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STUDIES ON THE DORMANCY OF SUGAR BEET FRUIT

A thesis submitted to the University of Glasgow

for the degree of

Master of Science

by

Tatiana Wikander

April

1977

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ACKNOWLEDGMENTS

I am grateful to Dr. J. R. Hillman and Professor M. B. Wilkins for their invaluable advice and encouragement, and to Dr. J. R. Hillman for his assistance in the preparation of the manuscript.

The work reported in this thesis was carried out at the University of Glasgow while the author was in receipt of a grant from the British Council.

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ABBREVIATIONS

ABA	abscisic acid
cpm	counts per minute
dpm	disintegrations per minute
Fig.	figure
GA ₃	gibberellic acid
% G	percentage of germination
H-D	Hydration-Dehydration
IAA	indole acetic acid
m.c.s.	metre-candle seconds
m.w.	molecular weight
s.e.	standard error
W	watt

SUMMARY

In this work some aspects concerning with the dispersal unit and its relationships of dormancy of Beta vulgaris are studied. Two batches of fruits were used and their viability assessed, showing that 90% of the fruits were capable of germinating.

The basic germination behaviour was determined using standard germination conditions at 25 °C. Sugar beet fruits germinated very slowly with maximum germination of 67% for one of the batches, whereas the other one only exhibited 39% germination. Light appear to exert no effect on germination.

The range of constant temperatures within which germination takes place was found to be 0 °C - 40 °C, with a maximum response between 5 °C - 15 °C. A study of imbibition showed that seeds imbibe rapidly, and the presence of the fruit coat did not delay germination.

The role of the fruit coat was investigated in experiments dealing with scarification treatments. Removal of the fruit coat lead to total germination, indicating that the seed itself is not innately dormant and that the presence of the fruit coat is largely responsible for delayed germination. The role of germination inhibitors was also investigated; however, no inhibition activity was noted in the extracts prepared from sugar beet fruits.

Other mechanisms of dormancy were investigated, specifically those mechanisms involving the restriction of gaseous interchange. Low oxygen concentrations were capable of promoting germination of true seed and scarified fruit. Intact fruit was markedly affected by oxygen concentrations, increasing concentrations considerably enhanced the germination percentages. These results, as well those of scarification, suggest that the fruit coat is an impediment to oxygen uptake. The effect of one respiratory inhibitor was tested - potassium cyanide - and it was found that it had no effect on intact fruit and only slightly inhibited germination of true seed and scarified fruit.

These findings can be taken as a mild confirmation of Robert's hypothesis of the existence of an oxidation reaction needed for germination, a system not directly connected but competing for oxygen with the cytochrome oxidase system.

Washing and drying the fruits prior to germination enhanced germination. When this treatment was repeated several times, there was an increase of germination with an increase of the number of cycles of hydration and dehydration.

Three germination promoters were tested on fruits and seeds. Treatments with gibberellic acid, indole acetic acid and kinetin had no effect on germination. Further studies on hormonal effects were carried out with abscisic acid. ABA modified the germination pattern of sugar beet; true

seed was inhibited whereas intact fruit was not affected by the chemical. These results tend to support the idea of a resistance to uptake imposed by the fruit coat.

This aspect was briefly considered with preliminary experiments concerning the use of stains and uptake of radioactive indole acetic acid. Fruits were placed in stains and sections of them revealed that the fruit material was externally stained, but the seed tissues remained unstained, although they were hydrated. The results on uptake of radioactivity IAA are not conclusive, but it was found very little radioactivity in the seed tissues, indicating a problem of uptake of the chemical.

This study has not resolved the question of the role of inhibitors in sugar beet, but gives some evidence that other mechanisms may be involved, in particular those concerned with gaseous interchange.

INTRODUCTION

One of the most universal characteristics of practically all existing plant species is that at least once during their life cycle they produce specialized cells, or multicellular bodies, that exhibit the phenomenon which has become known as dormancy. The term dormancy may be used to describe any stage in the life cycle, whether this is a regular phase in the developmental process or an imposed phase in which active growth is suspended for a period of time, and in which there is a reduced rate of metabolism as compared with the rate that occurs during the active stage of the life cycle. This may include the development of special structures often associated with dormancy such as seed, buds, and bulbs and various organs of vegetative propagation, or there may be merely the suspension of development without obvious morphogenetic changes, which may occur in hibernation.

Although dormancy is associated with an abrupt and almost simultaneous end of metabolic, synthetic and morphogenetic activities, it is not a static phase of the life cycle of a plant. Onset of dormancy appears to be much more than a mere cessation of these activities. Not only does it involve a sequence of events as orderly as that which is encountered in the formation of the dormant structures, if this is the case, but once the dormant condition is established, metabolic changes occur in the dormant structure during the passage of time and the external conditions under which dormancy takes place may also change. These changes in turn cause changes in the requirements for those external conditions needed for overcoming the state of dormancy.

Dormancy can be induced as a result of changing external factors, and often occurs when external factors are unfavorable to further development. Unfavorable external conditions, however, are not a necessary factor for the induction of dormancy, which usually takes place at the time when the external environment is in no apparent way unfavorable for active growth. Therefore, the imposition of dormancy is likely to be controlled endogenously and the ultimate control is probably located in the tissues of the mother plant. Nevertheless, there is evidence that in many plants the formation of the dormant structures is initiated under a more or less specific combination of external conditions. Thus, even when the morphological development of these structures and their physiological transition to the dormant state are under endogenous control, the primary timing mechanism which initiates these events depends on perception of and response to environmental signals, such as photoperiod or cold in the case of many flowering plants, or availability of minerals or light in many algae.

The reduction of the physiological activities that occurs in the dormant state is commonly associated with development of external protective tissues and with a reduction of hydration of the cytoplasm. These two factors combine to make the dormant structure much more resistant to unfavorable environments than the plant that produce them. In this sense dormancy has a survival value.

That dormant structures are resistant or tolerant to adverse environmental conditions guarantees not only the survival of the organism during the

critical periods, but also the survival of the specie through dormant propagation structures or organs.

Dormancy probably has evolved as a solution to the periodic, as well as non-periodic changes in the environment, which makes it difficult for the plant to function properly, or even exist, during certain critical periods.

The responses of organisms to a certain combination of environmental conditions are numerous, and thus in plants is commonly found that the structures or organs of propagation with dormant capacity are varied, some of them being spores, sclerotia of Fungi, seeds and bulbs. Seeds in particular, represent one of the most successful solutions to the problem and one of the major aspects of evolution. In flowering plants the seed consists of an embryo surrounded by one or more covering structures, the nature of these covering structures largely depending on the species. Functionally, the seed is a "propagule" or dispersal unit. In many species the seeds are liberated from the fruit and the isolated seeds become the dispersal units. In other species, however, the fruit may contain a single seed which is retained within the fruit coat (pericarp), the fruit itself being shed as a whole and becoming the dispersal unit.

The term dormancy applied specifically to the seed covers a wide variety of phenomena. Two basic kinds of dormancy can be distinguished in seeds. Firstly, dormancy may be due to purely external physical conditions of the environment, such as lack of available water or a range of temperature incom-

patible for physiological processes. Secondly, dormancy may be conditioned by the internal constitution of the seed; by its anatomical structure or to more subtle causes associated with internal physiological state of the seed (Torrey, 1967). Whether seeds possess either one or both of these kinds of rest depends upon environmental influences and/or heredity.

It should, however, be realized that the two influences are mutually dependent and sometimes cannot be separated. Nevertheless, the term seed dormancy usually refers to the failure of otherwise viable seed to recommence development when supplied with water and oxygen at the temperatures recognized as normally favorable for plant growth. This delay may last for a variable period of time under constant conditions and in some cases may continue indefinitely until some special condition is fulfilled. The overcoming of dormancy in seeds is the phenomenon named "germination", and it is defined by some authors (Evenari, 1965; Lang, 1965) as those processes starting with the imbibition of the dispersal unit and ending with the protrusion of the embryonic root which takes place inside the dispersal unit and prepares the embryo for normal growth. A more general definition would be "the sequential series of morphogenic events that result in the transformation of an embryo into a seedling". (Berlyn, 1972). Mayer and Poljakoff-Mayber (1963) have first defined germination as the consecutive number of steps which causes a quiescent seed, with a low water content, to show a rise in its general metabolic activity and to initiate the formation of a seedling from the embryo. It is a half-closed system, i.e., it is initiated when the quiescent embryo is reactivated, but the terminal end of the system is open

because the exact stage at which germination ends and seedling growth begins is undefined. Germination is indeed a remarkable process, involving cell division, cell expansion, and the formation of plant organs. Torrey (1967) recognizes four steps in germination: the physical absorption of water, called the imbibition phase; the hydration and activation of the chemical constituents of the seed, including enzymes; the division and/or enlargement of cells in the embryonic root; and finally the protrusion of the root through the seed coat.

Different general types of seed dormancy can be distinguished in which seed germination is restricted or prevented even despite apparently optimum environmental conditions (including an adequate water supply, aeration, and appropriate temperature for germination).

1. Dormancy due to seed coat restrictions.

The terms "seed coat" includes all the tissues surrounding the embryo. In the true seeds this consist of the testas and in some cases as endosperm and also nucellar membranes. The seed itself is sometimes also surrounded by tissues from the fruit, such as a dry, non-fleshy pericarp, which may be fused to the testa. Many plant families produce seeds with a hard seed coat which can cause dormancy in one of three ways. A hard coat may be impermeable to water, impermeable to gases, or both, or it may mechanically constrain the embryo.

The impermeability of the seed coat to water is one of the simplest but highly effective means of delaying germination. Only relatively few species have extremely thick and fairly waterproof seed coats, but this dormancy mechanism is exhibited by members of several families notably the Leguminosae, Malvaceae, Chenopodiaceae, Liliaceae, and Solanaceae (Barton, 1965; Wareing, 1969). The uptake of water is prevented by the testas, and the disruption of this layer is promptly followed by swelling due to water uptake, and germination commences almost immediately. There is usually much variation in the degree of impermeability to water within a particular batch of seeds; some seeds are capable of imbibing and germinating almost immediately, while water uptake in other seeds is delayed for a variable length of time, thereby spreading the production of seedlings over a period of time.

Frequently, seed coats are impermeable to gases despite the fact that seeds are permeable to water. This impermeability may be either towards carbon dioxide or oxygen or both. That such a differential permeability exists seems fairly well established, despite the apparently small differences in molecular diameter of the substances involved. Only very few artificial membranes are known which show such differential permeability. The classic example of impermeability to oxygen is provided by *Xanthium*. In *Xanthium* Crocker (1906) showed that the fruit contains two seeds, an upper, dormant seed and a lower non-dormant one. Crocker suggested that the delay in germination for the upper seed was due to a restriction of oxygen by the seed coat. Shull (1911) and Thornton (1935) showed that both seeds differ in their requirements for external oxygen pressure for germination. Thornton (1935) showed that oxygen

pressure for germination. Thornton (1935) showed that oxygen requirements for germination were greater for intact seeds than for excised embryos, thus indicating that the seed coat was impermeable to oxygen. Removal or damage to parts of the seed coats, or increasing the oxygen tensions of the surrounding air leads to an increase in the rate of embryo respiration in many species of seeds, and frequently results in germination, and the inference often made is that increased oxygen leads to an increase in the availability of energy by oxidation processes.

Mechanical resistance to embryo growth is probably a rare occurrence of an effect of the seed coat on germination. Indeed, when one considers the great pressures generated by growing plant organs, such as the breaking of concrete roadways and lifting of paving stones by plant organs growing beneath them, it is only among those seeds which remain enclosed within an extremely hard endocarp that one would look for cases of dormancy imposed by mechanical resistance to embryo growth. In fact, many such reported cases are caused by factors other than, or in addition to, mechanical constraint. Nevertheless, it is known that many seeds, or dispersal units, have extremely hard and durable structures. In Rosa, for instance, great mechanical pressures are needed to destroy the hard endocarps, but even in this case it has been shown that in addition to any mechanical resistance imposed on the embryo, the embryo itself is maintained in state of dormancy by other mechanisms. Jackson and Blundell (1965, 1966) have demonstrated that growth inhibitors are present in the stony remains of the pericarp in *Rosa* species.

Dormancy imposed by structures surrounding the embryo depends on the integrity of these structures, and germination can be brought out by the disruption of the seed coat and other enclosing tissues. In nature, the seed coat may be broken down or punctured by mechanical abrasion, microbial attack, passage through the digestive tracts of animals or exposure to alternating high and low temperatures, which, by expanding and contracting the seed coat, crack it. Under laboratory conditions and in agriculture other means of scarification have to be adopted. Procedures as scratching or scanning the seed coat, soaking in acids, or freezing and thawing the seeds may serve to break seed dormancy by disruption of the restrictive layers.

2. Dormancy due to immaturity of the embryo

In certain plants the seeds are shed before the embryo has reached its mature embryological development. Such seeds fail to germinate until the embryo attains full maturity either during the actual process of germination or they may mature as a preliminary to germination. In either case the changes only occur if the seeds are kept under conditions favorables to germination, and differentiation between the two types is extremely difficult. Among the plants in which immature embryos occur are the Orchidaceae and Orobanchaeae. Some other species posses embryos which may be differentiated when the seeds are shed, but when imbibed with water the embryo continues growth to a larger size before germination can take place, as it is in the case of Fraxinus (Steinbauer, 1937). In these case of immaturity of the embryo it is difficult to decide whether the embryo development is part of

the final stages of seed development or the initial stages of the germination process.

In contrast, in many seeds no visible anatomical or morphological changes occur in the embryo during after-ripening. In these cases it can be assumed that the process of after-ripening is the result of chemical or physical changes within the seed or the seed coat. The composition of the storage materials present in the seed may alter, the permeability of the seed coat may change, substances promoting germination may appear or inhibitory ones may disappear.

3. Dormancy due to chemical inhibitors

Many kinds of inhibitory chemicals are found in seeds and in several species they play an important part in the control of dormancy. The possible importance of substances capable of inhibit germination was first postulated by Molisch (1922) who suggested that the juices of fleshy fruits retard the germination of the seeds because of the presence of inhibiting substances. Subsequently it has been established that many fruits and seeds do, in fact, contain substances which are markedly inhibitory to growth and germination when extract is applied to suitable test objects. There is also evidence that these inhibitors are frequently present in non-succulent fruits, and may occur in the pericarp, endosperm, testa or embryo. The classic work of Evenari (1949) and Wareing (1965) on germination inhibitors provide us with an account of the occurrence of these substances, which are not confined to seeds or fruits, but can be also found in other part of the plant such as

leaves, bulbs, roots and tubers. Some species produce germination inhibitors which are specific in the sense that they are inhibitory only to the seeds of the plant producing the substance, but in general inhibitors are rather nonspecific and prevent germination of seeds shed by other plants. Thus, some species producing such compounds in their leaves will prevent germination of other seed species, which may fall into the leafy litter and the growth of neighboring plants (Mayer and Poljakoff-Mayber, 1963; Gray and Bonner, 1948; Mergen, 1959).

The types of chemical inhibitors are remarkably diverse, ranging from volatile compounds such as ammonia, ethylene and mustard oils, to materials like organic acids, unsaturated lactones, aldehydes, essential oils and alkaloids (Evenari, 1949). Varga (1957-a, b, d, 1958) divided the inhibitory substances in fruits into three classes: 1) short-chain, organic acids, 2) aromatic acids, 3) essential oils. The most common group are the aromatic acids, which may include salicylic, o- and p-coumaric, possibly also cinnamic and m-oxybenzoic acids and coumarin. It must be emphasized that in the majority of cases those compounds that appear to be germination inhibitors are primary inhibitors of growth, affecting processes of cell division and cell enlargement which are evident early in seed germination.

The presence of these inhibitory substances in seeds and fruits does not necessarily mean they are involved in the dormancy control mechanism. Wareing (1965) claims that there has been a tendency to assume too readily that the occurrence of inhibitors, as detected by in vitro tests, in some

part of the fruit or seed, implies that they have a functional role as inhibitors in vivo. In order to determine whether there is a causal relationship between the presence of inhibitors and delayed germination, Wareing claims it is necessary to investigate how far the state of dormancy remains correlated with the inhibitor level, when the latter is varied naturally or experimentally, and, conversely, how far differences in the state of dormancy are associated by differences in the level of inhibitors.

In those cases where inhibitors found in the dispersal units genuinely prevent germination, they perform a very important role in the ecology of the species. The prevention of germination of seeds inside the fruit ensures that one of the main biological functions of the seed, dispersal of the species over a wide area, will be fulfilled. Their presence will also prevent germination of unripened seeds. Frequently the function of inhibitors is to produce sporadic germination over extended periods of time: the inhibitors in the dispersal unit are removed by rain (Went, 1949; Soriano, 1953; Koller and Negbi, 1959) and/or animal or bacterial action which destroy the fruit and seed coat containing inhibitors (Burton, 1948), the process of removal taking place at different rates and thus causing irregular germination over an extended period. The leaching of germination inhibitors from the seeds of certain species can prevent the germination of seeds of other species in the near vicinity, and thus may confer a selective advantage on the first species in competition with others (Evenari, 1949, 1961; Koller, 1972).

Germination inhibitors, then, seem to play in more than a way an important role in the self-preservation of those species that produce them.

4. Requirements for chilling

Certain seeds will germinate only after incubation on a moist substrate at temperatures slightly above freezing (Wareing, 1969). The chilling requirement may be imposed by either the seed coat or the embryo itself. The range of seed showing a chilling requirement is very wide, and includes both woody and herbaceous plants. In some seeds there is an obligate requirement for chilling, as it is in Fraxinus, whereas others, as in Pinus, a period of pretreatment at chilling temperatures, although not essential, increases and hastens subsequent germination (Wareing and Phillips, 1970).

