STUDIES ON SEED DORMANCY AND GERMINATION IN Avena fatua (L).

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by

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Studies on the Physiology of germination in the genus Avena.

The germination requirements of grains of Avena sativa, Avena fatua and Avena ludoviciana have been investigated, and seed dormancy shown to develop with the attainment of ripeness in these species. The degree of dormancy was found to be transitory in A. sativa, and more intense and more persistent in the other two species.

Promotion of the subsequent germination was observed when grains of A. sativa were allowed to imbibe for several hours, subjected to a dehydra treatment and a period of dry storage before being returned to germination conditions. This was shown to be largely due to the retention of some of the physical and physiological changes which normally occur within the earl stages of germination. Embryo damage resulted when the same treatment was given to grains with growing embryos, the severity of the damage being related to the degree of morphological development of the embryo at the time of treatment. It was concluded that there is no initial period of imbibition which can be completely reversed by this treatment, and that there is no distinct lag between the commencement of imbibition and of the changes leading to the onset of growth.

The development of amylolytic enzymes in germinating grains of A. sativa has also been studied. Only b-Amylase was present in the dry grain but concurrently with, or shortly after the commencement of embryo growth a-Amylase began to be developed. General similarities were observe in the pattern of Amylase development in germinating grains of all three species investigated, but dormant grains of A. fatua and A. ludoviciana

showed no increased amylase development. The development of a-Amylase would appear to take place in the endosperm, but the growing embryo appear to play an essential role in its development.

Analyses of grains of A. sativa after 24 hours in germination conditions showed that a considerable utilisation of seed fats, and of some soluble carbohydrates has occurred. Starch hydrolysis was not evident until 24-48 hours in germination conditions.

Proteolytic activity of dry grains of A. sativa was found to be mainly located in the embryo tissues, and to undergo a several-fold increase durin the pre-germination period of imbibition. This development took place mainly in the embryo tissues, with diffusion to the endosperm occurring later. Germinating grains of A. fatua and A. ludoviciana showed a similar pattern of increasing proteolytic activity, but dormant grains showed no capacity for increased activity. Since this increased activity is a pre-germination change, and is potentially capable of restricting embryo growth it must be considered as a possible mechanism by which seed dormancy is enforced.

The presence of germination inhibiting materials in whole grains, and husks of A. sativa has been confirmed, and their activity shown against seed of A. sativa, Hordeum vulgare, Triticum aestivum, Linum usitatissimum, Brassica oleracea and Trifolium pratense. Previous investigators claimed that the action of these materials was due to their inhibition of the amylase enzymes of the grains, particularly a-Amylase. Tests with amylase preparations from germinated grains of A. sativa, and barley, and fungal an bacterial a-Amylases failed to confirm this finding. The possibility of

retarding germination by inhibiting amylases would appear to be doubtful i the light of our findings on amylase development and starch utilisation. Considerable inhibition of the activity of proteolytic enzymes from grains of A. sativa and Barley was however observed in the extract of A. sativa husks. The same water extracts of whole grains of A. sativa and A. fatua were found to be capable of inhibiting the germination of A. sativa grains and promoting the germination of A. fatua grains. The presence, and the amounts of these germination inhibiting and promoting materials in A. fatu could not be correlated with the degree of dormancy in the grains used for extraction.

CONTENTS

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General Introduction

Note: In recent years many reviews on Germination and Dormancy have been published. Full use will be made of this in the General Introduction; more pertinent references will be confined to discussion in the relevant sections of the thesis.

A seed is defined as a ripened ovule (Crocker & Barton 1957) and consists of an embryo with its surrounding coats; in many seeds, an additional structure, the endosperm, is present, developed from the double fusion nucleus. The dispersal unit of the Gramineae is technically a fruit - a caryopsis - in which the covering layers are developed from the fused pericarp and testa (Brown 1965). The embryonic axis, of radicle enclosed in the coleorhiza and plumule protected by the coleoptile, is medianly attached to the scutellum, which is generally regarded as the single cotyledon. The grain of Avena fatua (L) is a structure of this type. In addition, the whole caryopsis is enclosed at maturity in the hardened and persistent pales.

This unit-seeded fruit, including the pales, will be referred to as the seed in this physiological investigation.

The development of the seed on the parent plant can be divided into two phases. Immediately after fertilisation, the embryo undergoes a period of very active growth with the elaboration of new tissues and the accumulation of food materials. Enzyme activity (see Brennan 1960) and growth substance content (Carr & Skene 1963; Stoddart 1965) are high during this phase. The moisture content of barley ovaries decreases daily from 80% to 42% (Harlan & Pope 1923). At this point, seed development enters its second phase during which dry matter accumulation is interrupted, respiratory activity declines (Wager 1957) and the seeds dry out rapidly to air dryness.

If immature seeds are removed from the parent plant and set in favourable conditions for growth, development will frequently continue without interruption from ovule to seedling (Gill 1938; Fuchs 1941; Barton 1962; Negbi & Tamari 1963). This appearance of germinability before ripeness, is probably related to a distinct developmental stage when moisture content levels off (Hyde 1959). et al. In cereals, the appearance of germinability has also been correlated with the degree of desiccation the seed has undergone (Wellington 1956a; 1964).

Further desiccation of the seed as it matures on the plant results in a halt in seed ontogeny. When all growth and development has thus ceased, the seed is said to have reached full-ripeness and in this state can remain viable for some years (Barton 1961). The term 'Quiescent' has been applied to this state (Brown 1965). The quiescent seed is characterised by a low water content, suspended embryo development, and a high content of potential energy in the form of accumulated protein, carbohydrate and fats. It also displays a marked resistance to lethal factors in the environment, e.g. extremes of temperature.

Germination can be considered to be the processes leading to the resumption of embryo development (Toole et al. 1956; Evenari 1957) and is to be distinguished from the subsequent seedling growth, dependent on the utilisation of the food reserves. In oat, Brennan (1960) observed germination to be the extension of the radicle coils,

meristematic activity beginning some hours later. Keller and Negbi (1959) consider that germination is the elongation of existing cells, while growth involves the differentiation of newly-formed cells. With lettuce seed, evidence has been presented suggesting that germination is specifically connected with cell expansion (Haber 1960; Haber & Luippold 1960a, b).

Relatively little is known about the nature of the processes preceding the commencement of cell elongation. Brown (1965) reviews the course of events until the seedling is no longer dependent on food reserves.

A primary requirement for germination must be water. The uptake of water by a seed is biphasic: there is an initial rapid uptake by imbibitional forces; the second phase of rapid uptake - occurring at 18 hours at 20°C in oat (Drennan 1960) - develops as the cells become vacuolated and is physiologically controlled (Wellington & Durham 1961).

An increase in physiological activity parallels the increase in water content, and is manifest by the increase in respiratory activity. As measured by gaseous exchange, respiration follows a biphasic increase (James 1953; Stiles 1960a). The plateau period between the phases varies in duration according to the species, and it has been suggested that certain crucial biochemical events occur at this point (Mayer & Poljakoff-Mayber 1963a). In lettuce seed, entry into the second rise is controlled by the red/far-red light control mechanism (Evenari et al. 1955). The pathway of respiration changes during the course of germination: the early

stages of germination in peas involve an anaerobic respiration until the seed coats are ruptured (Spragg & Yemm 1959); in oat, a fat-based cyanide-sensitive respiration operates for the first 48 hours before changing to carbohydrate respiration (Drennan 1960); initially, lettuce seeds are respiring via the pentose pathway until glycolysis and the tricarboxylic acid cycle (TCA) take over as the main pathways (Mayer 1961). The generation of the TCA requires synthesis of the enzymes involved in the system, succinoxidase being one of the first to appear (Poljakoff-Mayber et al. 1958). Respiratory enzymes found in germinating seeds are reviewed by Brown (1965) and Mayer (1960). Oxidase systems reported to be operating in seed germination include (Toole et al. 1956; Mayer 1960):-

cytochrome oxidase;

ascorbic acid oxidase

and the phenolases.

Full metabolism of seeds does not develop as soon as water is taken up. Most enzyme systems are inactive and only appear as germination progresses. Studies of the activation of these systems in relation to the visible signs of germination indicate that the latter precede changes in enzyme activity (Wareing 1963). Koller et al. (1962) also conclude that organic nutrient release is unlikely to be concerned in the germination process. Drennan and Berrie (1962) observed cell elongation before there was a measurable rise in α-amylase activity in the endosperm. In wild oats, the increase in α-amylase activity depends on the unity of the embryo and

endosperm, suggesting that such increase in activity follows completion of preliminary growth processes in the embryo (Drennan 1960). Brown (1965) points out that, in barley, little use is made of the endosperm reserves during the first 48 hours.

Although it is doubtful whether starch hydrolysis by amylase activity is critical for germination, there is considerable utilisation of fats and some soluble carbohydrates before the onset of meristematic activity in oat (Drennan 1960); also, proteolytic activity of the embryo undergoes a several-fold increase during the pregermination period of imbibition. In lettuce seed, sucrose is the first substrate utilised in germination, lipid oxidation being a relatively late process (Poljakoff-Mayber 1952: quoted in Mayer 1960).

Detailed accounts of the biochemical changes during this period of development are given in the comprehensive reviews by Crocker and Barton (1957); Mayer (1960; 1961); Drennan (1960); Koller et al. (1962); Mayer and Poljakoff-Mayber (1963a, b); Brown (1965).

This breakdown of food reserves is accompanied by a movement of sugars, amino acids etc. to the growing regions and the appearance of synthesising activity in the scutellar region (Edelman, Shibko and Keys 1959; Edelman and Keys 1961).

Thus the establishment of a seedling results from the resumption of activity of the quiescent embryo and its continuation through to growth, utilising food reserves until a photosynthetic system is established.

The seed of many species will germinate readily when provided with conditions of temperature, aeration and moisture favourable for growth. That is, development from the ovule to the new plant can continue without interruption. This is distinct from the situation where a seed has attained full-ripeness or has been subjected to an unfavourable environment and will not germinate upon return to favourable conditions without a specific pre-treatment. Such seed are said to be dormant. Toole (1939: quoted in Evenari 1956) considers dormancy to be "any condition of viable seeds which makes them resistant to germination under environmental conditions ordinarily favourable for quick germination." Thurston (1960) reviews the use of the term, pointing out that there are three main states:-

Innate dormancy:	a genetic property of the ripened seed;
Induced dormancy:	persisting after a period of unfavourable conditions;
Enforced dormancy:	imposed by necessarily continuing unfavourable conditions.

Koller (1955) restricts the term to cases where the embryo does not germinate when excised from the surrounding tissues, describing other structures concerned in the block as co-factors.

Environmental factors involved in the breaking of dormancy can be considered under the general headings of light, temperature, gases and chemical effects. Each of these factors influences in

one or more ways the main mechanisms responsible for the imposition of the dormant condition on the living embryo, viz:

inhibiting covering layers;

underdeveloped physiological state;

presence or absence of substances affecting germination.

Seed Coverings

The inhibition caused by covering structures can arise in several ways. The testa of many species of leguminous seed is impermeable to water (Hyde 1954). Mechanical restriction of expansion of the embryo has been attributed to the seed coat of Amaranthus sp. (Crocker 1916). Gaseous exchange between the living tissues and the external atmosphere may be interrupted, physically (Atwood 1914; Brown 1940; Thornton 1945; Roberts 1961) or chemically (Pollock 1958). The coverings may also exert their control by affecting inhibitor relationships, either by carrying an inhibitor (Black & Wareing 1959; Miyamoto et al. 1961; Evenari 1957) or by preventing leaching or oxidation of the inhibitory substances (Black 1959; Wareing & Foda 1957).

The subject of seed covering effects is reviewed by Caldwell (1959), Koller et al. (1962), Vegis (1964), Lang (1965) and Barton (1965b).

Physiological Underdevelopment

The types of dormancy considered under this heading are equivalent, in many cases, to Barton's (1965a) embryo dormancy. The blocks to germination, manifest by specific environmental

requirements, become less strict as the seed ages in storage, suggesting a physiological maturing of the seed. Although the presence of the seed coat in an intact condition is usually necessary for this state to be imposed (e.g. Evenari & Neumann 1952) the change which occurs in the seed in enabling it to overcome the block to germination has not yet been shown to involve a change in the seed coat.

Radiation is an environmental factor concerned in the imposition or removal of such a block to germination. Reviews on the effects of light on plants are provided by Borthwick and Hendricks (1960, 1961), Heath and Vince (1962) and Mohr (1962, 1964) and, with specific regard to seed germination, by Evenari (1956, 1965) and Mayer (1960). The effects of light on seed germination are various. The stimulatory effect seems to be controlled by the phytochrome mechanism, red light promoting germination and far-red light reversing these effects (Borthwick et al. 1952). The inhibitory effects of light on germination are not so clearly understood; the greatest activity lies in the blue and far-red regions of the spectrum and a high energy reaction activated by these wavelengths has been proposed (see Mohr 1962). Isikawa (1962) reviews various types of response of seeds to light, indicating that the classical phytochrome control of lettuce seed represents a less complex system. A photoperiodic effect is also found in seed germination (Wareing 1959b). The response of seeds to light is markedly influenced

by temperature (Toole et al. 1955; Black & Wareing 1955; Siegel 1950; Stearns & Olsen 1958; Fuji & Isikawa 1961) suggesting that in seed germination, as in flowering, photocontrol commonly interacts with thermocontrol.

The temperatures at which seed will germinate provide another parameter which indicates the specificity of the required germination environment. Edwards (1932) reviews and discusses the concept of the optimum temperature. Long (1965) reviews the more recent literature. Maximal and minimal temperatures of germination vary from species to species, and also within a species depending on the age of the seed from harvest (Harrington 1923c). The temperature range for the germination of cereal seeds narrows during maturation of the seeds (Fuchs 1941); and freshly-harvested seeds often have a low maximum temperature which rises with increasing age from harvest (Atterberg 1907). Vegis (1963, 1964) has developed this concept and describes how the temperature range is often negatively correlated with the depth of dormancy. The effects of diurnal alternating temperatures in stimulating germination (Harrington 1923a) or substituting for a light requirement (Evenari 1956) and for which various mechanisms of action have been proposed (see Koller et al. 1962) also become less marked with increasing age of the seeds from harvest.

Growth Substances

An inherent failure of isolated embryos to germinate has been attributed by many authors to the presence of an inhibitory substance in the dormant tissue. The subject is adequately reviewed by Evenari (1957) and by Wareing (1965). Among naturally-occurring substances which have been found to have inhibitory properties are: ammonia, cyanide, unsaturated hydrocarbons, essential oils, mustard oils, alkaloids, unsaturated lactones, phenolic acids and a large number of unidentified compounds. The correlation between inhibitor content and depth of dormancy that exists for bud dormancy in woody species (Wareing 1959a; Libbert 1959; Vegis 1964) has not been definitely established for dormancy in seeds. In some cases there is correlation between the loss of dormancy and inhibitor disappearance (Black 1959; Delouche 1956; Soriano et al. 1964) or the appearance of inhibitors under unfavourable conditions for germination (Rollin 1958a; Mosheev 1936); other authors have been unable to demonstrate such correlations (Drennan 1960; May 1962).

Although immature seeds have proved to be a rich source of stimulatory compounds (Corcoran & Phinney 1962; Jones, MacMillan & Radley 1963), investigations into changes in levels of such substances during germination have met with limited success in lettuce seeds (Blumenthal-Goldschmidt & Lang 1960; Ikuma & Thimann 1960). But there are reports of the activity of

stimulatory compounds increasing during stratification (Frankland & Wareing 1962). Recent work on this aspect has developed the concept of the state of dormancy being determined by interaction between inhibitors and stimulators (Allen 1960; Eagles & Wareing 1962; Hemberg 1958; Naylor & Simpson 1961; Villiers, Frankland & Wareing 1963).

With the introduction of these concepts of chemical control of dormancy, effects of the application of chemicals to dormant seed are of interest. Gibberellic acid has been found to substitute for a light requirement (Lona 1956; Kahn, Goss & Smith 1957), for a cold treatment (Gray 1958; Barton et al. 1957; Rollin 1958b) and to reverse high temperature inhibition of germination (Toole & Cathey 1961). It has been shown that gibberellin can affect organic nutrient release via increased amylase activity (Paleg 1960, 1963; MacLeod & Miller 1962). Kinetin has been found to sensitise lettuce seeds to light (Miller 1958) and to reduce heat damage (Porto & Siegel 1960). Two other naturally-occurring compounds which have been investigated by exogenous application are the stimulatory substance, thiourea, and the inhibitor, coumarin. Reviews on their effects will be found in Mayer (1960, 1961) and in Poljakoff-Mayber & Mayer (1960): coumarin seems to inhibit lipid breakdown; thiourea can substitute for a cold treatment and seems to bring the TCA into operation at an early stage of lettuce seed germination. Other substances found to affect germination, for example, mannitol, dinitrophenol, 6-(substituted) aminopurines,

nitrates, are discussed in the review by Stiles (1960b).

From a consideration of this work, one is led to the conclusion that seed metabolism is not fixed and that these substances affect germination through various pathways and through various interactions with endogenous substances.

After-ripening

Although the processes leading to the loss of dormancy are speeded-up or reversed by the described treatments, dormancy also decreases with ageing of the seed in dry storage, as is manifest by the gradual relaxation of the strictness of the required environment and by a rise in the percentage germination under any one environment. The term "after-ripening" was first used by Crocker (1916) to describe dormancy-breaking changes during moist storage at low temperatures, but is now also used to refer to naturally-occurring processes which take place after harvest and remove dormancy. The subject is reviewed by Stokes (1965).

Flemion (1933) has described the effects of such "stratification", or moist low temperature storage, on dormant Rosaceous seeds, which give rise to dwarf plantlets if germinated without the cold treatment. Such a requirement is also common in temperate grasses, although, in this case, any germination which does occur in the absence of the treatment is normal. Some changes which occur during the treatment have been described: the "growth potential" of the embryo increases (Crocker & Barton

1957); fat digestion occurs (Vegis 1964); the respiratory quotient decreases (Harrington 1923b); phosphate acceptors increase (Pollock & Olney 1959); and changes occur in phosphates, nucleotides and TCA intermediates (Bradbeer & Colman 1963). Older workers considered the changes which occur to involve oxidation (Eckerson 1913; Harrington 1923b). More recent reports are concerned with the increase in activity of stimulatory factors (Frankland & Wareing 1962).

The changes which take place in seeds in dry storage, for example, disappearance of light and temperature sensitivity, have been suggested to be due to changes in coat permeability (Atwood 1914; Johnson 1935). Roberts (1963) suggests that the reaction(s) may involve a non-metabolic oxidation and Koller et al. (1962) also believe that inactivation of inhibitory substances may be involved.

It is possible that changes occurring during the after-ripening of dormant seeds are equivalent to changes which occur in non-dormant seeds during ripening on the mother plant.

Secondary Dormancy

If seeds which have the capacity for entering the dormant state are set to germinate in unfavourable conditions, not only is germination inhibited, but the seed fail to germinate upon return to optimum conditions. This state has been termed

secondary dormancy (Crocker 1916; Koller et al. 1962). Such a state can be induced by:-

Light - in Nigella sativa (Kinzel - quoted in Crocker 1916);

Darkness - in Chloris ciliata (Gassner - quoted in Crocker 1916);

High temperature - in lettuce (Borthwick & Robbins 1928);

Low oxygen - in Ambrosia trifolia (Davis 1930);

Carbon dioxide - in Sinapis alba (Kidd 1914);

Anaerobiosis - in Avena fatua (Naylor & Christie 1956);

In all these cases, it is the embryo which is affected, although other structures may have contributory roles. Mechanisms proposed to explain such phenomena are based on the production and accumulation of inhibitory intermediates by various pathways (Thornton 1945; Vegis 1956; Hay 1962).

Thus, there are well-balanced, sequential patterns of physiological and metabolic changes by which an embryo develops into an active plant, dormancy representing a block to, or change in, these patterns.

The seed habit of higher plants ensures the multiplication and dispersal of the species; the unique morphological and physiological adaptations operating during this phase of development endow the dispersal unit with resistance to adverse environments. The condition of dormancy allows seed to be dispersed in time, as well as in space. In nature, the moist

seed may be exposed to favourable conditions for germination followed by an unfavourable period for growth. Therefore, this ability to remain imbibed and viable, but ungerminated, can be regarded as an adaptive feature (Amen 1963; Koller 1964); Barton 1965a) and is no doubt subject to selection. Geographically dormancy is more frequently found in species of the temperate zone (Crocker 1916) where it affords the plant a means of controlling germination under a variable environment. It is also more common in wild species (Barton 1962) than in specially-selected cultivated plants.

In Avena sativa, the system of reactants involved in germination is complete and balanced, requiring only water to promote the change, under a wide variety of environments, from a quiescent embryo to a developed seedling. But Avena fatua, under apparently favourable conditions, does not germinate to the same extent. This dormancy is one of the main factors responsible for the success of this annual plant as a weed species, rendering ineffective cultural methods of control.

Lindsey (1956) has described the origins and varieties of the species. The distribution of wild oats in Britain has been surveyed by Thurston (1956, 1963) and its severity as a weed species in North America by Selleck (1961), Atwood (1914) and Barton (1962).

Any weed control programme must be based upon knowledge of the dormancy mechanisms operating in the species. Also, the

behaviour of seeds involves responses common to other plant processes. A study of these processes, using a material convenient to handle, such as seeds, may lead to a better understanding of the fundamental mechanisms controlling morphogenesis.

Therefore, this investigation is concerned with the characterisation and study of blocks to the germination processes of *Avena fatua*.

Part I: A Preliminary Survey of the Germination Behaviour of Avena fatua

In a study of dormancy, two approaches are possible; the environments in which the state of dormancy arises can be determined; and the factors required to remove the block can be examined. Inferences may then be made regarding the nature of the block.

Since temperature and light are environmental agencies which markedly affect seed dormancy, a survey of the germination behaviour of Avena fatua under various regimes of these factors was carried out. In Part I, the results of such work are described and presented as a statement of the problem.

Throughout this work, the term "dormant" refers to the seed population. The degree of dormancy in the population is described by the percentage germination occurring under a particular environment. Inherent in such a view will be the fact that, due to the natural variability among the seeds of a population, some germination will occur under any environment. However, this percentage may be altered by various treatments; that is, the population is changed and dormancy is said to have been increased or decreased. This view is, in reality, considering the physiological behaviour of the population under different environments.

