed Research Organisation, Oxford in England. This was not an inbred population but

was taken from a disjunctive population growing adjacent to a cultivated land. This is a grey-seeded ecotype which produces vast numbers of uniform seeds. Both these northern ecotypes required significantly lengthened photoperiods to induce them to flower in the winter at Stellenbosch. Vernalization for up to six weeks apparently did not alter the photoperiodic requirement.

The Kenyan ecotype was obtained from Mr. Patrick Watala of the Ugandan Agriculture Department. This ecotype contained a longer and a shorter straw subtype which did not differ in level of dormancy. However, the long straw type was selected out and later an in-bred population was derived which originated from a single plant selection. This type had a dark brown seed which was appreciably larger than the two northern ecotypes.

The Western Cape ecotype was derived from an isolated population on Rondebosch Common (which subsequently died out as a result of most of the weeds being harvested). This is a cream-coloured, large-seeded ecotype which does not set viable seed if grown in the summer or under long photoperiods. Fresh seed stocks of these ecotypes were maintained by growing the plants under glasshouse or growth chamber conditions at Stellenbosch.

If at all possible, any set of experiments was conducted with a single batch of seed as different batches of seed, even from homozygous populations derived from a single plant selection differed in their dormancy characteristics and enzyme activities. Seed was stored dry at sub-zero temperatures to maintain dormancy. This procedure was found to be successful and dormancy of the two northern ecotypes increased with time of storage under sub-zero conditions.

CHAPTER 4

THE EFFECT OF PLANT GROWTH SUBSTANCES AND THEIR INTERACTION WITH OTHER FACTORS ON THE DORMANCY OF *AVENA FATUA* SEED

4.1 INTRODUCTION

The control of wild oats by the use of dormancy breaking compounds, either on the parent plant or on seed reserves in the soil, has received little attention but Fay and Goricki (1978) met with limited success in depleting soil reserves of *A. fatua* seed by the application of potassium and sodium azide. Synergism between the different classes of dormancy breakers may suggest cheaper treatments to cause total destruction and/or germination of the wild oat seed burden in the soil, thus saving the cost of repeated annual applications of herbicides. A study of the interaction between the major plant hormones might reveal a simple method of preventing the formation of dormant seed thus providing another avenue of control other than the conventional herbicide approach. This hypothesis will consequently be examined in this study.

Reports on the promotion and inhibition of *A. fatua* germination by light abound in the literature (Simpson, 1978). Much of this confusion is certainly due to differences in physiological phytochrome-mediated reactions to light by ecotypes from different parts of the world as intimated by Hilton and Bitterli (1983). It thus seems necessary that the role of light and its interaction with plant growth substances must be re-examined.

4.2 MATERIALS AND METHODS

4.2.1 Seed source

Seed used in these experiments was harvested by hand from ecotypes of the various *Avena* species described in the previous chapter. The seed was harvested by hand from plants grown at Stellenbosch or Oxford in field plots or in glasshouses and stored at 4°C until required. In experiments where it was essential to preserve the initial dormancy level, the seed was first stored for two to three weeks at 4°C and thereafter transferred to sub-zero temperatures. Freshly harvested seed was damaged if stored directly at sub-zero temperatures due to the high moisture content.

4.2.2 Germination tests

Germination tests were conducted using 9 cm disposable plastic petri dishes lined with three layers of Whatman No. 1 filter paper and moistened with 6 cm^3 of distilled water or test solution. Three replicates of either 20 or 25 seeds were employed. Germination took place in the dark (unless otherwise stated) in temperature-controlled germination cabinets. To prevent evaporation of water or test solution, petri dishes were placed in polythene bags closed with a clip. Treatments which included volatile test solutions were incubated separately.

Germination was recorded at least three times a week and germinated seedlings removed with each count. Germination tests ran for three weeks and viability was tested by dehusking the seeds at the end of this period, pricking them and treating them with 10^{-3} mol dm^{-3} GA_3. The seeds were re-incubated and seed which had not germinated after 14 days of this treatment was considered non-viable (Peters, 1978).

4.2.3 Effect of GA_3 on the dormancy of maturing *A. fatua* seed

Panicles of *A. fatua* were cultured in solutions containing 5×10^{-6}, 5×10^{-5}, 5×10^{-4} and 5×10^{-3} mol dm^{-3} in tap water. The panicles used in this experiment were excised just below the peduncle node just after anthesis of the basal florets in the panicle. The panicles were subsequently cut just above the peduncle node and placed in the various solutions which had been neutralised with Na_2CO_3.

To examine the effect of GA_3 on intact panicles, the hormone was sprayed on mature plants at 50% anthesis at concentrations of 0, 10^{-5}, 10^{-4}, 10^{-3} and 10^{-2} mol dm^{-3}. The solutions were again neutralised and applied at a volume of 250 dm ha^{-3} together with 0,05% 7X wetting agent.

4.2.4 Effect of $GA_{4/7}$, IAA and kinetin on the germination of dehusked *A. ludoviciana* seed

Solutions of $GA_{4/7}$, IAA and kinetin were applied to freshly harvested, dehusked British *A. ludoviciana* seed at 0, 10^{-3}, 10^{-2} and 10^{-1} mol dm^{-3}. Combinations of the above concentrations were tested making 64 treatments in all. A second experiment was also carried out where the kinetin concentration was taken down to 10^{-7} mol dm^{-3} and the IAA concentration to 10^{-5} mol dm^{-3}.

4.2.5 Effect of $GA_{4/7}$, ABA and SD8339 on the germination of *A. fatua*

$GA_{4/7}$ was used in this experiment as it has been shown by Thomas *et al.* (1975) that $GA_{4/7}$ interacted more favourably with the other growth hormones than GA_3 when used to stimulate the germination of celery seed. The growth regulator

concentrations used in this experiment were GA_3: 0, 10^{-5}, 10^{-4}, 10^{-3} mol dm^{-3}; ABA: 0, 10^{-6}, 10^{-5}, 10^{-4} mol dm^{-3} and SD8339 (Adenine, N-benzyl-9-tetrahydro-2H-pyran-2-yl: a synthetic cytokinin) at 0, 10^{-5}, 10^{-4} and 10^{-3} mol dm^{-3}. All combinations of the above concentrations were used.

4.2.6 Effect of light, $GA_{4/7}$ and kinetin on the germination of *Avena fatua* seed which had been treated with water saturated with CO_2

An Oxfordshire ecotype of *A. fatua* was used for this experiment. The seed which initially gave 42% germination, was imbibed for 14 days under water which had been saturated with CO_2. Light and dark treatments were given by placing the petri dishes in transparent polythene bags or in light-proof plastic bags respectively. The light source was natural daylight in a glasshouse (May, Oxford). A factorial design incorporated light, $GA_{4/7}$ at 10^{-5} mol dm^{-3} and kinetin at 10^{-6} mol dm^{-3}.

4.2.7 Effect of light quality, $GA_{4/7}$ and kinetin on the germination of primary dormant *A. fatua* seed

Seeds were germinated in petri dishes as previously described. The red light source consisted of white fluorescent lamps (Philips T.L. 32) filtered with 1 layer each of Cinemoid No. 6 (primary red) and No.1 (yellow), giving and intensity of 0,45Jm^2 s^{-1} in the 600-700nm bands. The far-red light source was provided by 75W incandescent lamps with internal reflectors, filtered with 1 layer of Cinemoid No.20 (primary blue) and 2 layers of red cellophane. This source emitted no light at wavelength below 700nm. The calculated irradiance in the 700-800nm band was also approximately 0,45Jm^{-2} s^{-1}. Kinetin at 0, 10^{-7}, 10^{-6} and 10^{-5} mol dm^{-3}, $GA_{4/7}$ at 0 and 10^{-5} mol dm^{-3} and light quality were incorporated in a

factorial design experiment with 3 replicates. The experiment was carried out at 15°C.

4.2.8 Interaction between $GA_{4/7}$, GA_3 and other chemicals on the germination of *A. ludoviciana* and *A. fatua*

Several chemicals were tested for dormancy-breaking properties both on their own and in combination with sub-optimal GA_3 and $GA_{4/7}$. The chemicals used were: hydroxylamine hydrochloride, sodium azide, ethephon, daminozide and SD8339.

These compounds were tested at 0, 10^{-5}, 10^{-4}, 10^{-3} and 10^{-2} mol dm^{-3}. Application level of GA_3 and $GA_{4/7}$ was 10^{-4} mol dm^{-3}. The respiratory inhibitors azide and hydroxylamine hydrochloride showed strong synergism with $GA_{4/7}$ and further work was conducted with these chemicals. Preliminary trials were carried out with *A. ludoviciana* due to availability of suitable seed but pilot trials were carried out with U.K. *A. fatua*.

The concentration of hydroxylamine hydrochloride and sodium azide were adjusted according to the results of the preliminary screening. Hydroxylamine hydrochloride and sodium azide were tested at 0, 10^{-4}, 10^{-3} and 10^{-2} mol dm^{-3} with and without $GA_{4/7}$ at 2×10^{-5} mol dm^{-3}. Daminozide was applied at 0, 10^{-3}, 10^{-2} and 10^{-1} mol dm^{-3} and $GA_{4/7}$ at 10^{-6} and 10^{-5} mol dm^{-3}.

4.3 RESULTS AND DISCUSSION

4.3.1 Effect of GA_3 on the dormancy of maturing *A. fatua* seed

Only 13% of seed harvested from panicles cultured in 5×10^{-4}

and 5×10^{-3} mol dm^{-3} GA_3 were dormant (Table 4.1). The 5×10^{-5} mol dm^{-3} GA_3 was also effective and 57% germinated. Germination in the other treatments and the control was negligible. These results confirm those of Black and Naylor (1959) who found that *A. fatua* seed harvested from panicles grown in GA_3 solutions, were non-dormant.

Table 4.1 The effect of GA_3 taken up by excised panicles of U.K. *A. fatua* on dormancy of the seed produced

GA_3 (mol dm^{-3})	Germination
5×10^{-6}	5
5×10^{-5}	57
5×10^{-4}	87
5×10^{-3}	87
Control (water)	0
Control (whole plant)	3

LSD 6,1 (P = 0,05)

The next step in this investigation was to establish if foliar applications of GA_3 could be used to induce the production of non-dormant seed in intact plants. Wild oat plants were treated with foliar applications of the various GA_3 solutions in the milk dough stage.

The results of this experiment show that foliar applications of GA_3 were not nearly as effective in breaking dormancy of seeds produced as GA_3 taken up by panicles (Table 4.2). The highest concentration used (10^{-2} mol dm^{-3}) resulted in only 22% germination. The lower concentrations had no effect. This finding would seem to preclude the use of GA_3 as a practical method of inducing field populations of wild oats to

produce non-dormant seed.

Table 4.2 The effect of foliar applications of GA_3 to U.K. *A. fatua* plants on the dormancy of seed produced

GA_3 (mol dm^{-3})	Germination
10^{-5}	6
10^{-4}	3
10^{-3}	2
10^{-2}	22
Control (wetting agent)	0
Control (water)	3

LSD 1,2 (P = 0,05)

In addition to the above germination test (Table 4.2), seed from the plants sprayed with GA_3 was planted outside in Oxfordshire in the U.K. during August. Three replicates of 100 seeds each were placed on the surface and three replicates planted at a depth of 2 cm. The germination recorded is shown in Table 4.3.

The germination of the buried seed was very similar to that of the above experiment where the seed was germinated at 15°C in the dark. However, the seed placed on the surface responded differently. No significant reaction to the GA_3-treatment could be observed and all treatments gave more than 50% germination.

The reason for the differing behaviour of the seeds on the soil surface must be sought in the different conditions to which they were exposed. The experiment was carried out during the summer of 1976 during which the U.K. experienced

very warm, dry weather. This would result in rapid after-ripening of the seed on the soil surface. Also, as Hilton and Bitterli (1983) pointed out, light is stimulatory to the germination of U.K. ecotypes of wild oats and these results are consistent with their findings. The aspect of the influence of light on germination is discussed later (sections 4.3.4 - 4.3.6).

Table 4.3 The effect of foliar application of GA_3 to *A. fatua* plants on the dormancy of seed produced when sown outside under U.K. summer conditions

GA_3 (mol dm^{-3})	Germination (%)	
	Surface sown	Sown 2 cm deep
10^{-5}	59	5
10^{-4}	58	9
10^{-3}	56	7
10^{-2}	67	32
Control (wetting agent)	59	3
Control (unsprayed)	54	6

LSD 8,59 (P = 0,05)

4.3.2 The effect of $GA_{4/7}$, IAA and kinetin on the germination of dehusked *A. ludoviciana* seed

Apart from $GA_{4/7}$, no treatment or interaction between treatments were significant (Table 4.4).

Table 4.4 Analysis of variance on the effect of $GA_{4/7}$, IAA and kinetin on the germination of dehusked *A. ludoviciana* seed

Treatment[x]	F-value
$GA_{4/7}$ (GA)	31,01**
IAA (IAA)	0,52
Kinetin (K)	0,60
GA x IAA	0,06
GA x K	0,38
K x IAA	0,26
GA x K x IAA	0,52

** Significant at P = 0,01

x Concentrations of the growth regulators given in materials and methods

Further experiments (results not shown) where IAA and kinetin were applied at lower concentration, also failed to show any significant effect or interaction. These findings would seem to indicate that IAA and kinetin do not play an important role in the germination of *A. ludoviciana* when applied exogenously. However, with this type of experiment the penetration of the hormone to its site of action is always open to question. Kinetin especially, is notorius for its insolubility and lack of translocation within the plant. This aspect was investigated by applying the hormone dissolved in ethanol to the dry seed. The organic solvent was evaporated before germination was tested. Even under these conditions no effect could be observed. It is however, significant to note that the effect of $GA_{4/7}$ applied in this manner was significantly enhanced.

Sharma *et al.* (1976) found that kinetin and benzyladenine significantly stimulated the germination of Canadian *A. fatua*. Their findings are however open to criticism in that their experiments were carried out "on the lab bench" and that their seed was not very dormant (controls gave 28-42% germination). In addition, all the compounds they tested (KNO_3, $NaNO_3$, NH_4Cl and thiourea) gave a significant stimulation of germination. None of these compounds was found to stimulate the germination of dormant wild oats (Peters, 1978).

4.3.3 The effect of ABA, $GA_{4/7}$ and SD8339 on the germination of *A. fatua* seed

A variance analysis of the germination figures show that once again only $GA_{4/7}$ had any effect on germination (Table 4.5). Although ABA slowed germination down it did not reduce germination. On the contrary, over all treatments the highest germination figures were obtained at the highest concentration of the inhibitor. The result may seem surprising but Berrie *et al.* (1979) could find no relationship between endogenous ABA and dormancy in *A. sativa* or *A. fatua*.

4.3.4 The effect of light, $GA_{4/7}$ and kinetin on the germination of *A. fatua* previously treated with CO_2

The results of this experiment (Fig. 4.1) show that the germination of the untreated seed was completely inhibited in the light. Treatment of the seed with $GA_{4/7}$ resulted in 8% germination but with the addition of kinetin 49% of the seeds germinated. Overall, kinetin increased germination significantly. Although the $GA_{4/7}$ x kinetin interaction was not significant at the 5% level (only one degree of freedom) it was significant at the 10% level. The $GA_{4/7}$ x kinetin x light interaction was also significant (Table 4.6).

Table 4.5 Variance analysis of the effect of $GA_{4/7}$, ABA and SD8339 on the germination of *A. fatua* seed

Treatment[x]	F-value
SD8339 (SD)	$2,4^{ns}$
ABA	$1,16^{ns}$
$GA_{4/7}$ (GA)	$305,05^{**}$
SD x ABA	0,91
SD x GA	$1,14^{ns}$
ABA x $GA_{4/7}$	$1,2^{ns}$
ABA x GA x SD	$1,09^{ns}$

ns = non significant
** = significant (P = 0,01)
x Concentration of growth regulators given in materials and methods

Gibberellin-induced germination of light requiring lettuce seeds (Miller, 1956) and celery seeds (Thomas *et al.*, 1975) was greatly enhanced by kinetin if germination took place in the dark, but germination in the light was optimum with or without kinetin. It is surprising that in the case of *A. fatua*, kinetin stimulated germination of seed which was inhibited by light. Taylor and Simpson (1980) found that in fully after-ripened seeds of *A. fatua* there was marked reduction in the level of detectable cytokinins following imbibition and speculate that the hormone was being used up in the process leading to germination. They also found a positive correlation between the cytokinin and dormancy levels and suggested that this hormone is not a limiting factor for germination.

Table 4.6 Analysis of variance for the interaction of light, $GA_{4/7}$ and kinetin on the germination of U.K. *A. fatua* seed previously treated with CO_2

Treatment[x]		F-value
$GA_{4/7}$	(GA)	96,59**
Kinetin	(K)	5,25*
Light	(L)	111,11**
GA x K		3,18ns
GA x L		15,37**
K x L		3,17ns
GA x K x L		5,30*

ns = non significant
* = significant (P = 0,05)
** = significant (P = 0,01)
x Concentrations of the growth regulators given in materials and methods

Seeds used in this experiment (U.K. *A. fatua*) were induced into a dormant state by being imbibed in water saturated with CO_2 (see 4.2.6). The untreated seed which had not been imbibed was partially dormant (42% germination) whereas the seed induced into a dormant state was totally dormant. The CO_2-treatment obviously did not affect viability as two of the hormone treatments gave 100% germination. The CO_2-treatment may have influenced the reaction to light, as Hart and Berrie (1968) found that there was an interaction between CO_2 and light in partially dormant *A. fatua*. These authors demonstrated that at zero CO_2 and various proportions of O_2, white light inhibited germination of both entire and dehulled seed. At 3% CO_2 with the same range of O_2 concentrations, white light inhibited germination of entire

seed, but not dehulled seed. At 20% CO_2 there was a marked reduction in germination of all treatments, but light had no effect on either the entire or the dehulled seeds. The light/CO_2 interaction disappeared after the seed had been fully after-ripened. Anerobiosis might also have influenced the outcome of the present experiment as Hay and Cumming (1959) found that by the induction of dormancy by high temperatures, white light inhibited germination totally. This inhibition could be partially alleviated by removing the hulls.

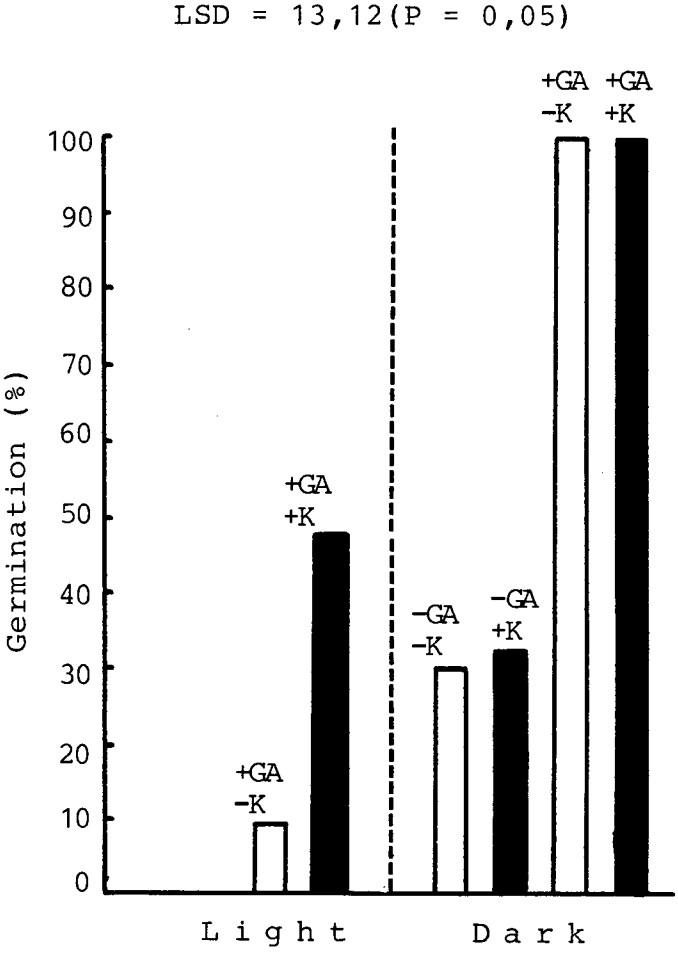

Fig. 4.1 Effect of light, $GA_{4/7}$ and kinetin on the germination of U.K. *A. fatua* seed (GA = $GA_{4/7}$ 10^{-5} mol dm^{-3}, K = Kinetin 10^{-6} mol dm^{-3})

4.3.5 The effect of light quality, $GA_{4/7}$ and kinetin on germination of primary dormant *A. fatua* seed

None of the levels of kinetin had any effect on germination nor was any interaction involving kinetin, significant (Table 4.7).

Table 4.7 Analysis of variance for the interaction of light quality, $GA_{4/7}$ and kinetin on the germination of U.K. *A. fatua* seed

Treatment[x]	F-value
Light (L)	147,9**
$GA_{4/7}$ (GA)	240,0**
Kinetin (K)	0,55
L x GA	27,09**
L x K	0,63
GA x K	0,58
L x GA x K	0,43

** = Significant at P = 0,01

x = Concentrations of the growth regulators given in materials and methods

Both light quality and $GA_{4/7}$, as well as the interaction between these two factors, significantly influenced germination.

The effect of light quality and $GA_{4/7}$-treatment (over all kinetin treatments) is illustrated in Fig. 4.2. Red light was stimulatory to germination. The stimulatory effect of $GA_{4/7}$ with far-red light or in the dark, could be equalled

by red light in the absence of $GA_{4/7}$ (24% germination). Red light was strongly sinergistic to $GA_{4/7}$ activity and $GA_{4/7}$ + red light resulted in 80% germination.

The inhibitory effect of light on the germination of dormant and semi-dormant *A. fatua* seed has been observed by many workers (Hay and Cumming, 1959; Hart and Berrie, 1968; Hsiao and Simpson, 1971 and Cairns 1974). However, as has been pointed out in previous experiments (section 3.2.4) and has been demonstrated by Adkins (1981) and Hilton and Bitterli (1983), U.K. wild oats seem to be peculiar in that germination is stimulated by light. Hsiao and Simpson (1971) showed that light was inhibitory to GA_3-stimulated germination. Quite the reverse was experienced in the present experiment. Hilton and Bitterli (1983) could also find no light-induced inhibition of GA_3-activity on the germination of U.K.*A. fatua*.

It is interesting to note that light-inhibition of germination in U.K. *A. fatua* as well as inhibition of $GA_{4/7}$-induced germination can be induced by imbibing the seed at low temperature in CO_2-saturated water (see section 4.3.4).

4.3.6 Interaction between $GA_{4/7}$ and other chemicals on the germination of *A. fatua* seed

In the absence of $GA_{4/7}$, hydroxylamine and azide had little if any effect on the germination (Fig. 4.3). However, both these compounds showed a synergistic effect with $GA_{4/7}$, particularly hydroxylamine, at the higher concentrations. In other less dormant seed batches (results not shown) azide showed the greater dormancy breaking effect.