This type of dormancy has been frequently associated with the presence of germination inhibitors in the embryo. If such embryos are excised and washed with water, germination often takes place, but if they are excised and held in a humid atmosphere without loss of water from the tissues, and where leaching of substances from the embryo is impossible, the embryo will remain dormant (Villiers, 1972). In the case of Xanthium pennsylvanicum (Wareing and Foda, 1957) leaching of excised embryos allowed germination, a decrease in inhibitor could be detected within the embryos, and germination inhibitors were detected in the water used for the soaking. That the germination of these leached embryos could not be ascribed merely to increased water uptake was shown by redrying the embryos back to their former wet weight before leaching, when germination still occurred.

It is difficult, however, to comprehend the relation between cause and effect, between low temperature treatments and dormancy imposed by inhibitors within the embryos. Moreover, because inhibitors are present and may vary in activity within the tissues of seeds it cannot be inferred that they are the agents responsible for the control of germination and growth.

5. Irradiation.

One of the most interesting forms of dormancy is that shown by seeds sensitive to irradiation. In a considerable number of species, exposure to irradiation is necessary for germination, e.g. Betula spp., Lepidium virginicum, Nicotiana tabacum, Amaranthus retroflexus, some varieties of Lactuca sativa, and many others. Those seeds which are promoted in their germination by irradiation are termed "positively photoblastic", while a small number of other such as Nigella damascena, Nemophila insignis and Phacelia tenacetifolia are inhibited by irradiation, and are termed "negatively photoblastic". The phenomenon of germination controlled by irradiation has received intensive study in recent years, and it has been described by Evenari in 1965.

Irradiation-sensitive seeds will only respond to irradiation after they have imbibed water. The level of irradiation required may actually be quite low. Measurements of Isikawa (1952) have shown for a yellow variety of tobacco that 100 m.c.s. (meter-candle seconds) were enough to obtain maximum germination, while lettuce seeds (var. Grand

Rapids) need between 400 - 600 m.c.s. for an appreciable promotion of germination (Eveneri, 1965).

The mechanism of the release of seed dormancy by irradiation has been shown to be similar to that controlling many other morphogenetic stages in the development of plants, and it was a study on the photocontrol of germination in the seeds of lettuce which gave the first clues to the energy receptor system in irradiation-controlled development (Borthwick et.al.,1952). The action spectrum for germination of lettuce seeds shows that red irradiation (660 nm) is most effective but that the stimulatory effect of red irradiation is reversed if it is immediately followed by far-red irradiation (730 nm). The explanation is that the pigment phytochrome in the form designated P660 is converted by absorption of red irradiation to P730 which promotes germination. It is photoreversible pigment; P730 reverts to P660 on the absorption of far-red irradiation. This photoreversion may be performed several times: whether or not the seed germinates depends on the final exposure of the sequence. Phytochrome is a chromoprotein whose two forms (P660 and P730) absorb maximally at the expected wavelengths. The study of the phytochrome has received great attention by several authors; a study of its isolation, structure and photochemical transformation has been done by Kroes (1970).

The difference between positively and negatively photoblastic seeds appears to lie in their relative sensitivities to red and far-red. Under natural conditions, seeds receive white irradiation which includes both red and

far-red; in positively photoblastic seeds the promotive effects of red evidently predominate, whereas in negative photoblastic seeds it is the far-red effect which is over-riding (Wareing, 1969).

Some chemical treatments are capable to replace the irradiation requirement of seeds. Chemicals such as potassium nitrate, thiourea, gibberellic acid and kinetin will promote germination in the dark of positively photoblastic seeds (Thompson and Kosar, 1938, 1939; Toole et al.; 1955; Kanh et al. 1956, 1957; Miller, 1956; Skinner et al., 1957; Evenari et al., 1958).

The effect of light in the control of germination under natural conditions is not always very clear to understand. Mayer and Poljakoff-Mayber (1963) suggested that a advantage arising from the regulation of germination by irradiation would be, in seeds having different irradiation requirements, in adapting them to their habitat. Thus, irradiation may prevent germination of seeds requiring irradiation when they are buried under soil or leaf litter, or promoted when they fall in the soil surface. Such behaviour may determine how a seedling will subsequently be able to establish itself.

The types of dormancy described are by no means mutually exclusive, and more than one mechanism for the imposition of dormancy may be possessed by any one species. Therefore, more than one treatment may be necessary in order to break seed dormancy.

The phenomenon of dormancy is so widely spread in nature that very early in his history man must have been aware of it. The primitive agricultural societies realised the value of dormant structures, and some of them, such as seeds, had a double value. Seed harvesting and storage not only provide a supply of food for unfavorable times, but also represented the guarantee for the crop of subsequent years. As civilization became more complex the range and quantity of edible seeds or seed-producing crop plants has been increased, resulting not only in a more varied human diet but in produce to ensure man's existence through times of famine.

One of the most valuable cultivated plants is Beta vulgaris. Although in origin of limited importance because the edible parts of the plant - roots or leaves depending on the variety - were of low nutritive value, its importance has exceeded to a large extent the original limit because its use as a raw material for sugar production. The cultivated beet of today is believed to have been derived from the wild beet which is a common seashore plant on certain coasts of the Mediterranean and N.W. Europe, and has been described as a cultivated crop by various classical authors. The species Beta vulgaris has been an object of research in more than one aspect, but the particular interest of this thesis is concerned with its dispersal unit and its relationships of dormancy.

It is a well known fact that the dispersal unit of Beta vulgaris (the so-called "seed-ball") do not germinate satisfactorily even under favorable germination conditions. The actual cause of delayed germination has been attributed to various factors; amongst one the most frequently causes found in the literature is the presence of inhibitory substances in the pericarp tissue of sugar beet. Water soluble extracts prepared from the seed balls have been found to retard the germination of sugar beet seeds and kill the radicles of emerged seedling. Moreover, the toxicity of these water extracts has been found to be non-specific, inhibiting the germination of other species. Washing the sugar beet seed balls before placing them to germinate will remove part of the toxic effect (Fröschell, 1939, 1940; Tolman and Stout, 1940).

The exact nature of the inhibitory substances in the water extracts is still not very clear, and numerous causes have been claimed to be responsible for the inhibitory effect. Stout and Tolman (1941) found inhibition to be largely due to the toxic action of ammonia, which is released from the nitrogenous compounds of the extract by enzymatic hydrolysis. Experimental data indicated that the toxic action of the released ammonia was not entirely due to the resulting increase of pH. Rehm (1953) agreed that free ammonia acted as a germination inhibitor but stated that this was formed by bacteria. Duym et.al. (1947) failed to detect any specific inhibitory substance, giving more importance to the osmotic effect of the extract. De Kock et.al. (1953) have claimed that the inhibitory effect of the extracts was due to an unsaturated yellow oil which is capable of inhibiting germination of various seeds. A similar oil was isolated by De Roubaix and Lazar (1960). Several intermediate products of lignin biosynthesis (water soluble phenolic acids) have

also been reported to be isolated from water extracts from sugar beet seed-balls (Massart, 1957; Van Sumere, 1960; Battle and Whittington, 1969-b).

Factor other than inhibitors can cause low germination in Beta vulgaris. Microbial factors has been also reported to produce delayed germination in sugar beet. Byford (1963) found that the number of seedlings that emerge in the field depends partially in the number of seed-balls contaminated with Pleospora betae - that a treatment with an ethyl mercury phosphate increased the emergence of seedling. Heydecker and Chetram (1971) also found a low germination due to microbial factors, claiming that the effect of bacteria was caused by competition between and seeds for oxygen.

Heydecker et al. (1971) provided some evidence that suggested that one of the main causes of delayed germination is the prevention of oxygen to the embryo by the fruit material: scarification treatments removing part of the fruit material improved germination and counteracted the effect of germination inhibitors present in the fruit. This effect of gaseous interchange was very early hinted by Garner and Sander in 1932, who reported that treatments of sugar beet balls with sulphuric acid leads to an increase in both rate of germination and total germination. They attributed the increase of germination to a greater permeability of the hard fruit wall, which allows the process connected with germination to take place more rapidly.

Other reasons have been claimed to be responsible for delayed sugar beet germination, e.g. an excess of water supply, high soil salinity or high

germination temperatures. These have to be discarded as dormancy mechanisms because they obviously are extrinsic factors to the system and can be easily controlled experimentally. It has to be emphasized, however, that these factors are of common occurrence in nature, where they can act as external dormancy mechanisms.

The main known causes of seed dormancy have been discussed with particular reference to Beta vulgaris. In this thesis an account is given of an investigation into certain factors which may regulate germination of sugar beet. The experiments are divided into six sections: the first deals with a characterization of the basic germination behaviour occurring under standard conditions; the second section examines the effect of the fruit coat; the third deals with water supply, the fourth oxygen and the fifth briefly considers the effect of specific chemicals. The last section deals with the uptake of substances through the fruit coat.

MATERIALS AND METHODS

a. Plant Material

Genetically monogerm fruits of Beta vulgaris L. variety Sharpes Klein E, rubbed and graded, were obtained from R. K. Gemmel and Co., Glasgow, U.K. Two batches of fruits were used, one batch was obtained in May of 1971 and the other one in March of 1972, both batches were stored in darkness at room temperature. The second batch had to serve as replacement upon exhaustion of stock of the former one. The first batch of fruits were treated with mercurial fungicide and an unspecified organo-chloride dressing, but it was not possible to obtain the second batch treated this way. This may have caused some difference on the germination percentages of both batches and this is accounted in the results. Except for this chemical treatment the fruits were morphologically identical. These two batches of fruits are named as Lot 1 (treated with fungicide) and Lot 2 (not treated).

The dispersal unit in polygerm varieties of Beta vulgaris is composed of several fruits united in a compact structure. Each fruit is a dry and indehiscent achene which tightly encloses only one seed. The "monogerm" varieties consist of single fruits. In the cultivation of sugar beet the polygerm varieties may lead to practical problems arising from the production of number of closely intertwined seedlings which are difficult to separate when the time comes to thin the crop to a series of single plants. There are no natural lines of cleavage in the sugar beet fruit and it does not easily

separate into portions each containing a single true seed. Many attempts have been made to obtain an artificially monogerm variety specially through the process of rubbing, a mechanized abrasive process devised to separate the individual seeds contained within polygerm fruit cluster. This process has some disadvantages: individual seed units are not always produced; it is known to cause, in certain instances, submicroscopic damage to some of the embryos (Chetram and Heydecker, 1967); it is not economically satisfactory and, finally, it does not solve the actual biological problem of obtaining a monogerm variety. The real solution to the problem was provided by the discovery of Savitsky (1950) of plants producing seed clusters containing a single seed, enabling the development of monogerm varieties.

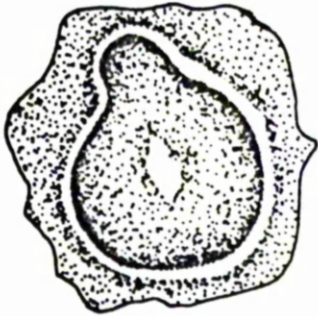
The natural monogerm fruit is a brown irregular body with remains of the calyx attached to it. The five-lobed, incurved calyx adheres to the base of the ovary and becomes a hard structure in the fruit. During rubbing this husk is taken away and the final product is the commercial "seed". The rubbed fruit is a very hard structure with a shape resembling a slightly elongated half sphere. The thickness of this half sphere is 2.4 ± 0.2 mm and its flat side is 3.9 ± 0.4 mm long and 3.6 ± 0.3 mm. It weighs 10.6 ± 1.4 mg (Fig. 1, A and B). The unit fruit-seed may be described as a cupula with a cap or lid which tightly covers the locule that contains the individual seed (Fig 1, C and D). When the seed germinates the radicle emerges pressing against the line of junction between the edge of the cap and the wall of the locule. The lignified fruit material is easily wetted and according to Heydecker et al. (1971) and excessive water supply causes a thin layer of mucilage to arise

all over the fruit material, including the cap and its edge.

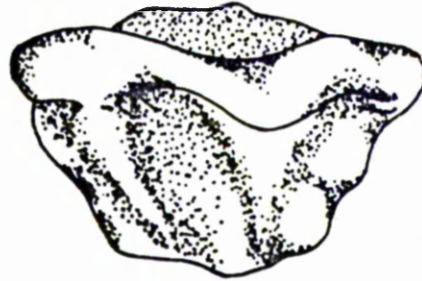
The true seed is a flat lentil-shaped structure, 2.6 ± 0.1 mm in diameter and 1.4 ± 0.2 mm thick. It weighs 3.8 ± 0.3 mg. It lies horizontally in the ovarian cavity and the funiculus is bent under it; when the seed is removed from the pericarp the funiculus is still intact and appears on the lower side of the seed as a small, sharply bent stalk. The embryo occupies a horizontal position (Fig. 1, E, F and G) and is curved around the perisperm, the lower part of the radicle is enclosed by a single layer of endosperm. The perisperm is a starchy storage tissue of nucellar origin, the embryo is rich in albuminous and oily storage material (Artschwager, 1927; Bennet and Esau, 1936). The mature embryo is protected by a seed coat consisting of two layers of thick-walled cells enclosing between them some thin-walled parenchyma. These two testas are derived from the two integuments of the ovule. The outer seed coat is very brittle and separates easily from the seed, its reddish color is due to the pigmentation of the outer layer.

- Figure 1.- Sugar beet fruit and seed.
- A and B = diagrams of the external appearance of the fruit.
 - C and D = drawings showing the seed inside of the fruit, in C fruit with the ovary cap removed, in D fruit transversely sectioned showing the position of the seed in the fruit locule.
 - E = sugar beet seed, longitudinally sectioned with respect to the embryo.
 - F = transverse section made along axis b in E.
 - G = transverse section made parallel to axis a in E.