Materials and Methods

Seed material from two sources was used: commercial samples, obtained through Hasler & Co. Ltd., Dunmow, Essex, from the threshings of cereal crops; and seed grown under natural conditions at Glasgow. Samples were stored in closed containers at 20°C or at -16°C until required. All populations were composed of at least three varieties, according to the classification of Hubbard (1954):

brown palea - var. pilosissima S. F. Gray;

grey palea - var. pilosa Syme;

yellow palea - var. glabrata Peterm.

It was found that there was variation in the germination percentages within samples. In addition to the mixture of genotypes in wild oat populations (Inman & Allard 1965), the degree of dormancy is correlated with the stage of ripening (Drennan 1960) and the position of the seeds in the panicle (Johnson 1935); and, in preliminary experiments at Glasgow, it has been found that the environment under which the seeds are ripened influences the depth of dormancy in the population. Allowance has been made for such variation by repeating each experiment at least three times and observing reproducible differences between specific treatments. The results reported in this thesis refer to germination percentages of representative trials. Seed from both sources behaved similarly, but were never mixed within one experiment.

Standard germination tests were carried out in 9 cm. petri

dishes on Whatman Seed Test paper, 9 cm. x 0.4 mm. Deionised water was supplied at the rates of 4 ml. per 25 seed, or 5 ml. per 50 seed; care was taken to keep the paper moist throughout an experiment.

The light source used was a 15 W, 240V tungsten bulb situated 8-12 inches from the petri dishes. The radiant energy was measured by a Kipp & Zonen compensated thermopile, not cosine corrected. Irradiation, 9" from the filament and after passing through the petri dish lid, was 6×10^3 ergs/sec/cm^2. The ratio of red/far-red = 1/1.12. Darkness was achieved by enclosing the dishes in cardboard boxes, previously tested for light-tightness.

Seeds were exposed to the stated temperatures $\pm 1^{\circ}$C, in thermostatically-controlled incubators.

Germination was counted as having occurred when the radicle was first observed, using a x14 binocular microscope. Such germinated seeds were removed from each dish. Counting of darkness-treated seeds was carried out under a green safe-light, previously tested for its lack of physiological effect on seed of Grand Rapids lettuce and wild oats. It was found that no further germination occurred after five days from the start of imbibition in air. Therefore, the total germination occurring within a week is considered to be a measure of the germination under a particular environment.

Statistical significance of the differences between treatments was determined by the contingency chi-squared test, using the

numbers of germinated and ungerminated seed in the totalled replicates for each treatment. Interactions were determined by analyses of variance on the angular transformations of the individual germination percentages.

Section A - TEMPERATURE EFFECTS

Experiment 1 - The germination of different samples of seed over a range of temperatures.

Two samples of seed, a) 4 months dry storage at 20°C, b) 60 months dry storage at 20°C, were tested under standard conditions in darkness, over a range of temperatures. Results of a typical trial, using seed of var. pilosa, are shown in Table 1; the percentages in each treatment are from three replicates of 50 seeds.

The other two varieties showed similar patterns of behaviour in being inhibited by high temperatures during the early stages of after-ripening.

Table 1

Final percentage germination of var. pilosa at the stated temperatures.

Seed type	Temperature			
	15°C	20°C	25°C	30°C
4 months from harvest	46	41	24	5
60 months from harvest	88	94	92	80

Figure 1: Patterns of germination after high temperature pretreatments.

Abscissa = hours germination.

Ordinate= % germination.

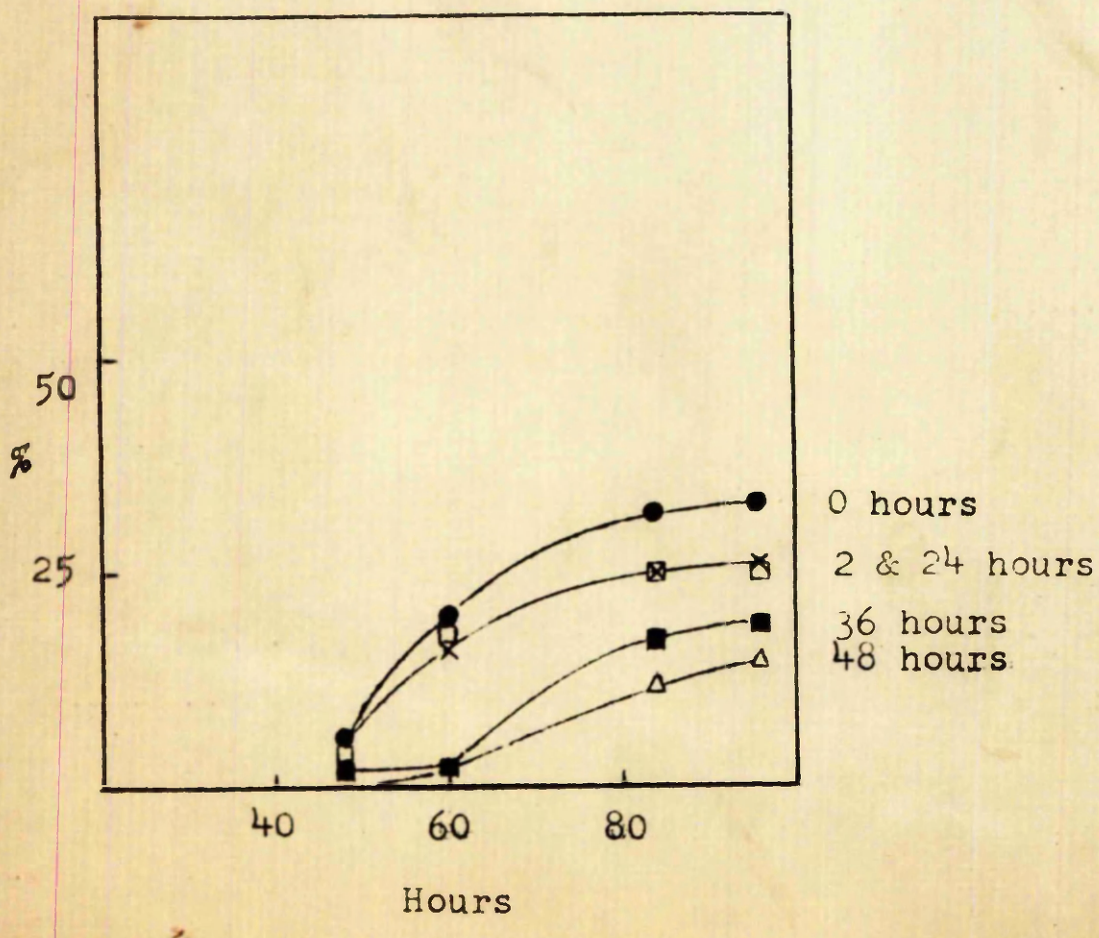
●: o hours at 30°C.

×: 2 hours

□: 24 hours

■: 36 hours

Δ: 48 hours



Experiment 2 - The germination of dormant seed after a high temperature pretreatment.

Seed which had been stored for 4 months at room temperature were imbibed in darkness at 30°C for various lengths of time before being placed to continue germination at 20°C, in darkness. Germination was recorded at time intervals from the start of imbibition.

The patterns of germination for var. pilosa are recorded in Table 2 and graphed in Figure 1.

Table 2

Germination at various times after high temperature pretreatments.

Period at 30°C	Percentage germination at:				
	48 hours	60 hours	84 hours	90 hours	15 days
0	6	20	32	33	34
2 hours	6	16	25	26	27
24 hours	4	17	25	25	26
36 hours	2	2	17	19	20
48 hours	0	2	12	15	17

X^2 (4df) for final count = 14.5**

Experiment 3 - The effects of a high temperature shock at various stages of imbibition.

The possibility of there being a specific stage during germination that is sensitive to high temperatures was investigated. Seed, 4 months from harvest, were set to germinate in darkness at 20°C; at specific times from the start of imbibition, the samples were placed as 30°C for 2 hours or for 6 hours and then returned to 20°C.

No effects of such high temperature shocks were manifest in the final germination percentages, as recorded in Table 3.

Table 3

Final percentages of germination after high temperature shocks.

Stage at which 30°C shock given	Duration of 30°C shock	
	2 hours	6 hours
No 30°C treatment	44	44
0 hours	38	--
8 hours	36	40
24 hours	42	38
36 hours	42	36
48 hours	36	--
	χ^2 (5df) = 5.96	χ^2 (3df) = 2.37

Experiment 4 - Non-reversal of high temperature inhibition by low temperatures.

Seeds were subjected to the high temperature inhibition by imbibing them for 1 or 2 days at 30°C, in darkness. They were then placed at 4°C for varying periods before being put into 20°C to complete their germination. No germination was apparent in any sample when it went into 20°C. The final counts recorded in Table 4 were made when there had been no further germination for 6 days. Both treatments can be seen to be inhibitory.

Table 4

Final germination percentages at 20°C after various temperature treatments.

Period at 4°C.	Period at 30°C		
	0	1 day	2 days
0	36	27	18
2 days	30	20	10
4 days	15	20	14
6 days	20	20	15

Analysis of Variance on the Angular Transformations

Source	df	S.S.	M.S.	V.R.
Replicates	2	30.54	15.27	N.S.
4°C	3	308.70	102.9	8.3**
30°C	2	214.16	107.08	8.7*
4°C x 30°C	6	156.39	26.15	N.S.
Residual	22	271.41	12.34	-
Total	35	981.70	-	-

Experiment 5 - Effects of low temperature pretreatment on germination in darkness.

"Stratification" in cereals generally requires 5-7 days at 5°C. Samples of wild oats were imbibed for 6 days at 5°C before being germinated at 20°C in darkness. This treatment was carried out on two seed types:-

a) less than 4 months from harvest;

b) more than 6 months from harvest.

The effect of such stratification in raising the germination of the 4 month sample and lowering the germination of the 6 month sample is shown by Table 5.

Table 5

Final germination percentages after stratification.

Seed Variety	Period at 4°C	Age of seed from harvest		X^2 (1df) "0" VS. "6" days	
		4 months	6 months	4 months	6 months
glabrata	0	20	40	4.5*	14.28**
	6 days	30	20		
pilosa	0	19	46	18.0**	8.15**
	6 days	42	30		
pilosissima	0	7	40	61.8**	8.62**
	6 days	49	24		

Discussion

Dormant A. fatua seeds respond to temperature in the typical manner of freshly harvested cereal seeds, as described by Atterberg (1907) and Fuchs (1941), in that the initial low maximum temperature for germination rises as the seeds age in dry storage (experiment 1). Greater inhibition of germination results from increasing lengths of exposure to high temperatures (experiment 2) and this seems to be a cumulative effect of high temperature on the metabolism of the seed rather than sensitivity to high temperature of a specific stage of the process (experiment 3). The high temperature inhibition results in a change of state in the seed, irreversible by a return to optimal or lower temperatures of the extent tested in experiment 4. This is in contrast to the behaviour of A. ludoviciana (Thurston 1954; Brennan 1960) and of lettuce seed (Borthwick & Robbins 1928). The inhibitory effects of supra-optimal temperatures on germination are well-documented (e.g. Davis 1930; Koller & Roth 1963) and it has been suggested that high temperature is conducive to the formation of an inhibitory situation (Vegis 1956) or substance (Borrie 1966) within the seed.

Although observations have been made with regard to the beneficial effects of over-wintering on graminaceous seed (Bibbey 1948), experimental work on the effects of a cold treatment has not been conclusive (Friesen & Shebeski 1961). The results of experiment 5 indicate that the effects of a cold treatment upon germination in darkness vary with the degree of dry storage which

the seeds have undergone: with increasing after-ripening stratification becomes inhibitory. These results are in good agreement with Kirkwood (1956) for A. sativa and Krueger (1941) for Impatiens balsamina. It may be that the apparently beneficial effects of a cold treatment upon relatively fresh seed, as shown in Table 5, result merely from the provision of a period during which reactions inhibited by high temperature can occur, rather than from the initiation of stimulatory reactions by low temperature.

Summary of Section A

1) The low maximum temperature for germination of dormant seed rises with storage.

2) High temperature inhibition is the manifestation of a change of state in the seed, irreversible by return to low temperatures.

3) Stratification will raise the germination of freshly-harvested seed; but has no effect on, or depresses, the germination of partially after-ripened seed.

Section B - EFFECTS OF RADIATION

1. Inhibition of Germination by Light

Experiment 6 - The germination response under different photoperiods.

Samples of var. *pilosa* - 4 months and 60 months from harvest - were subjected to various photoperiodic treatments at 20°C.

The results of a typical trial are reported in Table 6, as the final percentages of germination in 3 replicates of 50 seeds per treatment.

The other two varieties behaved similarly in showing increasing inhibition with lengthening photoperiods in the early stages of after-ripening.

Table 6

Final germination percentages under different photoperiods

Seed type	Daily hours of Light:						
	0h.	4h.	8h.	12h.	16h.	20h.	24h.
4 months	41	29	28	18	17	17	10
60 months	94	--	96	89	89	--	90

Experiment 7 - The germination response to increasing periods of irradiation.

Dormant samples of var. pilosa were exposed to light for varying periods of time from the start of imbibition at 20°C. They were then placed in darkness at the same temperature to continue their germination. After 7 days in darkness all germination had ceased.

The final counts reported in Table 7 indicate an increasing inhibition with increasing time in light - except for the germination occurring after 6 hours light which is significantly higher than the dark-germination.

Table 7

Final germination percentages after single exposures to light for different periods.

Hours of light	0	6h.	12h.	24h.	48h.	96h.	continuously
Final % germ.	52	68	40	44	12	20	16

Figure 2: Germination after exposure to light under nitrogen.

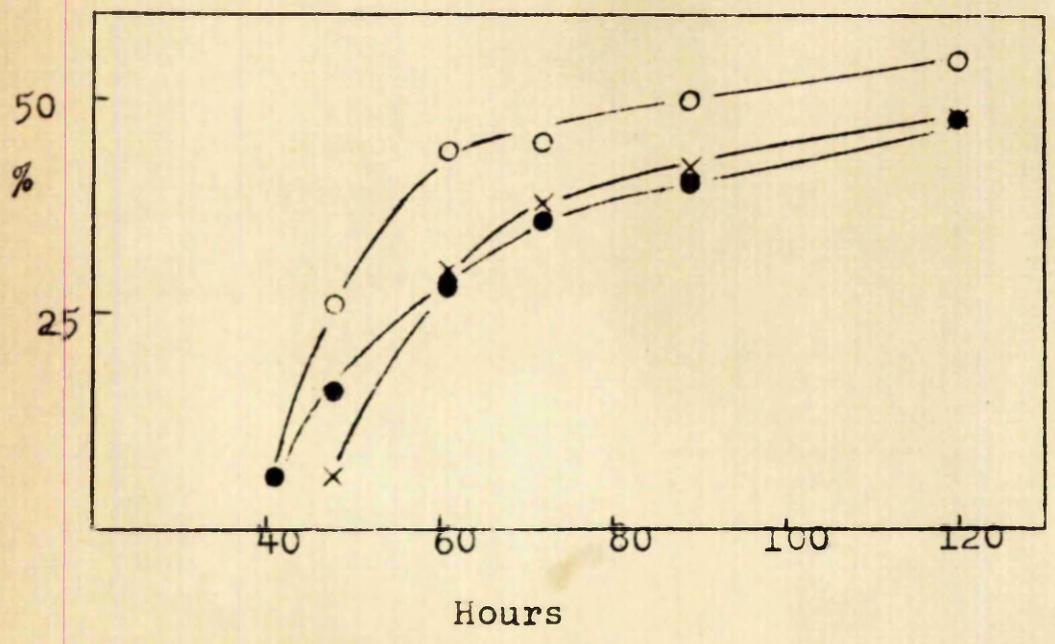
Abscissa = hours germination.

Ordinate = % germination.

x : standard germination;

● : 2 days darkness under N_2;

o : 2 days light under N_2.



Experiment 8 - The effects of exposure to irradiation under nitrogen.

Partially-dormant samples of var. pilosa (10 months from harvest) were imbibed in light or darkness under an atmosphere of nitrogen for 2 days at 20°C. (Full details of the method used for treating seeds with gas mixtures will be found in Part III.) After such treatment the seeds were germinated in petri dishes in darkness at 20°C.

The patterns of germination in a representative trial of three replicates of 25 seeds per treatment are recorded in Table 8 and graphed in Figure 2. The time intervals were measured from the point when the seeds were placed in air.

It can be seen that light has no inhibitory effect when seeds are imbibing under nitrogen; and the significance of the X^2 indicates that irradiation under these conditions may hasten germination.

Table 8

Germination after irradiation under nitrogen

Pretreatment	Percentage germination at:-						
	18h.	41h.	48h.	61h.	72h.	89h.	120h.
None	0	0	6	30	38	42	48
2 days Light in N_2	0	6	26	44	46	50	55
2 days Dark in N_2	0	6	16	28	36	41	48
X^2(1df) between N_2 =		5.3*	4.2*	1.1	0.00	0.06	

Discussion

The germination of a partially-dormant population of wild oats is inhibited by white light (Cumming & Hay 1958). There does not seem to be any photoperiodic effect at 20°C (experiment 6); the longer the period of exposure to light, the greater is the proportion of the population inhibited (experiment 7). The results also indicate that the prevention of germination by light is not reversed by returning the seeds to darkness. It can be demonstrated that such seed are not dead since they can be caused to germinate by removing their palea and pricking the caryopses. Therefore, the degree of dormancy in the population has been increased by light. Light does not increase the dormancy of fully-after-ripened seed (experiment 6).

The light inhibition of germination in Lemium amplexicaule (Baxter-Jones & Bailey 1956) and Nemophila insignis (Black & Wareing 1957) has been shown to be due to the far-red and blue regions of the spectrum, suggesting control by the high energy reaction (Mohr 1962). In wild oats, although a high degree of inhibition is induced by 2 days exposure to light in air (experiment 7), no such inhibition is induced by 2 days irradiation under nitrogen (experiment 8). Therefore, the light inhibition mechanism in wild oats does not seem to be controlled by phytochrome, since Ikuma & Thimann (1964) report that the change to the active form of phytochrome can occur under nitrogen.

Figure 3: Short irradiations of varying periods at different stages of imbibition.

Abscissa = hours germination.

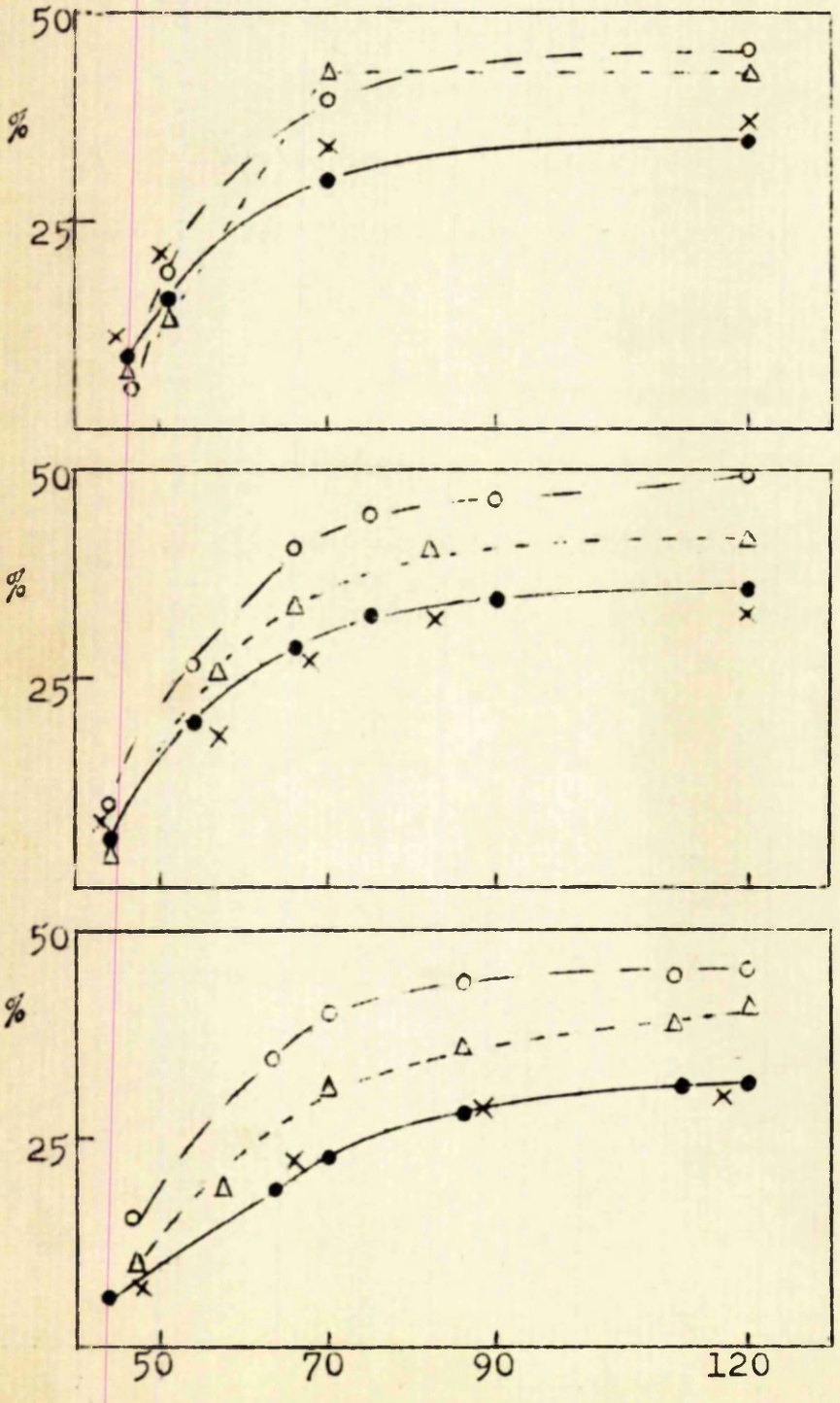
Ordinate = % germination.

●: standard dark germination;

○: light during 0-12 hours;

×: light during 12-24 hours;

△: light during 24-36 hours;



Irradiation period of 4 hours.

Irradiation period of 8 hours.

