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THE CHEMISTRY AND MODE OF ACTION
OF NATURALLY-OCCURRING GROWTH
REGULATORS FROM *AVENA*

A Thesis
Submitted by

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ABSTRACT

This thesis is concerned with the nature and function
of endogenous chemicals in the maintenance of oat seed
dormancy. The prime germination inhibitors within dormant
Avena spp. are characterised and their development pattern
during seed maturation and after-ripening established. Their
in vitro biological activities as inhibitors of germination
and reserve starch degradation are also shown.

Abscisic acid (ABA) and a group of saturated, medium-chain
length carboxylic acids (MCFA) have been detected in dormant
Avena seed. Levels of endogenous fatty acid and ABA were
monitored in developing grain of dormant and non-dormant oat
species. It is shown that the highest amounts of MCFA are
present within dormant seed, these levels decreasing during,
or prior to, that period of increasing seed germinability.
Reduced amounts of MCFA are associated with non-dormant grain.

It is shown that oat seed germination correlates well with
the endogenous level of MCFA, particularly nonanoic acid.
It is speculated that volatile fatty acids are lost from
dormant wild oat grain during dry storage by a natural
physical process involving gradual volatilisation.

ABA did not appear to be directly involved with oat
seed dormancy. Large variations in ABA content only occurred
in hydrated grain of both species, the level increasing
markedly during grain filling. As desiccation on the parent
plant proceeded the ABA content of seeds rapidly decreased.
At harvest the levels of free ABA in common and wild oat were
found to be similar, although their germinations were 65%

and 0%, respectively. It is shown that ABA is not efficacious as an inhibitor of germination in the common oat.

MCFA are detected in the β inhibitor mixtures isolated from a wide variety of plant material. It is proposed that they contribute to the inhibitory properties of this complex.

Fatty acids in the series pentanoic to undecanoic are tested for their physiological activity in seed germination. MCFA are found to inhibit seed germination and suppress gibberellin-induced amylolysis in barley at concentrations below $10^{-3}M$. In general, nonanoic acid is the most effective treatment. At a concentration of about $2 \times 10^{-3}M$ nonanoic acid reduces the germination of non-dormant oat seed by 50%. MCFA-induced inhibition of lettuce seed germination is shown to be similar to natural thermodormancy, being reversed by treatment with red light or applications of cytokinin or gibberellin.

It is speculated that MCFA impose their inhibitory action by modifying membrane function and/or enzyme activity within the plant cell.

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v

LIST OF ABBREVIATIONS

ABA	Abscisic acid
amu	atomic mass unit
cpm	counts per minute
cv	cultivar
2,4-D	2,4-Dichlorophenoxyacetic acid
dpm	disintegrations per minute
DW	dry weight
ed	external diameter
FFAP	Free Fatty Acid Phase
FID	Flame ionisation detector
FW	fresh weight
GA	Gibberellic acid
GCMS	Gas chromatography-Mass spectrometry
GLC	Gas-liquid chromatography
G-6-P	glucose-6-phosphate dehydrogenase
GRLS	Grand Rapids lettuce seed
LSD	Least significant difference
MCFA	Medium-chain length fatty acid(s)
MeABA	Methyl abscisate
6-P-G	6-phospho-gluconate dehydrogenase
POPOP	1,4-Bis-5-phenyloxazal-2-yl benzene
ppm	parts per million
PPO	2,5-diphenyl oxazale
PVP	polyvinyl polypyrrolidone
TLC	Thin-layer chromatography

CHAPTER I

INTRODUCTION

INTRODUCTION

THE IMPORTANCE OF THE SEED TO MAN

Throughout recorded history Mankind has depended upon
the dispersal units of higher plants as a means of food
supply.   Indeed, Heiser (1973) has proposed that
civilisations did not develop until a system of grain
cultivation had been established.   In prehistory man
existed as a hunter-gatherer but through the gradual
domestication of both animals and plants this life-style
slowly changed to that of food producer.   It would appear
that agriculture first originated in the Near East around
the semiarid mountainous areas near the river valleys of
Mesopotamia (Heiser 1973).   This was quickly followed by
the establishment of several other centres throughout the
globe.

Most plants are immobile having evolved a life-style
based upon a close association with the substrate (soil or
water) on which they grow.   Such an existence has necessitated
the development of propagules which, after developing on
the parent plant, are detached to disseminate the species.
The most dominant form of terrestrial vegetation are the
angiosperms which comprise roughly two-thirds of all plant
species.   Of particular importance to man within this group
is the family Gramineae which includes such cereals as
wheat, barley, rice, maize, oats and rye.   These grasses
produce dispersal units which are true fruits although they
are commonly referred to as seed or grain.

In a strict morphological sense seeds should be regarded as disseminules which separate from the pericarp when ripening on the parent plant. Those which include the pericarp or other floral structures should be referred to as fruits. Both structures serve the common biological function of species dispersal. A true seed is a mature fertilized ovule comprising an embryo and food supply surrounded by one, or more, protective coverings derived from the original integuments of the ovule. The embryo consists of one or more cotyledons, a plumule or stem growing point, a hypocotyl and the radicle, a rudimentary root. In some species the seed may contain an endosperm, either as a separate mass or a layer under the seed coat, or this tissue may be absent. The disarticulation of the mature seed from the parent plant results in a termination of its natural supply of water and nutrients. Thus the seed is usually disseminated as a desiccated structure in which metabolic reactions are reduced to a minimum. In this state the seed is significantly resistant to adverse factors in the environment.

Annual grasses, such as the cereals and related species, complete their development from seed germination to the production of mature fruits in one calendar year. It would be detrimental for an annual species to disseminate offspring displaying total and uniform emergence following dispersal. This characteristic would not promote survival of the species in the advent of unfavourable conditions resulting in the loss of one, or more, season's growth. Thus Nature must always be evolving a more efficient control over seed germination in order to optimize perpetuation of the species.

This is effected by ensuring adequate seed dispersal both in space and time. Natural selection is consequently directed towards those species which display indiscriminant seed emergence over an extended period.

Man, however, requires plants for cultivation which give maximum soil emergence combined with vigorous plant growth. This trend is clearly demonstrated in the cereals which have lost most of their natural dissemination characteristics which previously probably ensured the survival of the species. They have acquired, through selection, characteristics which increase their adaptability to cultivation. Certain unwanted plants have always been associated with those domesticated types on which man depends. Such species manifest characteristics which enable their survival and proliferation through normal farming methods, even though their presence results in decreased crop yields. Their ability to resist control measures is due, in part, to a characteristic feature of delayed seed germination which ensures the maintenance of a viable reserve in the soil.

DORMANCY

The propagules from some species remain viable for only a short period after dispersal. However, the majority of disseminules often lie for a prolonged term in the soil under ambient conditions favourable for germination and development. Such fruiting bodies can be regarded as maintaining an innate state of suspended growth between maturation on the parent plant and emergence in the soil. Viable seeds which do not germinate given an adequate water supply, a suitable temperature and a normal gaseous environment are referred to as dormant.

True dormancy must involve a mechanism whereby the dispersal unit has a genetic capacity to develop a block to active growth and metabolism. This innate dormancy must be contrasted with that where development is prevented by unfavourable external conditions, such as an insufficiency of water. In such cases, the seed may be regarded as merely quiescent because germination will occur once the basic conditions for growth are supplied. This state may be termed imposed or enforced dormancy (Harper 1957). When dormancy is imposed and this condition continues after the environmental influences have been modified, or the seed removed to a more favourable regime, then such a state is termed induced dormancy. In this condition the seed often requires exposure to a particular stimulus, such as specific light or temperature regimes, in order to instigate growth and development.

Dormancy has been defined by Amen (1974) as a "self-imposed transient suspension of growth and development

accompanied by partial metabolic arrest". A more
practical definition, however, is to regard seed dormancy
as a population phenomenon, such that a given sample of
viable dispersal units exhibiting a germinability below an
arbitrarily ascribed level (50%) would then be classed as
dormant. Different plant species may display considerable
variation in the relative depth of disseminule dormancy.
Whilst some will not emerge given a favourable environment,
others may germinate slowly or incompletely and yet display
normal sprouting when transferred to a different regime.

Other plant structures besides the seed, such as the
bud, bulb, rhizome and corm, often display an innate dormant
condition during some part of their life-cycle. In this
state they manifest an increased tolerance to harsh
environmental conditions. Many species have thus adapted
to survive regular periods of climatic stress by utilising
a distinct physiology combined with an almost complete
suspension of normal metabolism. The environmental factors
required to terminate dormancy often vary between species
and may involve seed-desiccation or exposure to a short
period of near freezing temperature. In this manner
development can be synchronized with the cyclic climatic
changes.

PREVIOUS LITERATURE

Several authors have presented extensive reviews
concerning the phenomenon of seed dormancy (Skene 1924,
Crocker and Barton 1953, Amen 1963, 1968, Vegis 1964,
Barton 1965 ab, Evenari 1965, Stokes 1965, Nikolaeva 1967,
Wareing 1969, Taylorson and Hendricks 1977).

From the literature it is clear that more than one factor may influence the resting-condition. The seed-coats may restrict diffussion of gases or water to the embryo, or germination may depend upon such environmental stimuli as a specific temperature treatment, light intensity or length of photoperiod. Mayer and Poljakoff-Mayber (1975) have presented a comprehensive review of such influences.

Because a detailed examination of all relevant literature is beyond the scope of this introduction, the author will restrict the review to that pertinent to the most recent hormonal/chemical theory of seed dormancy regulation. This hypothesis proposes that germination is controlled through a critical balance between certain endogenous chemicals. The reader is referred to the cited reviews for additional information concerning other aspects of seed dormancy regulation.

a) The influence of endogenous germination inhibitors:

Natural inhibitors of growth and germination are prevalent in seeds and fruits (Evenari 1949, Wareing 1965). They are extremely varied in chemical structure and have been suggested to play an important role in controlling developmental processes. Such compounds may be associated with the embryo, endosperm or covering structures of the seed or fruit. It is well known that seeds enclosed within fleshy types of fruit cannot germinate within the pericarp. The inhibitory action of fruit juices has been attributed partly to a high osmotic pressure and acidity (Evenari 1949) and to the presence of inhibitory compounds (Evenari 1949, Varga 1957).

Unidentified chemical compounds which prevent seed emergence have been extracted from the seed-coats of several species (Barton 1965b). Some photosensitive seeds have also been reported to contain specific substances in their external layers which are responsible for the photorequirement (Black and Wareing 1959).

One of the first to suggest that endogenous compounds might influence the regulation of seed germination was Köeckmann (1934), who extracted biologically active chemicals from seeds. Later, a detailed review by Evenari (1949) listed many species of fruit and seed which were found to contain non-specific germination inhibitors. The same author classified known inhibitory compounds into the mustard oils, organic acids, unsaturated lactones, aldehydes, essential oils, alkaloids, cyanogens, phenols, ethylene, ammonia-releasing compounds and inorganic salts.

Similarly, Varga (1957) after an exhaustive chromato-graphic study of fleshy fruit species classified three main groups of endogenous inhibitor, the short-chain organic acids, aromatic acids and the essential oils. It must be remembered that amongst such varied chemicals will be found compounds which are toxic to the seed. These may prevent germination when applied at a suitable concentration by the simple process of killing the plant tissue. Such substances cannot be regarded as dormancy-inducing. It is therefore convenient to consider only those chemicals which have a reversible inhibitory action on seed germination.

Amongst the secondary plant metabolites, a well-known group of substances inhibitory to germination are the

phenolic and flavanoid compounds which occur widely in seeds and fruits (Thimann 1972, Van Sumere *et al.* 1972). Unlike hormones, these chemicals only exert their inhibitory influence at relatively high concentrations (Zinsmeister and Hollmuller 1964). However, their ubiquitous nature indicates that such compounds may act as natural inhibitors of germination within the plant kingdom (Van Sumere 1960). Kefeli and Kadyrov (1971) proposed that the effect of phenolics on seed emergence is not hormonal but is due to some inhibitory influence on normal metabolic functions.

Cinnamic acids are naturally-occurring in both the free form, caffeic (I), coumaric (II), ferulic (III), and as related internal lactones, coumarin (IV) and scopoletin (V) (Thimann 1972). Akkerman and Veldstra (1947) have suggested that caffeic and ferulic acids are responsible for inhibiting the germination of tomato seeds within the fruit. However, these acids were not found to occur at sufficient concentration to account for such an effect.

Other authors have proposed that it is the combined inhibitory effect of coumarin together with several cinnamic and benzoic acid derivatives which is responsible for preventing seed emergence within fleshy fruits (Varga 1957). Hydroxy-cinnamic (VI) and vanillic (VII) acids, together with coumarin, have been detected in the husks of barley (Van Sumere *et al.* 1958) and lettuce seeds (Blumenthal-Goldschmidt 1961). Cook and Pollock (1954) suggested that a number of phenolic acids (*eg.* gallic (VIII), syringic (IX)

Caffeic acid

<u>trans</u>-3,4-dihydroxycinnamic acid

$$CH=CHCOOH$$
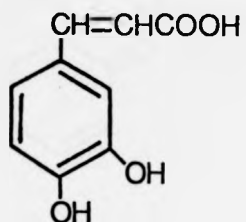
(I)

<u>p</u>-Coumaric acid

<u>trans</u>-4-hydroxycinnamic acid

$$CH=CHCOOH$$
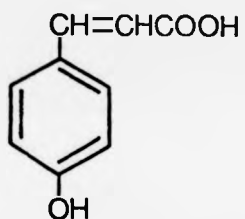
(II)

Ferulic acid

<u>trans</u>-4-hydroxy-3-methoxycinnamic acid

$$CH=CHCOOH$$

OCH_3

OH
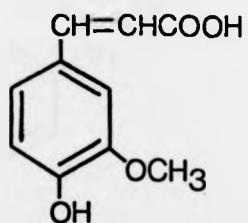
(III)

Coumarin

1,2-benzopyrone
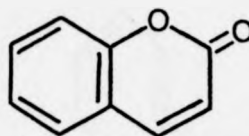
(IV)

Scopoletin

7-hydroxy-6-methoxy-1,2-benzopyrone
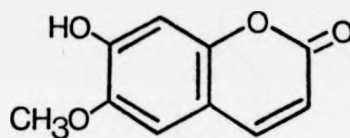
(V)

o-Coumaric acid

trans-2-hydroxycinnamic acid
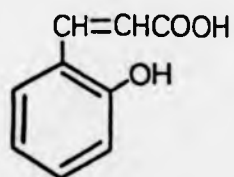
(VI)

Vanillic acid

4-Hydroxy-3-methoxybenzoic acid

COOH

OCH₃

OH
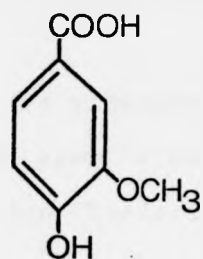
(VII)

Gallic acid

3,4,5-Trihydroxybenzoic acid

COOH

HO   OH

OH
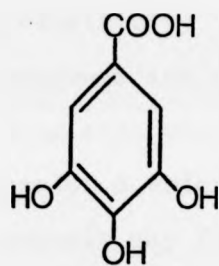
(VIII)

Syringic acid

4-Hydroxy-3,5-dimethoxyphenylacetic acid

CH₂COOH

CH₃O   OCH₃

OH
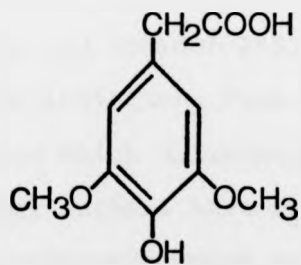
(IX)

and vanillic) were the active constituents responsible for
the biological activity of steeping-liquors produced from
malting barley.

Unsaturated lactones are widespread in the plant
kingdom and, in some cases, exert a marked inhibitory
influence on seed germination (Evenari 1949, Van Sumere *et al.*
1972). Coumarin, an internal lactone, is a potent emergence
inhibitor for a wide variety of seeds (Mayer and Poljakoff-
Mayber 1975). It can also induce light-sensitive dormancy
in some varieties of lettuce which do not normally require
light for germination (Nutile 1945). Berrie *et al.* (1968)
have studied the effect of several coumarin derivatives on
the induction of light-sensitivity in lettuce. They
concluded that these compounds act as anti-gibberellins
by competing for a binding site which is specific for the
lactone bridge. Because of a ubiquitous nature and potent
biological activity, coumarin may function as a natural
inhibitor of germination. It has, however, only been shown
to occur at inhibitory concentrations in *Trigonella arabica*
seeds (Lerner *et al.* 1959).

Phthalides, which occur in many seeds of the family
Umbelliferae, often show some degree of biological activity.
One of the most active isolated is n-butylidene-hexahydro-
phthalide (X) (Moewus and Schader 1951). Mitchell and Tolbert
(1968) have extracted inhibitors from the fruit of sugar-beet
and dormant wheat seed which inhibited markedly seed
germination. Several authors have reported other groups of
naturally-occurring compounds which prevent the germination
and growth of plants. These include terpenes (Muller 1965),

norditerpene lactones from *Podocarpus* sp. (Hayashi *et al.*
1968), cucurbitacins from seeds of the Cucurbitaceae (Guha
and Sen 1973), fatty acids (Bentley 1958, Komoto *et al.* 1972,
Ando and Tsukamoto 1974) and amino acids (Cleland 1963,
Smith and Fowden 1966).

Perhaps the most preponderant natural inhibitor of seed
emergence is abscisic acid (XI).   This chemical is widespread
within the plant kingdom as an endogenous component of plants,
plant organs and both dormant and non-dormant seeds.   A
sesquiterpene, it has been the subject of several comprehensive
reviews.   The reader is referred to these for further
details of history, isolation, chemistry and physiology
(Addicott and Lyon 1969, Wareing and Ryback 1970, Gross 1972,
Milborrow 1974, Leopold and Kriedeman 1975).

The first extraction of abscisic acid (ABA) was from
cotton fruit, as a substance which promoted leaf-abscission
in young cotton seedlings (Ohkuma *et al.* 1963).   Similar
isolations from other plant material soon followed (Robinson
and Wareing 1964, Rothwell and Wain 1964).   The active
constituents isolated from the differing species were shown
by Cornforth *et al.* (1965, 1966ab) to be identical.   In order
to avoid nomenclatory confusion the compound was named
abscisic acid by mutual consent (Addicott *et al.* 1968).
The stereochemistry of the acid, 3-methyl-5-(1'-hydroxy-4'-
oxo-2',6',6',-trimethyl-2'-cyclohexen-1'-yl)-*cis, trans*-2,4-
pentadienoic acid, was finally verified by a direct
correlation of naturally-occurring (+)-abscisic acid with
malic acid (Ryback 1972).   Later evidence also supported the
assigned S-configuration (Oritani *et al.* 1972, Harada 1973,
Koreeda *et al.* 1973, Mori 1973).   ABA is photosensitive and

n-butylidene-hexahydrophthalide

$$CHCH_2CH_2CH_3$$
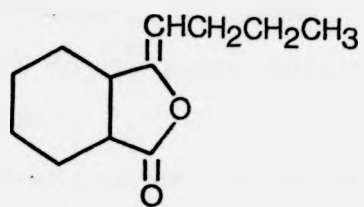
(X)

Abscisic acid
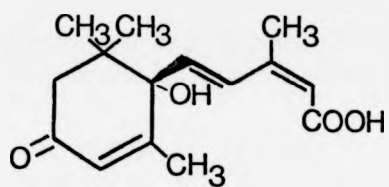
(XI)

Phaseic acid
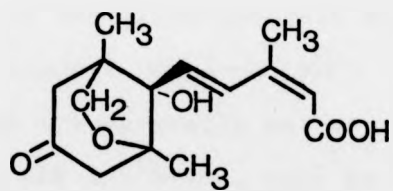
(XII)

exposure to ultraviolet light converts it to the 2-*trans*-4-*trans* isomer until an equilibrium is reached at approximately a 1:1 ratio (Mousseron-Canet *et al*. 1966). This isomer does not inhibit growth in bioassays which exclude light (Cornforth *et al*. 1965b).

Although no bioassay has been found to be totally specific for abscisic acid, several different techniques have been utilized to detect ABA-like activity. These include the accelerated abscission of certain petiolar explants (Addicott 1970), the inhibition of extension growth (Eagles and Wareing 1964, Koshimizu *et al*.1966, Wright and Hiron 1972), the inhibition of embryo growth or seed germination (Cornforth *et al*. 1966a, Gabr and Guttridge 1968), the antagonism of cytokinin and gibberellin-induced growth responses (Cornforth *et al*. 1966b), the inhibition of hydrolase synthesis in aleurone layers (Chrispeels and Varner 1967, Sivori *et al*. 1971) and some immunological responses (Fuchs *et al*. 1972). Tucker and Mansfield (1971) have reported that closure of leaf stomata is a quantitative response to ABA. The acid has also been found to affect the surface potential of root-sections (Tanada 1973).

Naturally-occurring analogues of abscisic acid, such as phaseic acid (XII) and dihydrophaseic acid, have been extracted from dry seeds (Milborrow 1969 , Walton *et al*. 1973). They are generally not as active as (+)-ABA. Conjugated forms of the inhibitor, such as glucosides, have been detected in some seeds (Koshimizu *et al*. 1968, Petrova and Nikolaeva 1974) and dormant embryos (Bulard *et al*.

1974).   Although also inhibitory, they have been found to
occur at a lower endogenous concentration to that of the
free acid (Milborrow 1974).   The same author has suggested
that the hydrolysis of bound-ABA by cell sap may release the
free form of the inhibitor (Milborrow 1970).   Endogenous
amounts of both free and bound-ABA have been correlated
with a termination of the resting-condition in dormant buds
(Harrison and Saunders 1975, Wright 1975).

b)   The influence of endogenous germination stimulators:

In several species seed emergence cannot be correlated
with a concomitant decrease in inhibitor content during
treatments, such as stratification, which break dormancy.
It has been proposed that these treatments stimulate
germination by inducing the production of an endogenous
growth promoter(s) which may be antagonistic to inhibitory
influences in the embryo (Flemion and de Silva 1960, Wareing
and Villiers 1961, Villiers and Wareing 1965, Frankland and
Wareing 1966).   The seeds from such parasitic species as
Striga sp. and *Orobanche* sp. require the presence of a
specific stimulant in order to germinate and develop.   These
chemicals, such as strigol (XIII), are exuded from the
roots of the host plant (Brown *et al.* 1952, Cook *et al.* 1966).
Exogenous application of diverse compounds may also
effectively terminate seed dormancy and, in some cases, these
chemicals may substitute for a natural dormancy-breaking
event (Mayer and Poljakoff-Mayber 1975).

c)   Plant Hormones:

Phytohormones include the gibberellins, auxins, cytokinins
and ethylene.   Thimann (1974) has reviewed the history of

Strigol
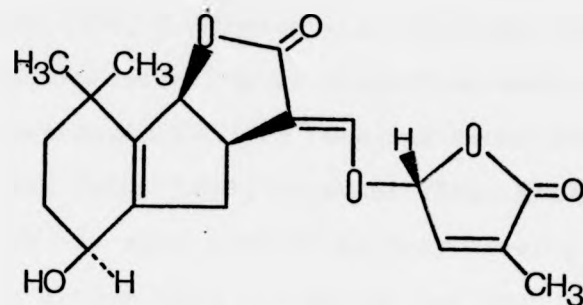
(XIII)

Gibberellic acid (GA$_3$)
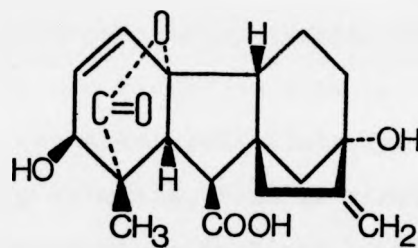
(XIV)

Gibberellin A$_7$
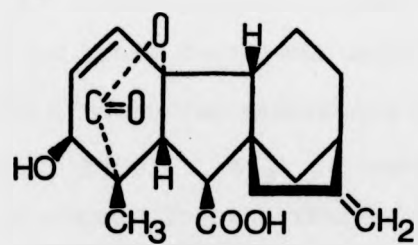
(XV)

phytohormone research, while several comprehensive reviews have been concerned with these plant hormones (Leopold and Kriedeman 1975, Taylorson and Hendricks 1977).

Gibberellins seem to be ubiquitous amongst higher plants and are found in both free and bound forms (MacMillan and Suter 1958, Murakami 1961, Hiraga *et al.* 1974). They are also present in many lower plants and fungi. The gibberellin literature has been recently comprehensively reviewed by Lang (1970), Jones (1973), MacMillan (1974) and Leopold and Kriedeman (1975). Phinney and West (1960) have reported the presence of gibberellin-like substances in several species of seed. They appear to be converted from a free to a bound form during fruit and seed ripening and Lang (1970) has suggested that this process may be reversed during germination.

Gibberellins are effective promoters of seed germination and can often substitute for a natural dormancy-breaking stimulus, such as stratification, dry after-ripening or exposure to light. The most commonly encountered member of the group is gibberellic acid, GA_3 (XIV), although other members, such as GA_7 (XV), may show greater specific physiological effects in certain bioassays (Overbeek 1966, Reeve and Crozier 1974). The acid can substitute for red light in promoting the dark germination of many light-requiring seeds (Evenari *et al.* 1958, Skinner *et al.* 1958, Toole and Cathy 1959). However the modes of action of these two treatments are dissimilar (Ikuma and Thimann 1960). GA_3 is more effective than red light in overcoming the thermodormancy of certain varieties of *Lactuca sativa L.* and can counteract the

inhibitory effect of a high osmotic pressure (Kahn 1960).
The metabolic basis for gibberellin action has yet to
be established (Jones 1973), although Mayer and Shain
(1974) have suggested that the hormone influences processes
associated with membrane formation.

Endogenous gibberellins are frequently produced
during stratification treatment and may be the means
whereby the innate character of delayed seed germination is
overcome. Although such events may suggest a natural
dormancy-breaking action, Webb *et al.*(1973 ab) have
reported that such increases may not be concomitant with
the advent of germination. The endogenous gibberellin
content of hazel seeds was found to increase only when
the stratified disseminules were placed at a favourable
temperature for germination (Williams *et al.*1973).
Similarly, Villiers and Wareing (1965) were unable to
detect gibberellin-like substances in stratified ash seed.

The group of phytohormones known as the auxins are
typified by indoleacetic acid (XVI) which is the commonest
naturally-occurring auxin in a wide range of plants, algae
and fungi. Auxins do not appear to function in the control
of seed dormancy and conflicting reports have been
concerned with their effect on seed germination. Exogenous
applications have resulted in both the promotion and
inhibition of emergence. The response pattern is dependent
on the seed species and auxin concentration utilised
(Poljakoff-Mayber 1958, Robertson *et al.*1976). Reviews by
Davies (1973) and Leopold and Kreidemann (1975) give a
detailed study of this group of biologically active

Indole-3-acetic acid (IAA)
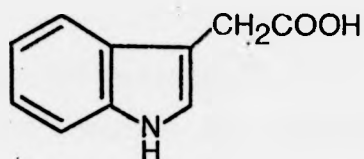
(XVI)

Zeatin

6-(4-hydroxy-3-methylbut-<u>trans</u>-2-enylamino)-purine
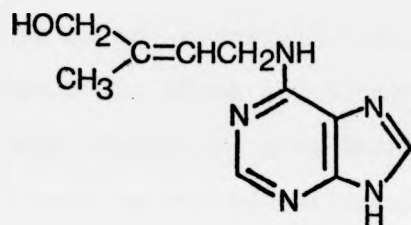
(XVII)

Kinetin

6-(furfurylamino)purine
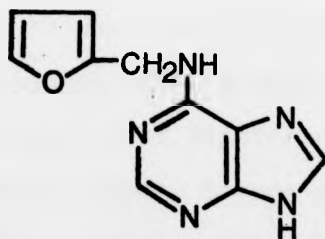
(XVIII)

compounds. Auxins play an important role in the cell-enlargement of stems and coleoptiles but, in general, dormant seeds are not responsive to applied physiological concentrations.

Natural cytokinins are derivatives of N^6 (Δ^2-isopentenyl) adenosine and these phytohormones can stimulate growth and differentiation in cultured callus tissue. Substances exerting cytokinin-like activity have been isolated from several plant species (Letham 1967) either as the free cytokinin or incorporated into tRNA (Hall 1973). Zeatin (XVII), the initial cytokinin hormone characterised in plants, has been detected in many species (cf. Fox 1969). Extensive reviews of the cytokinin literature have been published (Letham 1969, Skoog and Schmitz 1972, Hall 1973).

Usually large amounts of cytokinins are present in plant tissue undergoing active cell-division, such as young fruits or germinating seeds (Miller 1967, Gupta and Maheshwari 1970). Further, these chemicals are known to be metabolized rapidly in seeds at germination (Tzou *et al.* 1973). It has also been suggested that they are converted from a bound to a free form during the initiation of emergence (Van Staden 1973). Weiss (1960) has demonstrated that kinetin (XVIII) cannot fully substitute for red light in promoting the germination of light-requiring seeds. The chemical was found to merely induce germination at a reduced light intensity. Some authors have correlated the germinability of seeds undergoing stratification with changes in their endogenous cytokinin content (Webb *et al.* 1973ab, Kopecky *et al.* 1975).

Ethylene has long been recognised as a chemical active in the ripening of fleshy fruits (Denny 1924). The role of this gas within the plant kingdom has been extensively reviewed (Spencer 1969, Mapson and Hulme 1970, Yang 1974). Treatment of dormant structures, such as buds, seeds or tubers, with ethylene can lead to renewed growth (Vacha and Harvey 1927, Toole *et al*. 1964). This has led to the suggestion that endogenous ethylene is a natural dormancy-breaking agent (Esashi and Leopold 1969, Ketring and Morgan 1970). More of the phytohormone is evolved from nondormant rather than dormant seeds.

Ethylene is effective in overcoming dormancy, but its action is enhanced when in combination with other factors such as light (Olatoye and Hall 1973) or carbon dioxide (Negm *et al*. 1973). However, in some species exogenous ethylene cannot overcome seed dormancy (Abeles and Lonski 1969).

THE WILD OAT - A SERIOUS WEED PROBLEM

The present research stemmed from a long-standing
interest by the late Professor W Parker (Stirling) and
Dr A M M Berrie (Glasgow) into the nature of the deep
dormancy of mature *Avena fatua* L. seed.   This weed, the
common wild oat, is a major problem to temperate-zone
cereal producers (Chancellor and Peters 1970).   The wild
oats comprise *A. fatua* and certain varieties of *Avena
sterilis* L., the most important of which is usually
referred to as *Avena ludoviciana* Dur.   *A. fatua* and *A.
ludoviciana* are the most significant wild oats of northern
temperate zones.   The latter is the most winter-hardy of
all *A. sterilis* spp., as is reflected in its northerly
distribution and common name of Winter wild oat.

The presence of weeds in a commercial crop always
results in loss to the farmer through increased competition
with the crop, contamination of the harvested grain and
by acting as a reservoir for pests and diseases.   All of
these factors may contribute to a reduction in the quantity
and quality of the final harvest.   The wild oat can
complete development from seed emergence to seed dissemi-
nation between the sowing and harvesting of a commercial
grain crop.   This ability, combined with an extreme
longevity in the soil, has resulted in *A. fatua* becoming a
noxious weed to cereal producers throughout the globe.
The increased chemical control of broad-leaved weeds has
also contributed to its prevalence by reducing the natural

competition with other weed species (Fryer and Chancellor 1970).

Wild oats have been associated with cereal cultivation since the Bronze Age (Dadd 1957). *A. fatua* and *A. ludoviciana* appear to have originated in S.W. Asia and Asia Minor, respectively (Malzew 1930). In the main, their wide distribution can be attributed to the migrations of the Caucasian peoples with their cultivated grain crops (Hector 1936). Several surveys of the prevalence of wild oats in the United Kingdom have been recently undertaken (Elliot and Attwood 1970, Waterson and Davies 1973, Courtney 1974). Thurston (1974) reported the widespread occurrence of *A. fatua*, whereas *A. ludoviciana* was confined to a specific region near Oxford. Phillipson (1974) estimated the percentage of farming land infested with wild oats to vary from 15% in N. Ireland to 74% in S.W. England. The degree of infestation was found to vary markedly over the whole acreage. Nalewaja (1973) has estimated the significant wild oat problem in Northern USA.

THE WILD OAT - PHYSIOLOGY AND MORPHOLOGY

Wild oats are annuals of the family Gramineae. They have open panicles similar to the large loose ears of the cultivated oat but the fruits are smaller with strong geniculate awns. In Britain, Thurston (1957) has characterised twelve sub-species of *A. fatua* and ten of *A. ludoviciana*. Individual plants of both species are capable of setting seed as they are usually self-pollinated, each plant producing between 10 to 500 viable grains (Thurston 1956, Green and Helgeson 1957).

The main distinguishing feature between the two weeds is the mode of disarticulation of ripe grain in the spikelet. All seeds of *A. fatua* are separately dispersed. For *A. ludoviciana*, however, the lowermost and largest seed in each spikelet abscise from the pedicel. The intact spikelet is then disseminated as a discrete unit. (In the domesticated oat spikelets are retained on the parent plant at maturity and are separated during the threshing process).

The dispersed seed may become buried in the soil through hygroscopic action of the twisted awn on the lemma. This responds to changes in humidity by coiling and uncoiling and so drives the seed over the soil until it drops into a crack or becomes wedged under a stone. It appears that normal farming practises, rather than self-burial, are responsible for the incorporation of most wild oat seeds into the soil (Wilson and Cussans 1972).

A few seeds shed from any one growing season will germinate that same autumn. However, approximately 95% of mature viable *A. fatua* grain is dormant at the time of dispersal (Thurston 1956). Most of these abeyant propagules will germinate the following spring, between March and May, depending on the environmental conditions (Thurston 1961). For *A. ludoviciana* emergence mainly occurs during October to March (Thurston 1961). The seeds which remain in the soil can give rise to a series of seedlings over several succeeding years (Thurston 1956). Hence the period of an infestation, once established, can be very prolonged.

Seeds of *A. fatua* and *A.ludoviciana* have been found
to survive in the soil for 61 months and 33 months,
respectively (Thurston 1961).   Such survival is
dependent upon several factors, such as depth of burial
(Wilson and Cussans 1972), frequency of cultivation
(Whybrew 1964) and soil conditions (Lewis 1961).

THE WILD OAT - CULTURAL AND CHEMICAL CONTROL

The competitive effects of wild oats can be signi-
ficant and large losses of cultivated crops may result
from only light infestations (Bowden and Friesen 1967,
Chancellor and Peters 1974).   It is essential that any
method of weed control should encourage the emergence of
a maximum number of seedlings and prevent seed dissemin-
ation from the mature plant.   Such cultural practises
as deep-ploughing certainly stimulate germination and
reduce the reservoir of seed in the soil.   However,
unless combined with other control measures this may
result in an increased wild oat problem (Thurston 1954).

The cultivation of a particular sequence of "cleaning"
crops has frequently been recommended (Thurston 1956, Dadd
1957).   Another recommended control practise is to burn
the straw remaining from crops immediately after harvest,
in order to kill or break the dormancy of the disseminated
seed (Holroyd 1972).
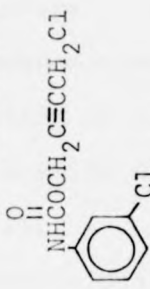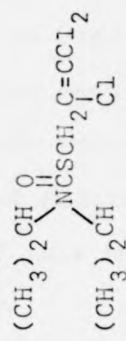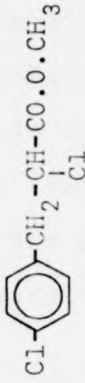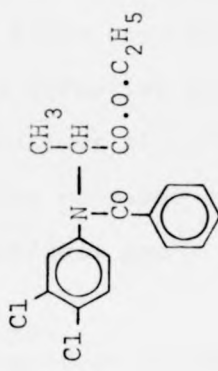
Delayed crop sowing in late spring allows the wild
oats to be destroyed by a shallow cultivation after
emergence (Selman 1968).   However this practise usually
results in a reduced grain yield.   Weed growth may be
kept to a minimum by selecting a sowing time, such as

late-autumn, which enables suitable crops to be well
established before the spring (Thurston 1956).   This
technique is obviously ineffective if the weeds germinate
with the winter sown crop.   Light infestations of up to
500 plants acre^{-1}are usually dealt with by hand-roguing
(Cherry 1968) and burning the collected weeds (Holyrood
1971).   Severe infestations normally require herbicide
treatment (Elliot 1972).

Two important herbicides, Triallate and Barban, are
widely used to control wild oats in commercial crops.
Both are carbamates (Table 1) and have a low mammalian
toxicity.   Triallate, marketed as Avadex BW, has
S-2,3,3-trichloroallyl di-isopropylthiocarbamate
as an active ingredient.   A preplant herbicide it is
applied before weed emergence and acts on the coleoptile
as it grows through the soil (Appleby *et al*. 1965), by
interfering with mitosis and cell extension (McKercher
et al. 1975).   This chemical is a selective treatment
for wild oats in broad-leaved crops and most cereals
(Banting 1963).

Barban, marketed as Carbyne, incorporates 4-chlorobut-
2-ynyl 3-chlorophenylcarbamate and is used as a post-
emergence spray for eliminating wild oats in many crops.
To be most effective the chemical must be applied at the
1-2½ leaf stage of seedling development (Holyrood 1960).
By interfering with cell division (Dubrovin 1959) it
restricts normal growth of the apical meristem causing
a cessation of plant growth and tillering (Holyrood) 1960).

TABLE 1: IMPORTANT HERBICIDES FOR WILD OAT CONTROL

Compound	Common Name	Structure	Use
4-chlorobut-2-ynyl 3-chlorophenyl-carbamate	Barban	$NHCOCH_2C{\equiv}CCH_2Cl$ (on chlorophenyl ring)	Postemergence control
S-2,3,3-trichloroallyl di-isopropylthio-carbamate	Triallate	$(CH_3)_2CH$ — $NCSCH_2\overset{\|}{C}=CCl_2$, $(CH_3)_2CH$, Cl	Preemergence control
Methyl 2-chloro-3(4-chlorophenyl) propionate	**chlor** fenprop-methyl	Cl—(ring)—$CH_2-CH-CO.O.CH_3$, Cl	Postemergence control
Ethyl benzoyl-N-(3,4-dichlorophenyl)-2-aminopropionate	benzoylprop-ethyl	(dichlorophenyl)$-N(-CO-phenyl)-CH(CH_3)-CO.O.C_2H_5$	Postemergence control

The haloalkanoic acid methyl 2-chloro-3-(4-chloro-phenyl) propionate (Table 1), commonly known as chlor-fenprop-methyl appears to be a very specific herbicide for *A. fatua*.

The aminopropionic acid ethyl ($\pm$)-2-(N-benzoyl-3,4-dichloroanilino) propionate (Table 1), known as benzoyl-prop-ethyl, may also be used to control *Avena* spp. in cultivated crops. The chemical is very effective when used as a postemergence treatment (Regnault *et al.* 1974). Severe stunting of oat plants is the prime response due to the application of this herbicide (Jeffcoat and Sampson 1973).

Such herbicides, and others, are therefore available for eliminating wild oats from cultivated land. Although these chemicals are effective if applied correctly, the cost may be significant and other factors, such as crop-sensitivity, may prevent their widespread use.

It would be possible to eradicate completely wild oats if all buried seed could be destroyed by the addition of toxins to the soil. The same result could be achieved by stimulating the germination of all dormant grains so that the weed seedlings may be eliminated by cultivation. However, unless a specific wild oat toxin is available, the latter alternative is preferable to the farmer and ecologist.

THE OBJECTIVES OF THE INVESTIGATION

The author's research during this study was concerned with the deep dormancy of wild oat seed manifest after maturation. Most recent theories propose that endogenous chemicals in the hull and/or caryopsis may play an important role in the regulation of seed dormancy in *Avena* spp. A high concentration of inhibitory compounds within the seed could prevent emergence. During dry after-ripening these chemicals could be metabolized to inactive forms and so allow germination to proceed under favourable conditions.

Naylor and Christie (1957) were able to reduce the germination of isolated wild oat caryopses in the presence of detached hulls. An aqueous hull extract has also been used to delay lettuce seed germination (Black and Naylor 1957). Black (1959) suggested that the main function of the hull was to prevent the leaching of water-soluble inhibitors from the caryopsis. A water-soluble inhibitor of germination and amylase activity has been detected by Elliott and Leopold (1953) in *A. sativa* cv. Victory. Extracts of both dormant and non-dormant wild oat seeds have prevented the growth of excised oat embryos (Kommedaht *et al.* 1958, Hay 1962).

The initial objective of the study was to characterise the major inhibitory chemical(s) in dormant *A. fatua* seed. Secondly, it was important to determine if any variation in endogenous inhibitor content occurred between *A. fatua* and *A. sativa* grains during maturation and post-harvest after-

ripening.   These two species show a marked difference
in germinability during this period, the cultivated oat
displaying a much weaker innate dormancy.   It was
hypothesised that an endogenous inhibitor having a
regulatory role would be significantly different, as
regards content and rate of variation, between the two
Avena spp.

In an attempt to characterize the putative
inhibitor(s) in the mature seed, a programme of chemical
extractions was initiated and monitored by a non-specific
bioassay.   Several limitations are inherent in this
approach to the determination of endogenous inhibitor
levels.   The biologically-active compounds may be
degraded during extraction and fractionation or they may
be significantly contaminated with other chemicals.
Alternatively, the isolated mixtures may be mutually
antagonistic or synergistic in any bioassay.   The commonly
used inhibition bioassays, such as the inhibition of seed
germination or extension growth, are non-specific and may
be completely unrelated to that physiological process
which the extracted inhibitor is suspected of regulating.
Few are capable of giving a linear dose response even
over a narrow concentration range, making quantitative
estimations of the growth-regulator extremely difficult.

Even if extraction and bioassay techniques are
optimal, a particular chemical should not be implicated as
regulating a given physiological phenomenon until
several important criteria have been met (Jacobs 1959,
Wareing 1965).   Development in dormant plant organs must
be prevented by some metabolic process.   However, the

presence of endogenous growth-regulators as extracted
in vitro, does not imply that they regulate dormancy *in vivo*.
Many seeds and fruits containing inhibitory chemicals
do not display a resting stage during their life-cycle.
Although biologically active plant metabolites are
widespread, it is clear that the majority are not
involved with the mechanism of dormancy control.

Wareing (1965) has suggested that to correlate seed
dormancy with inhibitor content requires a comparison
of the inhibitor levels associated with the various
degrees of dormancy, either by experimental or natural
manipulation.   In order to estimate the regulatory
nature of an extracted inhibitor, the following
questions must be posed.

1.   Is the depth of dormancy lessened when the inhibitor
     level is decreased by such treatments as leaching of
     the fruits and seeds?

2.   Can dormancy or delayed germination be imposed on
     non-dormant tissue by applying inhibitor experimentally?

3.   Is there any correlation between the state of dormancy
     of the embryo and the inhibitor level during embryo
     development or germination?

4.   What is the effect on the inhibitor level of various
     treatments which break dormancy, e.g. chilling,
     treatment with thiourea, etc?

5.   How far is the occurrence of inhibitors restricted
     to the species which show delayed germination?

The complexity of the dormancy response necessitates a
cautious attitude to the function of any endogenous plant-
growth inhibitor(s).   Only the most comprehensive studies

can unravel a chemical's true role and mode of interaction with the seed's metabolism.   With this in mind, it was decided to determine the characterized inhibitor(s) range of biological-activity and focus on those aspects which may suggest a regulatory capacity.

CHAPTER II

MATERIALS AND METHODS

MATERIALS AND METHODS

PART 1.   PLANT MATERIAL

Lettuce seed, *Lactuca sativa* L., cvs. Grand Rapids
and Great Lakes, were obtained from Page Seed Company,
Greene, New York, in 1972 and stored at -20° until
required.

Wild oat seed, *A. fatua* L., were either donated by
Hasler and Company, Dunmow, Essex (Lot 1, 1973), collected
locally (Lot 2, 1974) or grown on the university estate
(Lot3, 1975).

The common oat grain, *A. sativa* L., cv. Selma, was
either donated by W Thomson, The Steeds, Carse of Stirling,
(Lots 1 and 2, 1973 and 1974) or cultivated on the
university estate (Lot 3, 1975).

Both the wild and cultivated oat seeds (Lots 1 and
2) were stored under ordinary laboratory conditions until
required, while Lots 3 were stored at $20^{\circ}C$ in a constant
temperature growth-chamber under continual darkness.

The plant organs used for extraction of the "inhibitor-β"
complex were gathered locally between September 1974 and
March 1975 from wild-growing material.

Other plant materials used for extraction or bioassay
purposes were grown in the university glass-houses,
excluding seeds of barley (*Hordeum vulgare* L.), cv. Ymer,
which were obtained from James Gray and Son, Stirling.
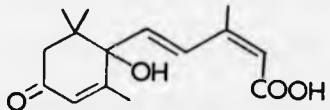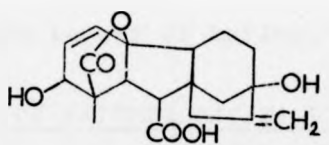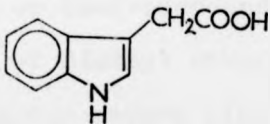
PART 2.   CHEMICALS

All chemicals and reagents used in this study were of Analar quality.   All solvents were distilled from glassware and solutions of test-compounds for bioassay were made up in either diethyl ether or distilled water.   Where dissolution in water proved difficult the test-compound was dissolved in 1.0ml ethanol and this was then made up to 250ml with water. At this concentration ethanol had no effect on any of the bioassays.   Of all the chemicals used only kinetin at 10^{-3}M could not be completely dissolved using this procedure and thus constituted a saturated solution approximating to 10^{-3}M.

The following chemicals were used as test-compounds in the various bioassays of this study:

Chemical	Trivial Name	Structure	Chain Length and Abbreviation	Supplier
Methanoic acid	Formic	HCO_2H	C_1	BDH Chemicals Ltd
Ethanoic acid	Acetic	CH_3CO_2H	C_2	"
Propanoic acid	Propionic	$CH_3CH_2CO_2H$	C_3	"
Butanoic acid	Butyric	$CH_3(CH_2)_2CO_2H$	C_4	"
Pentanoic acid	Valeric	$CH_3(CH_2)_3CO_2H$	C_5	"
Hexanoic acid	Caproic	$CH_3(CH_2)_4CO_2H$	C_6	"
Heptanoic acid	Enanthic	$CH_3(CH_2)_5CO_2H$	C_7	"
Octanoic acid	Caprylic	$CH_3(CH_2)_6CO_2H$	C_8	"
Nonanoic acid	Pelargonic	$CH_3(CH_2)_7CO_2H$	C_9	"

Chemical	Trivial Name	Structure	Chain Length and Abbreviation	Supplier
Decanoic acid	Capric	$CH_3(CH_2)_8CO_2H$	C_{10}	BDH Chemicals Ltd
Undecanoic acid		$CH_3(CH_2)_9CO_2H$	C_{11}	Aldrich Chem. Co. Ltd.
Dodecanoic acid	Lauric	$CH_3(CH_2)_{10}CO_2H$	C_{12}	BDH Biochemicals
Tridecanoic acid		$CH_3(CH_2)_{11}CO_2H$	C_{13}	Aldrich Chemical Co. Ltd.
Tetradecanoic acid	Myristic	$CH_3(CH_2)_{12}CO_2H$	C_{14}	BDH Biochemicals
Sodium [$1-^{14}C$] nonanoate		$CH_3(CH_2)_7{}^{14}CO_2Na$	$^{14}C_9$	The Radiochemical Centre, Amersham
DL-*cis, trans*-Abscisic acid			$(\pm)ABA$	Sigma, London Chemical Co. Ltd.
DL-*cis, trans*-[$2-^{14}C$] Abscisic acid			$(\pm)-ABA-2-^{14}C$	Hoffman La Roche
Gibberellic acid			GA_3 or GA	Sigma London, Chemical Co. Ltd.
Kinetin				Aldrich Chemical Co. Ltd.
Auxin			IAA	Sigma London, Chemical Co. Ltd.

PART 3.   BIOASSAYS

The physiological activities of the essential natural
products were characterised using a range of bioassays.
A simple lettuce seed germination test was utilised to
monitor the presence of general inhibitory activity within
the crude or fractionated plant extracts.   Because the
inhibitory chemicals were isolated from dormant *Avena* seeds,
their effect on the germination of non-dormant oat grains
was also required.   Specificity of the isolated plant-
growth regulators was estimated using bioassays involving
the induction of light sensitivity in certain species and
the ability to prevent the production and function of
hydrolytic enzymes within graminaceous seed.   The latter
effect was established by a determination of reducing sugars
and hydrolase activity released from endosperm pieces or
isolated aleurone layers of barley.

a)   INHIBITION OF *LACTUCA SATIVA L.*, cv. GREAT LAKES,
      SEED GERMINATION

Plant extracts or test-compounds were applied as a
known concentration of diethyl ether solution to Whatman
No. 3 qualitative filter-papers lining the bases of glass
petri dishes.   The volume and concentration of solution
used was appropriate to give, on evaporation of the solvent,
an amount which would result in the desired nominal molar
concentration when 1.0ml of distilled water was added to the
dish.   The crude plant extracts were assayed at 1000 ppm in
the dish.   The glass petri dishes were 4.5cm in diameter.

Seeds of *L. sativa*, cv. Great Lakes, were counted into lots of fifty and dispensed into 4.5cm petri dishes containing the test-solutions. The dishes were then placed into containers or wrapped in aluminium foil to exclude light. The grains were imbibed at a temperature of $20^O \pm 1^O C$ in a thermostatically controlled incubator. Germination as assessed by radicle emergence was scored after 24h.

b)   INHIBITION OF *AVENA FATUA L.* AND *AVENA SATIVA L.*, cv. SELMA, SEED GERMINATION

The test-compounds were applied in a solution of diethyl ether to Whatman No. 3 qualitative filter-papers lining the bases of 9cm diameter glass petri dishes. The volume and concentration of solution used was appropriate to give, on evaporation of the solvent, an amount which would result in the desired nominal molar concentration when 7.0ml of distilled water were added to the dish.

Seeds of the two *Avena* species were counted into lots of fifty and dispensed into dishes containing the test-solutions. The petri dishes were wrapped in aluminium foil to exclude light and the seeds imbibed at a temperature of $20^O \pm 1^O C$ in a thermostatically controlled incubator. Germination as assessed by radicle emergence was scored after 72h and 168h.

c)   INHIBITION OF *LACTUCA SATIVA L.*, cv. GRAND RAPIDS, SEED GERMINATION

The test-compounds were applied in a solution of diethyl ether to Whatman No. 3 qualitative filter-papers

lining the bases of 4.5cm diameter glass petri dishes.
The volume and concentration of solution used was appropriate
to give, on evaporation of the solvent, an amount which
would result in the desired nominal molar concentration when
1.0ml of distilled water was added to the dish.   Effects of
the germination stimulators, gibberellic acid and kinetin,
were assessed by substituting aqueous solutions of these
compounds for water in the experimental protocol.

Seeds of *L. sativa,* cv. Grand Rapids, were counted into
lots of fifty and dispensed into 4.5cm dishes containing the
test-solutions.   Manipulations were carried out in the
dark.   The petri dishes were enclosed in containers to
exclude light and the seeds imbibed at a temperature of
$20^{\circ} \pm 1^{\circ}C$ in a thermostatically controlled incubator.   After
4h of imbibition the grains were exposed to the appropriate
light-regime, if required.