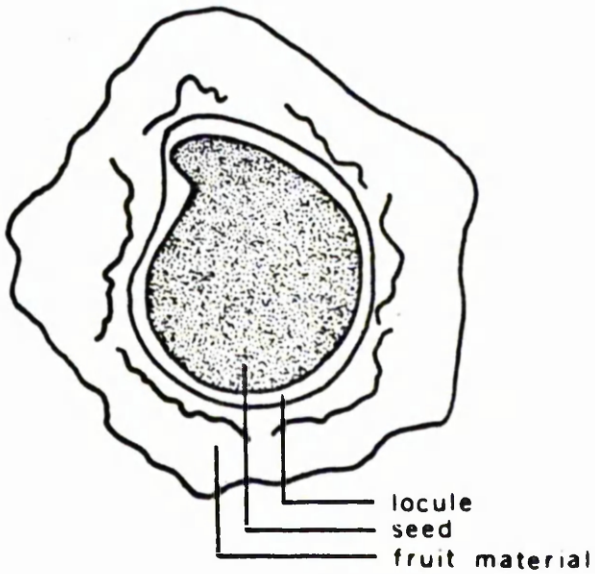
A



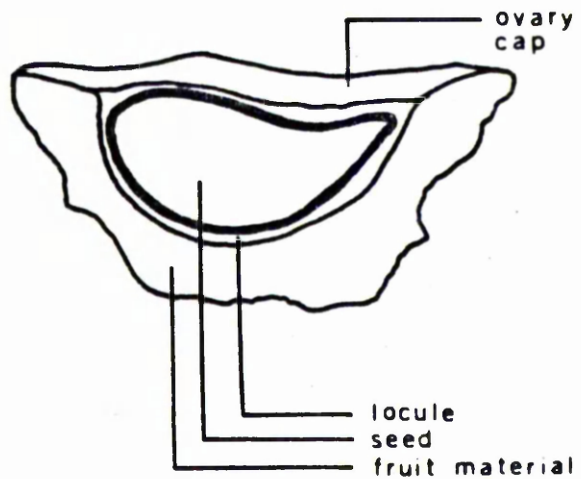
B

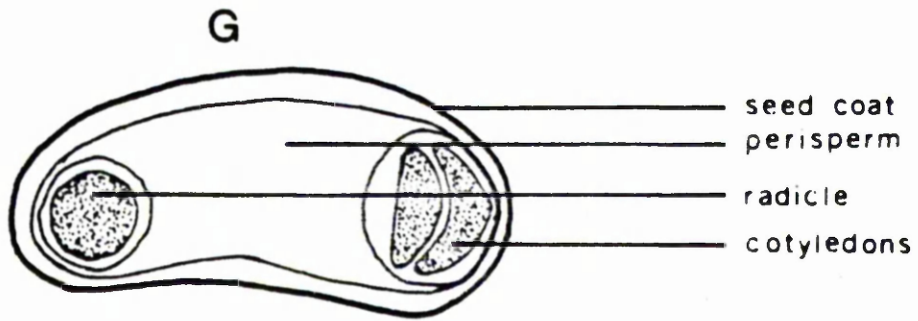
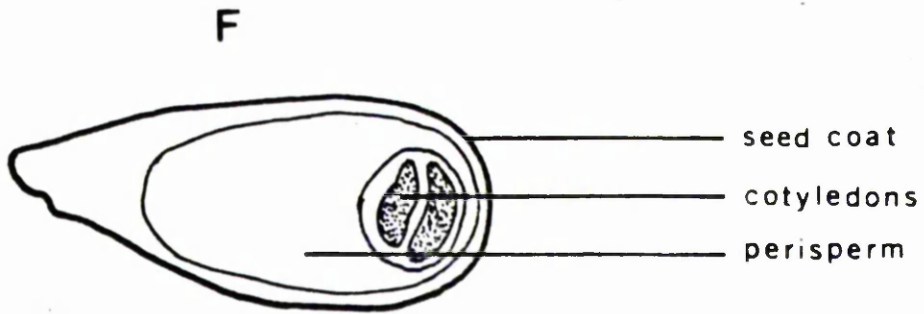
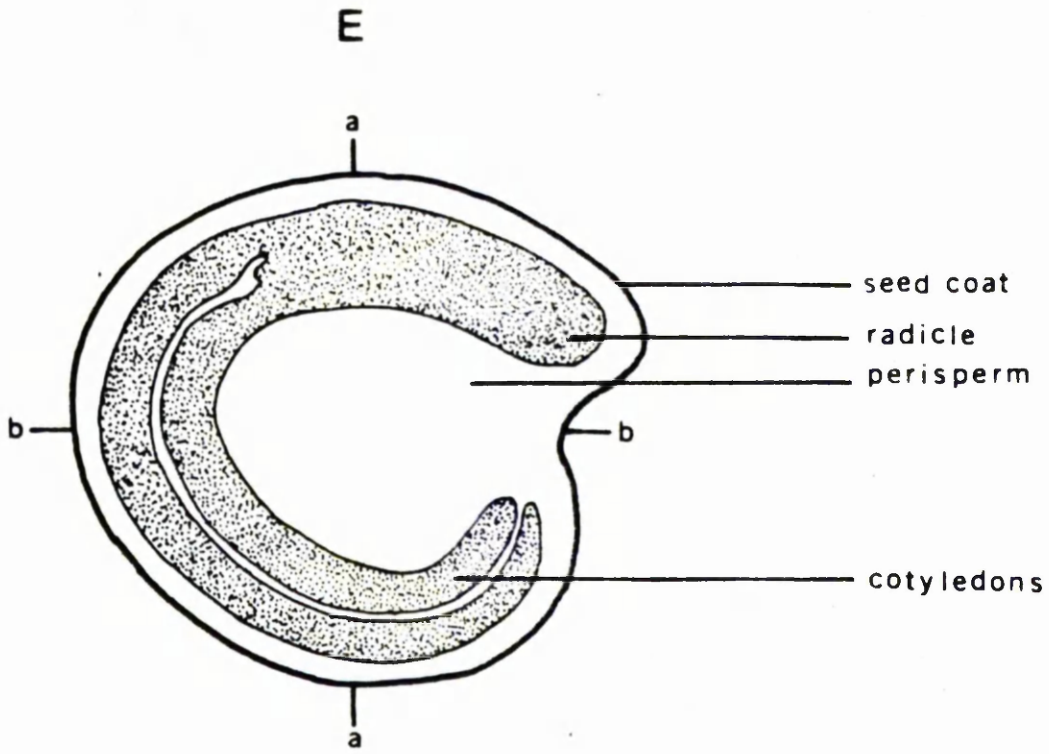


C



D





b) Germination Tests

The material was divided into three categories:

(i) Intact fruit.

The fruit was used as it came from the seedsman. No preliminary treatment was made.

(ii) Scarified fruit.

Unless otherwise stated the scarification consisted in the removal of the ovary cap, thus exposing a large surface of the seed to the surroundings, but leaving the seed inside of the fruit.

(iii) Seed or True seed.

The seed was excised from the fruit without removing the testas. This is a rather delicate operation since even a slight scratch of the testas could damage the seed and diminish the contact between embryo and perisperm. Both scarification and excision were made under a binocular microscope.

c) Standard Germination Conditions

Fruits and seeds were placed in 5-cm diameter plastic Petri dishes with 2 sheets of Whatman No. 1 filter paper firmly pressed in the bottom of each dish. Ten seeds or fruits were placed in each dish and soaked with 1.5 ml of water or test solution. A minimum of 10 Petri dishes (10 replicates) were used for each treatment, nevertheless there were 20 replicates in a large number of cases. The course of germination was followed by inspecting the fruits or seeds every 24 hours during the first three days, and thereafter the inspection was done once a week until no change in the germination values were observed. This usually occurs by the end of the third week, but in a few instances it was necessary to inspect the fruits up to the tenth week. Except for those experiments where fruits were exposed to irradiation, fruits and seeds were kept in continuous darkness in a growth cabinet at 25 ± 1 °C. Visible emergence of the radicle was taken as the criterion for germination. Full germination is defined as that level of germination occurring at the termination of the experiment. Total germination is the amount of germination expected from seed viability analysis.

d) Irradiation Source

Germination tests were carried out under irradiation from 2 Atlas 65/80 W super five, white fluorescent tubes lights, placed 3 ft above the level of the Petri dishes.

e) Statistical Analysis

For the analysis of the results an Olivetti Programma 101 desktop computer was used to calculate the standard error of the mean for each group of replicates as well as the Student's t index comparing the means of two samples.

Formulae used for standard error:

$$\text{s.e.} = \frac{\text{s.d.}}{n}$$

$$\text{s.d.} = \sqrt{\frac{n\sum x^2 - (\sum x)^2}{n(n-1)}}$$

- where:
- s.e. = standard error
 - s.d. = standard deviation
 - n = number of values (number of replicates)
 - x = variate value (percentages of germination)

On graphs a vertical bar through the datum point equal to twice the standard error is drawn, but where space is limited the bar is drawn only on one side of the point representing one standard error value.

Formulae used for the Student's t index:

$$M_1 = \frac{\Sigma X}{N_1} \quad ;$$

$$M_2 = \frac{\Sigma Y}{N_2} \quad ;$$

$$S_1^2 = \frac{N_1 \Sigma X^2 - (\Sigma X)^2}{N_1 (N_1 - 1)} \quad ;$$

$$S_2^2 = \frac{N_2 \Sigma Y^2 - (\Sigma Y)^2}{N_2 (N_2 - 1)}$$

$$\sigma = \sqrt{\frac{(N_1 - 1) S_1^2 + (N_2 - 1) S_2^2}{N_1 + N_2 - 2}}$$

$$t = \frac{M_1 - M_2}{\sigma \sqrt{1/N_1 + 1/N_2}}$$

where: M_1 = arithmetic mean of the first sample

M_2 = arithmetic mean of the second sample

S_1^2 = variance of the first sample

S_2^2 = variance of the second sample

N_1 = number of values in the first sample

N_2 = number of values in the second sample

x,y = variate values

f) Viability

The viability of the seeds was determined by the tetrazolium test. In this biochemical test the colourless tetrazolium is reduced in living cells by the action of dehydrogenase enzymes to form a red water-insoluble formazan product. The development of the red color is an evidence of the presence of active respiratory processes in which hydrogen radicals are transferred to the tetrazolium. The red color makes it possible to distinguish the living part of the seed from the colourless dead part. Lakon (1942; 1949) and Porter et al. (1947) have shown that the coloration of the seed embryo by tetrazolium is an indication of its viability.

A 1% aqueous solution of 2, 3, 5-triphenyl tetrazolium chloride at pH 6.4 was used. This reagent is light-sensitive and exposure to direct or diffused sunlight was avoided.

Procedure:

The fruits to be tested were soaked in water for 24 hours to permit absorption of water and thus facilitate the dissection of the seed. Following the soaking period each seed was bisected longitudinally

through the centre of the embryo so that each half had a part of the cotyledons and the radicle. One half of each seed was then placed in a petri dish and the solution of tetrazolim chloride was poured over the cut seeds until they were completely immersed. To avoid prolonged contact with the air, care was taken to insure that the seeds were always submersed in the solution. The petri dishes were kept for 12 hours in a dark cabinet at 25 °C. At the end of this immersion period each half seed was examined to determine the degree to which the parts of the embryo were stained carmine red.

The staining of the entire embryo was taken as viability criterion. Those seeds which only have stained the radicle or the cotyledons were considered as nonviable.

The percentage of viability was calculated from:

$$\% \text{ viability} = \frac{S}{\Sigma(S + U + E)} \times 100$$

Where S is the number of embryos completely stained, U is the number of embryos unstained or partially stained. In many cases empty fruits or fruits containing abnormal seeds were found and the number of these fruits is indicated by E.

g) Germination Pattern of Sugar Beet

The germination pattern of sugar beet was determined on intact fruits from Lot 1 and Lot 2 as well. For each batch there were two groups of replicates, one group was kept in the dark and the other group subjected to continuous irradiation. In both darkness and irradiation the fruits were soaked with water and maintained at a constant temperature of 25 °C. The source of radiation is described in page 27. Details of the procedure are in standard germination conditions.

h) Imbibition

The time course of imbibition was followed in fruits as well in seeds, using:

- a) Intact fruit (whole fruit enclosing the seed).
- b) Non-scarified seed (true seed with the testas).
- c) Scarified seed (true seed with the outer testa completely removed. A minute perforation in the perisperm area was made by means of a very small dissection needle).

In each case 50 fruits and seeds were used (50 replicates). They were placed to imbibe in 5-cm diameter plastic Petri dishes with 4 ml of water. Care was taken to avoid confusion of the individual fruits and seeds and thus making possible to identify each one of them separately. Except for relatively

brief operations concerning the weighing of the material, the Petri dishes were kept in the dark at 25 °C. Each fruit and seed was weighed dry and then placed in the Petri dish. After certain times -2, 4, 6, 8, 24 and 53 hours- they were removed from the dish, the excess of water of their surface dried up with filter paper, and weighed again. Afterwards they were replaced in the Petri dish. An analytical balance with a scale accuracy of 0.5 mg was used to weigh the fruits and seeds.

The imbibition curve is expressed as percentage of the initial weight (dry weight) of fruits and seeds at different times.

$$\% \text{ of initial weight} = \frac{\text{weight at different times} \times 100}{\text{Initial weight}}$$

As the experiment was performed with seeds and fruits which have not been killed, many of them germinated at 53 hours. The calculations of percentage of initial weight for this time were made taking into account only seeds and fruits not germinated. The figures of germination for 53 hours were 16% for intact fruit, and 70% for scarified and non-scarified seeds.

i) Effect of Temperature

Intact fruits of sugar beet taken from Lot 2 were soaked in water and kept in the dark at different constant temperatures of 0, 5, 10, 15, 20, 25, 30, 35 and 40 °C. Except from the modification in temperature the experiment was carried out under the standard germination conditions. The Petri dishes

were arranged in growth cabinets set at the required temperatures. These cabinets were checked every day and on two occasions a deviation of ± 1 °C from the desirable temperature was observed for the cabinet set at 40 °C. The deviation was corrected when necessary. Germination was recorded up to the tenth week, until full germination was obtained for all temperatures.

j) Scarification

Sugar beet fruits and seeds were scarified using 4 procedures in addition to the scarification treatments described in Materials and Methods, part b). All scarification treatments are specified for each experiment, and as long as it is expedient the nomenclature for the different treatments is maintained.

For matter of convenience the results are grouped as:

- A) Effect of scarification on fruits from Lot 1.
 - B) Effect of scarification on fruits seeds from Lot 1.
 - C) Effect of scarification on fruits and seeds from Lot. 2
- A) Effect of scarification on fruits from Lot 1.

Fruits only were used in the experiment, the material was divided into:

- 1) Intact fruit (whole fruit enclosing the seed).
- 2) Punctured fruit. The fruit was scarified making two or three small perforations in the ovary cap by means of a fine dissection needle.

Care was taken to make the perforations through the ovary cap only and avoiding the seed itself.

- 3) Scarified fruit. The ovary cap was removed, thereby exposing a large surface of the seed.
- 4) Ovary cap replaced. The ovary cap was removed, as in 'scarified fruit', but after removal it was replaced in its original position using gentle pressure, so that the line of junction between the edge of the ovary cap and the wall of the locule was disjointed, but the seed remained covered.
- 5) Scarified fruit + ovary cap. The fruit was scarified as in 'scarified fruit' but the ovary caps were arranged on the filter paper so they were in contact with the fruits.

In all cases the fruits were soaked with water and placed in continuous darkness at 25 °C, according to the standard germination conditions.

B) Effect of scarification on fruits and seed from Lot 1.

The material consisted of:

- 1) Intact fruit.
- 2) Scarified fruit.
- 3) Seed or True seed (the excised seed with the testas).
- 4) Scarified seed. The excised seed with the outer testa removed.

It differs from 'scarified seed' in the sense that the inner testa is not scarified.

Fruits and seeds were soaked with water and placed in the dark at 25 °C. An identical group of fruits and seeds was subjected to continuous irradiation at 25 °C.

C) Effect of scarification on fruits and seeds from Lot 2.

The material consisted of:

- 1) Intact fruit.
- 2) Scarified fruit.
- 3) Seed or True seed.

Fruits and seeds were soaked with water and placed in the dark at 25 °C.

k) Uptake of Radioactive IAA

The uptake of ^3H -5-IAA (specific activity 29 Ci/m M) into sugar beet fruits, and the leaching of the compound from fruits by water, were examined using liquid scintillation spectrometry. Radioactive indole acetic acid was obtained from CEA, France.

Fruits were incubated in 1.5 ml of 10^{-5} M radioactive IAA solution for 24 hours at 25 °C in the dark. An incubation time of 24 hours was selected because imbibition place within this period (see page 79) and longer incubation times will result in germination of the seed. After incubation, each individual fruit was subjected to 5 consecutives washings in 10 ml of distilled water.

Each washing had a duration of 5 minutes. From the water of these washing 0.1 ml was taken for the assay of radioactivity. After the last washing each fruit was carefully dissected and the isolated seed incubated in 1 ml of absolute ethyl alcohol for 24 hours in the dark at 25 °C. From the alcohol of incubation 0.1 ml was taken for the assay of radioactivity.

1) Assay of Radioactivity

The water from the washing and the alcohol of incubation were transferred to the scintillation vials. The water and the alcohol were removed at room temperature in a desiccator and then 10 ml of toluene containing 4 g/l of PPO (2,5-diphenyl oxazole, BDH Chemicals, Ltd., Poole, U.K.) added to each vial. The vials, after equilibration to 4 °C, were assayed on a Packard Tricarb Model 3380 liquid scintillation spectrometer.

In another identical group of samples obtained following the same experimental procedure, the water from the washings and the alcohol were transferred to the vials and 10 ml of the solution of PPO added. Four mls of absolute ethanol were then added to solubilise the water. No secondary fluor was added. The vials were then assayed as previously described.

The Tricarb Spectrometer was adjusted to a background subtraction of 64.3 and the result cpm (counts per minute) converted to dpm (disintegrations per minute) by the expression:

$$\text{dpm} = \frac{\text{cpm} \times 100}{\% \text{ efficiency}}$$

The percentage of efficiency was calculated from a quench correction curve for tritium for the liquid scintillation spectrometer.

m) Effect of Fruit Coat Extracts

The experiments are presented in three sections:

- A) Effect of the intact fruit water extract and the fruit coat extract on the germination of the true seed.
- B) Effect of the concentration of the intact fruit water extract.
- C) Effect of sterilization of the fruit coat extract.

Part A: Effect of the intact fruit water extract and the fruit coat extract on the germination of the true seed.

1) Preparations of the extract.

All extracts preparations and true seeds used in these experiments were from Lot 1.

a) Intact fruit water extract.

Intact fruit water extract was prepared by incubating 10 intact fruits in 1 ml distilled water for 48 hours in the

dark at 25 °C. After the incubation period, the extracts were filtered under vacuum on a Büchner funnel. In order to eliminate microorganisms that could be present in the extract as a result of leaching from the surface of the fruit, the extracts were further filtered through a Millipore filter, having a mean pore size of 0,22 µm. The extracts were used immediately after preparation.

b) Fruit coat powder.

Ten intact fruits were dissected, separating the seeds from the fruit coats. The empty fruit coats were finely ground using a mortar and pestle.

c) Empty fruit coat.

Intact fruits were dissected and the empty fruit coats reserved for the experiment.

2. Germination of true seeds.

Ten true seeds were dissected and placed in 5-cm petri dishes containing: a) 1.5 ml of intact fruit water extract; b) 1.5 ml of fruit coat powdered solution; c) 15 empty fruit coats, in close contact with the true seed, in 1.5 ml of distilled water.

Two groups of replicates were prepared for each treatment, one group being kept at 25 °C in the dark and the other being kept in continuous

irradiation at 25 °C for the required period of germination.

Part B: Effect of the concentration of the intact fruit water extract.

The extract were prepared as describe in Part A-a) with intact fruits from Lot 2 and the germination of intact fruit, scarified fruit and true seed was examined. Intact fruit water extracts were used at the following concentrations: 200% (preparing by extracting 20 fruits per ml), 100% (prepared as in Part A-a) , 75%, 50%, 25% an 0%. The germination of 10 intact fruits, scarified fruits and true seeds was examined at 25 °C in the dark, in 1,5 ml of extract at the required concentration.

Part C: Effect of sterilization of the fruit coat extract.

Fruits and seeds from Lot 2 were used for the germination test and for the preparation of the extracts. Ten intact fruits were dissected and the empty fruit coats slightly ground using a mortar an pestle. These empty fruits were incubated in 1 ml distilled water for 48 hours in the dark at 25 ° C. After the incubation period the extract was filtered on a Büchner funnel under vacuum and the divided into two aliquots. One aliquot was filtered through the Millipore filter in order to eliminate microorganisms (this is termed 'sterile extract') an the other one was used directly in the germination test (this is termed 'non-sterile extrac'). Both extracts were tested on the germination of intact fruit, scarified fruit and true seed.