Irradiation period of 12 hours.

2. Stimulation of Germination by Light

Experiment 9 - The effect of short periods of irradiation at various stages of imbibition.

Seed were set to germinate at 20°C in darkness. At different stages of germination, measured as time from the start of imbibition, they were exposed to light for various lengths of time, then returned to darkness.

A complete trial, using var. *pilosa* 8 months from harvest, is reported in Table 9, as final percentages of germination. Figure 3 shows the patterns of germination obtained after such treatments.

Table 9

Final germination percentages after different periods of irradiation at various stages of imbibition

Duration of irradiation	Stage of Imbibition		
	0-12 hrs.	12-24 hrs.	24-30 hrs.
0 (dark)	34	36	37
4 hours	46	39	46
8 hours	50	34	42
12 hours	46	32	40

Variance analysis of Angular transformations

Source	df	S.S.	M.S.	V.R.
Replicates	2	2.53	1.26	N.S.
Stage	2	167.64	83.82	9.7**
Amount	3	100.70	33.57	3.9*
Interaction	6	104.61	17.45	N.S.
Residual	22	190.04	8.64	-
Total	35	565.52	-	-

Discussion

The results of experiment 9 indicate that white light stimulates the germination of wild oats, if they are irradiated at the appropriate stage of imbibition, viz. during the first 12 hours at 20°C. The stimulatory effect of light may also be manifest when seeds are irradiated under nitrogen (cf. N_2 treatments in experiment 8).

Thus, light seems to evoke two responses in wild oat seeds. Cases are recorded where white light exerts opposite effects on the germination of other species (Soriano 1953; Isikawa 1957; Vose 1962; Negbi & Koller 1964); lettuce seed germination is inhibited by continuous red light (Scheibe & Lang 1965) and by blue light at specific stages of imbibition (Leggatt 1948; Evenari et al. 1957; Wareing & Black 1958). To explain these results, interaction of the phytochrome mechanism with the high energy reaction has been suggested. These two photomechanisms are also thought to interact in the cell elongation involved in lettuce hypocotyl growth (Evans et al. 1965) and in dodder hook opening (Lane & Kasperbauer 1965).

No facilities were available for investigating the action spectrum or the energy relationships of the light responses in order that they may have been shown to be controlled by one or by two photomechanisms. The light used in the reported experiments had more energy in the far-red than in the red regions. The data of Cumming & Hay (1963) indicate that red light has no inhibitory

action on wild oat germination under their conditions. Further experimentation with Red light may show that phytochrome is active in the germination of this species, together with an inhibitory photomechanism.

Summary of Section B

1) The germination of dormant wild oats is inhibited by white light; this inhibition disappears during after-ripening.
2) The inhibitory mechanism does not seem to be controlled by phytochrome; seed metabolism is blocked, such blockage resulting in secondary dormancy.
3) White light may also stimulate the germination of wild oats.

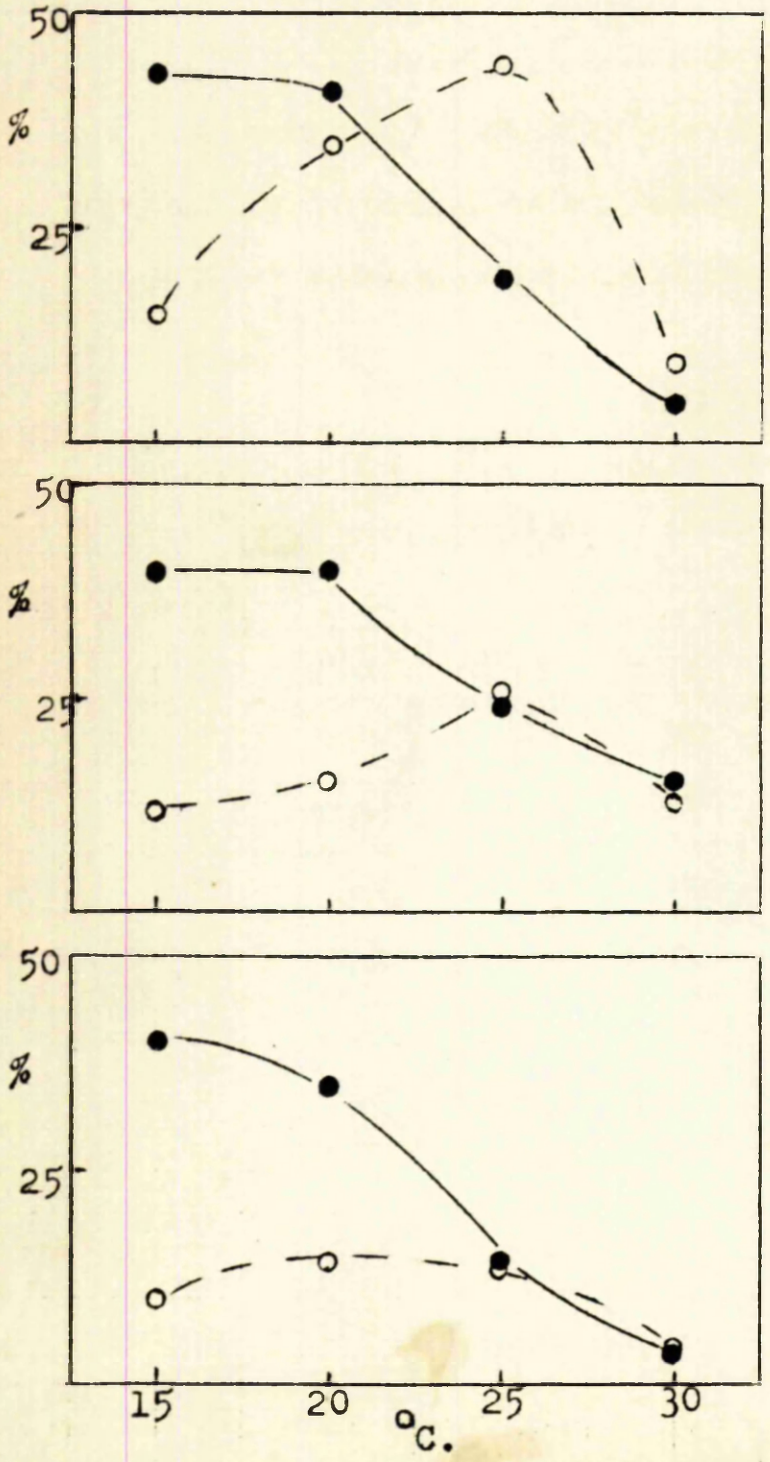
Figure 4: Final germination in light and darkness over a range of temperatures.

Abscissa = temperature.

Ordinate = % germination.

● : dark germination;

o : light germination.



var. glabrata

var. pilosa

var. pilosissima

Section C - LIGHT AND TEMPERATURE INTERACTIONS

Experiment 10 - **Germination in light and darkness over a range of temperatures.**

After 6 months storage at room temperature, seed were germinated in standard tests under light or darkness at four temperatures. The final germination percentages are indicative of the responses to such treatments. A trial of 4 replicates of 25 seeds per treatment is reported in Table 10; significant differences between light and darkness at a particular temperature are denoted by asterisks. The results are graphed in Figure 4.

Table 10

Final germination percentages in light and darkness over a range of temperatures.

Variety	Light	15°C	20°C	25°C	30°C
	L	15	35	44	9
glabrata	D	43**	41	19**	5
	L	12	15	25	12
pilosa	D	40**	40**	24	14
	L	10	14	15	3
pilosissima	D	40**	35**	15	4

Experiment 11 - The effects of temperature on the response to short irradiations.

Samples of var. pilosa were exposed to light during the first 8 hours of imbibition. The light exposures were carried out at 20°C, 30°C or 5°C, with appropriate controls of 8 hours imbibition at 30°C or 5°C in darkness. After treatment, all the samples were returned to darkness at 20°C for germination.

Thus, darkness at 20°C is the standard germination; 8 hours imbibition in darkness at 30°C or 5°C shows any temperature effect on germination; and 8 hours exposure to light at 30°C or 5°C are the treatments being tested, compared to these controls and 8 hours light at 20°C.

Samples were used which had undergone different periods of after-ripening and it will be seen that the system changes during after-ripening from a marked inhibition by low-temperature-light at 0-12 months after-ripening, to a stimulation of germination by high-temperature-light at 12-20 months of after-ripening.

Tables 11a and b show the final germination percentages after such treatments; the X^2 are calculated from contingency tests of the totalled replicates - 8 replicates of 50 seeds per treatment.

Table 11

a) Final germination percentages at 20°C, after a short irradiation at 30°C.

Treatment	Age from harvest:-		
	6 months	12 months	20 months
Continuous dark 20°C	46	50	50
8 hrs. dark 30°C	37	42	50
8 hrs. light 20°C	48	48	55
8 hrs. light 30°C	38	62	68
X^2 (3df)	6.41	10.08*	10.65*

b) Final germination percentages at 20°C, after a short irradiation at 5°C.

Treatment	6 months	12 months	20 months
Continuous dark 20°C	46	50	50
8 hrs. dark 5°C	42	44	40
8 hrs. light 20°C	48	48	55
8 hrs. light 5°C	30	36	44
X^2 (3df)	11.85**	7.28	0.98

Discussion

Seeds of the three varieties respond differently to light (experiment 10): pilosissima shows the greatest inhibition by light, and glabrata the least. Coffman & Stanton (1938), under unspecified light conditions, found the greatest dormancy in dark-coloured seeds. When germination is graphed against temperature, as in Figure 4, the behaviour of the three varieties can be seen to follow a similar pattern, indicating that the systems being studied may not be qualitatively different.

The changing response of the seeds towards light under different temperatures is further demonstrated in experiment 11: the stimulation by short irradiations is enhanced by high temperatures; and at an earlier stage of after-ripening, the short irradiations at low temperatures are inhibitory; the temperature shocks themselves, in darkness, do not affect the germination response.

Therefore, it could be argued that in a partially-dormant population of wild oat seed there is a system operating which causes light to have a stimulatory effect at high temperatures and an inhibitory effect at low temperatures. It is clear that the light responses are not indirect temperature effects caused by the radiation being absorbed in the form of heat; if this were so, and 15°C-20°C taken as optimal temperatures for germination, then at higher temperatures light would be expected to be much more inhibitory than darkness.

Light and temperature interactions are known to operate in the germination of many species. A light requirement for germination has been reported to increase with temperature (Koller & Negbi 1959; Fuji & Isikawa 1961; Koller & Roth 1964; Mayer & Poljakoff-Mayber 1963b). Cumming (1963) points out that the optimum photoperiod for germination in Chenopodium spp. increases with temperature. Such systems may be analagous to the case of lettuce seed, where supra-optimal temperatures divert metabolism into a germination pathway which requires to be activated by phytochrome. Conversely, Hutchings (1932) found the light requirement of Mimulus sp. to decrease with temperature and Black & Wareing (1955) suggested that the decreased light requirement of birch was due to high temperature favourably affecting the processes initiated by light. Toole et al. (1955) reported high temperature to result in increased sensitivity of the photomechanism to red light, and decreased sensitivity to far-red.

The experiments reported in this section do not allow distinction to be made between these three situations with respect to the light x temperature interactions in wild oat seeds, viz:

a) diversion of metabolism from a pathway inhibited by light to one stimulated by light with increasing temperature;

b) two simultaneous light responses, their associated reactions having different temperature coefficients;

c) one light reaction with a change in sensitivity of the response with temperature.

To resolve those possibilities, greater control of the quantity and quality of the radiation will be necessary.

Experiment 11 also indicates that this complex system changes with progressive after-ripening: fresh seed seem to have the inhibitory system predominating; with increased after-ripening, the stimulation by light becomes more obvious.

Experiment 12 - The effect of stratification on the light response.

Seeds of var. glabrata were subjected to a stratification period of 6 days at 5°C in darkness before being set to germinate in light or darkness at 20°C or 30°C. Because of the difference in the dark response to stratification with age from harvest, shown in experiment 5, seeds of different ages were tested.

The results of a typical test involving 3 replicates of 50 seeds are shown in Table 12; the behaviour of non-stratified seed is also shown for comparison.

It can be seen that stratification enhances the high temperature light stimulation after 7 months storage.

Table 12

Final germination percentages at 20°C and 30°C after stratification

Temperature of germination	Light	Age from harvest:-			
		3 months		7 months	
		Strat.	Non-strat.	Strat.	Non-strat.
20°C	L	22	2	12	36
	D	30	20	20	40
30°C	L	32	0	52	10
	D	26	0	13	5

Experiment 13 - The effect of stratification under different gaseous environments.

Seeds were placed on moist filter paper in stoppered conical flasks and a sequence of evacuation and flushing carried out until atmospheres of oxygen, carbon dioxide or nitrogen were obtained. After 6 days in darkness at 5°C, the seeds were removed from the flasks and germinated in light or darkness at 20°C or 30°C.

Three replicates of 50 seeds of var. pilosissima after 4 months dry storage were used.

In table 13, a comparison of the germination percentages indicates that air or oxygen are not necessary for the effects of stratification on the high temperature light stimulation to be manifest.

Table 13

Final germination percentages at 20°C & 30°C after stratification under different gases.

Germination conditions		Stratification atmosphere:				X^2(3df) between gases
		Air	O_2	N_2	CO_2	
20°C	L	10	10	14	20	8.65*
	D	28	29	35	45	10.74*
30°C	L	28	19	50	20	44.0**
	D	20	20	20	15	2.10
No stratification						
20°C	L	4				
	D	20				
30°C	L	2				
	D	1				

Discussion

The effects of stratification on subsequent germination in the light differ from its effects on dark germination (experiment 12): a cold treatment enhances the light stimulation at high temperatures, although this effect is only manifest in seed which have undergone some 7 months dry storage. Rollin & Martin (1961) note that low temperature brings the phytochrome system into operation in Phacelia tanacetifolia. Black & Wareing (1955) found that chilling removes the light requirement of birch seed.

In this respect of "readying" the seed for high temperature ligh stimulation, stratification in wild oats can be seen to result in a similar physiological state within the seed as does a period of some twenty months dry storage (cf. experiment 11). However, the differing responses to stratification, depending on the stage of after-ripening and subsequent germination conditions of the treated seeds, suggest that no one single reaction can explain the process and may account for the conflicting statements in the literature regarding the effects of stratification on wild oats (cf. Coffman & Stanton 1938; Friesen & Shebeski 1961).

The stratification effect does not seem to be operating via an "oxygen storage" mechanism, as suggested for seeds of apple (Harrington 1923b), rice (Roberts 1962) and reed canary grass (Vose 1962). Experiment 13 shows that the effect can operate in an atmosphere in which oxygen is absent.

Summary of Section C

1) Varieties of wild oat behave differently with respect to specific conditions, but follow a similar basic pattern of response to light and temperature.

2) Temperature modifies the response to light in that high temperatures favour light stimulation and low temperatures increase light inhibition of germination.

3) Stratification enhances the high temperature light stimulation of germination at the appropriate stage of after-ripening in dry storage.

4) Stratification does not depend on oxygen for its effects.

5) The light response changes during dry storage from a light inhibition towards a light stimulation.

General Conclusions from Part I

An obvious feature of the preceding work is the lack of a complete breaking of dormancy or inhibition of germination by any environment. Criticism can also be made of the drawing of conclusions from a sometimes slight difference between a treatment and its control. These features are believed to result from the multiplicity of dormancy mechanisms operating in this species and from the inherent genotypic and phenotypic variability in the populations used. However, all the reported effects are statistically significant and reproducible: as described in Materials and Methods, the reported effects have appeared in repeated trials.

The systems here described by their responses to light and temperature obviously form a network of processes.

The response to temperature is typical of freshly-harvested cereal seed, with a rising maximum during storage. The complex process of stratification is similar in its enhancement of light stimulation at high temperatures to after-ripening in dry storage; it is not an "oxygenation" effect.

Two light effects can be shown by dormant wild oat seed, the effects differing in the amounts of irradiation required to initiate the response, the temperature relations of the associated reactions and the stage of after-ripening at which each is most apparent.

It is probable that the slight stimulation by light results

from activation of a phytochrome mechanism. The prevention of germination by light may be an effect of the high energy reaction. This block to metabolism is imposed upon seed actively germinating in air and results in the entry of the seed into the state of secondary dormancy.

The mechanisms affected by irradiation and responsible for the inhibition of germination are investigated further in the following sections.

Part II: The Influence of the Seed Coverings on the Responses to Light and Temperature

There are two distinct covering structures around the wild oat grain: the pales, made up of collapsed, lignified cells; and the caryopsis wall, which, in Avena spp. (Bennett 1961) consists of the lignified remains of the ovary and ovule epidermal layers, interspersed by fatty material (Thurston 1963).

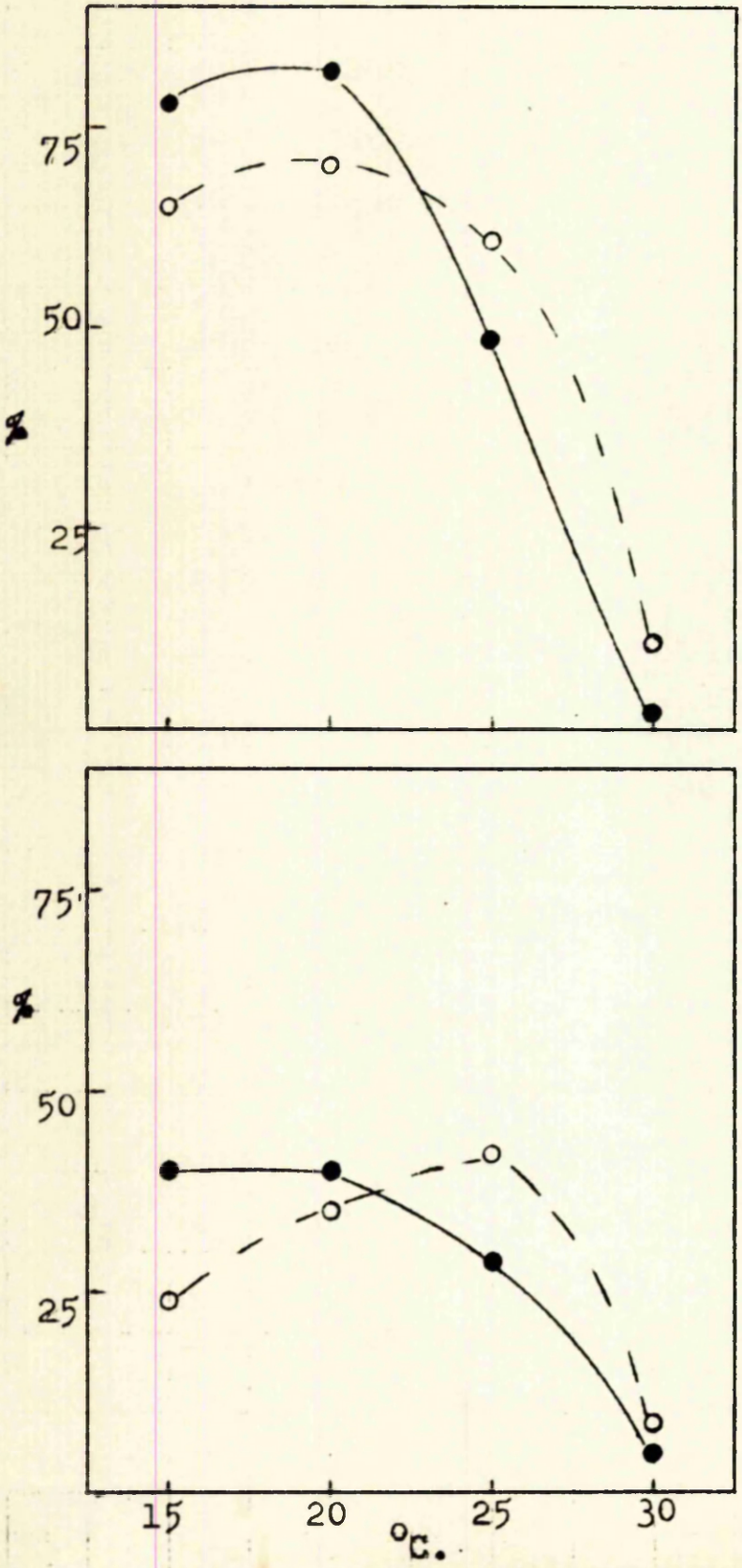
Germination in many seeds is promoted by removal or rupture of the structures enclosing the embryo and the response to light is often modified by such treatments (see Evenari 1965). The effects of disrupting the structural association between the coverings of the wild oat grain are described in Part II.

Materials and Methods

Seed treatments consisted of "pricking" or of "dehusking" the grain: pricking involves piercing the dorsal surface of the grain with a mounted needle, such that both the pales and the caryopsis are holed; removal of the pales is termed dehusking - only undamaged caryopses being used. Unless otherwise stated, these treatments were carried out on seed in the unimbibed state.

Standard germination tests were run under the described environments. Recording and presentation of the results are as in Part I.

Figure 5: Final germination under light x temperature of pricked and normal seed.



Abscissa=temperature.

Ordinate=% germ.

● : dark;

o : light;

Pricked seeds.

Normal seeds.

Section A - EFFECTS OF PRICKING

Experiment 14 - The effects of pricking on the response to light and temperature.

Seed of var. glabrata, 7 months from harvest, were pricked and set to germinate in light and darkness at four temperatures.

The final germination percentages of a typical trial are reported in Table 14 and illustrated in Figure 5. Comparison with the figures for non-pricked seed indicates that, although pricking raises the percentage germination, it does not radically alter the pattern of response to light and temperature at this stage of after-ripening.

Table 14

Germination of pricked seed under various Light/Temperature regimes

Seed type	Light	15°C	20°C	25°C	30°C
Pricked	L	65	70	61	11
	D	78	82	49	2
X^2 (1df) L vs. D		6.43*	5.87*	3.44	7.1**
Non-pricked	L	24	35	42	8
	D	40	40	39	5
X^2 (1df) Lus. D		6.6**	0.9	5.84*	0.8

Experiment 15 - The effects of pricking on seed at different stages of after-ripening.

Samples of var. pilosa were stored at -15°C or 20°C for 9 months from harvest. They were then pricked and set to germinate in light or darkness at 20°C.