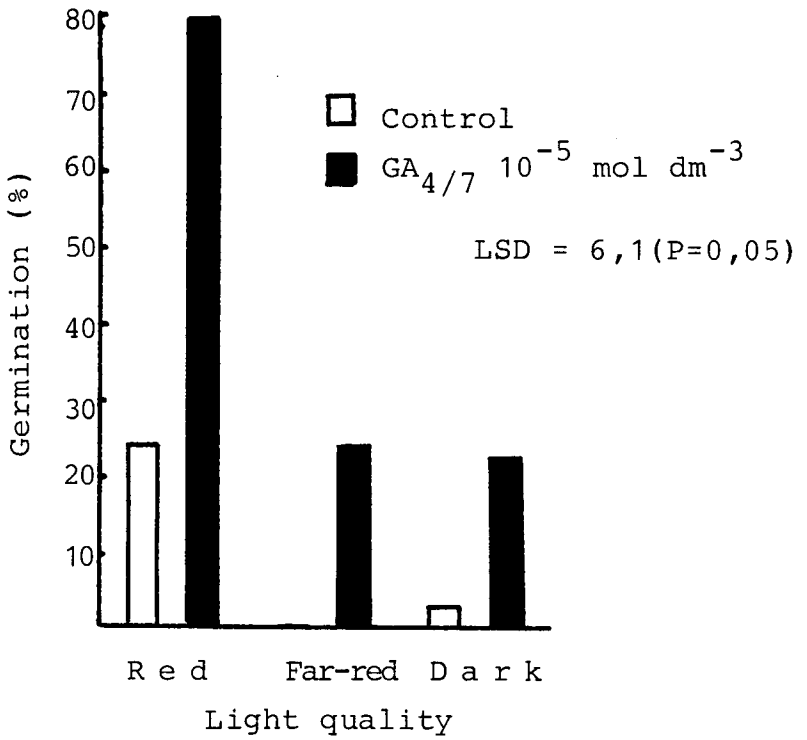

Fig. 4.2 The effect of light quality and $GA_{4/7}$ on the germination of U.K. *A. fatua*

This synergism between respiratory inhibitors and gibberellic acid points to different sites of action for the two types of compounds. Uphadyaya *et al.* (1982) also found a synergistic effect between GA_3 and azide. They explain the dormancy-breaking properties of azide in terms of an increased sythesis of gibberellin. This deduction was made on the basis that the stimulatory effect of azide could be completely inhibited by 2-chloroethyl trimethylammonium chloride (CCC), an inhibitor of gibberellin biosynthesis. However, they were unable to break the dormancy of the deeply dormant ecotype Montana 73 with azide. Dormancy in this line could however, be broken with GA_3. This result does not seem to be consistent with their hypothesis that azide stimulates gibberellin biosynthesis but it is explained in terms of the presence of a second metabolic block in Montana 73.

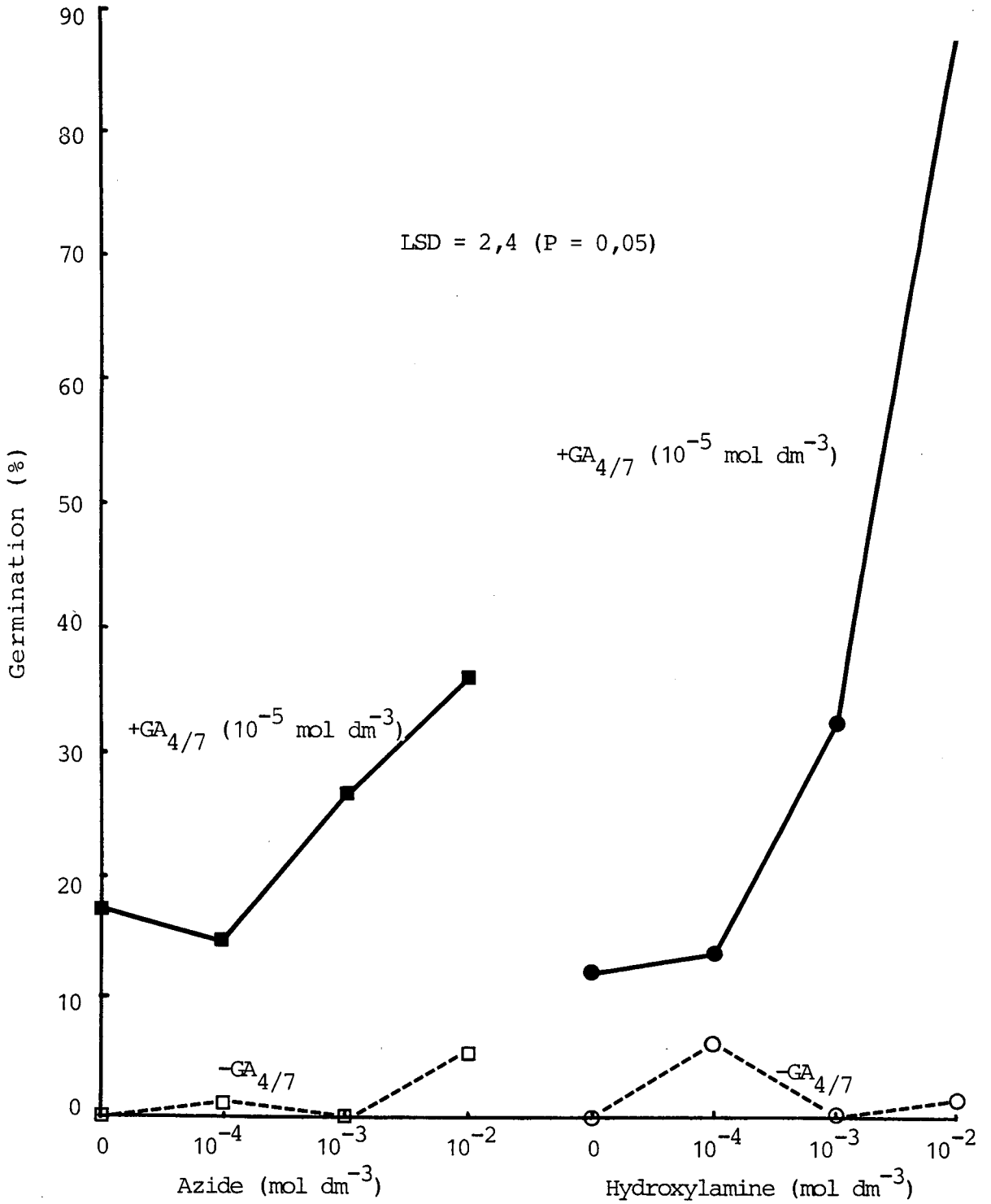

Fig. 4.3 Effect of interaction between azide and hydroxylamine and $GA_{4/7}$ on the germination of U.K. *A. fatua* seed

Another inconsistency in their reasoning is that SHAM is able to block azide stimulated germination but not GA_3-stimulated germination (Uphadyaya *et al.*, 1982, 1983).

It has been suggested that alternative respiration may play an important role in the control of seed dormancy (Esashi, 1979) and that respiratory inhibitors such as azide stimulate germination by increasing the role of the alternative respiratory pathway. Uphadyaya *et al.* (1983) obtained up to a 400% increase in respiratory activity in wild oat seed which had been treated with azide, but a similar stimulation of respiratory activity could be observed in Montana 73 without any stimulation of germination. This matter was investigated more fully later (see Chapter 6).

Daminozide also interacted with $GA_{4/7}$ to stimulate germination of *A. fatua* (Fig. 6.4). The combination of 10^{-1} mol dm^{-3} daminozide and 10^{-5} mol dm^{-3} $GA_{4/7}$ resulted in 95% germination whereas $GA_{4/7}$ on its own at the same concentration gave only 12% germination. Daminozide on its own had practically no effect on germination. $GA_{4/7}$ at 10^{-6} mol dm^{-3} also had no effect on germination but when combined with the highest concentration of daminozide (10^{-1} mol dm^{-3}) resulted in 52% germination.

These results may appear surprising, given the fact that daminozide is a known growth retardant which inhibits the biosynthesis of gibberellins (Cathy, 1964). However, Thomas *et al.* (1975) found appreciable stimulation of celery seed germination if daminozide was added to low concentrations of $GA_{4/7}$. Daminozide was found by Thomas *et al.* (1974) to increase the cytokinin content of celery seed and they explain the synergistic effect of daminozide and $GA_{4/7}$ on germination of celery seed in terms of an inactivation of an inhibitor which then allows $GA_{4/7}$ to stimulate germination. However, it would appear from experiment 4.3.2 that cytokinin *per se* does not influence *A. fatua* germination - at least not

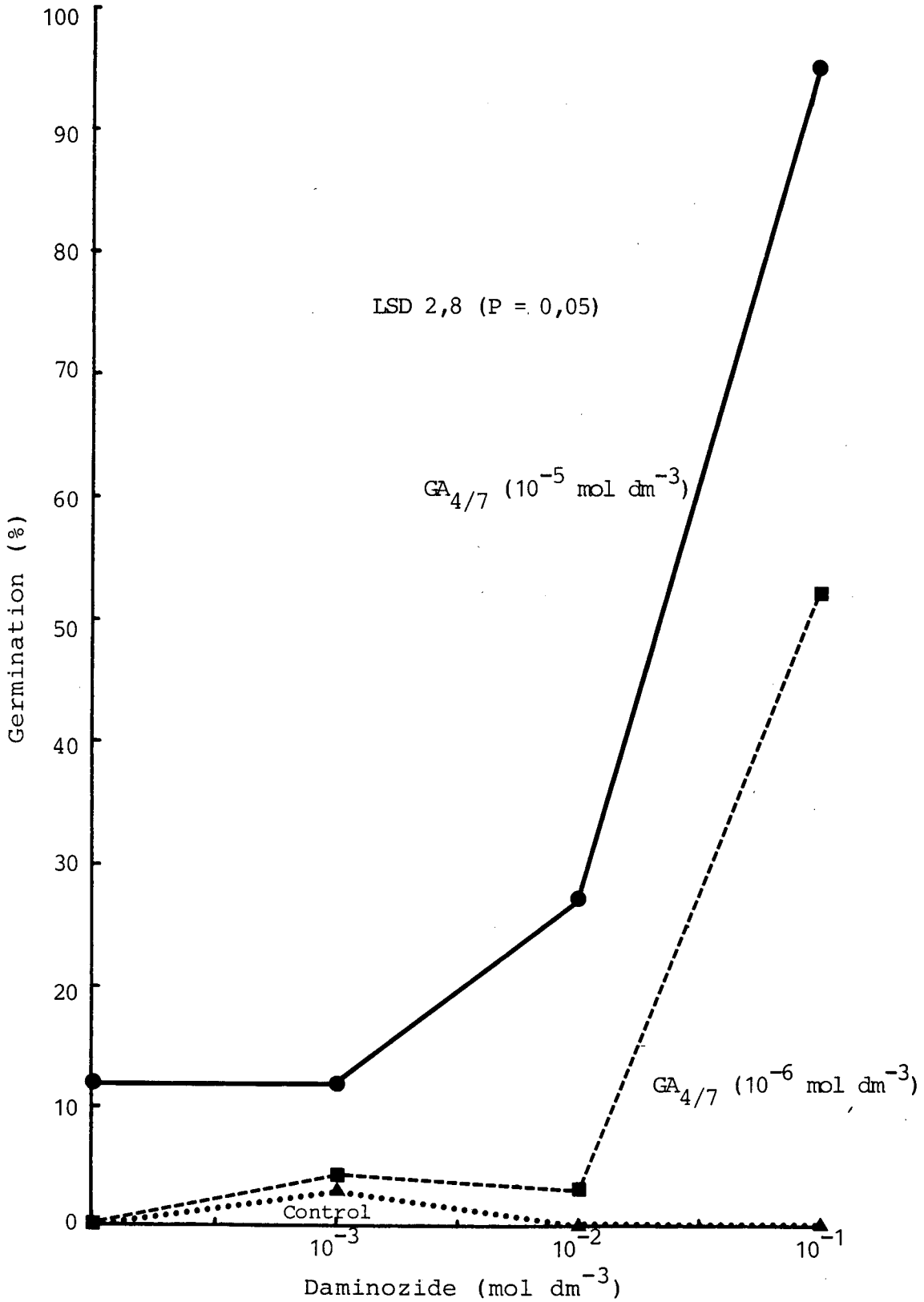

Fig. 4.4 Effect of the interaction between daminozide and $GA_{4/7}$ on the germination of U.K. *A. fatua* seed

in the classically demonstrated way whereby cytokinins either satisfy a light requirement or deactivate gibberellin inhibitors (Kahn, 1971).

The answer to the observed synergism between $GA_{4/7}$ and daminozide in the stimulation of *A. fatua* seed germination might be found in the work of Kuo and Pharis (1975) who found that the treatment of *Cupressus arizonica* seedlings resulted in an 11-fold increase of gibberellin-like compounds. The authors postulated that daminozide does not cause dwarfing by interfering with either gibberellin biosynthesis or activity but rather by an inhibition of auxin biosynthesis. They also suggested that daminozide may be involved in the interconversion of various gibberellins which show a higher biological activity. No gibberellin or IAA determinations were carried out in the present study and it is therefore not possible to say if daminozide had a similar mode of action in the stimulation of *A. fatua* seed germination but the dramatic and intriguing increase of $GA_{4/7}$ activity by this so-called growth inhibitor merits further investigation in the future.

CHAPTER 5

EFFECT OF VARIOUS SUGARS ON GIBBERELLIC ACID SENSITIVITY AND DORMANCY OF *AVENA FATUA* SEED

5.1 INTRODUCTION

The effect of environmental conditions experienced by wild oat seed during maturation has an important effect on the dormancy thereof (Sexsmith, 1969; Peters, 1978 and Sawhney and Naylor, 1979). Environmental variables such as temperature, photoperiod, moisture availability and fertility level of the soil all play a role in the determination of the dormancy status. The physiological basis of this phenotypical expression of dormancy has however, not been elucidated. This chapter is thus devoted to the study of one of the components of seed maturation which may influence dormancy viz. level of assimilates which are available to the developing seed.

5.2 MATERIALS AND METHODS

5.2.1 Culture of *A. fatua* panicles in various sucrose solutions

Plants from a non-dormant line of *A. fatua* originally derived from Kenya, were grown in a glasshouse at 21°/15°C day/night temperature during the winter at Stellenbosch. Panicles were grown in sucrose solutions according to the method employed by Nichols (1979). Panicles were excised from the plant just above the flag leaf node when the apical florets were in the soft dough stage. The basal florets on the panicle which matured much later had by this stage just been fertilized. The flag leaf lamina and leaf sheath were

removed before the panicles were placed in 250 cm^3 Erlenmeyer flasks containing the various sucrose solutions (see results and discussion for further details). The flasks were then placed in a growth chamber at 18°/25°C night/day temperature and exposed to a 12 h-photoperiod. Solutions were renewed and the peduncle tip recut (about 1 cm from the base) every two days. Ripe seed was harvested by gently tugging the awns. If any resistance to disarticulation was encountered, the seeds were left for a subsequent harvest. Seed was harvested daily and stored at 4°C in the dark until required.

5.2.2 α-Amylase synthesis by de-embryonated endosperm halves

The potential of de-embryonated endosperm halves, excised from seed harvested from the sugar-grown panicles, to synthesise GA_3-induced α-amylase was investigated. Extraction of the α-amylase was based on the method of Nichols (1979) and assayed according to the method of Barnes and Blakeney (1974). The seed was dehusked, cut in half and the distal endosperm half used to study the capacity to synthesise α-amylase in the presence of GA_3. The proximal embryo-containing halves of the seed were retained for other experiments involving the excised embryo.

Ten de-embryonated endosperm halves were transferred to 7 cm petri dishes lined with three Whatman No.1 filter papers and moistened with a solution containing 20 mmol dm^{-3} $Ca(NO_3)_2$ and 5×10^{-6} mol dm^{-3} GA_3. The de-embryonated endosperm halves were then incubated in the dark for 48 h at 30°C. Incubation was terminated by freezing.

Extraction of the enzyme commenced with the thawing of the contents of the petri dishes. The slurry was then macerated in a mortar and pestle together with 10 cm^3 of extraction buffer containing 58,4 mmol dm^{-3} sodium chloride and 1,14 mmol dm^{-3} calcium acetate. The slurry was then decanted

into 50 cm^3 Erlenmyer flasks and extracted on a shaker bath for 60 min at 30°C. The homogenate was then transferred to centrifuge tubes and centrifuged at 25 000 x g for 30 min at 1°C. Five cm^3 aliquots of the clear supernatant were then drawn off and decanted into 30 cm^3 test tubes containing a further 5 cm^3 of the sodium chloride/calcium acetate buffer.

α-Amylase activity was assayed according to the method of Barnes and Blakeney (1974). The extracts were incubated with a highly specific dye-labelled substrate ("Phadabas" tablets from Pharmacia, Uppsala, Sweden). These tablets consist of a substrate made by cross-linking partially hydrolized potato starch, using 1,4 butandiol-diglicidether as the cross-linking agent. The soluble starch is transformed into a three-dimensional insoluble lattice network which swells in water. The degree of swelling is regulated by the number of cross-linked bridges formed and this also controls the degree of susceptibility of the substrate to enzyme degradation. The substrate is labelled with Cibacron blue by covalent bonds. Each tablet contains 45 mg of dry starch and 25 mg of buffer ($Na_2PO_4.2H_2O$, KH_2PO_4 and NaCl) to give a final concentration of 0,2 mol dm^{-3} phosphate buffer, pH 7, and 0,05 mol dm^{-3} NaCl. The tablets also contain bovine serum albumin. α-Amylase hydrolizes the blue starch polymer into a water soluble blue dye which absorbs light at 620 nm (Grierson, 1981).

Incubation of the extracts took place at 50°C for various periods depending on the activity of the enzyme. The reaction was terminated by adding 1 cm^3 of 0,5 mol dm^{-3} NaOH to each test tube after which the tubes were shaken vigorously and left to stand overnight to allow the undigested substrate to settle. Alternatively the samples were centrifuged for 10 min at 20 000 x g. A 5 cm^3-aliquot of the supernatant was made up to 10 cm^3 with the above sodium chloride/calcium acetate buffer and the absorbance read at

620 nm.

The effect of exogenously applied sugars on the ability of de-embryonated endosperm halves to synthesize α-amylase in the presence of GA_3 was investigated under sterile conditions. De-embryonated endosperm halves derived from whole plants grown in the glasshouse at 21°/26°C night/day temperature, were incubated in the presence of various concentrations of the various sugars. De-embryonated half-seeds were sterilized in 1% NaOCl (diluted commercial "Jik") for 90 min. The seeds were subsequently washed 10 times with sterile water under sterile conditions. The seeds were then transferred to 50 cm^3 Erlenmyer flasks and incubated with 5 cm^3 of the various sugar solutions in 20 mmol dm^{-3} $Ca(NO_3)_2$ and 10^{-8} mol dm^{-3} GA_3 for 48 h on a shaker bath at 30°C.

All solutions were sterilized either by autoclaving or by filtration through a 0,25 µm Millipore filter and all manipulations up to and including the incubation period were conducted in a laminar-flow sterile cabinet. The determination of α-amylase activity was determined as previously described. α-Amylase activity was expressed as enzyme units where one unit of α-amylase activity is defined as the amount of enzyme catalyzing the hydrolysis of 1 µmol dm^{-3} glucosidic linkage at 50°C.

Germination studies were carried out as previously described.

5.2.3 α-Amylase synthesis by excised embryos with attached scutella

Excised embryos were obtained by first dehusking and imbibing the seed at room temperature in distilled water for 2 h to soften the tissues. The testa and pericarp were removed by making a lateral incision with a scalpel on both sides of

the base of the embryo below the radicle to a point just behind the distal extremity of the scutellum. The testa and pericarp, covering the embryo and scutellum, were then carefully peeled off with a pair of tweezers taking care not to damage the embryo. The tip of the scutellum was then gently lifted out of the endosperm with a dissecting needle. The needle was then moved slowly down under the scutellum towards the embryo, thus separating the scutellum and embryo from the endosperm. Any endosperm that still adhered to the scutellum was carefully removed with a scalpel. The embryos were then dried and stored at room temperature until needed.

The synthesis of α-amylase by whole embryos (embryo plus attached scutellum) was investigated by incubating them on an agarose/blue starch substrate. The α-amylase substrate was the same as that used in the "Phadabas" tablets, the composition of which has been previously described. However, this substrate was much finer and was specially designed by Pharmacia for use in agarose. The agarose/substrate mixture was formulated essentially as described by Hejgaard and Gibbons (1979) but was supplemented by GA_3 at 10^{-6} mol dm^{-3} and varying amounts of sucrose (see results and discussion). Sixty mg of agarose was dissolved in 6 cm^3 boiling 50 mmol dm^{-3} phosphate buffer (pH 6,9) containing 20 mmol dm^{-3} $CaCl_2$ and 16 mg of the blue starch substrate. The mixture was vigorously stirred to ensure uniform suspension and poured on to a 8 x 5 cm glass plate thereby forming a uniform 1,5 mm gel layer.

After the plates had cooled, the whole embryos were placed on the surface of the substrate with the scutellum facing downwards and covered with a second glass plate which was lightly pressed down to exclude air. The substrate containing the embryos was thus sandwiched between the two glass plates. The plates were then incubated in a humid atmosphere at 15°C for 120 hrs (shorter periods of incubation

at higher temperatures were also employed but the most reliable and reproducible results were obtained at 15°C). The enzyme reaction was stopped by immersing the plates in 5% acetic acid for 2 hrs. The dye released from the digested substrate diffused into the acetic acid solution and α-amylase activity was determined by measuring the diameter of the circular opaque zone of digested substrate surrounding the embryo by means of a slide calliper.

5.2.4 Determination of soluble sugars

Sugars were extracted according to a method based on that of Coble and Slife (1971). One hundred mg of milled dehusked *A. fatua* seed was extracted with 10 cm^3 of 50% ethanol at 70°C for 1 h. The suspension was centrifuged at 20 000 x g and a 5 cm^3 aliquot of the supernatant was drawn off and freeze dried overnight. The sugar was determined quantitatively according to the antrone method of Deywood as amended by Hansen and Moller (1975). The residue was redissolved in 10 cm^3 35% perchloric acid. A 0,2 aliquot was transferred to a test tube and 10 cm^3 of the anthrone reagent added (1 g anthrone dissolved in 500 cm^3 72% sulphuric acid and stored at 0°C). The test tubes were then placed in a boiling water bath for 10 min and the absorbance determined at 630 nm in a Hitachi spectrofotometer.

5.3 RESULTS AND DISCUSSION

5.3.1 Dormancy of *A. fatua* seed harvested from panicles grown in various concentrations of sucrose

Dormancy of *A. fatua* seed harvested from panicles grown in solutions containing 0; 0,075; 0,150; 0,225 and 0,300 mol dm^{-3} sucrose is presented in Fig. 5.1. Dormancy increased

progressively with increase in sucrose concentration. Forty eight percent of the seed harvested from panicles grown in tap water germinated compared to only 12% of those harvested from panicles grown in 0,3 mol dm^{-3} sugar solution. Viability of the seed was not affected by any of the treatments. In other similar experiments (results not shown), different ecotypes were grown in the sucrose solutions with very similar results.

Various authors (Sexsmith, 1969; Peters, 1978 and Sawhney and Naylor, 1982) have reported that *A. fatua* plants that are subject to drought stress during seed maturation, produce seed which are less dormant than the well-watered controls. The physiological basis of this phenomenon has not been investigated, but Peters (1978) found that the α-amylase content of drought-stressed seeds was approximately four times greater than in seed from well-watered control plants. He speculated that the level of α-amylase and other hydrolytic enzymes may have led to lesser dormancy in the drought-stressed seeds.