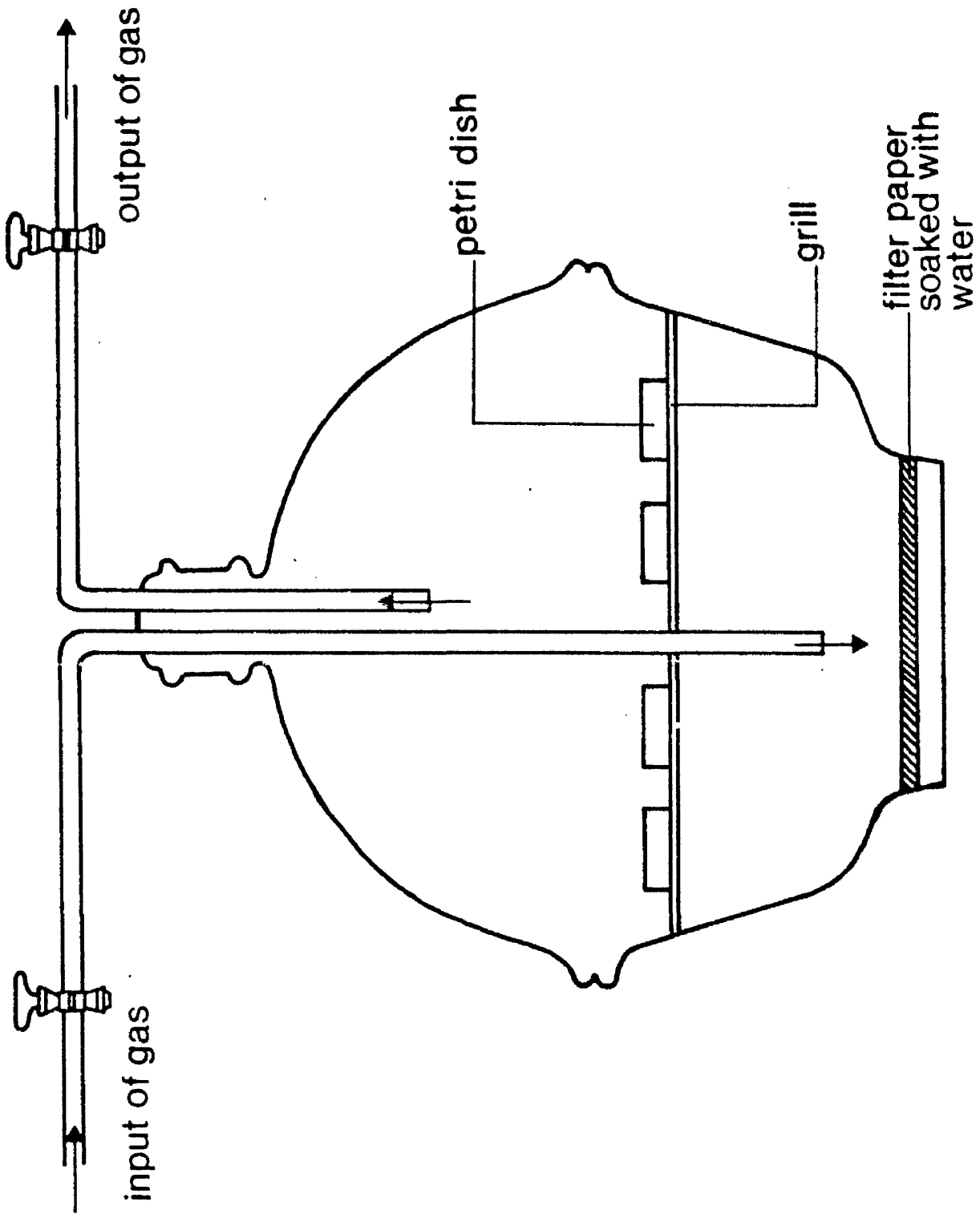
n) Effect of Oxygen and a Respiratory Inhibitor

Effect of oxygen

The effect of oxygen on sugar beet germination was tested by subjecting fruits and seeds to an atmosphere of oxygen at different tensions. Petri dishes were lined with 2 sheets of filter paper and soaked with 1.5 ml of distilled water, each dish containing 10 fruits of 10 seeds. The dishes were arranged over a grill situated inside the desiccator (See Fig. 2) and they were left open to allow the gas filling the desiccator to be in contact with the fruits and seeds. Several sheets of filter paper were placed in the bottom of the desiccator and completely soaked with water. The desiccator was sealed by a paraffin film and the stream of gas started. To count the number of germinated fruits and seeds the stream of gas was interrupted, the desiccator opened and the dishes examined. Afterwards the desiccator was resealed and the stream of gas resumed.

Interruption of the gas flow, although undesirable, considerably facilitated counting the germinating seeds, and preliminary experiments indicated that this interruption did not significantly affect the germination values obtained.

Figure 2.- Diagram of the desiccator and petri dishes used in the experiments of effect of oxygen on sugar beet germination.



Three sets of experiments were carried out employing this procedure:

- 1) Intact fruits from Lot 2 were placed in the desiccator and the stream of pure oxygen passed through it, thus the concentration was 100% at atmospheric pressure. The desiccator was kept in darkness at 25 °C.

A similar test was carried out in continuous irradiation.

- 2) Intact fruits from Lot 2 were arranged in several desiccators. One group of them were subjected to a stream of oxygen at 100%; a second group subjected to a nitrogen stream at 100% and in the third group the air was excluded from the desiccator. In this last group the desiccators and petri dishes were prepared as usual, but the input A (See Fig. 2) was closed and the output B connected to a vacuum pump, which was left working during 1 hour; after this period the output B was also closed. The operation was repeated every time the desiccator was opened to count the germinated fruits.

In all cases (100% oxygen, 100% nitrogen and vacuum) the desiccators were kept in darkness at 25 °C. The tests were done simultaneously.

- 3) Intact fruit, scarified fruit and true seed from Lot 2 were subjected to a stream of oxygen at different percentages.

These percentages were provided employing nitrogen as complementary gas, as follows:

100 %	oxygen	-	0%	nitrogen
50 %	"	-	50%	"
20 %	"	-	80%	"
10 %	"	-	90%	"
5 %	"	-	95%	"
0 %	"	-	100%	"

The experiment was carried out in darkness at 25 °C. All the gases, including the mixture of oxygen and nitrogen, were obtained from British Oxygen, Ltd.

Effect of Hydrogen Peroxide

The effect of hydrogen peroxide on sugar beet germination was tested on intact fruits from Lot 1. Ten intact fruits were placed in each Petri dish and 1.5 ml of the solution added to the dish. The hydrogen peroxide concentrations were: 2M, 1M, 10^{-1} M, 10^{-2} M, 10^{-3} M and a water control was also included. Germination conditions were darkness at 25 °C.

Effect of Potassium Cyanide

The effect of a respiratory inhibitor was examined. Potassium cyanide was tested on germination of intact fruit, sacrificed fruit and true seed from Lot 2. Ten fruits or seeds were placed in each Petri dish and 1,5 ml

of the solution added to the dish. The potassium cyanide concentrations were: 10^{-3} M, 10^{-4} M, 10^{-5} M, 10^{-6} M and 10^{-7} M. The germination test was carried in the dark at 25 °C.

o) Water Supply

The experiments are presented in three sections:

Part A: Hydration - Dehydration. Effect of different times of hydration on germination.

Part B: Accumulative effect of hydration - dehydration cycles.

Part C: Water sensitivity.

Part A: Hydration - Dehydration

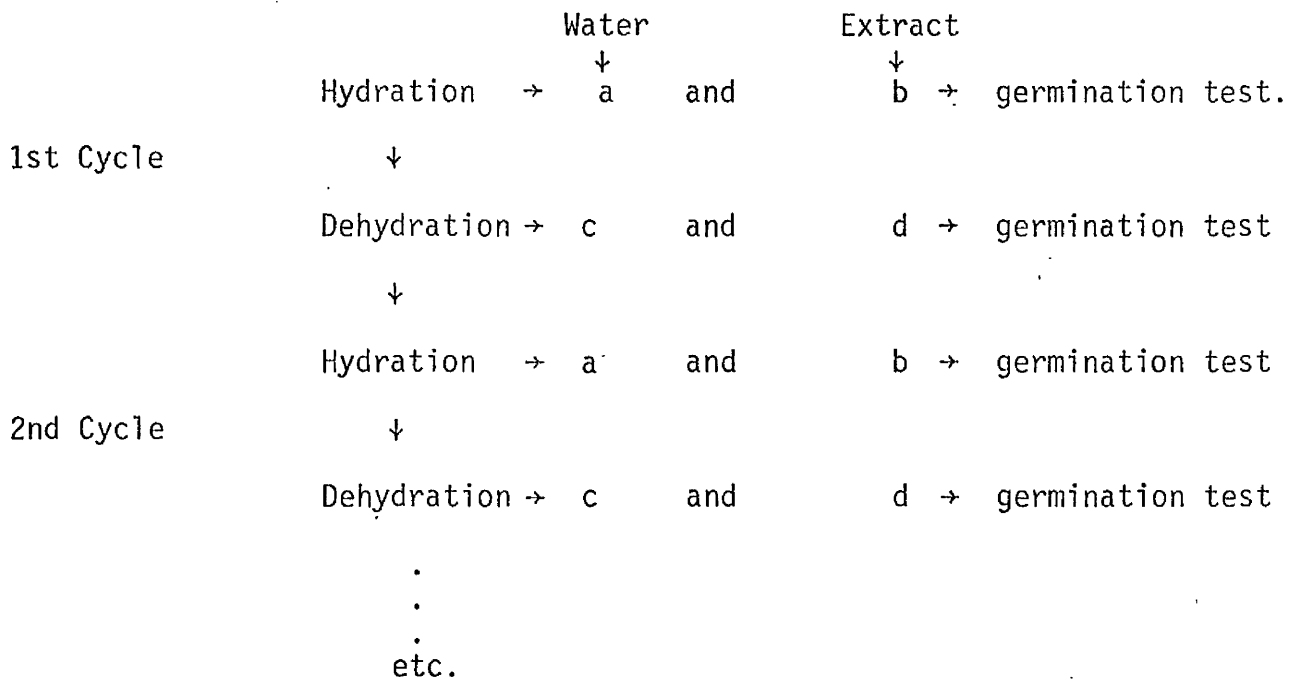
Intact fruits from Lot 2 were subjected to hydration in an excess of water for 2, 4, 8, 12 and 48 hours in the dark at 25 °C.

After the hydration periods the fruits were separated into two groups. In one group the fruits were placed to dry on filter paper at 25 °C in the dark, and were weighed every day until no further change in their weight was detected. The drying period was observed to take one week. In the second group, used as a control, the fruits were placed to germinate immediately after the hydration

period, without drying. In both groups the fruits were placed to germinate in the dark at 25 °C and soaked with 1.5 ml of water.

Part B: Accumulative effect of hydration - dehydration cycles

In this experiment the fruits were given several cycles of hydration - dehydration prior to germination. Intact fruits from Lot 1 were used, and subjected to a hydration period of two hours followed by drying of one week. Two hours of hydration followed by one week of drying was considered as one cycle and four cycles were given. In each cycle group of fruits were taken, according to the following scheme:



The germination test was carried out sowing the fruits in water or in intact fruit water extract prepared as previously described. The fruits

were placed to germinate in the dark at 25 ° C.

Two different methods of hydration were used:

- i) fruits continuously leaching with running water
- ii) fruits left to soak; 1 ml of water for every 10 fruits.

Part C: Water sensitivity

The sensitivity to water was tested by germinating intact fruits and true seed from Lot 1 in different quantities of water. Fruits and seeds were placed in Petri dishes and 0.5, 1, 1.5, 2, 3 and 4 ml of distilled water were added. The dishes were weighed initially and then every 24 hours to detect water loss by evaporation. Any such loss was made up by the addition of distilled water. The germination test was carried out in darkness at 25 °C.

p) Chemicals

Germination Promoters

The effect of three germination promoters was tested on germination of intact fruit, scarified fruit and true seed from Lot 1. The promoters used were gibberellic acid, indole acetic acid and kinetin. Each of these hormones were tested separately at concentration of 10^{-4} and 10^{-5} M. In

addition, the effect of combinations of two of the hormones was tested by the use of solutions prepared as follows: gibberellic acid/indole acetic acid; kinetin/indole acetic acid and gibberellic acid/kinetin. For all solutions a proportion of 50% to 50% of each hormone was used, and final concentration was 10^{-5} M. The Petri dishes contained 1.5 ml of the solutions and the experiment was carried out in the dark at 25 ° C.

Germination Inhibitor

Abscisic acid (ABA) was used as a germination inhibitor. The solutions were prepared with synthetical ABA, a racemic mixture of equals proportions of (+) -ABA and (-) -ABA. The concentrations were 10^{-6} M; 10^{-5} M; 10^{-4} M and 2×10^{-4} M; ABA was tested on the germination of intact fruit, scarified fruit and true seed from Lot 2. The petri dishes contained 1.5 ml of the solution and the experiment was carried out in the dark at 25 °C.

q) Stains

Intacts fruit from Lot 1 and 2 were used in this section, the stains employed were methylene blue, neutral red and hematoxylin, in aqueous solution at 0,1%.

The procedure consisted of placing the fruits in the petri dishes and adding to them 1,5 ml of the stain solution, after this the fruits were incubated during 72 and 96 hours in darkness at 25 °C. At the end of the 72 and 96 hourd period the fruits were washed to remove excess stain and dried

with filter paper. Longitudinal and transverse sections of the fruits were made, and observed under the microscope. Unimbibed fruits were stained by sectioning as before, followed by the addition of 1-2 drops of the solution directly to the tissues.

EXPERIMENTAL

Viability

The results of a germination test are more reliable when the viability of the seeds has been taken into account because this provides the maximum value of germination that can be expected for the particular batch of seeds under study. Viability not only provides information about the general condition of the seeds but also gives some knowledge about whether dormancy plays any role in the germination process, since high viability and low germination can be taken as an indication for dormancy.

Table 1 shows the results of an experiment in which the viability of the seeds from 592 fruits was tested with the method outlined in page 30. It can be seen that 92.6% of the fruits had viable seeds and only 3.0% non-viable seeds. Empty fruits or fruits with abnormal seeds amounted 4.4%. According to this result it could be expected that an experiment in which germination is tested on the same fruit will give germination values close to 93%. This high figure of germination was however very difficult to reach on germination experiments using intact fruits. In Fig 3, for instance, the results of a typical fruit germination experiment in standard conditions are plotted as function of time, after 21 days less than 60% of the fruits have germinated.

Mayer and Poljafoff-Mayber (1963) have pointed out that chemical methods devised to test viability are only partially satisfactory. According to these

1-a. Lot 1	S	U	E	T
Absolute Values	548	18	26	592
Percentages	92.6	3.0	4.4	100.0

1-b. Lot 2	S	U	E	T
Absolute Values	91	4	5	100
Percentages	91.0	4.0	5.0	100.0

Table 1.- Determination of viability of sugar beet seeds of Lot 1 (1-a) and Lot 2 (1-b) by staining with 1% solution of tetrazolium chloride.

- S: number of embryos stained
- U: number of embryos unstained
- E: number of empty fruits
- T: total number of fruits used; $T = \sum (S+U+E)$

More details in the text.

authors such tests can only check for one definite reaction which may be correlated with the eventual ability of the seed to germinate. In the present experiments the difference observed between viability and germination figures could be then explained in these terms, since some of the fruits could contain seeds that will give a positive result to the viability test and yet still be unable to germinate. This explanation does not however rule out the possibility of a mechanism of dormancy in connection with the low germination obtained for intact fruits, in addition the difference between germination and viability values are too large to be explained only on the basis of methodology. Evidencegiving support to the idea of a dormancy mechanism will be shown later.

Table 1 also shows that 4.4% of the fruits were empty or contained abnormal seeds. This fact introduces a small difference in the viability for the material when it is used as scarified fruit or true seed, because in these two cases the viability is slightly higher than the value for intact fruit. The reason for this is that during the process of scarification and excision only those fruits containing seeds are used and the empty ones are discarded. It is not possible to do the same when the material is used as intact fruit since there is no morphological nor structural difference between an empty fruit and a fruit containing a seed. This difference in the viability value ought to be taken into account when a particular treatment is applied to the material.

An identical viability test was carried out on fruits from Lot 2, but in this case only 100 fruits were tested. The results are shown in Table 1-b and are very similar those obtained for Lot 2.

Germination Pattern

In order to establish the basic germination behaviour of sugar beet, intact fruits were soaked with water and placed to germinate in continuous darkness or continuous irradiation at a constant temperature of 25 °C. The experiment was carried out on fruits from Lot 1 and Lot 2 to verify if both batches of fruits exhibited the same germination pattern. The results are expressed as time course of germination in Figs. 3, 4, 5 and 6. Each point of these curves represents the mean of eight experiments in which 10 petri dishes were used.

Figure 3 shows the germination in the dark of intact fruits from Lot 1. Germination was very slow for the first 24 hours, but increased rapidly for the next two weeks and the plateau of the curve was reached at 21 days. The highest germination figure obtained was 54% for 28 days. Fruits in continuous irradiation (Fig. 4) also exhibited slow germination for the first 24 hours and achieved the plateau of the curve at 21 days, with a figure of 67% for full germination at 28 days.

Figure 3.- Basic germination behaviour of sugar beet fruits. Time course of germination for intact fruit in darkness at 25 °C. Lot 1. In the abscissa percentages of germination \pm standard error, in the ordinate time in days.