In Table 15, the effects of pricking on the final percentages of germination in such populations are shown: those seeds which have been in dry storage at 20°C, and which have presumably undergone a greater degree of after-ripening, have their light inhibition negated by pricking.

Table 15

Final percentages of germination after pricking in seed at different stages of after-ripening

Seed type	Light	Pricked	χ^2(1df) L vs. D	Non-pricked	χ^2(1df) L vs. D
9 months at 20°C	L	75	1.05	20	29.8**
	D	81		58	
9 months at -15°C	L	51	14.8**	8	0.6**
	D	77		25	

Experiment 16 - Investigation of a possible mode of action of the pricking stimulation.

The effect of pricking may be related to an increased aeration of the dormant tissues. This was tested by comparing the germination of pricked seed with that of seeds in which the hole caused by pricking was covered with lanolin.

Variety pilosa, 7 months from harvest, was used: as each seed was pricked lanolin was immediately smeared over the hole, taking care not to spread the lanolin over the whole dorsal surface.

The final percentages of germination in Table 16 - from 3 replicates of 25 seeds per treatment - indicate that while lanolin does not affect the germination of non-pricked seed, it does reduce the germination of pricked seed.

Table 16

Final percentages of germination of seed pricked and smeared with lanolin

Germ. conditions	non-pricked seed	Non-pricked + lanolin	Pricked seed	Pricked +lanolin	X^2 (1df) "Prick." vs. "Prick+lan."
Light	16	14	66	45	6.9**
Dark	47	45	89	66	12.4**

Discussion

Pricking breaks the dormancy of wild oats to a certain extent, confirming the results of Atwood (1914). Germination in both light and darkness is raised, but the inhibitory effects of high temperature are still apparent (experiment 14).

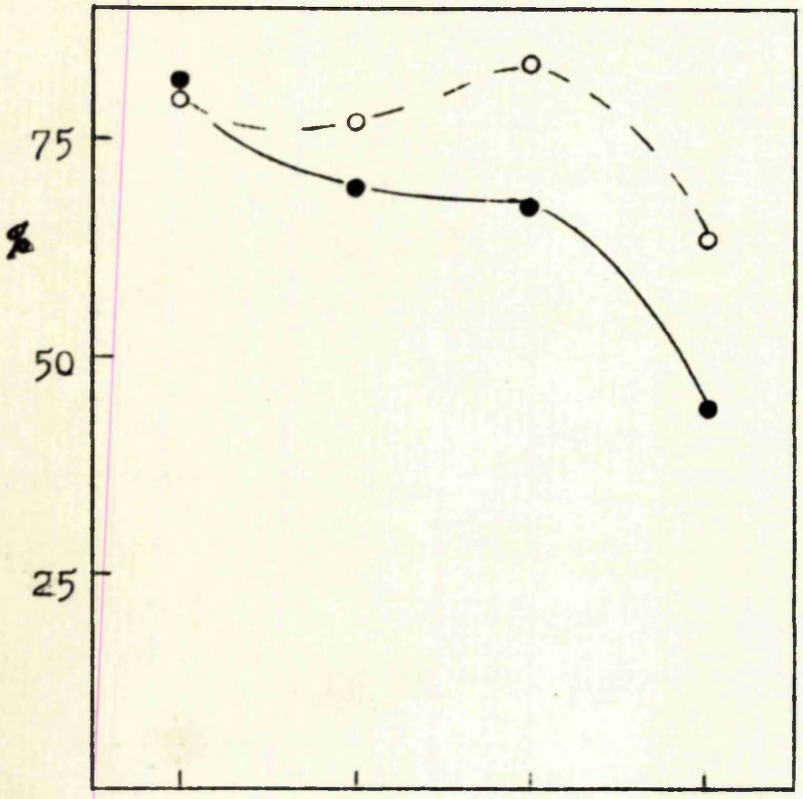
The inhibition of germination by light does not seem to be specifically counteracted by pricking: experiment 15 indicates that both after-ripening and pricking are necessary. Differences in after-ripening may account for the disagreement between these results and those of Cumming & Hay (1958), who found that pricking did remove the light inhibition. Legatt (1948) reported that pricking did not nullify the effects of blue light on lettuce seed germination.

If lanolin is smeared over the hole caused by pricking, the stimulation is significantly lowered (experiment 16). This suggests that at least part of the stimulation is brought about by an increased entry of air - or exit of a volatile inhibitor. However, even with lanolin over the hole, germination is significantly increased by pricking. Therefore, either lanolin is not such an efficient filter as the seles, or pricking has some other stimulatory effect. Consideration of the effects of "wounding" on living cells, indicates that the latter is likely.

Therefore, the light inhibition and high temperature inhibition of dormant wild oat seed does not depend on the structural integrity

of the palea in the early stages of after-ripening. With increased after-ripening, the light inhibition is negated by pricking; and at least part of such stimulation is lost if gaseous exchange between the caryopsis and the atmosphere is interrupted.

Figure 6: Final germination under light x temperature of dehusked and normal seed.



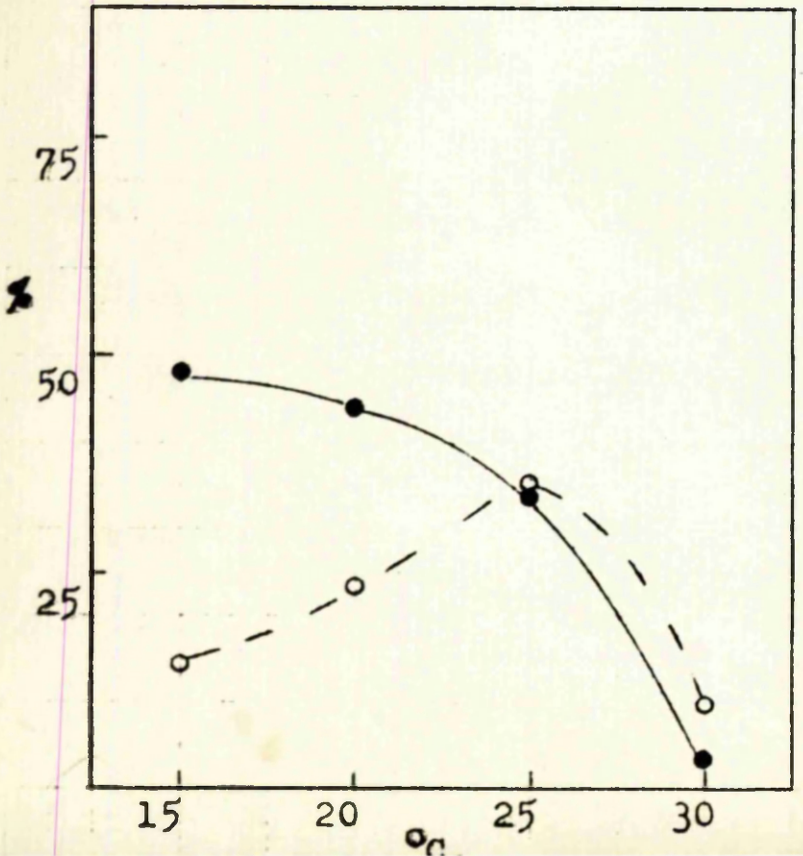
Abscissa=temperature.

Ordinate=% germ.

●: dark;

○: light;

Dehusked seeds.



Normal seeds.

Section B - REMOVAL OF THE PALES

Experiment 17 - The effects of dehusking on the responses to light and temperature.

Seed of var. *pilosa* were dehusked and germinated in light or darkness at four temperatures.

The final percentages of germination after such treatment are recorded in Table 17 and Figure 6. The chi-squareds are calculated from the totals of 3 replicates of 50 seeds per treatment.

Comparison with the germination of normal seed, indicates that removal of the pales negates the light inhibition and causes the light stimulation at higher temperatures to be more obvious.

Table 17

Final germination percentages of dehusked seed

Seed type	Light	15°C	20°C	25°C	30°C
Dehusked	L	80	77	84	64
Seed	D	82	70	69	44
χ^2 (1df) Lvs.D		NS.	NS.	9.0**	12**
Normal	L	14	23	35	10
Seed	D	40	45	34	4
χ^2 (1df) Lvs.D		41.8**	11.0**	NS.	4.1*

Experiment 18 - The effects of dehusking on seed at different stages of after-ripening.

Populations of var. pilosissima, which had been in dry storage at 20°C for 1 month and for 3 months from harvest, were dehusked and set to germinate in light or darkness at 20°C.

The final germination percentages in Table 18 show that dehusking only negates the light inhibition after the caryopses themselves have been in dry storage for longer than 1 month.

Table 18

Final germination percentages of caryopses after different periods in dry storage

Time from harvest	Light	%	X^2(1df) L.v.s.D.
1 month	L	39	5.38*
	D	51	
3 months	L	56	NS.
	D	55	

Experiment 19 - The effects of dehusking at different stages of imbibition.

Seeds of var. pilosissima, 6 months from harvest, were dehusked at various stages of imbibition:-

a) Dehusked and palea replaced around caryopses;

b) Dehusked in unimbibed condition;

c) Dehusked after 2 hours imbibition in light or dark;

d) Dehusked after 22 hours imbibition in light or dark.

Germination was continued in light or darkness at 20°C. The final percentages of germination recorded in Table 19 indicate that if the palea remain in position during imbibition, a state of dormancy is induced in the embryo.

Table 19

Stage at which dehusked	% germ. in:-		χ^2(1df) Lvs.D
	Light	Dark	
Palea replaced	31	47	9.4**
Before imbibition	73	76	0.6
2 hrs. imbibition	39	35	0.9
22 hrs. imbibition	29	35	5.2*

Discussion

Removal of the palea of wild oats permits more caryopses to germinate (experiment 17): the inhibition by light is not apparent, even at lower temperatures; and the stimulation by light at higher temperatures is greater, although there is still significant depression of germination by high temperature itself. The state of dormancy induced by the presence of the palea during imbibition is not negated by removal of the palea (experiment 19); and if the grains are irradiated during 22 hours of imbibition, more dormancy is induced in the population.

The manipulations involved in dehusking are not responsible for negating the light inhibition by damaging the caryopsis: in experiment 18, the treatment of replacing the palea around the caryopses reimposes the potentiality to be inhibited by light.

The light requirement of lettuce seed (Evenari & Neumann 1952) and of Betula sp. (Black & Wareing 1955) is negated by removal of the seed coverings; and the light inhibition of Phacelia tanacetifolia is removed (Arontjev 1930; Chen & Thimann 1965). Schulz & Klein (1963) also concluded that the light inhibition of Phacelia was related to the presence of the seed coverings.

The data of Hay & Cumming (1965) indicate that dehusking does not negate the light inhibition of wild oats. This anomaly may be explained, in part, by the results of experiment 18: the stage of after-ripening in dry storage determines the efficacy of dehusking in removing the light inhibition. But, within 3 months

from harvest - while there is still a high degree of dormancy and light inhibition in populations of normal and of pricked seed - removal of the palea does remove the light inhibition.

Table 20a

Final germination percentages after reciprocal transfers between pales and caryopses.

Age of Pales	Age of caryopses	% germ. in:-	
		Light	Dark
3 months	3 months	29	64
	15 months	43	56
15 months	3 months	44	88
	15 months	63	76

Table 20b

Analysis of variance of angular transformations of the data in (c).

Source	df	Sum Squares	Mean Square	Var. Ratio
Replicates	2	15.1	7.6	NS.
Pales	1	329.1	329.1	16.9**
Caryopses	1	17.3	17.3	NS.
Light	1	1717.3	1717.3	33.8**
P x C	1	0.3	0.3	NS.
P x L	1	5.5	5.5	NS.
C x L	1	287.5	287.5	5.6*
P x C x L	1	43.0	43.0	NS.
Residual	14	711.2	50.8	-
Total	23	3687.4	-	-

Experiment 20 - The effects of reciprocal transfers between the palea and caryopses of seed at different stages of after-ripening.

In an attempt to evaluate the contributions made by the palea and the caryopses to the phenomena of dormancy and light inhibition, reciprocal transfers were made between the palea and caryopses of seed after-ripened in dry storage for different lengths of time.

Seed of var. pilosa were used, after 3 and 15 months dry storage at 20°C:-

a) 15 month palea x 15 month caryopses (palea removed and replaced);

b) 15 month palea x 3 month caryopses;

c) 3 month palea x 15 month caryopses;

d) 3 month palea x 3 month caryopses.

Since there is less dormancy and light inhibition in 15 month-old seed, it was hoped to establish whether the properties of the palea or of the caryopses had changed during after-ripening.

The results of one such trial of 3 replicates of 25 seeds per treatment are reported in Table 20a as final germination percentages. The analysis of variance in Table 20b was carried out on angular transformations of the individual percentages.

Discussion

Experiment 20 perhaps expands and summarises the concepts developed in this section. The general inhibitory influence of the pales declines during after-ripening in dry storage; but the lack of interaction between the age of the pales and light indicates that the loss of light inhibition during after-ripening is not due to a change in some property of the pales. That the potentiality for light inhibition resides in the state of the caryopsis is borne out by the interaction between the age of the caryopses and light: fresh caryopses are inhibited by light but lose this characteristic during dry storage. The lack of other interactions, e.g. between the pales and the caryopses, suggests that any type of pale tested here can impose the light inhibition, if the caryopsis is in the appropriate physiological state.

This supposition is borne out by the results of experiment 21 where a light inhibition is induced in seed at a specific stage of after-ripening by a treatment which, it is thought, may act as an artificial husk. Other authors have also found the light responses of certain seeds to be reimposed by the presence of an unnatural covering, e.g. the light requirement of Chloris ciliata (Gassner 1911) and the light inhibition in P. tanacetifolia (Bohmer 1926) and Citrullus colocynthis (Koller et al. 1963).

The artificial coverings may be acting in two ways:

a) by preventing the exit of an inhibitor;

b) by interfering with gaseous exchange.

These possibilities will be investigated in the following sections.

Experiment 21 - The germination of caryopses under an artificial covering.

Dehusked caryopses of var. pilosissima, 6 months from harvest, were germinated in specially-prepared petri dishes: moist seed test paper carrying the caryopses was placed between two layers of clear polythene; the moisture sealed the edges of the polythene layers and the caryopses thus germinated in a confined space unexposed to the general atmosphere.

Final germination percentages are reported in Table 21 for 4 replicates of 25 seeds per treatment.

The polythene resulted in a significant depression of germination in the light, although not to the same extent as the natural coverings of the grain. Repeated tests at this age from harvest gave similar results; but the light inhibition by polythene could not be induced in caryopses at a different age from harvest.

Table 21

Final germination percentages of polythene-covered caryopses.

Light	Normal grains	Dehusked caryopses.	Dehusked + polythene
L	30	72	43
D	58	76	65
$\chi^{2}_{(1df)}$	30.3**	NS.	9.7**

General Conclusions from Part II

Dormancy in wild oats, and its associated phenomenon of light inhibition of germination, seems to depend upon a relationship between the palea and the caryopsis.

The palea have a general inhibitory influence upon germination. This influence is not the sole contributory factor in the light inhibition of germination.

Such inhibition by light depends also upon the physiological state of the caryopsis. In the early stages of after-ripening, the metabolism of the germinating naked caryopsis is blocked by light.

During after-ripening, the general inhibitory properties of the palea become weaker; also, the state of the caryopsis changes such that the germination metabolism is only sensitive to light when the caryopsis is enclosed by unbroken palea or by an artificial covering; with still further after-ripening, the capacity to be inhibited by light is lost by the caryopsis, although the palea may still be able to exert their general inhibitory effects in both light and darkness.

Part III: The Influence of the Gaseous Environment on Germination

Introduction

Regulation of dormancy in seeds has been ascribed to the action of oxygen (Thornton 1945; Vegis 1956) or of carbon dioxide (Kidd & West 1917). The effects of oxygen and carbon dioxide on germination are reviewed by Carr (1961).

Responses to variations in oxygen supply include both stimulation of germination by increased oxygen (Atwood 1914; Borthwick & Robbins 1928; Black 1959; Roberts 1962) and induction of dormancy by low oxygen tensions (Davis 1930a, b; Naylor & Christie 1956; Hay & Cumming 1959). Oxygen has also been reported to overcome the inhibitory effects of light on germination (Nolin 1958a; Vose 1962). However, the oxygen requirement for germination seems to vary between species (Taylor 1942; Siegel & Rosen 1962) and within a species, depending on the stage of after-ripening (Klessling 1911).

Carbon dioxide influences cell extension (Geisler 1963; Harrison 1965). The effects of this gas on germination also vary from species to species (Thornton 1935, 1936, 1944; Harrington & Crocker 1920). Recent accounts of its stimulatory action deal with relatively low concentrations of the gas, up to 5% (Ballard 1958; Toole et al. 1964).

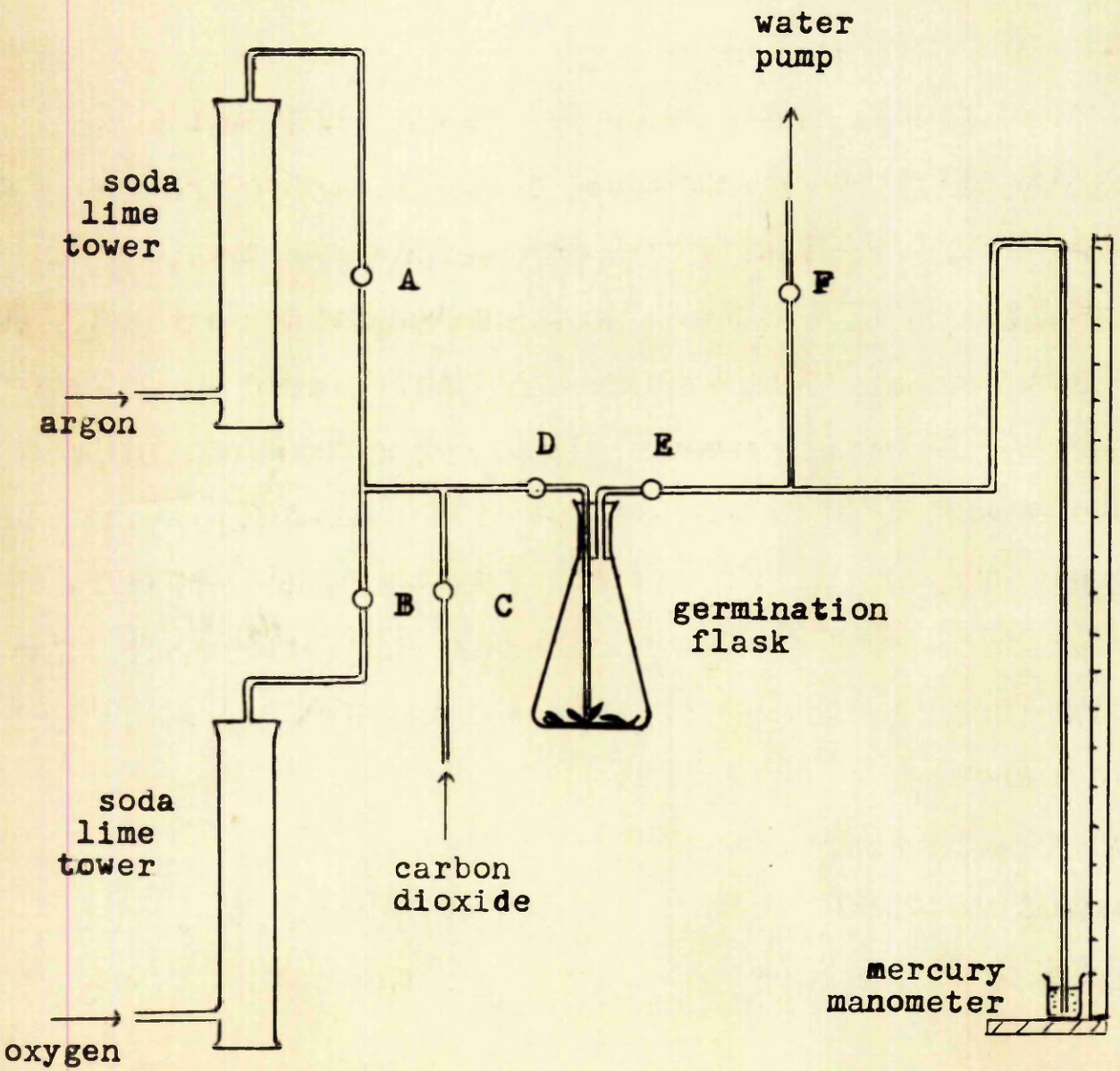
Dormancy in wild oats has been attributed to the impermeability of the seed coverings to oxygen (Atwood 1914; Johnson 1935). In other species, such effects of the seed coverings have also been inferred (Crocker 1906; Kidd & West 1920; Roberts 1961 ; Villiers

& Varoing 1964), and Brown (1940) has shown the membranes of cucumber seed to be differentially permeable to carbon dioxide and oxygen.

In Part II, the light inhibition of germination in wild oats was seen to be related to the presence of the palea. The palea could act by interfering with gaseous exchange; and light may modify the gaseous requirements of the imbibing seed. These possibilities are investigated in Part III by examining germination in light and darkness in various gaseous environments.

Figure 7: Apparatus used to prepare gas mixtures.

A,B,C,D,E,F, = screw clips.



Materials and Methods

Seeds were germinated on seed test paper under different gaseous environments in 250ml. conical flasks. In those treatments involving no carbon dioxide, containers of 2-3 gm. soda-lime - "Carbosorb" - were placed within the flasks and the gases used in the preparation of such mixtures passed through soda-lime towers. Cylinders of Argon, Oxygen and Carbon dioxide were obtained from British Oxygen Company.

The apparatus employed to obtain the gas mixtures is diagrammed in Figure 7:-

Fifty seeds were placed in the dry flask. With taps A, B, C closed, the system was evacuated to 10 mm. mercury using the water pump. Tap F was then closed and argon admitted through A to normal pressure. This procedure was repeated 3 times, after which 5 ml. of boiled, deionised water was quickly added to the seed test paper. The whole system was again purged twice with argon. The head of mercury which corresponded to a particular percentage by volume of a gas was calculated from the atmospheric pressure. The system was evacuated, argon admitted to the desired level, then oxygen to give its required proportion and finally carbon dioxide. For example:-

Atmospheric pressure = 70 cm.

Desired gas mixture = 70%A, 21%O_2, 3%CO_2.