Red light was generated by filtering the radiation
from a 200W tungsten filament lamp, run at mains voltage
(250V), through single layers of Cinemoid Numbers 1 and 6
used in combination.   Far-red light was generated using
the same source but filtering the radiation through
combined single layers of Cinemoid Numbers 1, 6 and 20.
In both cases, long wavelength radiation was removed by a
40cm water-filter.   Seeds were irradiated for 10min each
treatment and after exposure were returned to the germination
chamber until scored for germination.   The doses of
incident radiation, as measured by a Kipp and Zonen
compensated thermopile, were 436 mJ cm^{-2} for red light and
15 mJ cm^{-2} for far-red light, respectively.

Germination as assessed by radicle emergence was scored after 24h.

d) INHIBITION OF GIBBERELLIN-INDUCED AMYLOLYSIS IN BARLEY ENDOSPERM

Embryo-free half seeds were prepared according to the method of Coombe *et al.* (1967) from husked barley *(Hordeum vulgare,* cv. Ymer). The seeds were dehusked and sterilised in 50% v/v sulphuric acid for 3h at 20°C followed by ten washes using vigorous and repeated agitation with sterile, double-distilled water. All operations were performed under sterile conditions with sterilised apparatus. The dehusked, sterile seeds were then soaked in water for 20h at 3°C, following which they were cut transversely 4mm from the distal end to provide the barley endosperms. The embryo pieces were discarded.

1.0ml of the test-solution plus 0.05ml of gibberellin (Gibberellin *purum,* Fluka AG; 80% GA_3, 20% GA_1) solution were placed in each vial (4 replicates) giving a liquid depth of 2mm in the 24mm x 50mm tubes. All test solutions were buffered to pH 4.8 with 1M sodium hydroxide solution. The gibberellin solution was 8.8 x 10^{-6}M in GA with a concentration of 0.5g litre^{-1} in streptomycin. Two half seeds were added to each vial and incubated for 42h at 26°C. Gibberellin-induced starch hydrolysis was assessed by measuring the amount of reducing sugar released into the medium (1.05ml) by the pairs of embryo-free endosperm incubated in the presence and absence of gibberellin.

After the incubation period the endosperm pieces were removed and 9.0ml water added to each vial. 0.1ml aliquots

were removed from the mixtures and assayed for reducing
sugars using Somogyi's alkaline copper reagent (Somogyi
1952).   1.0ml of this reagent plus 0.9ml water were well
mixed with the aliquot in a test-tube.   The tubes were
covered with marbles and heated for 10min at $94^{\circ}C$, then
cooled in an ice-water mixture for 5min.   1.0ml of
Nelson's arsenomolybdate reagent (Nelson 1944) was then
added to each tube followed by 2.0ml water to give a final
volume of 5ml.   Each tube was vigorously agitated and the
optical density of the solution then determined at 560mμ
using a Bausch and Lomb Spectronic 700 spectrophotometer.
The optical densities were compared with appropriate
glucose standards.

PART 4. SAMPLING OF THE PLANT MATERIAL

Samples of *A. fatua* and *A. sativa,* cv. Selma, were
gathered from the experimental garden of the University
of Stirling at regular intervals between June and August
1975.   Random amounts were collected from different areas
of the field and combined to give a bulk sample of several
hundred grains.   Each sample was immediately assessed for
dry weight $grain^{-1}$, moisture content and the levels of
endogenous abscisic acid and medium-chain length fatty acids.
The whole fruit (caryopsis enclosed by the lemma and palea)
was examined sc that as the grain matured the caryopsis
became more significant.   Separation of the seed into its
respective parts was not attempted in this study.

PART 5. DRY WEIGHT AND MOISTURE CONTENT

The dry weight and moisture content of the samples were determined by the removal of endogenous water in a drying oven held at 60°C until the plant material had reached a constant weight (24h). Eight replicates for each sample were assessed.

PART 6. <u>STUDIES ON ENDOGENOUS PLANT GROWTH SUBSTANCES.</u>
<u>EXTRACTION, PURIFICATION AND QUANTITATION</u>

<u>ABSCISIC ACID (ABA)</u>

The extraction and purification procedure for the
assessment of ABA from plant material is illustrated in
Figure 1. Light was excluded from the extracts at all
times during the fractionation to prevent isomerisation of
the acid. The method was developed from that reported by
Berrie *et al.* (1975).

a) <u>EXTRACTION</u>

The plant material was homogenised in cold 80% aqueous
methanol. Abscisic acid was extracted from the ground
plant material by subjecting it to three soakings in the
same solvent at 4°C over a period of one week. The
methanolic extract was reduced to the aqueous phase by
rotary evaporation *in vacuo* at 30°C. The mixture was
acidified to pH2.0 by the addition of 10M sulphuric acid
(H_2SO_4) and then partitioned against three volumes of diethyl
ether. Constituent acids were removed from the resulting
ethereal phase (A) by three partitions with 5% sodium
bicarbonate solution (pH8.8). The resulting aqueous
alkaline phase was taken to pH2.0 with 10M sulphuric acid
and extracted three times with diethyl ether to obtain
Fraction 1, which contained free ABA from the plant tissue.

The organic phase (A') was taken to dryness *in vacuo*
and the residue hydrolysed with 0.1M sodium hydroxide
solution (750ml) at 60°C for one hour. After cooling, the
hydrolysed solution was shaken three times with diethyl ether,

GROUND TISSUE (xg)

8x ml 80% aqueous MeOH
at 4°C over 7 days

METHANOLIC EXTRACT

reduced in volume

AQUEOUS EXTRACT (pH 2.0)

3x ml ether

AQUEOUS
(discarded)   ORGANIC (A)

3x ml 5% NaHCO₃

ORGANIC (A)   AQUEOUS (pH 2.0)(B)

Hydrolysis at
60°C with 0.1M
NaOH

3x ml ether   3x ml ether

ORGANIC   AQUEOUS   AQUEOUS   ORGANIC
(discarded)   (pH 2.0)   (Fraction 1)

3x ml ether

AQUEOUS   ORGANIC
(discarded)   (Fractions 2 & 3)

SEPHADEX CHROMATOGRAPHY

POLYCLAR A.T. CHROMATOGRAPHY

PREPARATIVE T.L.C.

FINAL ABA FRACTIONS

METHYLATION

ABA QUANTITATION (G.L.C.)

Figure 1: Flow chart representation of the procedure used
for the quantitation of ABA from plant tissue.

the organic phase being discarded.   The remaining aqueous phase was then adjusted to pH2.0 with 10M sulphuric acid and extracted with three volumes of ether to obtain Fraction 2.   This solution contained free-ABA released by hydrolysis of the ether-soluble neutral components from solution A.

A third ABA fraction was obtained from the residual aqueous phase after ether partition of the acidified solution B (Figure 1).   This phase was neutralised with sodium hydroxide and made 0.1M in the base.   The resulting solution was heated at 60°C for one hour, cooled and shaken with three volumes of diethyl ether, the organic phase being discarded.   The aqueous phase was adjusted to pH2.0 with 10M sulphuric acid and extracted with ether to obtain Fraction 3.   This contained free-ABA released by hydrolysis of the water-soluble components from solution B.

b)   PURIFICATION

The free and alkali-labile ABA extracts were purified in a similar manner, using alkylated Sephadex chromatography, P.V.P. chromatography and preparative T.L.C.   Quantitation was by means of electron-capture G.L.C. after methylation of the purified extracts with diazomethane.

(i)   Alkylated Sephadex LH-20 Chromatography

According to the method of Brooks and Keates (1969). After removal of diethyl ether at 30°C *in vacuo,* the extracts were taken up in 5ml of 5% v/v benzene in methanol and applied to columns of alkylated Sephadex LH-20 (20cm x 2cm diameter).   The columns had been previously packed and washed with the same solvent and elution was set at a flow

rate of 1.25ml min^{-1}. To determine the elution profile of ABA a Gilson Automatic Fraction Collector was used to collect 80 x 2ml fractions, which were examined by a germination bioassay. Similarly ($\pm$)-ABA-2-^{14}C was utilized and eluted from the column. 10ml bulk fractions were collected and assessed for radioactivity using an Inter-technique SL3 liquid scintillation spectrometer after the addition of 10ml of a 2,5-diphenyl-oxazale(PPO)-toluene mixture (8.8g l^{-1}).

Figure 2 illustrates a typical result of the seed bioassay compared with the elution-profile of ^{14}C-ABA. The recovery of ^{14}C-ABA from this chromatographic system was determined as 98%. It was therefore decided to collect a bulk fraction of 60ml in further purifications, which would then be subjected to P.V.P. column chromatography.

(ii) Polyvinyl polypyrrolidone (P.V.P.) Chromatography

According to the method of Glenn *et al.* (1972). 15g of dry Polyclar AT powder (P.V.P.) (BDH Biochemicals) were thoroughly mixed with 200ml distilled water and the fines decanted off after a settling period of 15min. After six such procedures the slurry was poured into a 30cm x 2.0cm diameter glass-column employing a Number 3 porosity sinter overlain with acid-washed sand as a support. A fine layer of acid-washed sand was placed on top of the bed to prevent its disruption. The column was equilibrated with 0.1M phosphate buffer (pH7.0) at a flow rate of 250ml h^{-1} prior to chromatography.

3.0ml of phosphate buffer was added to each of the benzene-methanol solutions from the previous stage b(i) and
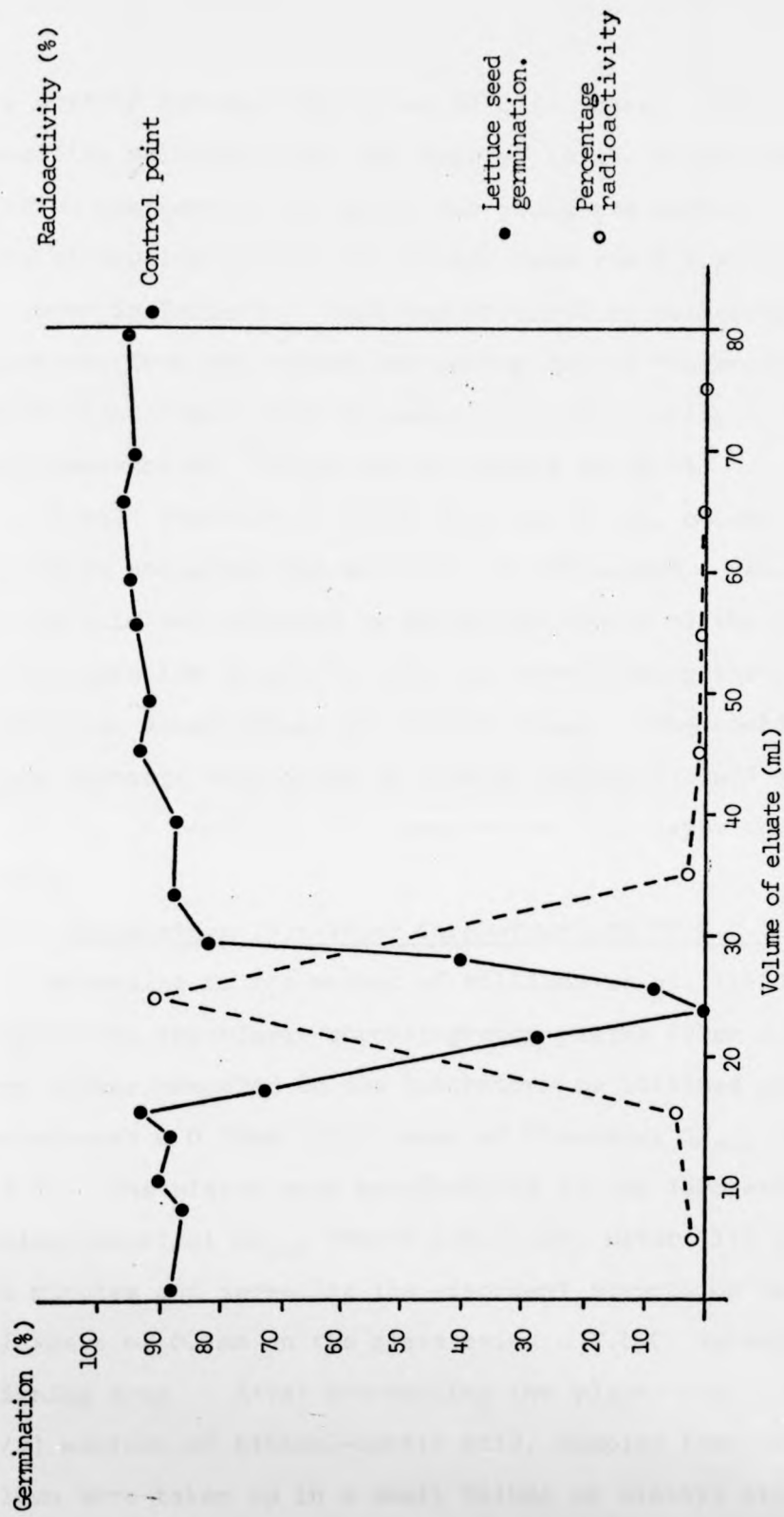
Figure 2: The elution profile of ABA from an alkylated Sephadex LH-20 column.

the organic solvent removed at 30°C *in vacuo*. The resulting solution (3ml) was applied to the column and elution was carried out using the phosphate buffer. A typical elution profile of ^{14}C-ABA from the P.V.P. column is shown in Table 2. This was obtained by collecting 20ml fractions from the column and adding 2ml of "Unisolve" (Koch-Light Labs) prior to scintillation counting. The mean recovery of ^{14}C-ABA was estimated at 98.5%.

A bulk fraction of 120ml from the P.V.P. column therefore contained the majority of ABA eluted. Recovery of the acid was effected by adjusting the pH of the fraction to 2.0 with 10M sulphuric acid and partitioning three times against an equal volume of diethyl ether. The combined ether extracts were taken to a small volume (1.0ml) at 30°C *in vacuo*, in readiness for preparative thin-layer chromatography.

(iii) Preparative Thin-layer Chromatography (T.L.C.)

According to the method of Williams *et al*. (1973). Preparative thin-layer chromatography plates (20cm x 20cm) were either prepared in the laboratory or obtained pre-coated with a 0.25mm thick layer of Kieselgel GF_{254} (Merck Ltd.). The plates were manufactured in the laboratory by mixing Kieselgel GF_{254} (Merck Ltd.) with water (1:2 w/v) for two minutes and spreading the adsorbent suspension to a thickness of 0.2mm on the glass using a T.L.C. spreader and aligning tray. After pre-washing the plates with a 98:2 (v/v) mixture of ethanol-acetic acid, samples from the P.V.P. column were taken up in a small volume of diethyl ether and applied as a 15cm narrow band, 2.5cm from the sides of the

TABLE   2

Elution profile of ^{14}C-ABA from P.V.P. column-chromatography

Fraction No.	Volume (cm^3)	% radioactivity
1	20	0.06
2	20	21.54
3	20	76.21
4	20	1.8
5	20	0.21
6	20	0.07
7	20	0.06
8	20	-

plate.   Markers of authentic ABA were placed on either
side of the base line.

The plates were developed in an ethyl acetate:
chloroform:acetic acid mixture (15:5:1, v/v) allowing the
solvent front to run 15cm from the origin.   The chromatograms
were then removed and visualised under U.V. light (254nm)
and the band lying between the two ABA markers carefully
scraped from the plate.   Adsorbed compounds were eluted
from the silica gel by means of Soxhlet extraction with 50ml
diethyl ether.   The organic solution was then reduced to
dryness and methylated prior to quantitation of ABA by
gas-liquid chromatography (G.L.C.).

At some stages qualitative T.L.C. was carried out
using 10cm x 5cm plates pre-coated with a 0.25mm layer of
Kieselgel GF_{254} (Merck Ltd.).   Experimental samples or
authentic standards were spotted 2cm from the bottom of the
plate, 1.5cm from the side and 1cm apart.   They were then
developed in the appropriate solvent-system to a height of
7.5cm from the origin.   The samples were recognised by
visualisation under U.V. light (254nm).

c)   METHYLATION

Methylation was accomplished with an ethereal solution
of diazomethane generated from the nitrosomethylurea
precursor, according to the method of Arndt (1943).   To
100ml of diethyl ether was added 30ml of 40% potassium
hydroxide and the mixture cooled to $4^{\circ}C$.   About 10g of
nitrosomethylurea was then added, in small portions over a
period of several minutes, with continual stirring.   The
deep yellow, ethereal solution was carefully decanted into
a clean flask.   The concentration of diazomethane was

approximately 2.8g per 100ml. The solution was stored at -20°C over pellets of potassium hydroxide to remove any residual water. The utmost care was taken in the preparation and use of this reagent, which is toxic and potentially explosive.

A slight excess of the diazomethane solution was added to each extract and the solution allowed to stand for about 20s. Excess reagent was then removed from the solution under a stream of nitrogen. Diethyl ether was used as a solvent to give a standard volume of 1.0ml before G.L.C.

d) QUANTITATIVE ANALYSIS OF ABSCISIC ACID BY GAS-LIQUID CHROMATOGRAPHY

Quantitative determination of ABA in the plant extracts was achieved by adaption of the electron capture gas chromatography technique described by Seeley and Powell (1970). The determinations were performed using a Perkin Elmer F17 gas chromatograph fitted with a Nickel-63 electron capture detector operated in the pulsed voltage mode.

Samples were chromatographed at 210°C on 1.5% w/w of FS-1265 (QF$_1$) supported on 60-80 mesh AW-DCMS Chromosorb W in a 5' x $\frac{1}{8}$" external diameter stainless steel column. The injector and detector temperatures were set at 275°C. In all cases the relative pulse width setting was 6, as determined from the optimization curve (Figure 3). Nitrogen at a flow rate of 60ml min^{-1} through the detector was used as a carrier; the inlet pressure was 300 KN/m^2. The G.L.C. column was obtained from Phase Separations Ltd. and conditioned for 48h at 220°C before use.

Percentage peak area
(relative to the maximum)
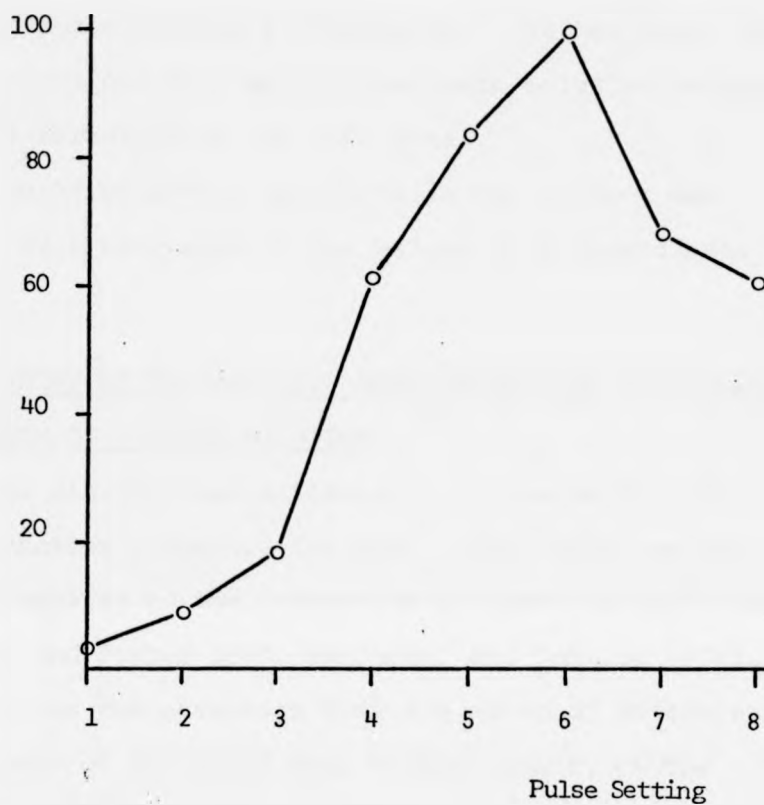
Figure 3:   Optimization curve for the electron capture detector

obtained by injecting a fixed amount of methyl abscisate

and vary ing the pulse setting.

Linearity of the detector response was established using standard solutions of the abscisic acid methyl ester. Concentrations of the inhibitor from 4.58×10^{-11} g μl^{-1} to 4.58×10^{-9} g μl^{-1} were used to determine the detector response at pulse setting 6 (Figure 4). It was found that repeated injections of a methyl abscisate solution resulted in a $\pm 2.65\%$ variation in the peak area.

The amount of methyl abscisate in the extract was determined by a reference to the values of standard peak areas.

e)   ASSESSMENT OF THE ABSCISIC ACID EXTRACTION PROCEDURE
     BY MEANS OF ISOTOPE DILUTION

Isotope dilution was utilized to determine the efficiency of the extraction procedure for ABA. The technique has been previously applied to the extraction of plant metabolites (Rittenberg and Foster 1940, Bandurski and Schulze 1974). This method has the advantage that the error of determination is independent of the yield and, within limits, of the concentration of ABA in the extract.

It was necessary to correct for loss of counting efficiency due to chemical and/or colour quenching effects of the plant extracts on the radio labelled source. These effects were assessed by means of a quenching curve obtained by using the external counting mode of an Intertechnique SL3 liquid scintillation spectrometer.

In this mode a γ-emitter source (^{137}Cs) was placed close to the detection chamber and the emitted radiation counted with the scintillating solution contained in the sample. Differing amounts of plant pigment and/or water, methanol or acetone were used as samples to give a variable
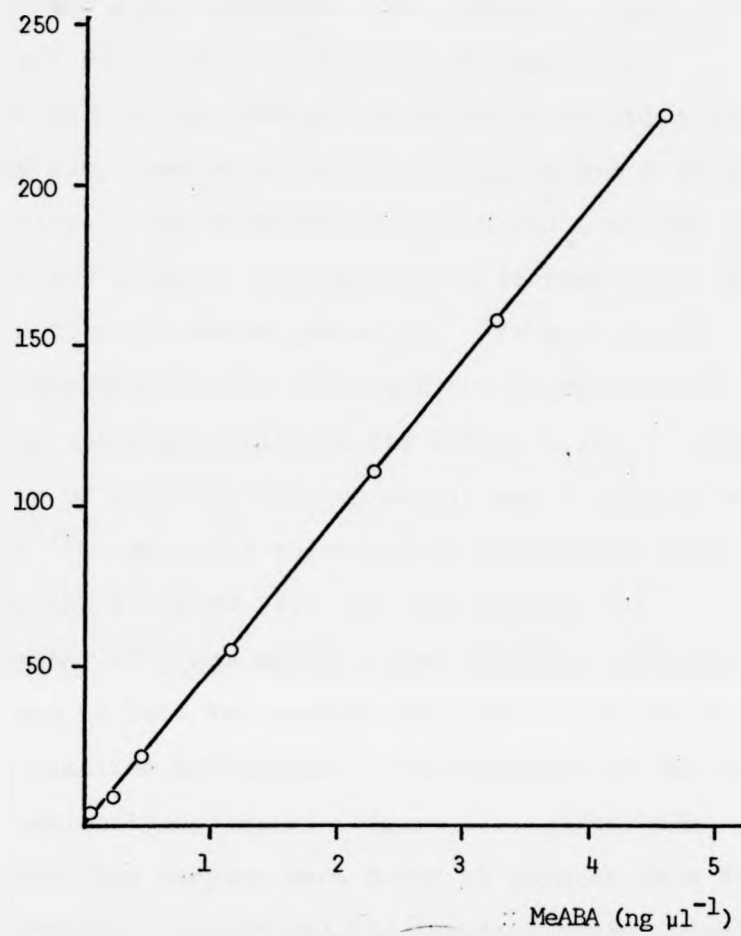
Figure 4: Relationship between the peak weight and amount of methyl abscisate injected at pulse setting 6.

quenching effect.   The quenching factor was mixed in a scintillation tube with 15ml of scintillator solution (4.4g PPO and 0.2g POPOP per litre toluene, Figure 5) and 0.1 μCi of $(\pm)$-ABA-2-^{14}C (5-10 mCi mmol^{-1}).

Counting of the external standard source plus sample or sample alone was effected in channels A and B of the spectrometer.   The standard ratio, ES_1/ES_2, of the count-rates for the external standard alone in channels A and B was a function of sample quenching.   It was possible to obtain a quenching curve (Figure 6) which represents the efficiency value according to the ratio ES_1/ES_2.   The efficiency of counting for any sample containing an unknown amount of ^{14}C-ABA could therefore be determined after computing the standard ratio of that sample.

$(\pm)$-ABA-2-^{14}C was added to the original methanol extract and to both the neutral and aqueous acidic phases prior to alkaline hydrolysis.   Purification of the extract was as previously described (Figure 1).   After ABA quantitation the samples were taken to dryness in a stream of dry nitrogen, counted and the standard ratio computed. By means of the quenching curve (Figure 6) the respective counting efficiencies were determined and the sample activities (dpm) calculated.

The percentage extraction of ABA by the isolation procedure could then be obtained:

$$\text{Percentage recovery} = \frac{\text{d.p.m. of extracted-ABA}}{\text{d.p.m. of added-ABA}} \times 100$$

This value enabled a quantitative determination of the amount of ABA in the plant extracts prior to fractionation.
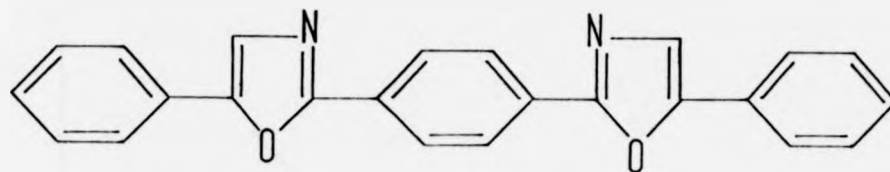
1,4-Bis-5-phenyloxazol-2-yl benzene (POPOP)
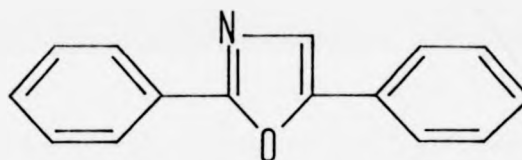
2,5-diphenyloxazole   (PPO)

Figure 5:   The chemical structures of the scintillators
utilized in the procedure.
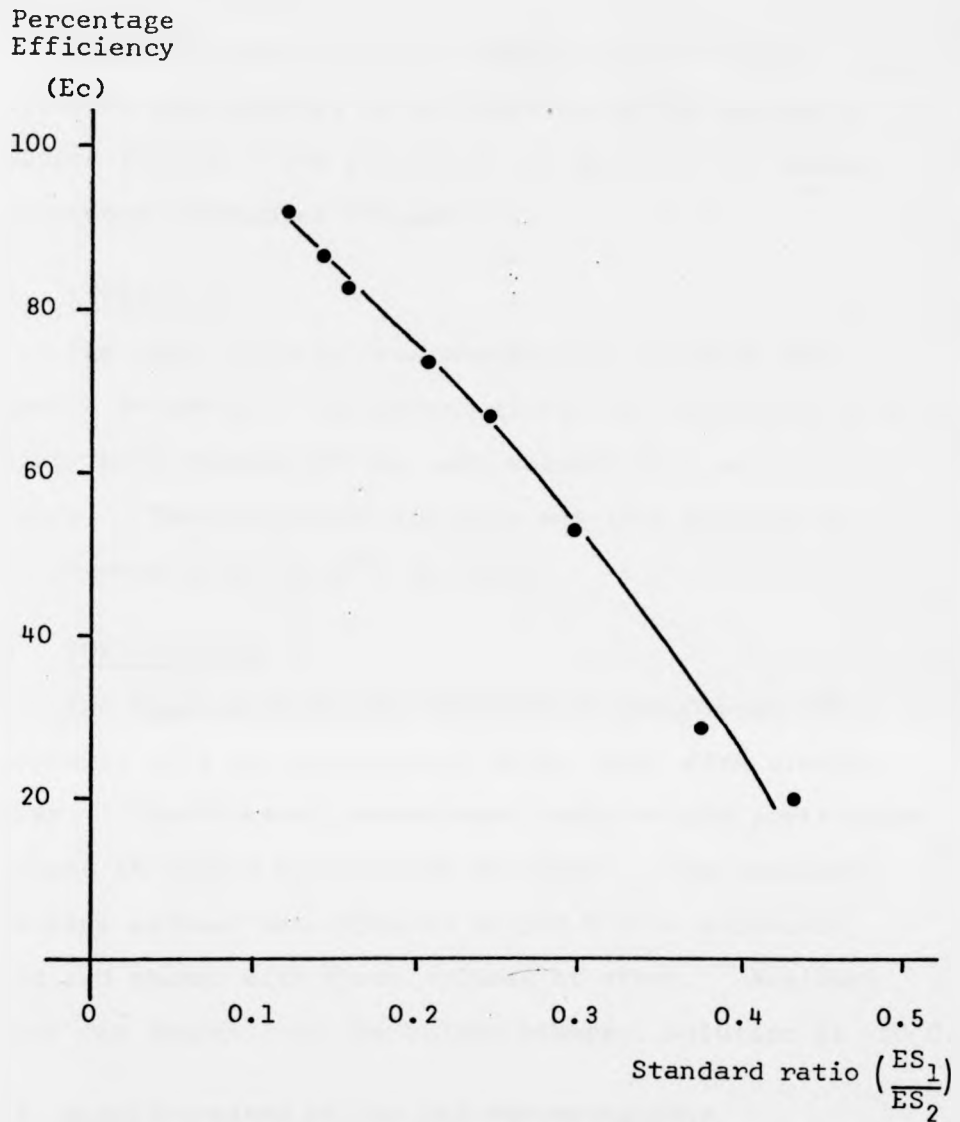
Figure 6:   Quenching curve for $(\pm)$-ABA-2-^{14}C $(0.1\mu Ci)$

obtained by using the external counting mode

(^{137}Cs) of an Intertechnique SL3 liquid

scintillation spectrometer.

β-INHIBITOR COMPLEX

The inhibitor-β mixture from the plant material was extracted and purified by an adaption of the method of Kefford (1954). The procedure was modified to remove extraneous substances (Figure 7).

a) EXTRACTION

The plant material was homogenised in cold, 80% aqueous methanol. The ground tissue was extracted at $4^{\circ}C$ using three changes of the same solvent over a period of 7 days. The methanolic solution was then reduced to the aqueous phase at $30^{\circ}C$ *in vacuo*.

b) PURIFICATION

The aqueous phase was adjusted to pH2.0 with 10M sulphuric acid and partitioned three times with diethyl ether. The ethereal phases were combined and partitioned against 5% sodium bicarbonate solution. The combined alkaline extract was adjusted to pH2.0 with sulphuric acid and shaken with three volumes of ether. Residual water was frozen from the bulked ethereal solution at $-20^{\circ}C$.

(i) Alkali-treated silica gel chromatography

According to the method of McCarthy and Duthie (1962). The ethereal solution from the previous procedure was applied as a small volume to a 14.5cm x 1.5cm diameter column of alkali-treated silica gel. 50ml of isopropanol containing approximately 50mg ml^{-1} potassium hydroxide was

GROUND PLANT MATERIAL (xg)

8x ml of 80% aqueous methanol
at $4^{o}C$ over 7 days

Methanolic extract

reduced in volume

Aqueous extract (pH 2.0)

3x ml ether

AQUEOUS   ORGANIC

3x ml 5% $NaHCO_3$

AQUEOUS (pH2.0)   ORGANIC

3x ml ether

AQUEOUS   ORGANIC

reduced in volume

ALKALI-TREATED SILICA
GEL CHROMATOGRAPHY

PAPER CHROMATOGRAPHY

Zone at R_f 0.5-0.7
eluted

INHIBITOR-β FRACTION

METHYLATION

MCFA QUANTITATION
(GLC)

Figure 7:   Flow chart representation of the procedure
used for the extraction and purification of the
inhibitor-β complex from plant material.

equilibrated with 30g of silica gel (100-200 mesh). The
isopropanol-KOH solution was obtained by refluxing
vigorously for 30min. 100ml diethyl ether were then
added to the mixture and the resulting slurry poured into
a 1.5cm diameter glass column employing a glass sinter
(porosity No 3) overlain with acid-washed sand as a support.
A fine layer of acid-washed sand was also placed on top of
the silica gel to prevent disruption. The extract was
applied after washing the column with 100ml diethyl ether.

Neutral components were eluted from the column with
100ml diethyl ether. The solvent flow rate was approximately
5ml min^{-1}. Free acidic compounds were then eluted with
100ml of 2% formic acid in diethyl ether (v/v), followed by
150ml of ether. The combined acid fraction was reduced
to a small volume (0.3ml) at 30°C *in vacuo*, prior to paper
chromatography.

(ii) Paper chromatography

The acid fraction was applied to a 10cm x 20cm sheet
of Whatman No 3MM chromatography paper as a narrow band
2cm from the base of the paper and 1cm from the sides.
Chromatograms were run according to the ascending method
in a glass tank maintained at room temperature. The
developing solvent was isopropanol-ammonia (0.88) -water
(10:1:1 by vol.) and this was allowed to run to a pencil
line 15cm from the origin. On completion the paper was
removed from the developing-tank and the zone between
R_f 0.5-0.7 inclusive was cut from the chromatogram. The
β-inhibitor complex was eluted from the adsorbent in a
Soxhlet by reflux with 20ml of methanol followed by diethyl
ether.

(iii) <u>Determination of medium-chain length fatty acids</u>

<u>in the β-inhibitor complex</u>

The solution of inhibitor-β was reduced to a small volume *in vacuo* and methylated using diazomethane reagent. Volatile fatty acids in the mixture were assessed by G.L.C. using a Perkin Elmer F11 gas chromatograph fitted with a flame ionisation detector.   Samples were chromatographed at $170^{\circ}C$ on 5% w/w of F.F.A.P. supported on Chromosorb G(AW-DMCS), 80-100 mesh, in a stainless steel column (6' x $\frac{1}{8}$" ed).

During operation of the chromatogram the gas pressures of air, hydrogen and nitrogen were all 140 KN/m^2.   The column-flow of the nitrogen carrier was 35ml min^{-1}.   The G.L.C. column was obtained from Phase Separations Ltd. and conditioned for 48h at $210^{\circ}C$ before use.   The quantities of methylated fatty acids in the plant extracts were determined by reference to standard values of the peak areas obtained with authentic samples.

MEDIUM-CHAIN LENGTH FATTY ACIDS

Fatty acids were extracted and purified using one of the following three procedures (A, B or C) which are summarised in Figures 8, 9, and 11, respectively.

a)   PROCEDURE A (Figure 8)

(i) Extraction and Solvent Partition

The plant material was homogenised in cold petroleum ether (B.P., 40°-60°C).   The ground tissue was extracted at 4°C using three changes of the same solvent over a period of 7 days.   The defatted material was then extracted at 4°C using three changes of methanol over a period of 7 days.   The methanolic solution was reduced at 30°C *in vacuo* and a volume of 5% sodium bicarbonate solution mixed with the residue.   The alkaline mixture was partitioned with three volumes of diethyl ether and the organic phase discarded.   The remaining aqueous phase was acidified to pH2.0 with 10M sulphuric acid.   This acidic solution was shaken three times with ether and the organic phase reduced at 30°C *in vacuo*.

(ii) Alkylated Sephadex LH-20 Chromatography

This was carried out as in the previous section for abscisic acid (Section b(i)).

(iii) Alkali-treated Silica gel Chromatography

According to the method of McCarthy and Duthie (1962).

Silica gel (100-200 mesh) (15g) was treated with 30ml of isopropanol containing approximately 50mg ml^{-1} of potassium hydroxide.   The isopropanol-KOH solution was obtained by vigorous reflux for 30min.   100ml of diethyl

GROUND PLANT MATERIAL (xg)

8x ml petroleum ether (40-60)
at 4°C over 7 days.

PETROLEUM
EXTRACT

DEFATTED TISSUE

8x ml methanol at 4°C
over 7 days

METHANOLIC EXTRACT

reduced in volume

AQUEOUS EXTRACT

2x ml 5% $NaHCO_3$

6x ml ether

AQUEOUS (pH 2.0)   ORGANIC

6x ml ether

AQUEOUS   ORGANIC

reduced in volume

SEPHADEX CHROMATOGRAPHY

ALKALI-TREATED SILICA
GEL CHROMATOGRAPHY

METHYLATION

MCFA QUANTITATION
(G.L.C.)

Figure 8:   Flow chart representation of a procedure
used (Method A) for the extraction and
purification of MCFA from plant material.

ether were then added to the mixture. The resulting
slurry was poured into a 1.5cm diameter glass column
employing a glass sinter (porosity No 3) overlain with
acid-washed sand as a support. A fine layer of acid-
washed sand was also placed on top of the silica gel to
prevent disruption. The resulting bed-dimensions were
7.5cm x 1.5cm. The plant extract was applied after
washing the column with 100ml diethyl ether.

The purified extract from the previous stage was
reduced to a small volume *in vacuo*. It was then taken up
with 1.0ml of diethyl ether and applied to the silica gel.
Neutral components were then eluted from the column with
100ml of the same solvent. The solvent flow rate was
approximately 5ml min^{-1}. Free acidic compounds were then
eluted with 50ml of 2% formic acid in diethyl ether (v/v),
followed by 100ml of ether. The combined acid fraction
was reduced in volume at $30^{o}C$ *in vacuo*, prior to esterifi-
cation and G.L.C. analysis.

b)   PROCEDURE B (Figure 9)

(i) Extraction and Solvent Partition

Plant material was homogenised in petroleum ether
(B.P., 40°-$60^{o}C$). The ground plant tissue was extracted
for 24h under reflux by means of a Soxhlet apparatus. The
defatted material was then extracted in a similar manner
with methanol. A volume of water was then added to the
alcoholic extract and the mixture reduced to the aqueous
phase at $30^{o}C$ *in vacuo*. The residue was diluted with water (to 2x ml)
and steam-distilled for three days with simultaneous

GROUND PLANT MATERIAL (xg)

4x ml petroleum ether
(40-60) over 24h.

PETROLEUM
EXTRACT

DEFATTED TISSUE

4x ml methanol until
extractant colourless

METHANOLIC EXTRACT

reduced in volume

AQUEOUS EXTRACT

steam distillation with
ether extraction for 3 days

4x ml 5% NaHCO$_3$

AQUEOUS (pH 2.0)   ORGANIC

6x ml ether

AQUEOUS   ORGANIC

reduced in volume

ALKALI-TREATED SILICA
GEL CHROMATOGRAPHY

METHYLATION

MCFA QUANTITATION
(G.L.C.)

Figure 9:   Flow chart representation of a procedure
            used (Method B) for the extraction and
            purification of MCFA from plant material.
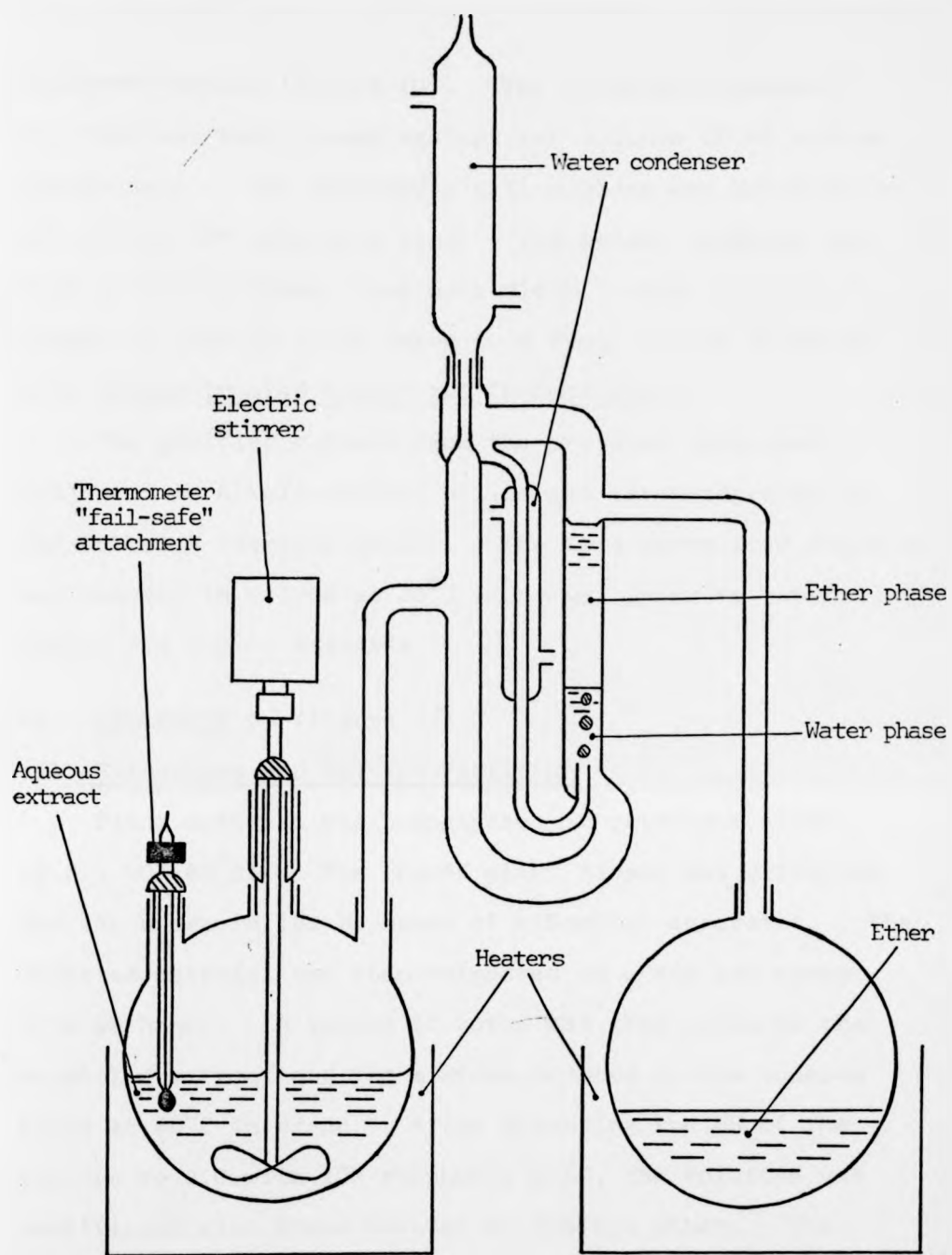
Figure 10: Apparatus used for the combined steam-distillation/
ether extraction of plant material (Method B).

ether-extraction (Figure 10). The resulting ethereal solution was partitioned against two volumes of 5% sodium bicarbonate. The combined alkaline phase was adjusted to pH2.0 with 10M sulphuric acid. The acidic solution was then extracted three times with diethyl ether and the resulting organic phase taken to a small volume *in vacuo*.

(ii) Alkali-treated Silica gel Chromatography

The purified extract from the previous stage was subjected to alkali-treated silica gel chromatography as for Method A (Section a(ii)). The free fatty acid fraction was reduced in volume at 30°C *in vacuo,* prior to esterification and G.L.C. analysis.

c) PROCEDURE C (Figure 11)

(i) Extraction and Solvent Partition

Plant material was homogenised in petroleum ether (B.P., 40°-60°C). The ground plant tissue was extracted . r 24h under reflux by means of a Soxhlet apparatus. The defatted material was then extracted in a similar manner with methanol. A volume of water was then added to the alcoholic extract and the mixture reduced to the aqueous phase at 30°C *in vacuo*. After adjusting the pH of the residue to 2.0 with 10M sulphuric acid, the solution was partitioned with three volumes of diethyl ether. The combined organic phase was then extracted three times with saturated sodium bicarbonate solution. The alkaline phase was taken to pH2.0 with 10M sulphuric acid and extracted with three volumes of ether. The resulting organic phase was then reduced to a small volume *in vacuo*.

GROUND PLANT MATERIAL (xg)

4x ml petroleum ether
(40-60) over 24h.

PETROLEUM   DEFATTED TISSUE
EXTRACT
                             4x ml methanol until
                             extractant colourless.

                    METHANOLIC EXTRACT

                             reduced in volume
                             x ml water added

                    AQUEOUS EXTRACT (pH 2.0)

                    3x ml ether

            ORGANIC   AQUEOUS

                    3x ml NaHCO$_3$ solution

            AQUEOUS (pH 2.0)   ORGANIC

                    3x ml ether

    AQUEOUS   ORGANIC

                             reduced in volume

                    ALKALI-TREATED SILICA
                    GEL CHROMATOGRAPHY

                    METHYLATION

                    MCFA QUANTITATION
                         (G.L.C.)

Figure 11:   Flow chart representation of a procedure
             used (Method C) for the extraction and
             purification of MCFA from plant material.

(ii) <u>Alkali-treated Silica gel Chromatography</u>

The purified extract from the previous stage was subjected to alkali-treated silica gel chromatography as for Method A (Section a(ii)). The free fatty acid fraction was reduced in volume at 30°C *in vacuo*, prior to esterification and G.L.C. analysis.

d) <u>METHYLATION</u>

Esterification of the purified free fatty acid fractions was carried out as described in Section (c) of the ABA procedure.

e) <u>QUANTITATIVE ANALYSIS OF MEDIUM-CHAIN LENGTH FATTY ACIDS BY GAS-LIQUID CHROMATOGRAPHY</u>

Volatile fatty acids were assessed using a Perkin Elmer F17 gas chromatograph fitted with a flame ionisation detector. Samples were chromatographed at 135°C on 5% w/w of F.F.A.P. supported on Chromosorb G(AW-DMCS), 80-100 mesh, in a stainless steel column (6'x$\frac{1}{8}$" ed). During operation of the chromatogram the gas pressures of air, hydrogen and nitrogen were respectively 165, 100 and 140 KN/m^2. The column-flow of the nitrogen carrier was 35ml min^{-1} and the injection temperature 175°C. The G.L.C. column was obtained from Phase Separations Ltd. and conditioned for 48h at 210°C before use. The quantities of methylated fatty acids in the plant extracts were determined by reference to standard values of the peak areas obtained with authentic samples. Repeated injections of a standard methyl nonanoate solution resulted in a variation of $\pm$5.4% in the peak areas. It had been established that

the F.I.D. showed a linearity of response to individual
fatty esters, acetate to tetradecanoate inclusive, over a
range of injected concentrations (Alam, private communication).

f)   ASSESSMENT OF THE EXTRACTION PROCEDURE FOR MEDIUM-CHAIN
     LENGTH FATTY ACIDS BY MEANS OF ISOTOPE DILUTION

Losses for nonanoic acid which occurred during the
extraction and purification procedures were determined by
isotope dilution.

As in the assessment of the ABA extraction procedure
(ABA Section (e)) it was necessary to correct for loss of
counting efficiency due to chemical and/or colour quenching
of the plant extracts on the radiolabelled source.   These
effects were assessed by means of a quenching curve
obtained by using the external counting mode of an
Intertechnique SL3 liquid scintillation spectrometer.

The quenching curve was determined in a similar manner
to that for ABA (ABA Section (e));   0.1 µCi of $1-^{14}C$
nonanoic acid (5-20 mCi $mmol^{-1}$) provided the radiolabelled
source.   By using the obtained relationship (Figure 12)
the efficiency of counting for any sample containing an
unknown amount of ^{14}C-nonanoic acid could be determined
after computing the standard ratio of that sample.

$1-^{14}C$-nonanoic acid (0.1 µCi) was added to the original
methanolic extract of the plant material.   Fractionation
of the extract was carried out by either Method A, B or C
(Figures 8, 9 and 11, respectively).   After MCFA
quantitation the samples were reduced to almost dryness in
a stream of dry nitrogen, counted and the standard ratio
computed.   By means of the quenching curve (Figure 12) the

(ii) <u>Alkali-treated Silica gel Chromatography</u>

The purified extract from the previous stage was
subjected to alkali-treated silica gel chromatography as
for Method A (Section a(ii)). The free fatty acid
fraction was reduced in volume at 30°C *in vacuo*, prior to
esterification and G.L.C. analysis.

d) <u>METHYLATION</u>

Esterification of the purified free fatty acid
fractions was carried out as described in Section (c)
of the ABA procedure.

e) <u>QUANTITATIVE ANALYSIS OF MEDIUM-CHAIN LENGTH FATTY</u>
<u>ACIDS BY GAS-LIQUID CHROMATOGRAPHY</u>

Volatile fatty acids were assessed using a Perkin
Elmer F17 gas chromatograph fitted with a flame ionisation
detector.   Samples were chromatographed at 135°C on 5% w/w
of F.F.A.P. supported on Chromosorb G(AW-DMCS), 80-100 mesh,
in a stainless steel column (6'x$\frac{1}{8}$" ed).   During operation
of the chromatogram the gas pressures of air, hydrogen and
nitrogen were respectively 165, 100 and 140 KN/m^2.   The
column-flow of the nitrogen carrier was 35ml min^{-1} and the
injection temperature 175°C.   The G.L.C. column was
obtained from Phase Separations Ltd. and conditioned for
48h at 210°C before use.   The quantities of methylated
fatty acids in the plant extracts were determined by
reference to standard values of the peak areas obtained
with authentic samples.   Repeated injections of a standard
methyl nonanoate solution resulted in a variation of
$\pm$5.4% in the peak areas.   It had been established that

the F.I.D. showed a linearity of response to individual
fatty esters, acetate to tetradecanoate inclusive, over a
range of injected concentrations (Alam, private communication).

f) ASSESSMENT OF THE EXTRACTION PROCEDURE FOR MEDIUM-CHAIN
    LENGTH FATTY ACIDS BY MEANS OF ISOTOPE DILUTION

Losses for nonanoic acid which occurred during the
extraction and purification procedures were determined by
isotope dilution.

As in the assessment of the ABA extraction procedure
(ABA Section (e)) it was necessary to correct for loss of
counting efficiency due to chemical and/or colour quenching
of the plant extracts on the radiolabelled source. These
effects were assessed by means of a quenching curve
obtained by using the external counting mode of an
Intertechnique SL3 liquid scintillation spectrometer.

The quenching curve was determined in a similar manner
to that for ABA (ABA Section (e)); 0.1 µCi of $1-^{14}C$
nonanoic acid (5-20 mCi $mmol^{-1}$) provided the radiolabelled
source. By using the obtained relationship (Figure 12)
the efficiency of counting for any sample containing an
unknown amount of ^{14}C-nonanoic acid could be determined
after computing the standard ratio of that sample.

$1-^{14}C$-nonanoic acid (0.1 µCi) was added to the original
methanolic extract of the plant material. Fractionation
of the extract was carried out by either Method A, B or C
(Figures 8, 9 and 11, respectively). After MCFA
quantitation the samples were reduced to almost dryness in
a stream of dry nitrogen, counted and the standard ratio
computed. By means of the quenching curve (Figure 12) the

Figure 12: Quenching curve for 1-^{14}C-nonanoic acid (0.1μCi) obtained by using the external counting mode (^{137}Cs) of an Intertechnique SL3 liquid scintillation spectrometer.

respective counting efficiencies were determined and the sample activities (dpm) calculated.