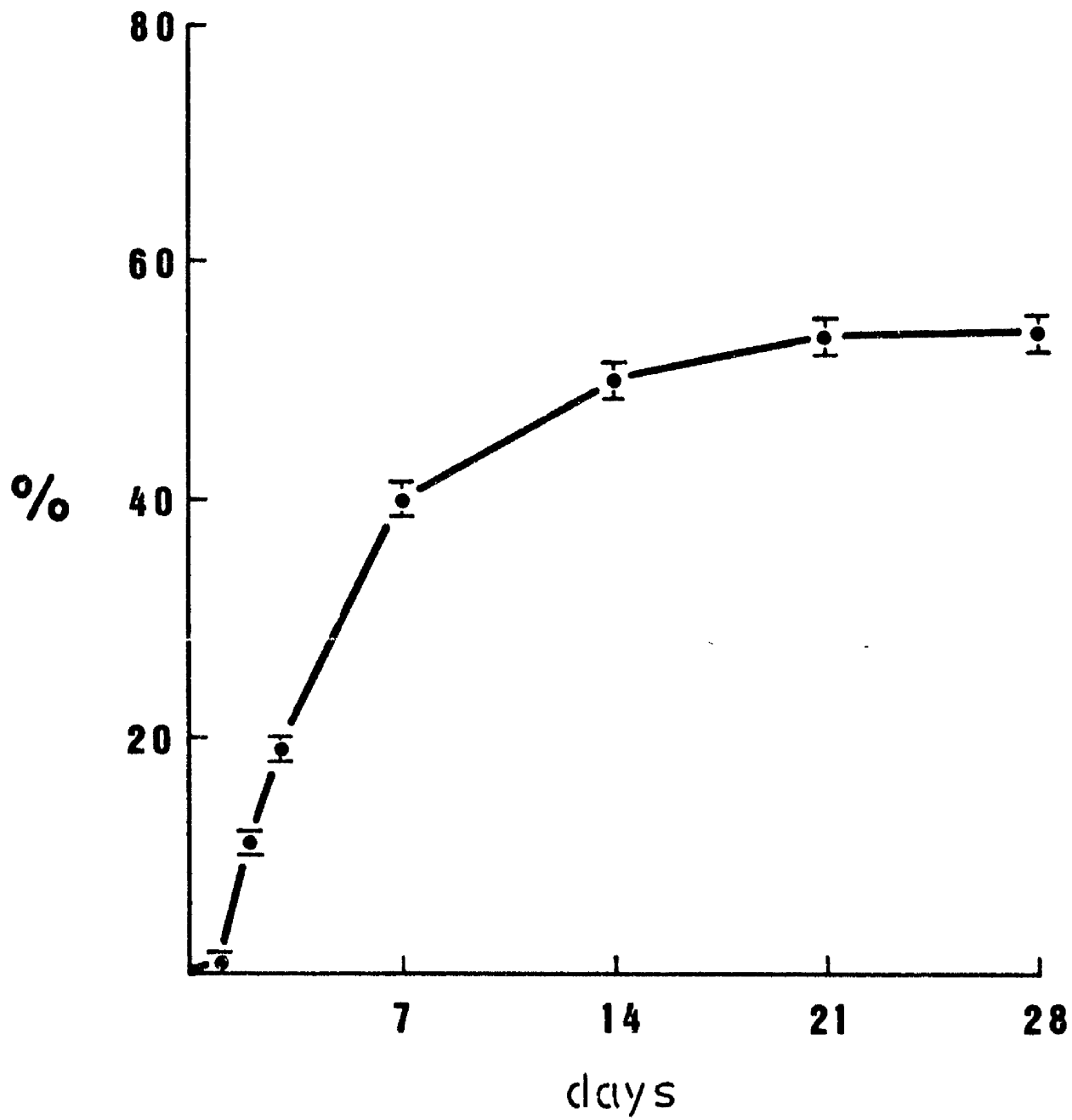


Figure 4.-

Basic germination behaviour of sugar beet fruits. Time course of germination for intact fruits in light at 25 ° C. Lot 1. In the abscissa percentages of germination \pm standard error, in the ordinate time in days.

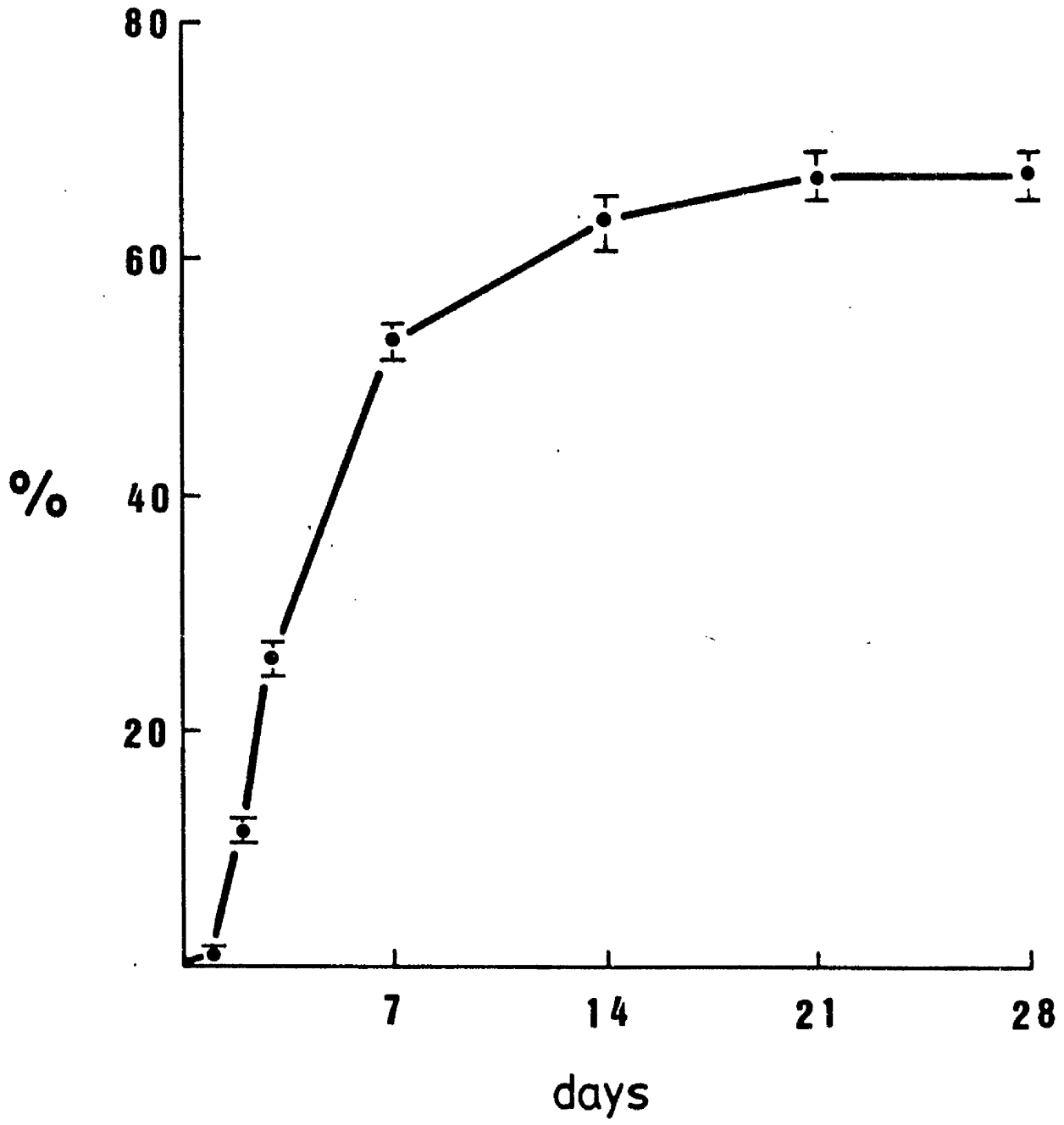


Figure 5.- Basic germination behaviour of sugar beet fruits. Time course of germination for intact fruit in darkness at 25 °C. Lot 2. In the abscissa percentages of germination \pm standard error, in the ordinate time in days.

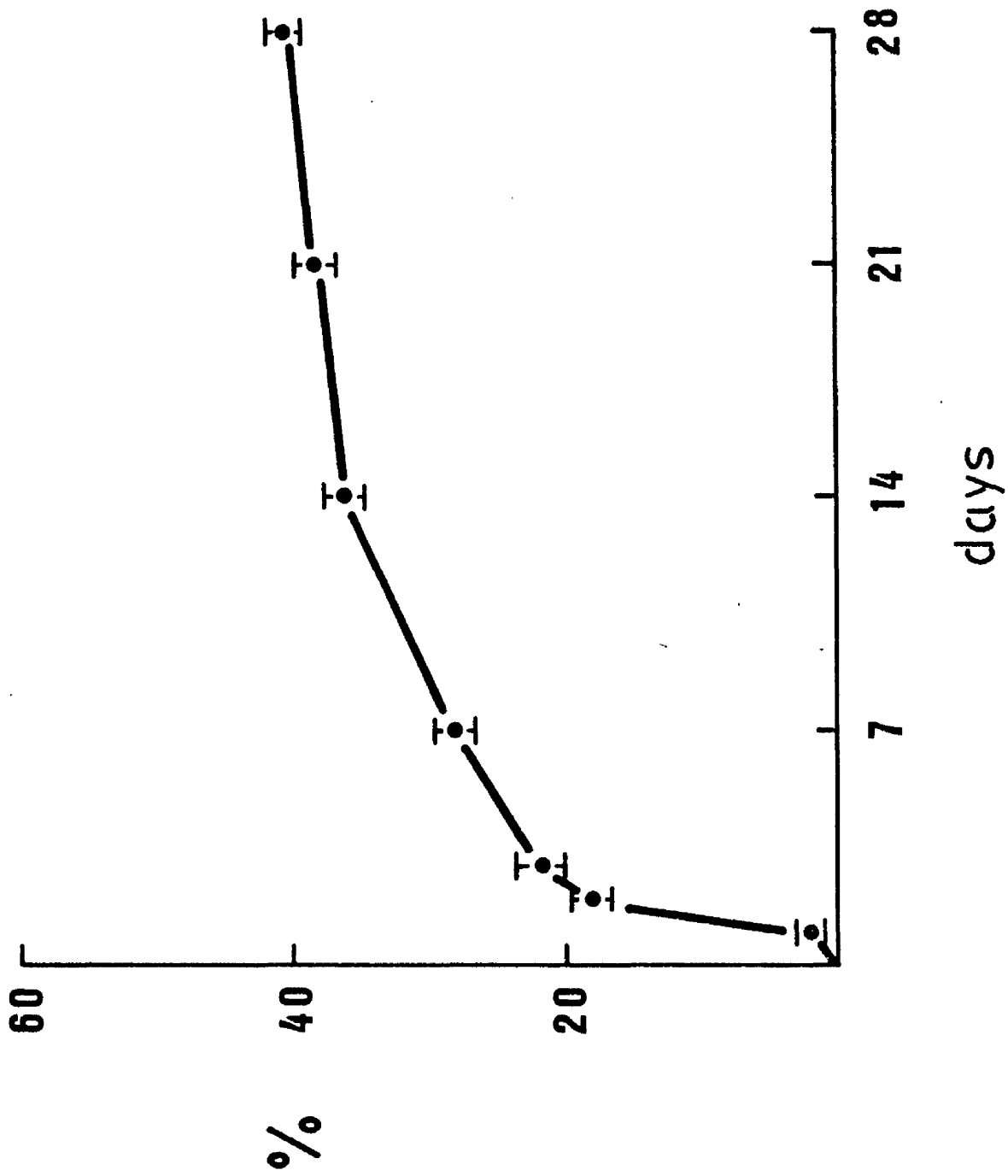
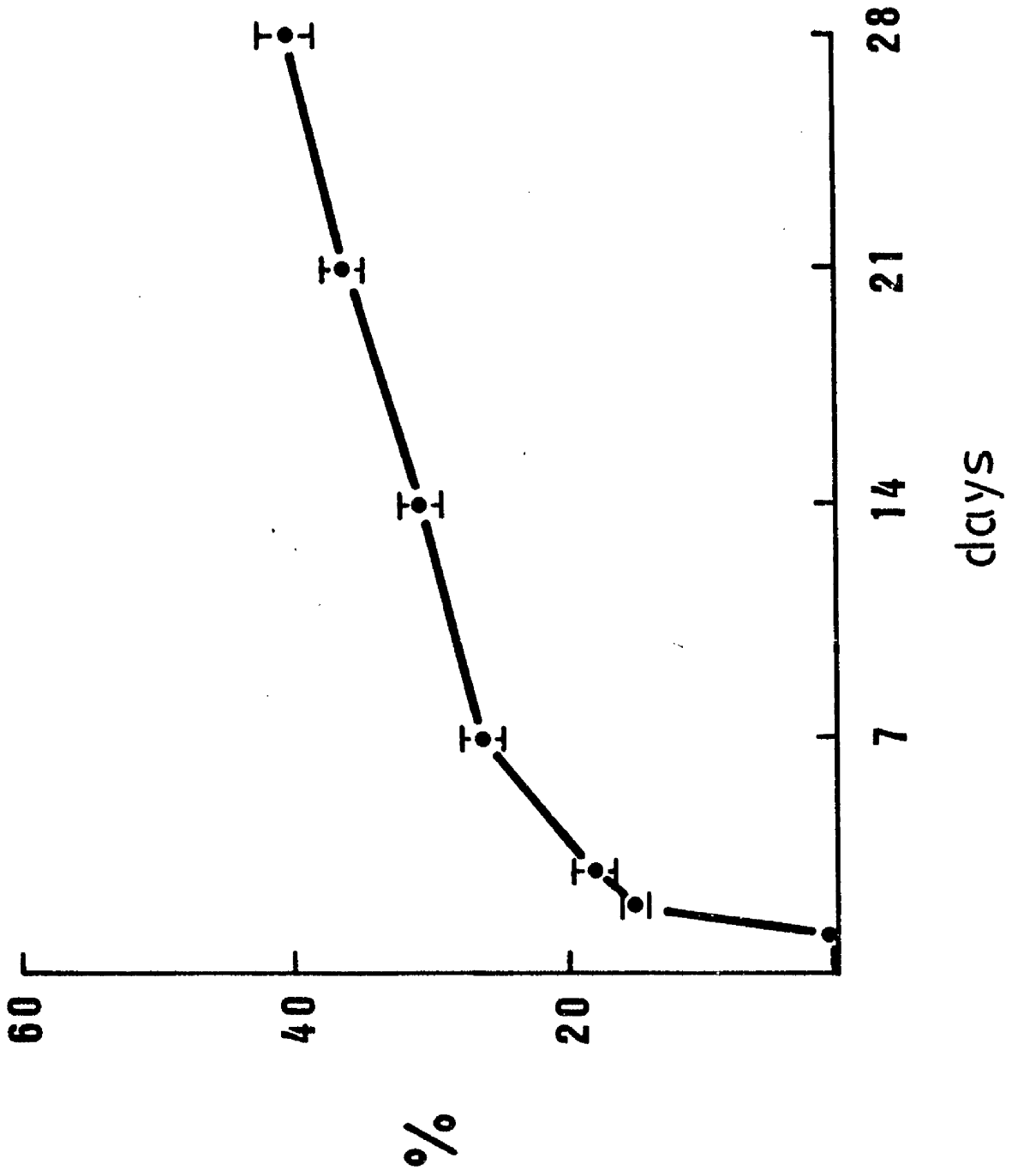


Figure 6.-

Basic germination behaviour of sugar beet fruits. Time course of germination for intact fruit in light at 25 °C. Lot 2. In the abscissa percentages of germination \pm standard error, in the ordinate time in days.



Light promotes germination not only increasing the value of full germination but slightly increasing the speed of the germination process, especially during the first week of the experiment. In spite of the similarities between the curves in Figs. 3 and 4 the differences in their values must be taken into account in the analysis of experiments performed with this lot of fruits. For each pair of germination values corresponding to 7, 14, 21 and 28 days in the curves of Figs. 3 and 4 the Student's t test was applied, and it was found that they were statistically different at a level of probability less than 0.1%. This indicates the existence of a promotive effect of the radiation on the germination of fruits from Lot 1.

It may be argued that this promotive effect of irradiation on sugar beet germination may be attributed to activation of the phytochrome system of the seed, assuming that such a system occurs in sugar beet seed. It should be pointed out, however, that this seed is protected from the environment by the fruit which is an opaque and relatively thick body, and it is unlikely, therefore, that the seed itself is irradiated. Furthermore, irradiance of seeds has no promotive effect on germination (see Section B of scarification). A second explanation for the described effect of irradiation on germination would be to assume the existence in the fruit material of a substance which would react to irradiation. This substance will be then transported to the seed tissues where it will promote germination. It is difficult, however, to think of a substance of this type in the non-living, lignified tissue of the fruit material. Still another explanation for this effect could be made in terms of an increase in tempe-

perature inside those petri dishes under irradiation. Measurements of temperature inside and outside of plastic petri dishes were made by Primrose (1972) in the same laboratory using the same growth cabinet and identical conditions of irradiation and temperature used in the particular experiment of this thesis. It was found that there was a mean temperature difference of $0.35\text{ }^{\circ}\text{C}$ ($\pm 0.05\text{ }^{\circ}\text{C}$) between the inside and outside the petri dish. The maximum difference found by Primrose at any time over a period of 5 weeks was $0.9\text{ }^{\circ}\text{C}$. Furthermore, experiments testing the effect of temperature on sugar beet germination (see page 66) proved that even an increase of $5\text{ }^{\circ}\text{C}$ over $25\text{ }^{\circ}\text{C}$ does not produce an increase in the germination percentages. A plausible explanation for this promotion may be found in the fact that those petri dishes placed under irradiation showed a certain degree of water evaporation higher than the evaporation in the petri dishes in darkness. This was due to the fact that those dishes subjected to irradiation were placed directly over the irradiation bench and consequently this favoured water evaporation. On the other hand, the dishes for experiments in darkness were wrapped in aluminium foil and tightly packed in a metallic container. Experiments where the fruits were incubated in variable quantities of water demonstrated the dramatic effect of water sensitivity: in these petri dishes the highest percentage of germination occurred using 1 ml of water. In view of the fact that 1.5 ml of water was used as a starting volume in this experiment, any evaporation occurring in the irradiation treatment would tend to cause a rise in germination as the optimum volume is achieved.