Equivalent heads of mg = 57.70 cm.A, 15.90 cm.O_2, 2.23 cm.C

after evacuation, F closed, argon admitted until manometer reads 57.76;

A closed, oxygen admitted until manometer reads 73.72 (57.70 + 15.96);

B closed, carbon dioxide admitted until manometer reads 76.0.

The air controls were treated in the same way, being purged with air instead of argon. Flasks were sealed by closing D and E, disengaged from the apparatus and placed in light or darkness at 20°C until final germination counts were made.

Treatment of the results is as before, analyses of variance being carried out on the angular transformations of the percentages of germination.

Experiment 22 - The effects variation in the gaseous environment on the germination of dormant whole grains and caryopses.

Seed of var. pilosa were germinated under gas mixtures in flasks by the method described. Whole grains and dehusked caryopses, 6 months from harvest, were used. The resulting germination percentages are shown overleaf in Tables 22a and 22b respectively; they are graphed in Figure 8 (see Discussion).

The data refer to a complete trial of only 2 replicates of 50 seed per treatment. However, each of the treatments has been repeated 6 times on other occasions with similar results; and analyses of this and other experiments have never shown significant differences between replicates within treatments. A large amount of space and equipment is involved in a single trial of all the treatments described. It was thought more valid to carry out complete trials a number of times with seemingly few replicates, rather than to use more replicates and compare different gas treatments tested on different occasions.

All analyses of variance showed similar results to those in Table 22c.

Table 22c

Analysis of variance of the populations in Tables 22a & b.

Source	df	S.S.	M.S.	Var. Ratio.
Replicates	1	2.2	2.2	NS
Oxygen	3	6,549.2	2,183.1	193.2**
CO_2	2	1,147.1	573.5	50.8**
Light	1	1,385.1	1,385.1	122.5**
Pales	1	11,792.6	11,792.6	1,043.6**
L x Pales	1	162.3	162.3	14.4**
CO_2 x Pales	2	78.5	39.2	3.5*
CO_2 x L	2	407.4	203.7	18.0**
CO_2 x O_2	6	88.7	14.8	NS
O_2 x Pales	3	53.9	17.9	NS
O_2 x L	3	106.5	35.5	3.1*
L x Pales X O_2	3	103.6	34.5	3.1*
L x CO_2 x O_2	6	141.1	23.5	NS
L x Pales x CO_2	2	51.3	25.6	NS
Pales x O_2 x CO_2	6	110.4	18.4	NS
L x Pales x O_2 x CO_2	6	370.7	61.8	5.5**
Residual	47	531.0	11.3	
Total	95	23,101.6	-	

Table 22a

Percentage germination of whole grains in O_2 x CO_2

$\%CO_2$	Light	% oxygen 10	20	50	80	Air
0	L	0	7	9	14	11
	D	7	28	41	37	26
3	L	2	22	19	17	
	D	2	21	41	39	
20	L	1	6	6	13	
	D	1	19	20	17	

Table 22b

Percentage germination of dehusked caryopses in O_2 x CO_2

$\%CO_2$	Light	% oxygen 10	20	50	80	Air
0	L	16	48	40	30	36
	D	23	60	66	58	56
3	L	25	59	72	60	
	D	38	66	61	71	
20	L	20	47	46	51	
	D	21	36	56	52	

Experiment 23 - Germination of caryopses in the presence and "absence" of carbon dioxide.

The germination patterns of caryopses, 6 months from harvest, were followed in environments containing adequate oxygen (21%) and either 3% CO_2 or 0% CO_2.

A large number of flasks, each with 25 imbibing caryopses in the appropriate gas mixture, were placed in light or darkness at 20°C. At specific time intervals, 3 such flasks were selected from each treatment, the percentage germination noted and the flasks discarded.

The results are given in Table 23 and graphed against time in Figure 9 (see Discussion).

Table 23

Percentage germination at stated time intervals of caryopses in 21% O_2/3% CO_2 or 21% O_2/0% CO_2.

Treatment		germination at:			
Gas	Light	32 hrs.	57 hrs.	72 hrs.	96 hrs.
21% O_2:	L	25	46	45	48
0% CO_2	D	45	60	68	73
21% O_2:	L	36	68	70	66
3% CO_2	D	41	60	68	72
χ^2 (3df)		7.6	9.08*	11.95**	13.8**

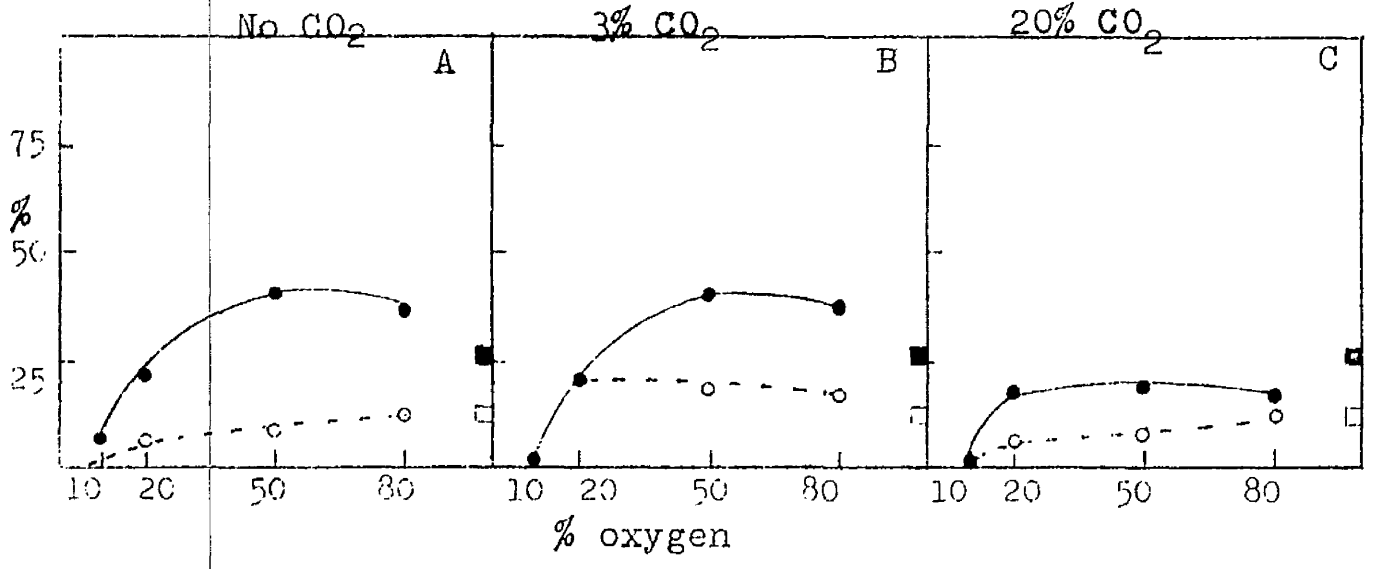
Figure 8: Final germination in O_2 x CO_2.

Abscissa = % oxygen.

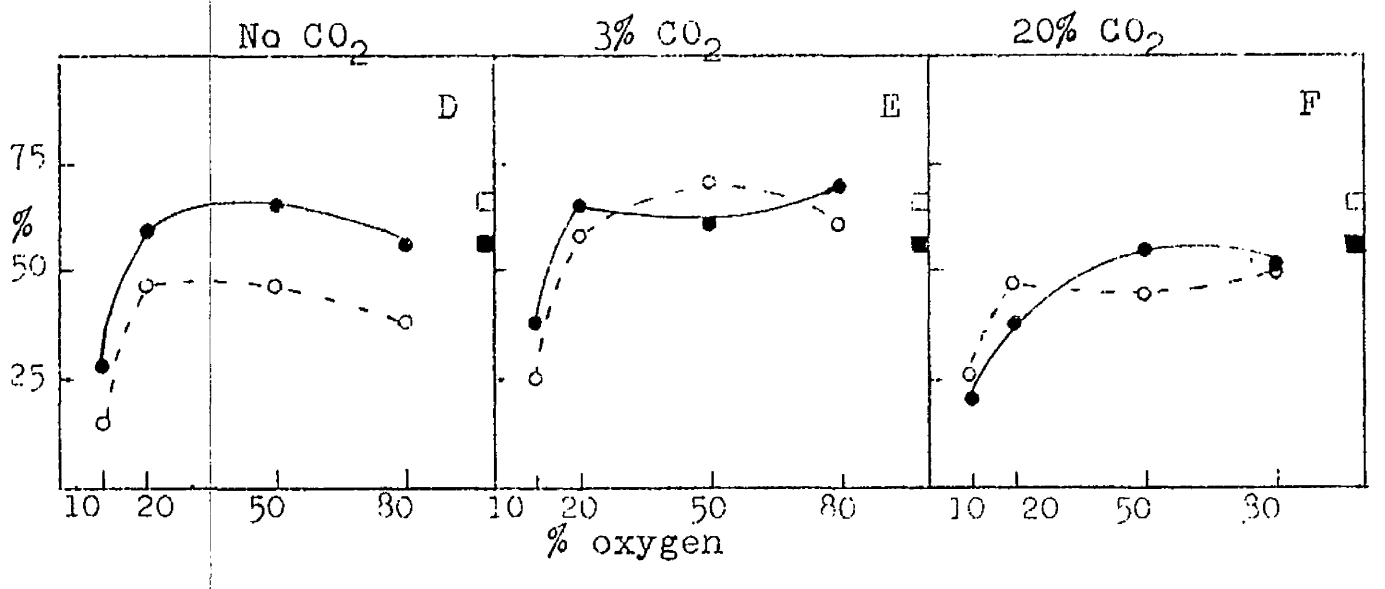
Ordinate = % germination.

● : dark germ.
○ : light germ.
■ : dark) in air.
□ : light)

Whole Grains.



Dehusked Caryopses.



Discussion

The composition of the gaseous environment does influence the germination of wild oat seed. All the variables tested - light, oxygen, carbon dioxide and the palea - interact in the germination system. Light and/or palea inhibit germination; oxygen and carbon dioxide modify the degree of inhibition. The data from experiments 22 and 23 do not allow the formulation of a unified model to account for the significance of all the interactions shown in Table 22c. However, in this investigation, the emphasis is on the mechanism of the light inhibition of germination and certain conclusions regarding this can be drawn.

From the data of experiment 22 (Fig. 8), it can be seen that oxygen is a necessary factor for the germination of this species: germination is depressed by oxygen tensions below that normally found in air; but stimulation of germination by oxygen levels greater than that in air does not occur in every condition tested, e.g.:-

1) The significant light x oxygen interaction is due to oxygen raising germination in darkness to a greater extent than it does germination in light.
2) Also, in the presence of the palea, in darkness, oxygen tensions greater than 20% result in an increase in germination (see Fig. 8A, B); but in the absence of the palea, such increased oxygen tensions do not appreciably increase the already high germination percentages

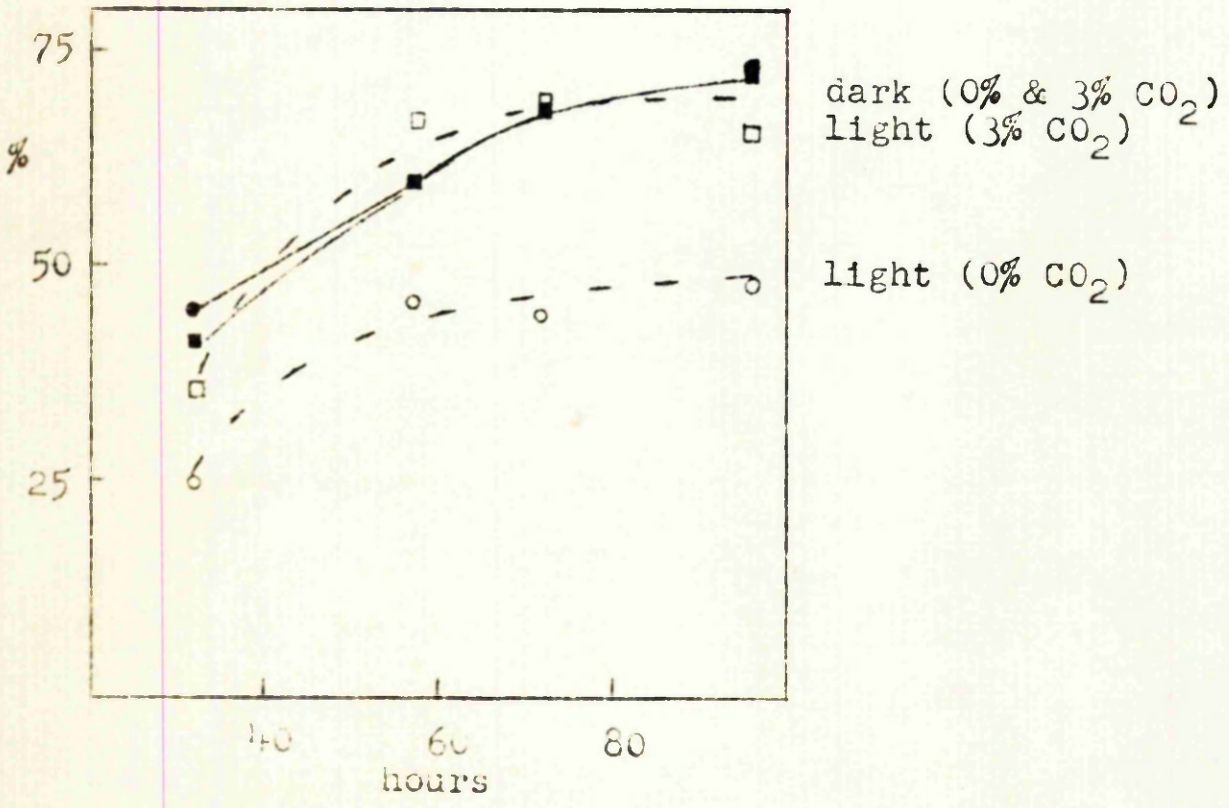
Figure 9: Germination pattern of caryopses in gas mixtures with and without CO_2.

Abscissa = hours germination.

Ordinate = % germination.

●: dark) - 0% CO_2
○: light)

■: dark) - 3% CO_2
□: light)



(Fig. 8, D, E.). This is presumably the basis for the significance of the light x oxygen x palea interaction.

From these facts, it is concluded that: a) contrary to the results of Rollin's (1953) investigations with P. tanacetifolia, oxygen does not reverse the light inhibition of wild oat germination; and b) oxygen can enhance the germination of wild oats, in darkness, by acting against the inhibitory properties of the palea.

Experiment 22 also indicates that carbon dioxide is active during germination: 3% carbon dioxide, at all tested oxygen levels, allows significantly more germination than atmospheres lacking such concentrations of carbon dioxide, and 20% carbon dioxide depresses germination, even in high oxygen concentrations (Fig. 8 C, F). The highly significant carbon dioxide x light interaction results from the light inhibition of germination in the absence of carbon dioxide (e.g. Fig. 8D). The palea also interact in this system by depressing the stimulatory action of 3% carbon dioxide; but 3% carbon dioxide does negate the light inhibition of germination in whole grains at 20% oxygen (Fig.8D). Experiment 23 illustrates the carbon dioxide effect on germination: changes in carbon dioxide level do not affect dark germination; but in the "absence" of carbon dioxide, the germination of a proportion of the population of caryopses is inhibited by light (Fig. 9).

Atwood (1914) attributed his results wholly to the effects of increased oxygen, but made no provision for the effects of respired carbon dioxide on ungerminated seed. The results reported here are in agreement with an oxygen stimulation of germination, under certain conditions. But it seems to be carbon dioxide which is involved in the negation of the light inhibition in this species.

The effects of the pales in intensifying the degree of light inhibition could arise from two mechanisms: a) by interfering with gaseous exchange between the caryopsis and the atmosphere; b) by modifying metabolism (chemically) such that carbon dioxide is a necessary factor for germination. The fact that an artificial covering of polythene (see Part II) can impose a light inhibition suggests that method a) is operating. However, since the artificial covering did not impose such a severe light inhibition, either it is not such an efficient filter, or method b) must also be considered.

The effect of light in inhibiting germination could derive from: i) an intensification of a CO_2-trapping property of the pales; ii) the imposition of a block to metabolism which can be overcome by carbon dioxide. Since a low or non-existent level of carbon dioxide results in a light inhibition of caryopses' germination even when the pales are absent, it would seem that

light does block the metabolism of germinating seed, such a block being overcome by carbon dioxide.

General Conclusions from Part III

Investigations on the effects of the ambient atmosphere on the germination of wild oat seed, reveal an extremely complex system to be operating in this species.

Oxygen is a necessary factor for germination; levels of oxygen greater than that in air are thought to stimulate germination by reducing inhibitory properties of the pales; but no tested concentration was effective in negating the light inhibition of germination.

Carbon dioxide also influences the germination of this species: a lack of carbon dioxide results in light being inhibitory; 3% carbon dioxide stimulates germination and removes the light inhibition; 20% carbon dioxide is inhibitory in both light and darkness.

The pales seem to exert their inhibitory effects on germination both by disrupting gaseous exchange and by influencing the germination metabolism in some other way.

Continuous irradiation of imbibing wild oat seed by white light is thought to block the germination metabolism; carbon dioxide seems to overcome this block.

Part IV: Observations on the Possible Role of Germination-regulating substances.

Introduction

Substances inhibitory to growth and germination are frequently found in the extracts of tissues which are dormant or which have been exposed to unfavourable conditions, although such relationships have not always been shown to be of a causal nature. The light inhibition of root growth (Masuda 1962; Pilet 1963) and of stem elongation (Engelsema & Meijer 1965) is correlated with an increased inhibitor content; in germinating rice seedlings, light is thought to enhance the accumulation of a growth inhibitor (Yoshimura & Tagawa 1961). Conversely, the application of seed extracts to various types of seed seems to result in a greater inhibition of germination in light than in darkness (Moshcov 1938; Froeschel 1940; Vose 1962); the significance of these observations may be lessened by the finding that salt solutions also exert this effect (Duym et al. 1947).

The role of elevated oxygen tensions in promoting germination is thought, in certain cases, to be concerned with the inactivation of inhibitors, rather than with effects on respiratory metabolism per se (Wareing & Foda 1957; Black 1959; see also Roberts 1964). In P. tanacetifolia, increased oxygen tensions are reported to negate the action of an inhibitor produced in light (Rollin 1958a). The seed coverings are also concerned in such effects.

Dormancy in A. fatua has been suggested to be due to the presence of an inhibitor (Naylor & Christie 1956). Chromatograms of the aqueous extracts of wild oat caryopses showed two inhibitory

regions in subsequent bioassays (Black 1959); Hay (1962) considers these to be general growth inhibitors and the palea to carry the germination-inhibiting fraction. However, no differences in the levels of such inhibitors could be found between dormant and non-dormant seed (Black 1959; Brennan 1960; Hay 1962). A dormancy mechanism based on the inhibition of the gibberellin-controlled utilisation of the endosperm food reserves has also been proposed (Naylor & Simpson 1961; Simpson & Naylor 1962).

The participation of the palea in the light inhibition of wild oat germination has been described in previous sections of the thesis. The light inhibition of the germination of dehusked caryopses is not as severe in the treatments involving an artificial covering or a lack of carbon dioxide, as it is when the palea enclose the caryopsis. Part IV reports preliminary investigations made with regard to the possible interaction of extractable, naturally-occurring inhibitory substances in the system(s) inhibited by light.

Materials and Methods

The basic procedure involved observing the effects of applying crude aqueous extracts to germinating caryopses.

Known amounts of seed or pales were ground and leached for 24 hours at 2°C. Microbial activity was suppressed during leaching by the addition of toluene; control experiments indicated that the amounts of toluene used had no effects on germination. Although no detailed examinations were made, there were no macroscopic signs of micro-organisms.

The pH of such leachates was 6.2. Fractionation of these aqueous extracts was not attempted. In certain cases, the tissue was filtered off and the extract concentrated under vacuum at 30°C; after Seitz filtration, known amounts of extract were tested against caryopses. In other experiments, seed test paper was placed over the mixture of seed material and leachate; caryopses were then set to germinate on this moist medium. Both methods gave similar results.

Standard germination tests were carried out in light or darkness at 20°C, in petri dishes or in conical flasks when the gaseous environment was also varied. Details of the types and amounts of extract tested are given in individual experiments.

Experiment 24 - The effects of aqueous extracts on the germination of caryopses under standard conditions.

Seeds were imbibed in light or darkness at 20°C for 30 hours. The palea and caryopses were then separated and aqueous extracts prepared. The palea and caryopses of unimbibed grains were also extracted. After concentration, aliquots were tested against 10 month old caryopses:-

500 palea (or caryopses) leached in 200 ml. water;

leachate concentrated to 50 ml.;

4 ml. leachate tested against each replicate of 25 caryopses, i.e. an equivalent of 40 palea (or caryopses) per dish of 25 caryopses.

Germination of the caryopses in such extracts was followed at 20°C in light or darkness; the germination percentages of 3 replicates per treatment are reported in Tables 24a and 24b. The differences between light and dark germination are only statistically significant when caryopses are germinated in extracts from the palea of light-imbibed grains; more concentrated extracts did not increase the light/dark differential. The extracts of caryopses did not result in differences between light and dark germination.

Table 24a

Germination of caryopses in extracts of palea

Type of extract	Light	% germination at:		
		42 hrs.	66 hrs.	110 hrs.
Water	L	24	66	73
	D	20	66	75
Uninbibed husks	L	24	52	59
	D	28	69	65
Light imbibed	L	12	48	50
	D	40*	62*	69*
Dark imbibed	L	28	56	60
	D	20	60	60

* denotes significant differences between light and darkness.

Table 24b

Caryopses germination in extracts of caryopses.

Type of extract	Light	% germination at:		
		42 hrs.	66 hrs.	96 hrs.
Water	L	30	68	76
	D	30	60	72
Uninbibed caryopses	L	36	64	66
	D	28	52	55
Light imbibed	L	33	72	77
	D	37	69	75
Dark imbibed	L	44	76	76
	D	47	74	74

Experiment 25 - The effects of husk extract on the germination of caryopses under low oxygen tensions.

Weighed amounts of powdered pales were leached in conical flasks for 24 hours. Caryopses, 12 months from harvest, were set to germinate on the covering seed test paper in atmospheres of air or 10% O_2/0.5% CO_2.