The percentage extraction of nonanoic acid by either procedure could be estimated.

$$\text{Percentage recovery} = \frac{\text{d.p.m. of extracted-}^{14}\text{C}_9}{\text{d.p.m. of added-}^{14}\text{C}_9} \times 100$$

These values enabled a quantitative determination of nonanoic acid in plant extracts prior to fractionation.

g) ASSESSMENT OF THE EXTRACTION PROCEDURE FOR MEDIUM-
    CHAIN LENGTH FATTY ACIDS BY MEANS OF A STANDARD
    SOLUTION

Losses of fatty acids which occurred during the
extraction and purification techniques for procedure C
(section c) were determined using a standard solution
containing known amounts of the acids.   Aliphatic acids
in the series hexanoic to decanoic were utilized in diethyl
ether.   Although this method is not as precise as isotope
dilution, it has the advantage that the percentage
recoveries of individual acids can be determined
simultaneously and without having to use expensive radio-
labelled compounds.

1.0ml aliquots of the standard solution were added to
water and worked up by the purification procedure for
method C (Figure 11).   After methylation of the final
acidic fraction the samples were made up to a volume of
1.0ml with ether.   1.0μl aliquots were assessed for fatty
acid content by means of G.L.C., as described in section (e).
Subsequent comparison of the chromatograms obtained from
the respective samples (either standard or fractionated
stock solution) enabled a calculation of the extraction
efficiency for each fatty acid.

$$\text{Percentage Recovery} = \frac{\text{Amount of assessed acid in purified fraction}}{\text{Amount of assessed acid in standard solution}} \times 100$$

PART 7.   COMBINED GAS CHROMATOGRAPHY - MASS SPECTROMETRY

OF ENDOGENOUS PLANT GROWTH SUBSTANCES

Confirmation of the tentative identification by G.L.C. of abscisic acid and medium-chain length fatty acids extracted from plant material was obtained using a combined gas-liquid chromatograph-mass spectrometer (G.C.M.S.).

The mass spectrometer employed a JEOL D100 single beam, double focusing instrument coupled to a JEOL Chromatograph, Model Number JGC 20K.   A two-stage jet-separator combined enrichment of the organic compound in the gas effluent entering the mass spectrometer, with a reduction in pressure at the GLC-MS interface.   Such a pressure drop is required because of the vast difference in operating pressures between the gas-liquid chromatograph (1 atmosphere) and the mass spectrometer (10^{-7} atmos at the ion-source).

For all operations the ion-source temperature was $140^{o}C$ with a separator temperature of $150^{o}C$ and an ionising voltage of 75eV.   The total ion-current was used to indicate the output from the G.L.C. and was displayed by a pen-recorder. The carrier gas, helium, was set for optimum flow (10ml min^{-1}) as monitored at the ion-source.

Mass spectral analyses of components in the plant extracts were accomplished by subjecting the methylated samples to gas-liquid chromatography and scanning the resulting peaks from 0-800 amu in 2 sec with the mass spectro-meter.   The peaks in the chromatograms which corresponded to known retention times of the authentic methylated acids, under identical conditions, were subjected to a full scan

to unequivocally identify the compounds.

The C.L.C. for abscisic acid utilised a stainless steel column (5' x $\frac{1}{8}$" ed) packed with 2.5% w/w of O.V.-1 Silicone supported on 80-100 mesh Chromosorb G (AW-DCMS). The oven temperature was 180°C with an injection temperature of 225°C. For the medium-chain length fatty acids the column was stainless steel (6' x $\frac{1}{8}$" ed) packed with 5% w/w of F.F.A.P. supported on 80-100 mesh Chromosorb G(AW-DCMS). The oven temperature in this case was 115°C with an injection temperature of 150°C.

CHAPTER III

RESULTS

RESULTS

PART 1: THE CHARACTERISATION OF PUTATIVE PLANT-GROWTH
REGULATORS FROM DORMANT SEED OF *AVENA* SPP.

Abscisic acid (ABA) was extracted from various plant
material by the procedure outlined in Figure 1. The
presence of methyl abscisate in the methylated extracts
from dormant seed of *A. sativa* cv Selma and *A. fatua* was
confirmed by gas chromatography-mass spectrometric
determination and by gas-liquid chromatographic comparison
against an authentic sample of the methylated racemic
acid. Peaks from the methylated plant extracts which
had the same retention times as, and which co-chromatographed
with, the authentic ABA derivatives on a stationary
phase of 2.5 percent OV-1 were examined by G.C.M.S.

In both species, the six most abundant ions in the
mass spectrum of the isolated derivative were comparable
with that obtained from methyl abscisate under identical
conditions (Table 3). The mass spectra were also in
good agreement with previously published spectra of the Me
ABA (Most *et al* 1970, Gray *et al.* 1974, Rivier *et al.* 1977).
Peaks from the methylated oat seed extracts were also
found to co-chromatograph with a *cis,trans/trans,trans-*
mixture of racemic methyl abscisate using a stationary
phase of 1.5 percent FS-1265, under the same conditions
(Figure 13). The 2-*cis-4-trans-* and 2-*trans-4-trans-*
isomers of the derivatised inhibitor were separated
under these conditions (Seeley and Powell 1970).

Table 3: The six most abundant ions (with m/e >41) in
the mass spectrum of *cis, trans*-methyl abscisate
extracted from dormant seeds of *Avena fatua* and
Avena sativa.

Ionising voltage 75eV.

Mass to Charge Ratio	I N T E N S I T I E S		
	A. fatua	*A. sativa*	Standard
91	25.9	26.7	16.5
125	14.4	31.5	29.4
134	32.3	36.8	31.6
162	34.7	36.8	34.3
190	100.0	100.0	100.0
191	11.9	15.3	16.2

Mass to Charge Ratio	Gray *et al* (1974) MeABA	Most *et al* (1970) MeABA	Rivier *et al* (1977) MeABA
91	19	14	7
125	34	28	28
134	42	24	26
162	41	25	29
190	100	100	100
191		10	7
260	17 (eV 70)		(eV 50)

Figure 13: Gas-liquid chromatogram of the 2-*cis*-4-*trans*-and 2-*trans*-4-*trans*- isomers of methyl abscisate.

Injection A comprised 1.0μl of a solution of Me-ABA extracted from dormant *Avena fatua*.

Injection B comprised 1.0μl of a standard solution of Me-ABA (91.2 pg/μl).

Stationary phase: 1.5% FS-1265 at 210°C

Figure 13:
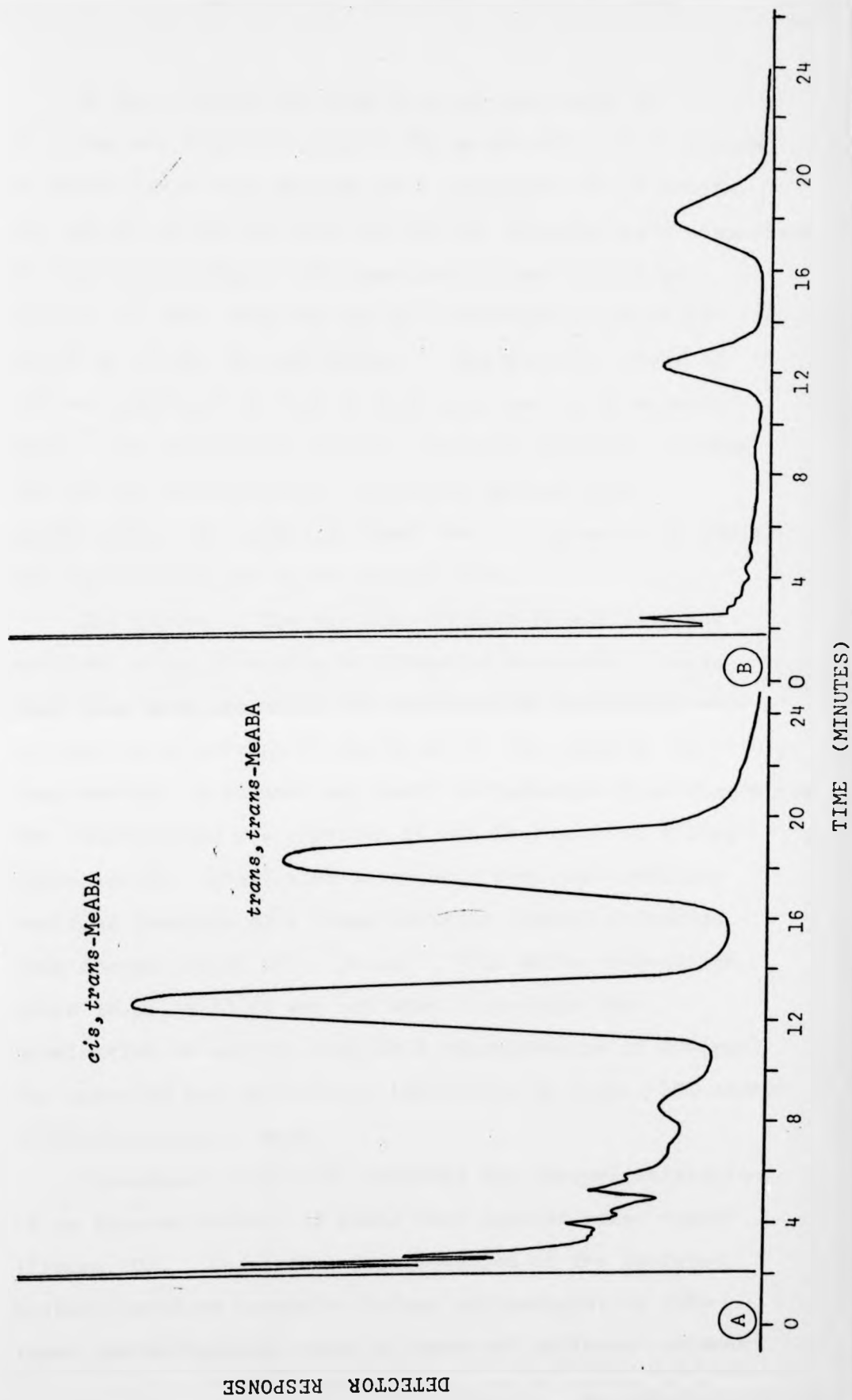
An ABA extract isolated from dormant seeds of
A. fatua was found to prevent the germination of *L. sativa*
cv Great Lakes when applied at a concentration of 500ppm.
The amount of ABA in this mixture was assessed by a comparison
of the intensities of the base peak at m/e 190 in an
aliquot of this fraction and in a standard solution of
MeABA by single ion monitoring.   The putative level of
ABA was assessed at 20μg of free acid per kg of dormant
seed.   By comparative bioassay between authentic (±)-ABA
and the oat seed extract, utilising lettuce seed
germination, the inhibitor level was estimated to be 130μg
ABA equivalents per kg of dormant seed.

The nature of the residual inhibitory activity was
realised after 18 months of intensive research.   During
this time many isolation and purification procedures were
invoked in an attempt to characterise the unknown inhibitory
component(s) in dormant oat seed. An indication of the character of
the inhibitor(s) was provided by the isolation of a long-
chain length, unsaturated fatty acid from the partially
purified fraction of a steam-volatile mixture extracted
from dormant seeds of *A. fatua*.   This white crystalline
solid (M.p. 52-53°C) was not able to prevent the
germination of lettuce seed at a concentration of 1000ppm.
The compound was tentatively identified as *trans* - 10, *trans*-
12-octadecadienoic acid.

Subsequent isolations involved the steam-distillation
of an aqueous extract of *Avena* seed against ether vapour
(Figure 10).   Sequential fractionation of the isolated
mixture involved extensive column and preparative thin-
layer chromatography, using a number of different solvent

systems.   Fractions which displayed the greatest biological activity, as monitored by a simple lettuce seed germination test, were then assessed for free fatty acid content. Both saturated and unsaturated fatty acids with carbon backbone numbers of between six and eighteen atoms were detected by GLC against authentic standards.   Acids in the series hexanoic to decanoic, inclusive, were determined to possess marked biological activity.

As a result of this work three isolation procedures were devised to extract and characterise medium-chain length fatty acids from plant material.   The procedures are summarised in Figures 8, 9 and 11.   Unequivocal identification of MCFA in the methylated extracts from dormant seeds of *A. sativa* cv Selma and *A. fatua* were obtained by GCMS determination and GLC comparison against authentic samples of the methyl fatty derivatives.

Components from the methylated plant extracts which had the same retention times as, and which co-chromatographed with, authentic methylated fatty acids, in the series hexanoic to decanoic, on a stationary phase of 5 percent FFAP were examined by GCMS.   In both species, the eight most abundant ions in the mass spectra of the isolated derivatives were in good agreement with those obtained with the authentic methyl esters under identical conditions (Table 4).

The base peak for each derivative was identified at m/e 74 and in each case the respective molecular ion was evident.   A base peak of m/e 74 is characteristic of saturated, straight-chain methyl esters in the series

Table 4: The eight most abundant ions (with m/e >40) in the mass spectrums of methylated medium-chain length fatty acids.

METHYL HEXANOATE

m/e	INTENSITIES		
	A. sativa	A. fatua	Standards
41	16.8	20.9	17.7
43	46.5	36.5	40.3
55	12.1	13.9	12.2
59	17.4	14.4	19.1
71	11.5	11.5	10.0
74	100.0	100.0	100.0
87	31.6	31.0	39.1
99	25.0	21.5	20.9

METHYL HEPTANOATE

m/e	INTENSITIES		
	A. sativa	A. fatua	Standards
41	17.8	20.5	17.6
43	33.0	35.6	32.2
55	16.1	15.1	13.0
59	14.8	15.7	13.3
74	100.0	100.0	100.0
87	36.5	31.1	33.9
101	14.2	13.4	13.3
113	17.8	14.5	14.6

METHYL OCTANOATE

m/e	INTENSITIES		
	A. sativa	A. fatua	Standards
41	16.1	21.4	17.8
43	23.9	22.4	21.4
55	16.8	16.8	15.4
57	14.3	16.2	15.0
59	11.4	12.0	11.0
74	100.0	100.0	100.0
87	44.1	38.5	37.8
127	15.1	11.6	11.5

METHYL NONANOATE

m/e	INTENSITIES		
	A. sativa	A. fatua	Standards
41	16.1	20.7	20.7
43	18.1	22.0	22.5
55	15.4	18.6	17.1
59	8.8	10.0	10.3
74	100.0	100.0	100.0
87	44.1	42.6	43.3
129	12.6	9.4	9.6
141	13.4	9.8	10.3

METHYL DECANOATE

m/e	INTENSITIES		
	A. sativa	A. fatua	Standards
41	26.2	20.8	21.7
43	24.8	23.1	23.5
55	22.8	19.8	17.9
69	11.8	10.1	9.2
74	100.0	100.0	100.0
87	52.8	46.9	49.1
143	20.7	14.4	15.5
155	10.5	10.0	9.2

C_6-C_{26} carbon backbone numbers. It arises by a McLafferty rearrangement through β-cleavage and the transfer of a γ-hydrogen atom, resulting in the formation of an enol ion with m/e 74. Components from the methylated oat seed extracts were also found to co-chromatograph with the authentic methyl fatty esters (C_6-C_{10}) using a 5 percent FFAP stationary phase and identical conditions (Figure 14).

Additional biological activity was also detected in the neutral fractions isolated from dormant seeds of the cultivated oat (Lots 1 and 2) by the procedures outlined in Figures 9 and 11. Thin-layer chromatography of this complex mixture resulted in the detection of biological activity associated with a specific area of the chromatogram and visualised under UV light (254nm). The degree of activity was assessed by inhibition of lettuce seed germination. A combination of preparative thin-layer and micro-column chromatography, using silica gel and several different solvent systems, resulted in the isolation of a partially-purified inhibitory mixture. As a final purification step the mixture was chromatographed on a 2mm layer of silica gel GF_{254} (Merck) using a developing system of benzene-ethyl acetate-methanol (50:5:2). The inhibitory area of the chromatogram as visualised in UV light (254nm) was found to lie between R_f 0.4-0.5. The components were eluted from the adsorbent with diethyl ether.

This fraction was markedly inhibitory to the germination of *L. sativa* cv Great Lakes when applied at a concentration of 500ppm (Table 5). However,

Figure 14:   Gas-liquid chromatogram of the methyl esters
              of medium-chain length fatty acids.

Injection A comprised 1.0μl of an ethereal extract of
dormant seed of *Avena sativa* cv Selma.

Injection B comprised 1.0μl of an ethereal solution of
the standard MCFA esters.

Stationary phase: 5% FFAP at 173°C.

DETECTOR RESPONSE

TIME (MINUTES)
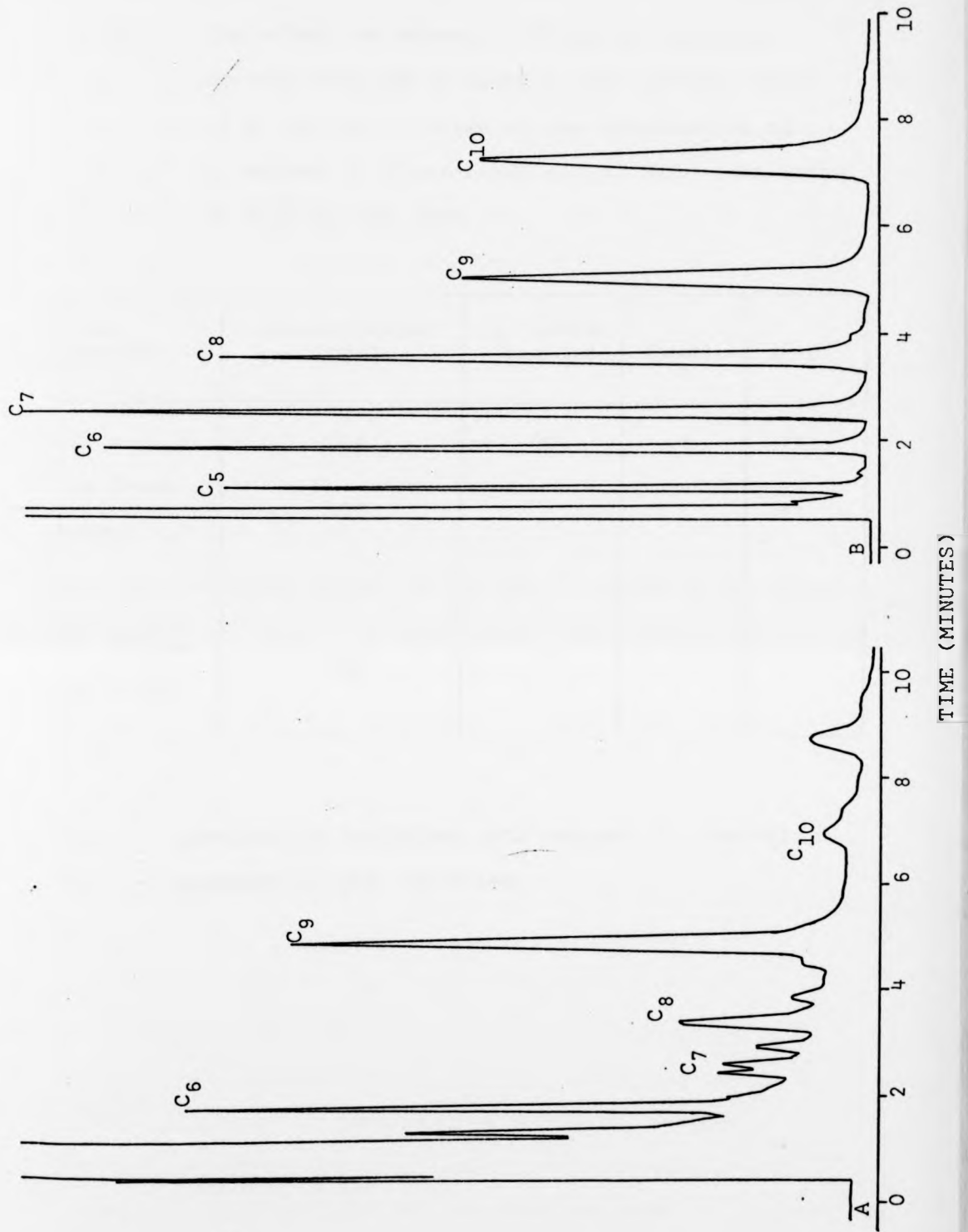
Table 5: The effect of phenol, 2,4-dichlorophenoxy
acetic acid and an extract from dormant seeds
of *A. sativa* cv Selma on the germination of
L. sativa cv Great Lakes and *A. sativa* cv Selma
at 21°C in the dark.

Seed Species	Concentration (ppm)	*A. sativa* extract	Phenol	2,4-D
L. sativa cv Great Lakes	500	††	††	††
	100	-	-	††
A. sativa cv Selma	200	-	-	-

††, - germination inhibited with respect to control.

-, - germination not inhibited.

at a concentration of 200ppm and 100ppm the extract did
not prevent the emergence of commercial oat and lettuce
seed, respectively (Table 5).   The infrared spectrum
(thin film) of the mixture had strong absorption bands
at 3540 cm^{-1} (hydroxyl), 1750 cm^{-1} (carbonyl), 1610 and
1510 cm^{-1} (aromatic) and 1265 and 1120 cm^{-1} (ester).
The ultraviolet spectrum was found to be sensitive to pH.
In neutral ethanol, maxima were detected at 211nm
(primary band) and 261nm (secondary band) assigned to
the phenol chromophore.   In weakly alkaline ethanolic
solution the secondary band was shifted to 288nm.   The
data suggested the presence of ester linkages and that
the components may be phenolic in nature.

A speculative identification of the inhibitory
compounds isolated from the neutral fraction extracted
from dormant *A. sativa* seed was obtained by GCMS
determination and GLC comparison.. Components in the plant
extract were examined by GCMS; the presence of phenol
and possible polychlorinated phenols were confirmed in
the inhibitory fraction (Table 6).   Subsequent
investigations utilising both GLC and TLC, in conjunction
with a lettuce seed germination test, supported the
characterisation of phenol but could not identify the
halogenated compounds.   Peaks from the methylated
extract were found to co-chromatograph with an authentic
sample of phenol but not with an authentic sample of
methyl 2,4-dichlorophenoxyacetate, using a stationary
phase of 5 percent FFAP and identical conditions (Figure
15).   Phenol was assessed as the principal component of
the inhibitory mixture by using a combination of GLC

TABLE 6:   THE EIGHT MOST ABUNDANT IONS (WITH M/E >40)

IN THE MASS SPECTRUM OF PHENOL AND METHYL

2, 4-DICHLORO-PHENOXY ACETATE

Chemical	Mass to charge ratio	Oat Extract-intensity	Mass to charge ratio	Standard-intensity
Phenol	38	8	38	21
	39	28	39	60
	40	9	40	27
	55	9	55	14
	63	9	63	15
	65	24	65	48
	66	43	66	83
	94	100	94	100
Me-2, 4-D	41	61	45	64
	43	47	111	46
	55	36	145	49
	171	37	175	76
	205	100	177	47
	206	51	199	100
	207	69	234	76
	208	36	236	49
	234	51		
	236	32		

Figure 15:   Gas-liquid chromatogram of a biologically-
active extract from dormant *Avena sativa* cv
Selma seeds.

Injection A comprised 1·0μl of an ethereal solution of the
extract.

Injection B comprised a mixture of solutions of phenol and
methylated 2,4-dichlorophenoxy acetic acid.

Stationary phase: 5% FFAP at 201°C
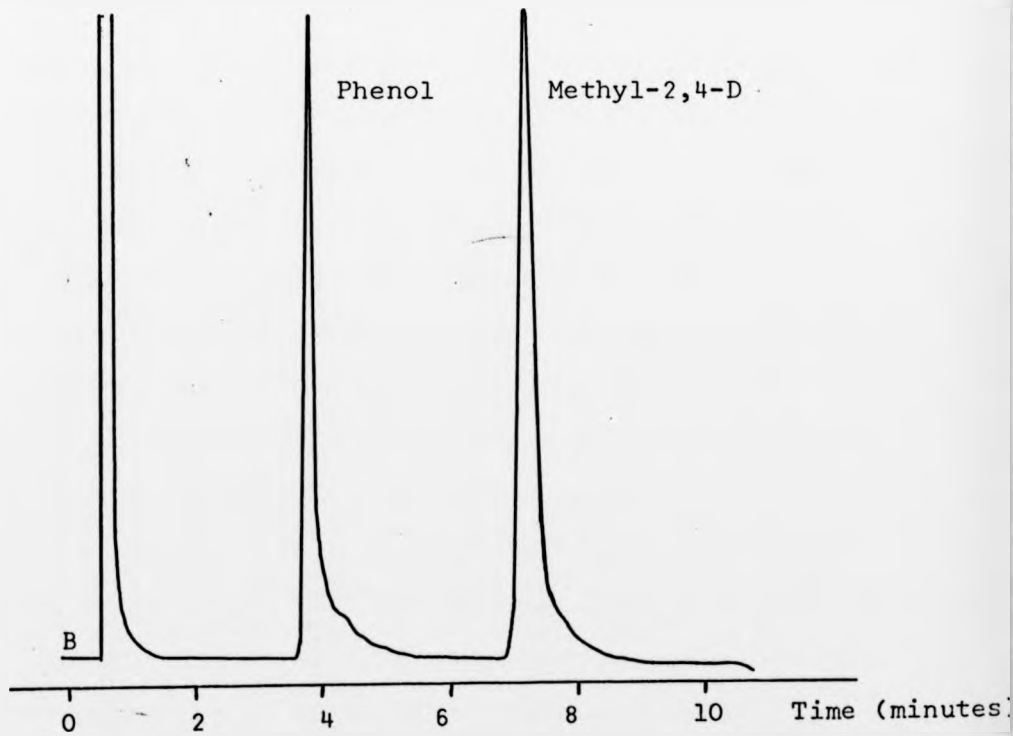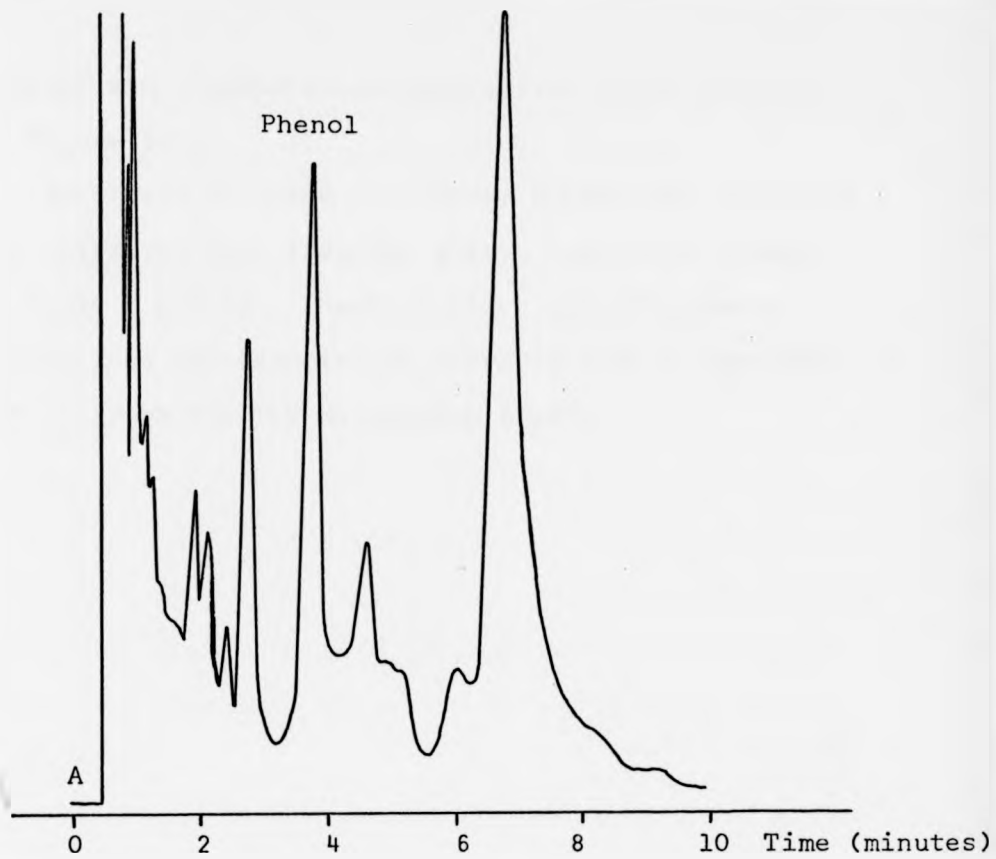
analysis and a comparative germination titre (Table 5 and Figure 15).

It should be noted that these inhibitory compounds were extracted only from the gifted samples of common oat (Lots 1 and 2). Such putative polychlorinated phenols were not detected in extracts from *A. sativa* (Lot 3) grown for the maturation study.

PART 2:   THE EFFECT OF MEDIUM-CHAIN LENGTH FATTY ACIDS ON

THE INHIBITION OF SEED GERMINATION

When buffered to pH 8.0 sodium salts of saturated
aliphatic acids with a chain length between 7-10 carbon atoms
were found to be potent inhibitors of germination.   These
acids significantly inhibited the germination of commercial
and wild oat (Lots 1), lettuce (cultivar Great Lakes), rape,
mustard and radish seed when applied at a concentration of
5×10^{-3}M (Table 7).   The degree of inhibition and acid
specificity was found to vary markedly between species.
In general, nonanoate was the most biologically active
treatment.

The longer fatty acids, both saturated and unsaturated,
with chain lengths of 13-18 carbon atoms were not
significantly inhibitory to the germination of these species
when applied as the sodium salt buffered to pH 8.0 (Table 7).

The effect of various concentrations of free MCFA
(C_5-C_{12}) on the germination of $L.$ $sativa$ cv Great Lakes
(Lot 1) at 20°C in the dark is shown in Figure 16.   It is
clear that in this experiment acids inducing the greatest
degree of inhibition occurred in the series hexanoic to
decanoic, inclusive.   Concentrations of the respective
acids between 1.5mM and 3.3mM were required to reduce the
seed germination by 50 percent with respect to a water
control after 24h at 20°C in the dark (Table 8 and Figure 17).

The relative pattern of inhibition was interesting.
The concentration of fatty acid required to reduce
germination by the designated amount was gradually decreased

Table 7: The effect of 5×10^{-3}M saturated aliphatic acids in the series butanoic (C_4) to octadecanoic (C_{18}) on the germination of various species at 20°C in the dark.

Fatty Acid	Percentage Germinations ($\pm$ s.e.m.)					
	Wild Oat	Common Oat	Radish	Mustard	Lettuce	Rape
Control	60$\pm$4.6	70$\pm$4.2	74$\pm$1.8	82$\pm$5.0	92$\pm$1.8	62$\pm$1.4
C_4	68$\pm$2.4	72$\pm$9.0	54$\pm$5.2	48$\pm$5.4	92$\pm$3.0	48$\pm$5.0
C_5	52$\pm$2.2	70$\pm$7.0	56$\pm$5.0	44$\pm$6.0	86$\pm$3.4	46$\pm$4.8
C_6	52$\pm$5.8	62$\pm$9.6	58$\pm$8.8	40$\pm$9.2	88$\pm$2.8	66$\pm$1.4
C_7	40$\pm$4.0	60$\pm$4.4	54$\pm$2.4	12$\pm$4.8	76$\pm$1.8	50$\pm$3.6
C_8	26$\pm$3.6	32$\pm$8.6	52$\pm$4.0	8$\pm$1.4	74$\pm$3.4	36$\pm$2.0
C_9	8$\pm$2.8	18$\pm$0.6	36$\pm$9.2	4$\pm$0.6	62$\pm$4.6	52$\pm$3.4
C_{10}	6$\pm$4.6	20$\pm$2.0	68$\pm$6.6	22$\pm$5.4	66$\pm$4.4	52$\pm$6.0
C_{11}	8$\pm$2.0	34$\pm$4.2	68$\pm$1.2	64$\pm$7.6	86$\pm$1.4	54$\pm$7.0
C_{12}	8$\pm$3.6	48$\pm$16.8	68$\pm$6.4	72$\pm$1.8	86$\pm$4.0	64$\pm$2.2
C_{13}	58$\pm$0.6	68$\pm$8.4	70$\pm$2.2	82$\pm$3.6	96$\pm$0.6	68$\pm$6.4
C_{14}	54$\pm$5.4	78$\pm$6.4	72$\pm$3.0	82$\pm$1.4	96$\pm$0.6	66$\pm$1.4
C_{15}	50$\pm$0.6	88$\pm$1.4	70$\pm$4.2	84$\pm$1.8	88$\pm$1.4	52$\pm$4.4
C_{16}	58$\pm$0.6	90$\pm$2.6	74$\pm$2.8	78$\pm$2.4	90$\pm$2.2	70$\pm$5.0
C_{17}	50$\pm$4.0	88$\pm$1.8	74$\pm$4.0	82$\pm$3.6	84$\pm$5.0	78$\pm$1.4
C_{18}	58$\pm$3.6	84$\pm$3.4	82$\pm$3.6	80$\pm$1.4	80$\pm$2.4	72$\pm$1.8

Before scoring germination the treatments (3 replicates) were imbibed for 72h, 24h, 42h, 28h, and 48h for oat, radish, mustard, lettuce and rape seed, respectively.

C_4-C_{14} acids were applied as the sodium salt in aqueous solution buffered to pH 8.0. C_{15}-C_{18} acids were applied in diethyl ether and after solvent evaporation the required volume of water (pH 8.0) was added to give the desired nominal concentration in the dish.
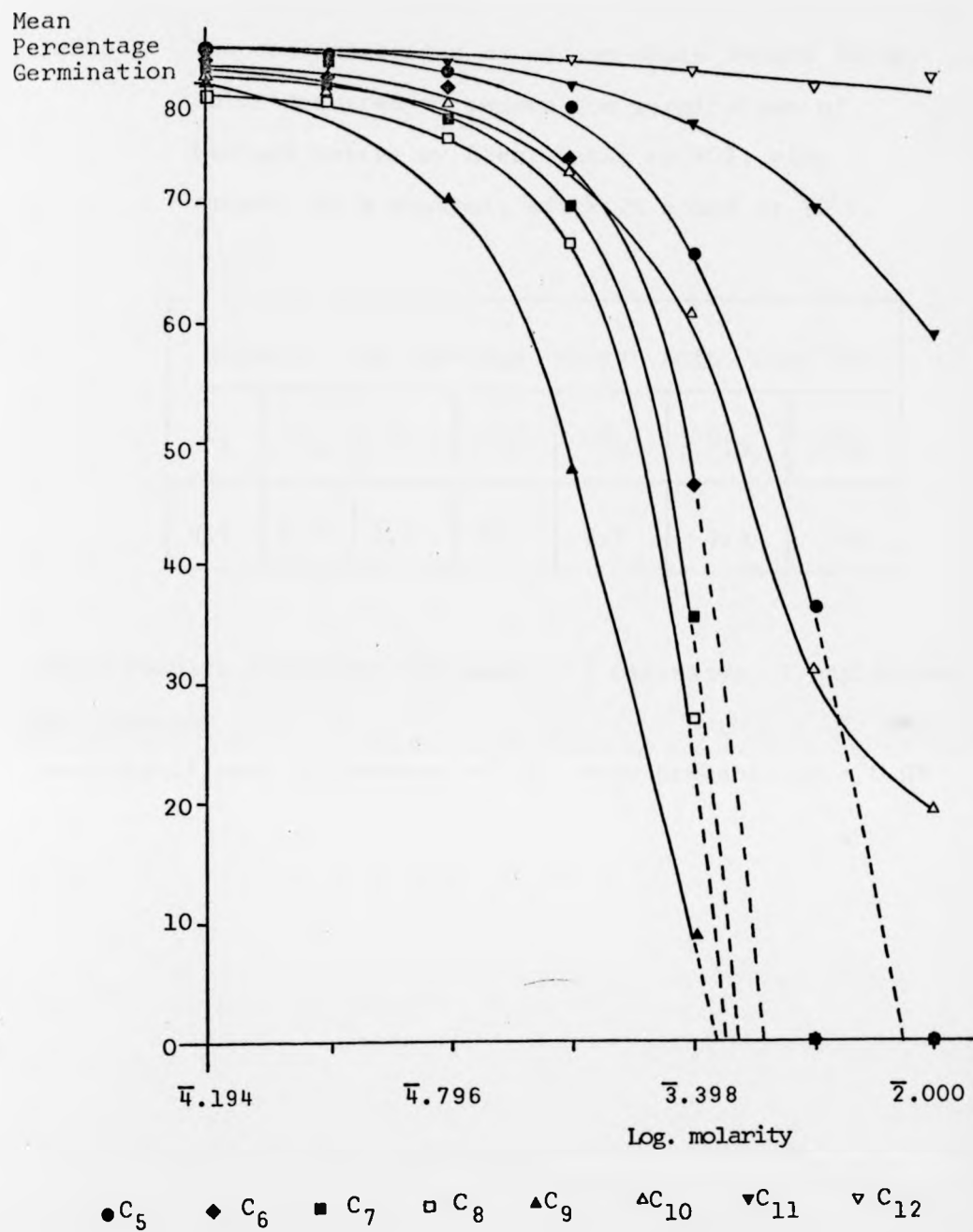
Figure 16: The effect of different concentrations of medium-chain length fatty acids on the germination of *Lactuca sativa* cv Great Lakes after 24 hours at 20°C.

Table 8: The concentrations of medium-chain length fatty
acids required to reduce the germination of
Lactuca sativa cv Great Lakes by 50%, with
respect to a control, after 24 hours at 20°C.

MOLARITY OF APPLIED FATTY ACID ($\times 10^{-3}$M)						
C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}
4.6	2.7	2.4	2.2	1.5	3.3	>10

These results represent the mean of 3 bioassays, 2 replicates
per bioassay

Least significant difference = 0.12, when probability = 0.05
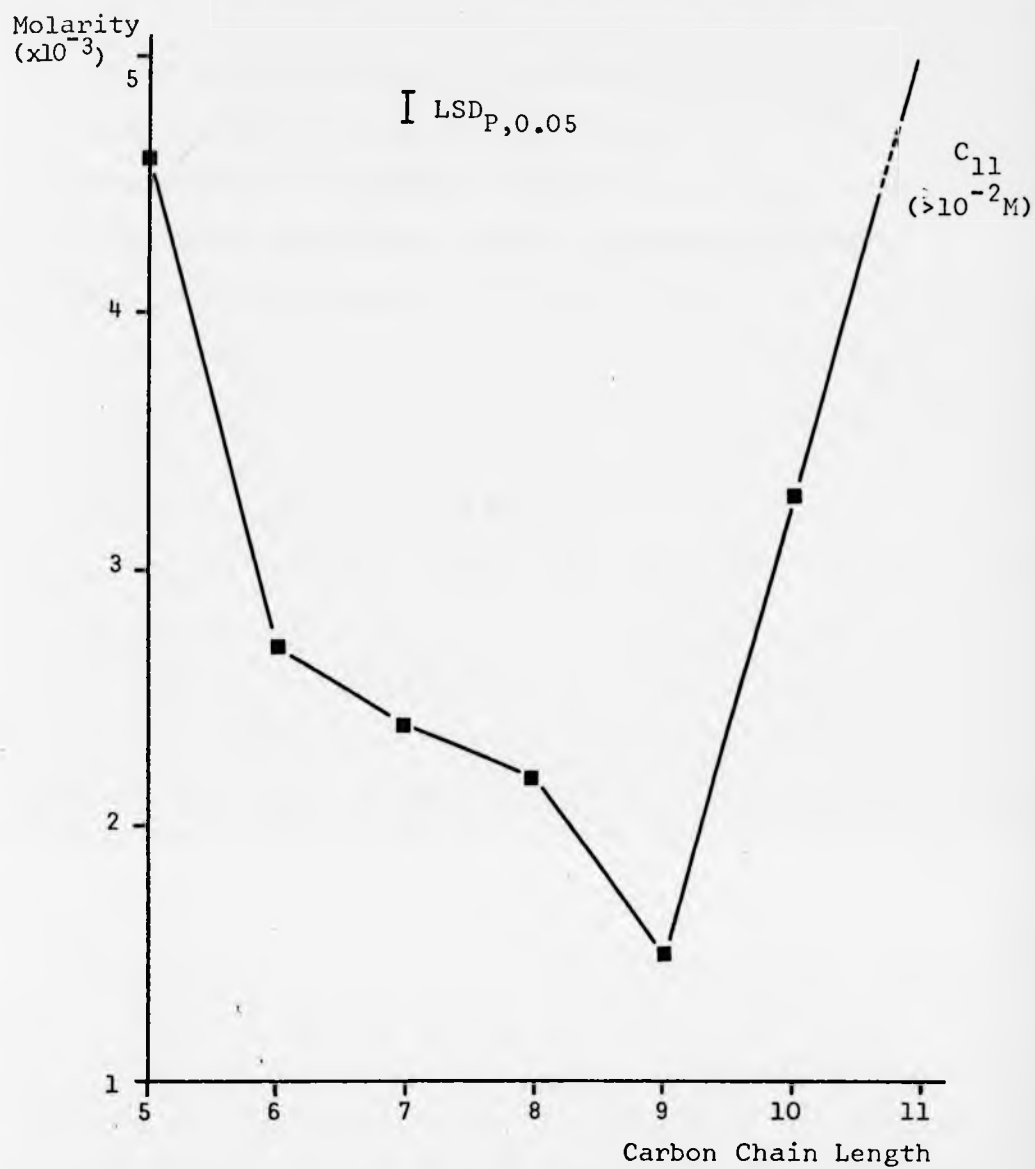
Figure 17: The concentrations of medium-chain length fatty acids required to reduce the germination of *Lactuca sativa* cv Great Lakes by 50% with respect to a control, after 24 hours at 20°C.

with increasing carbon chain length from 4.6mM (C_5) to a value of 1.5mM for nonanoic acid (Figure 17).   This treatment exerted the greatest inhibitory activity.   As the carbon chain length was further increased the degree of inhibition was relaxed.

PART 3: <u>DETERMINATION OF ANY DEGRADATIVE EFFECTS INCURRED</u>

<u>AS A RESULT OF THE EXTRACTION PROCEDURE FOR</u>

<u>MEDIUM-CHAIN LENGTH FATTY ACIDS</u>

The extraction of MCFA from plant material by a process involving steam distillation may possibly generate free fatty acids through the hydrolysis of bound-MCFA in the tissue.   It was important therefore to determine that these inhibitory compounds could be isolated by a procedure in which the final fraction was unlikely to contain free fatty acids derived from hydrolytic effects of the procedural work-up.

A quantity of dormant *A. sativa* cv Selma seed was soaked at $4^{o}C$ with three successive volumes of petroleum ether (40-60) over a period of 7 days.   This treatment was immediately followed by a similar extraction involving methanol.   The resulting methanolic extract was then reduced in volume and subjected to ether partition in both acidic and basic aqueous phases.   This treatment was followed by alkali-treated silica gel chromatography to effect a final purification of the plant extract (Figure 8).

As a parallel extraction, the same quantity of commercial oat was extracted in a Soxhlet apparatus with petroleum ether (40-60) followed by methanol.   The methanolic extract was reduced in volume and steam-distilled against ether vapour.   The resulting organic phase was partitioned against both acidic and basic aqueous phases, this being followed by base-treated silica gel chromatography (Figure 9).

The final fractions isolated using these two

extraction procedures contained free carboxylic acids in the series hexanoic to decanoic. Although the yield from the procedure utilising steam-distillation was reduced over that carried out at a lower temperature, similar amounts of endogenous MCFA were detected after correcting for losses incurred during the extractions.

Considering nonanoic acid, the procedure involving steam-distillation assessed the endogenous amount to be 440.4 ± 8.4 µg per kg seed while the cold-extraction estimated the putative level to be 627.4 ± 4.6 µg per kg seed.

As roughly comparable amounts of the inhibitor were estimated within the seed by two different isolation procedures, one of which was not inducive to the degradation of possible bound free fatty acids, it is therefore unlikely that free MCFA were artifacts of the isolation procedure.

PART 4:   A COMPARISON OF THE ISOLATION PROCEDURES FOR

ABSCISIC ACID AND MEDIUM-CHAIN LENGTH FATTY ACIDS

It was possible that the procedures used to isolate
ABA (Figure 1) and MCFA (Figure 8) may not have
distinguished between these two types of inhibitor.
Abscisic acid has been extracted from dormant seeds of
wild and cultivated oat by soaking in cold aqueous
methanol and assessed by GLC on a 1.5 percent FS-1265
stationary phase using electron capture detection (Figure 13).
This method of detection is not suitable for saturated
fatty acids.   The same seed extracts were therefore
examined for the presence of MCFA by GLC on a 5 percent
FFAP stationary phase using flame ionisation detection
(Figure 18).   From the results it is evident that the ABA
fraction also contained components which had the same
retention time as, and which co-chromatographed with,
authentic methyl esters in the series hexanoate to
decanoate.

The levels of free and conjugated ABA extracted from
dormant seeds of *A. sativa* cv Selma and *A. fatua* are
shown in Table 9.   The amounts of MCFA detected in these
ABA extracts are also indicated.   More than 1.5-times as
much free ABA (30.7 µg per kg) was estimated in seeds of
A. fatua than from the same weight of commercial oat seed
(18.6 µg per kg).   Considering bound-ABA, for both
fractions 2 and 3 more than 1.5-times as much was again
detected in the wild compared to the commercial oat seed.
The amount of inhibitor in fractions 2 and 3 (whether
isolated by hydrolysis of the ether-soluble mixture of

Figure 18: Gas-liquid chromatogram of an abscisic acid
extract from dormant *Avena fatua* seeds.

Injection A comprised 1.0µl of an ethereal solution of
the methylated ABA extract.

Injection B comprised 1.0µl of an ethereal solution of
standard methylated medium-chain length fatty acids.

Stationary phase: 5% FFAP at 173°C.

Detector Response

Detector Response

C_6 C_7

C_8

C_9

C_{10}

A

B

Time (minutes)
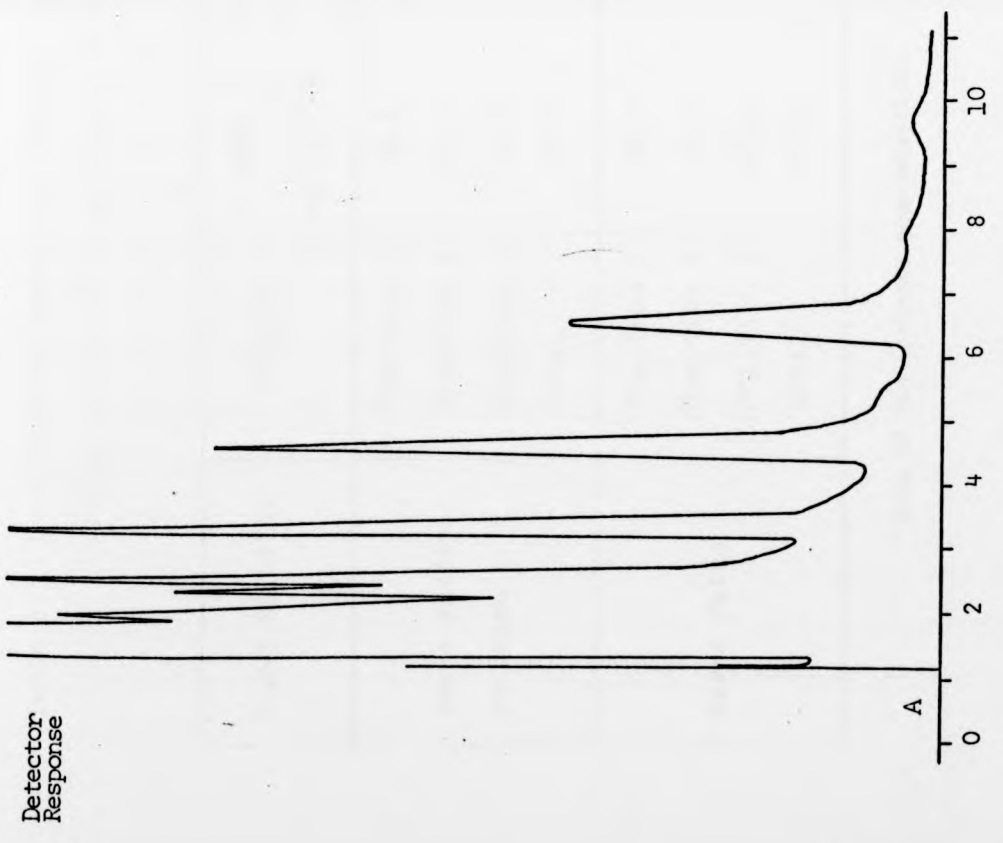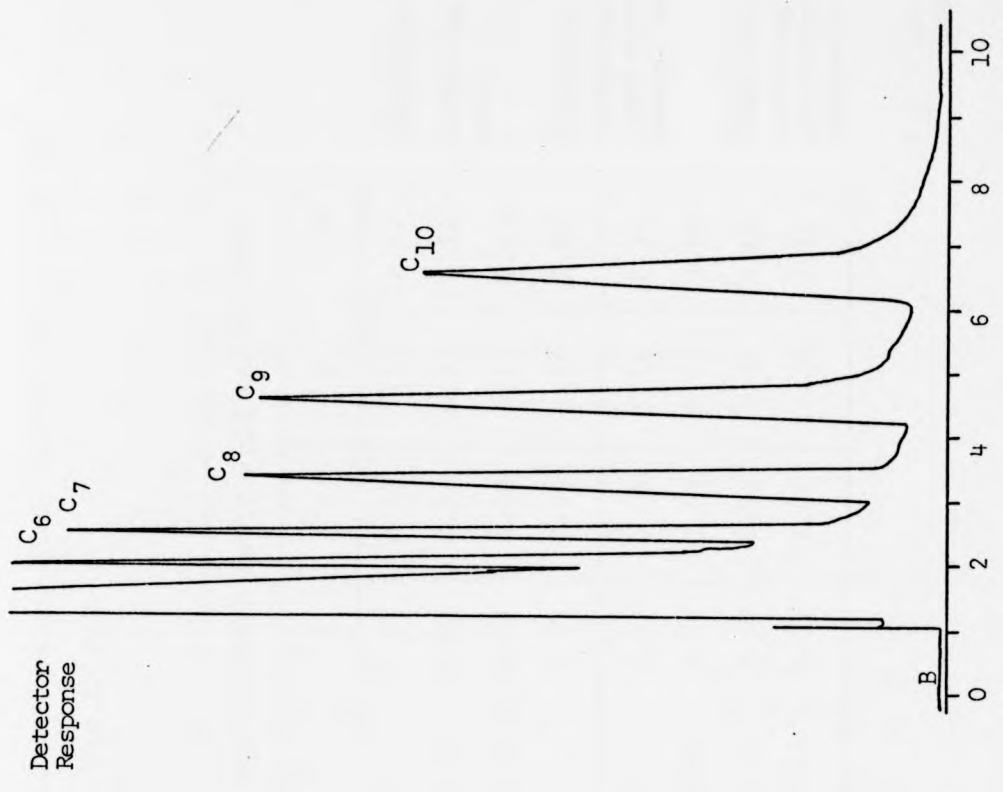
Table 9: Co-occurring levels of abscisic acid and medium-chain length fatty acids in dormant seeds of *A. sativa* cv Selma and *A. fatua*.