Figures 5 and 6 show respectively the germination course of fruits from Lot 2 subjected to continuous darkness or irradiation. In this particular instance almost no difference was found in the germination behaviour of fruits germinating in the dark or in irradiation. In both cases the plateau of the germination curve was reached at 21 days, the percentages of full germination was 37 - 39% and the speed of the germination was also the same. This batch produced a figure of full germination considerably lower than Lot 1, the highest value for full germination in Lot 2 being 39%, in contrast with a figure of 67% for germination in irradiation in Lot 1. This is not surprising because the nature of the environmental conditions during seed development has long been known to influence the subsequent pattern of germination of many seeds (Kidd and West, 1918). Sugar beet germination is largely dependent on the batch of fruits, partly because in agriculture the environment is frequently altered by sowing the crop or harvesting its seeds on different dates, or varying the quantities of fertilizer applied to the crop, and because there are genetic differences in batches coming from different harvests (Battle and Whittington, 1969-a; 1971). Moreover, Lot 2 was easily contaminated by microorganism which normally begin to develop by the second week once the fruit have been soaked. Although by the end of the second week most of the fruits have germinated, infection by microorganisms may modify germination.

In fact, it has been reported that the number of sugar beet seedlings that emerge in the field depends not only on the germination capacity of the fruits but also on the number of fruits contaminated with Pleospora betae Bjorling (Byford, 1963). Heydecker and co-workers in 1971 found that sugar beet germination is depressed by microbial factors and claimed that the

depression of germination is not due to a toxic product of bacterial metabolism but probably to a competition between bacteria and seeds for oxygen.

In relation to viability it is reasonable to expect that for Lot 2 where the highest germination figure was 67% and viability 92%, 25% of the fruits contain dormant seeds under the particular experimental conditions. It is more difficult to make an estimate of the degree and type of dormancy in Lot 2; viability was also 92% and full germination 39%, but for this batch it cannot be assumed that 50% of the fruits are dormant because germination could be affected by microbial infection.

Although there were differences in the germination behaviour of fruits from Lot 1 and 2, they respond in a similar way to a certain set of experimental conditions. There was a period of 24 hours before radicle emergence commenced, a minimum of three weeks were needed to achieve the plateau of germination curve; and full germination occurred at 28 days of incubation in both batches.

Effect of Temperature

Different seeds have different temperature ranges within they germinate. This range is not only determined by genetic differences between the species, but in the same species by varietal differences, source of seeds, as well age of seeds. In order to establish this range in Beta vulgaris, intact fruits taken from Lot 2 were soaked with water and placed to germinate in the dark at different constant temperatures, from 0 °C to 40 °C. The results are presented in Table 2 and they illustrate the effect of temperature on the

germination of sugar beet fruits; the values represent the germination percentages obtained for the time course of germination for the tested temperatures. From Table 2 the figures plotted in Figs. 7, 8 and 9 were taken.

Fig. 7 shows the percentages of germination for 28 days of treatment as a function of temperature; Fig. 8 also shows the germination percentages for each temperature, but in this case the figures are those obtained for full germination at 70 days. In Fig. 9 the minimum time required to achieve full germination for all temperatures have been plotted.

From Fig. 7 it can be seen that germination was very poor at 5 °C but increased very rapidly with temperature, producing a maximal value of 65% at 10 °C. A subsequent increase of temperature caused a slow decrease of germination, until it was prevented completely at 40 °C. This is a typical curve illustrating the effect of temperature on germination; the physiological processes leading to germination can only take place between certain temperature limits, these limits being 0 °C and 35 °C for sugar beet.

TIME (DAYS)	TEMPERATURE (°C)					
	0		5		10	
	%G	<u>±</u> s.e.	%G	<u>±</u> s.e.	%G	<u>±</u> s.e.
1	0.0	-	0.0	-	0.0	-
2	0.0	-	0.0	-	0.0	-
3	0.0	-	0.0	-	0.0	-
7	0.0	-	0.0	-	33.0	3.1
14	0.0	-	1.5	0.8	53.5	3.4
21	1.5	0.8	6.5	1.8	62.5	3.0
28	1.5	0.8	18.5	3.2	65.5	3.0
35	1.5	0.8	37.0	4.3	66.5	3.1
42	1.5	0.8	44.5	3.4	66.5	3.1
49	1.5	0.8	51.5	3.2	66.5	3.1
56	1.5	0.8	56.5	4.1	66.5	3.1
63	1.5	0.8	59.5	3.9	66.5	3.1
70	1.5	0.8	61.0	3.8	66.5	3.1

Table 2.- Percentages of germination of sugar beet fruits in darkness at different temperatures. Lot 1.

TIME (DAYS)	TEMPERATURE (°C)					
	15		20		25	
	%G	<u>±</u> s.e.	%G	<u>±</u> s.e.	%G	<u>±</u> s.e.
1	0.0	-	0.0	-	5.0	1.9
2	1.0	0.7	13.5	2.9	17.5	2.9
3	8.5	1.9	25.0	3.5	23.0	3.0
7	40.0	3.7	29.0	3.9	30.5	3.6
14	50.5	2.9	38.0	3.7	37.5	3.5
21	57.5	3.1	42.0	3.0	39.5	3.1
28	59.0	3.2	44.5	3.5	41.0	3.0
35	59.0	3.2	44.5	3.5	41.0	3.0
42	59.0	3.2	44.5	3.5	41.0	3.0
49	59.0	3.2	44.5	3.5	41.0	3.0
56	59.0	3.2	44.5	3.5	41.0	3.0
63	59.0	3.2	44.5	3.5	41.0	3.0
70	59.0	3.2	44.5	3.5	41.0	3.0

Table 2 (Continuation).- Percentages of germination of
sugar beet fruits in darkness
at different temperatures. Lot 1.

TIME (DAYS)	TEMPERATURE (°C)					
	30		35		40	
	%G	± s.e.	%G	± s.e.	%G	± s.e.
1	3.0	1.6	1.5	1.0	0.0	-
2	11.5	1.8	4.0	1.5	0.0	-
3	15.0	2.2	6.5	2.6	0.0	-
7	21.0	3.1	9.0	2.8	0.0	-
14	22.5	3.5	10.0	3.0	0.0	-
21	23.0	3.5	10.5	2.9	0.0	-
28	24.0	3.7	10.5	2.9	0.0	-
35	24.0	3.7	10.5	2.9	0.0	-
42	24.0	3.7	10.5	2.9	0.0	-
49	24.0	3.7	10.5	2.9	0.0	-
56	24.0	3.7	10.5	2.9	0.0	-
63	24.0	3.7	10.5	2.9	0.0	-
70	24.0	3.7	10.5	2.9	0.0	-

Table 2 (Continuation).- Percentages of germination of sugar beet fruits in darkness at different temperatures. Lot 1.

Figure 7.- Effect of temperature on the germination of intact fruit. The percentages of germination are the values obtained after 28 days treatment. Lot 2. In the abscissa percentages of germination \pm standard error, in ordinate temperature in °C.

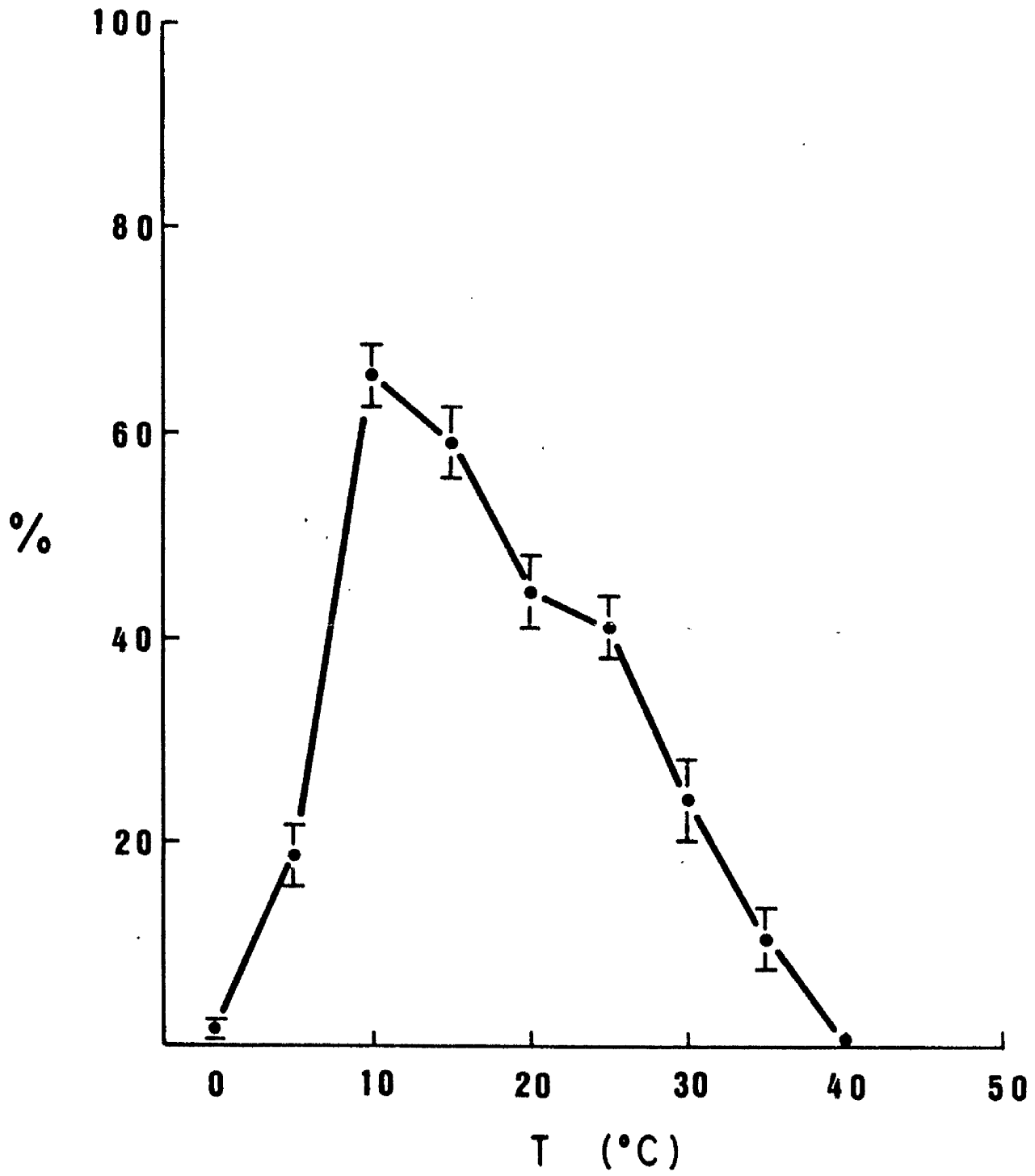


Figure 8.- Effect of temperature on germination of intact fruit. The percentages of germination are the values obtained after 70 days treatment. Lot 2. In the abscissa percentage of germination \pm standard error, in the ordinate temperature in °C.

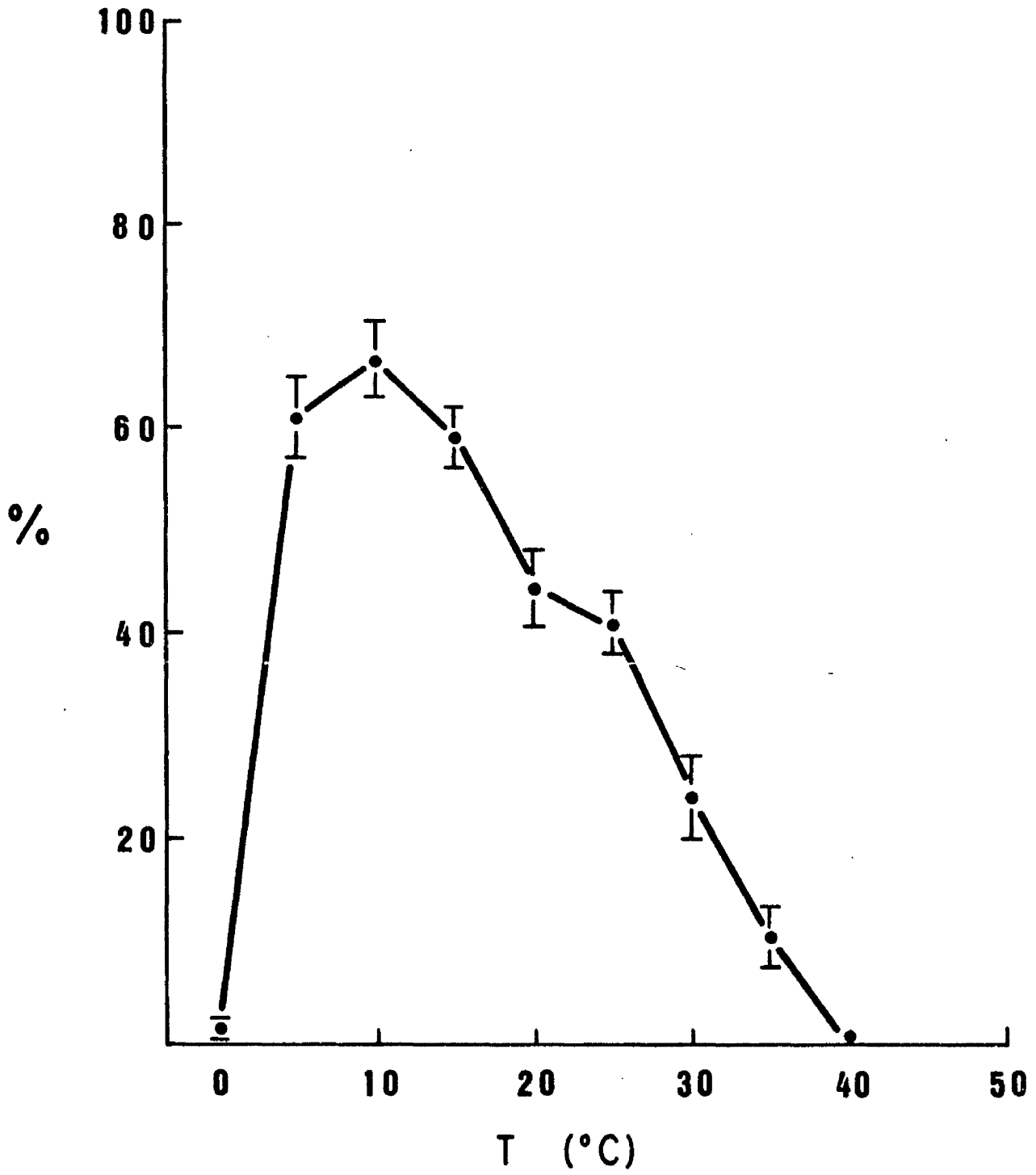
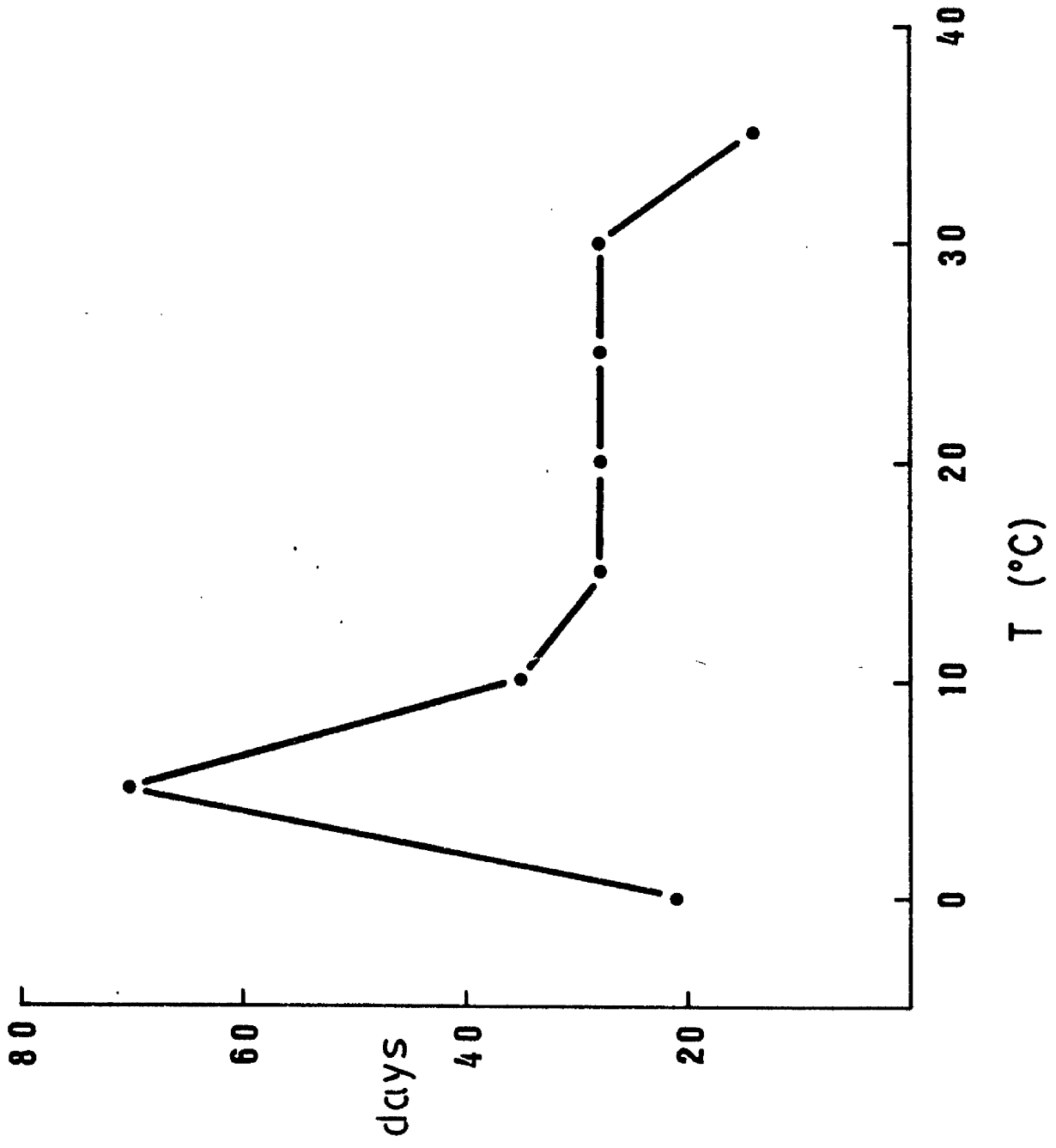


Figure 9.- Effect of temperature on the minimum time required to achieve full germination. Values taken from Table 2.