In the present experiment the increased osmotic concentration of the sucrose solutions could be expected to mimic the effect of moisture stress and thus cause the production of less dormant seed. However, the reverse was true and it would appear that sucrose was acting primarily as a physiological active substance and not as an osmoticum.

To test this hypothesis a further experiment was conducted. Other osmotically active substances such as mannitol and polyethylene glycol (PEG) were compared to sucrose in a panicle culture experiment. The dormancy of seed harvested from panicles grown in mannitol and PEG at 0,3 mol dm^{-3} was very similar to the water-grown controls but seeds harvested from panicles grown in 0,3 mol dm^{-3} sucrose were much more dormant, and similar in dormancy level to seed harvested from the whole plant controls (Fig. 5.2). The whole plant

control represents seed harvested from intact plants of the same batch from which the panicles for the other treatments were excised. All treatments including the whole plant control matured side by side in a glasshouse.

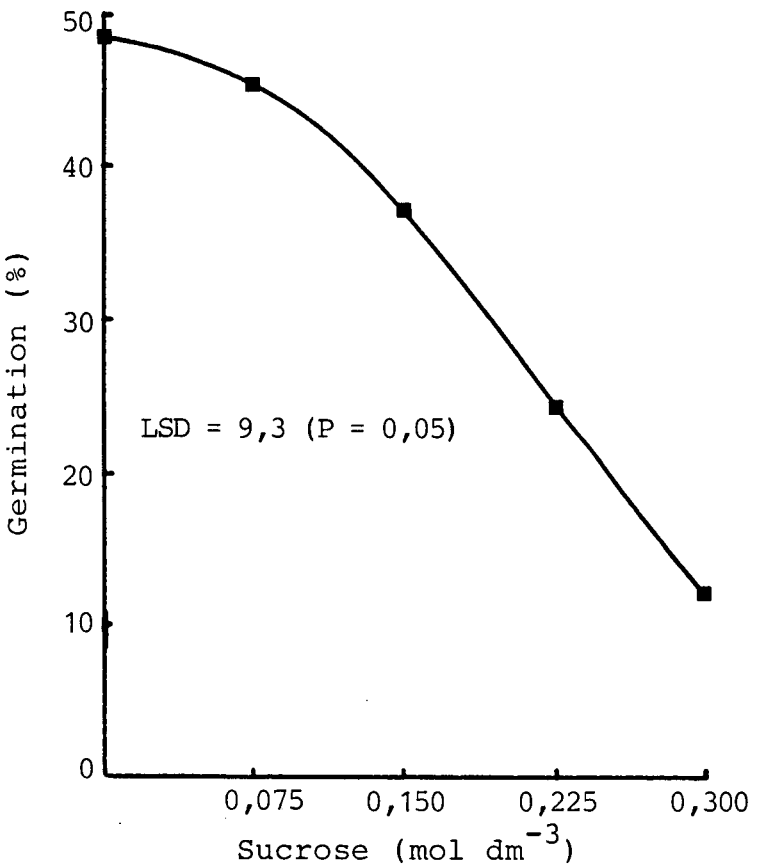

Fig. 5.1 Germination of *A. fatua* seed harvested from panicles grown in sucrose solutions.

These results illustrate clearly that sucrose was not acting primarily as an osmoticum. The mannitol, PEG and water control treatments could be expected to lead to a lower flux of assimilates entering the developing caryopsis - indeed seed from these three treatments were small although their viability was unimpaired. Seed formed on the panicles grown in the sugar solutions was plump and very similar in external morphological appearance to the seed formed on the whole plant controls, except thet the seed from the

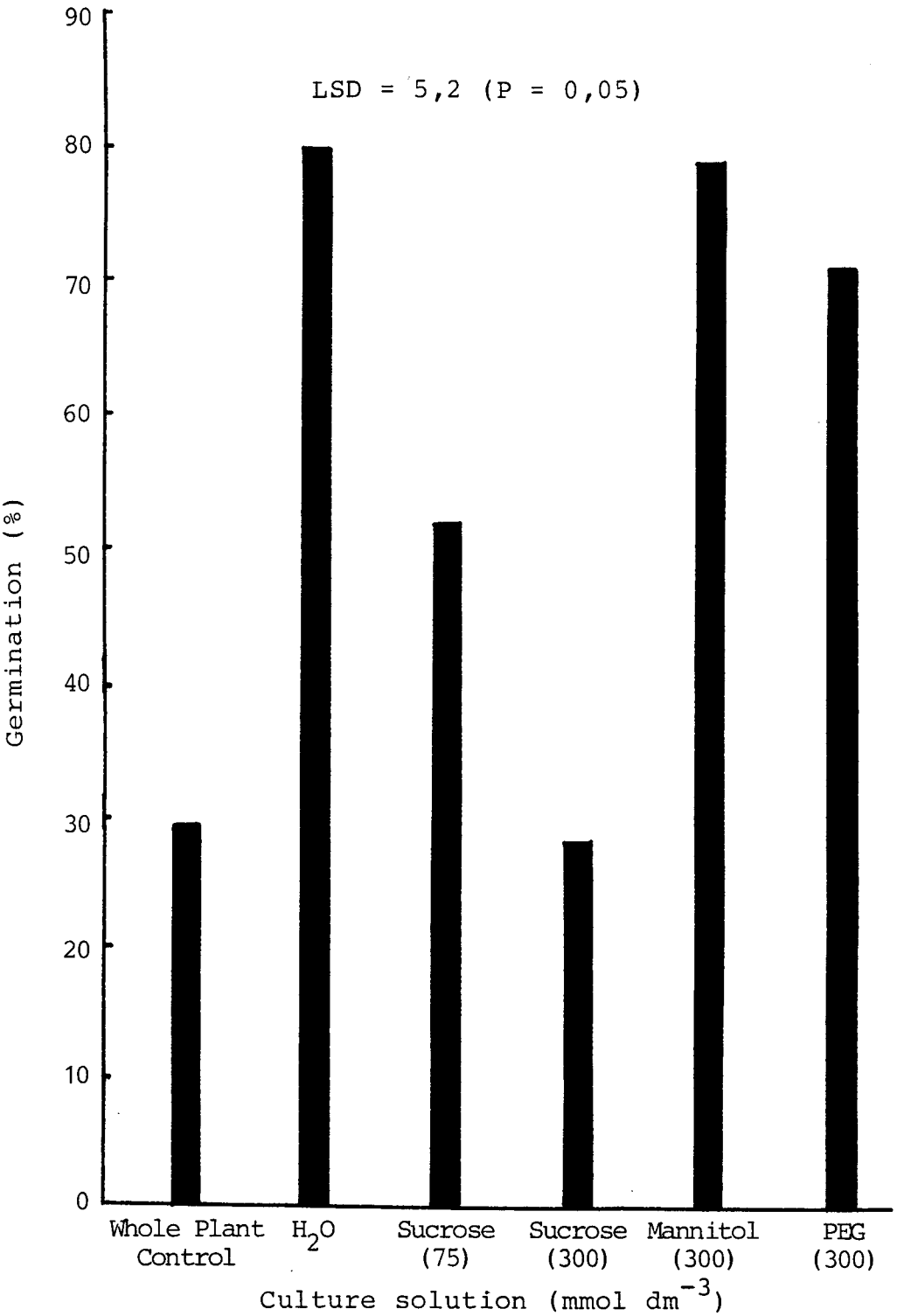

Fig. 5.2 Effect of culture solution on the dormancy of seeds produced by excised *A. fatua* panicles

0,3 mol dm^{-3} sucrose treatments was slightly plumper than those from the whole plant controls.

Dormancy in the sucrose-grown seed could be relieved by treatments commonly used to break dormancy in *A. fatua* seed. Seed harvested from the panicles grown in 0,3 mol dm^{-3} sucrose was fumigated with "Phostoxin" (see following chapter for further details), treated with GA_3 and dehusked and pricked. All these treatments were effective in breaking sucrose-imposed dormancy (Table 5.1). These results show that viability of the sucrose grown seed was unimpaired and that sucrose-imposed dormancy appeared to be somewhat different to conventional dormancy in that the GA_3-treatment was less successful in breaking dormancy than dehusking and pricking.

Table 5.1 The effect of three treatments on the breaking of dormancy of *A. fatua* seed harvested from panicles grown in a 0,3 mol dm^{-3} sucrose solution

Treatment	Germination (%)
Fumigation with "Phostoxin" for 48 hrs	80
GA_3 (10^{-3} mol dm^{-3})	85
Dehusking and pricking	100

Black and Naylor (1959) grew *A. fatua* panicles in water and GA_3 solutions and found that seed from the latter treatment were non-dormant compared to seed from the former which was dormant. An experiment was thus devised to determine if the dormancy-inducing effect of sucrose could be counteracted by the addition of GA_3 to the growing medium. The photosynthetic inhibitor simazine was also introduced into this trial to determine if the inhibition of photosynthesis

would affect the dormancy of the seed produced. The concentrations of the components of the culture medium was as follows (in mol dm^{-3}): sucrose, 10^{-3}; $GA_{4/7}$, 10^{-4} and simazine 1,4 x 10^{-5}. Germination of the freshly harvested seed was tested and the remainder of the seed was stored at 30°C in the light; conditions which could be expected to cause rapid after-ripening of the seed. Germination was subsequently recorded at monthly intervals for four months.

The results of this trial (Fig. 5.3) show that in the initial germination test the addition of sucrose to the $GA_{4/7}$ treatment (treatment 4) resulted in a highly significant reduction in the number of seeds germinating when compared to $GA_{4/7}$ on its own (treatment 5). The addition of simazine to the sucrose + $GA_{4/7}$ treatment (treatment 6) significantly increased germination when compared to the sucrose + $GA_{4/7}$ on its own but was still significantly lower than the $GA_{4/7}$ treatment (treatment 5).

When treatments 4 and 6 were compared one month after harvest, no significant difference could be observed. At the subsequent three samplings (2, 3 and 4 months after harvest) no difference between the three treatments containing $GA_{4/7}$ (treatments 4, 5 and 6) could be seen. Germination of treatment 1 (sucrose at 0,3 mol dm^{-3}) and treatment 7 (whole plant control) was similar throughout and although germination rose steadily with increasing time of storage, germination was always significantly lower than any of the $GA_{4/7}$ treatments. The water control (treatment 3) also produced significantly more dormant seed than the $GA_{4/7}$ treatments for the first two months but thereafter the two treatments did not differ significantly.

The most interesting treatment was the combination of sucrose and simazine which remained significantly more dormant than any of the other treatments throughout the experiment. It

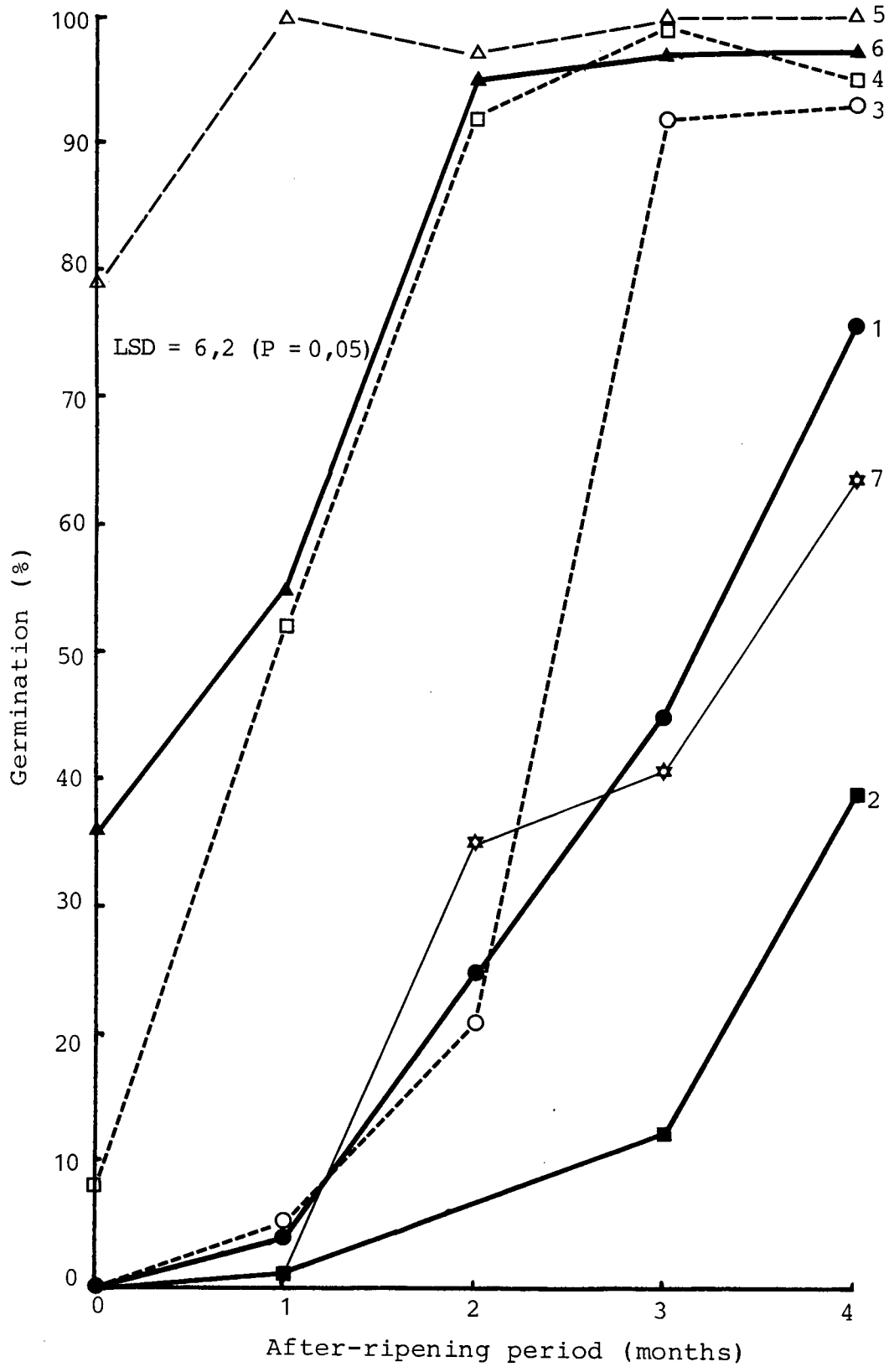

Fig. 5.3 Effect of duration of after-ripening on germination of *A. fatua* seed harvested from panicles grown in various solutions

1. Sucrose 0,3 mol dm^{-3} 2. Sucrose 0,3 mol dm^{-3} + simazine $1,4 \times 10^{-5}$ mol dm^{-3}
3. Water control 4. Sucrose 0,3 mol dm^{-3} + $GA_{4/7}$ 10^{-4} mol dm^{-3}
5. $GA_{4/7}$ 10^{-4} mol dm^{-3} 6. Sucrose 0,3 mol dm^{-3} + $GA_{4/7}$ 10^{-4} mol dm^{-3} + simazine $1,4 \times 10^{-5}$ mol dm^{-3} 7. Whole plant control

could be argued that this treatment, being entirely dependent on exogenous sucrose for its assimilates, had a sub-optimal carbohydrate balance but this would also apply to treatment 6 (sucrose + $GA_{4/7}$ + simazine) which was not very dormant. It is however, more likely that by stopping photosynthesis, the production of some germination-stimulating substance is interrupted. If this is exogenously applied as in treatment 6, the seed produced are non-dormant.

Some evidence to support this hypothesis comes from the work of Richardson (1979) who found that if he removed the glumes of the developing *A. fatua* seeds during maturation, dormancy was greater than in the untreated controls. He speculated that this might be as a result of a lack of germination-stimulating substance(s) which are produced in the glumes.

Simazine could also be acting as a cytokinin as Nader *et al.* (1975) found that simazine has appreciable cytokinin-like activity. The level of endogenous cytokinins in *A. fatua* seed was found by Taylor and Simpson (1980) to be inversely proportional to the dormancy level of the seed with cytokinin content declining with after-ripening. It appears from the work on the effect of cytokinins on the germination of *A. fatua* in Chapter 4 that exogenous applications of this plant growth substance do not affect germination, but cytokinins taken up during maturation, may well affect dormancy. Further work, using the above technique, should be undertaken to elucidate this matter.

The fact that the sucrose level available to the developing seed can affect dormancy, is very interesting as this would form the basis of a hypothesis which would explain why climatic conditions experienced by the wild oat mother plant can influence the level of the dormancy of seeds produced.

Precisely how an increased carbohydrate supply influences

the dormancy of seed is not known. Analysis of non-dormant seeds harvested from seed grown in distilled water and those grown in 0,3 mol dm^{-3} sucrose revealed that the total soluble sugar content was exactly the same in both batches of seed (3,88 mg g^{-1} seed).

5.3.2 Effect of sugars on the synthesis of α-amylase by de-embryonated endosperm halves of *A. fatua*

A pilot experiment was carried out to determine if exogenously applied sucrose could influence the ability of de-embryonated endosperm halves to produce α-amylase in the presence of GA_3. The results of this experiment (presented in Table 5.2) indicate that the presence of 0,3 mol dm^{-3} of sucrose strongly inhibited the ability of GA_3 to stimulate synthesis of α-amylase. This experiment was repeated on another line of *A. fatua* with very similar results.

Table 5.2 Effect of exogenous sucrose on the synthesis of α-amylase by de-embryonated endosperm halves (Pilot experiment)

Treatment	α-Amylase activity (mEu g^{-1} endosperm)	
	GA_3	
	10^{-4} mol dm^{-3}	10^{-3} mol dm^{-3}
Sucrose (0,3 mol dm^{-3})	6,9	13,4
Control	33,1	190,0

The next step in the investigation was to establish if other sugars had the same effect on GA_3-stimulated α-amylase production. Consequently, de-embryonated endosperm halves were incubated with GA_3 at 10^{-8} mol dm^{-3} and with glucose, fructose, maltose, sucrose or raffinose at 0, 0,1; 0,2; 0,3 and 0,4 mol dm^{-3}. The effect of different concentrations of these sugars on α-amylase synthesis by de-embryonated endosperm halves is illustrated in Fig. 5.4. These results show that both glucose and fructose showed an appreciable stimulation of α-amylase synthesis at 0,1 mol dm^{-3}. These sugars also stimulated synthesis of the enzyme at 0,2 and 0,3 mol dm^{-3} but were both inhibitory at 0,4 mol dm^{-3}. Maltose at 0,1 mol dm^{-3} showed a significant stimulation of α-amylase synthesis but became progressively more inhibitory at the higher concentrations. Sucrose showed only slight stimulation of α-amylase production at the 0,1 mol dm^{-1} level but was inhibitory at the higher concentrations. Raffinose proved inhibitory at all concentrations.

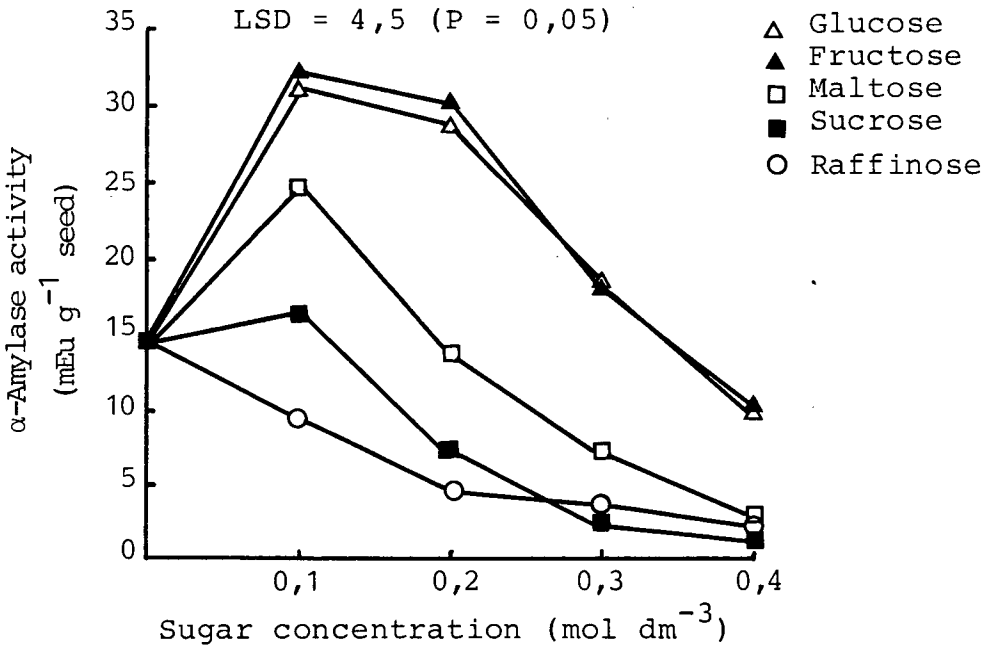

Fig. 5.4 Effect of sugar concentration on α-amylase synthesis by de-embryonated endosperm halves of *A. fatua* in the presence of 10^{-8} mol dm^{-3} GA_3.

As sucrose is by far the most important sugar during the development and filling of the cereal grain (Duffus and Cochrane, 1982) and also due to the fact that this sugar showed only a slight stimulation of α-amylase production at 0,1 mol dm^{-3}, the effect of lower concentrations of this sugar was examined (Fig. 5.5). These results show that the optimum concentration of sucrose for α-amylase synthesis lies between 0,025 and 0,075 mol dm^{-3}.

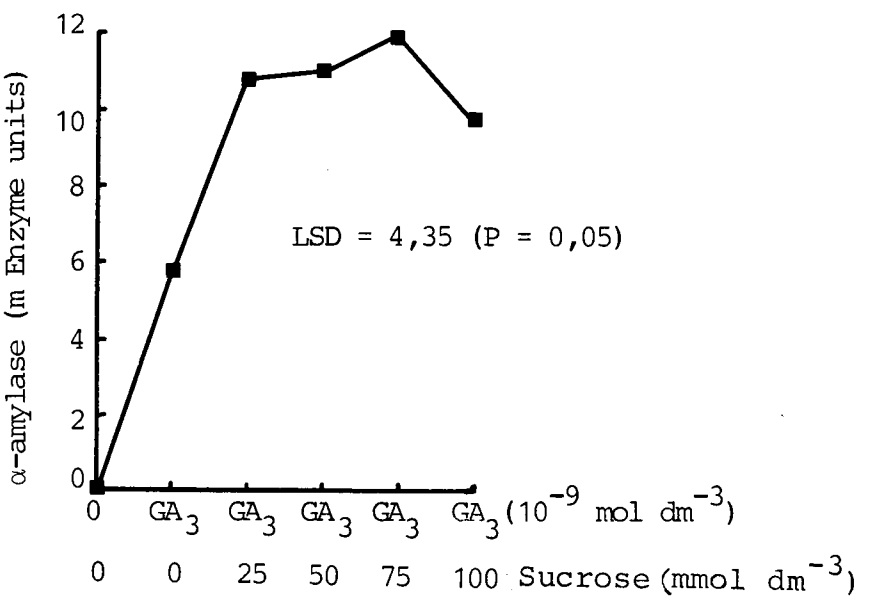

Fig. 5.5 Effect of low concentrations of sucrose on GA_3-stimulated α-amylase synthesis in de-embryonated endosperm halves.