PLANT MATERIAL	EXTRACT	ABA µg kg⁻¹FW	Medium-chain fatty acid (mg kg⁻¹FW)				
			C_6	C_7	C_8	C_9	C_{10}
Avena sativa cv Selma	Fraction 1	18.5	N.D.	N.D.	0.83	1.17	2.43
	Fraction 2	31.0	N.D.	N.D.	N.D.	0.23	0.32
	Fraction 3	11.9	N.D.	0.06	0.20	0.18	0.23
	Total	61.5	-	0.06	0.53	1.58	2.98
*Avena fatua**	Fraction 1	30.7	U.D.	0.37	1.07	1.18	0.94
	Fraction 2	45.0	U.D.	0.15	0.92	3.97	3.60
	Fraction 3	17.2	0.46	3.50	6.07	7.00	3.60
	Total	93.0	0.46	4.02	8.06	12.15	8.14

Fraction 1 containing free acids.

Fraction 2 containing hydrolysed ether-soluble neutral components.

Fraction 3 containing hydrolysed water-soluble components

N.D. - not detected

* Data of Don (private communication)

neutral components or from the solution containing water soluble components not partitioned into ether) must be derived from complexed inhibitor.

In both species the greatest amounts of ABA were obtained from the ether-soluble mixture of neutral components (Fraction 2) isolated during the course of the extraction. The amounts of this "neutral-bound" ABA were assessed to be 31.0 µg and 45.1 µg per kg seed for *A. sativa* and *A. fatua* respectively.

Regarding the associated amounts of MCFA, it is clear that the ABA isolation procedure results in an appreciable contamination of the final fraction. Generally the amounts of detected MCFA are greater in *A. fatua,* being increased 6-fold over that detected in the ABA fraction isolated from *A. sativa* cv. Selma. This may possibly be due to the greater number of seeds per unit weight associated with the former species.

The total free fatty acid contamination is about 4.0 mg MCFA per kg seed in both species and the individual acid levels, in the series hexanoic to decanoic, are roughly comparable. Due to the partition coefficients of the respective fatty acids between water and diethyl ether it is unlikely that their presence in Fractions 2 and 3 totally represent complexed material. However, it is clear from Table 9 that on comparison of the two oat species, increased amounts of MCFA were detected in Fractions 2 and 3 of the ABA extracts from *A. fatua.* Such levels may therefore suggest the presence of derivatised MCFA in dormant seed of this species.

PART 5: THE PRESENCE OF FATTY ACIDS IN THE INHIBITOR-β
COMPLEX FROM PLANT MATERIAL

Because MCFA were found to co-occur with ABA in certain
plant extracts it was possible that the aliphatic acids
were also present in the inhibitor-β complex extracted from
plant material. Abscisic acid is known to be an important
inhibitory factor in this mixture (Milborrow 1967).

Carboxylic acids in the series hexanoic to decanoic
were detected in the β-inhibitor complex extracted from
several plant species (Table 10). They were assessed by
GLC comparison on a stationary phase of 5 percent FFAP using
co-chromatography with authentic samples of the methyl
fatty esters. Although all the material examined contained
these inhibitors there was significant variation between
the levels detected. Dormant seeds of wild oat, dock and
ash, together with the overwintering buds of sycamore, all
contained greater levels of MCFA than non-dormant plant
material.

On average 13-times more free MCFA was associated
with the dormant structures than with those which were non-
dormant. Indeed, the amounts of endogenous acids
associated with actively growing material, such as
liverwort and ivy leaves, were found to be approximately
100-fold less than that present in the naturally dormant
structures.

It is clear that such relatively large amounts of
MCFA associated with the β-inhibitor complex from dormant
plant material could account for a large proportion of
the biological activity of the mixture.

Table 10: Medium-chain length fatty acids (mg kg^{-1} FW) detected in the inhibitor-β complex from various plant material.

	PLANT MATERIAL	C_6	C_7	C_8	C_9	C_{10}	C_{11}	Total
Dormant	Wild oat seed	8.93	23.92	22.48	19.51	13.98	-	88.92
	Dock seed	11.17	18.79	17.76	17.49	20.8	-	86.01
	Ash seed	9.48	10.87	12.46	11.71	8.99	-	53.51
	Sycamore buds	4.59	48.77	5.10	8.06	7.55	1.51	75.68
Non-dormant	French bean pods	0.40	0.49	0.90	0.95	0.45	-	3.19
	Broad bean seed	-	-	0.20	0.19	0.08	-	0.47
	Ivy leaves	0.15	0.09	0.11	-	-	-	0.35
	Willow catkins	0.24	3.16	1.57	0.32	1.15	-	6.55
	Potato skin	0.10	0.85	1.27	1.18	1.62	-	5.02
	Hollyberry flesh	0.09	0.09	0.42	0.26	-	-	0.86
	Moss	0.50	1.35	2.75	1.35	0.13	-	6.08
	Liverwort	0.11	0.10	0.17	0.06	0.11	-	0.55

PART 6:   THE EFFECT OF EXOGENOUS APPLICATIONS OF MEDIUM-
           CHAIN LENGTH FATTY ACIDS ON THE GERMINATION OF
           AVENA SPP.

Having identified MCFA as endogenous components of
dormant *Avena* spp., it was important to determine the
effect of exogenous applications of these acids on oat seed
germination.   Authentic samples of MCFA were applied to
non-dormant seeds of *A. fatua* and *A. sativa* cv Selma.
These species showed a significantly reduced percentage
germination in the presence of fatty acid sodium salts with
a carbon chain length of between six and twelve atoms
(Table 7).   Table 11 shows the exogenous concentrations of
free acids (C_5-C_{11}) required to reduce oat seed germination
by 50 percent after 72h and 148h incubation periods.

In this assay the most effective inhibitory acid was
nonanoic which reduced germination to 50 percent of that
obtained with a water control at applied concentrations of
between 1mM and 3mM.   Note that as time elapses the
deleterious effect of the inhibitors disappears, possibly
through loss of the volatile acids or by metabolic
degradation within the moist seed.

Valeric (C_5) and undecanoic (C_{11}) acids are the least
effective of those tested and at a concentration of 10^{-2}M
appear to exert little biological activity.   It must be
remembered, however, that the lower members of this series
of aliphatic acids are quite volatile and it is possible
that their applied concentrations did not remain constant
during the whole of the bioassay.   Furthermore, the
effective concentration in solution of the higher members

Table 11: The concentrations of medium-chain length fatty acids required to reduce the germination of *Avena fatua* and *Avena sativa* cv Selma by 50% with respect to a control, after 72 and 148 hours at $20^{\circ}C$.

Seed species and Imbibition time	Molarity of applied fatty acid ($\times 10^{-3}$M)						
	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}
A. fatua, 72 h	7.6	6.1	2.9	2.0	1.1	1.7	>10.0
A. fatua, 148 h	>10.0	10.0	5.6	3.0	1.6	3.1	>10.0
A. sativa, 72 h	11.3	8.0	4.8	4.3	2.1	3.3	>10.0
A. sativa, 148 h	>10.0	13.5	7.3	5.0	2.8	9.8	>10.0

These results represent the mean of 3 bioassays, 2 replicates per bioassay.

Least Significant Difference = 1.54, when Probability is 0.05.

* Data of Don (private communication)

cannot be positively verified because of their relative insolubility in water.

As with *L. sativa* cv Great Lakes (Part 2) the relative pattern of inhibition follows a distinctive trend (Figure 19). At the respective incubation times that concentration of fatty acid required to reduce seed germination by the designated amount was gradually decreased, with increasing carbon chain length, from about 10mM (C_5) to a minimum of 1.1 - 2.8mM for nonanoic acid. As the chain length was further increased the degree of inhibition was relaxed to reach a value of greater than 10^{-2}M for undecanoic acid. Note that similar exogenous concentrations of MCFA were required to reduce both lettuce and oat seed germination to 50 percent of that obtained with a water control (Table 8 and 11).

It would appear from these results that the wild oat was more susceptible to treatment with MCFA than the cultivated variety (Figure 19). The increased inhibitory effect of decanoic acid on the germination of *A. fatua* is especially noteable.

A concentration of 9.8mM decanoic acid was required to reduce *A. sativa* cv. Selma germination to 50 percent of that obtained with a water control, after 148h of imbibition. For *A. fatua* the corresponding concentration was found to be 3.1mM, a three-fold difference in the exogenous levels.

It should be noted that the active exogenous concentrations of the most effective MCFA (C_6-C_{10}) are of the same order as other recognised inhibitors of germination.

Molarity
(x10^{-3})
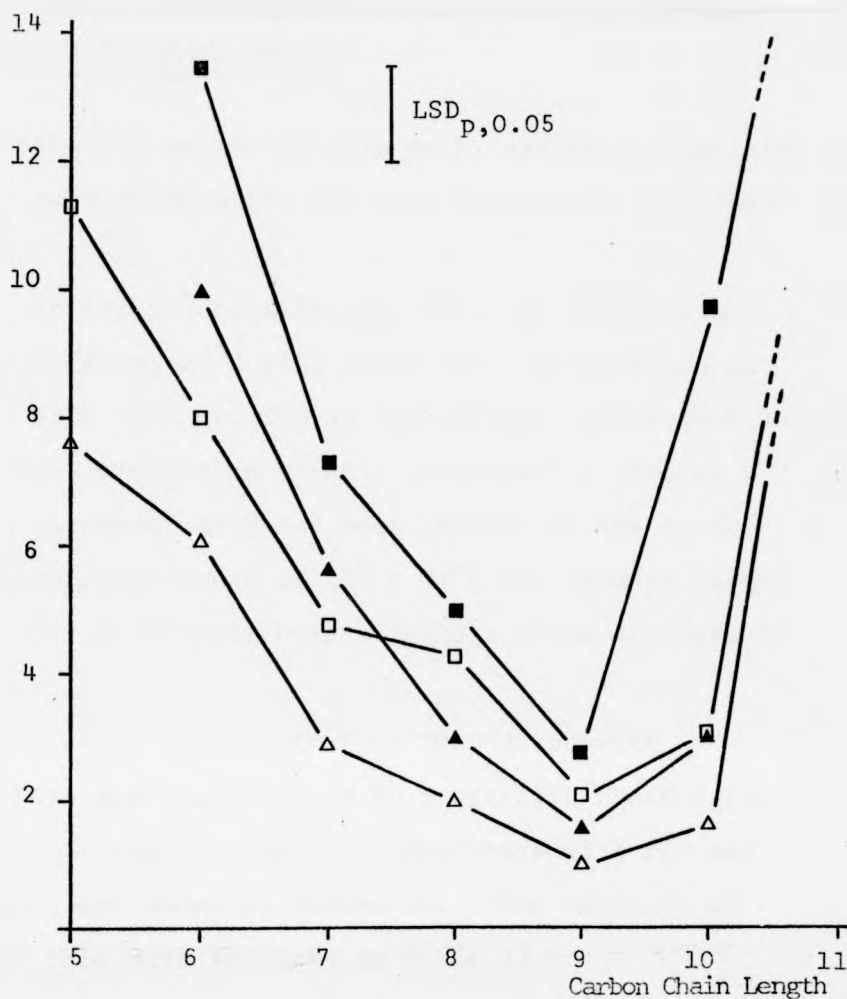
■ *A. sativa* cv Selma at 148h

□ *A. sativa* cv Selma at 72h

▲ *A. fatua* at 148h

△ *A. fatua* at 72h

Figure 19: The concentrations of medium-chain length fatty
acids required to reduce the germination of
Avena fatua and *Avena sativa* cv Selma by 50%
with respect to a control, after 72h and 148h.

PART 7: THE EFFECT OF EXOGENOUS APPLICATIONS OF MEDIUM-CHAIN

LENGTH FATTY ACIDS ON THE GERMINATION OF *LACTUCA*

SATIVA CV GRAND RAPIDS

The inhibitory action of MCFA on *L. sativa* cv Premiere
Great Lakes seed germination has been previously described
(Part 2).

For *L. sativa* cv Grand Rapids (GRLS) an imbibition
temperature of about 20°C will permit full germination in
the dark.   With this species as the ambient temperature is
increased dark germination usually decreases to zero at
which point thermodormancy has been imposed on the seed.
Over the temperature range of 25° - 35°C the seed is light-
sensitive, that is it will only germinate after exposure to
light.

The percentage germinations of GRLS dosed with
individual MCFA and then exposed to a specific photoregime,
comprising individual or combined treatments with red and
far-red light, are shown in Figure 20.   The analysis of
variance for this data is given in Table 12.

Replication of all treatments was good as indicated
from the low standard errors associated with each mean.   In
addition, variation between replicate treatments was found
to be insignificant (Table 12).   The germination of control
samples exposed to each photoregime were uniformly high and
were not influenced by the light treatments (Figure 20).

It is evident from the histograms that 6.25×10^{-4}M and
5.0×10^{-3}M concentrations of MCFA, in the series hexanoic
to decanoic, either totally or partially prevented the
germination of GRLS in the dark (Figure 20a).   In all cases

Figure 20:

The percentage germinations ($\pm$ s.e.m.) of *Lactuca sativa*
cv Grand Rapids, treated with medium-chain length fatty
acids and then exposed to a specific photoregime comprising
red light, or red followed by far- red light, or red
followed by far-red followed by red light.   The values
given represent the mean of 3 replicates, germination being
scored after 24 hours at 20°C.

Total dose of incident radiation:

Red (R)   436 mJ cm^{-2}

Far-red (Fr)   14.8 mJ cm^{-2}

□ 6.25×10^{-4}M treatment

■ 5.00×10^{-3}M treatment

LSD$_{p=0.05}$ = $\pm$ 2.7

Percentage Germination

a) DARK TREATMENT

I LSD$_{p=0.05}$

Carbon chain length
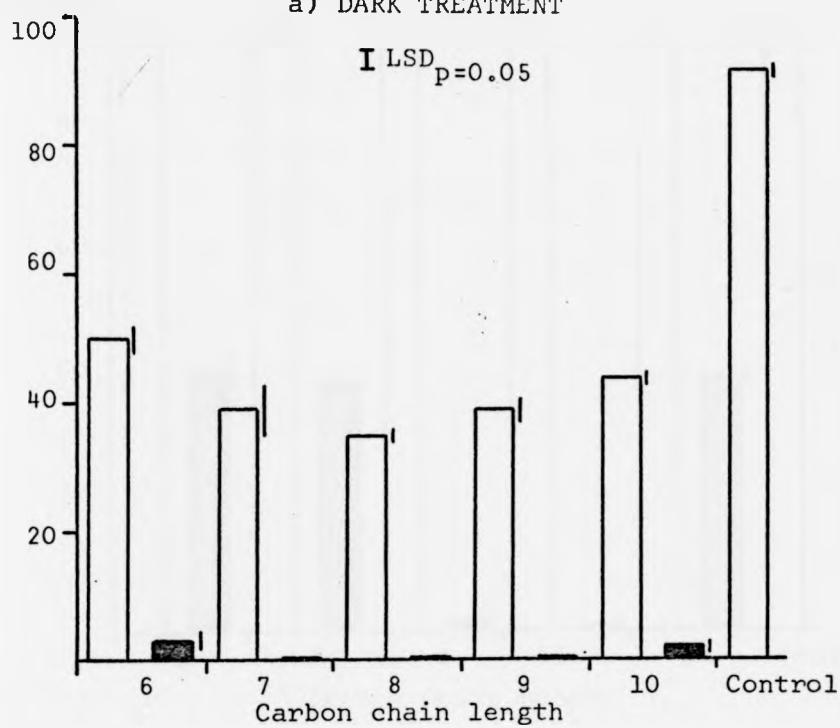
Percentage Germination

c) RED+FAR-RED TREATMENT

I LSD$_{p=0.05}$

Carbon chain length
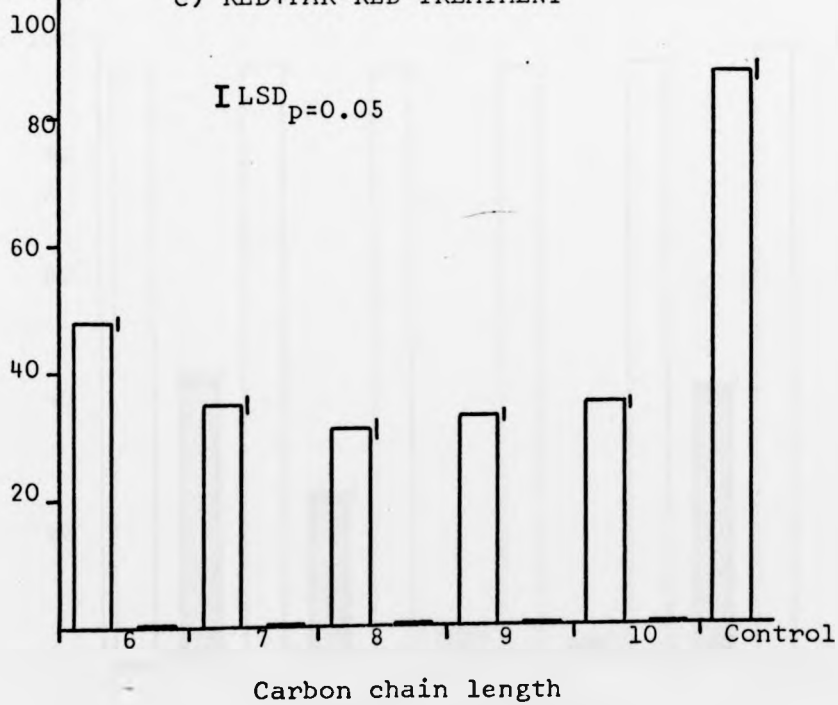
b) RED TREATMENT

$I_{LSD_{p=0.05}}$

Percentage Germination

Carbon Chain Length
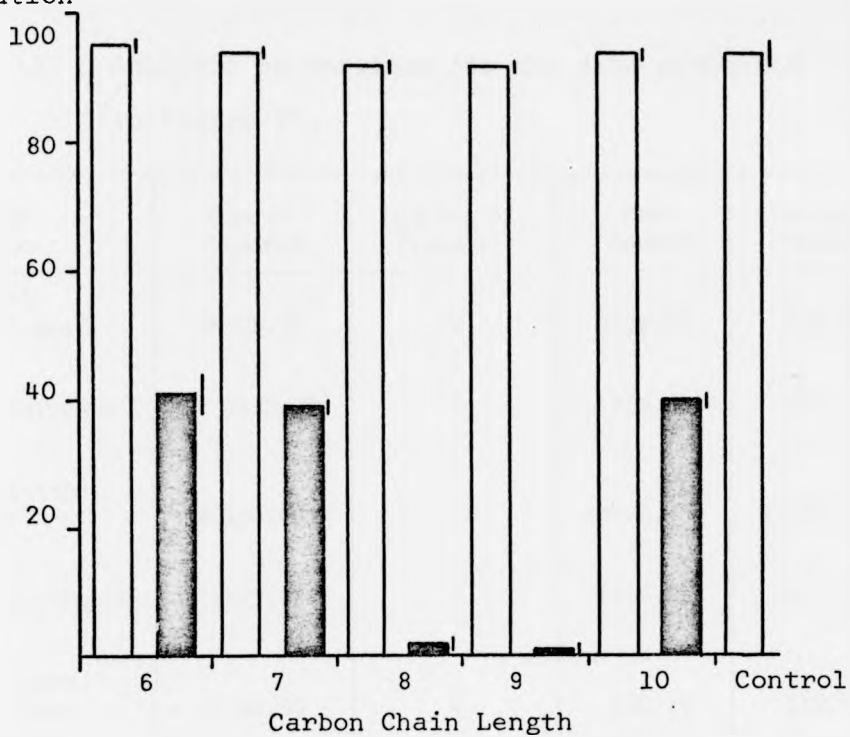
d) RED+FAR-RED+RED TREATMENT

$I_{LSD_{p=0.05}}$

Percentage Germination

Carbon Chain Length

Table 12:   Analysis of variance for the data presented
             in Figure 20.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Treatment Combinations	84708.67	39	2172.02	879.36
Acid treatments	2935.20	4	733.80	297.09
Concentration treatments	50130.48	1	50130.48	20295.74
Light treatments	25652.86	3	8550.95	3461.92
Acid x Concn. interaction	1184.98	4	296.25	119.94
Acid x light interaction	1222.41	12	101.87	41.24
Concn. x light interaction	1759.20	3	586.40	237.41
Acid x concn. x light interaction	4729.39	12	394.12	159.56
Replicates	5.18	2	2.59	1.05
Residual	192.29	78	2.47	
Total	84906.14	119		

the lower fatty acid concentration was less effective and the overall difference between the two concentrations was found to be highly significant (Table 12).

The inhibitory effect of acid treatment on GRLS germination was found to be totally or partially alleviated, depending on the particular treatment, by exposing the seed to a source of red light (Figure 20b). It was possible to reverse the promotive effect of red light by an additional exposure to far-red light (Figure 20c). Far-red light given after red irradiation reimposed acid sensitivity on the photosensitive seed. When a sequence of red, followed by far-red, followed by red light was given then germination was again stimulated in the presence of MCFA (Figure 20d).

At inhibitor concentrations of greater than $10^{-3}M$ phototreatment of the seed was not totally effective in overcoming the acid imposed inhibition of germination (Figures 20b and 20d). No germination was observed with $10^{-2}M$ applications of MCFA.

It is interesting to note the similarity between histograms representing the dark and red plus far-red light treatments (Figures 20a and 20c). Seed germination after both red and red followed by far-red, followed by red light treatments were also comparable (Figures 20b and 20d). The promotive effect of red light as a final irradiation treatment was clearly evident. The degree of germination stimulation was dependent upon both the concentration and chain length of applied inhibitor.

For individual acid regimes a final red light treatment induced comparable germinations for similar treatments that

had been previously exposed to either a dark or far-red photoregime (Figures 20b and 20d). In addition a final far-red irradiation overcame the action of red light and reduced the overall germinations to values comparable with a dark treatment (Figures 20a and 20c). The influence of photoregime as a source of variation was found to be highly significant (Table 12).

The effect of acid chain length on GRLS germination was not marked. At the lower concentration (6.25×10^{-4}M) little difference in the germinations was found between individual fatty acids (Figure 20). At the higher concentration (5.0×10^{-3}M) little difference was again evident between acid treatments after both the dark and red plus far-red light regimes, germinations being reduced (Figures 20a and 20c). However, following a final red irradiation which alleviates acid inhibition highly significant differences between MCFA treatments were evident (Figures 20b and 20d and Table 12).

The mean percentage germinations (1%-5%) obtained with octanoic and nonanoic acids were, in this case, not significantly different from each other but differed markedly from those germinations which occurred in the presence of hexanoic, heptanoic and decanoic acids (about 40%).

In this bioassay, therefore, octanoic and nonanoic acids exerted the greatest inhibitory activity on GRLS germination. It was only at a concentration of 5mM and after treatment with the promotive (red) photoregime that the influence of chain length was evident. This second order interaction is clearly indicated in Figures 20b and 20d and was found to be highly significant (Table 12).

Interactions between acid-concentration, acid-light and concentration-light were also found to be highly significant (Table 12). The acid-light interaction was not marked. This reflects the fact that it was only at the higher concentration that the acids showed differences in their effects in the different photoregimes.

These results suggest that GRLS are sensitised to light by MCFA treatment. The extent of light-sensitivity, or the degree to which germination was stimulated by light, was found to be largely dependent upon acid concentration. Germination was also influenced by the acid chain length at the active molarities. Application of MCFA appeared to lower the temperature at which thermodormancy was imposed on the seed. At $20^{o}C$ the seeds became light-sensitive and germination was promoted or inhibited by the appropriate photoregime, as occurs with naturally thermodormant lettuce seed.

The dark germination of light-sensitive lettuce seeds may be stimulated by treatment with either gibberellic acid or cytokinin. GRLS were dosed with the promoters in the presence of MCFA to determine whether they reduced acid-sensitivity of the seed.

At exogenous concentrations of $10^{-4}M$ and $10^{-3}M$ both gibberellic acid and kinetin were found to alleviate the acid imposed block to germination (Figures 21 and 22). The higher concentration of promotive compound was found to exert the greater stimulatory effect (Figures 21c and 22c). In addition, highly significant differences existed between the applied promoter concentrations (Tables 13 and 14).

Gibberellic acid enhanced MCFA-treated GRLS germination

Figure 21:

The percentage germinations ($\pm$ s.e.m.) of
Lactuca sativa cv Grand Rapids treated with medium-chain
length fatty acids and gibberellic acid at various
concentrations. The exogenous concentration of gibberellic
acid was 10^{-4}M or 10^{-3}M and that of the MCFA either 6.25 x
10^{-4}M or 5.00 x 10^{-3}M. The values given represent the
mean of 3 replicates, germination being scored after 24 hours
at 20°C in the dark.

☐ 6.25 x 10^{-4}M fatty acid treatment

■ 5.00 x 10^{-3}M fatty acid treatment

$LSD_{p,0.05}$ = $\pm$4.5

Percentage germination

a) O M Gibberellic acid

I LSD$_{p=0.05}$

Carbon chain length

6   7   8   9   10   Control
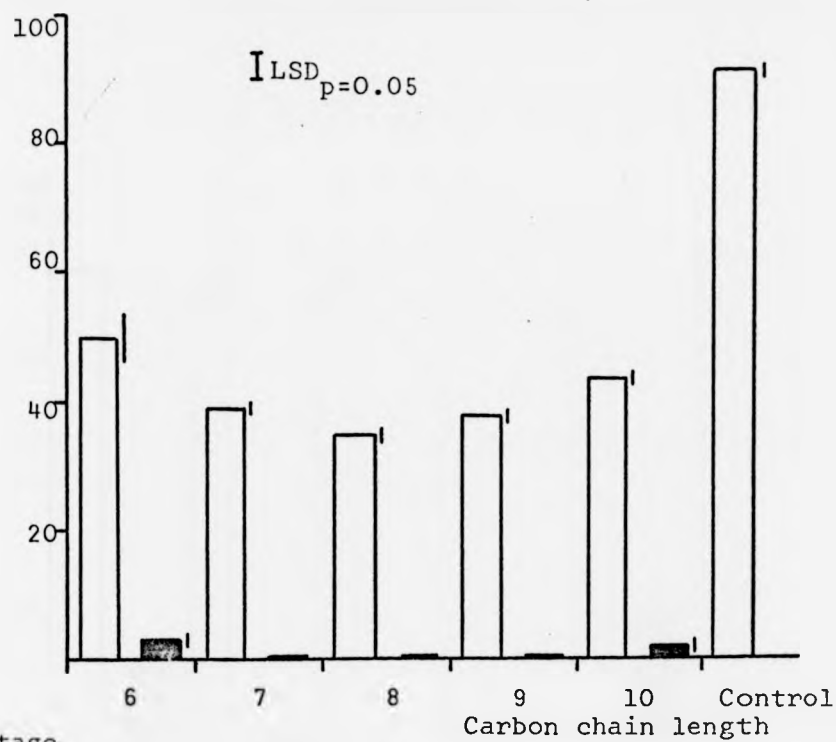
Percentage germination

b) 10^{-4}M Gibberellic acid

I LSD$_{p=0.05}$

6   7   8   9   10   Control

Carbon chain length

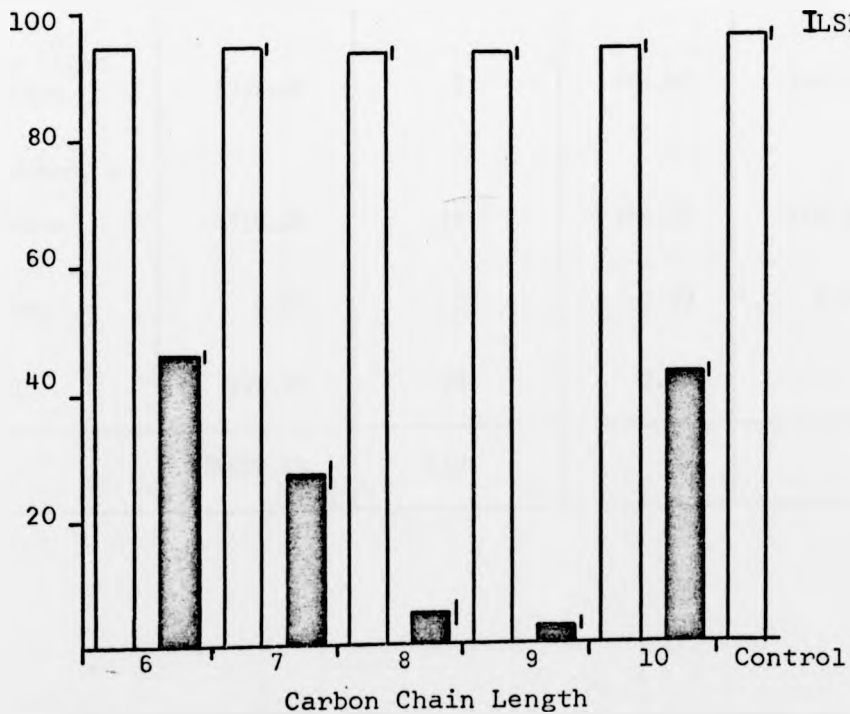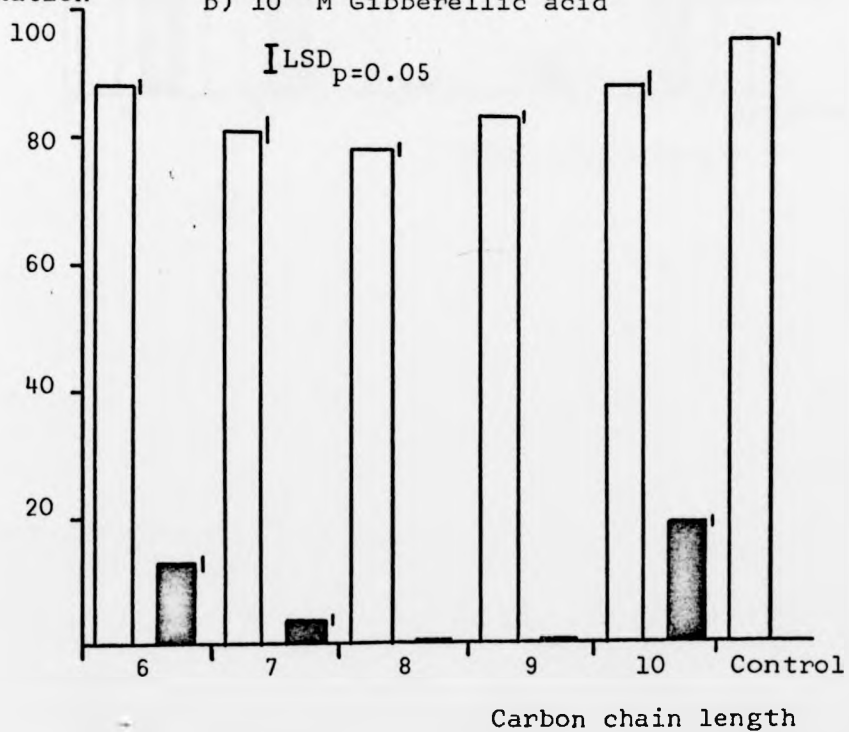c) 10^{-3}M Gibberellic acid
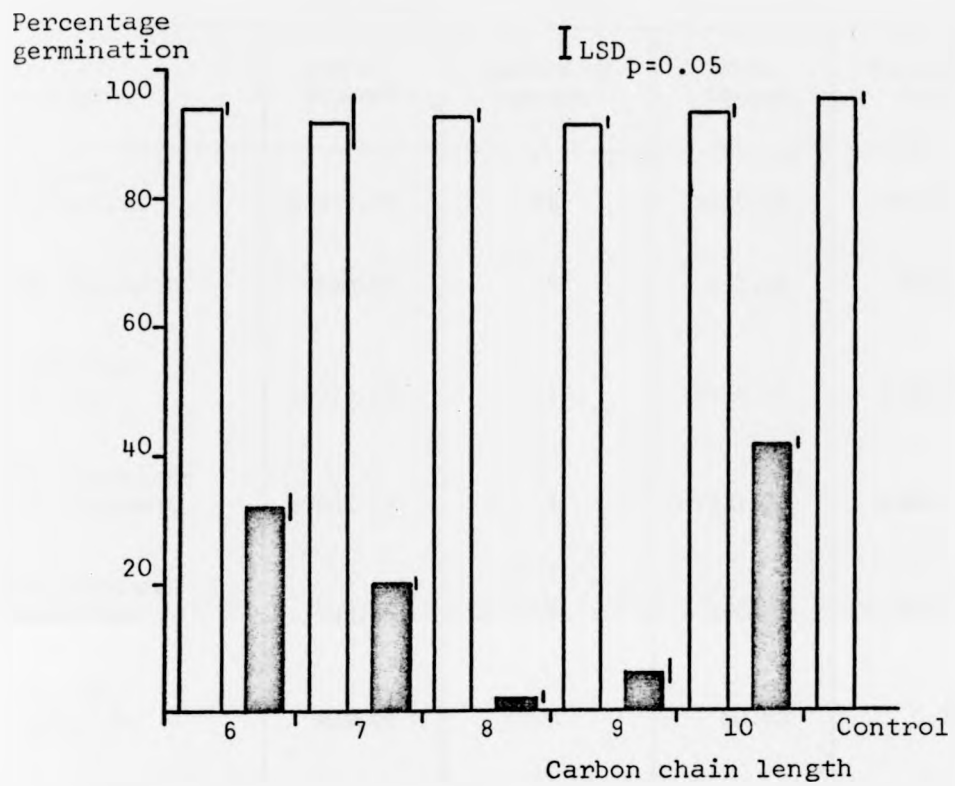
Table 13 : Analysis of variance for the data represented

in Figure 21.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Treatment Combinations	63673.16	29	2195.63	272.41
MCFA treatment	2083.42	4	520.86	64.62
Gibberellin treatment	10929.30	2	5464.65	678.00
MCFA concentration treatment	47431.23	1	47431.23	5884.77
Acid x Concn. interaction	742.84	4	185.71	23.04
Acid x GA_3 Interaction	403.42	8	50.43	6.26
Concn. x GA_3 interaction	1536.36	2	768.18	95.31
Acid x GA_3 x concn. interaction	546.59	8	68.32	8.48
Replicates	43.22	2	21.61	2.68
Residual	467.20	58	8.06	
Total	64183.58	89		

Figure 22:

The percentage germinations ($\pm$ s.e.m.) of *Lactuca sativa* cv Grand Rapids treated with medium-chain length fatty acids and kinetin at various concentrations. The exogenous concentration of kinetin was 10^{-4}M or 10^{-3}M and that of the MCFA either 6.25×10^{-4}M or 5.00×10^{-3}M. The values given represent the mean of three replicates, germination being scored after 24 hours at 20°C in the dark.

□ 6.25×10^{-4}M fatty acid treatment

■ 5.00×10^{-3}M fatty acid treatment

$LSD_{p,0.05} = \pm 3.92$

a) 0.0M Kinetin

Percentage germination

b) 10^{-4}M Kinetin

Percentage germination

c) 10^{-3}M Kinetin
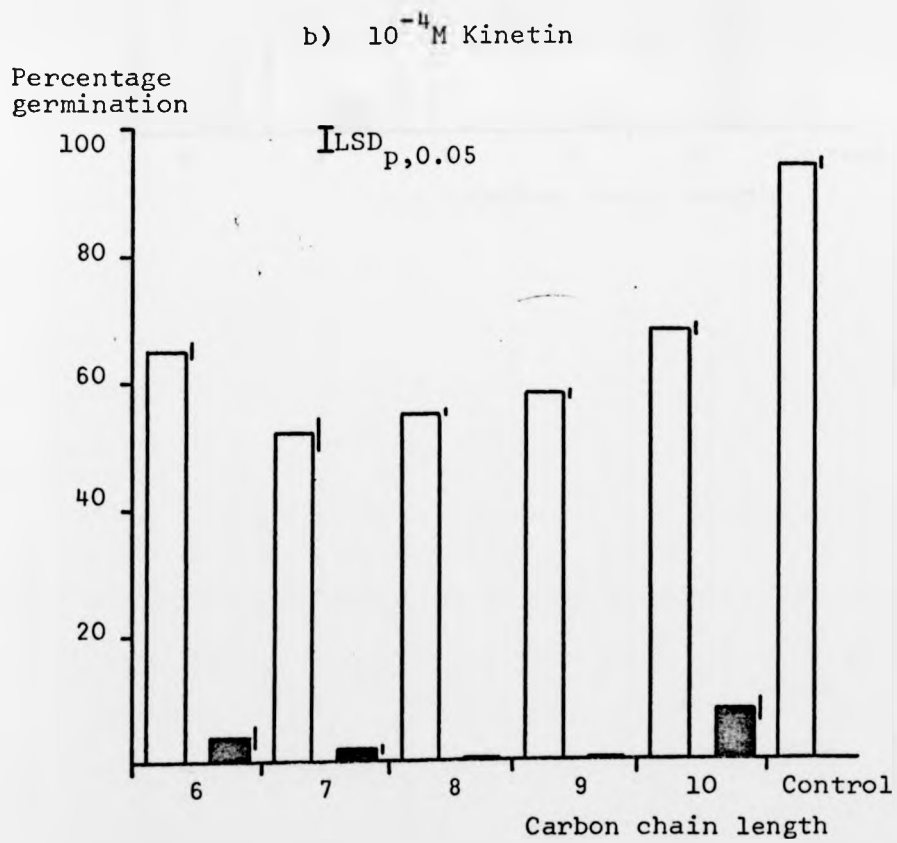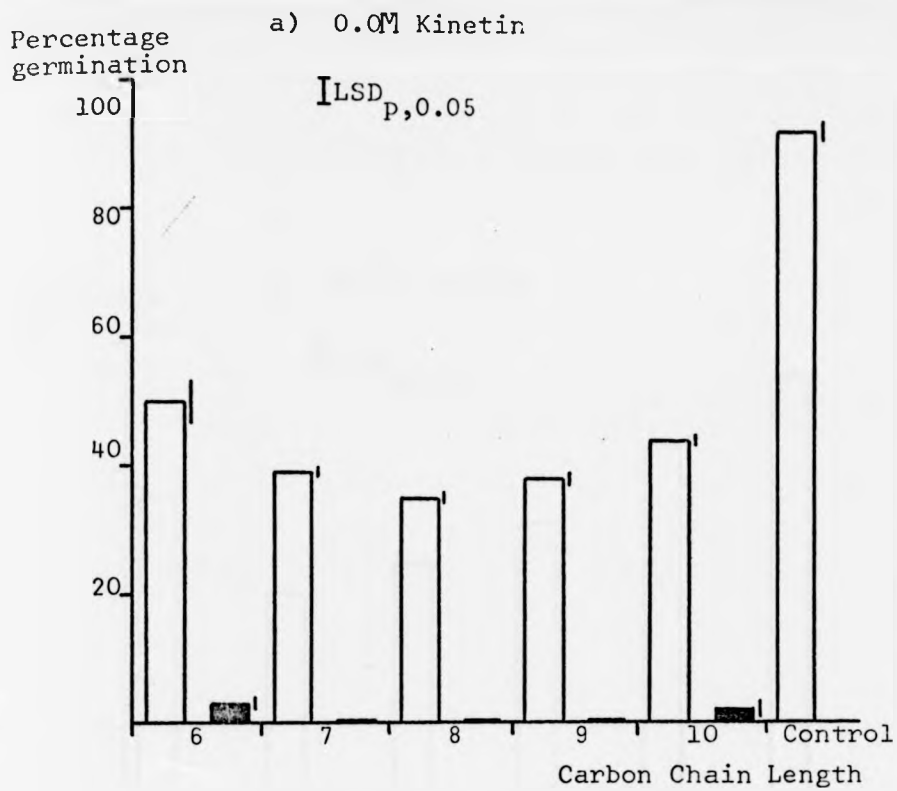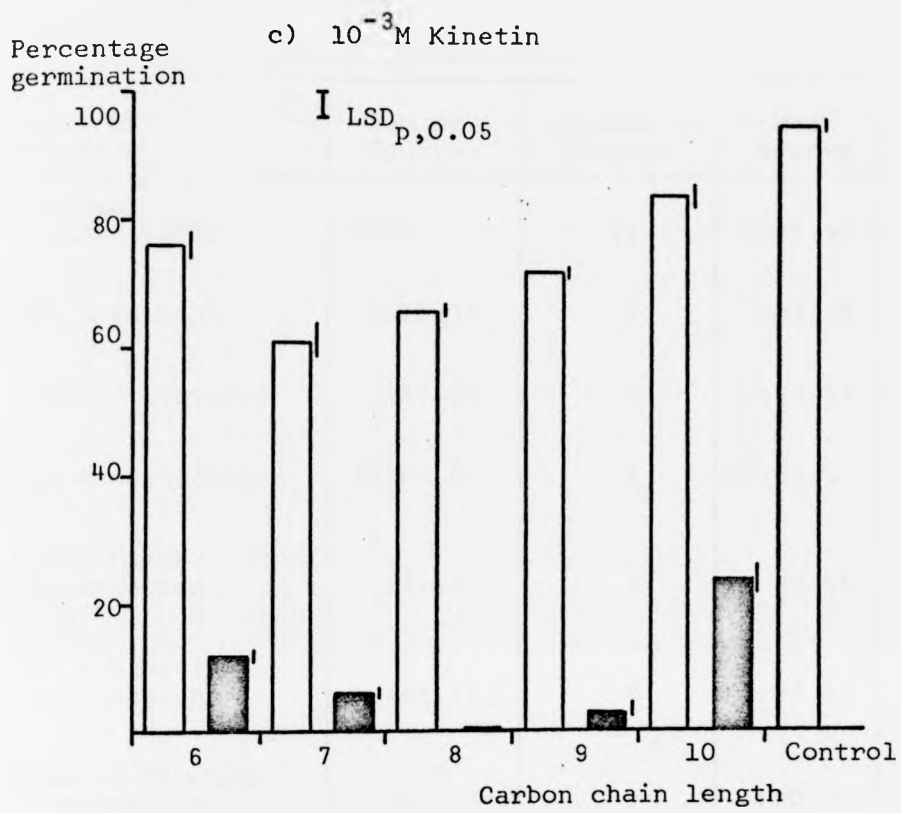
TABLE 14:   Analysis of variance for the data represented

in Figure 22.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Treatment Combinations	40887.14	29	1409.90	235.37
MCFA treatment	1615.17	4	403.79	67.41
Kinetin treatment	2867.06	2	1433.53	239.32
MCFA concentration	35368.0	1	35368.0	5904.51
Acid x Concn. Interaction	137.91	4	34.48	5.76
Acid x Kinetin Interaction	388.11	8	48.51	8.10
Concn. x Kinetin Interaction	360.35	2	180.17	30.08
MCFA x Kinetin x Concn. Interaction	150.54	8	18.82	3.14
Replicates	9.06	2	4.03	0.67
Residual	347.55	58	5.99	
Total	41243.75	89		

to a greater extent than equivalent concentrations of kinetin. At fatty acid concentrations of greater than 10^{-3}M neither chemical was totally effective in relieving the imposed dormancy. At 5mM concentrations of fatty acid the enhanced promotive action of gibberellin was 2-3 fold greater than kinetin (Figures 21 and 22).

Replication of all treatments was good (Tables 13 and 14) as indicated from the low standard errors associated with each germination mean (Figures 21 and 22). The germinations of control samples for each promoter treatment were found to be uniformly high (Figures 21 and 22). The higher concentration (5.0×10^{-3}M) of fatty acid was found to be the most effective inhibitory treatment, the overall difference between the two acid concentrations being highly significant (Tables 13 and 14).

The influence of differing acid chain lengths on the germination of promoter-treated GRLS was limited. At the lower concentration (6.25×10^{-4}M) little difference was observed between the germinations of individual fatty acid treatments (Figures 21 and 22). At this molarity the most inhibitory MCFA was found to be octanoic for gibberellin treatments and heptanoic for kinetin treatments. However, at the higher concentration the most active acids were found to be octanoic and nonanoic.

The differing effectiveness of acid chain length is shown most clearly in treatments incorporating the higher concentrations of MCFA (5mM) and either gibberellin or kinetin at 1mM (Figures 21c and 22c). The germinations decreased with increasing chain length from hexanoic to

octanoic acids and thereafter were increased for the higher chain lengths. Decanoic acid was found to be the least inhibitory of the MCFA series, in this bioassay. The mean percentage germinations (about 4%) of GRLS treated with 5mM concentrations of the most inhibitory acids, octanoic and nonanoic, and 1mM concentrations of kinetin or gibberellin were not significantly different from each other (Figures 21 and 22).

Thus, when dosed with the higher concentrations of both inhibitor and promoter highly significant differences between MCFA treatments were evident (Tables 13 and 14). This second order interaction was similar to that found with the germinations of MCFA-treated GRLS after treatment with red light (Figure 20).

Interactions between the various treatments, fatty acid-concentration, concentration-promoter and fatty acid-promoter, were also found to be highly significant (Tables 13 and 14). Again this reflects the importance of concentration to the germination effects of applied MCFA.

PART 8: THE SYNERGISTIC ACTION OF MEDIUM-CHAIN LENGTH
FATTY ACIDS ON SEED GERMINATION

1. *LACTUCA SATIVA* cv GREAT LAKES

The fatty acids characterised in this study always
occurred as complex mixtures and it was possible that some
degree of synergism existed between members of the series.
If this occurred an effect of the mixture would be greater
than the mean activity of the individual components at an
equivalent concentration. An extended study of all
possible combinations over a wide concentration range was
not attempted.

Table 15 indicates the percentage germinations of
L. sativa cv Great Lakes when exposed to paired combinations
of MCFA. It is clear that some mixtures at concentrations
of 2mM do display synergistic action under the test
conditions. If the inhibitory effect of a combination
were to be the average of the individual members activity,
then the expected germination would be given by the
bracketed values in Table 15. The actual germinations
obtained with paired combinations were compared with that
expected by means of a contingency x^2-test (Appendix II).

Combinations of the paired acids hexanoic-heptanoic,
heptanoic-octanoic, heptanoic-nonanoic and nonanoic-
decanoic had a significantly greater inhibitory influence
than was to be expected (Table 15). A combination of
heptanoic and nonanoic acids was found to exert the greatest
synergistic activity, in the order of a 2.5 fold decrease
in the expected germination. Even a comparison between the

TABLE 15: The effect of paired combinations of medium-
chain length fatty acids (at a total concentration
of 2mM) on the percentage germination of
Lactuca sativa cv. Great Lakes at 20°C.
Data of Don (private communication)

FATTY ACID ADDED AT 10^{-3}M	PERCENTAGE GERMINATION				
	6	7	8	9	10
6	64.3	33.3***	44.3	45.7	72.0
		(60.3)	(50.5)	(45.7)	(72.0)
7		56.3	34.3**	18.0***	64.0
			(46.5)	(41.7)	(68.0)
8			36.7	30.3	55.3
				(32.3)	(58.2)
9				27.0	42.7*
					(53.7)
10					79.7

Level of significance in comparing expected (bracketed)
value with observed value.

*** $P < 0.001$

** $0.001 < P < 0.01$

* $0.01 < P < 0.05$

emergence obtained with a mixture and the activity of the more inhibitory acid of the pair, reveals that combinations of hexanoic-heptanoic and heptanoic-nonanoic were still significantly more inhibitory than expected.

2. *AVENA SATIVA* cv SELMA

Synergistic inhibition was found to occur between saturated, medium-chain length fatty acids in the series hexanoic to decanoic when tested against lettuce seed germination (Table 15). However, a similar experiment involving *A. sativa* cv Selma could detect little synergistic activity between the acids.(Table 16). Only a combination of hexanoic and octanoic acids was found to result in a significantly different observed germination from that expected.

It would appear that mixtures of MCFA at concentrations of 2mM do not have a synergistic effect on commercial oat seed germination, under the test conditions used.

TABLE 16: The effects of paired combinations of medium-chain length fatty acids (at a total concentration of 2mM) on the percentage germination of *Avena Sativa* cv. Selma, imbibed at $20^{\circ}C$ for 72 hours.

FATTY ACID ADDED AT 2mM	PERCENTAGE GERMINATION				
	C_6	C_7	C_8	C_9	C_{10}
C_6	74.7	66.7 (64.7)	47.3* (61.3)	47.3 (57.3)	46.7 (57.3)
C_7		54.7	48.0 (51.3)	48.0 (47.7)	44.7 (47.3)
C_8			48.0	36.7 (44.0)	35.3 (44.0)
C_9				40.0	34.7 (40.0)
C_{10}					40.0

These results represent the mean of 3 bioassays, 2 replicates per bioassay.

Level of significance in comparing expected (bracketed) value with observed value.

* $0.01 < P < 0.05$

PART 9: A COMPARISON OF THE INHIBITORY EFFECTS OF NONANOIC

ACID AND ($\pm$)-ABSCISIC ACID ON THE GERMINATION OF

AVENA SATIVA

MCFA possess marked biological activity at physiological concentrations when assayed against the germinations of lettuce, *A. fatua* and *A. sativa* cv Selma seed.   They have also been found to co-occur with ABA in extracts from dormant plant material.   It was therefore of interest to determine the germination pattern of oat grain treated with these chemically disimilar plant-growth regulators.

A comparative germination titre was carried out at 20°C.   Cultivated oat seed was dosed with ABA and the most inhibitory MCFA, nonanoic acid, at different concentrations. Germination was assessed after 72h imbibition by scoring radicle protrusion.   The results for ABA and nonanoic acid are shown in Figures 23 and 24, respectively.

ABA at an applied concentration of 10^{-2}M could not prevent partial germination of the seed sample (Figure 23). Germination was found to be proportional to the logarithm of the inhibitor concentration over the molarity range 10^{-4}M to 10^{-2}M. For these treatments germination was decreased from 69 percent to 34 percent respectively (Figure 23).   The difference between the ABA concentration treatments was found to be highly significant (Table 17).

It is clear from Table 17 that the variation between bioassays was high, being significant at a probability of 0.01.   Therefore, in this experiment there was no appreciable difference between the ABA concentration treatments and the respective bioassays as a source of

Percentage Germination
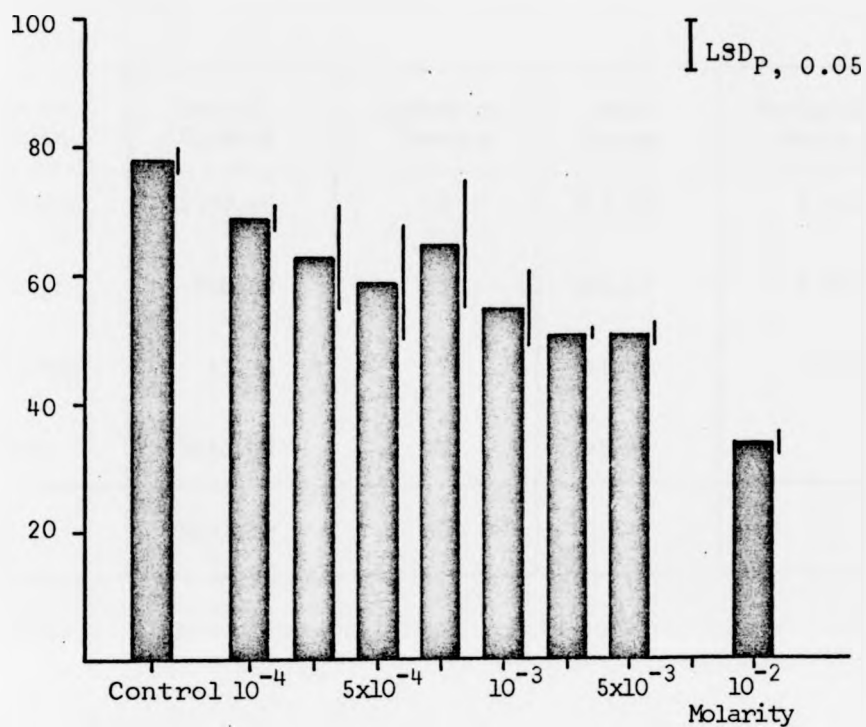
FIGURE 23:   The effect of various concentrations of abscisic acid on the germination of *Avena sativa* cv. Selma after 72 hours at 20°C.