Each replicate flask of an extract treatment contained:- 3 gm. (approx. 350) pales/10 ml. water/50 caryopses, (i.e. a more concentrated "extract" than in experiment 24). The percentage germination after 4 days in light or darkness is shown in Table 25 - 3 replicates per treatment. This more concentrated extract results in a more severe inhibition, especially under the low oxygen tensions, but no differences between light and dark germination are apparent.

Table 25

Germination of caryopses in husk extracts under low oxygen tensions.

Atmosphere	Extract	Light	% germ.
Air	Water	L	73
		D	64
	Extract	L	22
		D	25
10% O_2/0.5% CO_2	Water	L	42
		D	44
	Extract	L	3
		D	3

Experiment 26 - The effects of husk extract on the germination of caryopses in an atmosphere "lacking" carbon dioxide.

The procedure of experiment 25 was repeated, using similarly prepared "extract", under atmospheres of 21% O_2/3% CO_2 or 21% O_2/0% CO_2. The caryopses used were non-dormant after 2 years in dry storage - i.e., not inhibited by light and not responsive to a lack of carbon dioxide.

The results of a trial of 3 replicates per treatment are shown in Table 26; caryopses of this age from harvest seem more resistant to the inhibitory properties of the extract; a "lack" of carbon dioxide in itself gives no light/dark differential in germination; but a combination of "extract + no CO_2" results in a significant depression of germination in light.

Table 26

Germination of caryopses in husk extract under different carbon dioxide levels.

Atmosphere	Extract	Light	% germ.	X^2(1df) L. vs. D.
21% O_2/3% CO_2	Water	L	86	NS
		D	90	
	Extract	L	79	NS
		D	78	
21% O_2/0% CO_2	Water	L	83	NS
		D	83	
	Extract	L	56	14.09**
		D	82	

Discussion

It is recognised that the work reported in this section deals with very crude preparations; the extractable property of the palea whose effect is demonstrated in these experiments may not be due to a single or a unique substance. However, it is felt that another function can be ascribed to the palea, besides possible interruption of gaseous exchange.

In experiment 24, the extract of the palea from grains imbibed in light exerts an inhibitory influence on the germination of caryopses in light; this would seem to provide evidence for the action of an inhibitory factor, formed and active in light as reported for Rhoeelia (Rollin 1958a). But such effects, although reproducible, were not statistically significant in all trials, suggesting the participation of uncontrolled factors in the system.

The more concentrated leachate used in experiment 25 results in a more severe inhibition of germination. This effect is more marked in an atmosphere low in oxygen. Presumably this is a manifestation of the system described by Black (1959) in which increased oxygen resulted in a decrease in inhibitor. However, even in conditions of low oxygen, the leachate does not confer light sensitivity on germinating caryopses.

A similar leachate, in experiment 26, does cause light to be inhibitory in the absence of carbon dioxide; light, the leachate or the lack of carbon dioxide do not, in themselves inhibit the germination of caryopses after 2 years dry storage.

Thus, these experiments do not provide information on the mechanism by which light blocks the metabolism of germination in this species. However, the pales seem to carry extractable factor(s) which influence such metabolism: aqueous extracts have a general inhibitory influence in both light and darkness and increase the oxygen requirement of the germinating caryopsis, possibly by a system similar to the inhibitor-inactivation mechanisms described by Wareing and Foda (1957) and Black (1959); the leachate also confers a sensitivity to a lack of carbon dioxide on the germination metabolism in light. Further fractionation of the extract will be required to resolve the alternatives of: a) those two effects being attributable to a single substance (or set of substances) which can be inactivated by oxygen and whose effects on light metabolism can be negated by carbon dioxide; or b) different compounds being involved in the inhibition of germination in low oxygen and in the inhibition of germination in light. Also, it cannot be validly stated that such a proposed inhibitor is responsible for maintaining the state of dormancy in this species, since its action depends on specific conditions of: the caryopsis, irradiation, and the gaseous environment.

Summary of Part IV

1) The pales carry water-soluble factor(s) inhibitory to germination.

2) An inhibitory property is intensified under low oxygen tensions.

3) An inhibitory property participates in the metabolic system blocked by light.

Part V: The Relationship between Organic Acid Levels and Dormancy

Introduction

Carbon dioxide has been reported to affect permeability in Helianthus hypocotyl (Glinka & Reinhold 1962). But dormant and nondormant grains of A. fatua show no differences in their water uptake.

The pH of the germination medium can be varied from 3.0 to 7.0 without affecting the percentage germination. Harrison (1965) also concluded that the carbon dioxide stimulation of oat coleoptile elongation was not a pH effect.

It is possible that the carbon dioxide effect in wild oat germination is photosynthetic. This would extend the proposed mechanism of dormancy in this species involving sucrose metabolism (Naylor & Simpson 1961). However, in the literature on anatomical investigations of cereal grains, no descriptions of chloroplast-like bodies have been found; nor has chlorophyll been observed in seed extracts. Also, germination in white light is greater in 3% carbon dioxide than in the carbon dioxide levels of air; unless a low permeability of the seed tissues makes necessary relatively high levels of carbon dioxide, this does not suggest the operation of photosynthesis.

Therefore, although these arguments do not preclude the functioning of such systems, carbon dioxide is thought to affect metabolism in some other way.

Water uptake in various seeds is paralleled by the

activation of the enzymes of the tricarboxylic acid cycle (TCA) (Poljakoff-Mayber 1956; Mayer et al. 1957; Stanley 1957; Krupka & Towers 1958). The TCA also seems to be operative during the stratification of certain seed (Bradbeer & Colman 1963). In light-stimulated lettuce seed, the phytochrome mechanism influences respiration (Evenari et al. 1955), the development of the TCA (Poljakoff-Mayber & Evenari 1963) and the capacity for phosphorylation (Surrey & Gordon 1962). The effects of higher intensities of white light on respiration are controversial: Davis (1950) reviews the earlier literature and reports that such light does not influence respiration, as measured by gaseous exchange; but light seems to prevent the participation of newly-formed photosynthate in the TCA (Benson & Calvin 1950; Voigt et al.1951); Krotkov (1960) reviews other evidence for light inhibition of the TCA.

The glyoxylate pathway (Kornberg & Krebs 1957) is also capable of operating in seed metabolism; in seeds with a high fat content, both isocitritase and malate synthetase appear during germination (See Beevers 1961).

The enzymes concerned in the "dark-fixation" of carbon dioxide have been found in cereal seeds, notably wheat germ (Tchen & Vennesland 1955; Ochoa 1956). Carbon dioxide fixation has been described in the roots of barley seedlings (Pool 1953), in particles from barley roots (Graham & Young 1959), in lettuce seeds (Baber & Tolbert 1959) and in pollen spores (Goss 1963).

The operation of one, or both, of these respiratory pathways may be necessary during and for the germination of wild oats; dormancy and light inhibition of germination may be associated with blocks to these systems. Carbon dioxide could thus act by replenishment of the required intermediates through carboxylation of pyruvate or phosphoenolpyruvate to malate or oxaloacetate.

This hypothesis is investigated in Part V by observing the effects of applying organic acids to imbibing seeds, and by analysing the organic acid levels of seed populations of various degrees of dormancy.

Materials and Methods

Section A - The experiments in this section report the effects of applying certain tricarboxylic acid cycle intermediates to imbibing seeds. The organic acids were applied as sodium salts; acid solutions were adjusted to a stated pH with N/1 sodium hydroxide. It was considered inadvisable to use buffer solutions since these are, in the main, composed of substances whose lack of activity in the systems being investigated cannot be guaranteed.

Section B - Chemicals reported to inhibit respiration were also tested for their effects on germination: these include cyanide, dinitrophenol, malonic acid and fluoride. No buffer solutions were used with malonate for the reasons given above, the pH of such solutions being adjusted with sodium hydroxide. In the tests involving sodium fluoride, where the concentrations used reach high levels, a control of similar concentrations of sodium chloride was also included to allow distinction between osmotic effects and effects due to the fluoride ion.

Solutions of known concentration were applied to the seed test paper - 4 ml. per replicate of 25 seeds. Subsequent germination in light or darkness at 20°C is compared with germination in deionised water.

Recording and treatment of the data are as in previous sections.

Section A - EFFECTS OF ORGANIC ACIDS

Experiment 27 - The effects of malic and pyruvic acids at different concentrations and pH.

The test was carried out at concentrations of 10^{-3} & 10^{-5} M. at pH5 and pH9. Four ml. of solution were added to 25 seed of var. pilosa, 12 months from harvest.

The germination percentages at 4 days - from 3 replicates per treatments - are shown in Table 27; no differences are apparent between any of the acid treatments. Although not shown in the table, there were no differences in the rates of germination in the different treatments.

Table 27

Percentages of germination in solutions of malic and pyruvic acid

a) Malic acid

Conc. organic acid	pH 5		pH 9	
	L	D	L	D
Water	40	81	44	76
10^{-3} m	33	80	32	80
10^{-5} m	42	88	48	76

b) Pyruvic acid

Conc. organic acid	pH 5		pH 9	
	L	D	L	D
Water	40	81	44	76
10^{-3} m	48	80	40	76
10^{-5} m	37	72	40	68

Experiment 28 - The effects of various organic acids on the germination of grains and caryopses under different environments.

The acids succinic, malic, oxalacetic, pyruvic and citric were tested, at 10^{-2}M and at pH5, against various populations of var. pilosa:

a) normal seed, 4 months from harvest;

b) dehusked seed, 4 months from harvest;

c) dehusked seed, 12 months from harvest, in an atmosphere "lacking" carbon dioxide.

Four ml. of solution was applied per replicate dish, or flask, of 25 seed.

Germination percentages, representative of the behaviour under these treatments are shown in Tables 28a, 28b, 28c. The only statistically significant differences between a treatment and its corresponding water control are to be seen under an atmosphere lacking carbon dioxide (Table 28c): in darkness, malic, oxalacetic and pyruvic acids seem to stimulate germination slightly. This stimulatory effect could not be repeated.

Table 28a

Germination percentages of whole grains in various organic acids.

Light	water	pyruvic	citric	succinic	malic	oxal.
L	25	16	20	24	16	16
D	46	54	40	52	50	51
X^2(1df) for each "acid" vs. "water". L		2.5	0.5	NS	2.4	2.4
D		3.6	NS	NS	NS	NS

Table 28b

Germination percentages of dehusked caryopses in organic acids.

Light	water	pyruvic	citric	succinic	malic	oxal.
L	68	62	66	64	55	61
D	80	82	88	88	88	85

No statistical significance in the differences between a treatment and its water control.

Table 28c

Germination percentages of dehusked caryopses in various organic acids in an atmosphere lacking carbon dioxide.

Light	water	pyruvic	citric	succinic	malic	oxal.
L	58	68	55	54	68	60
D	56	78	56	63	78	76
X^2(1df) for each "acid" vs. "water" L		1.8	NS	NS	1.8	NS
D		7.6*	NS	NS	7.6*	5.76*

Discussion

These organic acids do not seem to influence germination, at the concentrations tested. In the reported experiments, the only statistically significant increases in germination are those shown in Table 28c; however, such effects were not apparent in subsequent repetitions of this particular experiment. If this is a real effect, such stimulation of dark germination by organic acids in atmospheres lacking carbon dioxide is difficult to relate to the suggested mode of action of carbon dioxide in overcoming the light inhibition of germination.

Brown (1907) has described a non-living, selectively-permeable membrane around the grains of cultivated cereals: acids and metal salts in aqueous solution were not taken up. It is possible that the apparent lack of effect of the organic acids is due to their lack of uptake by the grain. Valid discussion of the influence of applied organic acids on the system should therefore be postponed until this question is resolved. However, if the organic acids are being taken up and yet do not have any effect on germination, it would seem that it is not a lack of an organic acid which is responsible for dormancy or the light inhibition of germination.

Other authors have claimed effects of organic acids on germination: Gal (1938) reported inhibition, but Ruge - quoted in Fowden & Moses (1960) - found malic acid to increase germination slightly; Fowden & Moses also report the results of Russian workers in stimulating germination with succinic acid.

Section B - EFFECTS OF RESPIRATORY INHIBITORS

Experiment 29 - The effects of cyanide and dinitrophenol on wild oat germination.

Solutions of potassium cyanide, sodium cyanide and dinitrophenol were applied to seed test paper and whole grains set to germinate under standard conditions of light or darkness at 20°C. The seed used were 15 months from harvest and did not show severe dormancy or light inhibition.

The germination percentages shown in Tables 29a & b indicate that these compounds are not effective in inhibiting germination or intensifying the light inhibition, at the concentrations tested.

Table 29

Germination percentages of whole grains in cyanide and dinitrophenol.

a)

Compound	Light	Dark
Water	46	56
10^{-3}M KCN	48	64
10^{-3}M DNP	40	60

b)

Compound	Light	Dark
Water	50	74
10^{-2}M NaCN	48	80
10^{-3}M NaCN	52	74

No x^2s significant.

Experiment 30 - The effects of malonate on germination.

Malonate was applied to germinating seeds as both the sodium salt and as the free acid - in the latter case, at various pHs. A range of concentrations was tested on seed 20 months from harvest.

The results are demonstrated by the germination percentages shown in Table 30a, b, - from 4 replicates of 25 seed per treatment; repeated trials consistently gave an increased light inhibition at lower concentrations with no effects at higher concentrations.

Table 30

Percentage germination in various concentrations of malonate.

a) sodium malonate

pH	conc.	light	dark
4.2	water	45	75
4.3	10^{-2}M	44	81
4.3	10^{-3}M	24	63
4.3	10^{-4}M	45	73

b) malonic acid

pH	conc.	light	dark
4.4	water	46	72
2.5	10^{-2}M	43	68
3.2	10^{-3}M	41	73
4.1	10^{-4}M	31	69

Experiment 31 - The effects of sodium fluoride on germination.

Solutions of NaF were applied at 10^{-1}, 10^{-2} and 10^{-3} molar concentrations to seed 20 months from harvest - 4 ml. solution per 25 seed, 4 replicates per treatment. Similar concentrations of sodium chloride were also tested.

The results of a typical trial are shown in Table 31; sodium fluoride at high concentrations results in a severe inhibition of germination; at 10^{-2} molar, it increases the light inhibition and is ineffective at 10^{-3} molar. The correspondingly higher germination percentages in sodium chloride indicate that this effect is not wholly osmotic.

Table 31

Percentage germination in sodium fluoride solutions.

NaF

conc.	light	dark
water	45	62
10^{-1}M	0	0
10^{-2}M	36	62
10^{-3}M	36	61

NaCl

conc.	light	dark
water	45	62
10^{-1}M	36	62
10^{-2}M	44	64

The high level of NaF is not toxic: washing and pricking the seed after such treatment allows germination.

Discussion

The respiratory inhibitors, cyanide and dinitrophenol, have no effect on wild oat germination at the concentrations tested (experiment 29). This is in contrast to the stimulatory effects of cyanide on rice germination (Roberts 1964), although again it is possible that wild oats are not taking up these compounds.

The malonate results are confusing and probably best ignored until a more detailed study is made, except to note that it has been tested and inhibition of light germination can result. Malonate enters cells as the undissociated molecule or as the monovalent ion (Millerd 1960) and its inhibitory effects on respiration are only manifest at pH5 or lower. Although it was applied in solutions below pH5 (experiment 30) it is possible that the pH of the germination medium changes during imbibition.

The effects of sodium fluoride can perhaps be more easily accounted for (experiment 31). In contrast to its lack of effect on rice germination (Roberts 1964), sodium fluoride inhibits wild oat germination: high concentrations inhibit germination completely, not by an osmotic effect and not by killing the grains; lower levels of fluoride inhibit germination in light. This latter effect suggests that light and fluoride may be acting on the same system, although not necessarily at the same point. Fluoride is known to inhibit enolase, succinic dehydrogenase and adenosine-triphosphatase (see Beevers 1961); all these enzymes are concerned in respiratory systems.

Thus, the germination metabolism of wild oats seems to be different from that of rice where cyanide stimulates and fluoride has no effect on germination. Further work with other respiratory inhibitors is necessary before it can be conclusively stated that the fluoride inhibition of wild oat germination results from the dependence of wild oat germination in light upon the tricarboxylic acid cycle.

Summary of Sections A and B

1. In Section A, certain organic acids are shown to be without effects on the light inhibition of germination.
2. Cyanide and dinitrophenol do not affect germination.
3. Malonate can inhibit germination in the light - but the effect is variable, due to unknown factors.
4. Fluoride inhibits the germination of wild oats, especially in the light.

Section C - ANALYSIS OF ORGANIC ACID LEVELS

Material and Methods

1. Details of samples: Various types of seed were analysed:

a) Cultivated oats - this species shows no effects of carbon dioxide;

b) Glasgow-grown wild oats - after 3 and 10 months dry storage;

c) Commercially-obtained wild oats - after 10 and 24 months storage.

The levels of dormancy in such populations were determined by germination tests at each analysis.

Air-dry seed were weighed into 2 gm. batches; the numbers of seed in such batches were also recorded for expression of the results on a per seed basis. The seed were then extracted in the unimbibed state or set to germinate and analysed at various stages of imbibition.

Analyses were carried out in complete runs or trials incorporating a specified range of seed treatments or populations; it was possible to process 6 samples during a complete run lasting 5 days. It was decided to carry out the investigation, not by replication of treatments within a run, but by analysing a complete series of treatments a number of times to establish any differences between the treatments. This is thought to be justified, since it would not be biologically valid to compare, say, 6 replicates of one treatment with 6 replicates of a different treatment extracted on a different occasion: variation between

runs would arise both from differences in the seeds, due to after-ripening, and from undetected variations in technique.

As will be seen from the introductory experiment 32, the reproducibility between samples of the same population extracted at the same time is good. Comparison can justifiably be made of differences between treatments within a run. The reported differences between treatments have appeared in repetitions of the trials.

2. Extraction and purification: Imbibed seeds were plunged in 100 ml. of boiling 80% ethanol for 10 minutes; after being ground, the brei was returned to the 80% ethanol and leached at 20°C for a further 6-8 hours. Unimbibed samples were milled and similarly extracted in hot 80% ethanol.

The ethanolic extracts were reduced in volume by boiling. The remaining aqueous solutions were then centrifuged before being passed through ion exchange resins for preliminary purification. (In later experiments, similar results were obtained by passing the ethanolic extracts through the resins without reduction of volume by boiling).

Amino acids and other basic material was removed by passage through Zeocarb 225 in the H form (15 cm. x 0.5 cm.). The organic acid fraction was absorbed on anion exchange columns (Amberlite IR-4B, in the OH form, 15 cm. x 0.5 cm.). After washing, the acids were eluted with 2N ammonium hydroxide. The

excess NH_4OH was evaported off and solutions of the free organic acids obtained by a further passage through Zeocarb columns. This is essentially Method I of Ranson (1955).

The total volumes were noted and aliquots from each sample were titrated against N/10 NaOH to the end-point of phenol red to give a measure of total titratable acidity.

3. Chromatography: The remaining fractions of the samples were each divided into two lots, for thin layer and gas liquid chromatography.

a) TLC - After reduction to suitable volumes under vacuum, the samples were spotted onto silica gel plates, together with known standards, and developed in:

i) 96% ethanol: 20% ammonia (100:40) (Stahl 1964[5]);

or ii) methanol: 5N ammonia (80:20).

The location reagent was bromocresol green, as supplied by British Drug Houses.

b) GLC - The fractions were dried under vacuum and methylated with fresh diazomethane. The methyl esters of the organic acids were then taken up in chloroform for chromatography against similarly-treated standards on a Pye Argon Chromatograph, equipped with a preheater. Glass columns, 4 feet long, were used, modified for sample injection through a self-sealing septum. The stationary phase was 10% polyethylene glycol adipate on 85-100 mesh GasChromS. Operating conditions were:

gas inlet pressure = 10 lbs. psi.

gas flow rate = 50 ml./min.

column temperature = 125°C

detector voltage = 1000

nominal chart speed = 15 in./hr.

Further characterisation of the acids was obtained by elution of the Rf region of a specific acid from a developed thin layer plate and again identifying the suspected acid under the GLC system.

4. Quantitation: The procedures and amounts of reactants used were exactly similar between the treatments analysed during a complete run. Titration gives an indication of the total acidity, but it is not particularly meaningful without characterisation of the acids involved. With TLC, because the procedures were standardised, inspection of the areas and densities of the spots will indicate the acids involved; but, even with measurements, this method was found to be subjective and unreliable. Therefore, the main method was analysis of the peak areas on the GLC charts, using TLC and titration as checks.

Quantitative estimates of the methyl esters were made using an internal standard (Creech 1964). Over the range involved, their amounts are linearly proportional to their recorded peak areas, as measured by their altitude x width at half-height. However, variation in peak area, besides arising from variation in sample amount, also originates from variations in: injection technique, detector fluctuations and column adsorption (Horning

et al. 1963). Therefore, a constant amount of an internal standard - 1.8 µg nonadecane (a C-19 hydrocarbon) - was added to each sample; the areas of the acid peaks are expressed in relation to the peak area of this internal standard, i.e. the relative amounts of organic acids in the different samples

$$= \frac{\text{peak area of acid}}{\text{peak area of C-19}}.$$

Standard curves for malic and succinic acids were obtained by adding 1.8 µg nonadecane to varying amounts of the methyl esters of malic and succinic acids; a set of standard curves was prepared for each complete trial. This allows the results to be expressed as µg acid per seed. However, these are in no sense absolute values for the organic acid contents of seed; they are convenient units for expressing the results after standardised extractions.

A reconstruction experiment in which a known amount of malic acid was passed through the complete procedure showed the recovery value to be 68%. Blank extracts were also run and no peaks appeared on the GLC chart.

Figure 10: Typical GLC chart from wild oat extract.