Endosperm halves excised from the seed harvested in an earlier experiment (Fig. 5.1) were also tested for GA_3-stimulated α-amylase synthesis and the results are presented in Fig. 5.6. It is clear that here too, the increased sucrose concentration in which the panicles were grown inhibited the ability of de-embryonated endosperm halves to synthesis α-amylase in the presence of GA_3.

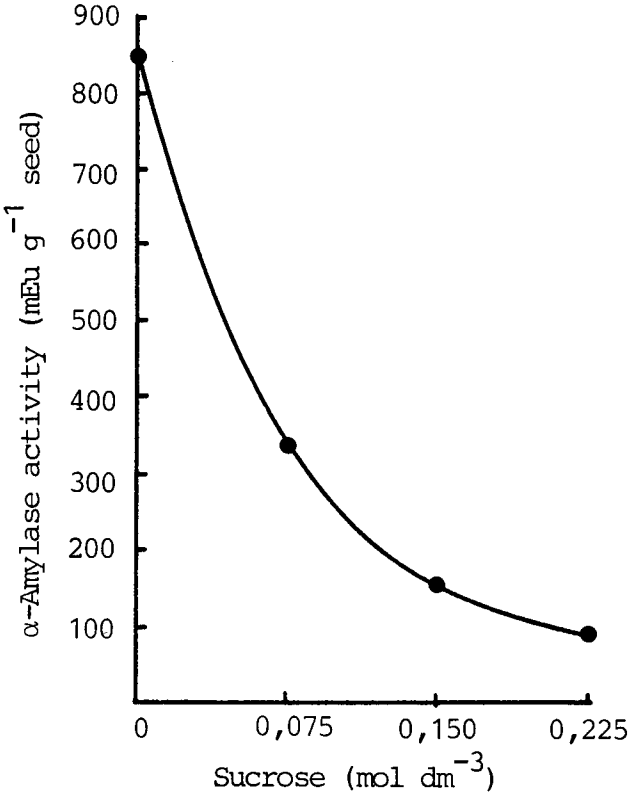

Fig. 5.6 Effect of sucrose in which *A. fatua* panicles were grown on the ability of de-embryonated endosperm halves, produced from the panicles, to synthesize α-amylase in the presence of 5×10^{-6} mol dm^{-3} GA_3.

5.3.3 Effect of sucrose on the synthesis of α-amylase by excised *A. fatua* embryos

The effect of the various concentrations of sucrose included in the agarose/blue starch on α-amylase synthesis is presented in Fig. 5.7. As in the case of the de-embryonated endosperm halves, α-amylase synthesis showed the same promotion/inhibition to increasing sucrose concentration. It is significant that the optimum sucrose concentration for α-amylase synthesis in excised embryos was the same for de-embryonated endosperm halves (75 mmol dm^{-3}).

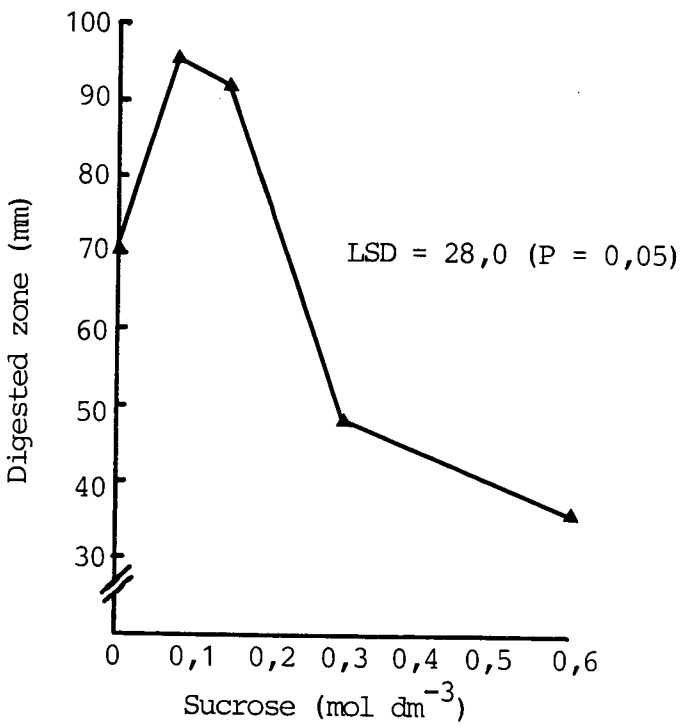

Fig. 5.7 Effect of sucrose on the synthesis of α-amylase by excised *A. fatua* embryos.

As previously stated the substrate contained 10^{-6} mol dm^{-3} GA_3. However, it was found that no difference in α-amylase synthesis occurred if GA_3 was omitted from the substrate mixture (results not shown). Similar results were reported by Simpson and Naylor (1962) who found that the embryo and scutellum of *A. fatua* were insensitive to GA_3 as regards α-amylase synthesis. Thus sucrose is able to stimulate the production of α-amylase in the embryo and scutellum in the absence of GA_3. Uphadyaya et al. (1981) found that the endosperms of some non-dormant lines of *A. fatua* could synthesize α-amylase in the absence of exogenous GA_3 and Nicholls (1983) found that in barley grown at high temperatures, the

aleurone layer synthesised α-amylase, independent of either embryo or exogenous GA_3. He also found that this facility to produce α-amylase, did not depend on high residual amounts of endogenous gibberellins in the aleurone tissue.

On the basis of the results presented above it is more than likely that the results of both Uphadyaya et al. (1981) and Mares (1983) could have been influenced by the assimilate flow during maturation. Future work should investigate this possibility fully.

5.4 CONCLUSIONS

Evidence is presented of a mechanism which controls both dormancy and GA_3-sensitivity of the endosperm in *A. fatua*. Many workers have been able to show that the environment under which the seed matures is able to influence the dormancy of the seed produced (Sexsmith, 1969; Peters, 1978; Walbot, 1978; Gutterman, 1981 and Sawhney and Naylor, 1982) but these authors have only speculated as to the possible physiological basis of this environmental control of dormancy. *In vitro* work on the culture of excised barley embryos by Cameron-Mills and Duffus (1980) showed that by increasing the sucrose concentration of the culture medium from 3 to 9%, caused a progressive decline in germination rate, while embryos grown in 12% sucrose completely failed to germinate and grew to immense proportions without reaching physiological maturity. Walton (1981) recorded the same effect if the culture medium contained ABA.

Duffus (1983) in her review on recent progress on the biochemistry of immature cereal grains, commented that the above observations suggested that the information required to prevent precocious germination comes from the environment external to the embryo rather than the embryo itself. The concentrations of sucrose used in these experiments were

well within physiological concentrations experienced during normal growth and development and thus any environmental stress situation could be expected to reduce the flow of assimilates to the developing seed.

The fact that all the sugars (with the exception of raffinose) stimulated α-amylase synthesis in de-embryonated endosperm halves at the lower concentrations would appear to indicate that they are operating as physiologically active compounds rather than osmotica as suggested by Jones and Armstrong (1971). This hypothesis is further strengthened by the fact that panicles grown in sucrose, produced more dormant seed than panicles grown in the same concentrations of biologically inactive osmotica.

Evidence to the affect that dormancy in *A. fatua* is not determined by endosperm factors but by genetic factors residing in the embryo, comes from the work of Naylor and Simpson (1961) who found that both sugar accumulation and utilization by the embryo were limiting factors and that they could be overcome by the exogenous application of GA_3. They did, however, find that sucrose promoted the germination of freshly harvested embryos but inhibited the germination of embryos that had been after-ripened for five months. The application of sucrose plus GA_3 had a strong sinergistic effect on freshly-harvested embryos but no synergism could be observed in embryos after-ripened for 18 months. They also found that leaching of the embryos enhanced the stimulatory effect of both GA_3 and sucrose.

Naylor and Simpson's findings and those of the present experiments show that the promotion/inhibition effect of sucrose, both on germination and α-amylase synthesis, is obviously concentration-related and given the fact that embryo sensitivity changes with stage of after-ripening, ecotype and environment under which the seed matured, it would be possible to obtain many different types of behaviour regarding

reaction to sucrose. For example, in a further panicle experiment (results not shown) the pH of the medium in which the panicles were grown was varied and seed harvested from panicles grown in 0,3 mol dm^{-3} sucrose at pH 7,5 were more dormant than those from panicles grown on the same concentration sucrose at lower pH.

The fact that sucrose was able to inhibit the promotive effect of GA_3 on germination in the panicle culture experiment (albeit only for a few months) is also interesting and shows that sucrose is acting as a physiological inhibitor and not as an osmoticum which would have the reverse effect (see Fig. 5.2). The question of how sucrose mediates its effect arises. It obviously does not act directly, as the sugar content of sugar-cultured and water-cultured seeds did not differ. The possibility that the sugar content of the embryo itself might be important cannot be excluded but it appears to be more likely that sucrose was acting via another compound or compounds. Cameron-Mills and Duffus (1980) found that during the early stages of grain development in cereals the endosperm sucrose levels are high. At the same time ABA levels are also high (King *et al.*, 1979). Duffus (1980) comments that these two events could combine to prevent germination. Various workers have tried to find a correlation between sucrose content and ABA. Coomb and Hale (1973) for example, found that in the grape, chemicals that delayed ripening also delayed ABA and sugar increases. However, nobody has yet been able to prove that high levels of sucrose or other sugars lead to an increased synthesis of ABA.

Evidence that ABA is not involved in the maintenance of dormancy in *Avena* comes from the work of Berrie *et al.* (1979) who found no relationship between endogenous ABA level and dormancy in either *A. fatua* or *A. sativa*. The hormone interaction work (Chapter 4) also shows that ABA does not seem to have a significant effect on wild oat dormancy.

It must therefore be concluded that the exact *modus operandi* of sugars in preventing germination and inhibiting the synthesis of hydrolytic enzymes is not known at this time but appears to form part of an involved mechanism which is under environmental control. These findings should provide the basis for further detailed investigations on how sugars, available to the developing seed, can affect the induction and maintenance of dormancy.

CHAPTER 6

EFFECT OF AMMONIA ON THE DORMANCY OF *AVENA FATUA* SEED

6.1 INTRODUCTION

Cairns (1974) noted that phosphine (PH_3) stimulated the germination of dormant seed of *A. fatua*. He based this finding on the fact that dormant seed which was infested with granary weevil (*Sitophilus granarius*) became non-dormant on fumigation with tablets containing aluminium phosphide ('Phostoxin', Degesch) which are commonly used to fumigate stored seed. These tablets give off PH_3 which is a strong respiratory inhibitor (Cherfurka *et al.*, 1976) but they also give off NH_3 and CO_2 which result from the decomposition of ammonium carbamate. Phosphine is spontaneously combustible with air (explosion point: 1,79% (v/v) in air) and this mixture of NH_3, CO_2 and PH_3 is designed to prevent spontaneous combustion (Anon, 1964). Phosphine is also a potent mammalian toxin (Monro, 1961).

Cairns (1974) and Cairns and de Villiers (1980) working on the effect of 'Phostoxin' fumigation on wild oat dormancy did not examine the relative effects of the three gases evolved by the tablets. It was thus considered necessary to examine the effect of PH_3, NH_3 and CO_2 individually and also all permutations of these three gases on the germination of dormant *A. fatua* seed.

6.2 MATERIALS AND METHODS

6.2.1 Gas preparation and treatment

6.2.1.1 Treatment of seed with 'Phostoxin'

Avena fatua seed was placed in a 2 dm^3 screw-top glass container. A vial containing 0,2 cm^3 water and a 'Phostoxin' tablet was placed in the container and the container sealed for certain fixed periods. The seeds were then aired for 72 h after which a germination test was carried out.

6.2.1.2 Preparation of pure phosphine

As previously stated, phosphine is spontaneously combustible with air and thus experiments in which a concentration of more than 1,79% (v/v) was carried out, were conducted with extreme caution to prevent explosions. Kashi and Bond (1975) developed a method of evolution of PH_3 which was designed to prevent spontaneous combustion. This method consisted of evolving the mixture of PH_3, NH_3 and CO_2 over water. Due to the greater solubility of NH_3 and CO_2, phosphine evolved in this way was relatively free of the other two gases. An additional advantage was that the phosphine was saturated with water vapour which prevented spontaneous oxidation of the gas.

Phosphine was evolved as follows: The bottom was cut off a 500 cm^3 volumetric flask. A 'Suba Seal' rubber septum was fitted to the narrow mouth of the flask. The flask was filled with water and a glass plate placed over the wide end of the flask to exclude all air. The flask was then placed in a 2 dm^3 glass beaker half filled with water. The glass plate was removed under the water and a 'Phostoxin' tablet was introduced into the flask under water. The phosphine gas so collected was kept over water and phosphine gas withdrawn by inserting the needle of a syringe into the rubber septum and withdrawing the required quantity of gas.

6.2.1.3 Preparation of NH_3 and treatment of seed

Ammonia was evolved by the action of NaOH on NH_4Cl:

$$NaOH + NH_4Cl = NaCl + H_2O + NH_3$$

Two methods were employed for the treatment of the seed with NH_3. Firstly, equimolar quantities of the two chemicals were placed in a 1 dm^3 screw-top container fitted with a glass stopcock. The top was screwed on and the stopcock connected to a polythene pipe fitted with a hypodermic needle at the other end. The evolving gas was used to inflate a balloon by introducing the needle through a rubber septum ("Suba Seal" stopper) fitted to the mouth of the balloon. The required quantity of gas was withdrawn from the balloon and injected through a rubber septum into a test tube containing the seeds to be treated. An equivalent quantity of air was first withdrawn from the test tubes to maintain atmospheric pressure in all tubes irrespective of the dose of NH_3 applied. At the end of the treatment period, the rubber septum stoppers were removed and the seed aired for 72 h before the germination or other tests were carried out.

The second method of NH_3 treatment involved the evolution of the gas *in situ* in the test tube containing the seed. A small vial containing 0,053 g of NH_4Cl was placed into a test tube. An excess of NaOH was added to the vial which was then covered with a metal cap. The wild oat seed was then introduced and the tube sealed as quickly as possible to prevent the evolving gas from escaping. After the treatment period the seed was taken out of the test tube and aired as above.

6.2.2 Permeability of seed

Membrane permeability of the fumigated seed was determined

by measuring the conductivity of electrolytes leached out of the seed. Duplicates or triplicate samples of either 25 or 50 seeds were weighed and placed in beakers containing 25 or 50 cm^3 of distilled water held at 25°C in a waterbath. Conductivity was monitored over an 8 h period but maximum differences between treatments occurred after 2½ hrs. Conductivity was determined on a Metrohm Herisau conductivity meter and was expressed as µ Siemens per gram seed.

6.2.3 Water uptake by seed

Water uptake by imbibed seed was determined by imbibing the seed for various periods of time, drying them rapidly using paper toweling and weighing them. The quantity of water absorbed by the seed was expressed as a percentage of the air dry seed.

6.2.4 Determination of the activity of the pentose phosphate pathway enzymes glucose-6-phosphate dehydrogenase and 6-phosphogluconic acid dehydrogenase

The enzyme assays were based on the methods of Uphadyaya *et al.* (1981). Whole seeds (25) were dehusked and homogenized at 4°C with a mortar and pestle with 5 cm^3 of a 10 mmol dm^{-3} phosphate buffer (pH 7,8) containing 1 mmol dm^{-3} EDTA and 1 mmol dm^{-3} β-mercaptoethanol. The homogenate was centrifuged at 20 000 x g in a Beckman cooled centrifuge fitted with a J-21 rotor at 0°C for 30 min. The supernatant was stored on ice and used as a source of the enzymes glucose-6-phosphate dehydrogenase (G6PDH) and 6-phosphogluconic acid dehydrogenase (6PGDH).

Activity of the enzymes were assayed as follows. An 0,3 cm^{-3} aliquot of enzyme extract was added to 3 cm^3 of 50 mmol dm^{-3} Tris-HCl buffer (pH 7,8) containing 50 mmol dm^{-3} glucose-6-phosphate (G6P) monosodium salt or 6-phosphogluconate (6PG) trisodium salt and 0,5 mmol dm^{-3} NADP. All these

chemicals were obtained from Sigma Chemical Corporation. The reduction of NADP to NADPH was determined on a Hitachi spectrophotometer at 340 nm. The change in A_{340} per min was measured and enzyme activity was determined by subtracting the change in absorbance of the reaction mixture without substrate (G6P or G6PG) from that of the reaction mixture with substrate. For each mole of G6P converted by G6PDH two moles of NADPH will be formed and the real activity of G6PDH will be half that measured. An appropriate correction was made. The activity of 6PGDH was always approximately six times higher than that of G6PDH. Enzyme activity was expressed as change in A_{340} min^{-1}.

6.2.5 Determination of α-amylase

α-Amylase was determined by the 'Phadabas' tablet method as described earlier in section 5.2.2. α-Amylase synthesis was monitored in either endosperm halves or embryo halves of seeds. Where endosperm halves were leached before determination of α-amylase activity, this was done under running tap water for 48 hrs. The method of incubation of endosperm halves was also as has been previously described. Further details will be given in results and discussion.

6.2.6 Determination of catalase

The method used to assay catalase activity is based on that developed by Bonnichsen *et al.* (1947) as reported by Colowick and Kaplan (1955). Whole dehusked seed (20) were homogenised at 4°C with a mortar and pestle with 5 cm^3 of a 0,01 mol dm^{-3} phosphate buffer (pH 6,8). The homogenate was centrifuged at 20 000 x g in a Beckman cooled centrifuge fitted with a J-21 rotor at 0°C for 20 min, filtered and stored on ice. All determinations were carried out within 2 hrs of extraction.

An aliquot (1 cm^3) of enzyme extract was rapidly pipetted into 5 cm^3 of 0,01 mol dm^{-3} H_2O_2 in the same phosphate buffer which was used for extraction. The enzyme extract was allowed to react for precisely 3 min at 25°C and the reaction was then stopped by the addition of 2 cm^3 0,5 mol dm^{-3} H_2SO_4. Control values were obtained by the addition of the H_2SO_4 before the addition of the enzyme extract. The H_2O_2 remaining in the substrate after the reaction had been terminated was determined by the addition of 0,5 cm^3 of 10% KI and one drop of 1% ammonium molybdate and, after the mixture had stood for 3 min at room temperature, titrating the liberated iodine with 5 mmol dm^{-3} $Na_2S_2O_3$. Starch (0,05%) was used to determine the end point of titration accurately. Enzyme activity was expressed as mmol dm^{-3} H_2O_2 consumed per min at 25°C.

6.2.7 Determination of peroxidase

Peroxidase was extracted from *A. fatua* seed using a method based on that of Noll (1983). Whole dehusked seeds (25) were homogenised with a mortar and pestle with 5 cm^3 10% sucrose solution. The enzyme was extracted for 30 min in centrifuge tubes on a shaker, whereafter the samples were centrifuged for 30 min at 20 000 x g. The clear supernatent was filtered through a Whatman No.1 filter paper and held on ice until assayed.

The assay method was according to that described in the Worthington Enzyme Manual (Anon, 1972) using o-dianisidine (Sigma) as the hydrogen donor. Peroxidase activity was measured as the change in absorbance at 460 nm in a Hitachi spectrophotometer and expressed as µmol H_2O_2 consumed per min at 25°C.

6.2.8 Effect of pH on germination

To determine if pH *per se* influenced germination, seeds were germinated in a range of buffered solutions ranging from pH 5-9. Phtalate/NaOH (0,1 mol dm^{-3}) was used for the range pH 5,0-5,5; KH_2PO_4/NaOH (0,1 mol dm^{-3}) for the range pH 6,5-7,0 and Tris/HCl (0,1 mol dm^{-3}) for the range pH 7,0-9,0. Seed were imbibed and germinated in the various buffered solutions for the full 21 day duration of the germination test.

6.2.9 Effect of other chemicals on the dormancy-breaking effect of NH_3

Avena fatua seed (Montana ecotype) was fumigated with NH_3 for 72 h as previously described in section 6.2.1.3 (method 1). Fumigated and control seeds were incubated with GA_3 at 10^{-4} mol dm^{-3}, NaN_3 at 10^{-3} mol dm^{-3}, IAA at 10^{-5} mol dm^{-3} and benzyladenine (BA) at 5×10^{-6} mol dm^{-3} in petri dishes at 15°C.

The effect of salicylhydroxamic acid (SHAM) on NH_3-stimulated germination was investigated by incubating the NH_3-treated seed with and without SHAM at 3×10^{-3} mol dm^{-3}. These treatments were applied to the dormant Montana ecotype and the relatively non-dormant Oxford ecotype.

6.2.10 Effect of NH_3, NaN_3 and SHAM on the respiratory activity of *A. fatua* seed

Ammonia-treated seed (Montana ecotype) and seed imbibed in 1 mmol dm^{-3} NaN_3 were incubated with or without 3 mmol dm^{-3} SHAM. After a 48 h incubation period, respiration of the seed was determined.

Prior to the incubation period, seed was surface sterilized

with 1% NaOCl and washed with distilled water. At the end of the 48 h incubation period, O_2 uptake was measured using a Yellow Springs Instruments O_2 electrode and a model 53 oxygen meter connected to a J.J. Instruments recorder. Twenty five seeds were stirred in 32 cm^3 distilled water in a 5023 Y.S.I. Macrobath water-jacketed glass container held at 25°C. Care was taken to exclude all air bubbles from the chamber before the commencement of the monitoring of O_2 uptake which took place over a 20 min period.

6.3 RESULTS AND DISCUSSION

6.3.1 Effect of 'Phostoxin' fumigation on the dormancy, permeability and viability of *A. fatua* seed

Germination, mortality and conductivity values obtained for seeds (*A. fatua* ex Stellenbosch) exposed to 'Phostoxin' fumigation for various periods are illustrated in Fig. 6.1. Only 9% of the untreated seed germinated. Germination increased rapidly with increased time of fumigation to a maximum of 92% after 24 h. Thereafter, germination declined to zero after 72 h fumigation with a concomitant increase in mortality. Similar results (not shown) were recorded for more dormant ecotypes, although the fumigation period for maximum germination was proportional to the dormancy of the seed (up to 96 h fumigation).

Fumigation also resulted in an increase in permeability of the seeds as determined by the conductivity of the leachate (Fig. 6.1). Maximum germination occurred at a conductivity reading of 238 µS. An increase in permeability beyond this point was obviously detrimental to the biological functions of the membranes and is reflected in the increase of mortality.