These results represent the mean ($\pm$s.e.m.) of three bioassays, 2 replicates per bioassay.

TABLE 17: Analysis of variance for the data represented in Figure 23.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Treatments	2777.46	8	347.18	8.03
Bioassays	516.83	2	258.42	5.98
Replicates	11.36	1	11.36	0.26
Residual	1815.48	42	43.28	
Total	5121.13	53		

Percentage
Germination
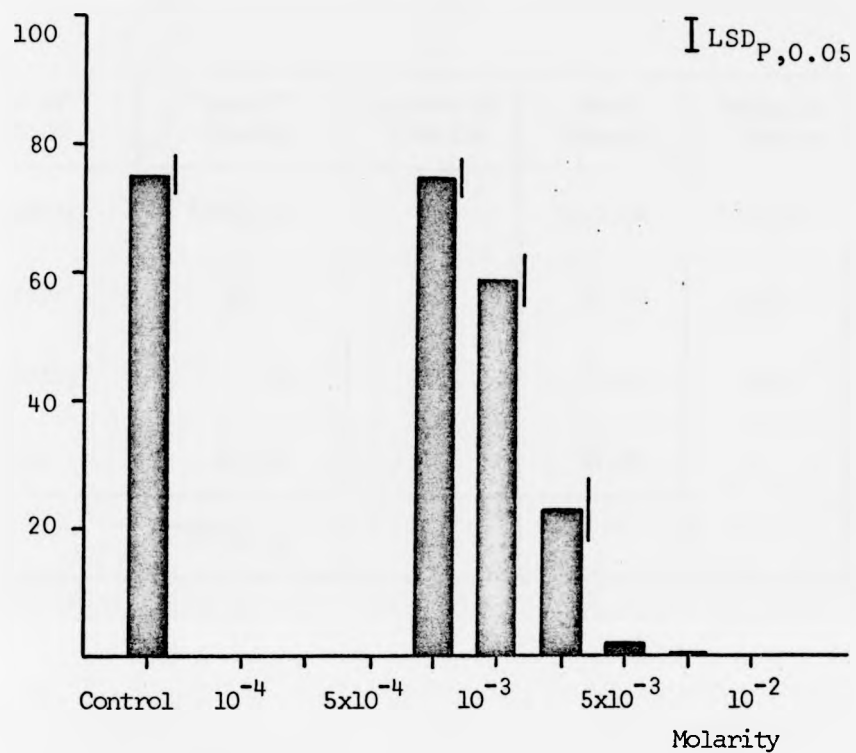
FIGURE 24:   The effect of various concentrations of
nonanoic acid on the germination of *Avena sativa*
cv. Selma after 72 hours at 20°C.

These results represent the mean (±s.e.m.) of three bioassays,
2 replicates per bioassay.

TABLE 18: Analysis of variance for the data represented in Figure 24.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Treatments	19555.19	5	3911.04	174.99
Bioassays	85.78	2	42.89	1.92
Replicates	1.58	1	1.58	0.07
Residual	603.41	27	22.35	
Total	20245.96	35		

variation. Thus, by comparing the variance ratios associated with treatments and bioassays, it may be suggested that ABA has little effect on the germination of common oat seed.

Although unable to inhibit germination, as monitored by radicle protrusion, abscisic acid was found to exert marked growth inhibitory activity. In most cases the radicle emerged from the grain but showed no further extension growth upon contact with the assay medium. For ABA-treated seeds only the radicle was generally visible after three days imbibition, no coleoptile growth being observed.

This phenomenon tended to make the ABA treatments difficult to score as indicated by the large standard errors associated with some germination means (Figure 23). However, there was no significant difference between the germinations of replicate treatments (Table 17). This effect was not seen with nonanoic acid, the coleoptiles from germinated seed generally being visible after 72h imbibition.

In contrast to ABA, nonanoic acid was found to exert a marked inhibitory effect on grain germination in this bioassay. Germination was decreased rapidly over the concentration range 7.5×10^{-4}M to 7.5×10^{-3}M, from 75 percent to 0 percent respectively (Figure 24). The germination pattern could therefore be represented as a threshold response. Variation between the nonanoic acid concentration treatments was found to be highly significant (Table 18). For the fatty acid, variation between replicates and the respective bioassays was not significant (Table 18).

PART 10:   VARIATION IN THE MEDIUM-CHAIN LENGTH FATTY ACID
AND ABSCISIC ACID CONTENTS OF *AVENA SATIVA* cv
SELMA AND *AVENA FATUA* DURING GRAIN DEVELOPMENT
AND AFTER-RIPENING

1.   SEED DEVELOPMENT

It was important to determine if a correlation
existed between the levels of MCFA or ABA and physiological
behaviour during the development of dormant and non-dormant
species of *Avena* seed.

The time-course variations in moisture content, fresh
weight and dry weight of *A. sativa* cv Selma and *A. fatua*
during grain maturation are shown in Tables 19 and 20,
respectively.   In both species there was a decline in
moisture content from almost 75 percent at anthesis to about
22 percent at the time of maturation.   There was a further
decline in the moisture content beyond harvest, stabilising
at a value of approximately 12 percent during dry storage.

Over the maturation period the rate of grain filling,
or increase in dry weight, was identical for both species.
(Figures 25 and 26).   However, the pattern of grain
filling was different.   In *A. sativa* grain filling proceeded
almost uniformly from anthesis until maturity at 63 days
after this time, a daily increase in weight of about 0.5mg
per seed (Figure 25).   In *A. fatua* noticeable grain filling
did not proceed until about 30 days from anthesis.   The
dry weight of individual grains then increased by 0.5mg per
day from about 2mg to 17mg at 60 days from anthesis
(Figure 26).

TABLE 19: The dry weight and percentage moisture content of grains of *Avena sativa* cv. Selma during maturation and subsequent dry storage. The development data represents the mean of 7 replicates ($\pm$ s.e.m.) and indicates the weight per 100 seeds. The germination data represents the mean of 3 replicates.

Days from anthesis	% age Germn.	FRESH WEIGHT(g)	DRY WEIGHT (g)	WATER CONTENT (g)	%age WATER CONTENT
0	0	5.08 $\pm$ .108	1.32 $\pm$ .020	3.77 $\pm$ .088	74
16	0	5.03 $\pm$ .177	1.81 $\pm$ .070	3.22 $\pm$ .100	64
23	0	5.70 $\pm$ .147	2.03 $\pm$ .047	3.66 $\pm$ .107	64
37	19	6.87 $\pm$ .256	3.19 $\pm$ .121	3.68 $\pm$ .143	54
44	29	7.48 $\pm$ .151	4.01 $\pm$ .097	3.47 $\pm$ .184	46
51	45	6.69 $\pm$ .104	4.27 $\pm$ .038	2.42 $\pm$ .093	36
58	69	6.01 $\pm$ .093	4.47 $\pm$ .101	1.55 $\pm$ .061	26
63	61	5.83 $\pm$ .102	4.52 $\pm$ .047	1.30 $\pm$ .070	22
70	65	5.20 $\pm$ .043	4.56 $\pm$ .061	0.64 $\pm$ .023	12
84	87	4.84 $\pm$ .090	4.33 $\pm$ .078	0.51 $\pm$ .018	10
313	96	4.37 $\pm$ .114	4.06 $\pm$ .113	0.31 $\pm$ .009	7

TABLE 20: The dry weight and percentage moisture content of grains of *Avena fatua* during maturation and subsequent dry storage. The development data represents the mean of 7 replicates (± s.e.m.) and indicates the weight per 100 seeds. The germination data represents the mean of 3 replicates.

Data of Dom (private communication) - obtained under parallel conditions

Days from anthesis	%age Germn.	FRESH WEIGHT(g)	DRY WEIGHT (g)	WATER CONTENT (g)	% age WATER CONTENT
0	0	0.45 ± .010	0.19 ± .010	0.33 ± .004	74
14	0	0.40 ± .007	0.16 ± .006	0.24 ± .004	60
29	0	0.41 ± .015	0.18 ± .005	0.23 ± .012	57
36	0	0.55 ± .017	0.26 ± .014	0.29 ± .009	52
46	0	2.05 ± .082	1.41 ± .065	0.65 ± .045	32
52	0	2.29 ± .060	1.70 ± .011	0.58 ± .050	26
59	0	2.35 ± .092	1.77 ± .066	0.57 ± .079	25
67	0	1.95 ± .046	1.62 ± .045	0.33 ± .036	17
81	0	1.86 ± .015	1.60 ± .006	0.26 ± .009	14
133	0	1.83 ± .012	1.59 ± .016	0.22 ± .004	13
175	0	1.82 ± .009	1.59 ± .003	0.23 ± .006	13
214	0	1.82 ± .009	1.58 ± .003	0.23 ± .007	13
242	8	1.81 ± .017	1.58 ± .010	0.22 ± .009	12
578	42	1.78 ± .017	1.54 ± .010	0.25 ± .007	14

Weight per 100 seeds (g)
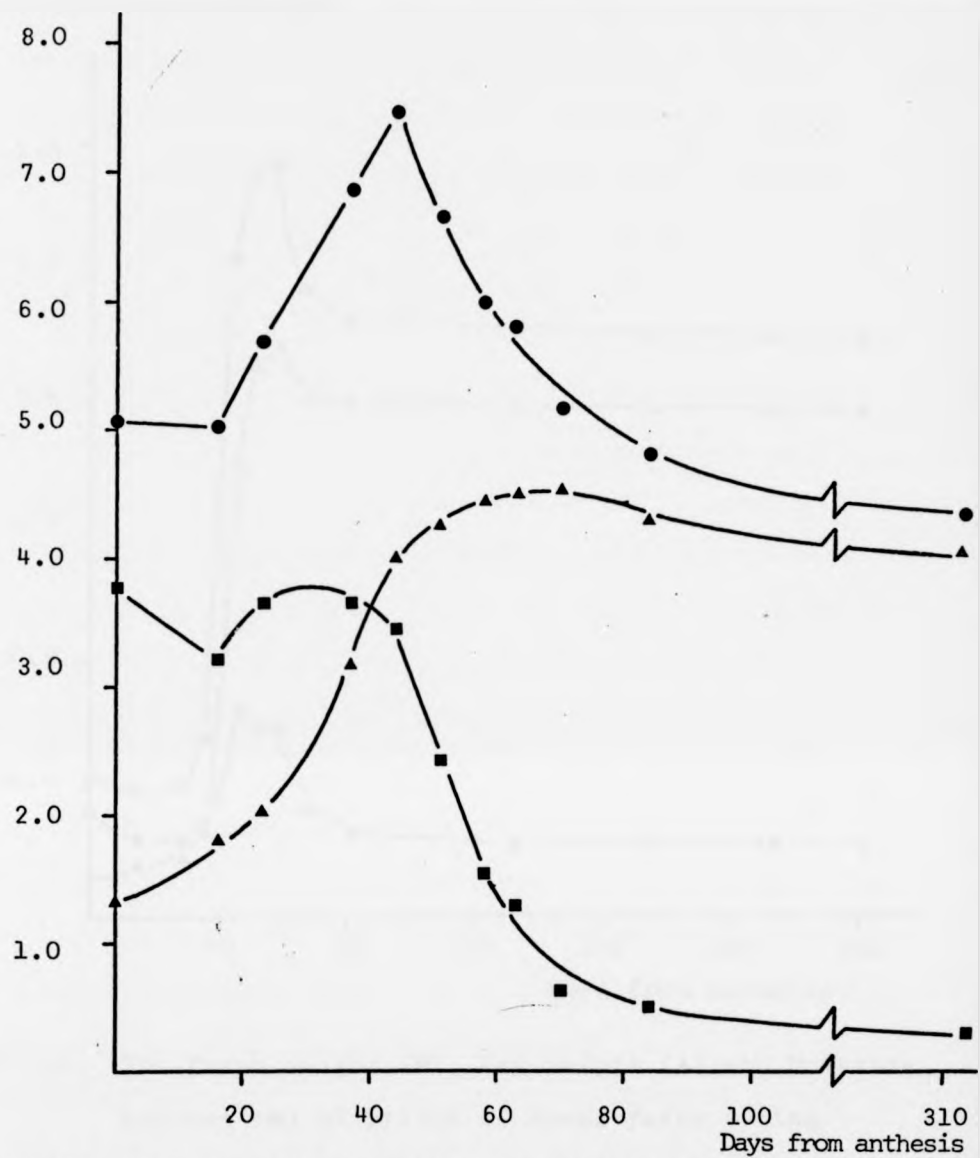
FIGURE 25: The Fresh weight (●), Dry weight (▲) and Moisture content (■) of grains of *Avena sativa* cv Selma during maturation and subsequent dry storage.

Weight per 100 seeds (g)
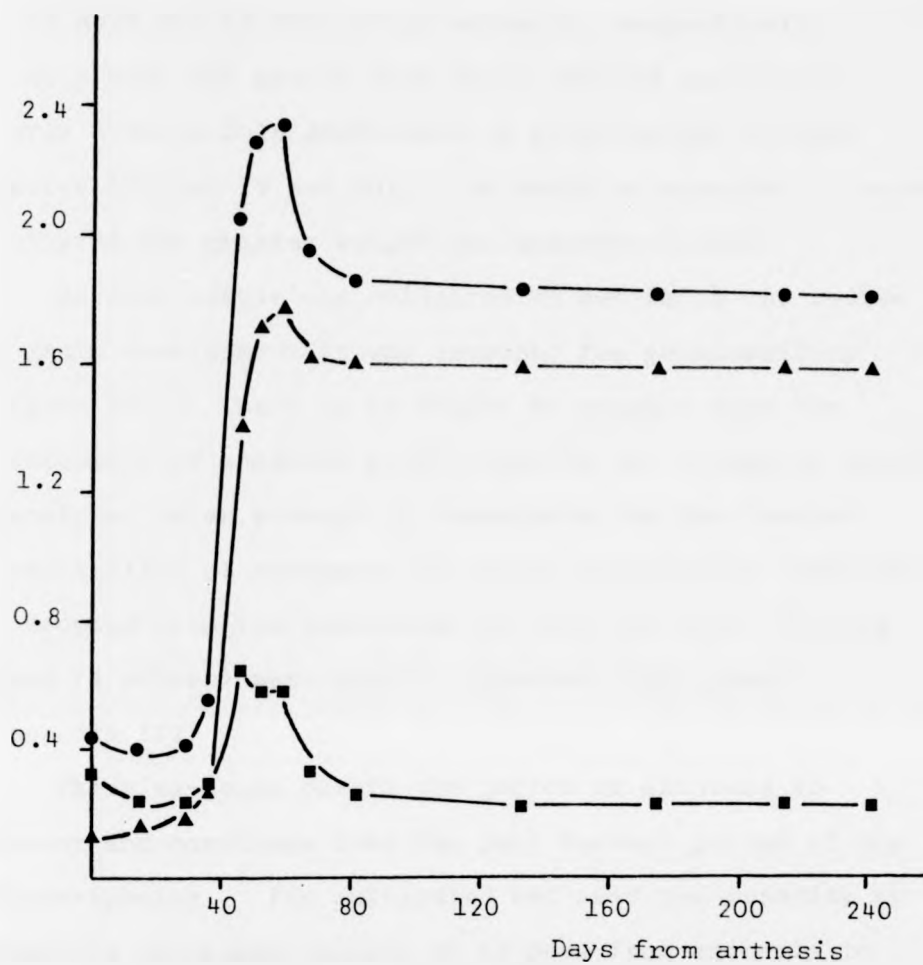
FIGURE 26: The Fresh weight (●), Dry weight (▲) and Moisture content (■) of grains of *Avena fatua* during maturation and subsequent dry storage.

Data of Don (private communication)

Seeds of the wild and cultivated oat were harvested at 67 days and 63 days after anthesis, respectively. At this time the grains were fully matured and showed nearly a three-fold difference in grain weight between species (Tables 19 and 20). As would be expected *A. sativa* exhibited the greater weight per individual seed.

As each sample was collected to determine the course of grain development it was assessed for germinability (Figure 27). There is no reason to suppose that the development of enhanced germination is not a regular trend. Therefore, in an attempt to compensate for the inherent unreliability of estimates of actual germination capacity, as deduced from the percentage germination data, running means (3 values) were used to construct this graph (Appendix II).

The time-scale covers the period of anthesis to harvest and continues into the post harvest period of dry after-ripening. For cultivated oat seed the capacity to germinate increased rapidly at 13 days from anthesis to reach a value of 65 percent germination at the time of harvest (Figure 27). This development occurred during maturation on the parent plant. A second phase of germination enhancement was associated with the post harvest period of dry storage and this may be considered the after-ripening stage.

In *A. sativa* germination reached a maximum value of 92 percent at about 130 days after anthesis. In contrast, *A. fatua* seeds did not show a propensity to germinate until

Percentage germination

Days from anthesis
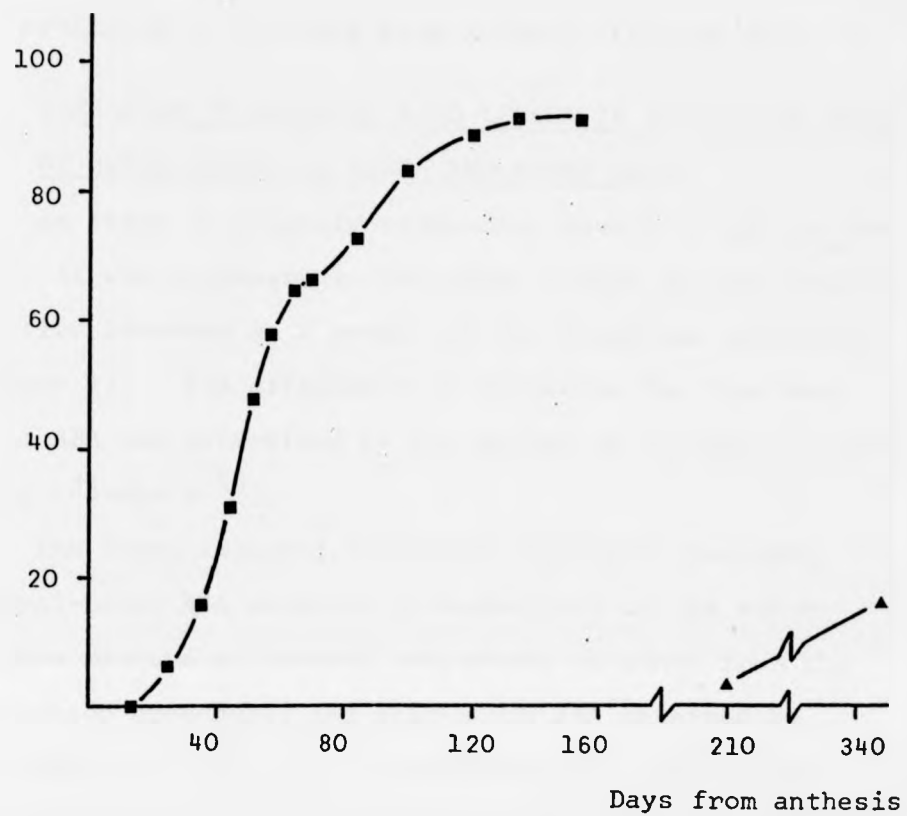
FIGURE 27:   The percentage germinations of *Avena fatua* (▲)
and *Avena sativa* cv. Selma (■) during maturation
and subsequent dry storage.
(Values given as the running-mean of 3
consecutive real observations.)

they have been stored in a dry condition for at least
4.5 months after harvest.   A germination of 3.5 percent
was estimated at 210 days from anthesis (Figure 27).

2.   VARIATION OF ABSCISIC ACID LEVELS IN DEVELOPING SEEDS
      OF *AVENA SATIVA* cv SELMA AND *AVENA FATUA*

In order to quantify endogenous levels of ABA in the
grain it was necessary to determine losses of the inhibitor
fraction incurred as a result of the isolation procedure
(Figure 1).   The efficiency of isolation for free and
bound ABA was determined by the method of isotope dilution
using $(\pm)$-ABA-2-^{14}C.

The three isolated fractions comprised free ABA,
neutral-bound ABA obtained by hydrolysis of the ether-
soluble mixture of neutral components obtained from the
extraction procedure, and acid-bound ABA obtained by
hydrolysis of the solution containing any water-soluble
components which did not partition into diethyl ether.
Losses in the order of 50 percent were estimated for each
of these fractions.

It should be expected that any natural developmental
phenomenon would involve a process of gradual change.   A
time-course programme of extractions from growing plant
material is often subject to errors imposed through short-
term variations in the ambient environmental conditions.
In order to effect a degree of compensation for such events
and to indicate the general developmental trend such data
may be expressed as running-means.   This treatment provides
a mean of three sequential real values throughout the time-

course study and reduces the impact of any abnormal variation on the development pattern.

Tables 21 and 22 express the real ABA data (Appendix III) as a running-mean (3 values) and indicate the estimated endogenous levels of free and bound inhibitor in seeds of *A. sativa* and *A. fatua*, respectively. ABA levels were found to vary markedly in both species during maturation.

In the cultivated oat after anthesis there was a very marked decrease in the ABA content over a period of 8 days (Table 21). The total inhibitor level at 28 days after anthesis was 97.9μg per kg grain and decreased to 32.1μg per kg at 36 days from anthesis. Overall there was a 7-fold decrease in the total ABA content from 97.9μg to 14.2μg per kg seed at 57 days after anthesis (Table 21). At harvest the level of total ABA had increased slightly (28.9μg) but subsequently decreased to 18.0μg per kg seed at 70 days from anthesis.

For *A. sativa* development trends for the ABA fractions were generally similar to that for total ABA (Table 21). Variation in the levels of neutral-bound ABA were, however, not marked. At the first measurement after anthesis (28 days)levels of free and acid-bound ABA were 24.9μg and 60.2μg per kg seed respectively. These amounts decreased rapidly over the succeeding 8 days to 8.5μg and 13.7μg per kg seed, respectively. Thereafter the endogenous inhibitor remained at comparable or slightly reduced levels during subsequent maturation (Figure 28). It is clear that the amount of free ABA present declined 15-fold during the

TABLE 21: Percentage germinations and levels of ABA in grain of *Avena sativa* cv. Selma during maturation and subsequent dry storage. The data is corrected for extraction losses and given as running-means of three consecutive observations for the dried seed.

Days from anthesis	%age Germn.	FREE ABA			"NEUTRAL" BOUND			"ACID" BOUND			TOTAL	
		µgkg⁻¹	Pg grain⁻¹	Rel. Content	µgkg⁻¹	Pg grain⁻¹	Rel. Content	µgkg⁻¹	Pg grain⁻¹	Rel. Content	µgkg⁻¹	Pg grain⁻¹
28	6.2	24.9	551	.25	12.8	324	.13	60.2	1274	.62	97.9	2149
36	15.6	8.5	260	.27	9.9	324	.31	13.7	392	.42	32.1	976
44	30.6	9.0	329	.35	8.6	301	.34	8.0	259	.31	25.6	889
51	47.4	9.0	391	.49	6.7	287	.36	2.8	123	.15	18.5	801
57	58.5	7.9	347	.56	4.1	182	.29	2.2	100	.15	14.2	629
64	65.3	6.7	299	.23	16.2	734	.56	6.0	271	.21	28.9	1304
70	66.6	1.7	78	.09	12.5	570	.70	3.8	172	.21	18.0	820

TABLE 22: Percentage germination and levels of ABA in grain of *Avena fatua* during maturation and subsequent dry storage. The data is corrected for extraction losses and given as running-means of three consecutive observations for the dried seed.

Data of Don (Appendix III/Table II) - obtained under parallel conditions

Days from Anthesis	% age Germ.	FREE ABA			"NEUTRAL" BOUND			"ACID" BOUND			TOTAL	
		ugkg^{-1}	pg grain^{-1}	Rel. Content	ugkg^{-1}	pg grain^{-1}	Rel. Content	ugkg^{-1}	pg grain^{-1}	Rel. Content	ugkg^{-1}	pg grain^{-1}
26	0	56.1	101	.47	18.6	32	.16	43.7	74	.37	118.4	207
37	0	70.4	451	.57	20.1	157	.16	32.5	257	.27	123.0	865
45	0	34.5	468	.52	11.9	167	.18	19.9	280	.30	66.3	915
52	0	40.0	610	.54	12.2	179	.17	21.5	320	.29	73.7	1109
59	0	16.3	281	.64	3.3	56	.13	5.7	99	.23	25.3	436
69	0	17.6	297	.75	2.6	44	.11	3.4	59	.14	23.6	400
94	0	14.3	229	.79	2.4	39	.13	1.4	27	.08	18.1	295
130	0	15.0	240	.81	1.6	26	.09	1.9	30	.10	18.5	296
174	0	10.6	168	.82	.8	13	.06	1.6	25	.12	13.0	206

ABA Content ($\mu g k g^{-1}$ seed)
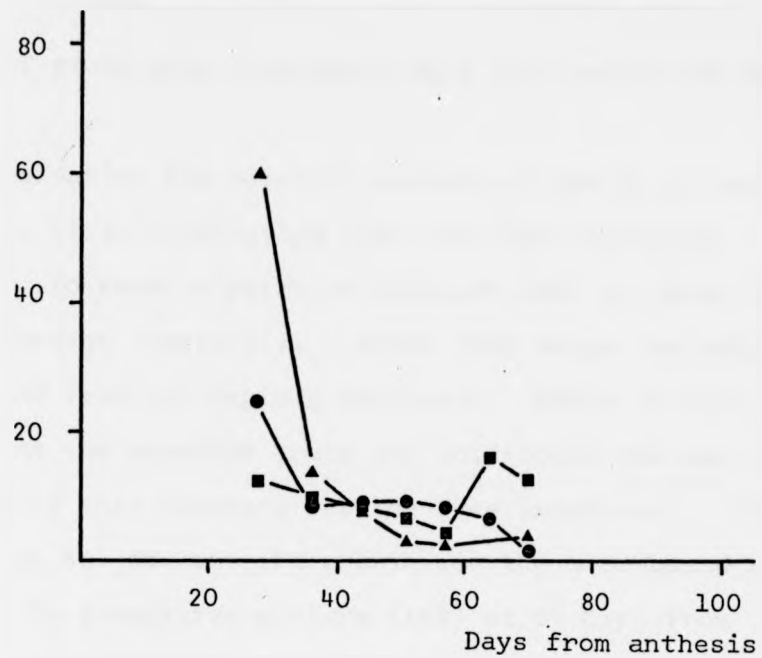
ABA Content (pg grain^{-1})

FIGURE 28: Levels of Free (●), Acid-bound (▲) and Neutral-bound (■) ABA in grain of *Avena sativa* cv. Selma during maturation and subsequent dry storage.

period of study when considered on a unit weight of seed basis.

Considering the relative amounts of ABA in *A. sativa* extracts, it is interesting that free ABA increased gradually to reach a relative maximum (56%) at about 5 days before harvest (Table 21). After this stage the relative content of free ABA rapidly declined. Table 21 also shows that the relative trend for acid-bound ABA was the opposite of that assessed for the free inhibitor. From a value of 62 percent after anthesis the acid-bound fraction declined to a relative minimum (15%) at 57 days from anthesis and thereafter increased slightly. The relative level of neutral-bound ABA increased throughout the period of study with a sharp rise occurring during the final stages of maturation (Table 21). The data suggests that interconversion may have occurred between the respective ABA fractions.

When considering the amount of inhibitor per kg of seed there was a 5-fold decrease in the total ABA content of the wild oat, from 118.4µg at the first measurement after anthesis to 23.6µg at harvest (67 days from anthesis). There was also a subsequent decline during four months of dry storage to a value of 13.0µg per kg grain at 174 days after anthesis (Table 22).

For *A. fatua*, as with *A. sativa*, the development trends for the respective ABA fractions were comparable with that found for total ABA (Table 22 and Figure 29). At the first measurement after anthesis (26 days) levels of free, acid-bound and neutral-bound ABA were 56.1µg,

ABA Content (μgkg^{-1})

Days from anthesis

ABA Content (pg grain^{-1})

Days from anthesis
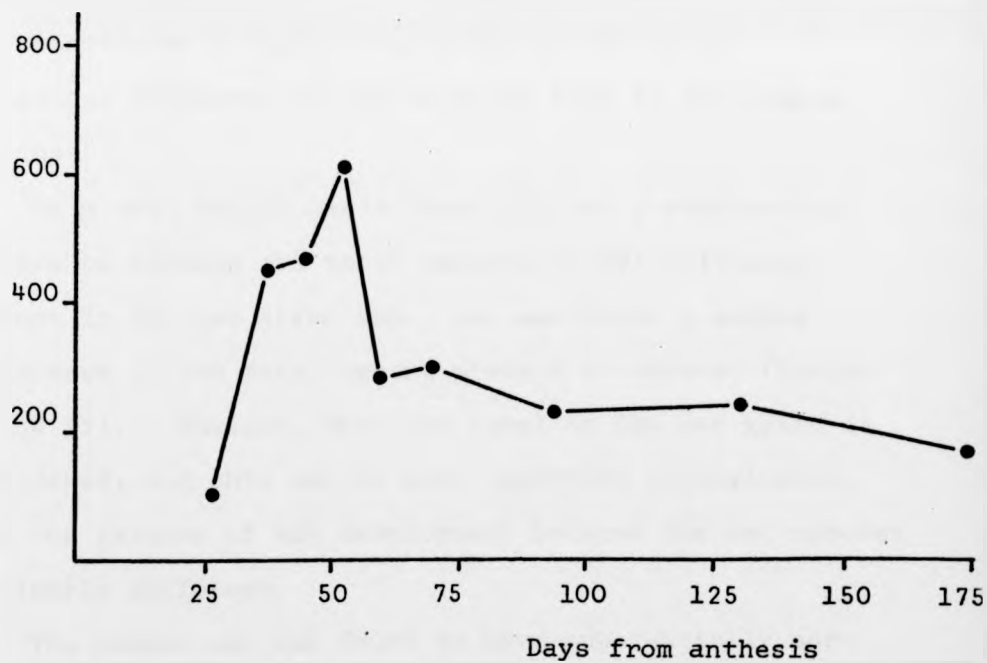
FIGURE 29:   Levels of Free (●), Acid-bound (▲) and
Neutral-bound (■) ABA in grain of *Avena fatua*
during maturation and subsequent dry storage.
Data of Don (private communication)

43.7µg and 18.6µg per kg seed, respectively. These amounts declined to 17.6µg, 3.4µg and 2.6µg per kg seed, respectively, at harvest. On a unit weight of seed basis Figure 29 shows that the level of free ABA in the wild oat decreased 5.5-fold over the maturation and dry storage period.

Considering the relative amounts of ABA in extracts of *A. fatua*, it is interesting that free inhibitor increased from a value of 47 percent after anthesis to 82 percent during dry storage, simultaneous with a decline in bound inhibitor (Table 22). The relative change in neutral-bound ABA was again not marked. Acid-bound ABA decreased from 37 percent at 26 days after anthesis to 12 percent at 174 days from anthesis. It should be noted that free ABA constituted a higher proportion of the total endogenous inhibitor in the wild oat than in the common species.

On a unit weight basis there was not a substantial difference between the total amounts of ABA initially present in the two *Avena* spp., nor was there a marked difference in the total amount present at harvest (Tables 21 and 22). However, when the level of ABA per grain is considered, and this may be more important biologically, then the pattern of ABA development between the oat species is clearly different.

The common oat was found to have substantially more endogenous ABA present during the early stages of development which subsequently declined during maturation (Table 21 and Figure 28). The total ABA content in

individual seeds of *A. sativa* decreased rapidly from 2149pg, as assessed at the first measurement after anthesis to 976pg at 36 days from anthesis. During maturation the inhibitor level was further reduced to 629pg per grain at 57 days. Table 21 indicates that at harvest (63 days) the total ABA content per grain increased to 1304pg and subsequently declined during dry storage.

Similar trends were evident for both acid-bound and neutral-bound fractions during maturation and dry storage of the common oat (Table 21). Changes in the amount of neutral-bound ABA were not marked. The development trend for free ABA was slightly different from the other fractions (Table 21). At 28 days from anthesis the free ABA content of individual seeds was 551pg. Figure 28 shows that this level declined rapidly, subsequently increasing to 391pg at 51 days after anthesis. The free inhibitor then declined during maturation and dry storage to 78pg per grain at 70 days from anthesis.

Compared to *A. sativa* there was initally less ABA present in individual seeds of *A. fatua* at the first measurement after anthesis. Total ABA increased rapidly from 207pg at 26 days to 1109pg at 52 days after anthesis. As shown in Table 22, this amount subsequently declined to 436pg per seed at 59 days from anthesis and decreased a further two-fold during the final stages of maturation and dry storage.

Amounts of free, neutral-bound and acid-bound ABA in individual seeds of the wild oat were 101pg, 32pg and 74pg,

respectively, at the first measurement after anthesis
(Table 22).   At 7 days before harvest the levels had
increased to reach respective maxima of 610pg, 179pg and
320pg.   Thereafter the endogenous fractions rapidly
declined during the final stage of seed maturation and
this process continued in the post harvest period (Table
22).

Thus levels of free ABA per grain start high and
decline in the common oat eventually becoming very low
(Figure 28).   In contrast, it is evident from Figure 29
that in the wild oat free ABA was initially present in a
small amount.   The level increased and subsequently
declined during maturation.   However, at no time during
development in the two species was there an order of
magnitude difference between the respective ABA fractions.
In seeds of both species the amount of free ABA present
peaked at 52 days after anthesis and as desiccation on the
parent plant proceeded the inhibitor level decreased
dramatically.

It is clear from Table 21 that a rapid decline in
endogenous free ABA occurred in the common oat between 28
and 36 days from anthesis, and during this period germinability
increased from about 6 percent to 16 percent.   It could be
postulated therefore that ABA may regulate the germination of
A. sativa cv Selma.   It might be expected that germination
would also occur in the wild oat when the free ABA level
declined.   Although free ABA at harvest had decreased to a
similar amount (about 298pg seed^{-1}) in both species, their
germinabilities were quite different being 65 percent and

O percent for the common and wild oat, respectively (Tables 21 and 22). Additionally, *A. fatua* germination was stimulated by prolonged dry storage during which free ABA did not exhibit a marked decrease (Figure 29).

On the basis of this data endogenous ABA does not appear to be directly involved in the control of oat seed dormancy, unless the two species studied have markedly different responsitivities to the inhibitor. In both *A. sativa* and *A. fatua* an increase in extractable free ABA was associated with periods of rapid grain filling and decreased amounts with the mature desiccated seed. The greatest changes in ABA levels only occurred when the seed was hydrated.

McWha (1975) found that endogenous free ABA in seeds of wheat increased to a maximum during the initial 40 days of development. King (1976) suggested that the dramatic rise (forty-fold) in the ABA content of developing wheat seeds, in the period prior to rapid desiccation, prevented precocious sprouting.

3. VARIATION OF MEDIUM-CHAIN LENGTH FATTY ACID LEVELS IN DEVELOPING SEEDS OF *AVENA SATIVA* cv SELMA AND *AVENA FATUA*

In order to quantify endogenous levels of MCFA in the grain it was necessary to determine losses of individual aliphatic acids, in the series hexanoic to decanoic, incurred as a result of the isolation procedures (Figures 9 and 11). Efficiencies for MCFA extractions were determined by the method of isotope dilution using n- $1\text{-}^{14}C$ -nonanoic

acid and by utilising a standard MCFA solution (Appendix IV).

Tables 23 and 24 express as a running-mean (3 values)
the real MCFA data (Appendix III) for dried seeds of
A. *sativa* cv Selma and A. *fatua*, respectively.   Both species
have similar levels of total MCFA (about 24mg per kg DW)
some 25 days after anthesis.   After this stage of
development the pattern of fatty acid change was markedly
different.

In A. *sativa* total MCFA subsequently declined to a
value of 13.6mg per kg grain at harvest (Table 23).   Seven
days after harvest the level had decreased a further four-
fold and remained at about 4mg per kg DW during prolonged
dry storage.   Table 23 indicates that germination
simultaneously increased from 0 percent at 13 days to 92
percent at 134 days from anthesis.

For the wild oat there was a rapid increase in total
endogenous MCFA after the 25 days of development to a
maximum of 277.14mg per kg seed at harvest (Table 24).
Subsequently the level declined to 36.83mg at 345 days
after anthesis.   Table 24 shows that the MCFA trend for
A. *fatua* was accompanied by a reduction in seed dormancy,
as indicated by the 3 percent germination at 210 days from
anthesis.

Therefore, on a unit weight of dry seed basis the
highest levels of total MCFA occurred in the common oat
during the early stages of seed development and declined
during maturation.   For the wild oat the greatest amounts
of total MCFA were associated with the mature seed of
harvest.

Table 23: The percentage germination and levels of MCFA in grains of *Avena sativa* cv Selma during maturation and subsequent dry storage. The data is corrected for extraction losses and given as a running-mean of three consecutive observations for the dry seed.

UD - undetected

Days from Anthesis	Percentage Germination	Hexanoic			Heptanoic			Octanoic		
		mg kg⁻¹	ng grain⁻¹	Rel Cont	mg kg⁻¹	ng grain⁻¹	Rel Cont	mg kg⁻¹	ng grain⁻¹	Rel Cont
13	0	5.81	106	.17	1.61	27	.05	0.72	15	.02
25	6	4.82	100	.19	1.09	24	.04	0.72	15	.03
35	16	3.86	85	.16	0.87	20	.04	1.01	26	.04
44	31	0.55	19	.04	1.15	47	.09	0.37	15	.03
51	48	0.80	35	.04	1.01	43	.05	0.58	24	.03
57	58	1.50	67	.08	1.03	44	.06	0.43	19	.02
64	65	1.99	90	.15	0.13	6	.01	0.50	23	.04
70	67	2.33	105	.63	0.34	15	.09	0.49	22	.13
84	73	2.29	101	.62	0.38	17	.10	0.44	19	.12
100	83	3.01	131	.67	0.57	25	.13	0.29	13	.06
120	89	2.34	100	.67	0.42	18	.12	0.11	5	.03
134	92	2.12	91	.60	0.58	25	.16	0.25	11	.07
154	91	2.53	107	.58	0.58	25	.13	0.44	19	.10

Table 23 (Continued)

Days from Anthesis	Nonanoic			Decanoic			Total MCFA	
	mg kg^{-1}	ng grain^{-1}	Rel Cont	mg kg^{-1}	ng grain^{-1}	Rel Cont	mg kg^{-1}	ng grain^{-1}
13	8.45	161	.24	18.59	306	.53	35.18	615
25	7.45	152	.29	11.92	262	.46	26.00	553
35	7.11	159	.29	11.89	337	.48	24.74	627
44	1.23	49	.10	9.55	373	.74	12.85	503
51	1.01	42	.05	17.74	769	.84	21.14	913
57	0.44	19	.02	14.57	644	.81	17.97	793
64	0.17	8	.01	10.81	483	.79	13.60	610
70	0.28	12	.08	0.25	11	.34	3.69	165
84	0.39	17	.11	UD	–	–	3.50	154
100	0.62	27	.14	UD	–	–	4.49	196
120	0.64	27	.18	UD	–	–	3.51	150
134	0.57	24	.16	UD	–	–	3.52	151
154	0.78	33	.18	UD	–	–	4.33	184

Table 24: The percentage germination and levels of MCFA in grains of *Avena fatua* during maturation and subsequent dry storage. The data is corrected for extraction losses and given as a running-mean of three consecutive observations for the dry seed.

Days from Anthesis	Percentage Germination	Hexanoic			Heptanoic			Octanoic		
		mg kg^{-1}	ng grain^{-1}	Rel Cont	mg kg^{-1}	ng grain^{-1}	Rel Cont	mg kg^{-1}	ng grain^{-1}	Rel Cont
26	0	0.79	2	.03	0.48	1	.02	0.44	1	.02
37	0	1.69	16	.03	0.87	7	.01	1.06	11	.02
45	0	5.11	76	.05	2.65	39	.03	4.60	75	.05
52	0	8.35	142	.05	10.54	184	.06	10.34	178	.06
59	0	9.65	165	.04	26.14	437	.11	14.09	240	.06
69	0	8.99	151	.03	37.00	609	.13	16.06	268	.06
94	0	8.19	131	.04	35.13	565	.15	14.86	238	.06
130	0	7.31	117	.04	21.23	339	.12	12.93	206	.07
174	0	5.28	84	.04	9.23	147	.07	8.39	133	.07
210	3	2.56	41	.03	2.91	46	.04	4.39	70	.06
345	17	1.29	20	.04	0.91	14	.02	2.03	32	.06

Data of Don (Appendix III/ Table IV) - obtained under parallel conditions

Table 24 (Continued)

Days from Anthesis	Nonanoic			Decanoic			Total MCFA	
	mg kg⁻¹	ng grain⁻¹	Rel Cont	mg kg⁻¹	ng grain⁻¹	Rel Cont	mg kg⁻¹	ng grain⁻¹
26	3.32	8	.15	17.71	42	.78	22.74	54
37	10.83	117	.17	50.35	540	.78	64.80	691
45	24.40	354	.24	63.66	801	.63	100.42	1345
52	54.70	932	.29	101.74	1663	.55	185.69	3099
59	77.05	1310	.33	105.38	1797	.45	232.31	3949
69	83.38	1397	.30	131.71	2202	.48	277.14	4627
94	60.57	974	.26	112.21	1801	.49	230.96	3709
130	41.27	659	.23	98.96	1580	.54	181.70	2901
174	24.07	383	.20	76.33	1214	.62	123.30	1961
210	14.17	225	.18	53.41	848	.69	77.44	1230
345	3.55	56	.10	29.05	459	.79	36.83	581

Table 24 (Continued)

Days from Anthesis	Nonanoic			Decanoic			Total MCFA	
	mg kg^{-1}	ng grain^{-1}	Rel Cont	mg kg^{-1}	ng grain^{-1}	Rel Cont	mg kg^{-1}	ng grain^{-1}
26	3.32	8	.15	17.71	42	.78	22.74	54
37	10.83	117	.17	50.35	540	.78	64.80	691
45	24.40	354	.24	63.66	801	.63	100.42	1345
52	54.70	932	.29	101.74	1663	.55	185.69	3099
59	77.05	1310	.33	105.38	1797	.45	232.31	3949
69	83.38	1397	.30	131.71	2202	.48	277.14	4627
94	60.57	974	.26	112.21	1801	.49	230.96	3709
130	41.27	659	.23	98.96	1580	.54	181.70	2901
174	24.07	383	.20	76.33	1214	.62	123.30	1961
210	14.17	225	.18	53.41	848	.69	77.44	1230
345	3.55	56	.10	29.05	459	.79	36.83	581

For individual fatty acids, levels of hexanoic, nonanoic
and decanoic acids in *A. sativa* displayed the greatest
variation (Table 23 and Figure 30).   Nonanoic acid was
assessed at 8.45mg per kg DW at 13 days after anthesis,
before germination was detected in the sample.   This level
subsequently decreased eight-fold to 1.01mg at 51 days,
seed germination being then 48 percent.   Figure 30 shows
that after a further two-fold reduction nonanoic acid
remained at about 0.5mg per kg dry seed.   Decanoic acid
was assessed at 18.59 mg per kg grain at the first measurement
after anthesis (Table 23).   This level declined and then
increased to peak at 17.74mg after 51 days.   During
subsequent maturation and dry storage decanoic acid
decreased to levels which could not be detected.

In *A. fatua* the greatest variation occurred with
levels of heptanoic, nonanoic and decanoic acids (Table 24).
The trends were similar to that of total MCFA.   Hexanoic
and octanoic acids did not vary markedly.   Nonanoic acid
increased rapidly from 3.32mg per kg seed at 26 days from
anthesis to a maximum of 83.38mg at harvest.   Figure 31
indicates that the level subsequently decreased to 3.55mg
per kg DW at 345 days after anthesis.

Endogenous amounts of decanoic acid were in the order
of 2-5 times higher than those for nonanoic but followed a
similar development trend (Table 24).   The level increased
by 2.65mg per day to peak at harvest (131.71mg per kg),
following which it declined at about 0.37mg per day to a low
amount after five months dry storage (Figure 31).   It is
evident from Table 24 that substantially decreased amounts

MCFA Content (mg kg^{-1} seed)

FIGURE 30: The levels of nonanoic (▲) and decanoic (■) acids in grains of *Avena sativa* cv. Selma during maturation and subsequent dry storage.

MCFA Content (mg kg^{-1} seed)
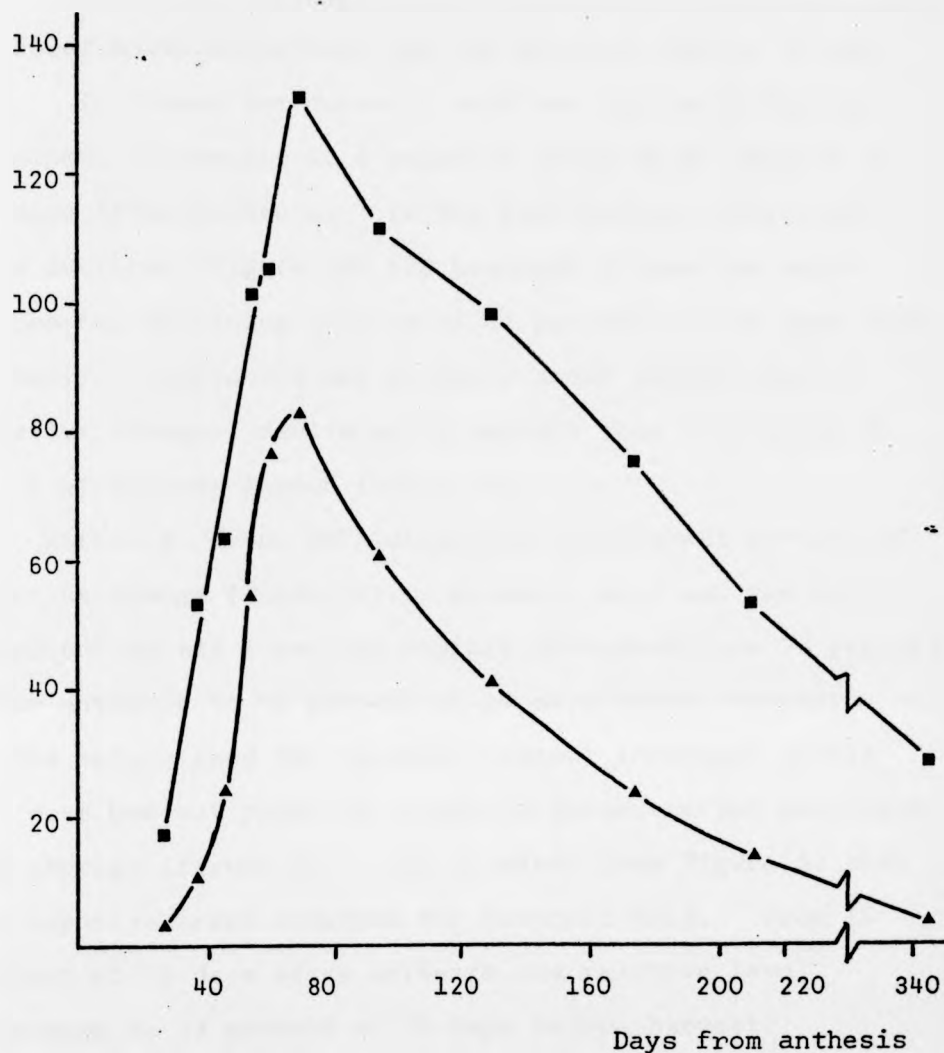
FIGURE 31:   The levels of nonanoic (▲) and decanoic (■) acids in
grains of *Avena fatua* during maturation and
subsequent dry storage.

Data of Don (Appendix III/ Table IV)

of MCFA were associated with non dormant grain.

Variation in the relative contents of individual acids differed markedly between the two species (Tables 23 and 24). In common oat decanoic acid was initially the most prominent increasing to a relative level of 84 percent at 51 days after anthesis. In the post harvest phase this acid declined (Figure 32) and hexanoic became the major component, attaining a value of 67 percent at 120 days from anthesis. Heptanoic and nonanoic acids showed similar relative changes, declining to harvest then increasing in the post harvest period (Table 23).

Within *A. fatua* MCFA displayed a different pattern of relative change (Table 24). Decanoic acid was the major component at all times and rapidly decreased from 78 percent after anthesis to 45 percent at 10 days before harvest. In the mature seed the relative content increased during the post harvest phase to attain 79 percent after prolonged dry storage (Figure 33). It is clear from Figure 33 that the opposite trend occurred for nonanoic acid. From 15 percent at 26 days after anthesis the relative level increased to 33 percent at 10 days before harvest. Thereafter the acid declined to the initial levels during dry storage.

In the wild oat relative levels of heptanoic acid showed a pattern similar to that of nonanoic, but in this species hexanoic, heptanoic and octanoic acids were all minor components (Table 24). In neither *Avena* spp. was octanoic acid present in relatively large amounts (Tables 23 and 24).

Relative Content

FIGURE 32:   The relative levels of nonanoic (▲) and decanoic (■) acids in grains of *Avena sativa* cv. Selma during maturation and subsequent dry storage.

Relative Content

FIGURE 33: The relative levels of nonanoic (▲) and
decanoic (■) acids in grains of *Avena fatua*
during maturation and subsequent dry storage.

Data of Don (Appendix III/ Table IV)

It is probably most meaningful biologically to consider the levels of MCFA per grain. On this basis the greatest amounts of total fatty acid occurred in the common oat during the later stages of seed maturation (Table 23). The acids increased to a maximum of 913ng at 51 days after anthesis, following which they declined to about 150ng per grain during dry storage.

As shown in Table 23, at the first measurement after anthesis 306ng of decanoic acid were assessed within individual seed of *A. sativa*. The acid rapidly increased to peak at 51 days from anthesis (769ng per seed). During subsequent maturation and dry storage the level declined dramatically to become undetectable (Figure 34).

It is clear from Figure 34 that the development pattern for nonanoic acid was quite different. The highest level was detected in cultivated oat after anthesis (161ng per grain) when no germination was evident. By harvest the acid level had rapidly declined to 8ng at which point germination was 65 percent (Table 23). During dry after-ripening the acid increased slightly to about 25ng per seed. This was accompanied by a further relaxation in seed dormancy, germination being 92 percent at 134 days.

Amounts of hexanoic, heptanoic and octanoic acids per seed did not vary markedly during maturation and dry storage of the common oat, although heptanoic showed a marked maximum at 57 days from anthesis (Table 23).