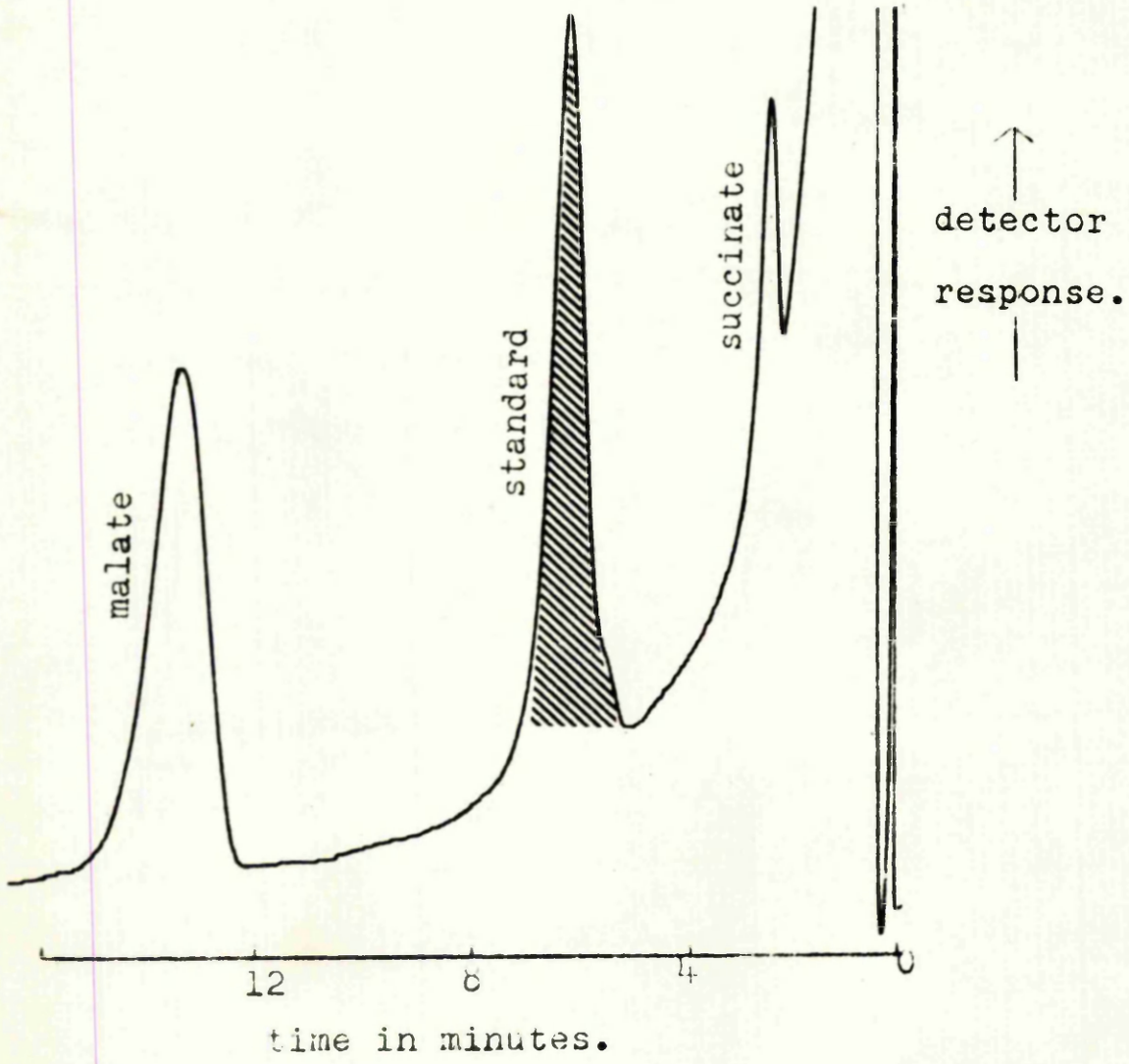
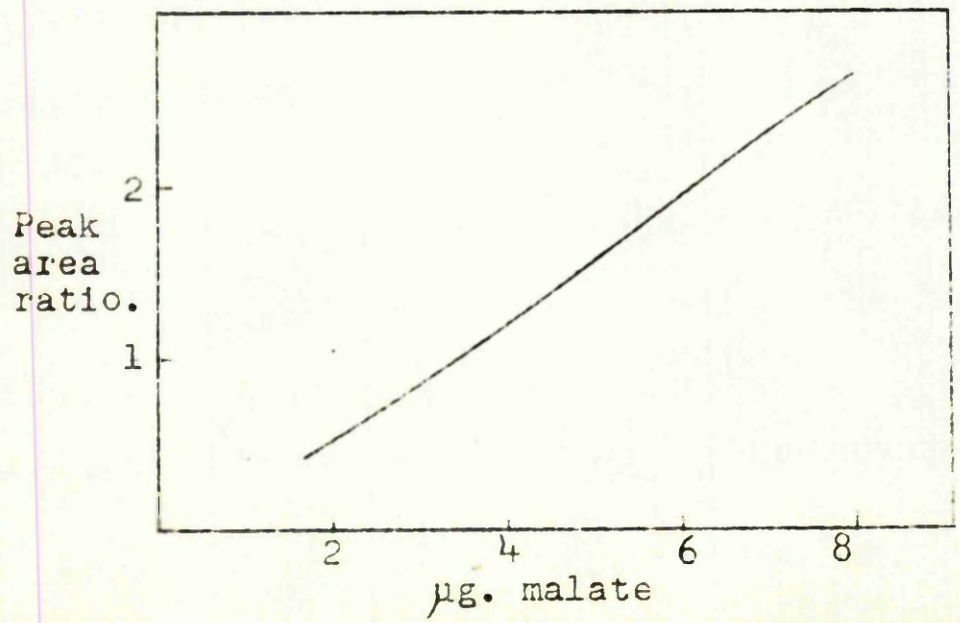


Figure 11: A malate standard curve.



Experiment 32 - Introductory experiment: analysis of the organic acid levels in samples from a single population.

This experiment provides an introduction to the type of results obtained and also demonstrates the reproducibility of the GLC technique within a single extraction series.

The seed population sampled was almost non-dormant - 70% germination under standard conditions of darkness at 20°C. Samples of various seed numbers were extracted and the levels of the methyl esters of the organic acids, succinic and malic, estimated in measured aliquots of the extracts using the GLC technique. The results, in terms of malic acid per seed, show good agreement between these replicate samples (Table 32b): the high level of malic acid, 11-13 µg/seed, and high ratio of malic/succinic are typical of nondormant seed.

Figure 10 illustrates a typical GLC recording: succinate appears on the solvent front, and malate just behind the internal standard; fumarate lies between the solvent front and succinate; citrate comes approx. 10 inches behind malate in this system; but these last two acids were never detected in seed extracts.

The peak areas are reported in Table 32a, in relation to the peak area of nonadecane. From the malic standard curve - e.g. Figure 11 - the levels of the methyl esters of malate in the extracts can be estimated as µg acid (Table 32b); the amounts in the original extracts, and thus per seed, can then be calculated.

Table 32a

Peak areas of the samples from one population.

Sample type		Measured area			Peak area ratio		Mal/Succ.
wt.	Number	C-10	Mal.	Succ.	Mal.	Succ.	
2 gm.	85	3.92	4.50	0.74	1.16	0.19	6
	89	3.98	5.77	0.76	1.42	0.19	7
	91	4.82	6.64	1.24	1.38	0.25	5
1 gm.	44	5.06	3.19	0.40	0.68	0.08	8
3 gm.	125	5.08	8.0	1.50	1.58	0.29	5.5

Table 32b

Calculation of malate per seed, from the peak area ratios

Seed No.	Peak ratio	µg Malate	µg in extract	µg per seed
85	1.16	4.0	1000	12.0
89	1.42	4.7	1175	13.1
91	1.38	4.6	1150	12.6
44	0.68	2.4	587	13.0
125	1.58	6.9	1475	11.8

Experiment 33 - Populations: analysis of malic and succinic acids in various populations.

The populations, and their codes, were:-

1) sativa - cultivated oats, nondormant and no CO_2 effects;
2) primary - primary grains of the 1964 Glasgow crop of wild oats, after 10 months storage at 20°C;
3) secondary - secondary, or distal, grains of the same crop;
4) 1964C - primary grains of the 1964 commercial sample of wild oats, after 12 months storage;
5) 1963C - primary grains of the 1963 commercial sample, after 24 months storage.

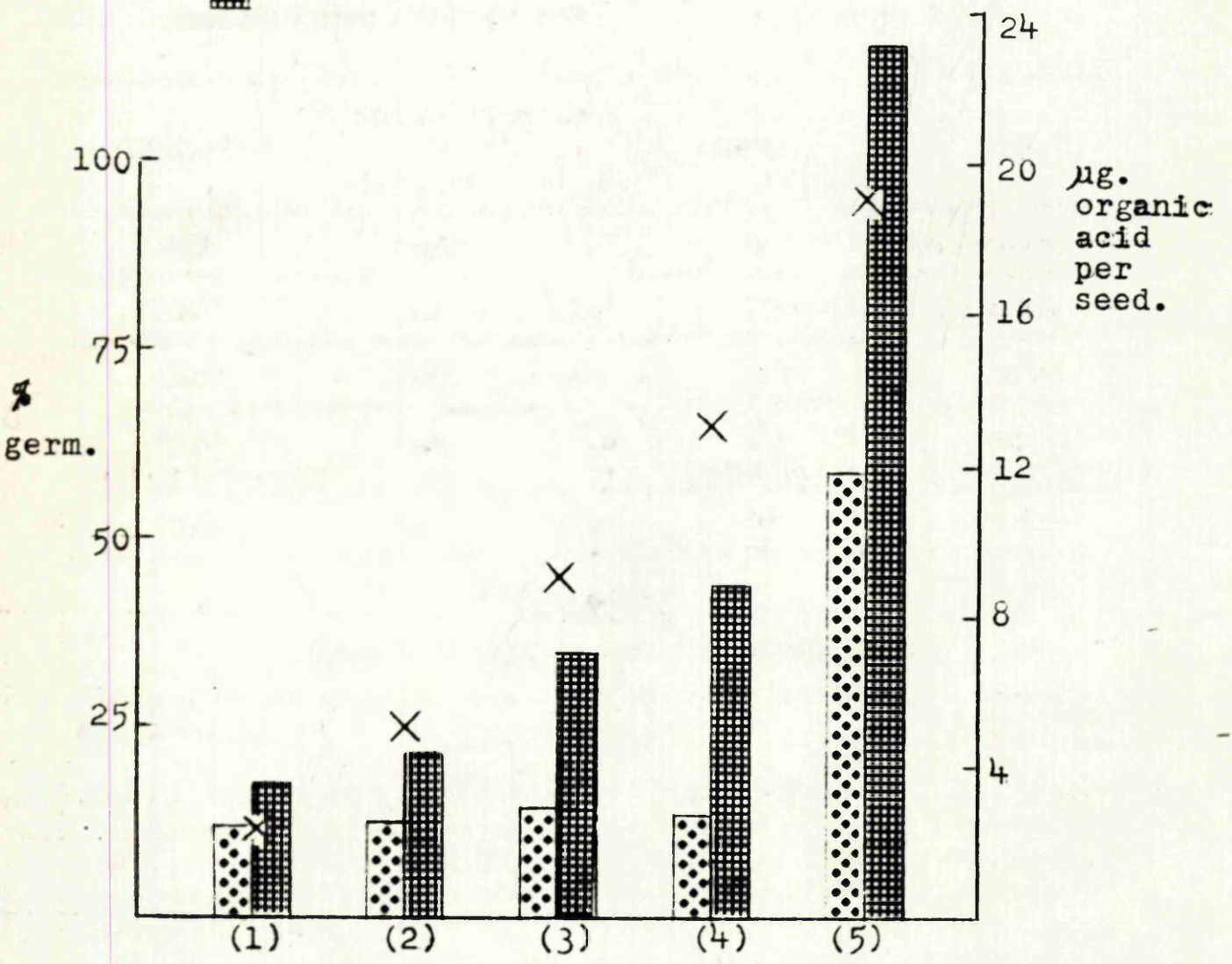
These populations were dormant to various degrees, as shown by their germination percentages in darkness at 20°C.

They were extracted in the unimbibed state:-

a) wild oats - 100 grains processed to give a final extract volume of 0.7 ml.; aliquots of 0.15 ml. dried and methylated; methyl esters taken up in 24 µl chloroform and 2 µl injected.
b) cultivated oats - 56 grains processed to give 0.7 ml. of extract; 0.17 ml. dried and methylated; methyl esters taken up in 144 µl $CHCl_3$ and 2 µl injected on to the column.

The peak area ratios are recorded in Table 33a: differences in the ratio of malic/succinic are apparent, due to increasing

Figure 12: Levels of organic acids and germinability in various seed populations.



(1) - secondary grains, Glasgow crop, 10 months storage;

(2) - primary grains, Glasgow crop, 10 months storage;

(3) - commercial sample, 12 months storage;

(4) - different commercial sample, 24 months storage;

(5) - non-dormant cultivated oats (A. sativa).

malic with decreasing dormancy. In Table 33b, the peak area ratios are converted to µg acid per seed; a large difference in the malic acid contents of cultivated and wild oats is apparent.

These differences are illustrated in the histogram of Figure 12; some are also demonstrated in Plate 1, (p.113a)

Table 33a

GLC peak area ratios from various populations

Population	% germ.	peak area ratio		mal./succ.
		malic	succinic	
secondary	12	2.1	0.3	2.6
primary	25	2.7	0.3	3.3
1964C	45	5.2	1.0	5.2
1963C	65	6.1	0.9	6.8
sativa	95	1.36	0.4	3.4

Table 33b

Conversion to µg acid per seed.

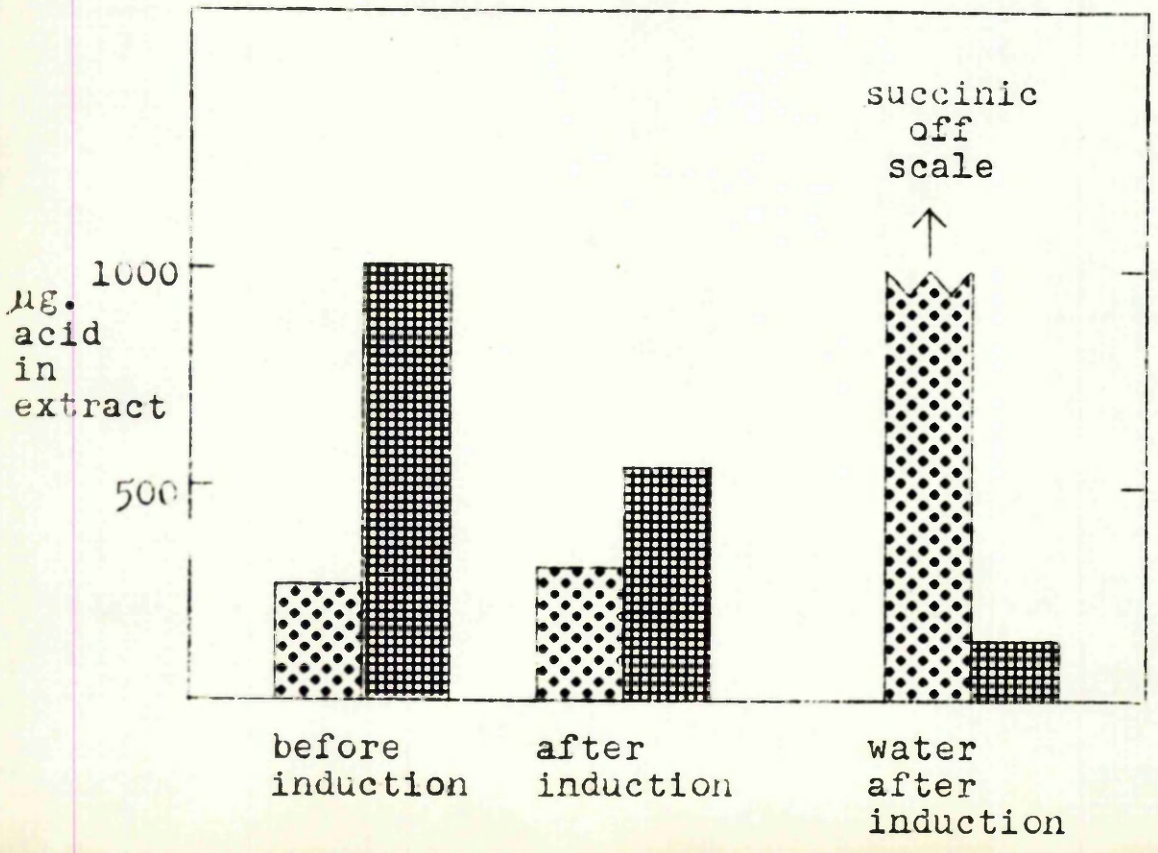
Population		%	µg malate			µg succinate		
type	no.	germ.	injected	extract	seed	injected	extract	seed
sec.	100	12	6.2	350	3.5	4.3	242	2.4
prim.	100	25	7.6	430	4.3	4.5	254	2.5
1964C	100	45	13.5	705	7.0	5.2	293	2.9
1963C	100	65	15.5	876	8.8	4.8	271	2.7
sativa	56	95	4.5	1296	23.1	2.3	660	11.8

Figure 13: Level of malic / succinic before and after dormancy induction under water.

Abscissa = type of extract.
Ordinate = total µg. acid in standardised extracts.

: succinic

: malic



Experiment 34 - Dormancy Induction: I. Analysis of the change in the levels of malic and succinic acids after dormancy induction under water.

If wild oats are submerged under water for 4-6 days, they enter the state of secondary dormancy (Hay & Cumming 1959).*

A 2 gm. sample of seed, 24 months from harvest, was subjected to this treatment. The levels of malic and succinic acid after such treatment can be compared with the levels in untreated seed in Table 34: the level of malic acid has dropped; succinic acid has increased and there is a very large amount of succinic in the induction water - presumably leached from the seed since the deionised water used did not carry either of the acids.

The data are shown in Figure 13. Plate 1 also illustrates the effect. (p.113a).

Table 34.

Levels of malic/succinic after dormancy induction

Treatment	peak area ratio malate	peak area ratio succinate	malate/ succinate	total µg malate	total µg succinate
Untreated	7.1	0.9	7.9	1003.9	270.7
After induction	3.5	1.0	3.5	547.2	305.3
Induction water	0.6	>7.0+	?	149.1	large

+succinate off scale

* This technique was found to give similar results with our material.

Experiment 35 - Dormancy Induction: II. Analysis of the levels of malic and succinic acids during the induction of dormancy under argon.

Dormancy can also be induced by imbibing wild oats under nitrogen for 4-6 days (Naylor & Christie 1956). In order to correlate the loss in germinability with changes in the levels of malic/succinic, a series of extractions was made during the induction process.

Flasks of seeds imbibing under argon were set up in darkness at 20°C. At specific time intervals from the start of imbibition, 3 flasks were removed and the contents analysed: a proportion of such seed was passed through the standard extraction and GLC process; the remaining seed were dehusked and the caryopses set to germinate under darkness in petri dishes to measure any changes in germinability which had occurred under argon.

The germination results are given in Table 35a, as the final percentages of germination out of groups of 75 caryopses. The results of the organic acid analyses are given in Table 35b. In Figure 14, these changes with time are illustrated.

In addition, 100 palea were put through the extraction process, to see if the amount of acid lost during induction, could be accounted for by leaching.

Figure 14: Changes in germinability and malic / succinic during dormancy-induction under argon.

Abscissa = hours under argon before germination.
Ordinate = µg. acid per seed; % germ. after treatment.

× : germination;

● : succinate;

○ : malate.

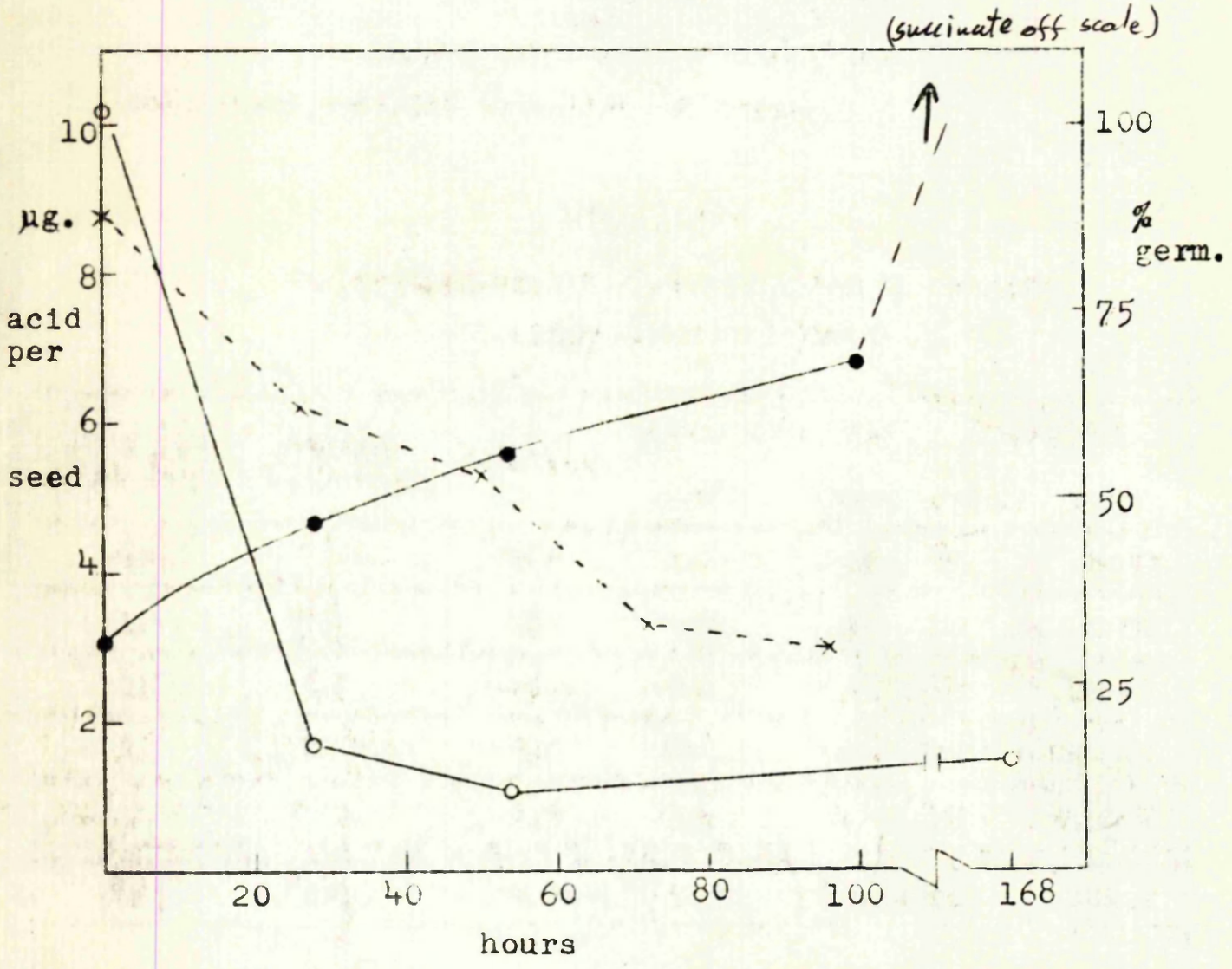


Table 35a

Final germination percentages after dormancy induction under argon for various periods.