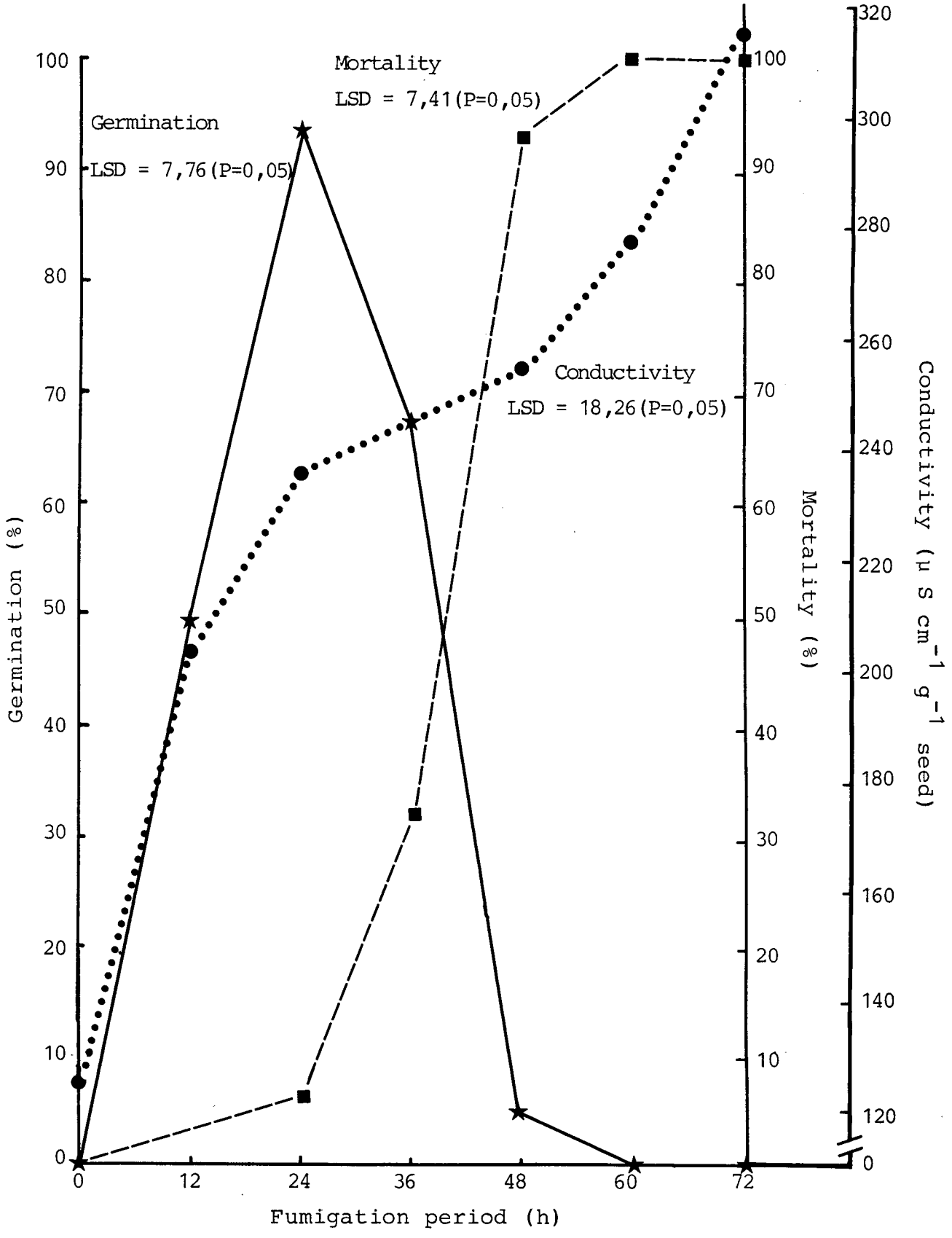

Fig. 6.1 Effect of 'Phostoxin' fumigation period on the germination, viability and permeability of *A. fatua* seed.

Various studies on the effect of O_2 and CO_2 on the germination of *A. fatua* seed have been carried out (Simpson, 1978) whereas the role of ethylene in wild oat dormancy and germination was examined by Adkins and Ross (1981) but this study is the first report on the release of dormancy by the gaseous treatment of dry seed. Although the increase of seed permeability is important, it cannot be assumed that the release from dormancy was exclusively via this factor only as dormancy in the more dormant ecotypes could not be broken by puncturing the testas; a procedure which led to an appreciable increase in conductivity of the leachate (results not shown). The fumigation must have led to other changes in the embryo which caused dormancy to be broken. These changes will be discussed later.

6.3.2 Identification of the active ingredient in the mixture of gases evolved by the 'Phostoxin' tablet

As previously stated, the 'Phostoxin' tablet gives off a mixture of PH_3, NH_3 and CO_2. These gases were injected separately into test tubes containing seeds (as previously described) in the same proportions as they occur in the gas mixture which is given off by the 'Phostoxin' tablet viz 4 : 2 : 1. From the results (Fig. 6.2) it is clear that germination was significantly stimulated only in those treatments containing NH_3. The slight stimulatory effect of PH_3 was not significant and the other two gases had no stimulatory effect on germination. The slight increase in germination in the PH_3 + NH_3 treatment was also negligible.

Although it could be expected that PH_3 (a strong respiratory inhibitor, Kashi and Cherfurka, 1976) would break dormancy, it had no effect, and NH_3 was thus identified as the gas which broke seed dormancy. This is the first report of NH_3 affecting the dormancy of seed.

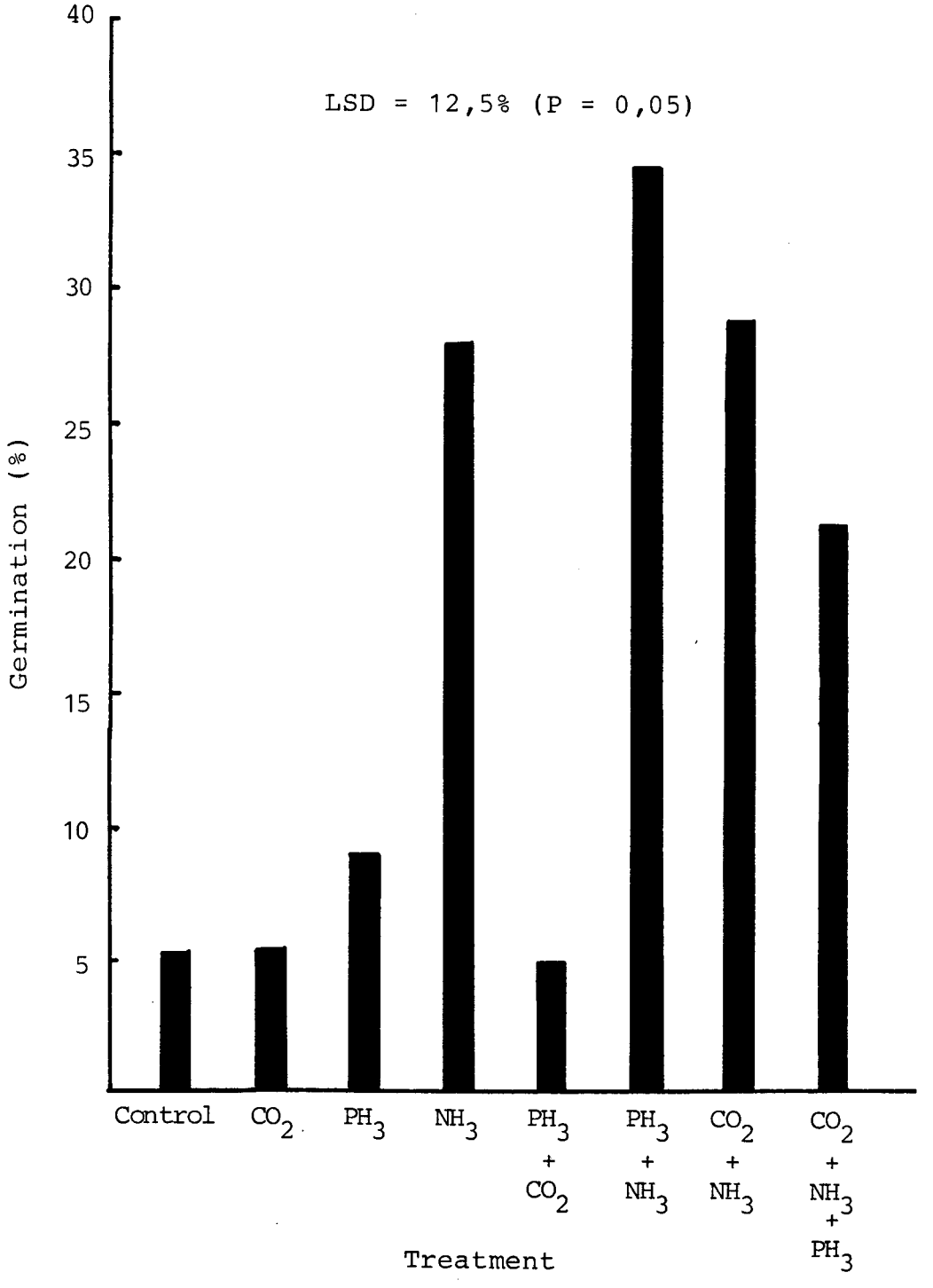

Fig. 6.2 Effect of various gaseous treatment of air-dry *A. fatua* seed on subsequent germination

The implication of this finding is far-reaching as many seeds which are commercially used, have long dormancy periods which create problems in their use. For example, some barley cultivars have long dormancy periods which neccesitate long periods of after-ripening and in some cases special treatment with GA_3 to ensure uniform and rapid germination. Another interesting case concerns the storage of wheat seed for practically a whole year to ensure that the seed sown is not dormant. In the Eastern United States wheat stands on the land for up to 11 months, and less than a month elapses between sowing and harvesting (Macguire, personal communication). The harvesting period is subject to regular downpours and the cultivars used there have been selected for long dormancy periods to counter pre-harvest sprouting. If NH_3 (a relatively cheap and easily attainable gas) could be used to relieve the dormancy of the wheat seed, this would obviate the expense of storing the wheat for a year.

The implications for weed control are also obvious. Povilaitis (1956) suggested that the application of chemicals to the soil to stimulate weed seed germination might be an alternative method of weed control and would result in the weed population being destroyed in one season rather than by repeated annual application of herbicides as is the case at the moment. The only chemicals which have been successfully used for this purpose are ethylene and various other chemicals used to stimulate the germination of the parasitic weed *Striga asiatica* (Egley and Dale, 1970). Liquid ammonia is now being used as a fertilizer in South Africa and other parts of the world (Theron, personal communication) and it would be very interesting to observe changes in the emergence pattern of weed seeds in the coming years.

6.3.3 Effect of NH_3 on the dormancy, permeability and viability of *A. fatua* (Oxford ecotype)

An experiment similar to experiment 6.2.1 but using NH_3

instead of PH_3, was carried out to determine if an increase in dosage rate of the gas would lead to increased germination and permeability. Method 1 of section 6.2.1.3 was used for NH_3 preparation and the treatment was carried out in a 30 cm^3 test tube.

The results (Fig. 6.3) show that both germination and conductivity increased to reach a maximum at 8 and 10 cm^3 per test tube respectively. A small percentage of the seed was non-viable at 10, 12 and 14 cm^3 NH_3 per 30 cm^3 tube.

These results are very similar to those obtained with the 'Phostoxin' tablet and although this experiment was not carried out with pure PH_3 (due to the extreme hazardous nature of the gas at these concentrations) it can be assumed that of the three gases released from the 'Phostoxin' tablet only NH_3 has any effect on permeability.

Whether the increased permeability of the seed had any direct bearing on the loss of dormancy could not be ascertained from this experiment but it is reasonable to assume that an increased permeability would lead to an increased rate of leaching and also an increased O_2 level to aid oxidative reactions involved in the germinative process. The hypothesis that increased permeability promotes germination via increased water uptake is examined in the following experiment.

6.3.4 Effect of NH_3 treatment on water uptake by *A. fatua* (Montana) seed

McIntyre and Hsiao (1983) found that dormancy of the intact seed of *A. fatua* was primarily due to the failure of the embryo to take up water in sufficient quantities for the induction of germination. To test the validity of this hypothesis, the water uptake of whole seeds treated with

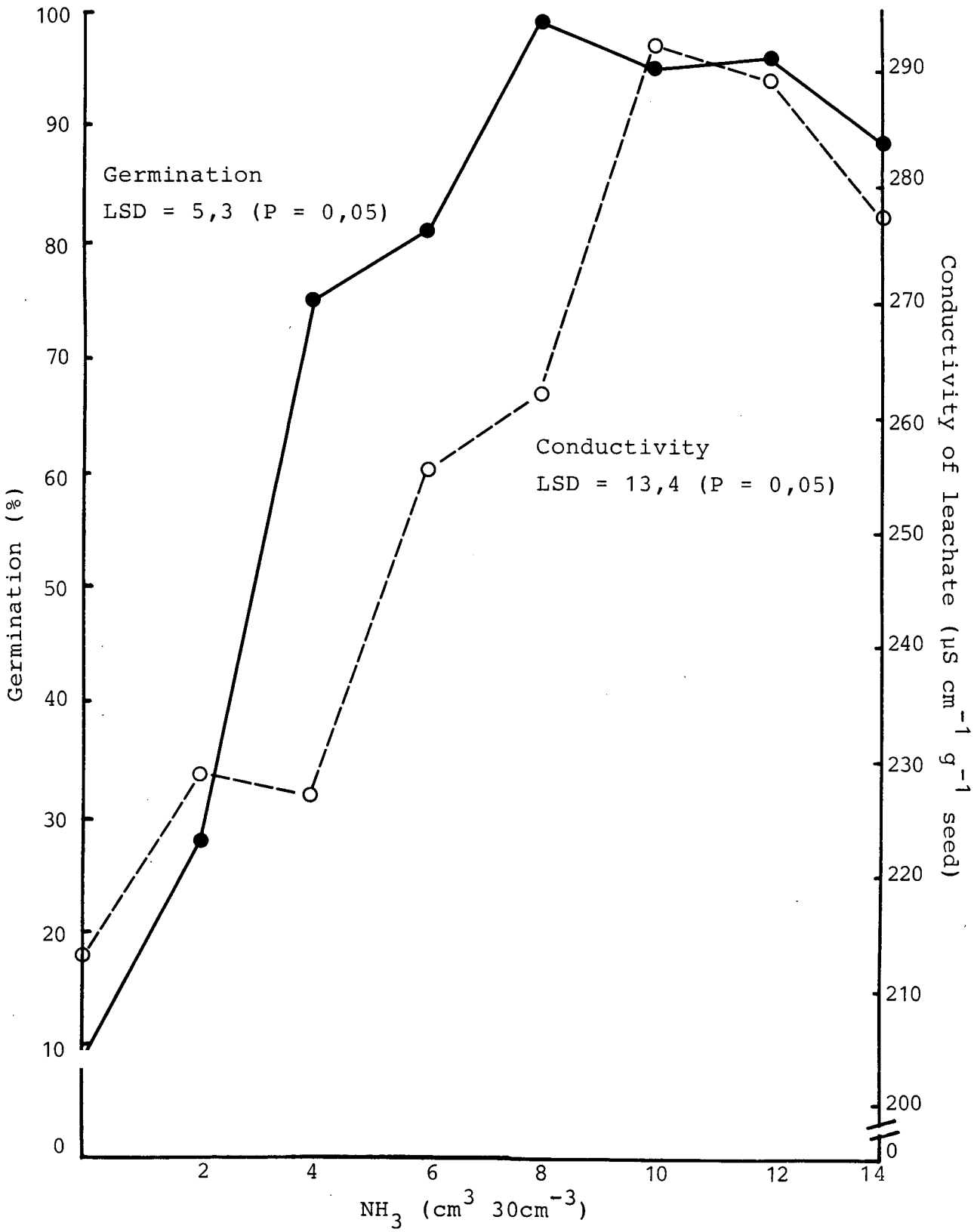

Fig. 6.3 Effect of NH_3 dosage rate on germination and permeability of *A. fatua* seed

different periods of exposure to a fixed concentration of NH_3, was examined.

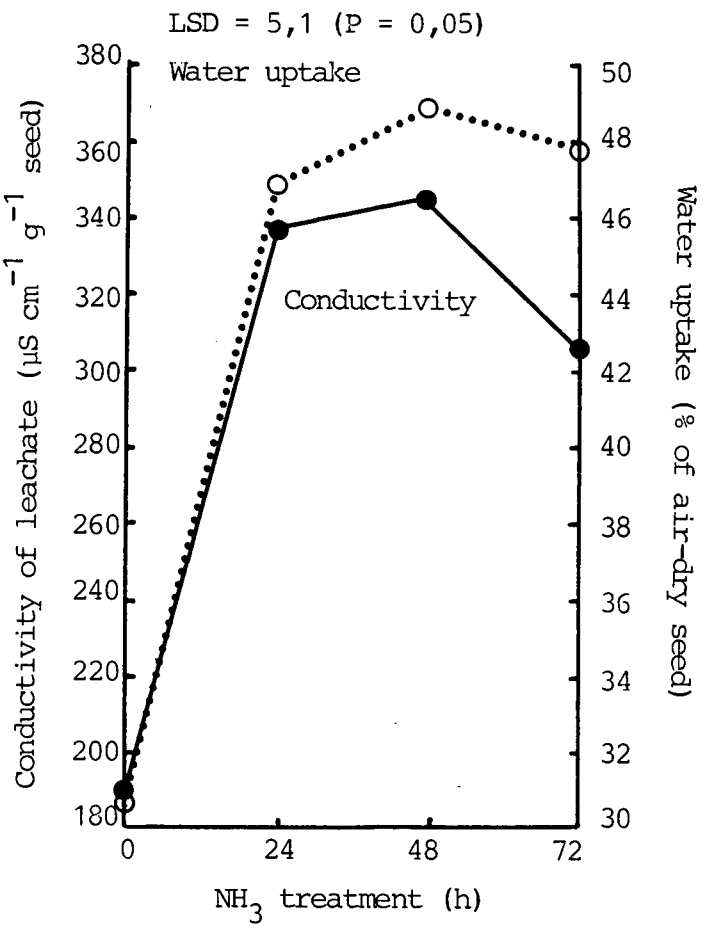

Fig. 6.4 Effect of time of exposure to NH_3 on the permeability and water uptake of *A. fatua* seed

The results of this experiment are presented in Fig. 6.4 and show that water uptake and increase of conductivity are closely related. Although no excised embryo studies were carried out, McIntyre and Hsiao (1983) showed that there is a close correlation between embryo water content and that of the intact seed. These authors speculated that the process of after-ripening in *A. fatua* involves a process of increased

desiccation during dry storage which may cause structural changes which affect the hydraulic conductivity of the tissues enclosing the embryo. While these studies are interesting they cannot explain how NH_3 can relieve dormancy of deeply dormant ecotypes such as Montana 73 (the line used in this particular study) where dormancy resides in the embryo and where dormancy is not influenced by pricking the caryopsis or excision of the embryo. Both these treatments were found by McIntyre and Hsiao (1983) to increase water content of the embryo and cause germination in the *A. fatua* lines they used.

It must therefore be concluded that although the increase in permeability and the increase in water uptake by *A. fatua* seeds treated with NH_3 may contribute to loss of dormancy, it is not the key to loss of dormancy.

6.3.5 Effect of after-ripening on the dormancy of NH_3-treated seed of *A. fatua* (Montana)

The effect of after-ripening on the mortality and germination of NH_3-treated and control seeds is shown in Fig. 6.5. Germination of the NH_3-treated seeds rose from 41% to 76% over the 12 week after-ripening period. No germination took place in the untreated control seed.

Incubation of NH_3-treated seed immediately after the NH_3-containing test tubes were opened, resulted in 17% of the seed being non-viable. Germination at this stage was calculated on the total number of seeds and not the number of viable seeds. If calculated on the basis of viable seeds, the percentage germination would rise from 41% to 51%. Germination declined by 1% in seeds incubated one week after NH_3-treatment but thereafter rose almost linearly until the eighth week. The rate of germination increase tended to flatten out as after-ripening proceeded. No germination took place in the untreated seed.

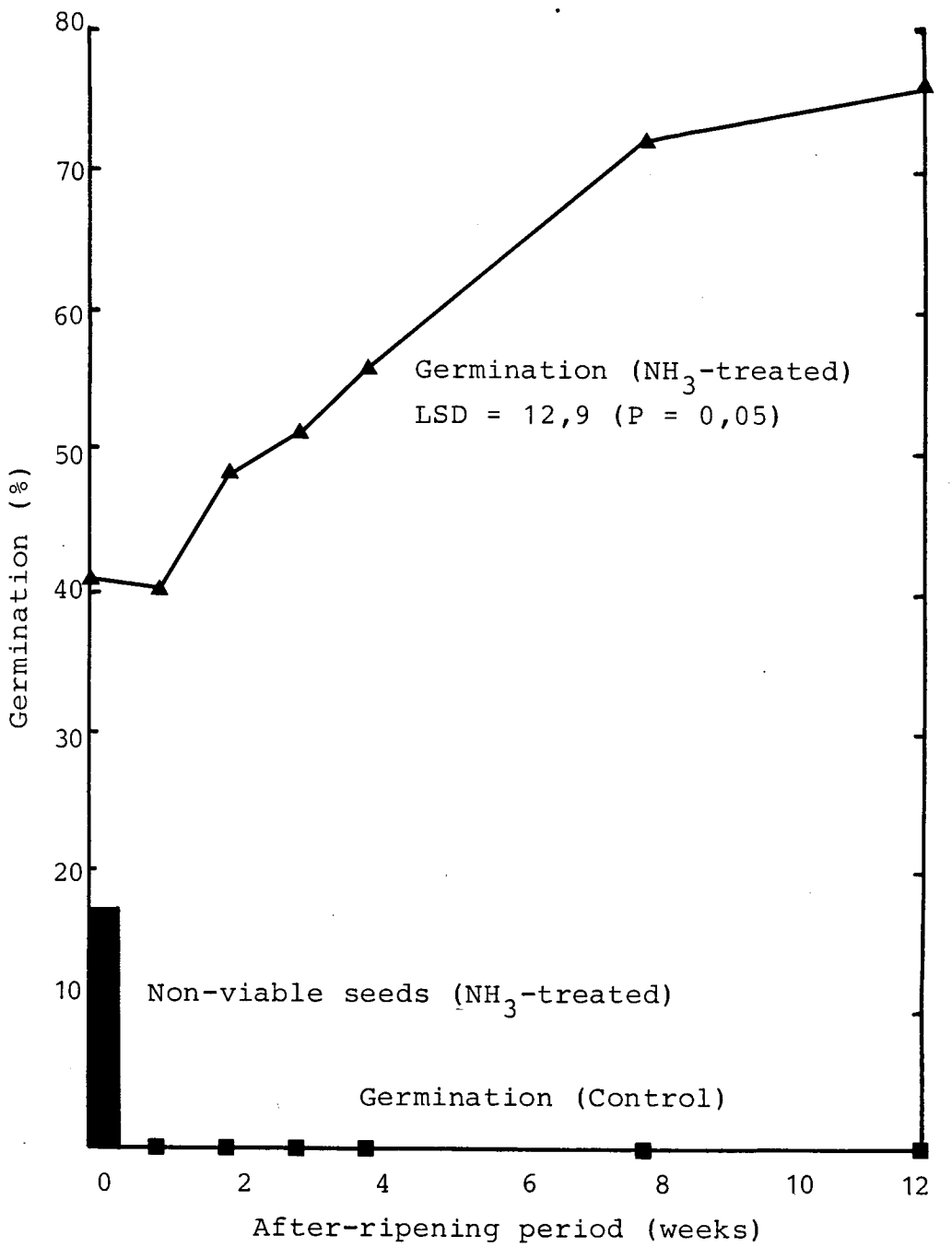

Fig. 6.5 Effect of NH_3-treatment and after-ripening period on mortality and germination of *A. fatua* seed

This experiment shows that the dormancy-breaking effect of NH_3 is not a transient phenomenon and the germinability of seed improves with time after treatment. The high percentage of non-viable seed in the treatment which was incubated immediately after exposure to NH_3 was undoubtedly due to NH_3 still present in the seed. Imbibed seed exposed for any length of time to NH_3 was rendered totally non-viable (results not shown) and it is thus necessary to air treated seed for at least 48 h before being set to germinate.