On an individual seed basis the development trend for MCFA in wild oat was unlike that obtained for common oat (Table 24). The acid level increased from 54ng after
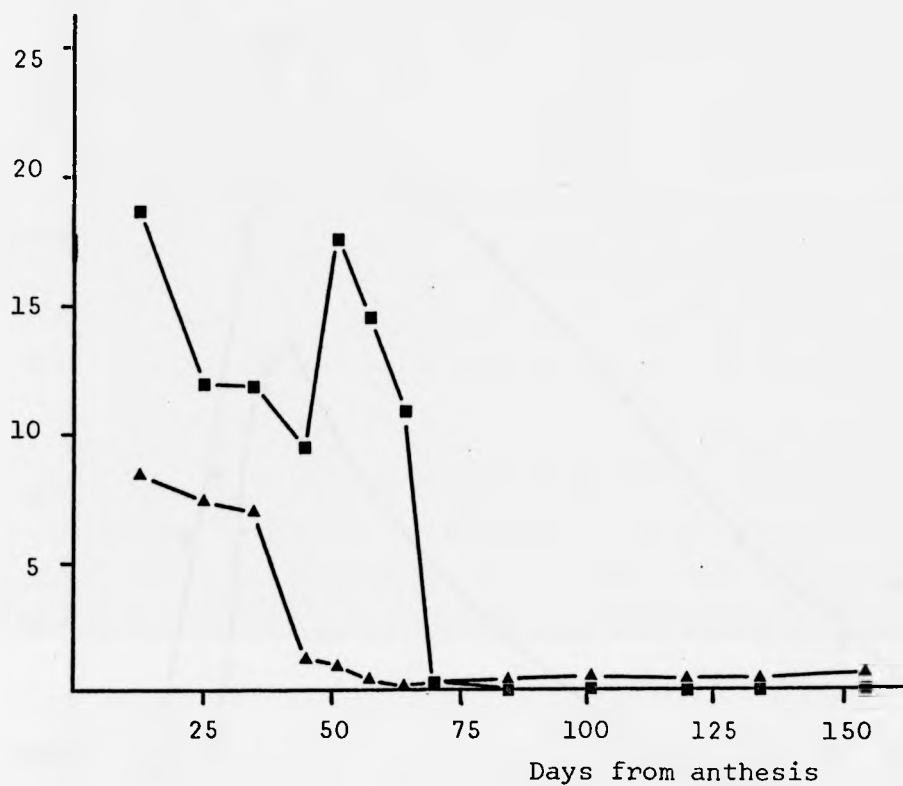
MCFA Content (ng grain^{-1})
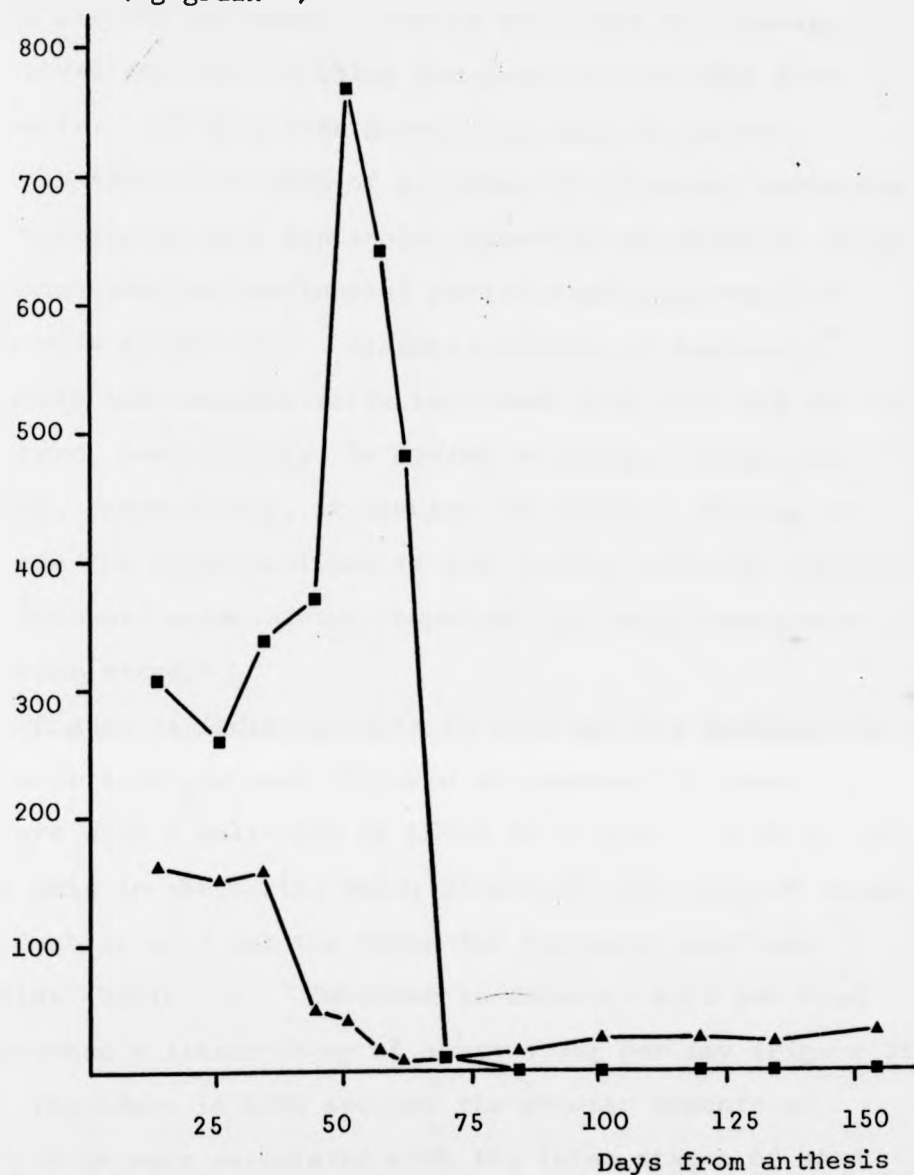
FIGURE 34: The levels of nonanoic (▲) and decanoic (■) acids in individual grains of *Avena sativa* cv. Selma during maturation and subsequent dry storage.

anthesis to 4627ng at harvest, a rate of approximately
106ng per day per seed.   During prolonged dry storage
the level declined to 581ng per grain at 345 days from
anthesis.   At this time germination was 17 percent.

In individual seed of *A. fatua* the greatest variation
was associated with heptanoic, nonanoic and decanoic acids,
although similar development patterns were observed for
each MCFA (Table 24).   Assessed amounts of heptanoic,
nonanoic and decanoic acids increased from 1ng, 8ng and 42ng
per seed, respectively, to maxima of 609ng, 1397ng and
2202ng, respectively, at harvest (67 days).   During dry
storage the acids declined to low levels, although nonanoic
and decanoic acids always comprised the major components of
the seed extract.

Figure 35 indicates that in wild oat the decrease of
nonanoic acid per seed followed an exponential decay
pattern with a half-life of about 56.5 days.   This is the
only acid in the series which displayed this type of decay,
although in wild oat the trend for heptanoic acid was
similar (Table 24).   Decrease in decanoic acid per seed
approached a linear decay of about 6.3ng per day (Figure 35).

Therefore in both species the greater amounts of
total MCFA were associated with the later stages of seed
desiccation.   Compared with *A. sativa*, *A fatua* had lower
endogenous amounts of MCFA during the early stages of
grain filling (Tables 23 and 24).   However, as the grains
matured and enlarged the acid levels increased dramatically
until at harvest wild oat contained far more (7.5-fold)
MCFA than the cultivated oat.   On a unit weight of seed
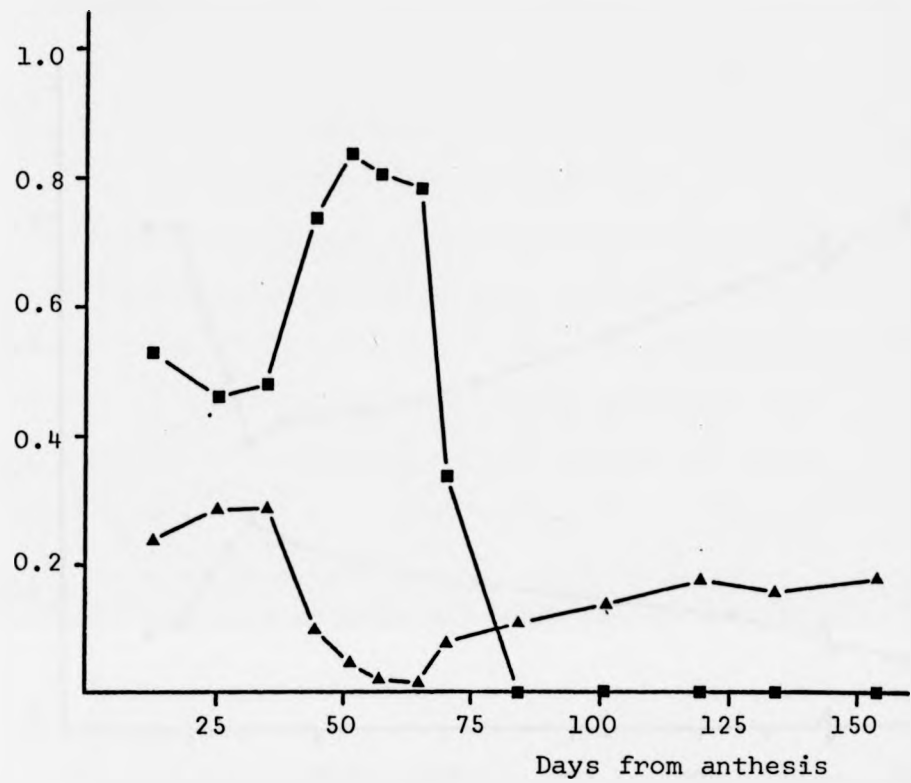
MCFA Content (ng grain^{-1})
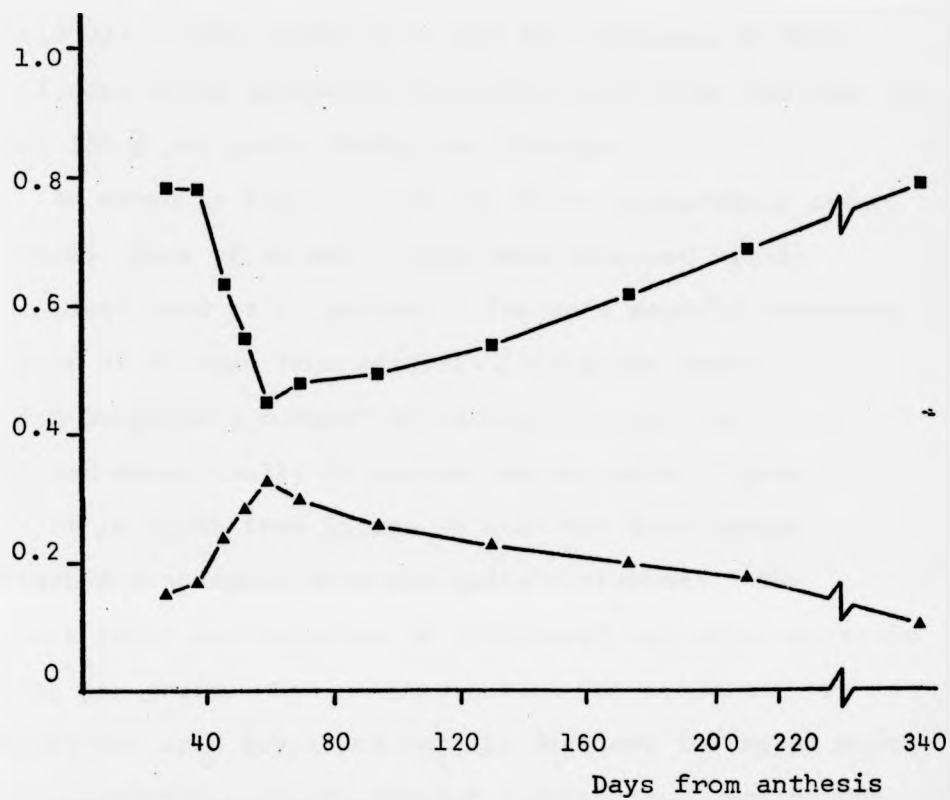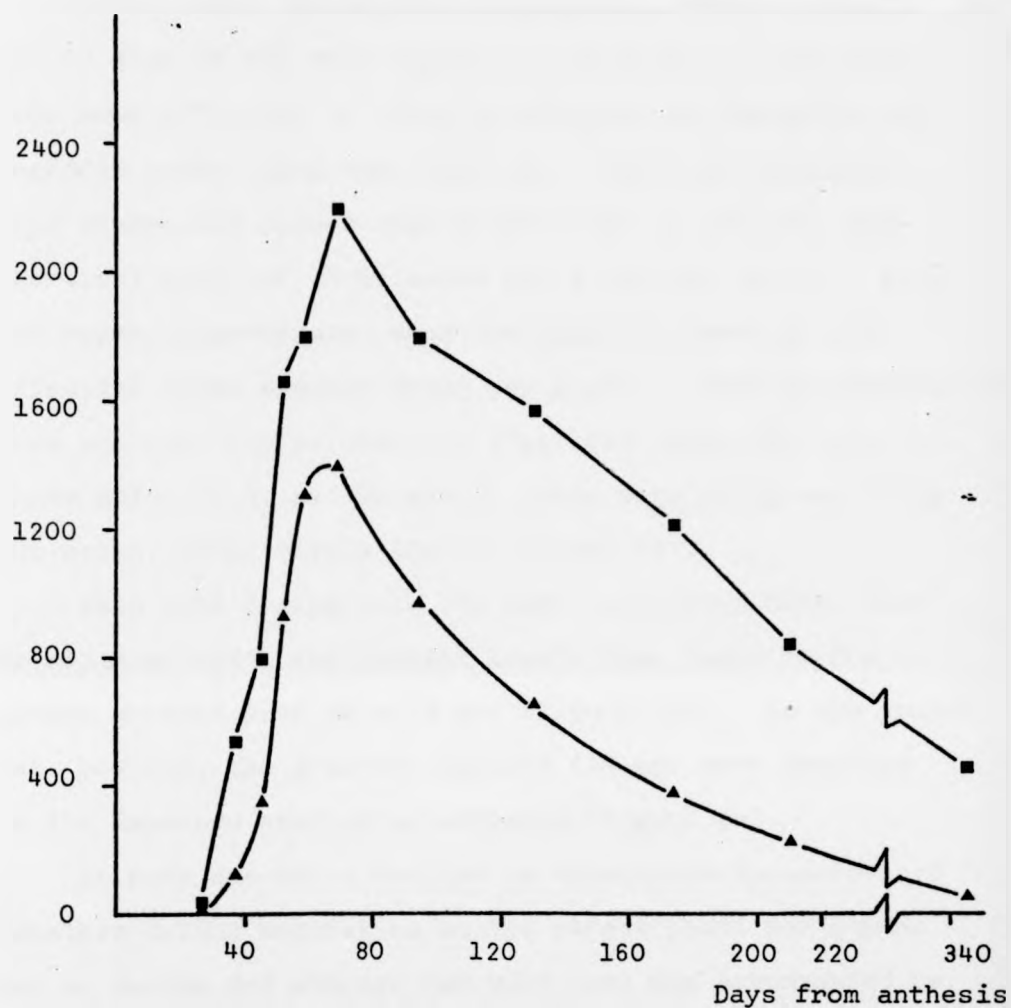

FIGURE 35:   The levels of nonanoic (▲) and decanoic (■)
             acids in individual grains of *Avena fatua*
             during maturation and subsequent dry storage.

             Data of Don (Appendix III/ Table IV)

basis there was a 20-fold difference between MCFA contents
of the species at harvest.

It has been shown previously in this study (Parts 2
and 6) that of the MCFA heptanoic, octanoic and nonanoic
were most effective in reducing germination, hexanoic and
decanoic acids being much less so.   Table 23 indicates
that common oat showed some germination (6 percent) when
the total level of these acids was 191ng per grain.   Wild
oat began to germinate when the combined level of the
effective acids reached 341ng per grain.   Similar germinations
were achieved (16 percent and 17percent) when the level of
these acids in *A. sativa* and *A. fatua* were 205ng and 102ng
per grain, respectively (Tables 23 and 24).

When considering only the most inhibitory MCFA, that
is nonanoic acid, the highest levels were found in the
mature dormant seed of wild oat (Figure 35).   In the common
oat, however, the greatest amounts (161ng) were detected
in the immature seed after anthesis (Figure 34).

In both species a decline in endogenous nonanoic acid
(whether during maturation on the parent plant for common
oat or during dry storage for wild oat) was accompanied by
increased germination.   After prolonged dry storage (about
9 months) the nonanoic acid level (56ng per grain) in wild
oat giving 17 percent germination was found to be comparable
with the acid level (159ng per grain) in common oat giving
16 percent germination.

Thus while it may not be possible to correlate ABA
content with the physiological state of the seed an excellent
correlation can be made between fatty acid content and
germinability.

PART 11: THE INHIBITION OF GIBBERELLIN-INDUCED AMYLOLYSIS
IN BARLEY ENDOSPERM BY MEDIUM-CHAIN LENGTH
FATTY ACIDS

It was of interest to determine the effect of MCFA and
ABA on the liberation of reducing sugars from embryo-free
half seeds of barley. It is known that ABA can markedly
inhibit this process (Addicott *et al.* 1966).

It is clear from Figure 36 that in the presence of
gibberellic acid (4.2 x 10^{-7}M) and water half seeds of
H. vulgare cv Ymer released substantial amounts (6.66mg
glucose equivalents) of simple carbohydrates into the
incubation medium. In the absence of gibberellin much
reduced sugar levels (0.33mg glucose equivalents) were
detected in the medium (Figure 36).

The reducing sugar content of media were estimated by
a colorimetric assay (P33). The amount of simple
carbohydrate associated with each treatment of paired
barley half seeds was calculated in mg per glucose
equivalents by direct comparison with standard glucose
solutions. Figure 36 represents the influence of saturated
fatty acids (acetic to undecanoic), at concentrations of
10^{-3}M and buffered to pH 4.8, on gibberellin-induced
amylolysis in barley endosperm. The endosperm pieces were
incubated in pairs for 42 hours at 26°C in the presence of
gibberellic acid (4.2 x 10^{-7}M).

At 10^{-3}M both valeric and nonanoic acids were found to
be most effective in reversing the stimulatory influence of
gibberellin. The amount of reducing sugar released into
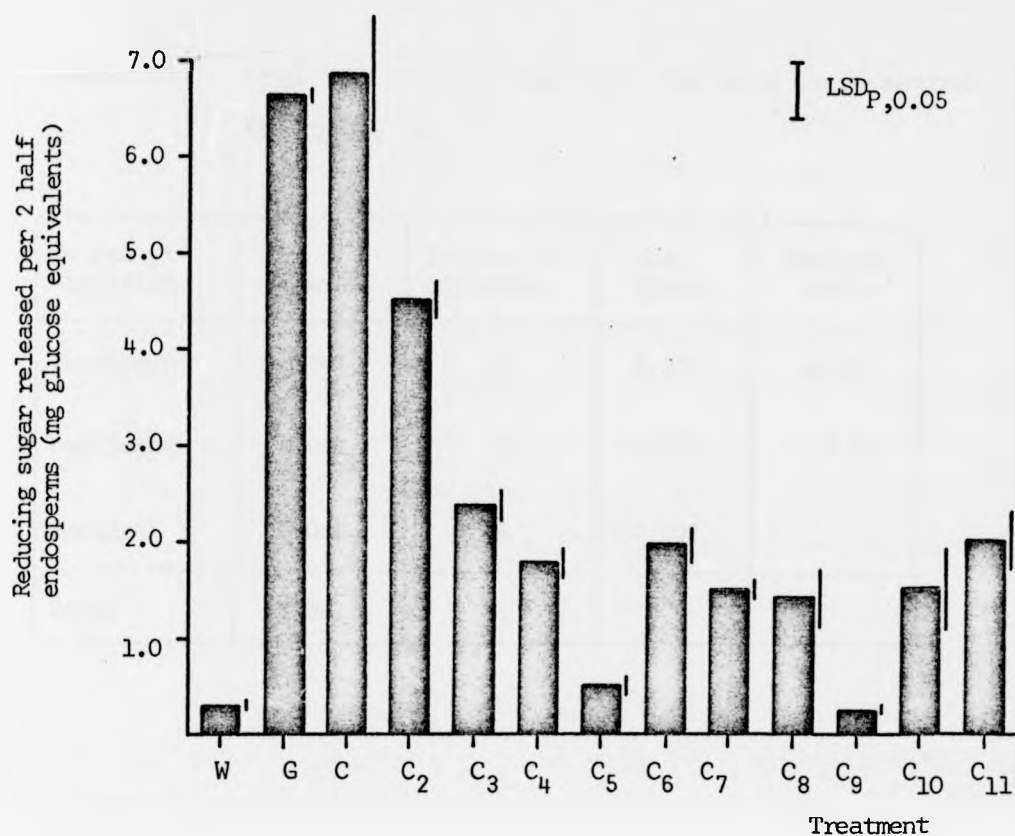
W - water alone

G - 4.2×10^{-7}M Gibberellin in water

C - 4.2×10^{-7}M Gibberellin in 10^{-3}M citrate buffer, pH 4.8

C_2-C_{11} - 4.2×10^{-7}M Gibberellin in 10^{-3}M acetate (C_2), propionate (C_3), ..., undecanoate (C_{11}) buffers, all at pH 4.8.

FIGURE 36:   Influence of 10^{-3}M medium-chain length fatty acids on the gibberellin-induced amylolysis of barley endosperm.

The values represent the amount of reducing sugar released into the incubation medium by embryo-free half endosperms incubated in pairs (4 replicates) with MCFA and gibberellic acid for 42 hours at 26°C.

TABLE 25:   Analysis of variance for the data represented
in Figure 36.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Treatments	2.741	12	0.228	114.00
Replicates	0.022	3	0.007	3.50
Residual	0.098	36	0.002	
Total	2.861	51		

the medium was assessed as 0.52mg and 0.27mg glucose equivalents for valerate and nonanoate, respectively (Figure 36). The sugar content of the citrate treatment (6.87mg) was not significantly different from that of the water control (6.6mg).

Table 25 indicates that at a concentration of 10^{-3}M highly significant differences occurred between the acid treatments. At a probability of 0.05 variation between the four replicate treatments (10^{-3}M) was also found to be significant. Inconsistent replication is also indicated by the large standard errors associated with some treatment means (Figure 36).

The influence of the most effective MCFA was superimposed on a general enhancement with increasing backbone number of the inhibition of sugar production obtained with the acetic acid treatment (Figure 36). The maximum biological activity was evident for nonanoate with subsequent decreasing inhibition at longer chain lengths (decanoic and undecanoic acids). All aliphatic acids tested inhibited amylolysis to some extent, even acetate caused a 30 percent reduction in sugar release from paired half seeds, a phenomenon first noted by Paleg (1960).

Figure 37 represents the effect of MCFA and ABA at concentrations of 10^{-4}M and buffered to pH4.8 on the release of reducing sugars from barley endosperm pieces. At this concentration ABA was found to be the most effective for reversing the gibberellin effect. The amount of sugar released into the incubation medium (0.26mg) was lower than that obtained with water alone (0.33mg).
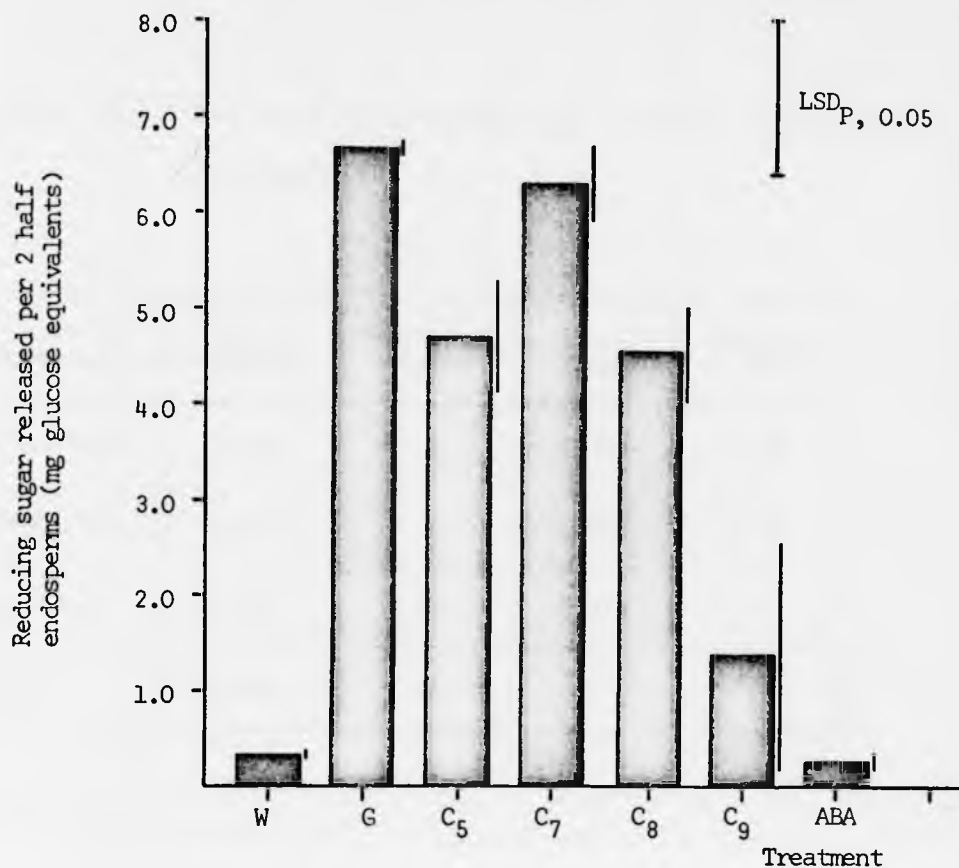
W - Water alone

G - 4.2×10^{-7} M Gibberellin in water

C_5-ABA - 4.2×10^{-7} M Gibberellin in 10^{-4} M

      valerate(C_5), heptanoate (C_7), ..., abscisate (ABA) buffers, all

      at pH 4.8.

FIGURE 37: Influence of 10^{-4} M medium-chain length fatty acids and
abscisic acid on the gibberellin-induced amylolysis of
barley endosperm. The values represent the amount of
reducing sugar released into the incubation medium by
embryo-free half endosperms incubated in pairs (4 replicates)
with gibberellic acid and either MCFA or ABA for 42 hours at
26°C.

TABLE 26:   Analysis of variance for the data represented in Figure 37.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Treatments	2.146	6	0.358	22.38
Replicates	0.014	3	0.005	0.31
Residual	0.289	18	0.016	
Total	2.449	27		

However, at a probability of 0.05 the nonanoate treatment (1.36mg glucose equivalents released) was not significantly different from the ABA value (Figure 37). At 10^{-4}M valerate, heptanoate and octanoate treatments only partially reversed the influence of gibberellic acid. Thus highly significant differences were detected between the acid treatments at this concentration (Table 26).

Dose response data for ABA and the four most inhibitory fatty acids (valeric, heptanoic, octanoic and nonanoic) are represented in Figures 38, 39, 40, 41 and 42. All treatments were buffered to pH 4.8 and incubated with 4.2×10^{-7}M gibberellic acid. The corresponding analyses of variance (Tables 27, 28, 29, 30 and 31) show that highly significant treatment differences occurred for the dilution range of each inhibitor. Variation between replicate treatments was not significant.

It is clear from the histograms that nonanoate was the most effective fatty acid treatment for preventing reducing sugar release from endosperm pieces (Figure 42). At 10^{-5}M, and below, all MCFA were ineffective inhibitors of gibberellin-induced amylolysis in barley half seeds. At these concentrations the fatty acid treatments were not significantly different (probability 5 percent) from a water control (6.66mg glucose equivalents released).

At 10^{-4}M MCFA were, in all cases, only partially effective in reversing the gibberellin effect. However, the difference between treatments of nonanoate (1.36mg glucose equivalents released) and water alone (0.33mg released) was not significantly different at a probability
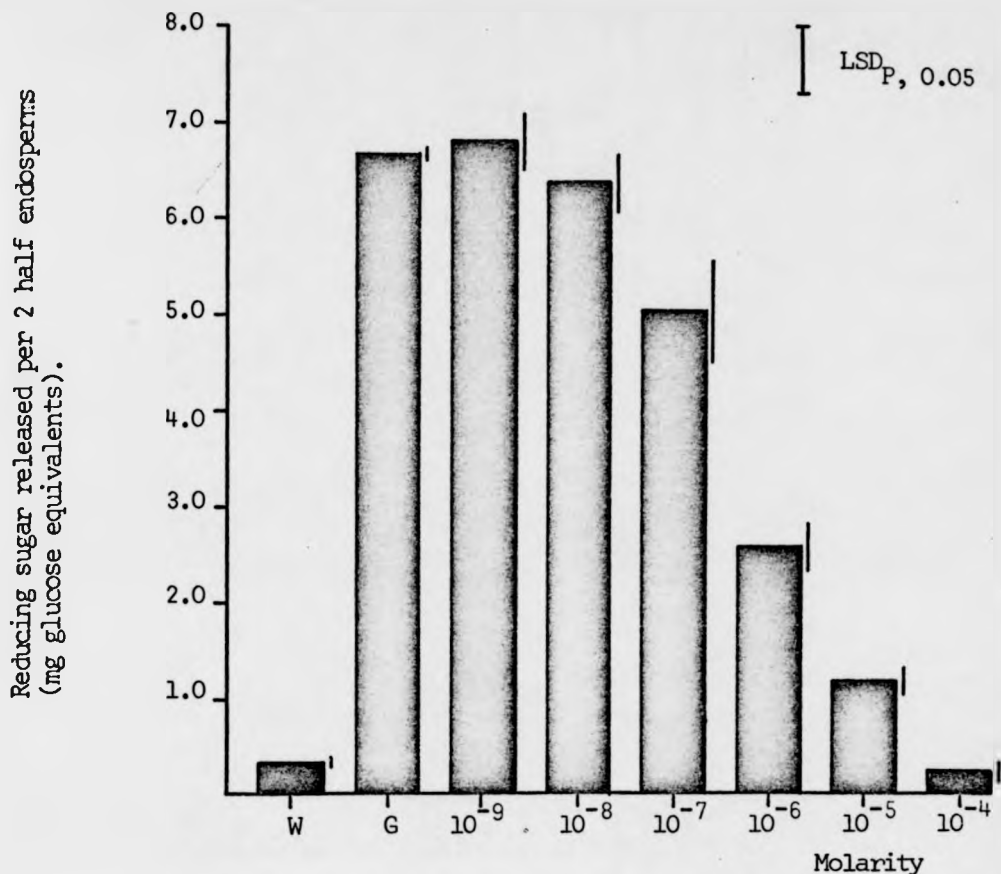
W – water alone

G – 4.2×10^{-7} M Gibberellin in water

10^{-9}-10^{-4} – 4.2×10^{-7} M Gibberellin in 10^{-9}-10^{-4}M   abscisate buffers, all at pH 4.8.

FIGURE 38:   The concentration dependence of abscisic acid inhibition of gibberellin-induced amylolysis in barley endosperm.   The values represent the amount of reducing sugar released into the incubation medium by embryo-free half endosperms incubated in pairs (4 replicates) with gibberellic and abscisic acids for 42 hours at 26°C.
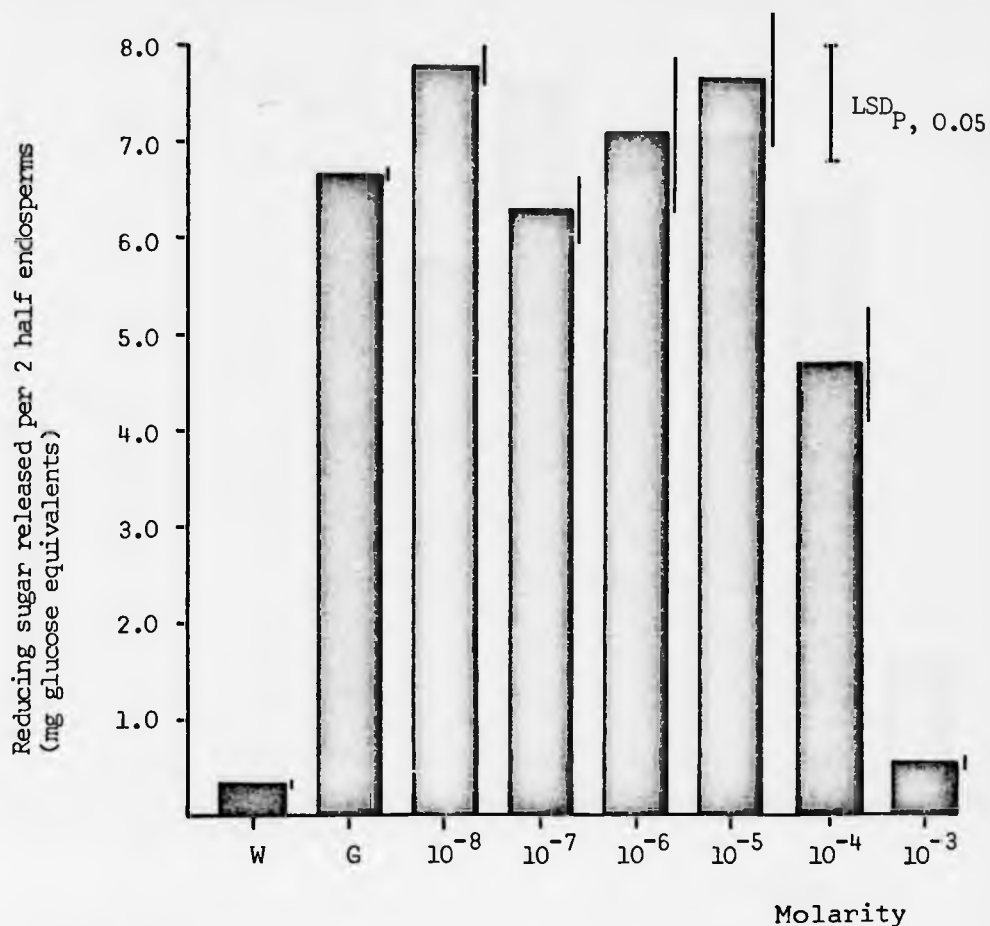
W - Water alone

G - 4.2×10^{-7} M Gibberellin in water

10^{-8}-10^{-3} - 4.2×10^{-7} M Gibberellin in 10^{-8} M to 10^{-3} M valerate buffers, all at pH 4.8.

FIGURE 39: The concentration dependence of valeric acid inhibition of gibberellin-induced amylolysis in barley endosperm. The values represent the amount of reducing sugar released into the incubation medium by embryo-free half endosperms incubated in pairs (4 replicates) with gibberellic and valeric acids for 42 hours at 26°C.

W - Water alone

G - 4.2 x 10^{-7}M Gibberellin in water

10^{-8} - 10^{-3} - 4.2 x 10^{-7}M Gibberellin in 10^{-8}M to 10^{-3}M heptanoate

buffers, all at pH 4.8.

FIGURE 40:   The concentration dependence of heptanoic acid inhibition

of gibberellin-induced amylolysis in barley endosperm.

The values represent the amount of reducing sugar released

into the incubation medium by embryo-free half endosperms

incubated in pairs (4 replicates) with gibberellic and

heptanoic acids for 42 hours at 26°C.

W - Water alone

G - 4.2x10^{-7}M Gibberellin in water

10^{-8}-10^{-3} - 4.2x10^{-7}M Gibberellin in 10^{-8}M to 10^{-3}M octanoate buffers, all at pH 4.8.

FIGURE 41:   The concentration dependence of octanoic acid inhibition of gibberellin-induced amylolysis in barley endosperm.   The values represent the amount of reducing sugar released into the incubation medium by embryo-free half endosperms incubated in pairs (4 replicates) with gibberellic and octanoic acids for 42 hours at 26°C.

W - Water alone

G - 4.2x10^{-7}M Gibberellin in water

10^{-8}-10^{-3} - 4.2x10^{-7}M Gibberellin in 10^{-8}M to 10^{-3}M nonanoate buffers, all at pH 4.8.

FIGURE 42:   The concentration dependence of nonanoic acid inhibition of gibberellic-induced amylolysis in barley endosperm.   The values represent the amount of reducing sugar released into the incubation medium by embryo-free half endosperms incubated in pairs (4 replicates) with gibberellic and nonanoic acids for 42 hours at 26°C.

TABLE 27:   Analysis of variance for the data represented in Figure 38.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Treatments	2.754	7	0.393	119.61
Replicates	0.014	3	0.005	1.67
Residual	0.069	21	0.003	
Total	2.837	31		

TABLE 28: Analysis of variance for the data represented
in Figure 39.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Treatments	3.084	7	0.441	49.9
Replicates	0.056	3	0.019	2.11
Residual	0.186	21	0.009	
Total	3.326	31		

TABLE 29:   Analysis of variance for the data represented
in Figure 40.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Treatments	2.441	7	0.349	34.90
Replicates	0.036	3	0.012	1.20
Residual	0.207	21	0.010	
Total	2.684	31		

TABLE 30:   Analysis of variance for the data represented

in Figure 41.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Treatments	2.810	7	0.401	22.28
Replicates	0.028	3	0.009	0.50
Residual	0.369	21	0.018	
Total	3.207	31		

TABLE 31: Analysis of variance for the data represented in Figure 42.

Source of Variation	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Treatments	3.174	7	0.453	22.65
Replicates	0.049	3	0.016	0.94
Residual	0.354	21	0.017	
Total	3.577	31		

of 0.05 (Figure 37).

The most striking feature of the data is that difference between the dose-response curves for MCFA and ABA. Abscisate inhibition is total at a concentration of 10^{-4}M (0.26mg glucose equivalents released) but absent at 10^{-8}M (6.37mg released). At intermediate concentrations the response is proportional to the logarithm of the concentration (Figure 38). On the other hand for the most effective acids, MCFA inhibition was total at a concentration of 10^{-3}M (0.27mg sugar released for nonanoate) but non-existent at 10^{-5}M (6.73mg released for nonanoate), Figure 42. Such a response may be represented as a threshold effect (Figures 39 to 42).

Experiments involving barley endosperm pieces did not differentiate between several possible inhibitory actions of MCFA in this system. Such acid effects may be associated with the production of gibberellin-induced hydrolase, with the actual hydrolase activity *in vitro* or with a possible acid-induced modification of reserve starch structure. This latter action may increase the substrates resistance to enzymic attack.

By utilising isolated barley aleurone layers it was possible to examine MCFA inhibition of gibberellin-induced synthesis and release of hydrolase from this secretory tissue. Any interaction between MCFA and endosperm starch could also be estimated.

It has been found (Buller and Reid, unpublished), in experiments carefully controlled to eliminate fatty acid effects upon amylase activity in the assay system,

that MCFA with chain lengths of seven, eight and nine carbon atoms completely inhibit the release of amylase activity from gibberellin-treated aleurone layers of *H. vulgare* cv Ymer at a concentration of 5×10^{-4}M. This suggests that MCFA can interfere, directly or indirectly, with amylase induction or release.

However, the aliphatic acids have also been determined to inhibit the activity of amylolytic enzymes. Nonanoate at 10^{-3}M reduced by approximately 60%, relative to a citrate control, the rate of production of reducing equivalents from a solution of potato starch by crude, soluble hydrolase prepared from germinated barley (Harris and Reid, unpublished). Thus it would appear that MCFA can inhibit both the production and activity of hydrolase from gibberellin-treated barley aleurone layers.

CHAPTER IV

DISCUSSION

DISCUSSION

The regulatory mechanisms which induce and maintain
the dormancy of those seeds manifesting a resting-condition
during their life-cycle remain obscure, although many
theories have been proposed to account for the phenomenon
of innate seed dormancy.

It has been suggested that the condition results
from a restricted gas supply to the embryo.   If a suitable
gas regime is not available then certain biochemical
pathways may become switched to mechanisms which actively
prevent germination.   In most cases this process is not
the *a priori* cause of dormancy, although it may reinforce
the innate condition of suspended growth.

Removal of hulls can stimulate the germination of
dormant *A. fatua* (Baker and Leighty 1958, Hay and Cumming
1959) and *A. sativa* (Schwendiman and Shands 1943).
Germination of abeyant wild oat grains can also be enhanced
by exposure to an increased oxygen pressure (Johnson 1935a,
Baker and Leighty 1958) or by treatments which rupture the
seed coat (Naylor and Christie 1957).

Although the covering structures must influence the
germination process, regulatory mechanisms other than
gaseous diffusion are apparent in delayed germination
(Naylor and Christie 1957, Simmonds and Simpson 1971).
Current evidence suggests that the dormancy regulating
mechanism lies within the embryo itself.   Johnson (1935b)
demonstrated by genetic work involving the crossing of
A. fatua and *A. sativa* strains, that the resting condition

was an inherited recessive character solely a property of the embryo and/or endosperm. Simpson (1965) and Andrews and Simpson (1969) have reported the presence of true-embryo dormancy in wild oat. The inception of dormancy usually takes place on the parent plant.

In the genus *Avena* there is a wide range of dormancy patterns. The cultivated oat (*A. sativa*) may display a post harvest dormancy of only a few weeks duration which can be easily overcome by a period of warm dry storage. The Spring wild oat (*A. fatua*) exhibits a prolonged dormancy period and may require after-ripening for up to three years in order to encourage maximum germination (Banting 1966). The first and larger (primary) propagule from a normal 2-seeded spikelet of *A. fatua* is much less dormant than the smaller distal seed and may germinate at low temperatures soon after dispersal (Johnson 1935a). The Winter wild oat (*A. ludoviciana*) also displays a period of profound dormancy, especially regarding the upper grain of the disseminule (Morgan and Berrie 1970).

The germinability of *A. fatua* seeds may be influenced by several factors including age of the grain (Johnson 1935a), conditions of storage (Thurston 1962), their sensitivity to light (Hart and Berrie 1966) and the ambient temperature at which they are imbibed. Wild oats may germinate between 2-35°C (Koch 1968). Chancellor and Peters (1972) found that under natural conditions emergence occurred when the soil temperature rose to 6-7°C.

Recently it has become evident that plant-growth regulators play an important role in the vegetative kingdom.

It is through the mediation of such substances that plants may translate environmental cues, such as photoperiod, temperature or water stress, into the internal regulatory control mechanisms of growth and development. Current hypotheses attribute the dormant condition to endogenous hormonal influences which regulate its nature and extent.

Much literature has been concerned with the endogenous inhibitor content of various seed species during treatments which terminate an innate resting condition. Several authors have correlated germinability with a decline of inhibitor levels within dormant seeds (Wareing 1965). Abscisic acid has particularly been implicated in the regulation of seed dormancy (Addicott and Lyon 1969).

Many researchers, however, have been unable to obtain such a correlation and it has been suggested that substances which promote germination, such as gibberellins and cytokinins, are produced within the seed during dormancy-breaking treatments. These promoters would antagonise endogenous inhibitory influences and thereby stimulate emergence (Villiers and Wareing 1960, Frankland and Wareing 1966). Dormancy-breaking treatments, such as stratification, may alter a critical balance between promoters and inhibitors of growth in favour of the former chemicals, so that germination ensues. Amen (1968) suggested that seed dormancy in all species depends to some extent on this inhibitor-promoter mechanism.

Many physiological effects of ABA within the plant kingdom have been well documented (Addicott and Lyon 1969, Millborrow 1974). This chemical has also been found in

many seeds and fruits.   Exogenous treatment of seeds with
ABA may prolong dormancy or induce this condition in non-
dormant species (Sondheimer and Galson 1966, Gabr and
Guttridge 1968, Holm and Miller 1972).   A continuous supply
of inhibitor is required for the response to be maintained
(Summer and Lyon 1967).

The presence of this potent plant-growth regulator
in dormant seeds of numerous species has led to the claim
that it may function in the control of delayed germination,
by interacting with stimulatory plant hormones (Villiers
1968, Khan 1975).

Some of the literature does not concur with a
regulatory role for ABA.   Several authors have been unable
to account for the inhibitory activity of a plant extract
by ABA content alone (Dorffling 1970, Webb and Wareing
1972).   When subjected to treatments which broke dormancy,
the germinability of lettuce seeds did not always correlate
with a decrease in levels of endogenous ABA (Braun and Khan
1975, Berrie and Robertson 1976).   Bex (1972) suggested
that the inhibitor did not prevent initiation of lettuce
seed germination only subsequent growth of the radicle.
ABA treatment did not prevent light-induced rupture of the
husks and initial radicle enlargement in *Chenopodium album* L.
(Karssen 1976).

Most *et al* (1970) proposed that ABA was the principle
inhibitory substance in immature carob fruit;   Corocoran
(1970) did not support this finding.   Such contradictory
reports probably result from the use of differing plant
material and indicate that enormous variation in endogenous
ABA can occur between related tissues subjected to different

environmental conditions or modes of storage.

In the present study abscisic acid was not an efficient inhibitor of *A. sativa* germination (Results, Part 9). The acid appeared to act as a potent inhibitor of both radicle and coleoptile growth after initial rupture of the seed-coats. Ando and Tsukamoto (1974) reported that the efficacy of ABA as an inhibitor of *Avena* coleoptile growth was not absolute even at a concentration of 10^{-3}M. It would be difficult therefore to propose a regulatory role for ABA in the dormancy of this species.

Having detected highest levels of ABA in maturing seeds of wheat, King (1976) suggested that the endogenous inhibitor prevented precocious germination in that period prior to seed desiccation. A similar hypothesis may be proposed for both *A. sativa* cv Selma and *A. fatua*.

Evidence of a regulatory role for ABA in seed dormancy has been provided by disseminules which require a period of stratification in order to stimulate germination. Putative amounts of the acid have been correlated with germinability in several species (Irving 1968, Martin *et al* 1969, Rudnicki *et al* 1969, Willemson and Rice 1972, Petrova and Nikolaeva 1974).

However, some literature does not corroborate such a hormonal function. The ABA content of *Corylus avellana* L. and *Fraxinus americana* L. seeds decreased during imbibition at both warm and low temperatures (Williams *et al* 1973, Sondheimer *et al* 1974). Low temperatures potentiated germination while elevated temperatures did not. Additionally, Sondheimer *et al* (1974) found that both dormant and non-dormant

embryos of *F. americana* rapidly metabolised ABA to phaseic acid and related compounds under both temperature regimes. In these species, therefore, stratification does not appear essential for the development of metabolic activity and the different germinabilities between dormant and non-dormant embryos was not dependent on any overt change in ABA metabolism.

High levels of abscisic acid were associated with the testa and pericarp of freshly harvested hazel nuts (Williams *et al* 1973). It was proposed that the inhibitor prevented growth of the initially non-dormant embryo prior to the onset of true dormancy. ABA did not appear to influence regulation of the resting state after the development of embryo dormancy. Walton and Sondheimer (1972) reported embryo growth in *Fraxinus* seed in the presence of high endogenous levels of ABA.

Although ABA is an important and potent inhibitor of plant growth processes, Millborrow (1974) suggested that its widespread occurrence and ease of characterisation often led to the presence of other endogenous inhibitors being overlooked. It was suggested that the total biological activity of a plant extract be quantified by comparison with a standard range of (+)-ABA concentrations. This allows the actual (+)-ABA level to be compared with the extracts activity in "ABA-equivalents" (Most *et al* 1970). The presence of other inhibitory compounds in the plant extract may then be realised.

Abscisic acid has been detected in dormant *Avena* spp. By using the comparative titre technique based on a germination

bioassay and single-ion monitoring, it was established that the amount of ABA in the plant extract was insufficient to account for its biological activity *in vitro*.. This method allows the discrimination of an ABA pattern of inhibition from a non-ABA pattern and is was clear that the inhibitor(s) from *Avena* spp. had a titre not comparable to ABA.

An extract displaying inhibitory activity of a different nature was also isolated from *A. sativa* cv Selma (Results, Part 1).   In this case the active constituents were characterised as phenol and possible polychlorinated phenols.   From control extractions it was established that the presence of these compounds did not result from the experimental protocol.   It was possible that they occurred within the mature grain due to the degradation of a commercial herbicide of the 2,4-D type (XIX).   This chemical is widely used for the control of broad-leaved weeds amongst cereal crops and the gifted oat samples may have been treated with the herbicide during development.

This interesting observation was not followed by further research as such chemicals have no direct involvement with the innate seed dormancy of *Avena* spp. However, it serves as a caution with regard to the use of "field" material.

The nature of the principle inhibitory components extracted from wild and common oat were subsequently determined to be a group of medium-chain length, saturated fatty acids.

MCFA have previously been reported to possess growth inhibitory properties, including the reduction of lateral shoot

(2,4-Dichlorophenoxy)acetic acid

OCH₂COOH structure on a benzene ring with Cl at positions 2 and 4
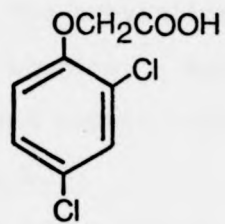
(XIX)

Salicylic acid

2-hydroxybenzoic acid

COOH and OH structure on a benzene ring
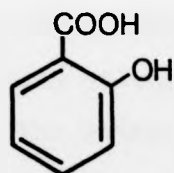
(XX)

growth (Tso 1964) and the prevention of mustard seed germination (Le Poidevin 1965). Clarke and Humphreys (1970) have characterised short and medium-chain length carboxylic acids as the active constituents from pea haulm exudate responsible for the prevention of extension growth and germination in some species. Ando and Tsukamoto (1974) isolated both abscisic and capric acids from dormant bulbs of *Iris hollandica* L.

MCFA are inhibitors of bacterial growth (Nieman 1954) and much literature also concerns their effect on microbial growth and spore germination. Garrett and Robinson (1969) have proposed that nonanoic acid may act as an endogenous inhibitor of spore germination in many fungal species. The effect of these aliphatic acids on mammalian physiology and their teratogenic and toxic action to insects is also well documented.

Cathey *et al* (1966) reported that certain medium-chain fatty acid esters were selectively toxic to meristematic plant tissue. The esters exerted a phytotoxic action on axillary buds of tobacco with chain lengths of 8, 9, 10 and 12 carbon atoms being most effective, without causing damage to the mature leaf or stem tissue (Tso *et al* 1965). Hole and Hardwick (1978) used a formulation of pentanoic and hexanoic acids to promote the drying of *Phaseolus vulgaris* seeds before harvest. Stowe and Hudson (1969) reported that certain lipids and associated compounds possessed growth regulating properties. Fatty acid derivatives (C_{14}-C_{22}) with marked biological activity have been isolated from rape and alder pollen (Mitchell *et al* 1970).

In the present study MCFA in the series hexanoic to decanoic had a significant inhibitory effect on the germination of *L. sativa* (Results, Part 2). Nonanoic acid exerted the greater inhibitory action. These aliphatic acids were also detected in the "ABA fractions" extracted from dormant seeds cf *A.sativa* and *A. fatua* (Results, Part 4). This finding should serve as a caution with regard to the significance of small amounts of ABA isolated from dormant plant material.

Bennet-Clark and Kefford (1953) found that a weakly acidic complex of growth inhibiting substances was widespread. It prevented both coleoptile and root growth. Varga (1957) proposed that this inhibitor-β complex comprised mainly aromatic acids and several (such as salicylic (XX) and coumaric) were characterised. ABA may also be partially responsible for its marked biological activity (Milborrow 1967, Holst 1971).