The performance of the physiological process improves as temperature is increased from the lower limit until the optimal temperature - between 10 and 15 °C - is reached, above which any further increase of temperature results in reduced performance reaching zero at the upper limit. A 'time factor' has been recognized in this process (Mayer and Poljakoff-Mayber, 1963; Lang 1965; Koller, 1972). As incubation is prolonged the optimum shifts to lower temperatures and becomes less sharply defined. If the reactions are measured as a function of temperature over a relatively short time interval, as it is in Fig. 7 where the germination percentages are those for 28 days, the optimal temperature is frequently higher than is measured over a longer interval. This can be seen in Fig. 8, where percentages of full germination at 70 days are expressed as a function of temperature. It shows that the lower and upper temperature limits remains the same but best germination is now obtained between 5 and 15 °C. At 5 °C germination reached 60% in contrast with Fig. 7 where the same temperature produced less than 20%. The shift of optimal temperature is due to slow germination at low temperature still taking place when the experiment at higher temperatures was considered as terminated. A way to visualize this slow germination is plotting the time to achieve full germination for all the temperatures tested (Fig. 9). At temperature between 15 °C and 35 °C fruits germinated in 28 days, while 70 days were needed to achieve full germination at 5 °C.

It is not possible to speak in terms of one accurate temperature for sugar beet but of a range of optimum temperatures and to determine this range one must take into account the values of full germination. This range proved to be 5-15 °C and it is in disagreement with temperatures reported as optimal for sugar beet. Constant temperatures of 20 ° C and 25 ° C have been reported to produce maximum germination for monogerm sugar beet (Francois and Goodin, 1972; Hoover and Gooding 1966). Radke and Bauer (1961) found that the optimal root temperature for sugar beet emergence was within the range of 25-35 °C.

Although 5-15 °C was found to produce the highest germination 25 ° C was chosen as germination temperature in the performance of the subsequent experiments. Some considerations were taken into account to decide the germination temperature. 25 °C had the advantage of producing half of the maximum figure of germination expected for the fruits, and this can be of some importance when a treatment which is expected to promote germination is applied to the fruits because a wide range of promotion is possible. It had also the advantage that changes of 5 °C above or below 25 °C did not cause drastic variation of the germination percentages and finally only four weeks are required to achieve full germination at this temperature.




Imbibition

The first process that occurs during germination is the absorption of water, called the imbibition phase. This is a physical process related to the properties of biocolloids, although very important physiological and biochemical events also take place during this phase, among them rise in respiratory rate and synthesis of nucleic acids and proteins (Black, 1970). Barton et al. (1971) have shown that profound quantitative and qualitative changes in RNA metabolism occur in Indian rice grass seeds during early hours of soaking and these changes may also occur in a seed which is unable to germinate under laboratory conditions. Imbibition is dependent on several factor viz: availability of water in the medium, temperature, kind of seed, and the presence of seed or fruit coats.

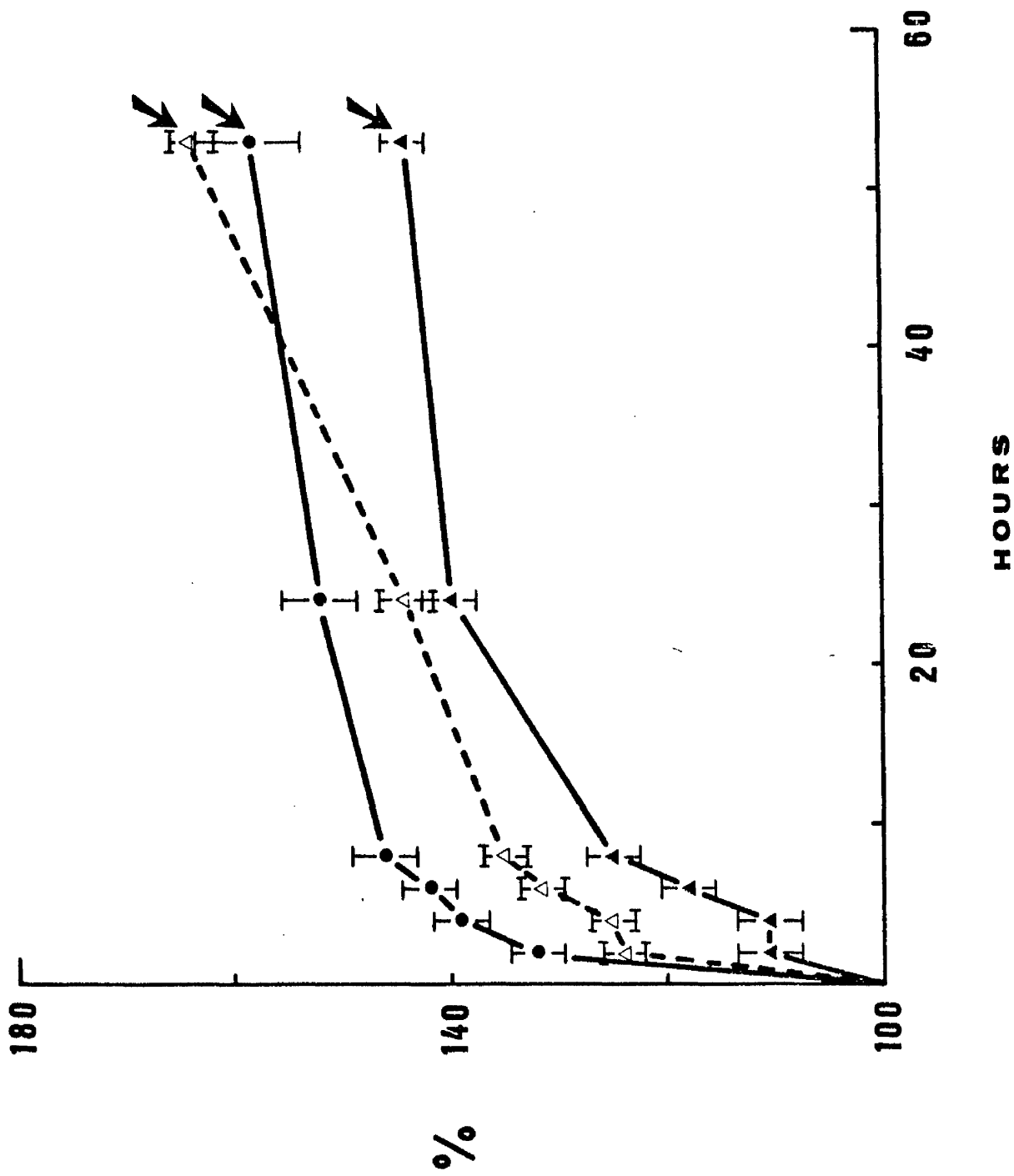
In Beta vulgaris it is of especial significance to determine the extent of imbibition because the seed is enclosed by a fruit with a thick and hard wall, and this wall is likely to interfere with water uptake by the seed or embryo.

Sugar beet fruits, scarified and non-scarified seeds were placed to imbibe at 25 °C in the dark, and their imbibition course was followed for 53 hours (Fig 10). In all these cases most imbibition took place within the first 24 hours. In the intact fruit there was a rapid uptake of water during the first 8 hours, stabilizing at 24 hours; by the end of the imbibition period the fruits had increased their original weight by 55%. Non-scarified

Figure 10.- Imbibition course of sugar beet fruits and seeds at 25 °C. The values are the percentage increase of initial weight at different times. In the abscissa percentage increase of weight \pm standard error, in the ordinate time hours.

keys:  Intact fruit
  Non scarified seed
  Scarified seed

The arrow denotes the commencement of radicle emergence.



seeds only imbibed 40% of their original weight, but the shape of the imbibition curve is similar to the fruits in the sense that rapid water uptake occurs during the first 8 hours with a stabilization at 24 hours. A different curve is obtained with scarified seeds; during the first 24 hours they imbibed more water than non-scarified seeds but less than the fruits on both a percentage and absolute basis.

On the other hand, water uptake for the last hours of the experiment equaled the water uptake of the fruits. It is also noticeable that no stabilization of the curve occurred at 53 hours, the time at which 70% of the seeds had already germinated. Water uptake in seeds often ceases for several hours at the end of the imbibition period (Ching, 1972), and then resumes at the time of radicle emergence. This period of recess occurs in intact fruits and non-scarified seeds, but in scarified seeds water is gradually absorbed into the seed, the process of imbibition and germination apparently being a continuous one. It can be argued that the lack of stabilization in scarified fruit is only a mathematical error, because the percentages of initial weight for 53 hours were calculated discarding many of the seeds which were taken into account for previous percentages. This is unlikely, however, because the imbibition curves for intact fruit and non-scarified seed were calculated in the same way and nevertheless, there is a stabilization of their curves at 24 hours.

It is to be expected that the fruits imbibe the largest quantities of water, because water has to be absorbed not only by the seed but by the thick fruit coat. Although the fruit coat affects the amount of water to be imbibed,

it does not delay the imbibition period, as it can be seen by comparing the results obtained for intact fruits and non-scarified seeds. In both cases the duration of imbibition is 24 hours. When only the fruit coat is absent, as it is in non-scarified seeds, less water is imbibed by the seed, although the pattern remains the same. If both the fruit and seed coat are absent, as it is in scarified seed, then the seed absorbs, on a percentage basis, as much water as the whole fruit. This last point suggests that the seed coat acts as a limiting factor - not a barrier - to water uptake. It may be assumed that in intact fruits water is rapidly absorbed by the fruit material and thus become available to the seed, but its further uptake into the seed is regulated by the seed testas. Consequently, the water uptake in the excised seed follows the same pattern than in intact fruit. When the physical integrity of the seed coats is disrupted there is no limiting factor to water uptake and imbibition becomes a continuous and gradual process. A criticism to this assumption is that it would be necessary to follow the imbibition course for the fruit material separately, to verify the rapidity of water uptake by the fruit material, and these data are not available in the experimental results.

Another criticism is that is not possible to assess which of the two testas of sugar beet is regulating the water uptake. In non-scarified seed both testas are present and in scarified seed the scarification affected both of them, the outer testa was completely removed and the inner testa was punctured.

In conclusion, imbibition is not inhibited by the fruit or seed coats: seed will imbibe water and eventually germinate, but their coats restrict the

quantities of water taken up into to the system.

Scarification

Many seeds fail to germinate because the restrictions imposed by the structures surrounding the embryo. The restrictions may be purely mechanical resistance to embryo growth (Crocker and Davis, 1914; Baker, 1948) or may be due to low permeability to gases or water, or both (Crocker, 1906; Johnson 1935; Brown, 1940; Visser, 1954, Khudairi, 1956; Black and Wareing, 1959; Rizzini, 1970-a,-b; Webb and Wareing, 1972), and in some cases the restriction is due to the inhibitory action of substances within the fruit or seed coat (Evenari, 1949; Tolman and Stout, 1940; Stout and Tolman, 1941; Duym et al. 1947, Rehm, 1953; De Kock and Hunter, 1950; De Kock et al., 1953; Van Sumere, 1960; Wareing, 1965; Edwards, 1969; Brown and Van Staden, 1971; Van Staden and Brown, 1972).

The promotive effect of the disruption of the covering structures on germination of some seeds has been known for some time, although in some cases the exact nature of this effect is unknown or not clearly understood (Sharpe and Merrill, 1942; Evenari, 1965). Many methods have been used to eliminate the hard coat effect and thus promote germination; among the best known ones are mechanical abrasion or impact, high and low temperature treatments, and chemical treatment to remove or dissolve the impermeable portions of the coat (Barton, 1965).

In Beta vulgaris where the embryo is enclosed by structures that belong not only to the seed but are derived from the fruit as well, it is relevant to ascertain the effect of these structures on germination. In doing fruits and seeds were subjected to diverse treatments of mechanical scarification. Only mechanical scarification was used because the other scarification treatments may introduce new variables to the system which secondary effects may be difficult to elucidate.

The results are grouped into three sections (see Parts A, B, and C) in which the scarification treatments are specified.

Part A: Effect of Scarification on fruits Lot 1

Sugar beet fruits were scarified as described in pages 26 and 34 and placed in the dark at 25 °C. The results are presented in Figs. 11 and 12; in both Figs. the germination percentages are expressed as a function of incubation time. Fig. 11 shows the germination of intact fruit and fruits scarified in three different ways. The intact fruit germinated very poorly achieving 35% germination at 21 days, with an early stabilization of the germination curve at 7 days. An enhancement of the germination was obtained when the fruits were scarified, but the treatment used to scarify them affected their response. Better germination was observed when the degree of scarification was larger. If the scarification consisted only in small perforations of the ovary cap ('punctured fruit') or the contact of the seed with its medium is reduced to the edge of the ovary cap ('ovary cap replaced'), an

increase in the speed of germination is obtained in both cases, with a value of 70% germination occurring at 21 days. This value represents a 35% increase over the intact fruit. When the extent of the scarification is even larger, as it is in 'scarified fruit' where the ovary cap has been completely removed, a better germination response is obtained. Germination increases very rapidly for the first three days and a figure of full germination of 90% is noted at 21 days. Considering that viability for 'scarified fruit' is 97% then almost complete germination occurs when the ovary cap is removed. This treatment produced an increase of 55% germination over the intact fruit and 20% increase over the two other scarification treatments.

It can be argued that the enhancement of germination does not depend on the extent of scarification and that the difference of 20% between the scarification treatments is merely caused by the presence or absence of the ovary cap in the petri-dish, which is absent in 'scarified fruit' but present in the other two cases. This objection may be reasonable in view that one of the best known causes that produces poor germination in sugar beet is the existence of inhibitory substances in the fruit, in the perianth and pericarp tissues rather than the true seed (Abu-Shakra and Aqil, 1969; Battle and Whittington 1969-b). Consequently, the exclusion of any part of the fruit may decrease the effect of these inhibitory substances. To verify this point a control experiment was carried using 'scarified fruit' under similar conditions, but maintaining the ovary cap in the petri-dish in contact with the fruit (Fig.12). No difference between the germination curves for 'scarified fruit' with or without the cap in the petri-dish was obtained.