Ammonia thus causes irrevocable biochemical and/or physical changes in the seed and after-ripening proceeds at a much faster rate after NH_3-treatment. *Avena sterilis* seed has been stored for more than two years after treatment with NH_3 without any loss of viability (results not shown). Ammonia treatment of dormant seed thus offers an extremely practical, yet a safe way to break the dormancy of seed for experimental and/or commercial use. Seedlings resulting from NH_3-treated seed were normal in every respect (growth habit, anthesis, seed set and seed dormancy were identical to control plants) and could be used for experiments with wild oat herbicides.

6.3.6 Effect of NH_3 on dormancy of various *A. fatua* ecotypes

The effect of NH_3 treatment on four wild oat ecotypes from different ecological regions of the world is presented in Fig. 6.6. The NH_3-treatments stimulated all ecotypes to germinate to a greater or lesser extent. In the relatively non-dormant Kenya ecotype 4 cm^3 NH_3 resulted in 100% germination. Germination at the higher dosage rates did not differ significantly from this treatment. Germination of the Oxford ecotype reached a maximum at 8 cm^3 and thereafter remained constant. The very dormant Montana ecotypes was almost unaffected by the 4 cm^3 treatment and maximum germination (84%) was obtained at the highest dose (12 cm^3).

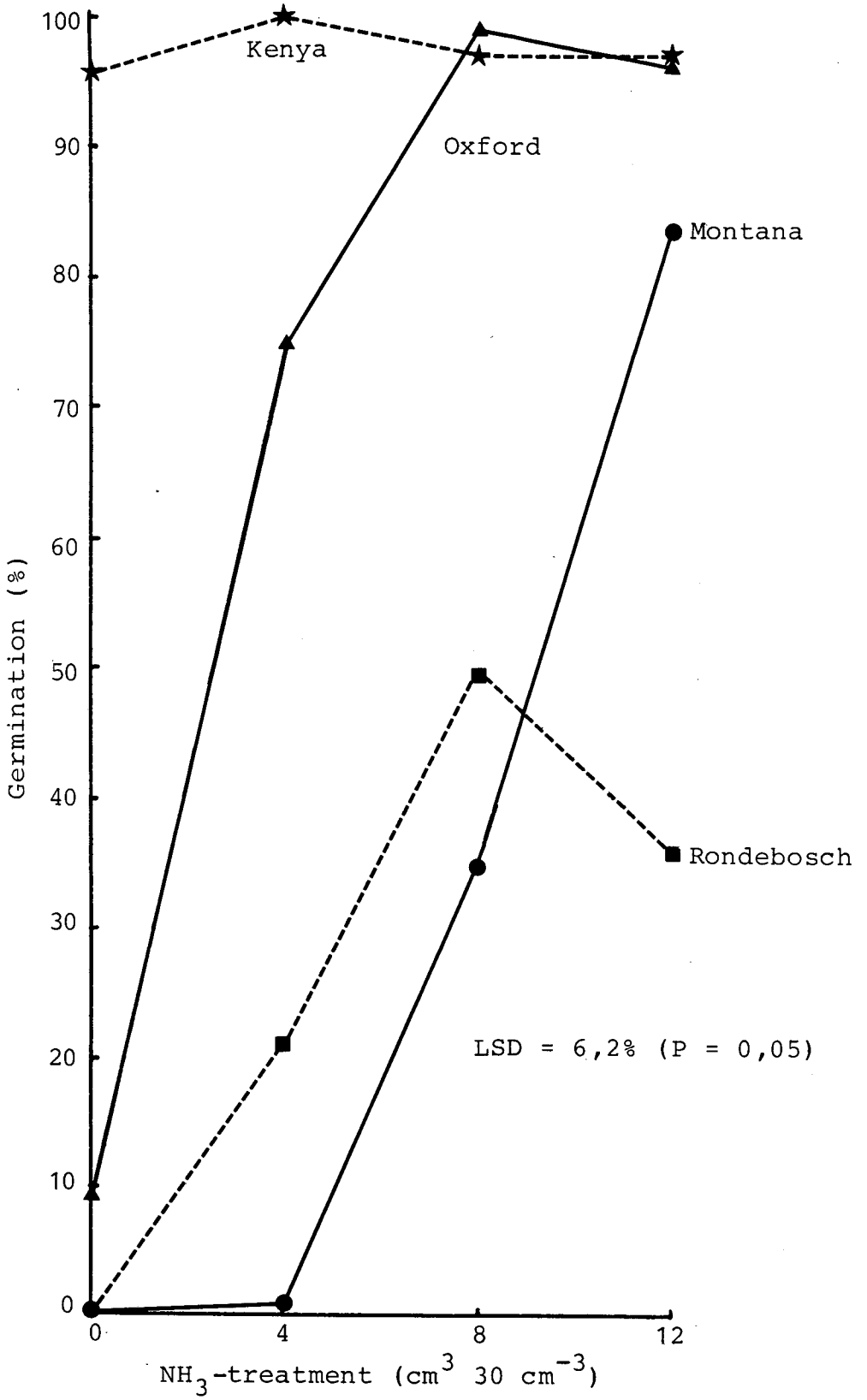

Fig. 6.6 Effect of NH_3 on the germination of various *A. fatua* ecotypes

Optimum germination in the Rondebosch ecotype was obtained at 8 cm^3 but germination decreased thereafter.

Many similar experiments were conducted with very much the same results. Generally speaking, the more dormant ecotypes required a higher dosage for maximum germination. The Rondebosch ecotype showed a sensitivity at the higher dosage rates which cannot be explained at this stage but it is interesting to note that this ecotype was cream in colour (Montana and Kenya were dark brown and Oxford grey) but whether or not this affected its sensitivity to NH_3 is not known at this stage as other cream-seeded ecotypes have not been tested. The Oxford ecotype always gave the largest germination increases at low doses of NH_3.

The fact that NH_3 successfully broke the dormancy of a range of ecotypes from different parts of the world and differing in dormancy levels establishes the gas as a true dormancy breaking compound.

6.3.7 Effect of NH_3 on the germination of other weed species

If NH_3 could stimulate the germination of a wide range of weed species it could be used to encourage germination of dormant weed seed in the soil. The gas was consequently tested on freshly harvested dormant seed of several species occurring on Welgevallen experimental farm at Stellenbosch.

The effect of the gas on the germination of seed of four common monocotyledonous weeds is illustrated in Fig. 6.7. All species responded to treatment by the gas and germination was significantly stimulated. Except for *Hordium murinum* which showed a slight decrease in germination at the highest dose (12 cm^3 30 cm^{-3}) the highest germination figures were obtained at 12 cm^3 30 cm^{-3}.

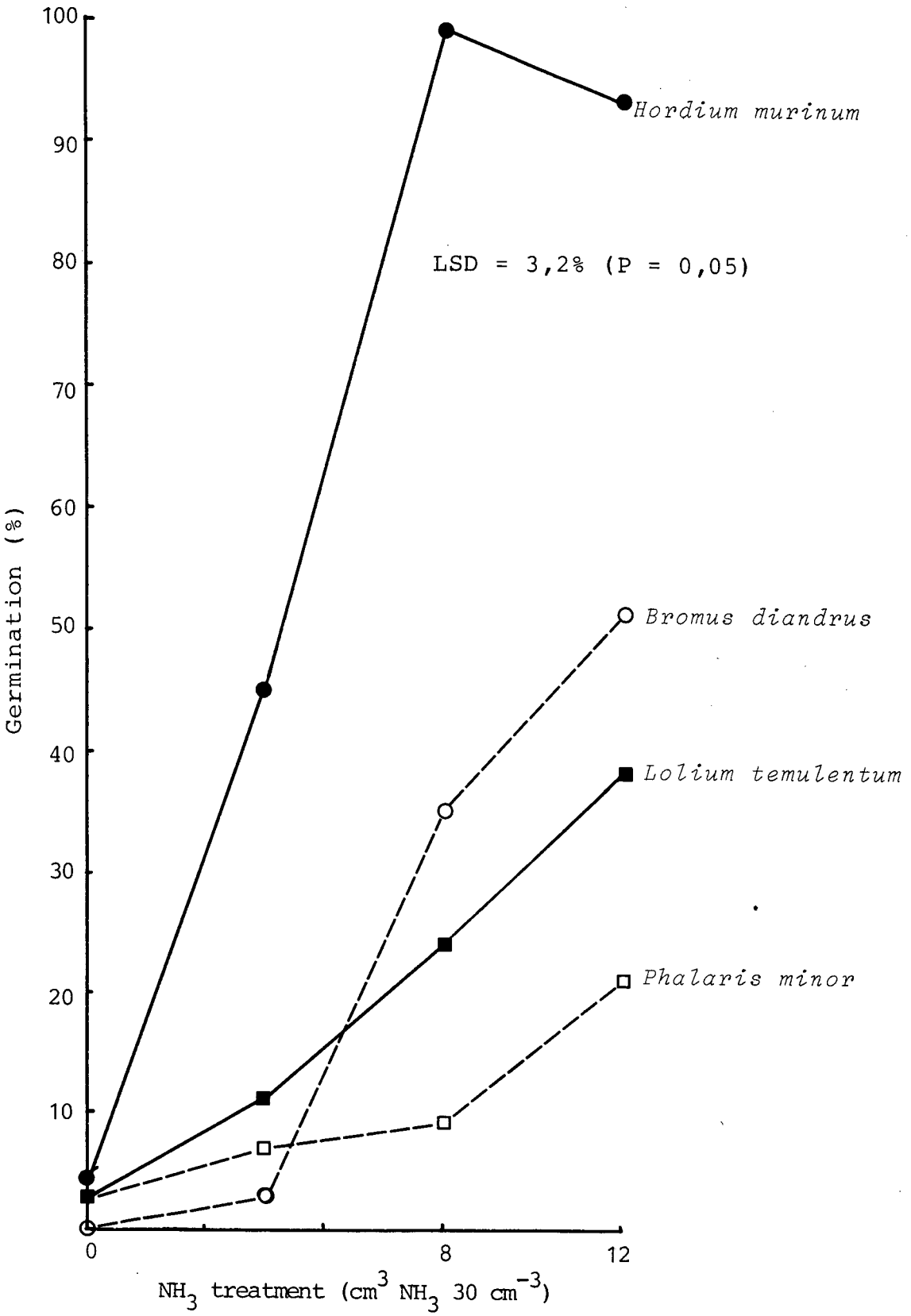

Fig. 6.7 Effect of NH_3 on the germination of various graminaceous weed species

No stimulation of germination with NH_3 was obtained with any of the dicotyledonous species tested viz. *Arctotheca calendula*, *Emex australis*, *Hypericum perforatum*, *Polygonum aviculare*, *Raphanus raphanistrum* and *Spergula arvensis*. Although this list is by no means comprehensive, it appears as if NH_3 does not effect germination of dicotyledonous seeds. In some cases, as with *Emex australis*, a slight inhibition of germination was noticed, even at the lower doses. Consequently even lower doses were used but to no avail.

This experiment established that the stimulatory effect of NH_3 on germination is not confined to one species (*A. fatua*) but is probably ubiquitous in the family *Gramineae* but does not extend to other families. However, not much time has been devoted to the effect of NH_3 on other species as this does not fall within the scope of this study but as in the case of other newly discovered dormancy breaking compounds, should be extensively evaluated both under laboratory and field conditions.

6.3.8 Effect of pH of the germination medium on dormancy of *A. fatua* seed

To establish if NH_3 was affecting dormancy via a pH effect, *A. fatua* (Oxford ecotype) was germinated in a range of solutions varying in pH from 5,0-9,0 as previously described. The results of this trial are illustrated in Table 6.1 and show that the highest germination was attained in the distilled water control. No pattern could be established except that a neutral to slightly alkaline pH seemed to be optimum for germination. It cannot however, be conclusively stated that this experiment is proof that NH_3 does not break dormancy via a change in internal pH. Ammonia gas applied to imbibed seed in concentrations necessary to break dormancy kills the seeds almost instantly. The above experiment was thus conducted under conditions which would

lead to rapid loss of viability if the seeds were treated with NH_3 and does not therefore have a direct bearing on the mode of action of NH_3. Further work using for example micro-electrodes, will have to be conducted to illucidate this problem.

Table 6.1 Effect of pH of the germination medium on the dormancy of *A. fatua* (Oxford ecotype)

pH	Germination (%)	pH	Germination (%)
5,0	9	7,5	19
5,5	12	8,0	7
6,0	8	8,5	5
6,5	5	9,0	4
7,0	16	6,4(Dist. H_2O)	4

LSD = 1,02 (P = 0,05)

6.3.9 Effect of NH_3 on the activities of the pentose phosphate pathway dehydrogenases in germinating *A. fatua* seed

The activity of G6PDH in control and NH_3-treated *A. fatua* seed (Montana) during a 144 h incubation period, is illustrated in Fig. 6.9. No difference in the activity of this enzyme could be observed before the initiation of germination after 72 h incubation. No germination took place in the control seeds. Thereafter, the NH_3-treated seed showed a slight increase in activity whereas the activity in the untreated seeds declined.

In Fig. 6.10 the activity of 6PGDH over the same incubation period is illustrated. The activity of the enzyme was

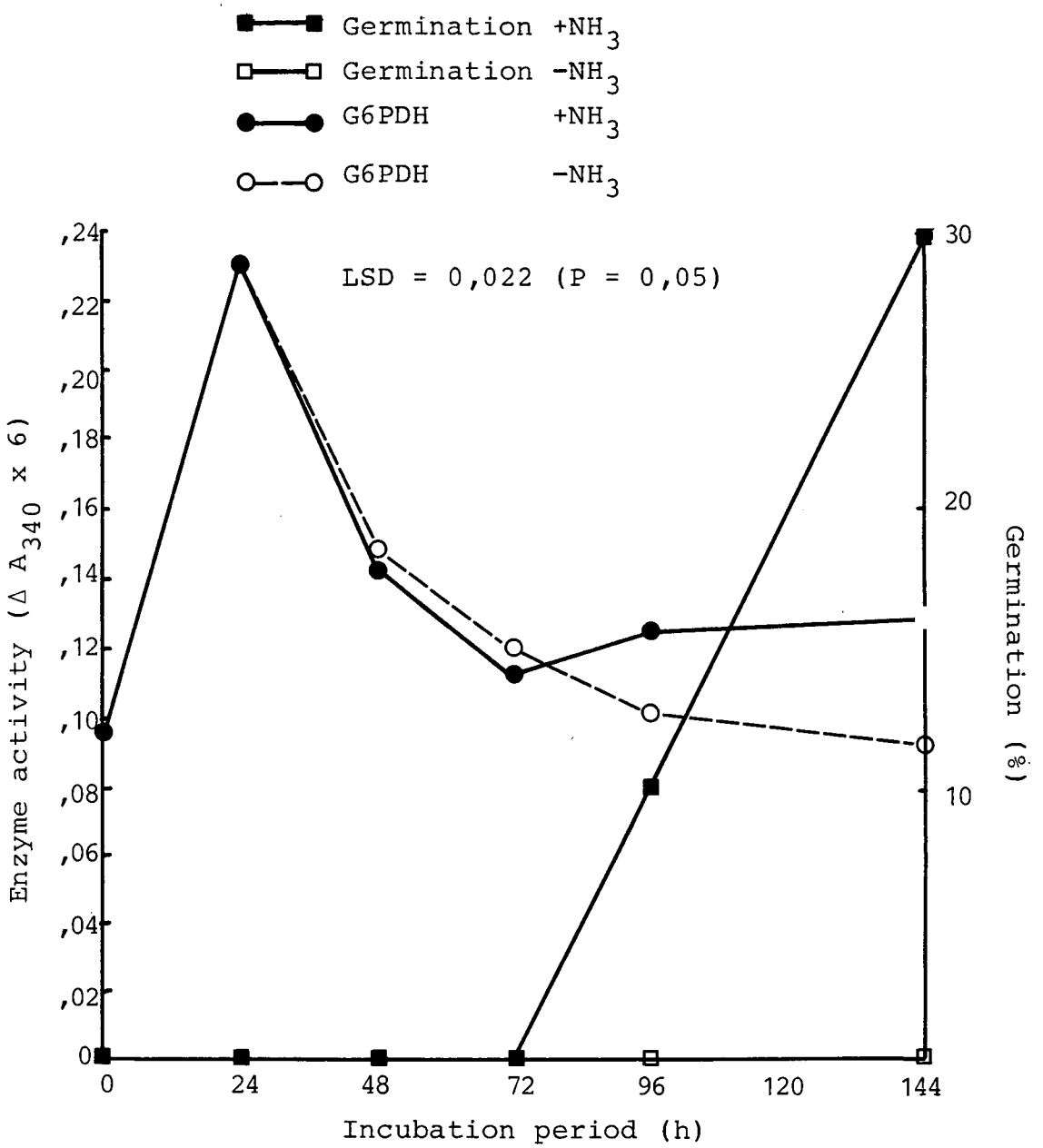

Fig. 6.8 Effect of NH_3 on germination and changes in G6PDH activity in *A. fatua* seed (Montana)

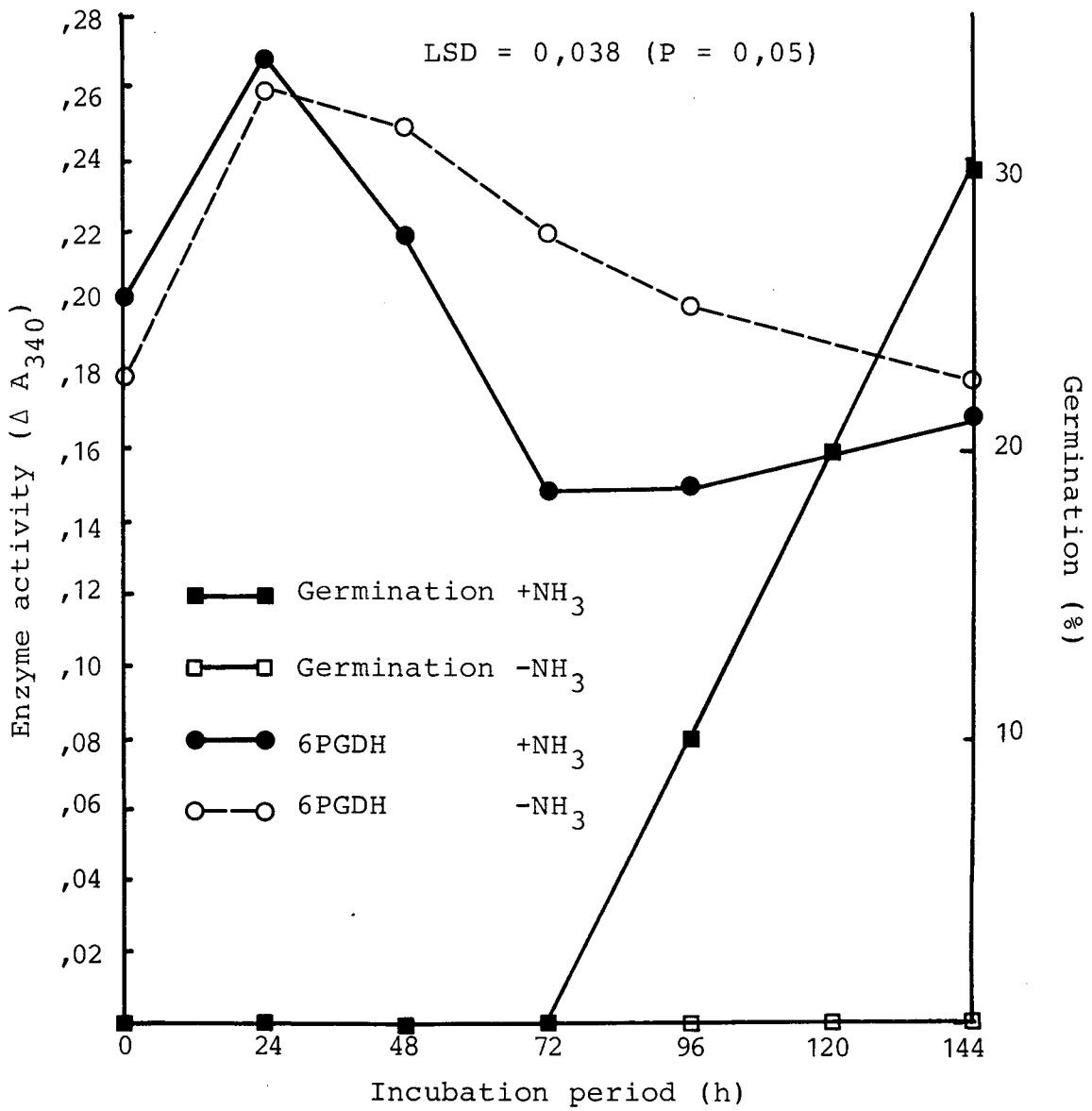

Fig. 6.9 Effect of NH_3-treatment on germination and changes in G6PDH activity in *A. fatua* seed (Montana)

higher in the control seeds which failed to germinate. A slight increase in 6PGDH-activity was noticed in the NH_3-treated seeds once germination had been initiated.

The above results indicate that there is no increased activity in the pentose phosphate pathway prior to the initiation of germination. Evidence that increased participation of the pentose phosphate pathway was related to the loss of dormancy in *A. fatua* was first presented by Simmonds and Simpson (1971). Using the C_6/C_1-ratio method they found that if dormancy in the embryo was broken either by after-ripening or by the application of GA_3, the C_6/C_1-ratio shifted towards a greater participation of the pentose phosphate pathway. Further work by these authors (Simmonds and Simpson, 1972) indicated that malonic acid, a competitive inhibitor of the Krebs cycle, increased germination and also shifted the respiratory metabolism towards greater participation of the pentose phosphate pathway. Roberts (1969) reviewed the field of oxidative processes involved in the germinative process and stressed the importance of an active pentose phosphate pathway in the germination of many seeds. The role of respiratory inhibitors was also explained in terms of their inhibition of cytochrome oxidase which presumably resulted in more oxygen being available for oxidases associated with the pentose phosphate pathway.

Evidence on the significance of the pentose phosphate pathway in dormancy of *A. fatua* was also presented by Kovacs and Simpson (1976) who found that in steeped non-dormant seeds the activity of G6PDH doubled while the activity declined in the dormant seeds. These authors concluded that their evidence was consistent with the hypothesis that the pentose phosphate pathway was involved in the loss of dormancy in *A. fatua*.

More recently, however, Adkins and Ross (1981) found that if *A. fatua* seeds were stored at 5°C (a situation where dormancy

is maintained) the activity of the pentose phosphate dehydrogenases increased to a greater extent than when seeds were stored at 25°C (which led to a rapid loss of dormancy). They could therefore, find no connection between dormancy breakage and increased activity of the pentose phosphate pathway.