Fatty acids have previously been detected in the β inhibitor complex. Bentley (1958) tentatively attributed a phytotoxic effect of the mixture in an *Avena* straight growth bioassay to the presence of fatty acids at greater than physiological concentrations. Housley and Taylor (1958) also reported the phytotoxicity of a complex extracted from the skins of dormant potato tubers. Coleoptile sections of wheat were found to lose turgidity when treated with β inhibitor. It appeared to modify membrane permeability. The effect was attributed in part to the presence of heptane 1,7 dicarboxylic acid (XXI) and a number of unidentified aliphatic acids.

Nonanedioic acid

$$HOOCCH_2(CH_2)_5CH_2COOH$$
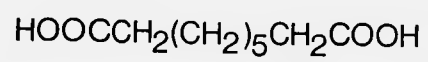
(XXI)

The relatively high levels of MCFA associated with the inhibitor-β must account for a large proportion of its biological activity. Certainly their presence at high concentrations would account for the observations of Bentley (1958) and Housley and Taylor (1958).

Regarding the biological activity of MCFA the response of *L. sativa* cv Grand Rapids to applications of these acids was interesting (Results, Part 7). Lettuce seeds become dormant when imbibed at supraoptimal temperatures although within a temperature range of $20-35^{\circ}C$ germination may be promoted by exposure to light.

The phenomenon of light effects in seed germination is known as photoblastism and emergence may be either promoted or inhibited by white light. Cumming and Hay (1958) reported that white, blue or far-red radiation prevented the germination of partially dormant *A. fatua* seeds.

Positive responses to light show an action peak in the red region of the spectrum (600-690nm). Germination is then stimulated by red light (R) and inhibited by far-red light (FR, 720-780nm) (Flint and McAlister 1935, 1937). The promotive effect of red light may be completely reversed by far-red treatment and *vice versa*, as reported by Borthwick *et al* (1952) for seeds of *L. sativa*.

Such effects result from a reversible photoreaction which depends on the absorption of either red or far-red light. Butler *et al* (1960) reported that this was carried out by a photoreversible pigment which they termed phytochrome. Phytochrome has been found in many plants and its photo-

reversibility observed *in vivo*. Chemically phytochrome
appears to be a tetrapyrrole which may be membrane bound
(Haupt and Weisenseel 1976). Jaffe and Galston (1967)
demonstrated that variation in membrane permeability can
be a phytochrome-mediated effect. The photomorphogenetic
controls and short-term responses mediated by phytochrome
are indeed suggestive of rapid changes in the biophysical
properties of membranes, which influence permeability
(Hendrick and Borthwick 1967).

In this study the inhibitory effect of MCFA on GRLS
germination was antagonised by exposure to red radiation,
that is the seeds became positively photoblastic. The acids
appeared to induce a requirement for phytochrome-mediated
reactions in lettuce species which normally germinated
without exposure to light. MCFA effectively lowered the
temperature at which thermodormancy was imposed on the seed.
The dormancy induced as a result of inhibitor treatment was
comparable to natural thermodormancy, the seeds displaying
true positive photoblastism under phytochrome control.
Berrie *et al* (1968) reported that photoblastism may be
induced in lettuce by exposure of the imbibed seed to
unsaturated lactones.

Khan (1971) has suggested that light sensitivity in
lettuce could depend on a critical balance between germination
inhibitors and promoters within the seed. Dark germination
of light-sensitive lettuce seeds can indeed be promoted by
treatment with either gibberellic acid or cytokinin (Kahn
et al 1957, Weiss 1960). However, unlike GA, cytokinin
cannot fully substitute for red light in overcoming the

phytochrome-mediated block to germination.   It would
appear that the two chemicals have quite different sites of
action within the seed (Ikuma and Thimann 1963).

Gibberellic acid and cytokinin were found to antagonise,
totally or partially, the fatty acid inhibition of lettuce
seed germination, with GA having the greater promotive
effect (Results, Part 7).

While MCFA-inhibition of GRLS germination can to some
extent be reversed by individual treatments of light,
gibberellin or cytokinin, ABA, on the other hand, completely
prevents GRLS germination in both dark and light and totally
antagonises the promotive influence of GA (Khan 1968,
Sankla and Sankla 1968).   The ABA-induced block to seed
germination may be reversed by treatment with combinations
of either cytokinin and red light or gibberellic acid and
kinetin (Khan *et al* 1971, Bewley and Fountain 1972).   A
totally different pattern of inhibition for ABA and nonanoic
acid (the most biologically active MCFA) was also observed
with the germination of *A. sativa* cv Selma (Results, Part 9).

Some authors have reported that the inhibitory effects
of low concentrations of ABA ($<6\mu M$) may be reversed by GA
alone (Aspinall *et al* 1967, Poggi-Pellegrin and Bulard 1976).

Abscisic acid can influence the stomatal control
mechanism (Kriedemann *et al* 1972) and accumulate in plants
during periods of water stress (Wright and Hiron 1969).
Therefore levels of endogenous ABA may correlate with water
loss during grain desiccation on the parent plant.   The
prime role of the inhibitor within the seed may be solely
concerned with water relations and not with the regulation

of seed dormancy.

In this respect estimated levels of endogenous ABA in both wild and common oat seed did not suggest a correlation between ABA content and the process of desiccation (Results, Part 10). As drying proceeded on the parent plant the amount of free ABA in grains of *A. sativa* cv Selma and *A. fatua* declined dramatically during the final stages of maturation. Thus, far from observing an increasing level of endogenous ABA during desiccation, as might be expected, the seed ABA content actually declined rapidly in association with natural drying of the grain.

These observations were in accordance with those of McWha (1975) and King (1976) in wheat. Endogenous levels of ABA were found to be highest in individual grains up to 40 days after anthesis and subsequently declined during natural seed desiccation. It was suggested (King 1976) that accumulation of ABA during the later stages of grain growth prevented precocious germination and premature hydrolysis of starch reserves in the unripe, but morpho-logically mature, grain.

On an individual grain basis, amounts of free ABA in wild and common oat were comparable and could not be correlated with germinability. It would appear, therefore, that ABA is not involved in the regulation of oat seed dormancy unless the two species have markedly different levels of sensitivity to the inhibitor. Several other studies have been unable to conclude a hormonal role for ABA in the maintenance of seed dormancy.

In his extensive review Milborrow (1974) suggested that

short-term variations in environmental conditions could significantly affect the level cf ABA in plant material. Indeed, as the inhibitor in leaves subjected to water stress increases 40-fold over that of normal tissue (Wright and Hiron 1969) a correlation of endogenous amounts with the dormant condition may be suspect.

The so-called "bound" ABA fractions isolated in this study are unlikely to be involved in the regulation of seed germination. Their pattern of variation was similar to that of the free acid, rapidly declining from high endogenous levels as the seed matured. These derivatised forms may provide a ready precursor for a rapid release of free ABA during periods of water stress. However, Milborrow and Noddle (1970) suggested that increases in inhibitor during such periods resulted from synthesis rather than release from a bound form. In fact, Powell and Seeley (1974) proposed that the esterification mechanism was a means for inactivating free ABA.

Although the ABA derivatives were not characterised, the "acid-bound" fraction was probably a polar, water-soluble glucose ester. This naturally-occurring derivative has half the biological activity of the free acid (Koshimizu *et al* 1968). The "neutral-bound" fraction was most likely an artifact of the extraction procedure. In acidic methanol the glucose ester of ABA transesterifies to give methyl abscisate and glucose (Milborrow 1974).

An interesting comparison can be made between levels of MCFA in wild and common oat seed during development and

dry storage.   The development pattern for MCFA in individual grains was found to be quite different between the two species (Results, Part 10).   High endogenous amounts of MCFA, particularly heptanoic, nonanoic and decanoic, were associated with dormant *Avena* grain.   By contrast, non-dormant seeds contained relatively low levels of these inhibitory acids.   In particular, the nonanoic acid content of seeds appeared to correlate well with germinability in both species.

Although it may be possible to postulate the involvement of certain MCFA in the regulation of seed dormancy, their observed decline may merely result from the preparation for germination.   That stimulus (i) which potentiates the seed for emergence, enabling it to germinate on imbibition, could alter grain metabolism so that MCFA are degraded.

In this respect levels of volatile acid within *A. fatua* were interesting.   In the wild oat nonanoic acid followed an exponential decay pattern during after-ripening.   It is possible, therefore, that the stimulatory effect of dry storage on grain germination resulted from a physical process involving volatilisation of nonanoic acid.   Inhibitor may diffuse to the seed surface and from there pass into the environment.   Such a process could explain the observed exponential decay pattern.

In the dry seed conditions are not inducive to the occurrence of metabolic reactions, intercellular diffusion of substrates and enzymes being restricted.   Therefore, it may be more appropriate to suggest such a physical process

to account for inhibitor loss from desiccated seed.
The volatile acids may gradually diffuse from dormant
Avena grain until a critical endogenous level has been
reached at which germination could occur.   It may
therefore be possible to predict which samples of *A. fatua*
are non-dormant by monitoring amounts of endogenous
nonanoic acid.

Although loss of MCFA occurred from *Avena* seed during
development and/or after-ripening, either by volatilisation
or degradation, it was possible that some interchange
occurred between the acids themselves.   This was
particularly noticeable for nonanoic and decanoic acids.
It was usually observed in both species that while the
relative level of nonanoic acid decreased the relative
amount of decanoic acid increased, and *vice versa*.   This
phenomenon may be another possible mechanism for activation,
or deactivation, of nonanoic acid *in vivo*.

This type of study which involves extraction of
endogenous chemicals from plant material may suffer from
several limitations which should be noted.   Due to a lack
of knowledge regarding the precise relationship between
seed metabolism and the determinants of dormancy, experiments
which monitor the metabolism of chemicals during treatments
which stimulate germination are difficult to interpret.
Emergence may result from an unrealised influence on grain
metabolism which stimulates fatty acid metabolic
pathways to produce the MCFA trends observed in this study.
Regarding seed metabolism it is difficult to separate cause
from effect and a correlation of MCFA levels with

germinability may merely be a causal result of dry storage.

However, previous literature suggests that endogenous chemicals do function in the regulation of *A. fatua* dormancy (Naylor and Christie 1957, Black and Naylor 1957, Black 1959). Hay (1962) proposed that the inhibitor(s) may act as an electron-acceptor and block the electron transport chain at some point where there is no alternative biochemical pathway. Naylor and Simpson (1961) attributed wild oat seed dormancy to antagonism between germination inhibitors and promoters. Simpson (1966) proposed that dry storage may effect the gradual reduction of an endogenous inhibitor which blocks production of gibberellic acid in dormant embryos.

Gibberellic acid can stimulate germination in dormant seeds of *Avena* spp. (Hay and Cumming 1959, Simpson 1965, Koch 1968). Black and Naylor (1959) found that non-dormant seeds developed from wild oat inflorescences which had been allowed to take up GA during maturation. Simpson (1965) detected a growth stimulating factor in *A. fatua* seed which increased in concentration during prolonged after-ripening. He proposed that dry storage allowed the gradual oxidation of an endogenous inhibitor which prevented production of a GA-like chemical in the embryo.

In this study MCFA-induced inhibition of lettuce seed germination was antagonised by GA-treatment (Results, Part 7). The acids also counteracted the stimulatory effect of gibberellin on hydrolase production from barley endosperm pieces (Results, Part 11). Such observations tend to support the proposal that volatile fatty acids may prevent

the production and/or utilisation of GA-like compounds within dormant *Avena* grain.

Khan *et al* (1964) detected a germination inhibitor in barley which prevented release of gibberellin-induced hydrolase from aleurone tissue (Bruin and Tolbert 1965). Elliott and Leopold (1953) also reported the presence of an inhibitor of germination and amylase activity in *A. sativa* seed.

During the initial stages of development in the family Gramineae, gibberellins are released from the embryo (Yomo and Iinuma 1966) and induce the synthesis and secretion of hydrolases from the aleurone cells comprising the outermost layer of the endosperm. These enzymes degrade reserve starch to maltose and glucose and reserve proteins to amino acids. The degradation products are translocated to the embryo and supply the energy requirements for active growth. In barley gibberellic acid stimulates production of hydrolases from intact and isolated aleurone tissue (Varner 1964).

Prior to this study ABA was the only naturally-occurring plant growth inhibitor reported to prevent gibberellin-induced amylolysis in barley half seeds or α-amylase production in isolated aleurone layers (Addicott *et al* 1966, Chrispeels and Varner 1967). Indeed, the former physiological response has been used as a bioassay for the inhibitor (Sivori *et al* 1971). However, at physiological concentrations the inhibitory effects of abscisate and nonanoate on GA-induced amylolysis were comparable, although the respective germination titres displayed quite different patterns of dose response.

The threshold response obtained for MCFA activity may be important in the Gramineae for regulation of such processes as germination or release from dormancy. It would appear that plant hormone activity is generally dependent on concentration rather than the target-tissue specificity observed with the mammalian hormones. Thus metabolic regulation in higher plants is probably more hormone-concentration dependent, most tissues being responsive to a range of growth regulators.

Caution is required, however, when assessing the significance of tissue response data obtained from exogenous applications of plant-growth regulators. In such experiments an active chemical may affect a totally different target site than operates in nature. In addition, naturally-occurring regulators may be compartmentalised within the cell and may not be readily available at an active site.

Development of hydrolase activity in seeds of *Avena* spp. has been extensively studied. Naylor (1966) reported that enzyme production from aleurone tissue of *A. fatua* was completely dependent upon gibberellins from the embryo. The common oat was found to be more self-regulating as regards hydrolase synthesis. Drennan and Berrie (1962) suggested that onset of endosperm activity in *Avena* spp. was initiated in response to a stimulus from the embryo. Chen and Chang (1972) and Chen and Park (1973) confirmed that development of hydrolase activity was a post-germinative phenomenon.

Chen and Varner (1970) reported that dormant seeds of wild oat synthesised protein at a similar rate to non-dormant

grain. Chen (1972) demonstrated that *de novo* protein synthesis occurred in dormant seed of *A. fatua* during dry storage. Such metabolic activity may explain the observed changes in enzymes and other endogenous chemicals during dry after-ripening. Delayed germination in *A. fatua* appears attributable therefore to a specific metabolic block rather than to repression of the genetic apparatus (Chen and Varner 1970).

Development of α-amylase activity in barley aleurone layers is known to be inhibited by uncouplers of oxidative phosphorylation (Varner 1964). Many chemicals can suppress coupling of electron flow to the synthesis of ATP without affecting electron transport. Such uncouplers may be regarded as replacing phosphate or ADP in the coupled reactions of ATP synthetase. Blondin and Green (1975) presented a model for the coupling of electron flow to the cyclical transport of an uncoupler ion across cell membranes. In this process uncouplers act as a co-ion for Mg^{2+}, as proton carriers, and as anions which are soluble in the membrane phase.

Thus, the chemical nature of MCFA indicates that these aliphatic acids may be effective uncouplers of oxidative phosphorylation (Lehninger 1951, Scholefield 1956). Fatty acids are known to prevent the ATP-Pi exchange reaction, an important step in the synthesis of ATP (Ahmed and Scholefield 1960, Falcone *et al* 1960). In this respect, it is interesting that rapid synthesis of ATP is an early event (30min imbibition) in the germination of wheat embryos (Obendorf and Marcus 1974).

Marinos and Hemberg (1960) suggested that the
β inhibitor complex from potato may uncouple oxidation
from phosphorylation and thereby deprive the dormant tuber
of energy for growth.   Recently, Hemberg (1978) has
suggested that ABA uncouples oxidative phosphorylation
in potato pith tissue.   High concentrations of inhibitor-β
caused an inhibition of ATP synthesis and light-induced
electron transport in photosynthesising *Scenedesmus* cells
(Tillberg 1968).

It would appear from the literature that an endogenous
inhibitor blocks two metabolic pathways in dormant seed
of *A. fatua* and that dry storage overcomes these blocks by
stimulating the production of endogenous gibberellin.
Thus germination of dormant caryopses may be influenced by
two separate mechanisms, both regulated by an endogenous
inhibitor which prevents biosynthesis of a promoter
concerned with translocation and utilisation of the seed's
food reserve.

Naylor and Simpson (1961) suggested that gibberellic
acid has two distinct target tissues in wild oat seed.
One, sited in the embryo, was concerned with inducing
germination while the other, in the aleurone layer, was
associated with the mobilisation of reserve carbohydrates.
An early action of GA appears to be a stimulation of
protein synthesis and mRNA production in the embryo (Chen
and Park 1973).   Simpson (1965) determined that embryos
from dormant *A. fatua* could be grown in a medium of sucrose,
amino acids and gibberellin.   As the grain after-ripened
dependence on exogenous GA decreased, accompanied by a

progressive increase in endogenous GA-like activity.

In *A. fatua* the pentose phosphate pathway seems to
have an important role in seed germination. Simmonds
and Simpson (1971) reported that dormant and non-dormant
embryos respired at a same rate, gibberellic acid
stimulating germination without affecting oxygen
consumption. During dry storage seed metabolism was
found to change from the glycolytic pathway to an increased
degradation of glucose by pentose phosphate metabolism.
This transition may be an essential step in the preparation
for germination and could provide a means for dormancy
regulation.

Kovacs and Simpson (1976) proposed that control of the
pentose phosphate and glycolysis-tricarboxylic acid pathways
was important in wild oat seed dormancy. A prerequisite
for germination appeared to be an ability of the seed to
stimulate the activities of glucose-6-phosphate dehydrogenase
and 6-phospho-gluconate dehydrogenase. These enzymes
catalyze the initial steps of the phosphogluconate oxidative
pathway. Ashihara and Komamine (1974) reported that G-6-P
dehydrogenase, the first enzyme in the pathway, limits the
degradation of glucose during the preliminary stages of
germination. It may therefore provide a convenient site
for the regulation of oat seed dormancy. In this respect,
it is interesting that application of GA to dormant
A. fatua stimulated G-6-P dehydrogenase activity compared to
6-P-G dehydrogenase.

It is tempting to speculate that gibberellic acid
influences oat seed germination by allosterically regulating

those enzymes associated with the pentose phosphate pathway (Simmonds and Simpson 1971). MCFA may antagonise the action of GA in this system by combining as a negative effector ligand at an allosteric site on the enzyme complex (Figure 43). In the active conformation the activator molecule (GA or cytokinin) is envisaged as having locked the protein into a configuration in which substrate can be bound at the active site.

However, one may also propose that MCFA act in a similar manner (allosterically) at some point in the metabolic pathway concerned with gibberellin biosynthesis itself. Clearly, control of this pathway would provide simple and effective regulation of seed germination in *Avena* spp.

MCFA have been reported as metabolic regulators in rat liver (Weber *et al* 1966). In this case enzymes associated with glucose degradation were inhibited by aliphatic acid treatment. Wachsmuth (1965) found that aliphatic acids with even side-chains inhibited liver alcoholdehydrogenase more than acids with an odd side-chain. Lewis and Johnson (1968) suggested that in certain fungi volatile fatty acids inhibited specific enzyme systems capable of limiting the tricarboxylic acid cycle.

A fatty acid anion-albumin complex has been detected in mixtures of short-chain length fatty acids and serum albumin (Ballou *et al* 1945). Bull and Breese (1967) stressed the importance of interaction between the acid hydrocarbon chain and hydrophobic amino acid residues of the protein. Binding of acids with egg albumin was found

Active Enzyme
(unstable)

Inactive Enzyme

Active Enzyme
(stable)

Inactive enzyme
(stable)

Negative Effector (ABA, MCFA)

Substrate

Positive Effector (GA, cytokinin)
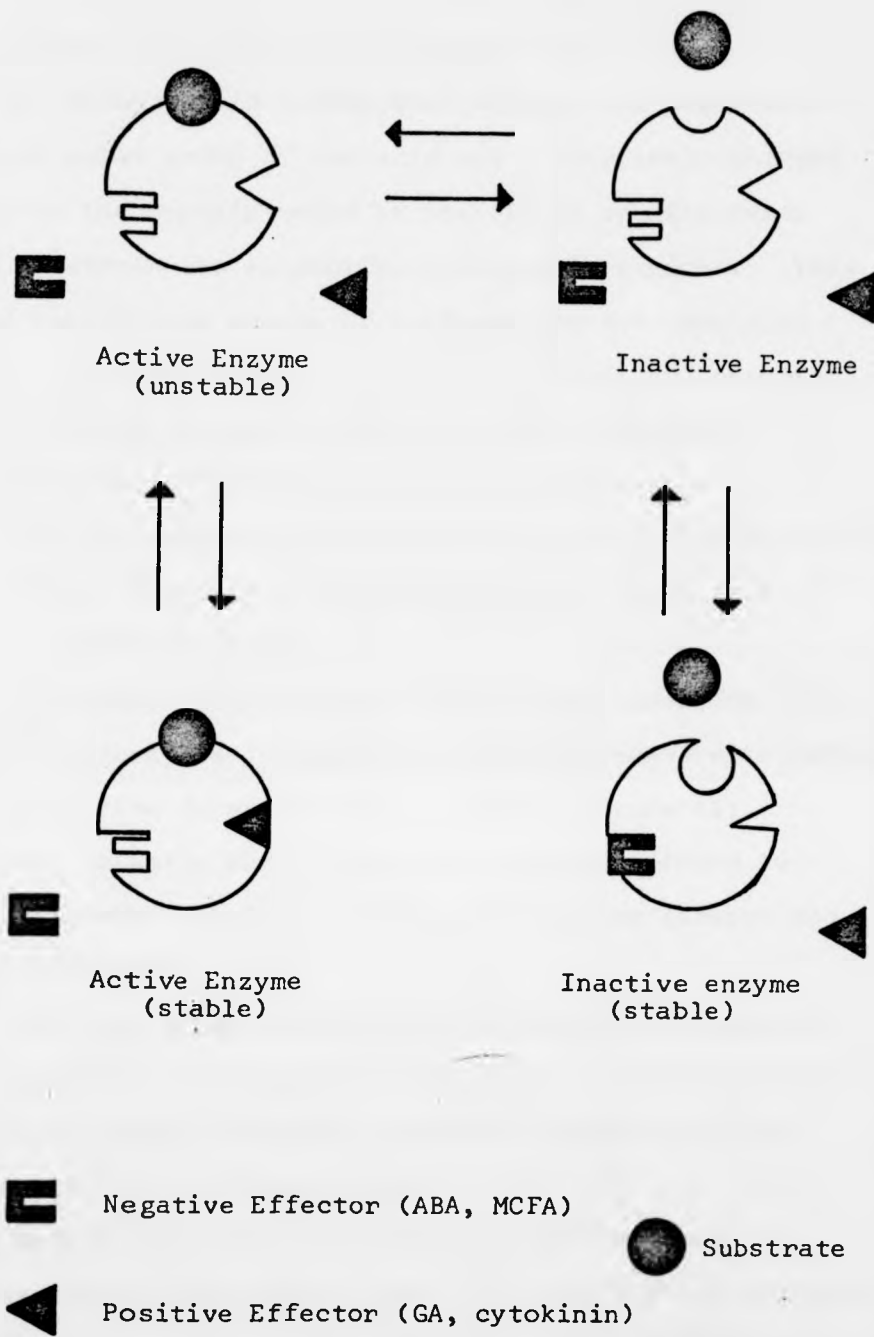
FIGURE 43 :   The possible allosteric inhibition by MCFA of
a regulatory enzyme complex.

to increase from acetic to heptanoic acid.

A MCFA-protein interaction between the negatively charged polar group of the acid and a positively charged group on the protein could be stabilised by dispersion forces between the respective hydrophobic regions. This could result in a change of conformation and catalytic behaviour of the enzyme. Chabaud *et al* (1969) reported that naturally-occurring ABA complexed with fungal α-amylase and altered its physical properties.

It is therefore possible to rationalise MCFA inhibition of cellular activity on a metabolic rather than purely physico-chemical level.

Obviously such allosteric regulation involving MCFA must be reversible in order to allow interconversion between the respective forms of enzyme complex (Figure 43). However, volatile fatty acids have also been found to interact with protein in a disruptive manner (Ibanez and Herskovits 1976).

Apart from depressing cellular activity by possible competitive or non-competitive effects on enzyme-mediated reactions, MCFA also exert a powerful influence on the permeability of cell membranes.

Methyl decanoate affected the ultrastructure of chrysanthemum cells (Nelson and Reid 1971), while Fay and Farías (1977) demonstrated that the growth inhibitory action of decanoic acid on *Escherichia coli* involved the disruption of outer membrane integrity. Willmer *et al* (1978) found that MCFA treatment increased cell leakage in *Beta vulgaris* L. root tissue.

Certain hydrocarbons are known to promote the effect of applied auxin (Stowe and Dotts 1971). It was proposed that such molecules disrupt membrane function by inserting between choline esters. MCFA do not appear to conform to this model being half the chain length of those compounds showing the greatest activity.

Fatty acids comprise both hydrophobic and hydrophilic groups which are suited chemically for binding membrane lipoprotein. An initial attraction between the carboxyl group on the acid and constituent choline and ethanolamine groups in the membrane may position the molecule at the lipid-water interface so that the alkane moiety is absorbed into the membrane lipids, causing a degree of disruption. Many plant hormones contain hydrophilic moieties and may influence membrane function in a similar fashion. The lipophilic nature of a regulator molecule may determine both its transmembrane transport and the extent of interaction at a hydrophobic site.

In a series of saturated monocarboxylic acids (C_1-C_9), Reynolds (1975) found that inhibition of lettuce seed germination increased in a non-linear fashion with the number of carbon atoms in the molecule. The biological activity did not correlate with branching of the hydrocarbon chain, or with the pK or pH of the applied acid. However, over a range of organic compounds a positive correlation was obtained with the lipophilic nature of the molecule. This type of relationship between biological activity and lipophilicity, also displayed by MCFA in this study, may be indicative of interactions involving the cell membrane.

An important action of plant hormones in the processes

of seed germination appear to involve cellular and/or membrane permeability (Taylorson and Hendricks 1977). Van Steveninck (1976) proposed that plant hormones control ion transport across cell membranes.   This effect may depend upon regulation of ion pump activities and/or membrane permeability, resulting from a direct interaction of growth regulators with the membrane lipid molecules.

Marrè (1977) outlined an interesting model for the hormonal control of ion transport in plants based on a fusicoccin-sensitive H^+/K^+ exchange system.   Fusicoccin (XXII) appeared to directly activate a plasmalemma-bound ATPase mediating the electrogenic proton/K^+ antiport. These membrane-bound proteins appear of importance in intracellular cation transport.   Hayer *et al* (1971) suggested that auxin stimulated growth by activating either a membrane-bound ATPase or the cell membrane proton pump.   The auxin receptor site may be located on the plasmalemma (Hertel *et al* 1972).

ABA is known to influence auxin-induced proton secretion (Rayle and Johnson 1973).   It can also increase rapidly the permeability to water of the cell membrane and affect ion pump activity (Glinka and Reinhold 1971, Kriedemann *et al* 1972).

The stimulatory action of gibberellic acid may also depend on the selective permeability of cellular membranes (Wood *et al* 1972).   Marrè (1977) suggested that the enhanced seed germination resulted from an increased K^+ uptake.   A molecular association between gibberellin and phosphatidyl choline, a common membrane component, has been

Fusicoccin
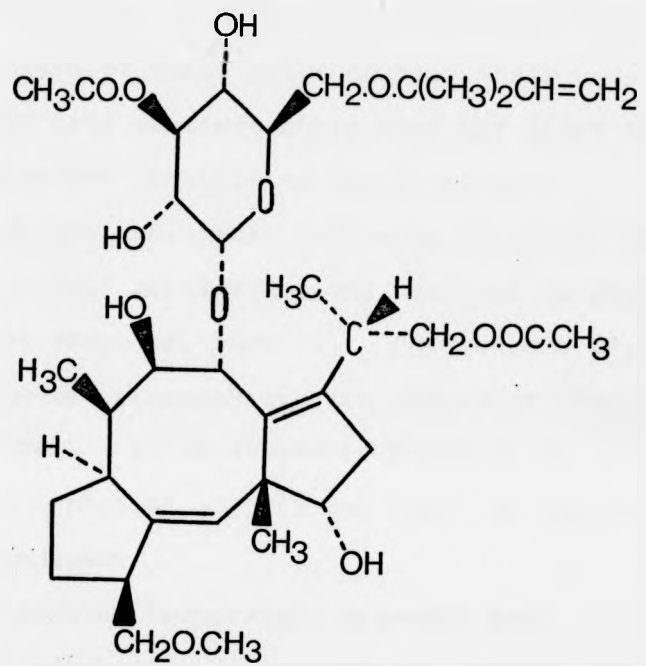
(XXII)

found to alter the energy requirements for membrane transport
(Wood *et al* 1974, Wood and Paleg 1974). Browning and Saunders
(1977)   also reported the intimate association of
certain gibberellins with membrane-bound proteins.

It may be tentatively proposed that MCFA could perturb,
directly or indirectly, an active site on a membrane-bound
macromolecule with either an enzymic or structural function.
The lipophilic nature of these acids enables their
penetration of the cell membrane where they may alter the
conformation and active function of bound protein.

In this study the inhibitory influence of physiological
amounts of MCFA on GRLS germination was overcome by phytochrome-
mediated reactions (Results, Part 7).   Phytochrome (P_{fr})
is known to influence transmembrane ion transport (Haupt
and Weisenseel 1976).   It is therefore possible to
rationalise the interaction of MCFA and light at the level
of the cellular membrane.

In GRLS an elevated temperature prevents seed
germination in the dark and this phenomenon appears to be
associated with increased membrane fluidity as the temperature
rises.   Germination may then be promoted through phytochrome-
mediated reactions within a narrow temperature range.
Such reactions may modify membrane structure in a manner
which allows optimal transmembrane transport, active
metabolism and subsequent germination.

A striking similarity was found between the effects
of elevated temperature and MCFA on GRLS germination.
Thus, the fatty acids may also increase membrane fluidity
and prevent germination.   However, gibberellic acid is

known to increase the fluidity of model membranes (Wood and Paleg 1972) and this hormone is antagonistic to the inhibitory action of MCFA.

One could therefore speculate that action of the respective plant-growth regulators may be localised at discrete regions of the cellular or organelle membrane. Membrane function could thus be modified at particular regions within the cell and manifest the characteristic response associated with the individual growth regulator. Such a hypothesis may be more in keeping with the concept of hormonal action.

Hormonal effects on the limiting membranes of plant cell organelles may regulate the transport of metabolites between cellular compartments and integrate the biochemical reactions required for seed germination. Dormancy may depend upon an imbalance between endogenous growth hormones which results in a discontinuity between related metabolic reactions required to drive the germination process.

CHAPTER V

SUMMARY AND CONCLUSIONS

SUMMARY AND CONCLUSIONS

As a result of this study it is proposed that the
biological activity of MCFA may provide a basis for seed
dormancy regulation in *A. sativa* and *A. fatua*.
Endogenous volatile acids, particularly nonanoic, could
be of importance in suppressing the germination of these
species.   It was found that high levels of MCFA were
associated with dormant seed, these amounts decreasing
during, or prior to, a period of increasing germinability.
Reduced levels of endogenous fatty acids were associated
with non-dormant grain of both species.

In common oat, endogenous MCFA decreased from a
maximum value of 913ng per grain during the later stages
of seed maturation to a level of about 150ng per seed.
This reduction probably involved metabolic degradation.
A. sativa cv Selma was not dormant at harvest, germination
being greater than 50 percent.

It was found that the wild oat, on the other hand,
accumulated these acids during seed development to a
maximum value of 4627ng per grain at harvest.   In this
species dormancy was overcome after 18 months of dry storage,
during which the total MCFA level declined to 581ng per seed.
Compared to the common oat, seeds of the wild variety
contained lower endogenous amounts of fatty acids during
the early stages of grain filling.   These levels rapidly
increased during maturation until at harvest they contained
7.5-fold more MCFA than *A. sativa*.

Levels of endogenous acids in *A. fatua* increased
throughout seed maturation and appeared to be lost from the

grain during after-ripening, possibly by a natural physical process involving gradual volatilisation. For nonanoic acid this process was estimated to proceed with a half life of 56.5 days. The fact that ripening is accelerated at high temperatures would tend to support such an "evaporation theory".

It was found that in both species a decline in endogenous MCFA, particularly nonanoic, was accompanied by increased germination. In wild oat the highest levels of nonanoic acid were detected in the mature dormant seed, while for the common variety they were associated with the immature seed after anthesis. The nonanoic acid contents of wild and cultivated oat seed displaying 17% and 16% germinations, respectively, were found to be comparable at about 100ng per grain. Generally, levels of hexanoic, heptanoic and octanoic acids did not vary markedly during maturation and dry storage of either species.

In conclusion, an excellent correlation was made between MCFA content and seed germinability.

These fatty acids were also detected in the β-inhibitor mixture isolated from a wide variety of dormant plant material. There was substantially more (13-fold) MCFA associated with the complex extracted from dormant structures than from non-dormant material. It is proposed that the acids contribute markedly to the mixtures inhibitory properties.

Saturated fatty acids in the series valeric to undecanoic were tested for their physiological activity. At physiological concentrations they inhibited germination

in several species, including both the common and wild
oat, and suppressed gibberellin-induced amylolysis in
barley endosperm.   In general nonanoic acid was the
most effective treatment, the other MCFA being less
biologically active.

At a concentration of about 2×10^{-3} M nonanoic acid
reduced the germination of non-dormant oat grain by 50
percent.   Lettuce seed germination was more sensitive
to applied acid, activity being observed at a concentration
of 6.25×10^{-4} M.   The inhibition was similar to that
induced by elevated temperatures in that it could be
reversed by treatment with red light or applications of
cytokinin or gibberellin.   Gibberellic acid was more
effective in overcoming the MCFA-imposed inhibition of
germination.

Abscisic acid did not appear to be directly involved
with the dormancy of *A. sativa* cv Selma or *A. fatua* seed.
The development pattern for ABA was assessed during maturation
and dry storage of both species.   Large changes in ABA
levels only occurred in the hydrated grain, rapid increases
being associated with the period of grain filling.
Endogenous ABA did not correlate with germinability during
seed development and after-ripening.

In seeds of both species the maximum amount of free
ABA (approximately 500pg per seed) was estimated within
grain at 52 days after anthesis.   As desiccation on the
parent plant proceeded their inhibitor content declined
dramatically.   The free acid decreased to similar levels
(300pg per seed).in both species at harvest, although their

germinabilities were then quite different being 65 percent and 0 percent for the common and wild oat respectively.

It is suggested that endogenous ABA may prevent precocious germination and the premature hydrolysis of starch reserves while the grain is developing on the parent plant. Certainly it has been found, in this and other studies, that ABA is not efficacious as an inhibitor of germination in mature oat seed.

From the literature it would appear that delayed germination in *Avena* spp. results from a specific metabolic block. rather than to repression of the genetic apparatus. Production of endogenous gibberellic acid seems to be required to overcome this block to growth and development in the dormant oat embryo.

MCFA are known to uncouple oxidation from phosphory- lation and ABA has also been reported to similarly affect the oxidative process. Fatty acids may antagonise the promotive influence of GA in the embryo by limiting the enzymes concerned with its biosynthesis. Volatile acids could inhibit enzyme activity *in vivo* either allosterically or by direct competitive effects at an active centre on an enzyme complex. MCFA are known to suppress enzyme activity *in vitro* and to prevent the production and/or release of hydrolase from barley aleurone tissue.

Recently, it has become evident that most plant-growth regulator responses may be mediated through transmembrane transport. Clearly germination involves changes in membrane composition or permeability. MCFA are known to alter membrane permeability and it is proposed that their

physiological effects could be associated with this process.   They may interact in a non-specific manner with constituent membrane lipids and thereby alter membrane function.   Alternatively they may compete with the promotive plant hormones (gibberellin or cytokinin) at discrete active sites such as protein bound to the membrane surface of plant cell organelles.

A wide variety of chemically dissimilar plant-growth regulators are known and some have been previously described. Those important to plant growth and development may function *in vivo* by altering the physical properties of lipid moieties and thereby influence membrane permeability and the intracellular transport of materials.   Alternatively a change in membrane fluidity could impair or activate membrane-bound enzymes, such as ATPase or adenyl cyclase.

REFERENCES

REFERENCES

ABELES, F.B. and LONSKI, J. 1969. Plant Physiol. 44:277.

ADDICOTT, F.T. 1970. Biol. Rev. 45:485.

————— , OKHUMA, K., SMITH, O.E., THIESSEN, W.E. 1966
Advan. Chem. Ser. 53:97.

————— , LYON, J.L., OHKUMA, K., THIESSEN, W.E., CARNS,
H.R., SMITH, O.E., CORNFORTH, J.W., MILBORROW,
B.V., RYBACK, G. and WAREING, P.F. 1968. Science
159:1493

————— and LYON, J.L. 1969. Ann.Rev.Plant.Physiol. 20:139.

AHMED, K. and SCHOLEFIELD, P.G. 1960. Nature 186:1046.

AKKERMAN, A.M. and VELDSTRA, H. 1947. Rec.Trav.Chim.
Pays.Bas. 66:411.

AMEN, R.D. 1963. Amer.Sci. 51:408.

————— 1968. Botan.Rev. 34:1.

————— 1974. Trans.Amer.Micros.Soc. 93:593.

ANDO, T. and TSUKAMOTO, Y. 1974. Phytochemistry 13:1031.

ANDREWS, C.J. and SIMPSON, G.M. 1969. Canad.J.Botany 47:1841.

APPLEBY, A.P., FURTICK, W.R., and FANG, S.C. 1965. Weed
Res. 5:115.

ARNDT, F. 1943. In: Organic Syntheses (Blatt, A.H., ed.)
Coll.Vol. 2:166. J. Wiley and Sons, London.

ASHIHARA, H. and KOMAMINE, A. 1974. Plant Science Lett.
2:331.

ASPINALL, D., PALEG, L.G., ADDICOTT, F.T. 1967. Aust.J.
Biol.Sci. 20:869.

BAILEY, N.T.J. 1969. Statistical Methods in Biology.
Hodder and Stoughton.

BAKER, L.O. and LEIGHTY, D.H. 1958. Proc. 16th west.
Weed Control Conf. 69.

BALLOU, G.A., BOYER, P.D., LUCK, J.M. 1945. J.Biol.Chem. 159:111.

BANDURSKI, R. and SCHULZE, A. 1974. Plant Physiol. 54:257.

BANTING, J.D. 1963. Res. Rep. west Sect. Canad.Nat, Weed Comm. 57.

———— 1966. Canad.J.Plant.Sci. 46:469.

BARTON, L.V. 1965a. Encycl.Plant.Physiol. 15:699.

———— 1965b. *Ibid*. 15:727.

BENNET-CLARK, T.A. and KEFFORD, N.P. 1953. Nature 171:645.

BENTLEY, J.A. 1958. Ann.Rev.Plant Physiol. 9:47.

BERRIE, A.M.M., PARKER, W., KNIGHTS, B.A. and HENDRIE, M.R. 1968. Phytochemistry 7:567

————, DON, R., BULLER, D., ALAM, M. and PARKER, W. 1975. Plant Science Lett. 6:163.

———— and ROBERTSON, J. 1976. Planta(Berl.) 131:211.

BEWLEY, J.D. and FOUNTAIN, D.W. 1972. Planta (Berl.) 102:368.

BEX, J.H.M. 1972. Acta.Bot.Neerl. 21:203.

BLACK, M. 1959. Canad.J.Bot. 37:393.

———— and NAYLOR, J.M. 1957. Res.Rep.west Sect.Canad. Nat. Weed Comm. 130.

———— and ———— 1959. Nature 184:468.

———— and WAREING, P.F. 1959. J.Exp.Bot. 10:134.

BLONDIN, G.A. and GREEN, D.E. 1975. Chem.Eng.News,Nov. 26.

BLUMENTHAL-GOLDSCHMIDT, S. 1961. Bull.Res.Coun. Israel D. 9:187.

BORTHWICK, H.A.,HENDRICKS, S.B., PARKER, N.W., TOOLE, H.E. and TOOLE, V. 1952. Proc.Nat.Acad.Sci. USA 38:662.

BOWDEN, B.A. and FRIESEN, G. 1967. Weed Res. 7:349.

BRAUN, J.W. and KHAN, A.A. 1975. Plant Physiol. 56:731.

BROOKS, C.J.W. and KEATES, R.A.B. 1969. J.Chromatog. 44:509.

BROWN, R., GREENWOOD, A.D., JOHNSON, A.W., LANDSDOWN, A.R.,

LONG, A.G. and SUNDERLAND, N. 1952. Biochem.J. 52:571.

BROWNING, G. and SAUNDERS, P.F. 1977. Nature 265:375.

BRUIN, W.J. and TOLBERT, N.E. 1965. Plant Physiol. 40:xxxviii.

BULARD, C., BARTHE, P., GARELLO, G. and LE PAGE-DEGIVRY,

M.T. 1974. C.R. Acad. Sci.Ser.D 278:2145.

BULL, H.B. and BREESE, K. 1967. Arch.Biochem.Biophys. 120:303.

BUTLER, W.L., HENDRICKS, S.B. and SIEGELMAN, H.W. 1960.

Plant Physiol. 35 (Supple):xxxii

CATHEY, H.M.,STEFFENS, G.L., STUART, N.W. and ZIMMERMAN, R.H.

1966, Science 153:1382.

CHABAUD, J.P.,MOUSSERON-CANET, M. and DURAND, B. 1969.

C.R. Acad.Sci.Ser.C 269:865.

CHANCELLOR, R.J. and PETERS, N.C.B. 1972.   Proc. 11th Brit.

Weed Control Conf. 218.

————— and —————   1974.   Weed Res. 14:197.

CHEN, S.S.C. 1972. Naturwissenschaften 59:123.

————— and VARNER, J.E. 1970. Plant Physiol. 46:108.

————— and CHANG, J.L.L. 1972. *Ibid*. 49:441.

————— and PARK, W.M. 1973. *Ibid*. 52:174.

CHERRY, M. 1968. Farmer and Stockbreeder 82, 4078:48.

CHRISPEELS, M.J. and VARNER, J.E. 1967. Plant Physiol. 42:1008.

CLARKE, E.G.C. and HUMPHREY, D.J. 1970. J.Sci.Food Agric.

21:225.

CLELAND, R. 1963. Nature 200:908.

COOK, A.H. and POLLOCK, J.R.A. 1954. J.Inst.Brew. 60:300.

COOK, C.E., WHICHARD, L.P., TURNER, B., WALL, M.E. and

EGLEY, G.H. 1966. Science 154:1189.

COOMBE, B.G., COHEN, D. and PALEG, L.G. 1967. Plant Physiol.

42:105.

CORCORAN, M.R. 1970. Planta (Berl) 95:95.

CORNFORTH, J.W., MILBORROW, B.V., RYBACK, G. and WAREING, P.F.
1965a. Nature 205:1269.

————— , ————— and RYBACK, G. 1965b. *Ibid.* 206:715.

————— , —————,RYBACK, G. and WAREING, P.F. 1966a.
Tetrahedron 8 (Suppl): 603.

————— , —————,—————, ROTHWELL, K. and WAIN, R.L.
1966b. Nature 211:742.

COURTNEY, A.D. 1974. Agriculture North Ire. 49:23.

CROCKER, W. and BARTON, L.V. 1953. Physiology of Seeds, 114-139.
Chronica Botanica Co., Mass.

CUMMING, B.G. and HAY, J.R. 1958. Nature 182:609.

DADD, C.V. 1957. Field Crop Abstr. 10:1.

DAVIES, P.J. 1973. Bot. Review. 39:139.

DENNY, F.E. 1924. J. Agric. Res. 27:757.

DÖRFFLING,K. 1970. Planta (Berl) 93:243.

DRENNAN, D.S.H. and BERRIE, A.M.M. 1962. New Phytol. 61:1.

DUBROVIN, K.P. 1959. Proc.Jt. 16th North Cent. and 10th West
Canad. Weed Control Conf. 15.

EAGLES, C.F. and WAREING, P.F. 1964. Physiol. Plantarum 17:697.

ELLIOTT, B.B. and LEOPOLD, A.C. 1953. Physiol. Plantarum 6:65.

ELLIOTT, J.G. 1972. Proc. 11th Brit.Weed Control Conf. 3:965.

————————— and ATTWOOD, P.J. 1970. Tech.Rep.Agric. Res.
Coun. Weed Res. Orgn. 16:1.

ESASHI, Y. and LEOPOLD, A.C. 1969. Plant Physiol. 44:1470.

EVENARI, M. 1949. Bot. Review. 15:153.

————————— 1965. Encycl. Plant Physiol. 15:804.

—————————, NEUMAN, G., BLUMENTHAL-GOLDSCHMIDT, S., MAYER, A.M.
and POLJAKOFF-MAYBER, A. 1958. Bull. Res. Coun. Israel D.
6:65.

FALCONE, A.B., SHRAGO, E. and MAO, R.L. 1960. Federation Proc.
19:38.

FAY, J.P. and FARIAS, R.N. 1977. J.Bacteriology 132:790.

FISHER, SIR R.A. and YATES, F. 1967. Statistical tables
        for biological, agricultural and medical research,
        Oliver and Boyd.

FLEMION, F. and DE SILVA, D.S. 1960. Contr.Boyce Thompson
        Inst. 20:365.

FLINT, L.H. and MCALISTER, E.D. 1935. Smithson. Misc.Coll. 94:1.
———— and ———— 1937. *Ibid.* 96:1

FOX, J.E. 1969. In: Physiology of Plant Growth and
        Development (Wilkins, M.B.,ed.) pp 85-123.
        McGraw-Hill, London.

FRANKLAND, B. and WAREING, P.F. 1966. J.Exp.Bot. 17:596.

FRYER, J.D. and CHANCELLOR, R.J. 1970. Proc. 10th Brit. Weed
        Control Conf. 958.

FUCHS, Y., MAYAK, S., FUCHS, S. 1972. Planta (Berl.) 103:117.

GABR, O.M.K. and GUTTRIDGE, C.G. 1968. Planta (Berl.) 78:305.

GARRETT, M.K. and ROBINSON, P.M. 1969. Arch.Mikrobiol. 67:370.

GLEN, J.L., KUO, C.C., DURLEY, R.C. and PHARIS, R.P. 1972.
        Phytochemistry 11:345.

GLINKA, Z. and REINHOLD, L. 1971. Plant Physiol. 48:103.

GRAY, R.T., MALLABY, R., RYBACK, G. and WILLIAMS, V.P. 1974.
        J.Chem.Soc.Perkin II:919.

GREEN, J.G. and HELGESON, E.A. 1957. Proc. 14th North Cent.
        Weed Control Conf. 5.

GROSS, D. 1972. Pharmazie 27:619.

GUHA, J. and SEN, S.P. 1973. Nat.New Biol. 244:223.

GUPTA, G.R.P. and MAHESHWARI, S.C. 1970. Plant Physiol. 45:14.

HAGER, A., MENZEL, H. and KRAUSS, A. 1971. Planta (Berl.) 100:47.

HALL, R.H. 1973. Ann.Rev.Plant Physiol. 24:415.

HARADA, N. 1973. J.Amer.Chem.Soc. 95:240.

HARPER, J.L. 1957. Proc. 4th Int. Congr. Crop Protection
Hamburg 415.

HARRISON, M.A. and SAUNDERS, P.F. 1975. Planta (Berl) 123:291.

HART, J.W. and BERRIE, A.M.M. 1966. Physiol. Plantarum 19:1020.

HAUPT, W. and WEISENSEEL, M.H. 1976. In: Light and Plant
Development (Smith, H., ed.) pp 63-74. Butterworths,
London.

HAY, J.R. and CUMMING, B.G. 1959. Weeds 7:34.

HAYASHI, Y., TAKAHASHI, S., ONA, H. and SAKAN, T. 1968.
Tetrahedron Lett. 17:2071.

HECTOR, J.M. 1936. Introduction to the Botany of Field Crops. 1.
S. African Agric. Series 15, Johannesburg.

HEISER, C.B. 1973. Seed to Civilization. pp 243. W H Freeman
and Co., San Francisco.

HEMBERG, T. 1978. Physiol. Plantarum 43:63.

HENDRICKS, S.B. and BORTHWICK, H.A. 1967. Proc.Nat.Acad.Sci.
USA 58:2125.

HERTEL, R., ST. THOMSON, K. and RUSSO, V.E.A. 1972.
Planta (Berl.) 107:325.

HIRAGA, K., YOKOTA, T., MUROFUSHI, N. and TAKAHASHI, N. 1974.
Agric.Biol.Chem. 38:2511.

HOLE, C.C. and HARDWICK, R.C. 1978. Ann.Appl.Biol. 88:421.

HOLM, R.E. and MILLER, M.R. 1972. Weed Sci. 20:150.

HOLROYD, J. 1960. Proc. 5th Brit. Weed Control Conf. 487.

——— 1971. Arable Farm. 5:42

——— 1972. Rep.Agric.Res.Coun. Weed Res.Orgn. (4) 1969-71:50.

HOLST, U. 1971. Physiol. Plantarum 24:392.

HOUSLEY, S. and TAYLOR, W.C. 1958. J.Exp.Bot. 9:458.

IBANEZ, V.S. and HERSKOVITS, T.T. 1976. Biochemistry 15:5708.

IKUMA, H. and THIMANN, K.V. 1960. Plant Physiol. 35:557.

———— and ———— 1963. Plant Cell Physiol. 4:113.

IRVING, R.M. 1968. Plant Physiol 43(Suppl.) : S-49.

JACOBS, W.P. 1959. Development. Biol. 1:527.

JAFFE, M.J. and GALSTON, A.W. 1967. Planta (Berl) 77:135.

JEFFCOAT, B. and SAMPSON, A.J. 1973. 25ste Symp.Fytopharm.

        Fytiatrie Meded Fak. Landb. Gent. 38:941.

JOHNSON, L.P.V. 1935a. Canad.J.Res. C-D 13:283.

———— 1935b. *Ibid.* 13:367.

JONES, R.L. 1973. Ann.Rev.Plant Physiol. 24:571.

KARSSEN, C.M. 1976. Physiol. Plantarum 36:259.

KEFELI, V.I. and KADYROV, C.S. 1971. Ann.Rev.Plant Physiol.

        22:185.

KEFFORD, N.P. 1954. J.Exp.Bot. 6:129.

KETRING, D.L. and MORGAN, P.W. 1970. Plant Physiol 45:268.

KAHN, A. 1960. Plant Physiol. 35:333.

————, GROSS, J.A. and SMITH, D.E. 1957. Science 125:645.

KHAN, A.A. 1968. Plant Physiol. 43:1463.

———— 1975. Bot. Review 41:391.

————,TOLBERT, N.E. and MITCHELL, E.D. 1964. Plant Physiol.

        39 (Suppl):xxviii.

————, HEIT, C.E., WATERS, E.C., ANOJULU, C.C. and

        ANDERSON, L. 1971. Search Agric. (Geneva, N.Y.) 1:1.

KING, R.W. 1976. Planta (Berl.) 132:43.

KOCH, W. 1968. Proc. 9th Brit. Weed Control Conf. 14.

KÖECKEMANN, A. 1934. Ber.Deut.Botan.Ges. 52:523.

KOMMEDAHL, T., DEVAY, J.E. and CHRISTENSEN, C.M., 1958.

        Weeds 6:12.

KOMOTO, N., IKEGAMI, S. and TAMURA, S. 1972. Agric.Biol.Chem. 36:2547.

KOPECKY, F., SEBANEK, J. and BLAZKOVA, J. 1975. Biol. Plantarum 17:81.

KOREEDA, M., WEISS, G. and NAKANISHI, K. 1973. J.Amer.Chem. Soc. 95:239.