Time under argon	% germ.	x^2(1df)
None	88	
26 hours	82	14.2**
50 hours	83	1.7
72 hours	33	5.6*
96 hours	30	NS

Note: x^2 refers to adjacent treatments.

Table 35b

Changes in malic/succinic during dormancy induction under argon

Treatment		Peak area ratio		mal/succ	µg mal. per seed	µg succ. per seed
type	no.	mal.	succ.			
None	89	2.8	0.4	7.0	10.2	3.1
28 hrs.	85	1.0	0.6	1.7	1.7	4.7
54 hrs.	86	0.9	0.7	1.3	1.1	5.6
100 hrs.	89	0.7	0.9	0.8	↓ OFF SCALE	6.8
7 days	93	1.0	1.0	0.5	1.5	↑ OFF SCALE
Poles	100	1.6	0.1	16.0	8.8	0.8

No organic acids found on seed test paper after imbibition under 100% argon.

Experiment 36 - Germination: Levels of malic and succinic acid during germination.

Batches of seed, 12 months from harvest, were set to germinate in darkness at 20°C. At the stated times, samples of 100 seeds were processed; the population extracted at 58 hours was 6% germinated. A sample was also extracted after 58 hours imbibition in light; none of this population had germinated.* Final %.

The relative levels of malic/succinic during such imbibition can be seen in Table 36; Plate I also illustrates the effect.(p.113).

Table 36

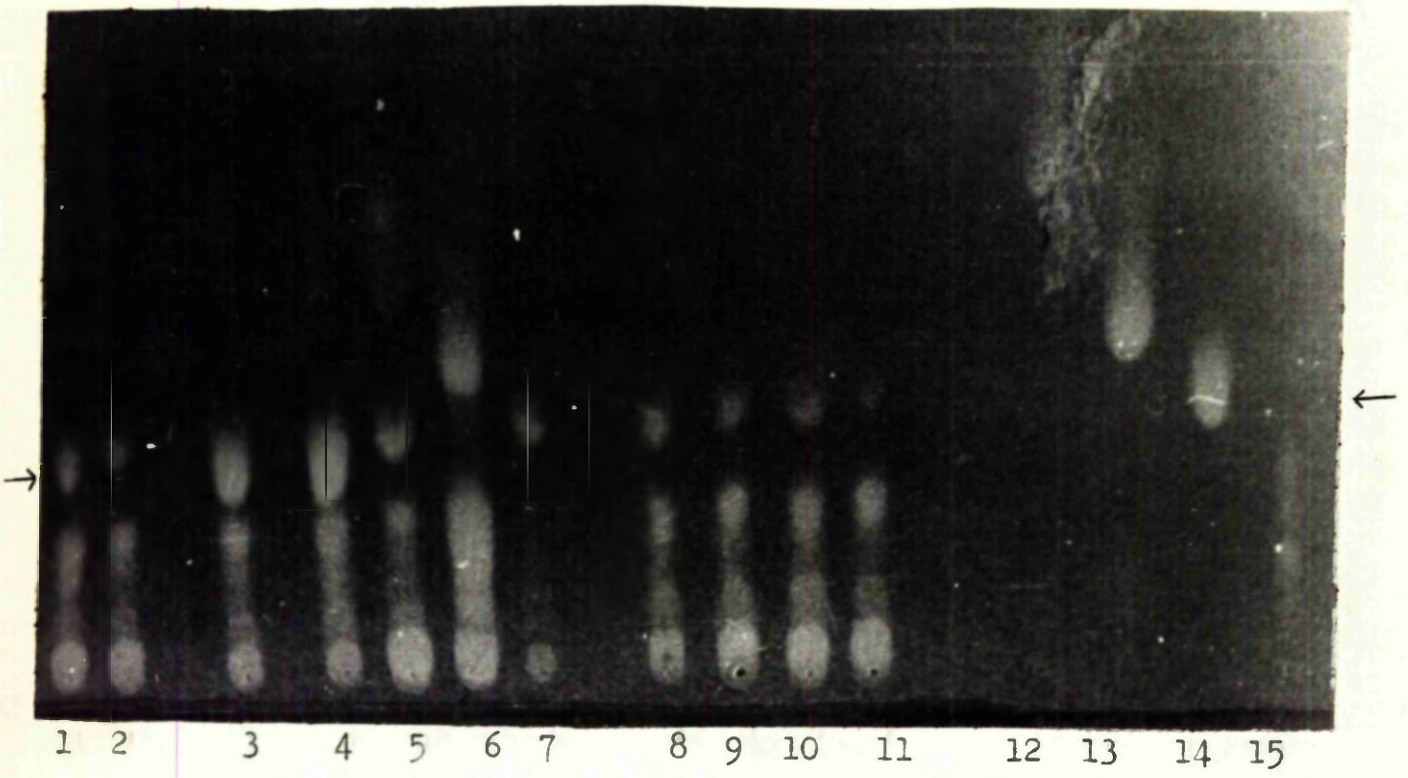
Levels of malic/succinic during imbibition

Imbibition period	peak area ratio malate	peak area ratio succinate	malate/ succinate	µg.mal. per seed	µg.succ. per seed
dry seeds	3.2	0.8	4	3.4	2.6
12 hrs. dark	2.3	0.4+	5	2.7	1.9+
30 hrs. dark	1.6	0.9	1.6	2.0	2.9
58 hrs. dark	2.5	0.9	2.8	2.7	2.9
58 hrs. light	1.0	0.3	3.0	1.4	1.1

+This drop in succinic only occurred in one trial; in other trials, the level did not change during imbibition in darkness.

* Final percentages of germination of these populations in darkness and light were 66% and 18% respectively.

Plate 1: Thin layer chromatogram of standardised extracts from various wild oat populations.



(1) - primary grains of Glasgow crop.
(2) - secondary grains of Glasgow crop.

(3) - 1964 commercial crop after 12 months storage.
(4) - 1963 commercial crop after 24 months storage.
(5) - 1963 commercial crop after dormancy induction.
(6) - deionised water after use in dormancy induction.
(7) - pales of wild oat grains (amounts not comparable).

(8) - imbibed in darkness for 12 hours.
(9) - imbibed in darkness for 30 hours.
(10) - imbibed in darkness for 58 hours.
(11) - imbibed in light for 58 hours.

(12) - fumaric acid.
(13) - succinic acid.
(14) - malic acid.
(15) - citric acid.

Discussion

The procedures described here detect malic and succinic acids in populations of oat seed, in contrast to the results of Holton & Noll (1955). About 30% of the malic acid in a grain is present in the palea (experiment 35). The results of experiment 32 indicate that the techniques are reliable in establishing the relative levels of these acids in replicate samples from the same population.

1) Populations: Differences in the level of malic acid and in the ratio of malic/succinic are apparent in the populations examined in experiment 33, between:

a) the two species of Avena;

b) wild oat samples from different localities;

c) wild oat seed from different positions within a spikelet;

d) seed after-ripened in dry storage for different lengths of time.

These differences correspond to differences in the levels of dormancy in these populations: the less dormant a population, the higher the level of malic acid; succinic acid does not seem to vary to the same extent. Correlations of germinability with organic acid content are well-documented in the review by Fowden & Moses (1960); citric acid has been found to be correlated with germinability in cereals (Taufel & Pohloudek-Fabini 1955b). No citric acid was observed in these wild oat extractions. Atwood (1914) noted a rise in acidity in wild oats with increasing after-ripening. This may be significant in relation to our observation

of a higher level of malic acid in the 24 month-stored 1963 sample of wild oats (Table 33). However, it should be noted that these samples were harvested in a different year from the 12 month-stored samples; thus, it is not established that this increase in malic acid represents a change during dry storage. Nor is it established that these differences between these populations examined do result from differences in the organic acid content of caryopses, rather than differences between the pales - due, for example, to differences in CO_2 fixation during ripening.

However, there does seem to be a correlation between germinability of a population and the level of malic acid in the seeds of that population.

2) Dormancy Induction: When dormancy is artificially induced in wild oats under conditions of anoxia, the level of malic acid falls, and succinic rises (experiments 34, 35): from a comparison of the levels of malic/succinic in the pales and in grains after treatment (Table 35), it seems unlikely that the decrease in malic acid is due solely to the amounts leached from the pales during imbibition; also, in experiment 34, the amount of malic acid in the water does not account wholly for the decrease in the level in the grain. Therefore, it is considered that the decrease in malic and increase in succinic reflect metabolic events occurring during the induction of dormancy.

Naylor & Christie (1956) describe a fall in respiration - measured by gaseous exchange - during dormancy induction; they conclude that, since germinability fell on the second day and respiration on the third day, a respiratory block did not cause the increase in dormancy. The results of experiment 35 show that, under our conditions, both germinability and the level of malic acid decrease after only one day under inducing conditions; further analyses may give a more clear idea of the change in acid content in relation to the change in germinability, but it is perhaps significant that in the first 20 hours the level of malic acid has fallen to its lowest level, whereas the germinability continues to fall for 3-4 days.

3) Germination: During standard imbibition under aerobic conditions, the level of malic acid also falls slightly (experiment 36); in darkness, the level rises again at about the time of radicle appearance; in light, the levels of malic and succinic acids both fall to very low levels. However, the ratio of malic/succinic stays constant in the light, suggesting that light affects a point in the system different from that acted upon by the dormancy-inducing treatments.

During the early stages of cereal germination, transaminase and decarboxylase activity bring about a decrease in ketoglutaric acid (Linko & Milner 1959); the level of citric acid has also been found first to fall during imbibition, then to rise rapidly

(Taufel & Pohloudek-Fabini 1955a). Our results parallel these findings with regard to malic acid level - although a more detailed account of the pattern of changes occurring during imbibition will be necessary before any valid conclusions may be drawn.

These changes in the levels of malic and succinic acids are open to various interpretations, which are discussed in the following pages.

It is considered that these variations in malic/succinic represent significant events in the reaction sequence:

succinic $\rightleftharpoons$ fumaric $\rightleftharpoons$ malic.

These reactions occur in both the tricarboxylic and glyoxylate pathways and are dependent upon dehydrogenase activity. In this respect, dormant wild oats are characterised by a lack of dehydrogenase activity (May 1962). Also, succinic dehydrogenase, although undetectable in dry lettuce seeds, becomes apparent during germination (Meyer et al. 1957).

The reducing conditions to which the seed are subject during the artificial induction of dormancy could block these systems either by inhibiting the action of the appropriate enzyme directly or by resulting in the reduction of a necessary cofactor or hydrogen acceptor. The subsequent change in the organic acid levels could then arise from:

a) a block at some point in the succinic/fumaric/malic chain. Thus, continued metabolism of the malic acid through, say, the

TCA, would lead to a build-up of succinic.

or b) a direct reversal of the reactions malic ⟶ succinic.

These concepts are similar to that proposed by Hay (1962), but postulated on the basis of a more defined system.

The inhibition of germination by high levels of carbon dioxide (see Part III) may be explained on the basis of such a system since levels of CO_2 greater than 10% are known to influence the levels of succinic acid (Hanson et al. 1960) by inhibiting the action of succinic dehydrogenase (Bendall et al. 1960).

The change in the acid levels under light, and the light inhibition of germination, may also be related to a similar type of situation, although the drop in the levels of both acids suggests that a different point in the system is affected. This change could arise from:

a) a light-stimulated channeling of succinic acid into another metabolic pathway;

or b) a block in the reaction chain at some point before the succinic/fumaric/malic sequence. Graham & Walker (1962) suggest that light affects the distribution of labelled carbon in the TCA by maintaining a high level of reduced nucleotide; Brown & Weiss (1959) claim the participation of a "photosynthetic reductant" in respiration. Such phenomena could be responsible for blocking the reaction:

$$\text{oketoglutarate} \longrightarrow \text{succinyl.CoA.}$$

Unfortunately, our procedure does not detect keto acids. However, Krupka & Towers (1956) report an accumulation of oketoglutarate during wheat germination; their data indicate a faster decline of oketoglutarate in darkness than in light.

The carbon dioxide effect in negating the light inhibition in wild oats could thus be due to its action as a metabolite in ensuring a continued supply of a required organic acid. The apparent lack of effect of exogenous organic acids (see section A) does not substantiate this, although no studies were made of the uptake of these compounds. However, carbon dioxide could act in the system in an alternative manner; since the carboxylation of

pyruvate ——→ malate

requires reduced pyridine nucleotide (Davies et al. 1964), any inhibiting production of reduced nucleotide could be negated.

Summary of section C

1. Techniques are described for the detection and estimation of the levels of malic and succinic acids.
2. The level of malic acid is shown to be inversely correlated with the degree of dormancy in a population of wild oat seeds.
3. When dormancy is artificially induced, the level of malic acid falls and succinic rises.

4. Imbibition under light results in a depression of the levels of both acids.

5. Possible metabolic pathways which could result in these events are suggested as lines for future investigations.

General Conclusions from Part V

A preliminary investigation has been made of some respiratory intermediates in wild oats. The results suggest that certain organic acids play a key role, or reflect significant changes, in the dormancy of this species.

A high level of germinability in a population is correlated with a high level of malic acid; when dormancy is induced, the level of malic acid falls, and that of succinic acid rises, inhibition under light results in a reduction in the levels of both acids.

It is pointed out that dehydrogenases will be concerned in certain of these changes, in particular succinic dehydrogenase, and it is suggested that environments which inhibit germination may do so by inhibiting this enzyme or by maintaining required cofactors in the reduced state.

The effects of the respiratory inhibitor, sodium fluoride, agree with this hypothesis: it is known to be capable of inhibiting succinic dehydrogenase and is shown here to inhibit germination, especially in light. High levels of carbon dioxide also inhibit germination and are known to inhibit succinic dehydrogenase.

Attempts to remove such a proposed block to metabolism by the application of organic acids were not successful. However, no valid conclusions can be drawn from these negative results since this apparent lack of effect could be due to:

a) their lack of uptake by the imbibing seeds; or

b) the fact that a low level of an organic acid is not the cause of the light inhibition, the changes in organic acid level here described being the results of changes in enzyme activity or cofactor state.

The relevance of these findings to the work of other authors has been discussed and it is suggested that a study of enzyme activities and substrate levels with regard to those metabolic pathways may prove valuable in establishing the causal factors in the dormancy of this species.

General Discussion and Summary Of Conclusions

One of the most striking aspects of the behaviour of dormant wild oat seed lies in their response to the radiation from a tungsten filament bulb. Although a stimulatory effect can be observed under certain conditions, continuous irradiation of imbibing seed prevents their germination and results in their subsequent entry into the state of secondary dormancy. Such inhibition of germination is probably related to an inhibition of cell elongation. Light inhibition of cell elongation is well known; an example can be seen in the effects of white light on shoot growth. It has often been proposed that light controls development by influencing the hormonal balance of an organ, but there are relatively few studies on the biochemical pathways involved.

The response of wild oat grains to light, changes during dry storage: for a relatively short period after harvest, the light inhibition effect resides in the caryopses itself; with increasing after-ripening, the palea become an important factor in the light inhibition system.

A lack of carbon dioxide in the ambient atmosphere was found to enhance the light inhibition. (Carbon dioxide also seems to be involed in the stratification effect (see Part I) - although no detailed experiments were carried out on this aspect). It is possible that the palea act to exclude carbon dioxide from the imbibing embryo, either by acting as barriers to, or by chemically removing, the carbon dioxide.

However, preliminary investigations into the effects on germination of crude aqueous extracts of the palea have indicated that extractable factor(s) can also act in the inhibition of the system: the palea carry a general inhibitory property, active in both light and dark germination and whose effects are intensified under low oxygen tensions; they also carry an extractable factor which interacts with light and a lack of carbon dioxide to prevent germination. The action of such factor(s) may account for the fact that, although a lack of carbon dioxide intensifies the light inhibition, increased levels of carbon dioxide were not very successful in negating the light inhibition of whole grains - where both light and the "inhibitor" are preventing germination. Further work is required to characterise these factors and to resolve their interactions with oxygen and carbon dioxide. It is suggested that, while oxygen may inactivate an inhibitor - as proposed by other authors - carbon dioxide overcomes the effects of such inhibitors by its action on the germination metabolism.

The results of analyses of the levels of certain organic acids in seed populations of different degrees of dormancy have indicated a metabolic pathway which may be important during germination: malic acid seems to be correlated with the level of germinability in a population; an early change during the induction of dormancy involves a decrease in the level of malic acid and an increase in succinic acid, and when seeds are imbibed

under light, the levels of both acids drop markedly.

The effects of certain chemicals on germination also indicate that blocks to metabolic pathways involving malic and succinic acids can affect germination: sodium fluoride inhibits germination in the light; this substance is known to inhibit succinic dehydrogenase. An extension of this work should include tests for the possible reversal of the inhibitory effects of such compounds by carbon dioxide. It would also be significant if application of such compounds, or of the naturally-occurring inhibitory fractions in the palea, was found to result in changes in the organic acid levels similar to those occurring during the induction of dormancy or during imbibition under light.

It is therefore postulated that the germination of wild oats in the light is dependent upon a respiratory pathway involving malic and succinic acids. Light and certain other unfavourable environmental factors block this system or divert metabolism into another pathway leading to the induction of secondary dormancy.

The work of other authors is cited to suggest possible mechanisms by which such control could be achieved. These involve inhibition of enzymes (for example, succinic dehydrogenase) or the production of an unfavourable ratio of reduced/oxidised cofactors (for example, pyridine nucleotides).

The tricarboxylic acid cycle is a respiratory pathway providing energy for growth processes; a perhaps more important function it performs is to provide, through transamination of certain of its intermediates, amino acids. These building blocks of proteins may be important metabolites in the development of an active embryo.

The glyoxylate cycle is another respiratory pathway which may also have a synthesising role: by providing a supply of acetylcoenzymeA, it is thought to be capable of acting as a link between fat and carbohydrate metabolism. Vegis (1956) relates dormancy and the fat content of an organ; and Brennan (1960) considers fat utilisation to be an early phase of embryo development.

The reaction sequence discussed in this thesis occurs in both of these cycles. Malate could thus be a key compound in systems involving enzyme synthesis or fat-breakdown: it could provide an "open-end" to the reaction chains by being continually synthesised via the carboxylation of pyruvate. Again, in the dormancy system proposed by Naylor & Simpson (1961) and involving blocks to sucrose production and utilisation, malate synthesis could provide a pathway for overcoming such blocks to the breakdown of endosperm carbohydrate.

Alternatively, as suggested by the apparent lack of effect of exogenously applied malic acid, the described changes in organic acid level may merely be the manifestation of more

of more fundamental changes in the germination metabolism, involving changes in enzyme activity or cofactor level.

However, it is considered that the changes in organic acid levels represent significant events in the germination metabolism of this species. Their relationship with the effects of certain environmental factors suggest a possible dormancy mechanism which is open to experimental verification through study of enzyme activity and substrate levels. Besides resolving any causal relationships between these changes and dormancy, such study may suggest pathways by which other developmental processes in higher plants could be controlled.

Studies on Seed Dormancy and Germination in *Avena fatua* (L)

Summary

The behaviour of partially dormant populations of wild oat seed was studied under various regimes of light and temperature. Their response to temperature is typical of freshly-harvested cereal seed, with an optimum temperature for germination of 20°C. Low temperature treatments (stratification) are not effective in reversing the high temperature (30°C) inhibition of germination in darkness. Temperature is also important in determining the response of these seeds to white light. Continuous irradiation inhibits germination at all temperatures, but with the greatest difference between light and dark germination at the relatively low temperature of 15°C. Short exposures to white light (eight hours) have a stimulatory effect on germination, such effects being most marked when the irradiation is carried out at 30°C.

The light inhibition of germination depends upon a relationship between the caryopsis and the palea. That is, in the later stages of after-ripening, the germination metabolism is only blocked by light when the caryopsis is enclosed by the palea. An artificial seed covering, for example polythene, also confers such light sensitivity on the caryopsis, although not to the same extent.

Further investigation showed that if carbon dioxide is excluded from the ambient atmosphere, the light inhibition of germination is intensified. Also, a high level of carbon dioxide tends to negate any light inhibition of germination at atmospheric oxygen concentrations. A possible mechanism of action of the pelon could lie in their acting as a barrier to gas flow between the caryopsis and the atmosphere. However, preliminary observations have indicated the presence in the pelon of water-soluble, extractable factors which can influence the germination of the caryopsis. There is a factor(s), inhibitory in both light and darkness, whose effects are intensified by a lack of oxygen; an aqueous extract of the pelon also inhibits the germination of caryopses in the light, such effects being negated by carbon dioxide.

The respiratory inhibitor, sodium fluoride, inhibits the germination of this species, especially in the light. Cyanide and dinitrophenol are ineffective.

It is postulated that light inhibits the germination of wild oats by influencing a respiratory pathway, and that carbon dioxide is effective through replenishment of required intermediates by carboxylation of pyruvate or phosphoenolpyruvate. The results of analyses of the levels of certain organic acids in seed populations of various degrees of dormancy support this view. The level of malic acid seems to be correlated with the level of germinability in a population. The artificial induction of

dormancy results in a decrease in the level of malic acid and an increase in succinic acid. And the levels of both acids fall when seeds are subjected to irradiation by white light.

Exogenously applied organic acids do not stimulate germination or negate the light inhibition, although this could be due to their lack of uptake by the seeds. If such organic acids are taken up and yet are ineffective, then the dormancy of wild oats cannot be due to the lack of a certain organic acid. The described changes in organic acid level would thus be the manifestation of changes in enzyme activity or cofactor level occurring during the changes in the dormancy state of this species.

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