Further evidence as to the non-participation of the pentose phosphate pathway in loss of dormancy was presented by Uphadyaya *et al.* (1981) who investigated the level of G6PDH and 6PGDH in genetically dormant and non-dormant lines of *A. fatua*. They found that dehydrogenase activity in the embryos isolated from dormant lines remained constant during the first 48 h of incubation, but in the non-dormant lines there was a sharp increase in the level of the two enzymes, but well after the onset of germination. They also found that when GA_3 was applied to break dormancy of dormant embryos, there was no increase in the activity of the two enzymes before germination had visibly been initiated. They concluded that the activity of the pentose phosphate pathway had nothing to do with the release of *A. fatua* seed from dormancy but was a post-germinative phenomenon.

Work by Satoh and Esashi (1980) working on cocklebur (*Xanthium pensylvanicum*) also found that neither the C_6/C_1-ratios or the activity of G6PDH or 6PGDH differed in dormant and non-dormant seed.

The results of the present study support the findings of Adkins and Ross (1981) and Uphadyaya *et al.* (1981) in that, yet another method of inducing dormant *A. fatua* seed to germinate, does not influence the activity of the pentose phosphate pathway. It thus is apparent that NH_3 does not stimulate germination via increased participation of the pentose phosphate pathway.

6.3.10 Effect of NH_3 on the synthesis of α-amylase in germinating half-seeds of *A. fatua* (Montana)

The effect of NH_3 on the synthesis of α-amylase in embryonated half-seeds of *A. fatua* (Montana) during a 96 h germination period is shown in Fig. 6.10. The synthesis of α-amylase could be detected in the untreated control seeds.

The results of this experiment were not unexpected as it has been demonstrated that the development of α-amylase is a post-germinative phenomenon (Drennan and Berrie, 1962). However, these authors found that the development of amylase activity only increased well after appreciable germination had occurred (75%). In Fig. 6.11 it is clear that germination did not precede α-amylase synthesis; both processes occurred simultaneously.

Chen and Chang (1972) (also working with the dormant Montana ecotype) found that if seeds were incubated with 0,1 mmol dm^{-3} GA_3, germination preceeded an increase in α-amylase activity by 24 h. During this period germination increased from 5 to 100% without any increase in α-amylase activity.

From the above discussion it is evident that NH_3 stimulates the synthesis of α-amylase in germinating seeds of wild oats earlier than when germination is stimulated by GA_3 or when naturally non-dormant seeds germinate. It might be argued that the methods used by Drennan and Berrie (1962) for the assay of α-amylase (starch hydrolysis) were not sensitive enough to determine early synthesis of α-amylase but the same cannot be said of the method used by Chen and Chang (1972) who used $H_2{}^{18}O$ density labelling method which is highly sensitive.

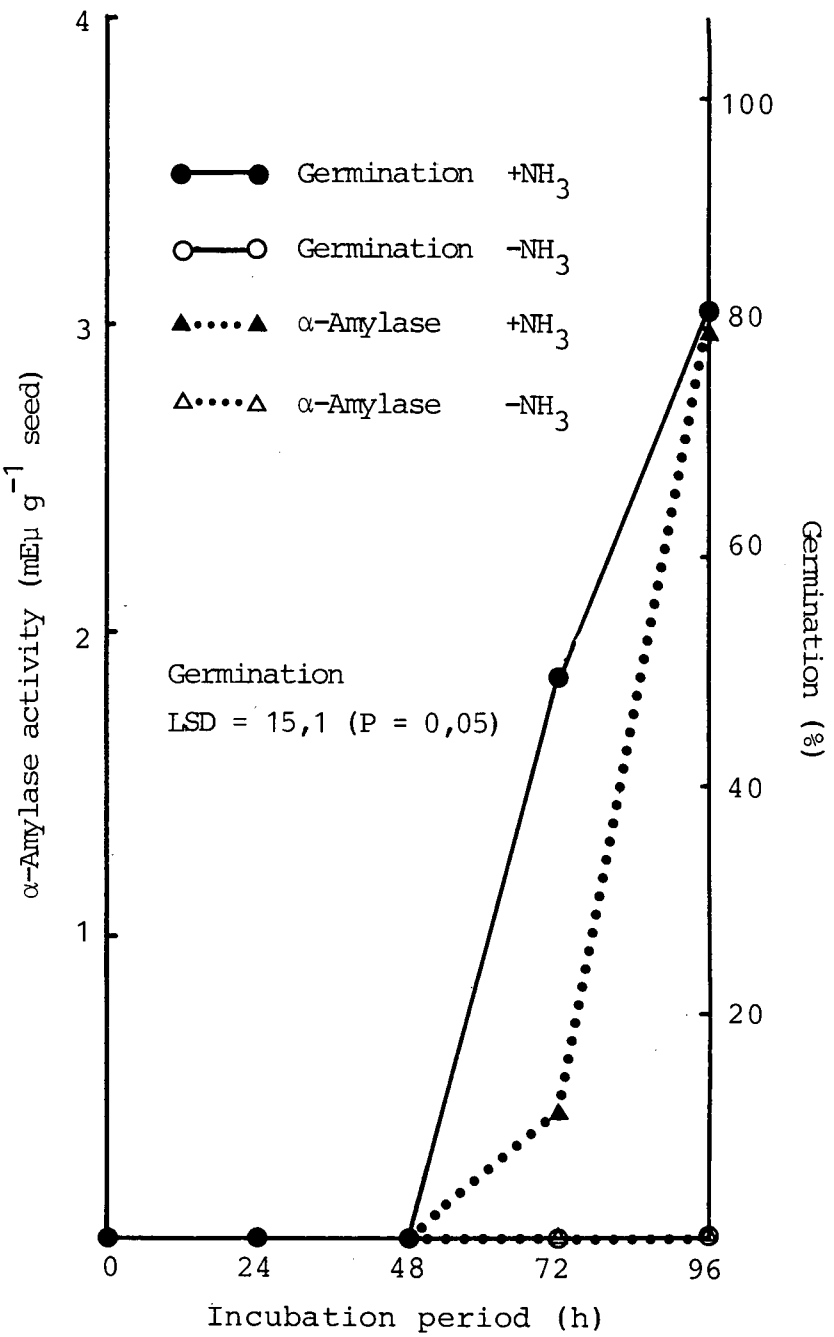

Fig. 6.10 Effect of NH_3-treatment on germination and α-amylase synthesis of embryonated *A. fatua* half seeds (Montana)

6.3.11 The effect of NH_3 on GA_3-mediated α-amylase synthesis in washed and unwashed de-embryonated endosperm halves of *A. fatua* seed (Montana)

α-Amylase synthesis in unwashed de-embryonated endosperm halves pre-treated with NH_3 did not differ significantly from that of the control endosperm halves (Fig. 6.11). It is, however, interesting to note that the unwashed NH_3-treated endosperm halves consistantly produced less α-amylase than the controls and it was found that in some cases at high dosage rates of NH_3, the synthesis of α-amylase was completely inhibited (results not shown).

However, in endosperm halves that had been washed under running tap water for 48 h, pre-treatment by NH_3 led to a massive increase in α-amylase synthesis (Fig. 6.11). This effect was seen in all experiments carried out and was even more accentuated in endosperm halves that had been pre-treated with the 'Phostoxin' tablet. α-Amylase synthesis in the washed controls did not differ from that of the unwashed controls.

The mechanism involved in the stimulation of α-amylase synthesis in the washed NH_3- pre-treated seeds is not known at this stage but several possibilities may be considered. Ammonia may release bound inhibitors that are leached out with washing. Ammonia may also cause the GA_3 to reach the site of action more effectively by increasing permeability but the fact that unwashed NH_3-treated seed did not show an increase in α-amylase synthesis would seem to preclude this hypothesis. However, the NH_3 which remains in the seed, may be inhibitory to α-amylase synthesis and this inhibitory effect could be removed by washing the NH_3 out of the seed. α-Amylase synthesis has an optimum pH of between 6,5 and 7,0 (Hejgaard and Gibbons, 1979) and the internal pH of the seed rose to 8,1 after treatment with NH_3. The pH of untreated seed was between pH 6,8 and 7,0. The internal pH was determined by macerating the seed in a small volume of distilled

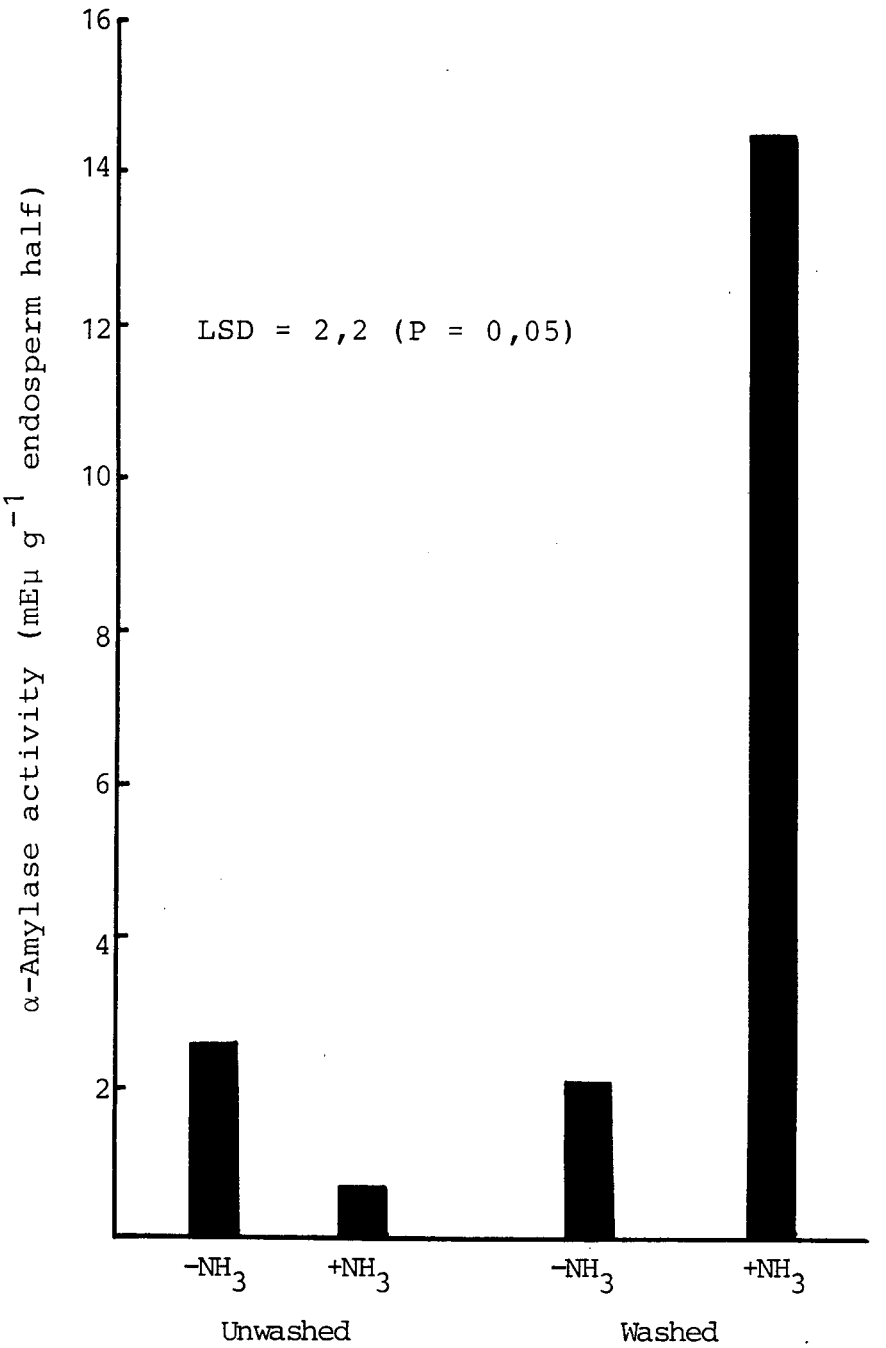

Fig. 6.11 Effect of NH_3-treatment on GA_3-stimulated α-amylase synthesis in de-embryonated endosperm halves of *A. fatua* seed (Montana) as affected by washing of the seed under running tap water prior to incubation

water and determining the pH of the slurry.

The possibility that NH_3-treatment may lead to an increased production of ethylene should also be considered as Eastwell (1981) found that this gas caused an increased diffusion of bound α-amylase out of barley aleurone tissue. However, no increase in ethylene production in the NH_3-treated seed could be observed (results not shown) and further work will have to be done to elucidate this huge increase in α-amylase synthesis by NH_3-treated seeds which have been washed.

6.3.12 The effect of NH_3 on peroxidase and catalase activity

The effect of NH_3 on peroxidase and catalase in embryo-containing half-seeds of *A. fatua* (Montana) is presented in Figs. 6.12 and 6.13 respectively. Peroxidase activity was stimulated significantly in the dry seeds (0 h incubation period) and up to an including the first 24 h of incubation. The difference in peroxidase level between the NH_3-treated and control seeds became progressively less and peroxidase levels were identical after 36 h of incubation. Germination in the NH_3-treated seed had however, already been initiated at this stage. No germination took place in the untreated control seeds.

Catalase activity however, was inhibited in the NH_3-treated seeds during the first 24 h of incubation (Fig. 6.13). Although this inhibition could not be shown to be statistically significant at the 5% level, it was observed in all four experiments conducted on this aspect and thus must be considered as being a true effect of NH_3-treatment. After the initiation of germination after 24 h, catalase activity of the NH_3-treated seeds increased sharply while activity in the control (dormant) seeds declined further.

Hendricks and Taylorson (1974) showed that nitrites, azides

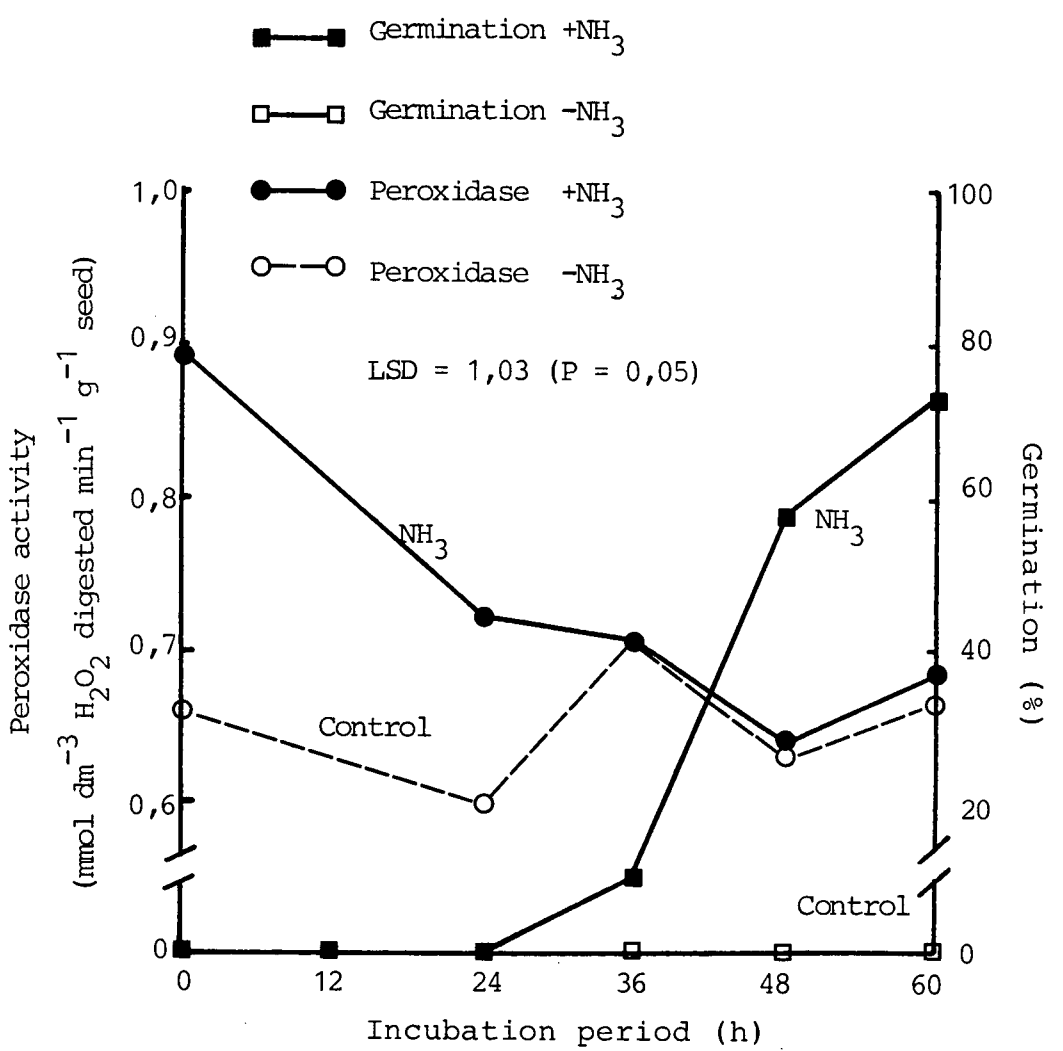

Fig. 6.12 Effect of NH_3-treatment on germination and peroxidase activity of *A. fatua* seed (Montana)

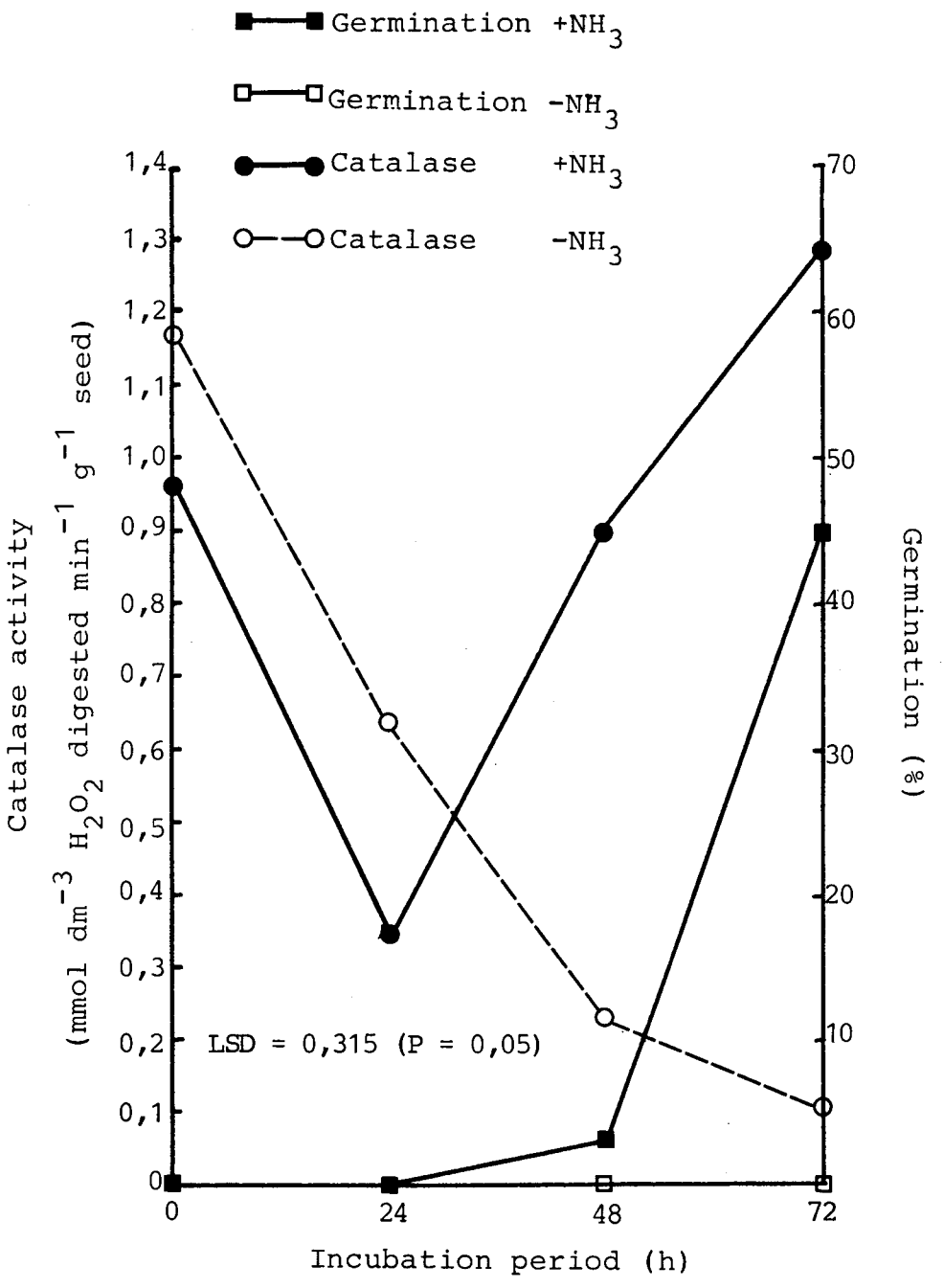

Fig. 6.13 Effect of NH_3-treatment on the germination and development of catalase activity

and hydroxylamines stimulated seed germination by inhibition of catalase activity. They however, found that ammonium salts to be less effective in the promotion of germination and promoted germination only slightly in only two of the species tested viz. *Beta vulgaris* and *B. verna*.

Hendricks and Taylorson (1975) developed their hypothesis further when they again found stimulation of germination of dormant seeds of *Lactuca sativa* and *Amaranthus albus* by solutions of thiourea, sodium nitrite and hydroxylamine salts. They contended that the mechanism of action was via catalase inhibition which spares metabolically derived H_2O_2. This is then used for the oxidation of reduced NADPH required as the oxidant in the pentose phosphate pathway. They postulated that the metabolic system used involves the enzymes peroxidase and pyridine-nucluotide-quinone-oxidoreductase which they found to be present in the quiscent seed prior to incubation.

Esashi *et al.* (1979) presented evidence pointing to the non-involvement of catalase-inhibition in the stimulation of germination by KCN, NH_2OH, $NaNO_3$ and $CS(NH_2)_2$. Working on cocklebur seeds (*Xanthium pensylvanicum*) they found that, in agreement with the results of Hendricks and Taylorson (1975), all the above mentioned chemicals inhibited catalase activity but that the degree of enzyme inhibition was not correlated to the stimulation of germination.

The results and conclusions drawn by Esashi *et al.* (1979) can be criticised on the grounds that they used non-dormant upper seed which were incapable of germinating at 25°C because of their 'lower germination potential' which was insuffient to overcome coat-imposed dormancy. Presumably this statement meant tnat the seed was subject to a form of thermo-dormancy and that the seeds could germinate at lower temperatures, as they later state that chilling resulted in germination without any increase in catalase activity. They

also found that GA_3 stimulated germination with no effect on catalase activity. The fact that GA_3 and chilling led to a breaking of dormancy without inhibiting catalase activity, cannot be used to prove that the inhibition of catalase by other compounds does not play a role in dormancy release. Simpson (1984), in a review article on wild oat dormancy, states that there are obviously a number of metabolic points where dormancy can be broken by various artificial agents and that these different agents do not neccessarily have the same mode of action. This hypothesis gains credence from the fact that dormancy in wild oats can be broken by a wide range of substances such as GA_3, azide and NH_3, which bear no apparant structural of physiological similarities with each other. The hypothesis of Esashi *et al.* (1979) that the inhibition of catalase activity does not lead to a stimulation of germination must be questioned on the grounds of insufficient evidence.