KOSHIMIZU, K., FUKUI, H., MITSUI,T. and OGAWA, Y., 1966. Agric.Biol.Chem. 30:941.

————, INUI, M., FUKUI, H. and MITSUI, T. 1968. *Ibid.* 32:789.

KOVACS, M.I. and SIMPSON, G.M. 1976. Phytochemistry 15:455.

KRIEDEMANN, P.E., LOVEYS, B.R., FULLER, G.L. and LEOPOLD, A.C. 1972. Plant Physiol. 49:842.

LANG, A. 1970. Ann.Rev.Plant Physiol. 21:537.

LEHNINGER, A.L. 1951. In: A Symposium on the Role of Phosphorus in Metabolism of Plants and Animals (McElroy, W.D. and Glass, B.,eds.) 1:350. John Hopkins Press.

LEOPOLD, A.C. and KRIEDEMANN, P.E. 1975. Plant Growth and Development (2nd Edition). pp. 545. McGraw-Hill Book Co.

LE POIDEVIN, N. 1965. Phytochemistry 4:525.

LERNER, H.R., MAYER, A.M. and EVENARI, M. 1959. Physiol Plantarum 12:245.

LETHAM, D.S. 1967. Ann.Rev.Plant Physiol. 18:349.

———— 1969. BioScience 19:309.

LEWIS, J. 1961. Proc.Int.Seed Test.Assoc. 26:68.

LEWIS, H.L. and JOHNSON, G.T. 1968. Mycologia 60:384.

MACMILLAN, J. 1974. Recent Adv. Phytochem. 7:1.

———— and SUTER, P.J. 1958. Naturwissenschaften 45:46.

MALZEW, A.I. 1930. Bull.Appl.Bot.Genet.Plant Breed.Suppl. 38:473.

MAPSON, L.W. and HULME, A.C. 1970. Progr.Phytochem. 2:343.

MARINOS, N.G. and HEMBERG, T. 1960. Physiol. Plantarum 13:571.

MARRE, E. 1977. In: Plant Growth Regulation. Proc. 9th Int. Conf. Plant Growth Subst. (Pilet, P.E.,ed.) pp 54-66. Springer-Verlag.

MARTIN, G.C., MASON, M.I.R. and FORDE, H.I. 1969. J.Amer. Soc.Hort.Sci. 94:13.

MAYER, A.M. and SHAIN, Y. 1974. Ann.Rev.Plant Physiol. 25:167.

———— and POLJAKOFF-MAYBER, A. 1975. In: The Germination of Seeds (Wareing, P.F. and Galson, A.Y.,eds.) pp46-75 Pergamon Press.

McCARTHY, R.D. and DUTHIE, A.H. 1962. J.Lipid Res. 3:117.

McKERCHER, R.B., ASHFORD, R. and MORGAN, R.E. 1975. Weed Sci. 23:823.

McWHA, J.A., 1975. J.Exp.Bot. 26:823.

MILBORROW, B.V., 1967. Planta.(Berl.) 76:93.

———— 1969. Chem.Commun. 966.

———— 1970. J.Exp.Bot. 21:17.

———— 1974. Ann.Rev.Plant Physiol. 25:259.

———— and NODDLE, R.C. 1970. Biochem. J. 119:727.

MILLER, C.O. 1967. Ann. N.Y. Acad. Sci. 144:251.

MITCHELL, E.D. and TOLBERT, N.E. 1968. Biochemistry 7:1019.

MITCHELL, J.W., MANDAVA, N., WORLEY, J.F., PLIMMER, J.R. and SMITH, M.V. 1970. Nature 225:1065.

MOEWUS, F. and SCHADER, E. 1951. Ber.Dtsch.Bot.Ges. 64:124.

MORGAN, S.F. and BERRIE, A.M.M. 1970. Nature 228:1225.

MORI, K. 1973. Tetrahedron Lett. 28:2635.

MOST, B.H., GASKIN, P. and MACMILLAN, J. 1970. Planta(Berl.) 92:41.

MOUSSERON-CANET, M., MANI, J.C., DALLE, J.P. and OLIVE, J.L.
     1966. Bull.Soc.Chim.France 12:3874.

MULLER, C.H. 1965. Bull. Torrey Bot.Club 92:38.

MURAKAMI, Y. 1961. Bot.Mag. Tokyo 74:424.

NALEWAJA, J.D. 1973. North Dak. Farm.Res. 31:3.

NAYLOR, J.M. 1966. Canad.J.Bot. 44:19.

———— and CHRISTIE, L.A. 1957. Proc. 10th Meet. West Sect.
     Nat. Weed Comm. Canad. 56.

———— and SIMPSON, G.M. 1961. Canad.J.Bot. 39:281.

NEGM, F.B., SMITH, O.E. and KUMAMOTO, J. 1973. Plant.Physiol.
     51:1089.

NELSON, W. 1944. J.Biol.Chem. 153:375.

NELSON, P.V. and REID, R.K. 1971. Amer.J.Bot. 58:249.

NIEMAN, C. 1954. Bact.Review 13:147.

NIKOLAEVA, M.G. 1967. Physiology of Deep Dormancy in Seeds.
     (Konovalov, I.N., ed.) pp 219. Acad.Sci. USSR, V.L.
     Komarov Bot. Inst.

NUTILE, G.E. 1945. Plant Physiol. 20:433

OBENDORF, R.L. and MARCUS, A. 1974. Plant Physiol. 53:779.

OHKUMA, K., LYON, J.L., ADDICOTT, F.T. and SMITH, O.E.
     1963. Science 142:1592.

OLATOYE, S.T. and HALL, M.A. 1973. In: Seed Ecology (Heydecker,
     W.,ed.) pp 233-49. Penn. State Univ. Press.

ORITANI, T., YAMASHITA, K. and MEGURO, H. 1972. Agric. Biol.
     Chem. 36:885.

OVERBEEK, J. 1966. Science 152:721.

PALEG, L. 1960. Plant Physiol. 35:293.

PETROVA, V.N. and NIKOLAEVA, M.G. 1974. Fiziol. Rast. 21:343.

PHILLIPSON, A. 1974. Weed Res. 14:123.

PHINNEY, B.O. and WEST, C.A. 1960. Ann.Rev. Plant Physiol.
11:411.

POGGI-PELLEGRIN, M. and BULARD, C. 1976. Physiol.Plantarum
36:40.

POLJAKOFF-MAYBER, A. 1958. Bull.Res.Coun. (Israel) D 6:78.

POWELL, L.E. and SEELEY, S.D. 1974. J.Amer.Soc.Hort.Sci. 99:439.

RAYLE, D.L. and JOHNSON, K.D. 1973. Plant Physiol. 51(Suppl):2.

REEVE, D.R. and CROZIER, A. 1974. J.Exp.Bot. 25:431.

RAYLE, D.L. and JOHNSON, K.D. 1973. Plant Physiol. 51(Suppl):2.

REEVE, D.R. and CROZIER, A. 1974. J.Exp.Bot. 25:431.

REGNAULT, Y., LOUBARESSE, J.P. and MOUILLAC, A. 1974.
Proc. 12th Brit. Weed Control Conf. 2:581.

REYNOLDS, T. 1975. Ann.Botany 39:797.

RITTENBERG, D. and FOSTER, G.L. 1940. J.Biol.Chem. 133:737.

RIVIER, L., MILON, H. and PILET, P.E. 1977. Planta (Berl.)
134:23.

ROBERTSON, J., HILLMAN, J.R. and BERRIE, A.M.M. 1976.
Planta(Berl.) 131:309.

ROBINSON, P.M. and WAREING, P.F. 1964. Physiol. Plantarum 17:314.

ROTHWELL, K. and WAIN, R.L. 1964. In: Régulateurs Naturels
de la Croissance Végétale, 363-375 (C.N.R.S., Paris,
748pp).

RUDNICKI, R., BORECKA, H. and PIENIAZEK, J. 1969. Planta(Berl.)
86:63.

RYBACK, G. 1972. Chem. Commun. 1190.

SANKHLA, N. and SANKHLA, D. 1968. Physiol.Plantarum 21:190.

SCHOLEFIELD, P.G. 1956. Canad.J.Biochem.Physiol. 34:1227.

SCHWENDIMAN, A. and SHANDS, H.L. 1943. J.Amer.Soc.Agron. 35:681.

SEELEY, S.D. and POWELL, L.E. 1970. Anal.Biochem. 35:530.

SELMAN, M. 1968. Proc.9th Brit.Weed Control Conf. 84.

SIMMONDS, J.A. and SIMPSON, G.M. 1971. Canad.J.Bot. 49:1833.

SIMPSON, G.M. 1965. Canad.J.Bot. 43:792.

———— 1966. *Ibid.* 44:115.

SIVORI, E.M., SONVICO, V. and FERNANDEZ, N.O. 1971. Plant
        Cell Physiol. 12:993.

SKENE, M. 1924. The Biology of Flowering Plants, 422-431
        Sidgwick and Jackson Ltd.,London.

SKINNER, C.G., TALBERT, F.D. and SHIVE, W. 1958. Plant
        Physiol. 33:190.

SKOOG, F. and SCHMITZ, R.Y. 1972. In: Plant Physiology, a
        Treatise (Steward, F.C., ed.) 6B:181. Academic Press.

SMITH, I.K. and FOWDEN, L. 1966. J.Exp.Bot. 17:750.

SOMOGYI, M. 1952. J.Biol.Chem. 195:19.

SONDHEIMER, E. and GALSON, E.C. 1966. Plant Physiol. 41:1397.

————, GALSON, E.C., TINELLI, E. and WALTON, D.C. 1974.
        Plant Physiol. 54:803.

SPENCER, M. 1969. Fortschr.Chem.Org.Naturst. 27:32.

STOKES, P. 1965. Encycl.Plant Physiol. 15:746.

STOWE, B.B. and HUDSON, V.W. 1969. Plant Physiol. 44:1051

———— and DOTTS, M.A. 1971. *Ibid.* 48:559.

SUMNER, D.C. and LYON, J.L. 1967. Planta(Berl.) 75:28.

TANADA, T. 1973. Plant Physiol. 51:150.

TAYLORSON, R.B. and HENDRICKS, S.B. 1977. Ann.Rev. Plant
        Physiol. 28:331.

THIMANN, K.V. 1972. In: Plant Physiology, a Treatise
        (Steward, F.C., ed.) 6B:129. Academic Press.

———— 1974. Plant Physiol. 54:450.

THURSTON, J.M. 1954. Ann.Appl.Biol. 41:619.

———— 1956. J.Roy.Agric.Soc. 117:43.

THURSTON, J.M. 1957. J.Agric.Sci.Cambr. 49:259.

———— 1961. Weed Res. 1:19.

———— 1962. *Ibid*. 2:122.

———— 1974. Rep.Rothamsted Exp.Statn. 1973:106.

TILLBERG, J-E. 1968. Zeit.Pflanzenphysiol. 59:305.

TOOLE, V.K. and CATHY, H.M. 1959. Plant Physiol. 34(Suppl):16.

————, BAILEY, W.K. and TOOLE, E.H. 1964. *Ibid*. 39:822.

TSO, T.C. 1964. Nature 202:511.

————, STEFFENS, G.L. and ENGLEHAUPT, M.E. 1965. J.Agric.
Food Chem. 13:78.

TUCKER, D.J. and MANSFIELD, T.A. 1971. Planta (Berl.) 98:157.

TZOU, D.S.,GALSON, E.C. and SONDHEIMER, E. 1973. Plant
Physiol. 51:894.

VACHA, G.A. and HARVEY, R.B. 1927. Plant Physiol. 2:187

VAN STADEN, J. 1973. Physiol.Plantarum 28:222.

VAN STEVENINCK, R.F.M. 1976. In: Encycl. Plant Physiol.New
Ser., Transport in Plants II, Part B, Tissues and
Organs (Lüttge, U. and Pitman, M.G., eds.) pp307-342.
Springer.

VAN SUMERE, C.F. 1960. In: Phenolics in Plants in Health
and Disease (Pridham, J.B., ed.) pp 25-34 Pergamon Press.

————, HILDERSON, H. and MASSART, L. 1958. Naturwissen-
schaften 45:292.

————, COTTENIE, J., DE GREEF, J. and KINT, J. 1972.
Rec.Adv. Phytochem. 4:165.

VARGA, M. 1957. Acta Biologica 3:213.

VARNER, J.E. 1964. Plant Physiol. 39:413.

VEGIS, A. 1964. Ann.Rev. Plant Physiol. 15:185.

VILLIERS, T.A. 1968. Planta(Berl) 82: 342.

VILLIERS, T.A. and WAREING, P.F. 1965. J.Exp.Bot. 16:519.

WACHSMUTH, E.D. 1965. Nature 208:942.

WALTON, D.C. and SONDHEIMER, E. 1972. Plant Physiol. 49:285.

————— , DORN, B. and FEY, J. 1973. Planta(Berl.) 112:87.

WAREING, P.F. 1965. Encycl.Plant Physiol. 15:909.

————— 1969. In: The Physiology of Plant Growth and
        Development (Wilkins, M.B., ed.) pp 605-644. McGraw-Hill.

————— and VILLIERS, T.A. 1961. In: Plant Growth Regulation.
        (International Conference on Plant Growth Regulation,
        1959) pp 95-107. Iowa State Univ. Press.

————— and RYBACK, G. 1970. Endeavour, 19:84.

WATERSON, H.A. and DAVIES, G.J. 1973. Weed Res. 13:192.

WEBB, D.P. and WAREING, P.F. 1972. Planta(Berl.) 104:115.

————— , VAN STADEN, J. and WAREING, P.F. 1973a. J.Exp.Bot.
        24:105.

————— , ————— and ————— 1973b *Ibid*. 24:741.

WEBER, G., CONVERY, H.J.H., LEA, M.A. and STAMM, N.B. 1966.
        Science 154:1357.

WEISS, J. 1960. C.R..Acad.Sci. 251:125.

WHYBREW, J.E. 1964. Proc. 7th Brit.Weed Control Conf. 614.

WILLEMSEN, R.W. and RICE, E.L. 1972. Amer.J.Bot. 59:248.

WILLIAMS, P.M., ROSS, J.D. and BRADBEER, J.W. 1973. Planta(Berl.)
        110:303.

WILLMER, C.M., DON, R. and PARKER, W. 1978. Planta(Berl.) 139:281.

WILSON, B.J. and CUSSANS, G.W. 1972. Proc. 11th Brit. Weed
        Control Conf. 234.

WOOD, A. and PALEG, L.G. 1972. Plant.Physiol. 50:103.

————— and ————— 1974. Austr.J.Plant Physiol. 1:31.

————— , PALEG, L.G. and SAWHNEY, R. 1972. In: Plant Growth
        Substances, 1970. Proc.Int.Congr.Growth Subst.,
        Canberra (Carr, D.J., ed.) pp 37-43,Springer-Verlag.

WOOD, A., PALEG, L.G. and SPOTSWOOD, T.M. 1974. Austr.J.
Plant Physiol. 1:167.

WRIGHT, S.T.C. 1975. J.Exp.Bot. 26:161.

—— and HIRON, R.W.P. 1969. Nature 224:719.

—— and ——1972. In: Plant Growth Substances, 1970.
Proc.Int.Congr.Growth Subst., Canberra (Carr,D.J.,
ed.) pp 291-298. Springer-Verlag.

YANG, S.F. 1974. Rec.Adv.Phytochem. 7:131.

YOMO, H. and Iinuma, H. 1966. Planta(Berl.) 71:113.

ZINSMEISTER, H.D. and HOLLMULLER, W. 1964. Planta(Berl.)
63:133.

APPENDIX I

Solubilities of Saturated Fatty Acids in the Series
Caproic to Stearic at 20°C

ACID	g acid per 100g water
Caproic	0.968
Heptanoic	0.244
Caprylic	0.068
Nonanoic	0.026
Capric	0.015
Undecanoic	0.0093
Lauric	0.0055
Tridecanoic	0.0033
Myristic	0.0020
Pentadecanoic	0.0012
Palmitic	0.00072
Heptadecanoic	0.00042
Stearic	0.00029

APPENDIX II

STATISTICAL ANALYSIS OF EXPERIMENTAL DATA

When scoring germination it is only possible to
determine it in terms of "yes" or "no". Thus the germination
of a seed population can be regarded as binominal, showing
a symmetry at the extremes of the distribution. Statistics
are generally only applied to data which can be fitted to a
normal distribution, that is, one which is symmetrical about
a mean. Therefore, the germination percentages must be
transformed using an 'arc sine transformation' before
statistical analyses can be applied to the germination data.
Using the angular transformation tables of Fisher and Yates
(1967) the distribution can be converted from binominal to
normal.

a) MEAN:

The mean was calculated using the following expression:

$$\text{Mean } (\bar{x}) = \frac{\Sigma x}{n}$$

where Σx is the sum of the individual values of each
observation (x) and n the number of observations.

RUNNING-MEAN:

It should be expected that any natural developmental
phenomenon would involve a process of gradual change. A
time-course programme of extractions of growing plant
material is often subject to errors imposed through short-
term variations in the ambient environmental conditions.
In order to effect a degree of compensation for such events,
which may modify the developmental trend, the experimental
data may be expressed as a running-mean. This treatment
provides a mean of three sequential real-values throughout
the time course study and reduces the impact of any

short-term abnormal variations on the developmental pattern.

b)   VARIANCE:

The variance was calculated using the following equation:

$$\text{Variance } (s^2) \quad = \quad \frac{\Sigma \ (x-\overline{x})^2}{n-1}$$

where x is the value for each individual observation and n is the number of observations.

c)   STANDARD DEVIATION,   STANDARD ERROR AND CONFIDENCE LIMITS:

The standard deviation (s) $= \sqrt{s^2}.$

The standard error is obtained from the following expression:

$$\text{Standard Error (S.E.)} = \frac{s}{\sqrt{n}} \ .$$

Confidence limits for the mean $(\overline{x})$ can then be set as follows:

$$\overline{x} \ \pm \ d_{0.05} \ \text{x S.E.} \quad \text{(when n>30)}$$

$$\text{or} \quad \overline{x} \ \pm \ t_{0.05} \ \text{x S.E.} \quad \text{(when n< 30)}$$

where $t_{0.05}$ is the "students" t-value taken from the tables of Bailey (1969).   When the probability (P) is 0.05 and there are (n-1) degrees of freedom then $d_{0.05}$ has a value of 1.96.   In this case the probability of observing a departure from the mean of more than 1.96 standard deviations is 5%.

Example:

The following example illustrates the use of statistical analyses for determining the confidence limits for the mean of a series of peak areas obtained by G.L.C. of a methyl

nonanoate solution. The following areas were assessed using an Albright spinning-disc planimeter:

> 1.92, 2.57, 2.28, 2.21, 2.00, 1.95, 2.15
>
> 2.50, 2.12, 2.25, 2.27, 2.48, 2.34, 2.09

The Standard Error for the mean of the observations is 0.05, where n is 14 and s is 0.20.

Confidence Limits can then be set for this mean:

$$= t_{0.05} \times \frac{s}{\sqrt{n}} = \pm 0.12$$

where $t_{0.05}$ is 2.16 from the t-distribution.

Therefore, the mean with its confidence limit can be written:

$$\bar{x} = 2.22 \pm 0.12 \quad \text{or} \quad 2.22 \pm 5.4\%$$

d) $\underline{x^2 - \text{TESTS OF GOODNESS-OF-FIT}}$

x^2 was determined from the following expression:

$$x^2 = \Sigma \frac{(O-E)^2}{E}$$

where 'O' is the observed result and 'E' the expected result.

For example, treatment of Great Lakes lettuce seed with 2mM hexanoic acid, 2mM heptanoic acid or both of these acids in combination gave the results shown in Table I.

The goodness-of-fit for the data could then be calculated as follows:

$$x^2 = \frac{(30-62.5)^2}{62.5} + \frac{(32-57)^2}{57} + \frac{(38-61.5)^2}{61.5} = 36.8$$

The probability of observing a x^2-value of 36.8 with two degrees of freedom (r-1)(c-1), where r is the number of rows and c the number of columns in the contingency table, is less than 0.001 from the x^2 distribution (Bailey 1969).

TABLE I

The effect of hexanoic and heptanoic acids, alone or in combination at 2mM, on the germination of *Lactuca sativa* L., cv. Great Lakes, at 20°C in the dark

TREATMENT	PERCENTAGE GERMINATION			
	1	2	3	Mean
C_6	68	60	65	64.3
C_7	57	54	58	56.3
$C_6 + C_7$ (observed)	30	32	38	33.3
$C_6 + C_7$ (expected)	62.5	57	61.5	60.3

Thus the observed values can be regarded as significantly different from that expected.

e)   ANALYSIS OF VARIANCE:

Experiments reported in this study utilised either a "randomised block design" or a "factorial design". Examples of analyses of variance for the two types of experiment are set out below.

(i)   Randomised block design.

The data from a randomised block designed experiment is usually set out as in Table II. From these results the analysis of variance table can be calculated (Table III). Significance of the variance ratios is tested using "Variance-Ratio (F) Distributions" (Bailey 1969), where the degrees of freedom of the error factor and the relevant "source" are used.

The treatment means, $\bar{x}$, each have an associated standard error $(^s/\sqrt{b})$, where b represents the number of blocks) and confidence limits can be set for this mean as outlined previously, section (c). To be significant (probability, P, 0.05) the difference between two treatment means must exceed the least significant difference (LSD), where:

$$LSD = d_{0.05} \times {}^s\sqrt{\frac{2}{b}} \quad or \quad t_{0.05} \times {}^s\sqrt{\frac{2}{b}},$$

using the value $d_{0.05}$ or $t_{0.05}$ where appropriate. The degrees of freedom are equal to that for the error $[(t-1)(b-1)]$, where t represents the number of treatments.

Example:

Consider an experiment where the effect of MCFA on the germination of Great Lakes lettuce seed at $20^{\circ}C$ was

TABLE II

General table of data for a randomised block design

Treatments	1 2-----j-----b	Treatment Totals	Treatment Means
1	x_{11} x_{12}---x_{1j}---x_{1b}	T_1	$\bar{x}_1$
2	x_{21} x_{22}---x_{2j}---x_{2b}	T_2	$\bar{x}_2$
i	x_{i1} x_{i2}---x_{ij}---x_{ib}	T_i	$\bar{x}_i$
t	x_{t1} x_{t2}---x_{tj}---x_{tb}	T_t	$\bar{x}_t$
Block Totals	B_1 B_2 ---B_j ---B_b	G	$\bar{x} = {}^{G}\!/_{bt}$

TABLE III

General table of analysis of variance for an experiment based on the randomised block design

Source of Variance	Degrees of Freedom	Sums of Squares	Mean Square	Variance Ratio
Total	$bt-1$	$\Sigma x_{ij}^2 - c$		
No. of Treatments (t)	$t-1$	$\dfrac{\Sigma T_i^2}{b} - c$	M_T	$\dfrac{M_T}{s^2}$
No. of Blocks (b)	$b-1$	$\dfrac{\Sigma B_j^2}{t} - c$	M_B	$\dfrac{M_B}{s^2}$
Error	$(t-1)(b-1)$	Z	s^2	

where:- $c = \dfrac{G^2}{bt}$

$$Z = (\Sigma x_{ij}^2 - c) - \left(\frac{\Sigma T_i^2}{b} - c\right) + \left(\frac{\Sigma B_j^2}{t} - c\right)$$

and $\quad M_T = \dfrac{\dfrac{\Sigma T_i^2}{b} - c}{t-1} \quad ; \quad M_B = \dfrac{\dfrac{\Sigma B_j^2}{t} - c}{b-1}$

and $\quad s^2 = \dfrac{Z}{(t-1)(b-1)}$

examined at six concentrations. The acids tested were in the series pentanoic to dodecanoic, inclusive, and the concentrations were:

A, 10^{-2}M

B, 5×10^{-3}M

C, 2.5×10^{-3}M

D, 1.25×10^{-3}M

E, 6.25×10^{-4}M

F, 3.12×10^{-4}M

The experimental results are indicated in Table IV, each being the mean of two bioassays. The angularly transformed results are shown in Table V. The analysis of variance table could then be calculated (Table VI).

From Table V, $G = 8058.77$

$c = \dfrac{G^2}{bt}$ where t is 49 and b is 3, $\therefore$ c = 441794.38

From all data:

$\Sigma x_{ij}^2 = 572530.96$, $\therefore$ $\Sigma x_{ij}^2 - c = 130736.58$

$\Sigma T_i^2 = 1714833.06$, $\therefore$ $\dfrac{\Sigma T_i^2}{b} - c = 129816.64$

$\Sigma B_j^2 = 21649205.78$, $\therefore$ $\dfrac{\Sigma B_j^2}{t} - c = 26.15$

$\therefore$ Z = 130736.58 - 129816.64 - 26.15 = 893.79

and the variance, $s^2 = \dfrac{Z}{(t-1)(b-1)} = 9.31$

and the standard deviation, s = 3.05

The standard error of the difference between any two treatments can be represented by the expression:

S.E. $= \pm\ s\sqrt{\dfrac{2}{b}} = \pm\ 2.5$

But to be significant the difference between any two means must exceed the least significant difference (LSD) where:

LSD $= \pm\ d_{0.05} \times\ s\sqrt{\dfrac{2}{b}} = \pm 4.9$

at a 5% probability.

TABLE IV

The effect of various concentrations of MCFA on the
germination of *Lactuca sativa* L., cv.   Great Lakes
after 24h at 20°C.

| Acid | Treatment | PERCENTAGE GERMINATION | | | |
		1	2	3	Mean
C_5	A	0	0	0	0
	B	50	35	26	37
	C	73	85	86	81.3
	D	93	95	93	93.7
	E	95	95	95	95.0
	F	95	96	92	94.3
C_6	A	0	0	0	0
	B	0	0	0	0
	C	54	48	52	51.3
	D	92	89	93	91.3
	E	97	93	95	95
	F	95	95	98	96
C_7	A	0	0	0	0
	B	0	0	0	0
	C	39	29	29	32.3
	D	91	85	85	87
	E	99	96	95	96.7
	F	96	97	97	96.7
C_8	A	0	0	0	0
	B	0	0	0	0
	C	9	24	28	20.3
	D	87	81	82	83.3
	E	94	96	96	94.7
	F	93	95	98	95.3

TABLE IV (contd.)

Acid	Treatment	PERCENTAGE GERMINATION			
		1	2	3	Mean
C_9	A	0	0	0	0
	B	0	0	0	0
	C	2	2	3	2.3
	D	61	49	57	52.3
	E	91	84	88	87.7
	F	93	97	96	95.3
C_{10}	A	6	15	13	11.3
	B	21	26	30	25.7
	C	73	77	75	75
	D	95	85	90	90
	E	95	95	97	95.7
	F	99	96	97	97.3
C_{11}	A	74	69	76	73
	B	84	90	88	87.3
	C	95	93	94	94
	D	95	95	96	95.3
	E	97	99	98	98
	F	95	95	97	95.7
C_{12}	A	96	95	94	95
	B	98	93	96	95.7
	C	95	93	98	95.3
	D	95	97	97	96.3
	E	96	97	96	96.3
	F	96	97	98	97
Control		99	96	99	98

TABLE   V

The Angular Transformation of the Percentage Germinations
shown in Table IV

Acid	Treatment	ANGULAR TRANSFORMATION			
		REPLICATES			Total (Σx)
		1	2	3	
C_5	A	4.05	4.05	4.05	12.15
	B	45.00	36.27	30.66	111.93
	C	58.69	67.21	68.03	193.93
	D	74.66	77.08	74.66	226.40
	E	77.08	77.08	75.82	229.98
	F	77.08	78.46	73.57	229.11
C_6	A	4.05	4.05	4.05	12.15
	B	4.05	4.05	4.05	12.15
	C	42.29	43.85	46.15	132.29
	D	73.57	70.63	74.66	218.86
	E	80.03	74.66	77.08	231.77
	F	77.08	77.08	84.26	238.42
C_7	A	4.05	4.05	4.05	12.15
	B	4.05	4.05	4.05	12.15
	C	38.65	32.58	32.58	103.81
	D	72.54	67.21	67.21	206.96
	E	84.26	78.46	77.08	239.8
	F	78.46	80.03	80.03	238.52
C_8	A	4.05	4.05	4.05	12.15
	B	4.05	4.05	4.05	12.15
	C	17.46	29.33	31.95	78.74
	D	68. 87	64.16	64.90	197.93
	E	75.82	78.46	78.46	232.74
	F	74.66	77.08	81.87	233.61

TABLE   V (contd.)

Acid	Treatment	ANGULAR TRANSFORMATION			
		REPLICATES			Total (Σx)
		1	2	3	
C_9	A	4.05	4.05	4.05	12.15
	B	4.05	4.05	4.05	12.15
	C	8.13	8.13	9.97	26.23
	D	51.35	40.48	49.02	140.85
	E	72.54	66.42	69.73	208.69
	F	74.66	80.03	78.46	233.15
C_{10}	A	14.18	22.79	21.03	58.00
	B	22.27	30.66	33.21	91.14
	C	58.69	61.34	60.00	180.03
	D	77.08	67.21	71.71	215.86
	E	77.08	77.08	80.03	234.19
	F	84.26	78.46	80.03	242.75
C_{11}	A	59.34	56.17	60.67	176.18
	B	66.42	71.75	69.73	207.72
	C	77.08	74.66	75.82	227.56
	D	77.08	77.08	78.03	232.19
	E	80.03	84.26	81.87	246.16
	F	77.08	77.08	80.03	234.19
C_{12}	A	78. 46	77.08	75.82	231.36
	B	81.87	74.66	77.08	233.61
	C	77.08	74.66	81.87	233.61
	D	77.08	80.03	80.03	237.14
	E	75.82	80.03	78.46	234.31
	F	75.82	80.03	81.87	237.72
Control		84.26	78.46	84.26	246.98
Column Totals (Σx)		26 80.31	2664.45	2714.01	8058.77

TABLE VI

Analysis of variance for the data represented in Table V

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
Treatments,t	129816.64	48	2704.5	290.5
Replicates,b	26.15	2	13.1	1.4
Error	893.79	96	9.3	
Total	130736.58	146		

By consulting the percentage points of the variance-ratio (F) distribution (Bailey 1969), no significant difference was found between the three replicate treatments (blocks). However, it is clear from Table VI that highly significant differences (probability 0.001) occurred between the various fatty acid concentrations.

(ii) <u>Multi Factorial Design</u>

A number of experiments have employed a factorial design in which two distinct treatment variables were tested at various "levels". A factorial design combines these two variables in all possible combinations. Abstracted and actual experimental data are represented in Tables VII and IX respectively.

In order to determine the analysis of variance the following six entities were calculated:

<u>Total</u>:
$$x_{111}^2 + \text{---} + x_{11j}^2 + \text{---} + x_{ihj}^2 + \text{---} + x_{tqb}^2 \qquad - \enspace \textcircled{1}$$

<u>Treatment A</u>:
$$(Z_1^2 + \text{---} + Z_i^2 + \text{---} + Z_t^2) \times \frac{1}{qb} \qquad - \enspace \textcircled{2}$$

<u>Treatment B</u>:
$$(Y_{11} + \text{---} + Y_{1j} + \text{---} + Y_{1b})^2 + \text{---} + (Y_{h1} + \text{---}$$
$$+ Y_{hj} + \text{---} + Y_{hb})^2 + \text{---} + (Y_{q1} + \text{---} + Y_{qj} + \text{---} +$$
$$Y_{qb})^2 \times \frac{1}{tb} \qquad - \enspace \textcircled{3}$$

TABLE VII

General table of data for a factorial design.

A \ Treatments B	1 Block 1---j---b	h Block 1---j---b	q Block 1---j---b	Totals
1	x_{111}---x_{11j}---x_{11b}	x_{1h1}---x_{1hj}---x_{1hb}	x_{1q1}---x_{1qj}---x_{1qb}	z_1
i	x_{i11}---x_{i1j}---x_{i1b}	x_{ih1} x_{ihj} x_{ihb}	x_{iq1}---x_{iqj}---x_{iqb}	z_i
t	x_{t11}---x_{t1j}---x_{t1b}	x_{th1}---x_{thj}---x_{thb}	x_{tq1}---x_{tqj}---x_{tqb}	z_t
Totals	Y_{11} --- Y_{1j} --- Y_{1b}	Y_{h1} --- Y_{hj} --- Y_{hb}	Y_{q1} --- Y_{qj} --- Y_{qb}	G

Interaction:

$$(x_{111} + \text{---} + x_{11j} + \text{---} + x_{11b})^2 + \text{---} + (x_{1h1} +$$
$$\text{---} + x_{1hj} + \text{---} + x_{1hb})^2 + \text{---} + \text{---} + \text{---} +$$
$$(x_{tq1} + \text{---} + x_{tqj} + \text{---} + x_{tqb})^2 \times \frac{1}{b} \qquad - \quad \text{(4)}$$

Blocks:

$$(Y_{11} + \text{---} + Y_{h1} + \text{---} + Y_{q1})^2 + \text{---} + (Y_{1j} + \text{---}$$
$$+ Y_{hj} + \text{---} Y_{qj})^2 + \text{---} + (Y_{1b} + \text{---} + Y_{hb} \text{---}$$
$$+ Y_{qb})^2 \times \frac{1}{qt} \qquad - \quad \text{(5)}$$

Correction Factor: $\quad \dfrac{G^2}{qtb} \qquad\qquad - \quad \text{(6)}$

The analysis of variance table can then be drawn up
(Table VIII). Standard errors, confidence limits of
treatment means, variance ratios and the least significant
difference between treatment means were calculated as
previously described, section e(i).

Example:

Consider an experiment where the effect of both MCFA
and gibberellic acid on the germination of Grand Rapids
lettuce seed at $20^{\circ}C$ was examined at various concentrations.
The experimental percentage germinations and the angular
transformations are indicated in Tables IX and X,
respectively.

Calculations:

Total: Sum of the squares of all values was
calculated to be 243792.35 ((1)).

Treatment ∴: Fatty acids in the series hexanoic to
decanoic, inclusive, were tested at concentrations of

TABLE VIII

General table of analysis of variance for an experiment based on the factorial design

Source of Variance	Degrees of Freedom	Sums of Squares	Mean Square	Variance Ratio
Total	$bqt-1$	$①-⑥$		
Treatment A	$t-1$	$②-⑥$	$\dfrac{②-⑥}{(t-1)}$, (M)	$\dfrac{M}{Q}$
Treatment B	$q-1$	$③-⑥$	$\dfrac{③-⑥}{(q-1)}$, (N)	$\dfrac{N}{Q}$
Interaction	$(q-1)(t-1)$	$(④-⑥)-\left[(②-⑥)+(③-⑥)\right]$	$\dfrac{(④-⑥)-\left[(②-⑥)+(③-⑥)\right]}{(q-1)(t-1)}$, (O)	$\dfrac{O}{Q}$
Blocks	$b-1$	$⑤-⑥$	$\dfrac{⑤-⑥}{b-1}$, (P)	$\dfrac{P}{Q}$
Error	$(bqt-1)-\left[(t-1)+(q-1)+(q-1)(t-1)+(b-1)\right]$, (A)	$(①-⑥)-\left[(②-⑥)+(④-⑥)(②-⑥)+(③-⑥)+(⑤-⑥)\right]$, (B)	$\dfrac{B}{A}$, (Q)	

TABLE IX

The effect of various concentrations of MCFA and Gibberellic
acid on the germination of *Lactuca sativa L.*, cv.
Grand Rapids, after 24h at 20°C.

GA TREATMENT MCFA		PERCENTAGE GERMINATION								
		0.0M GA			10^{-4}M GA			10^{-3}M GA		
		a	b	c	a	b	c	a	b	c
Control	0	90	94	92	96	96	93	94	96	98
C_6	5×10^{-3}M	2	2	5	15	11	13	35	29	32
	6.25×10^{-4}M	55	43	52	86	87	91	95	92	95
C_7	5×10^{-3}M	0	0	0	5	5	2	20	19	21
	6.25×10^{-4}M	38	38	41	84	83	76	98	93	85
C_8	5×10^{-3}M	0	0	0	0	0	0	4	2	0
	6.25×10^{-4}M	36	36	33	79	80	75	91	95	93
C_9	5×10^{-3}M	0	0	0	0	0	0	7	9	2
	6.25×10^{-4}M	37	38	39	84	85	80	92	93	91
C_{10}	5×10^{-3}M	0	5	1	18	20	19	42	41	43
	6.25×10^{-4}M	45	45	42	84	90	90	96	95	91

TABLE X   The Angular Transformation of the Percentage Germinations shown in Table IX

TREATMENT MCFA	GA	0.0M GA			10⁻⁴ M GA			10⁻³ M GA			Column Total (Σx)
		a	b	c	a	b	c	a	b	c	
Control	0	71.57	75.82	73.57	78.46	78.46	74.66	75.82	78.46	81.87	688.69
C₆	5x10⁻³M	8.13	8.13	12.92	22.79	19.37	21.13	36.27	32.58	34.45	195.77
	6.25x10⁻⁴M	47.87	40.98	46.15	68.03	68.87	72.54	77.08	73.58	77.08	572.17
C₇	5x10⁻³M	4.05	4.05	4.05	12.92	12.92	8.13	26.57	25.84	27.27	125.80
	6.25x10⁻⁴M	38.06	38.06	39.82	66.42	65.65	60.67	81.87	74.66	67.21	532.42
C₈	5x10⁻³M	4.05	4.05	4.05	4.05	4.05	4.05	11.54	8.13	4.05	48.02
	6.25x10⁻⁴M	36.87	36.87	39.84	62.73	63.43	60.00	81.87	74.66	67.21	523.46
C₉	5x10⁻³M	4.05	4.05	4.05	4.05	4.05	4.05	15.34	17.46	8.13	65.23
	6.25x10⁻⁴M	27.46	38.06	38.65	66.42	67.21	63.43	73.57	74.66	72.54	532.00
C₁₀	5x10⁻³M	4.05	12.92	5.74	25.10	26.57	25.84	40.40	39.82	40.98	221.42
	6.25x10⁻⁴M	42.13	42.13	40.40	66.42	71.57	71.57	78.46	77.08	72.54	562.30
Column Total (Σx)		298.29	305.12	309.22	477.39	482.15	466.07	598.79	576.92	553.33	4067.28

ANGULAR TRANSFORMATION

6.25×10^{-4} M and 5×10^{-3} M. The subtotals for treatment A (MCFA) were calculated by summing all values (9) at any one fatty acid concentration. These subtotals were then squared and summed. This total was divided by the number of treatments for each concentration.

$$\therefore \quad \textcircled{2} = \frac{1}{9} (688.9^2 + --- + 48.02^2 + --- + 562.3^2)$$
$$= 229788.70$$

Treatment B: Gibberellic acid was simultaneously tested at concentrations of zero molar, 10^{-4} and 10^{-3} M. As for Treatment A, the subtotals were calculated by summing all the values at any one concentration. These subtotals were squared, summed and the total divided by the number of observations for each subtotal.

$$\therefore \quad \textcircled{3} = \frac{1}{33} \left[(298.29+305.12+309.22)^2 + (477.39+482.15+466.07)^2 \right.$$
$$\left. + (598.79+576.92+553.33)^2 \right]$$
$$= 177419.29.$$

Interaction: This entity is represented by the total of the values for each treatment, there being 33 treatments in this experiment. Each set of three bioassays which make one treatment (a, b and c) were added to form a subtotal and these values were squared and summed over all treatments. The total obtained was divided by the number of replicates.

$$\therefore \quad \textcircled{4} = \frac{1}{3} \left[(71.57+75.82+73.57)^2 + --- + (66.42+65.65+ \right.$$
$$\left. 60.67)^2 + --- + (78.46+77.08+72.54)^2 \right]$$
$$= 243244.86.$$

Blocks: Three replicates were carried out for each treatment. The columns for each bioassay, a, b or c, were totalled, these subtotals then being squared and summed.

This figure was then divided by the total number of values which contributed to each subtotal.

$$\therefore \; \boxed{5} \; = \frac{1}{33} \left[(298.29+477.39+598.79)^2 + (305.12+482.15+ \right.$$
$$576.92)^2 + (309.22+466.07+553.33)^2 \left. \right]$$
$$= 167133.73$$

Correction Factor: $\dfrac{(\text{Total of all values})^2}{\text{Total number of values}}$

$$\therefore \; \boxed{6} \; = (4067.28)^2 \; x \frac{1}{99} \; = 167098.65$$

The degrees of freedom, sum of squares, mean square and the variance ratio for these factors were then calculated as demonstrated in Table VIII. The analysis of variance table could then be tabulated, Table XI. No significant difference was found between the three replicates for each treatment, but highly significant differences (P 0.001) occurred in other sources of variance.

The variance was found to be 8.01 with a standard deviation of 2.83. The standard error (S.E.) of each mean was then determined, where:

$$\text{S.E.} = \pm \frac{s}{\sqrt{b}} = \pm 1.63$$

Similarly, the standard error of the difference between any two treatments can be represented:

$$\text{S.E.} = \pm s\sqrt{\frac{2}{b}} = \pm 2.31$$

Confidence limits can be set for these values; the value of the mean ($\bar{x}$) with its confidence limit being:

$$\bar{x} \pm d_{0.05} \; x \frac{s}{\sqrt{b}} = \bar{x} \pm 3.19$$

To be significant the difference between any two means must exceed the least significant difference (LSD), where:

$$\text{LSD} = d_{0.05} \; x \; s\sqrt{\frac{2}{b}} = \pm 4.53$$

at a probability (P) of 5%

TABLE XI: Analysis of variance for the data represented

in Table X

Source of Variance	Sum of Squares	Degrees of Freedom	Mean Square	Variance Ratio
MCFA treatment	62690.05	10	6269.01	783.0
GA treatment	10320.64	2	5160.32	644.5
Interaction	3135.52	20	156.77	19.6
Replicates	35.08	2	17.54	2.2
Error	512.41	64	8.01	
Total	76693.70	98		

APPENDIX III

TABLE I. Percentage germination and levels of ABA in grain of *A. sativa* cv Selma during maturation and subsequent dry storage. (Values given are corrected for extraction losses and are the calculated amounts for individual observations for the dry seed). UD, undetected.

Days from Anthesis	%age Germ.	FREE ABA			"NEUTRAL" BOUND			"ACID" BOUND			TOTAL	
		µgkg⁻¹	pg grain⁻¹	Rel. Content	µgkg⁻¹	pg grain⁻¹	Rel. Content	µgkg⁻¹	pg grain⁻¹	Rel. Content	µgkg⁻¹	pg grain⁻¹
19	0	52.3	1005	.25	16.4	315	.08	141.2	2714	.67	209.9	4034
27	0	7.3	170	.25	5.3	123	.18	17.2	399	.57	29.8	692.0
37	18.7	15.0	478	.28	16.7	532	.31	22.2	708	.41	53.9	1718
44	29.3	3.3	132	.26	7.9	317	.61	1.7	68	.13	12.9	517
51	45.0	8.8	376	.88	1.23	53	.12	UD	-	-	10.0	429
58	69.3	14.9	666	.46	11.03	493	.34	6.7	299	.20	32.6	1458
63	61.3	UD	-	-	UD	-	-	UD	-	-	UD	-
70	65.3	5.1	232	.09	37.5	1709	.70	11.3	.515	.21	53.9	2457
77	73.3	UD	-	-	UD	-	-	UD	-	-	UD	-

TABLE II. Percentage germination and levels of ABA in grain of *A. fatua* during maturation and subsequent dry storage. (Values given are corrected for extraction losses and are the calculated amounts for individual observations for the dry seed).

Days from Anthesis	%age Germ.	FREE ABA			"NEUTRAL" BOUND			"ACID" BOUND			TOTAL	
		µgkg⁻¹	pg grain⁻¹	Rel. Content	µgkg⁻¹	pg grain⁻¹	Rel. Content	µgkg⁻¹	pg grain⁻¹	Rel. Content	µgkg⁻¹	pg grain⁻¹
14	0	35.9	57	.25	25.0	40	.18	81.9	131	.57	142.8	228
29	0	123.6	221	.62	29.3	52	.15	46.8	84	.23	199.7	357
36	0	8.8	23	.69	1.6	4	.12	2.4	6	.19	12.8	33
46	0	78.8	1109	.50	29.4	414	.19	48.3	680	.31	156.5	2203
52	0	15.9	271	.53	4.8	82	.16	9.0	154	.30	29.7	507
59	0	25.4	450	.73	2.3	41	.07	7.1	126	.20	34.8	617
67	0	7.5	121	.66	2.8	45	.25	1.0	16	.09	11.3	182
81	0	19.9	319	.80	2.8	45	.11	2.1	34	.09	24.8	398
133	0	15.4	246	.81	1.7	27	.09	2.0	32	.10	19.1	305
175	0	9.8	156	.84	.4	6	.03	1.5	24	.13	11.7	186
214	0	6.5	103	.79	.4	6	.05	1.3	21	.16	8.2	130

Data of Don (private communication) - obtained under parallel conditions

Table III: The percentage germination and dry weight levels of MCFA in grains of *Avena sativa* cv Selma during maturation and subsequent dry storage. (Values given are corrected for extraction losses).

UD - undetected

Days from Anthesis	Percentage Germination	Hexanoic			Heptanoic			Octanoic		
		mg kg⁻¹	ng grain⁻¹	Rel Cont	mg kg⁻¹	ng grain⁻¹	Rel Cont	mg kg⁻¹	ng grain⁻¹	Rel Cont
0	0	4.18	55	.11	2.05	27	.06	UD	-	-
16	0	3.16	57	.18	0.73	13	.04	UD	-	-
23	0	10.09	205	.20	2.04	41	.04	2.15	44	.04
37	19	1.21	39	.13	0.50	16	.05	UD	-	-
44	29	0.27	11	.02	0.06	2	.00	0.88	35	.07
51	45	0.17	7	.01	2.89	124	.18	0.23	10	.01
58	69	1.95	87	.06	0.08	4	.00	0.63	28	.02
63	61	2.37	107	.62	0.13	6	.03	0.44	20	.12
70	65	1.65	75	.62	0.17	8	.06	0.43	20	.16
77	73	2.97	132	.64	0.73	32	.16	0.61	27	.13
105	81	2.26	97	.70	0.25	11	.08	0.27	12	.08
119	96	3.79	163	.67	0.74	32	.13	UD	-	-
135	90	0.97	41	.59	0.26	11	.16	0.05	2	.03
148	90	1.60	68	.49	0.73	31	.22	0.71	30	.22
180	94	5.02	211	.62	0.75	32	.09	0.57	24	.07

(Refer to Appendix IV for MCFA extraction efficiencies)

Table III (Continued)

Days from Anthesis	Nonanoic			Decanoic			Total MCFA	
	mg kg⁻¹	ng grain⁻¹	Rel Cont	mg kg⁻¹	ng grain⁻¹	Rel Cont	mg kg⁻¹	ng grain⁻¹
0	3.63	48	.10	27.12	358	.73	36.98	488
16	2.86	52	.17	10.34	187	.61	17.09	309
23	18.85	383	.37	18.31	372	.36	51.44	1045
37	0.65	21	.07	7.12	227	.75	9.48	303
44	1.83	73	.14	10.25	411	.77	13.29	532
51	1.21	52	.08	11.27	482	.71	15.77	675
58	UD	-	-	31.69	1416	.92	34.35	1535
63	0.12	5	.03	0.75	34	.20	3.81	172
70	0.40	18	.15	UD	-	-	2.65	121
77	0.31	14	.07	UD	-	-	4.62	205
105	0.45	19	.14	UD	-	-	3.23	139
119	1.09	47	.19	UD	-	-	5.62	242
135	0.37	16	.22	UD	-	-	1.65	70
148	0.25	11	.08	UD	-	-	3.29	140
180	1.73	73	.21	UD	-	-	8.07	340

Table IV: The percentage germination and dry weight levels of MCFA in grains of *Avena fatua* during maturation and subsequent dry storage. (Values given are corrected for extraction losses).

*Sample from a different population

Days from Anthesis	Percentage Germination	Hexanoic			Heptanoic			Octanoic		
		mg kg^{-1}	ng grain^{-1}	Rel Cont	mg kg^{-1}	ng grain^{-1}	Rel Cont	mg kg^{-1}	ng grain^{-1}	Rel Cont
14	0	0.29	1	.03	0.16	0	.01	0.32	1	.03
29	0	0.38	1	.04	0.17	0	.02	0.76	1	.09
36	0	1.71	5	.04	1.12	3	.02	0.25	1	.01
46	0	2.99	42	.02	1.31	18	.01	2.18	31	.02
52	0	10.63	181	.09	5.52	94	.05	11.37	194	.09
59	0	11.44	203	.04	24.78	439	.08	17.46	310	.06
67	0	6.89	111	.02	48.11	778	.17	13.45	218	.05
81	0	8.63	138	.03	38.11	610	.15	17.27	277	.07
133	0	9.04	144	.05	19.16	306	.12	13.85	221	.08
175	0	4.25	68	.03	6.43	102	.05	7.66	122	.06
214	0	2.54	40	.03	2.09	33	.03	3.65	58	.05
242	8	0.88	14	.03	0.21	3	.01	1.87	30	.07
*578	42	0.44	7	.06	0.43	7	.06	0.57	9	.08

Continued ...

Data of Don (private communication) - obtained under parallel conditions

Table IV (Continued)

Days from Anthesis	Nonanoic			Decanoic			Total MCFA	
	mg kg^{-1}	ng grain^{-1}	Rel Cont	mg kg^{-1}	ng grain^{-1}	Rel Cont	mg kg^{-1}	ng grain^{-1}
14	0.80	1	.07	9.38	15	.86	10.95	18
29	0.95	2	.11	6.60	12	.74	8.86	16
36	8.20	22	.17	37.15	98	.77	48.43	129
46	23.35	329	.17	107.29	1510	.78	137.12	1930
52	41.65	710	.36	46.53	794	.40	115.70	1973
59	99.15	1758	.33	151.39	2684	.50	304.22	5394
67	90.35	1462	.33	118.23	1913	.43	277.03	4482
81	60.65	971	.24	125.52	2010	.50	250.18	4006
133	30.70	490	.19	92.88	1481	.56	165.63	2642
175	32.45	516	.25	78.47	1248	.61	129.26	2056
214	9.05	143	.12	57.64	914	.77	74.97	1188
242	1.00	16	.04	24.13	382	.86	28.09	445
578	0.60	9	.08	5.38	83	.73	7.42	115

APPENDIX IV

Table I: The Percentage Recovery
of MCFA, in the series hexanoic to
decanoic, by the procedure summarised
in Figure 9

Fatty Acid	Percentage Recovery
Hexanoic	19.1 ± 1.6
Heptanoic	15.6 ± 1.0
Octanoic	7.0 ± 0.4
Nonanoic	3.4 ± 0.5
Decanoic	1.2 ± 0.3

The data represents the mean of 3
replicates (± s.e.m.)

Table II. The Percentage Recovery
of MCFA, in the series hexanoic to
decanoic, by the procedure summarised
in Figure 11

Fatty Acid	Percentage Recovery
Hexanoic	78.4 ± 3.0
Heptanoic	71.7 ± 2.3
Octanoic	52.9 ± 0.7
Nonanoic	22.4 ± 0.6
Decanoic	6.5 ± 0.2

The data represents the mean of 3
replicates (± s.e.m.)