However, the hypothesis of Hendricks and Taylorson (1975) is also erroneous as it has been shown by Uphadyaya *et al.* (1981) and Adkins and Ross (1981) and the present study (6.2.10) that stimulation of the pentose phosphate pathway activity does not lead to germination but rather that the respiratory pathway becomes operative after germination has been initiated. Thus, the sparing of H_2O_2 by catalase inhibition and the consequential increased reduction by peroxidase must be linked to a system unrelated to the pentose phosphate pathway.

Although no work has previously been done on *A. fatua* regarding the relationship between catalase and peroxidase activity and dormancy, Noll (1983) found that in wheat, peroxidase activity increased with both chilling and GA_3 treatment. Olusuyi (1973) presented evidence that the oxidation of NADPH could be limited in dormant barley seeds which, by implication again, suggests that the oxidation of this coenzyme via peroxidase could play a key role in the release from dormancy.

Another possibility is that the inhibition of catalase, resulting in a sparing of H_2O_2, might in some way affect the hormonal balance of the seed by causing an inactivation of IAA. Although the involvement of IAA in the germinative process is still to be demonstrated (Roberts, 1983) it has been shown that H_2O_2 is particularly effective in causing the inactivation of IAA (Omran, 1977). It is significant that after the germinative process had been initiated (Fig. 6.14) there was a rapid increase in catalase activity. These results would be consistent with the hypothesis that the destruction of H_2O_2 via catalase would bring about a rise in the level of IAA which is essential for cell elongation during the onset of growth. It is also very significant that plant tissue, when damaged by some or other stress factor, causes a sudden increase in H_2O_2 content (Patterson and Meyers, 1973). Omran and Eisen (as quoted by Omran, 1977) found a drop in catalase activity and a rise in peroxidase activity when bean seedlings were exposed to 5°C for 24 h. Ammonia treatment of *A. fatua* and other seeds can also be considered as a stress factor and damage to the membranes undoubtedly occurred. This is borne out by increased leakage of electrolytes.

It must be concluded that the NH_3-treatment leads to an increased level of H_2O_2 in the seed via reduced catalase activity before the onset of visible germination but the precise *modus operandi* of the endogenous H_2O_2 build up and how it stimulates germination, remains to be elucidated. The significance of the peroxidase build up is also not clear. Although this enzyme can use H_2O_2 as a substrate, the native substrate of this enzyme in the germinating seed is not known (Noll, 1983).

6.3.13 Effect of interaction of NH_3 with other compounds on dormancy

Ammonia-treated and control seeds (Montana ecotype) were

incubated with various chemicals which could be expected to play a role in germination. The concentrations of the chemicals were chosen so that they would have very little effect on germination on their own. Any interaction between these compounds and NH_3 would thus be clearly evident.

The results of this experiment, presented in Table 6.2, show that with the exception of GA_3 no significant interaction between NH_3 and the other chemicals could be observed. The lack of interaction between azide and NH_3 is surprising as it was subsequently found (see 6.2.16) that NH_3 stimulated the alternative respiratory pathway. Uphadyaya *et al.* (1983) have shown that azide breaks dormancy by increasing the participation of the alternative respiratory pathway. In fact, azide was found in the present study to be a most unsatisfactory dormancy breaker as it not only failed to stimulate germination in several experiments, but actually inhibited germination in at least two experiments (results not shown). In addition, germinating seedlings in the azide-treatments were abnormal with short stubby discoloured roots and/or total lack of shoot development. In one experiment, azide at 10^{-3} mol dm^{-3} did in fact stimulate germination significantly but the batch of seed used (partially after-ripened Oxford ecotype) was only semi-dormant (23% of the untreated control seeds germinated) and it is significant that Uphadyaya *et al.* (1982) were able to achieve dormancy-breaking with azide only in relatively non-dormant lines. Uphadyaya *et al.* (1982) could not break dormancy in the very dormant Montana 73 line (the same ecotype was used in the present experiment) with azide.

The positive interaction between NH_3 and GA_3 could be the result of either a better penetration of the plant growth regulator due to the increased permeability of the seed coat (see Fig. 6.4) or due to different sites of action. The precise mode of action of GA_3 in dormancy breaking is still not completely understood but the idea that gibberellin might also work via modulation of membranes is fast gaining

ground (Khan and Saminy, 1982).

Table 6.2 Interaction between NH_3 and other dormancy-breaking chemicals

Treatment (mol dm^{-3})	Germination (%)	
	$-NH_3$	$+NH_3$
NaN_3 (10^{-3})	0	44
GA_3 (10^{-4})	0	64
IAA (10^{-5})	0	41
BA (5×10^{-5})	0	44
Control	0	48

LSD = 8,4 (P = 0,05)

The effect of salicylic hydroxamic acid (SHAM) on NH_3- and GA_3-stimulated germination is presented in Table 6.3. Salicylic hydroxamic acid did not affect either GA_3- or NH_3-stimulated germination in the Oxford ecotype but significantly inhibited the effect of NH_3 and GA_3 in the Montana ecotype.

Uphadyaya *et al.* (1982) found that azide-induced germination in certain lines of *A. fatua* could be completely inhibited by 3 mmol dm^{-3} SHAM, but that SHAM failed to inhibit germination in non-dormant seed and also seed which had been treated with GA_3. In the present experiment SHAM inhibited both GA_3- and NH_3-stimulated germination.

Simmonds and Simpson (1971) found that GA_3 did not affect the respiratory behaviour of *A. fatua* and the fact that SHAM is able to inhibit GA_3-stimulated germination, may point to

other less specific effects of SHAM. Although Schonbaum *et al.* (1971) found that the hydroxamic acids were specific inhibitors of the alternative respiratory pathway, Rich *et al.* (1978) found that the substituted hydroxamic acids were not as specific as originally suggested and that a number of redox enzymes e.g. tyrosinase and peroxidase were inhibited by the hydroxamic acids. They found that 1 mmol dm^{-3} SHAM inhibited horseradish peroxidase activity by more than 90%. Rich *et al.* (1978) also found that lipoxygenase activity was also inhibited by SHAM. Parish and Leopold (1978) and Goldstein *et al.* (1981) found that the increased oxygen uptake associated with lipoxygenase activity was cyanide-insensitive but sensitive to inhibition by SHAM. This implies that SHAM-sensitive respiration is not neccessarily an indication of alternative respiratory pathway activity.

Table 6.3 Effect of SHAM on GA_3- and NH_3-stimulated germination in different *A. fatua* ecotypes

Treatment	Germination (%)			
	Oxford		Montana	
	-SHAM	+SHAM*	-SHAM	+SHAM*
NH_3 (12 cm^3/30 cm^3)	77	72	56	33
GA_3 (10^{-3} mol/dm^3)	83	84	99	77
Control (untreated)	31	34	0	0
Mean	63,7	63,3	51,7	36,7

*SHAM at 3 mmol dm^{-3}

LSD = 10,57 (P = 0,05)

Peroxidase appears to play a role in NH_3-stimulated germination (Fig. 6.13) and it is thus not surprising that SHAM

inhibited NH_3-stimulated germination (Montana). It is not known whether peroxidase plays a role in GA_3-stimulated germination and it cannot, therefore, be concluded that SHAM also inhibits GA_3-stimulated germination in this way.

Notwithstanding the above, SHAM remains a potent inhibitor of the alternative respiratory pathway and the partial inhibition of NH_3-stimulated germination by SHAM points to the fact that NH_3 may also inhibit conventional respiration and by so doing, increase the participation of the alternative respiratory pathway as has been demonstrated for azide by Uphadyaya *et al.* (1983). The inability of SHAM to inhibit either GA_3- or NH_3-stimulated germination in the partially dormant Oxford seed point to the fact that a metabolic block had probably been overcome during the process of after-ripening.

6.3.14 Effect of NH_3, NaN_3 and SHAM on the respiratory activity of *A. fatua* seed (Montana ecotype)

Both azide and NH_3 stimulated respiratory activity of *A. fatua* (Montana) seeds (Fig. 6.1). Azide however, only stimulated respiration by 25% whereas NH_3 stimulated respiration by 81%. Salicylic hydroxamic acid (SHAM) at 3 mmol dm^{-3} had no significant effect on respiration of the control seeds but inhibited respiration in the azide and NH_3 treatments by 12% and 17% respectively.

Uphadyaya *et al.* (1983) also found SHAM to have little effect on the respiration of control *A. fatua* seeds (Montana ecotype) but that SHAM at 10 mmol dm^{-3} inhibited azide-stimulated respiration by as much as 350%. Uphadyaya *et al.* (1982) had previously demonstrated that SHAM also completely inhibited azide-stimulated germination in several less dormant lines of Canadian *A. fatua* but that SHAM had no effect on germination of genetically non-dormant and after-ripened

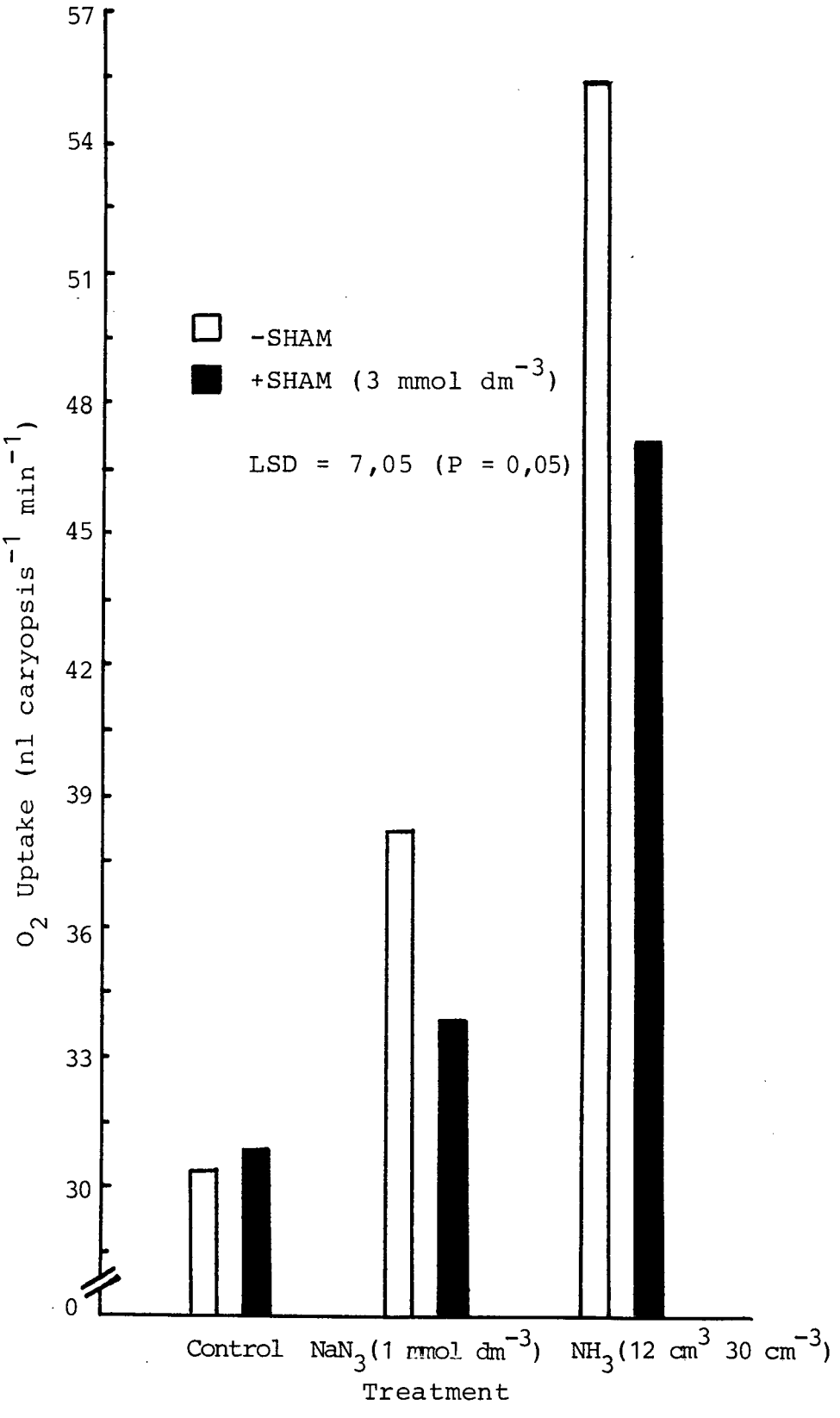

Fig. 6.14 Effect of azide and ammonia on the respiratory rate of *A. fatua* (Montana) as affected by the alternative respiratory pathway inhibitor SHAM

dormant lines.

The lower concentration of SHAM used in the present experiment and the fact that the seeds were not dehulled (Uphadyaya *et al.* (1983) dehulled their seeds before treatment), probably explains why SHAM inhibited azide and NH_3-stimulated germination to a lesser extent. As has been pointed out in the previous experiment, SHAM is not as specific as was originally thought and results using high concentrations must be open to question.

The fact that NH_3 stimulates respiration to a far greater extent than azide goes a long way in explaining its superiority over azide as a dormancy-breaker. The superiority of NH_3 as a dormancy-breaker is underlined by the fact that dormancy in the very dormant Montana 73 line could be broken, whereas azide had no effect on germination in this line even though respiratory activity was stimulated (Uphadyaya *et al.*, 1983).

On the evidence presented above it is by no means certain that the increase in respiratory activity is exclusively via the alternative respiratory pathway. Lambers (1980) stated that, in general, the alternative respiratory pathway is only operative when the cytochrome pathway is saturated either by saturation of one of the carriers or by constraint of oxidative phosphorylation. There is evidence to the effect that ammonium effectively inhibits conventional respiration (Vines and Wedding, 1960). The site of toxicity is thought to be in the electron-transport system where NADH oxidation is inhibited (Haynes and Goh, 1978).

However, other workers in this field have disputed the claim that ammonium inhibits conventional respiration. Wakiuchi *et al.* (1971) found that glycolytic and TCA-cycle enzymes were higher in cucumber plants grown with 11,1 mmol dm^{-3} ammonium compared to those grown with 1,1 mmol dm^{-3} ammonium.

It is impossible to determine the relative contributions of the conventional and alternative pathways on the basis of the results presented in the present study and a detailed study on the lines of that conducted by Theologis and Laties (1978), who described a method to establish the relative contributions of the two pathways, will have to be carried out to determine the mode of action of NH_3-stimulated respiration.

In a review article on wild oat dormancy, Simpson (1984) compared the relative efficacy of various wild oat dormancy-breaking compounds. Dormancy-breaking compounds were listed in ascending order of efficiency viz. gibberellin, ethanol, cyanide and azide and nitrates. In deeply dormant seed dormancy could only be broken by gibberellin. The other dormancy-breaking compounds could break dormancy in less dormant seed lines or in dormant seeds which had undergone a period of after-ripening.

Adkins *et al.* (1984a) found that nitrate, nitrite and hydroxylamine could break dormancy in some dormant lines of *A. fatua* provided that they had been after-ripened for 6 weeks. However, 6-week-old seed of the very dormant Montana 73 line did not respond to these nitrogenous compounds and had to be after-ripened for 6 months before these compounds were able to stimulate germination. It is interesting to note that these workers could not stimulate germination significantly in any line with ammonium chloride. Ammonia was not tested.

Adkins *et al.* (1984b) reported that both nitrate and nitrite stimulated respiration significantly but that ammonium salts did not. This increased respiratory activity induced by nitrate and nitrite could not be inhibited by SHAM showing that the alternative respiratory pathway was not involved.

The dormancy-breaking properties of nitrate and nitrite are explained by Adkins *et al.* (1984 a&b) in terms of the ability

of these two compounds to act as electron acceptors and thus take part in the oxidation of NADH. The inability of NH_4^+ to stimulate germination is explained in terms of its inability to act as an electron acceptor.

The dormancy-breaking effect of NH_3, even on freshly harvested seed of the highly dormant Montana, is difficult to reconcile with the hypothesis proposed for nitrate and nitrite by Adkins *et al.* (1984 a&b). Ammonia cannot act as an electron acceptor and thus increase respiration by helping to oxidise NADH. That NO_3^-, NO_2^-, NH_3 and NH_4^+ should differ so widely in their dormancy-breaking activity is an enigma, the solving of which could well provide the answer to many as yet un-answered questions on release from dormancy.

The possibility that NH_3 broke dormancy by acting as an anaesthetic in the same way as was demonstrated for ethanol by Taylorson and Hendricks (1979) was also considered but the fact that NH_3-treatment led to the irreversible loss of dormancy would seem to negate this. If seed was still non-dormant long after NH_3 treatment, it seems improbable that enough NH_3 could remain in the seed to cause an anaesthetic effect. In addition, no reference to NH_3 as an anaesthetic could be found in the literature.

It is postulated that dormancy release by NH_3 is mediated via a combination of the physiological effects observed. Increased permeability of the seed coat and the consequent increase of water uptake by the seed, inhibition of catalase activity, stimulation of peroxidase activity and the marked stimulation of respiratory activity are all factors that can contribute to the loss of dormancy. The discovery of NH_3 as a dormancy-breaker, inferior only to gibberellic acid in graminaceous seeds, will hopefully stimulate further physiological research on the mechanism of dormancy-breaking by this gas.

Finally, the use of NH_3 as a dormancy-breaking agent would seem to have the following practical advantages.

1. Dry seed can be treated, aired and then stored until needed. Other chemicals used to break dormancy have to be applied in an aqueous solution with the obvious disadvantage that germination is initiated and the seed has to be planted immediately. Certain organic growth regulators such as gibberellins and cytokinins can, however, be applied in organic solvents (Rao, 1976).

2. Ammonia is a relatively non-toxic gas and seedlings which result from NH_3-treated seed are normal in every respect. Wild oat seed treated with GA_3, produce seedlings which are elongated and weak and cannot be used for experimental work such as the screening of herbicides. Azide-treated seed produce seedlings with short stubby roots and abnormal shoots.

3. Ammonia-treatment is easy to apply and no special equipment is needed. If the gas is not available in cylinders it can be easily prepared from common laboratory reagents as previously described. Seed that is stored in silos can be easily fumigated by injection of the gas. The fact that NH_3 can also break dormancy in barley is very interesting as newly harvested barley is dormant and has to be stored for several months before it is suitable for malting purposes (Grierson, 1981).

4. Recently, there has been an increase in the use of liquid ammonia as a fertilizer (Theron, A.A. personal communication) and it would be interesting to ascertain if there was an increase in the germination of graminaceous weeds subsequent to the application of the ammonia. If germination is in fact stimulated with field applications of NH_3, it would offer the first practical

method of ridding the soil of its seed bank of germinaceous weeds. This aspect should be fully investigated in a further study.

CHAPTER 7

SUMMARY

7.1 Characterization of *Avena* ecotypes from different ecological regions

In a study of the growth habit and dormancy of different populations of naturalised ecotypes of *Avena* species, it was established that both germinative and photoperiodic behaviour was related to the climatic conditions prevailing at the centre of origin of the naturalised ecotype.

Growing temperature had no effect on dormancy of seed produced over all ecotypes, but ecotypes from temperate zones were less dormant when grown at 26°/20°C day/night temperature than when grown at 18°/12° day/night temperature. Over all ecotypes, significantly more seed germinated when incubated at 5°C than at 15°C.

White light inhibited the germination of Canadian and South African ecotypes of *A. fatua* but stimulated the germination of a U.K. ecotype. This divergent reaction to light can explain the many inconsistensies in literature concerning the effect of light on *Avena* germination. The reaction to light is explained in relation to the cultural practices employed in the countries of origin.

7.2 Effect of plant growth substances and their interaction with other factors on the dormancy of *A. fatua*

Gibberellic acid taken up by developing panicles was far more effective in preventing the onset of dormancy in the seed than was a foliar application of the hormone. Gibberellic acid (both GA_3 and $GA_{4/7}$) was the only plant growth regulator found to stimulate the germination of dormant wild oat

seed when applied exogenously to mature seed. However, in seed previously treated with CO_2 under anaerobic conditions, the addition of kinetin greatly enhanced the stimulatory effect of GA_3 on germination in the light.

Red light was also found to increase the stimulatory effect of $GA_{4/7}$ on germination of dormant U.K. *A. fatua* seed. Both far-red light and darkness were inhibitory to $GA_{4/7}$ - stimulated germination.

Azide, hydroxylamine and daminozide all proved to be synergistic to gibberellic acid - stimulated germination.

7.3 Effect of various sugars on dormancy and gibberellic acid sensitivity in *A. fatua* seed

Dormancy of *A. fatua* seed produced from excised panicles grown in sugar solutions was proportional to the concentration of the sugar. However, when panicles were grown in other osmotically active substances such as mannitol and PEG, it was found that the dormancy of the seed produced was inversely proportional to the concentration of the osmoticum. These results are related to the physiological role of sugars in the plant and the stress conditions imposed by high concentrations of osmoticum.

The addition of GA_3 to the sugar growth medium did not prevent the induction of dormancy in the seeds produced but these seeds became non-dormant after a much shorter period of after-ripening when compared to seed grown in sugar alone. The addition of the photosynthetic inhibitor simazine to the growth medium containing sucrose, led to the production of even more dormant seed.

Sugar concentration also affected the ability of de-embryonated endosperm halves to synthesise α-amylase. Low concentrations of the various sugars were stimulatory to

α-amylase synthesis but high concentrations completely inhibited α-amylase synthesis. This phenomenon is probably related to feedback inhibition by the sugars on the production of the hydrolytic enzyme.

The capacity of excised embryos with attached scutella to synthesise α-amylase was similarly influenced by the concentration of sugars in the medium. Low concentrations stimulated α-amylase synthesis while high concentrations were again inhibitory. The optimum sucrose concentration of α-amylase synthesis in both de-embryonated endosperm halves and excised embryos with attached scutella was found to be in the order of 75 mmol dm^{-3}.

7.4 Effect of NH_3 on the dormancy of *A. fatua* seed

On fumigation by 'Phostoxin' tablets it was found that dormant *A. fatua* seed became non-dormant. The active ingredient in the mixture of gases produced by this tablet was found to be NH_3. The other two gases PH_3 and CO_2 had very little, if any, effect on *A. fatua* dormancy. Treatment of air dry *A. fatua* seed with NH_3 resulted in a loss of dormancy. Ammonia also broke dormancy in all graminaceous seed tested but had no effect on dormancy of any of the dycotyledonous seeds tested.

Ammonia treatment of the seed was found to increase the permeability of the seed coat with the concomitant increase in water uptake. Treated seed could be stored for at least 3 months with germination improving with the length of the after-ripening period. *Avena sterilis* seed, which had been stored for more than two years after NH_3-treatment, showed no loss of viability.

The pentose phosphate pathway enzymes glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase were

unaffected by the NH_3-treatment. However, treated seeds had a higher peroxidase level during the first 24 h of incubation but catalase activity decreased over the same period.

With the exception of gibberellic acid, which showed a positive interaction with NH_3, other plant growth regulators and an inhibitor of the conventional respiratory pathway (NaN_3) showed no effect on NH_3-stimulated germination. However, the alternative respiratory pathway inhibitor SHAM inhibited NH_3-stimulated germination by 17%.

Ammonia was also shown to have increased respiratory activity in treated seeds that had been incubated for 48 h by 81% while azide-treatment only resulted in a 25% increase in respiration.

The breaking of dormancy with NH_3 is explained in terms of the combined effect of increased permeability and water uptake, increased peroxidase activity, lower catalase level and an increased respiratory tempo.

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