Figure 11.- Effect of scarification on sugar beet fruits.
Part A. Time course of germination for fruits from Lot 1 in the dark at 25 ° C. In the abscissa percentages of germination \pm standard error in the ordinate time in days.

key: ● Intact fruit
 ▲ Punctured fruit
 ○ Scarified fruit
 ■ Ovary cap replaced

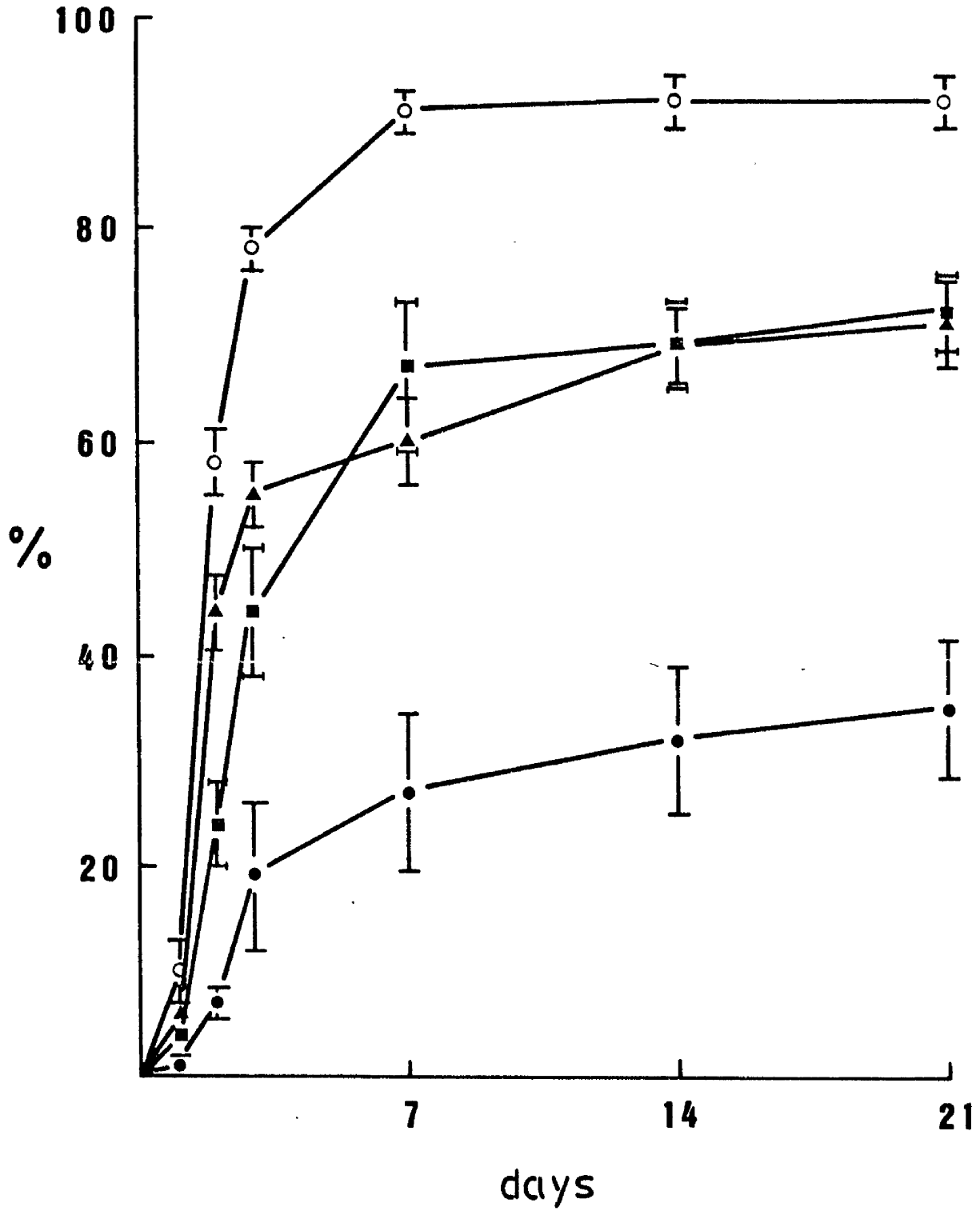
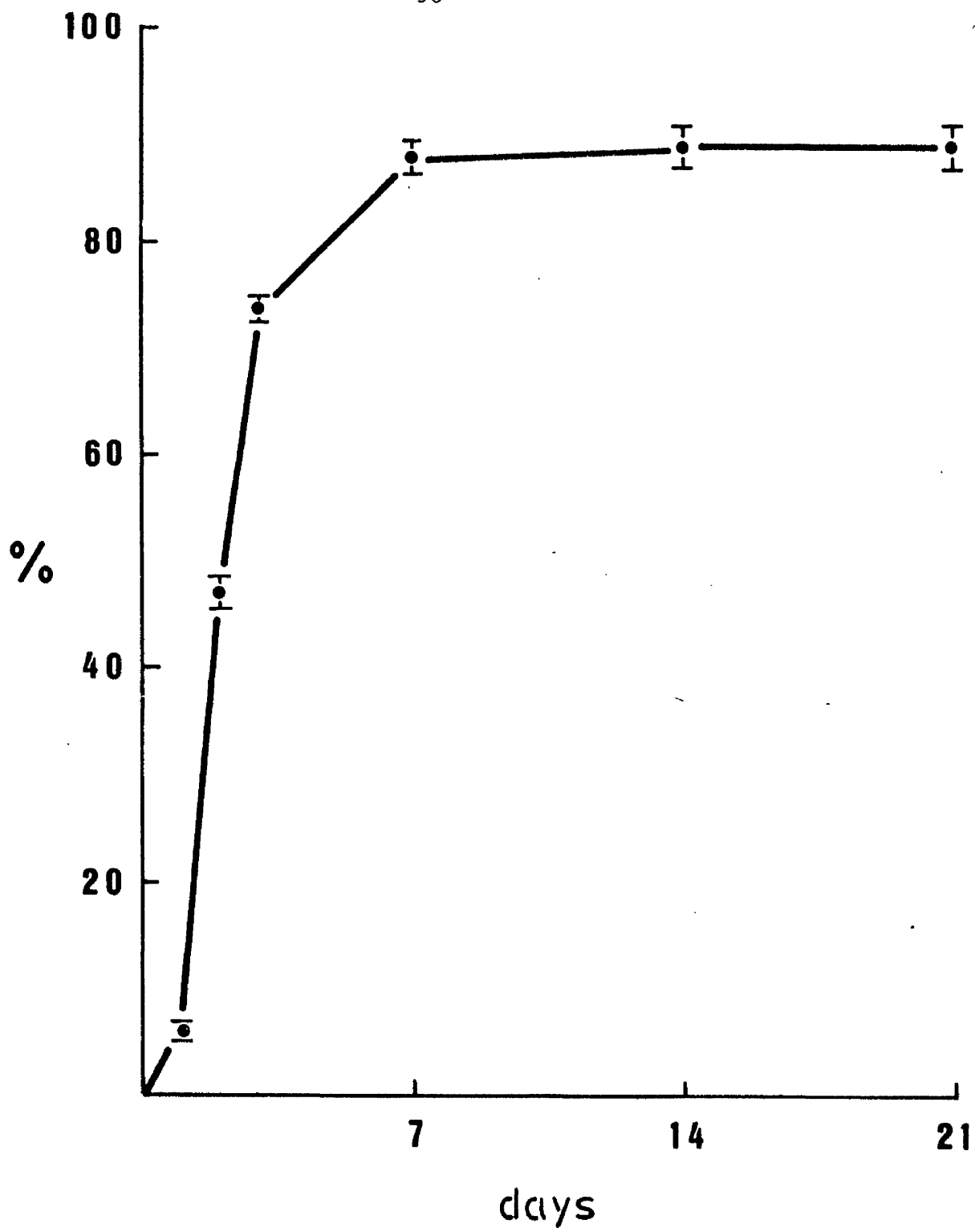


Figure 12.- Effect of scarification on sugar beet fruits.
Part A. Time course of germination of
"scarified fruit + ovary cap" in the dark
at 25 °C. Lot 1. In the abscissa percentages
of germination \pm standard error, in the ordinate
time in days.



Now, the germination percentages of intact fruit in Fig. 11 were inexplicably lower than those obtained for the basic germination behaviour of the same batch of fruits in the dark (Fig. 3). This low germination in Fig. 11 could lead to a false impression of promotion by the scarification treatments, because if in the analysis of the results the values of Fig. 3 are taken into account the enhancement by scarification will not be as drastic as shown in Fig. 11. Nevertheless, despite allowance for a deviation from basic germination behaviour, scarification leads to a pronounced enhancement of germination - 15% for 'ovary cap replaced' and 'punctured fruit' and 35% for 'scarified fruit'.

Part B: Effect of Scarification of Fruits and Seeds from Lot 1

The material used is described in page 35, and it was placed in the dark at 25 °C. An identical group of replicates were subjected to continuous irradiation at 25 °C, because this batch of fruits was seen to be influenced, directly or indirectly, by irradiation.

The results are presented as time coursed of germination in Figs. 13 and 14. Fig. 13 shows the germination of 'intact fruit', 'scarified fruit', 'true seed' and 'scarified seed' in the dark. Intact fruit achieved the plateau of the curve at 7 days, with 56% germination at 21 days. A very different response was obtained for 'scarified fruit', 'true seed', and 'scarified seed', viz: germination was very rapid for the first three days, the plateau of the curve was reached at 7 days, and a high value of full germination of 90 - 96% at 21 days, i.e. almost total germination. All these treatments significantly enhanced germination, not only by raising the full germination by 35 - 40% over that of intact fruit, but also by accelerating the speed of the process. No differences were observed between scarification treatments. 'True seed' and 'scarified seed' show the same germination pattern; moreover, the germination of 'scarified fruit' is similar to excised seed, suggesting that the extent of the scarification in these cases is above the limit required to produce the maximum germination expected.

Figure 13.- Effect of scarification on sugar beet fruits and seeds. Part B. Time course of germination for fruits and seeds from Lot 1 in the dark at 25 ° C. In the abscissa percentages of germination \pm standard error, in the ordinate time in days.

key: ● Intact fruit
 ○ Scarified Fruit
 ▲ True seed
 △ Scarified seed

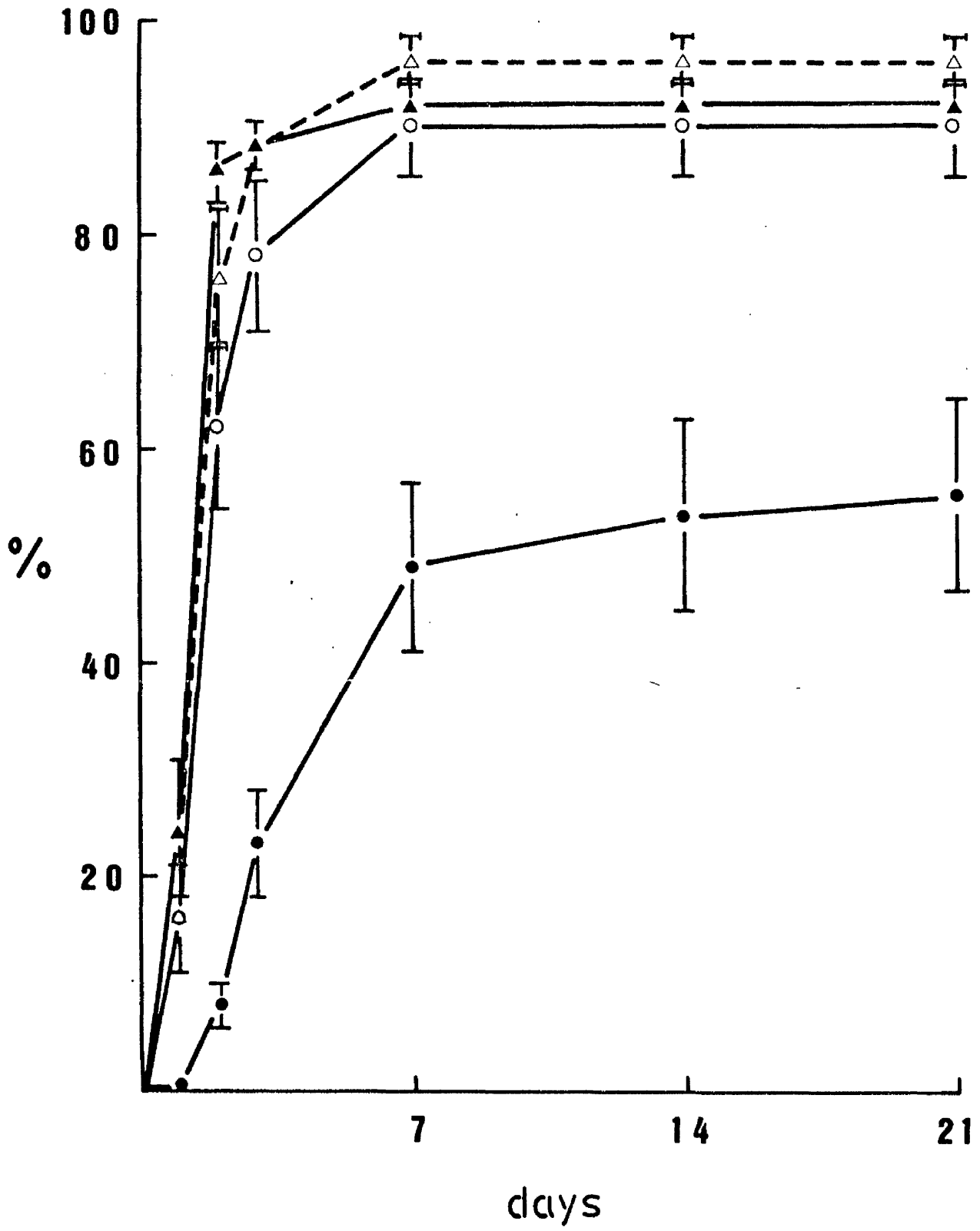


Figure 14.- Effect of scarification on sugar beet fruits and seeds.
Part B. Time course of germination for fruits and seeds from Lot 1 under continuous irradiation at 25 °C. In the abscissa percentages of germination \pm standard error, in the ordinate time in days.

key: ● Intact fruit
 ○ Scarified fruit
 ▲ True seed
 △ Scarified seed

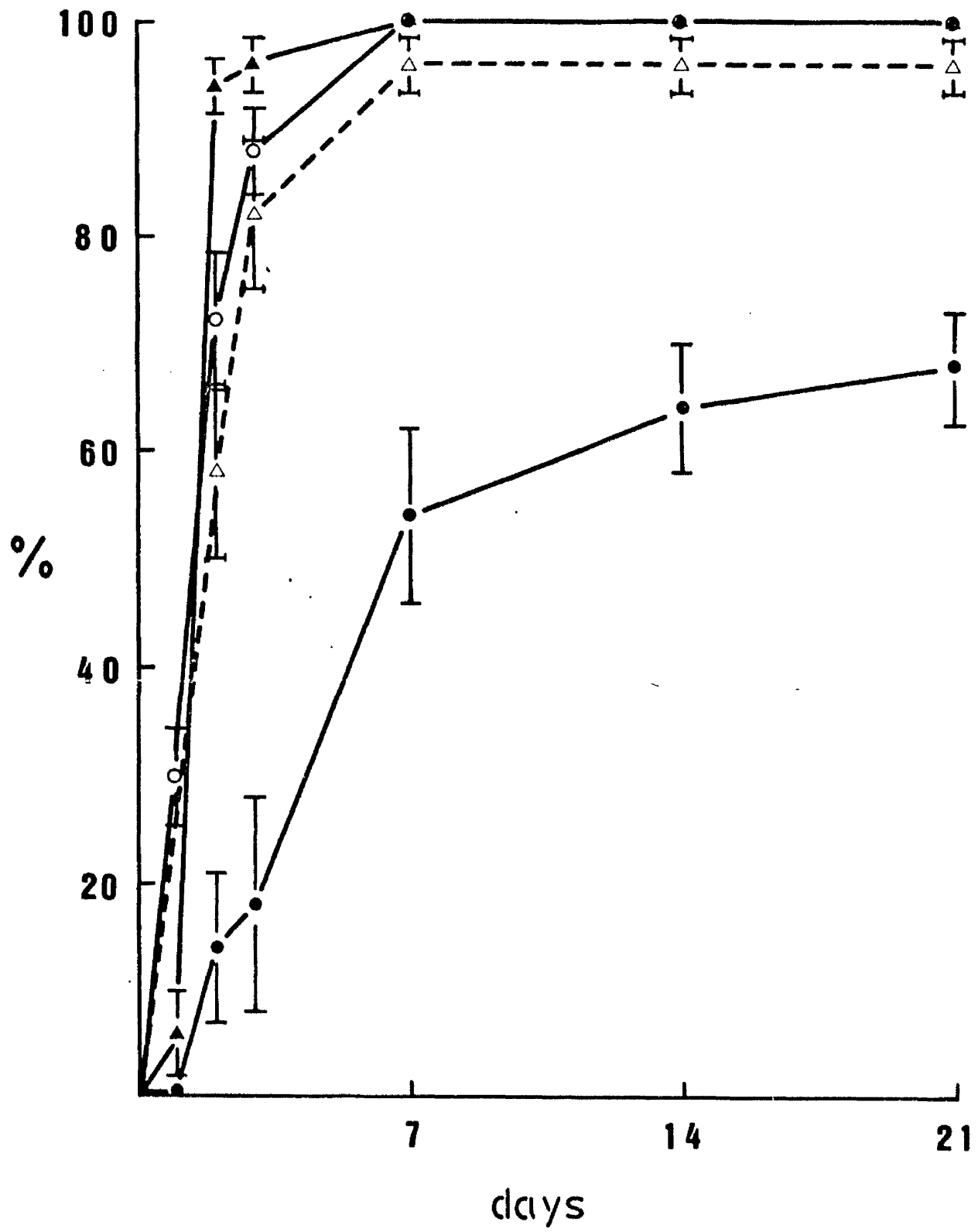


Fig 14 shows the germination of 'intact fruit', scarified fruit', 'true seed' and 'scarified seed' in continuous irradiation. 'Intact fruit' exhibited the germination pattern expected for this batch under these particular experimental conditions, with 68% (full germination) at 21 days. The scarification treatments produced the same germination response; in all cases very rapid germination occurred during the first three days of incubation, the plateau of the curve was reached at 7 days and full germination was 96 - 100%. The germination of irradiated 'intact fruit' was better than in the dark, and this effect has been already discussed in "Basic Germination Behaviour". The germination pattern of 'scarified fruit', 'true seed' and scarified seed' were similar whether or not they are irradiated.

Although the figures of full germination in light were slightly higher than those obtained in the dark (Fig. 13), the Student's t test showed no significant differences at a level of probability higher than 5% between each pair of germination values for 'scarified fruit', 'true seed' and 'scarified seed' at 21 days.

Scarification of the fruit leads to a promotion of germination, which is also affected by irradiation, but any slight enhancement due to irradiation may simple reflect the higher germination of irradiated 'intact fruit'.

Part C: Effect of Scarification on Fruits and Seeds from Lot 2

Only 'intact fruit', 'scarified fruit' and 'true seed' were used for the experiment. The material was placed in the dark at 25 °C and no irradi-

ation control was done in this case. The effect of scarification on this batch of fruits and seeds is shown in Fig. 15, where the values of germination are expressed as a function of incubation time. The germination of intact fruit follows the expected pattern under these experimental conditions, the germination percentages being considerably lower than Lot 1, with a maximum figure of 42% at 28 days. When the fruit was scarified ('scarified fruit') the speed of germination greatly increased at the beginning of the experiment, the curve reaching its plateau at 7 days with full germination of 89% at 28 days. The 'true see' showed the same germination pattern as 'scarified fruit' although full germination was higher, the figure being 97%.

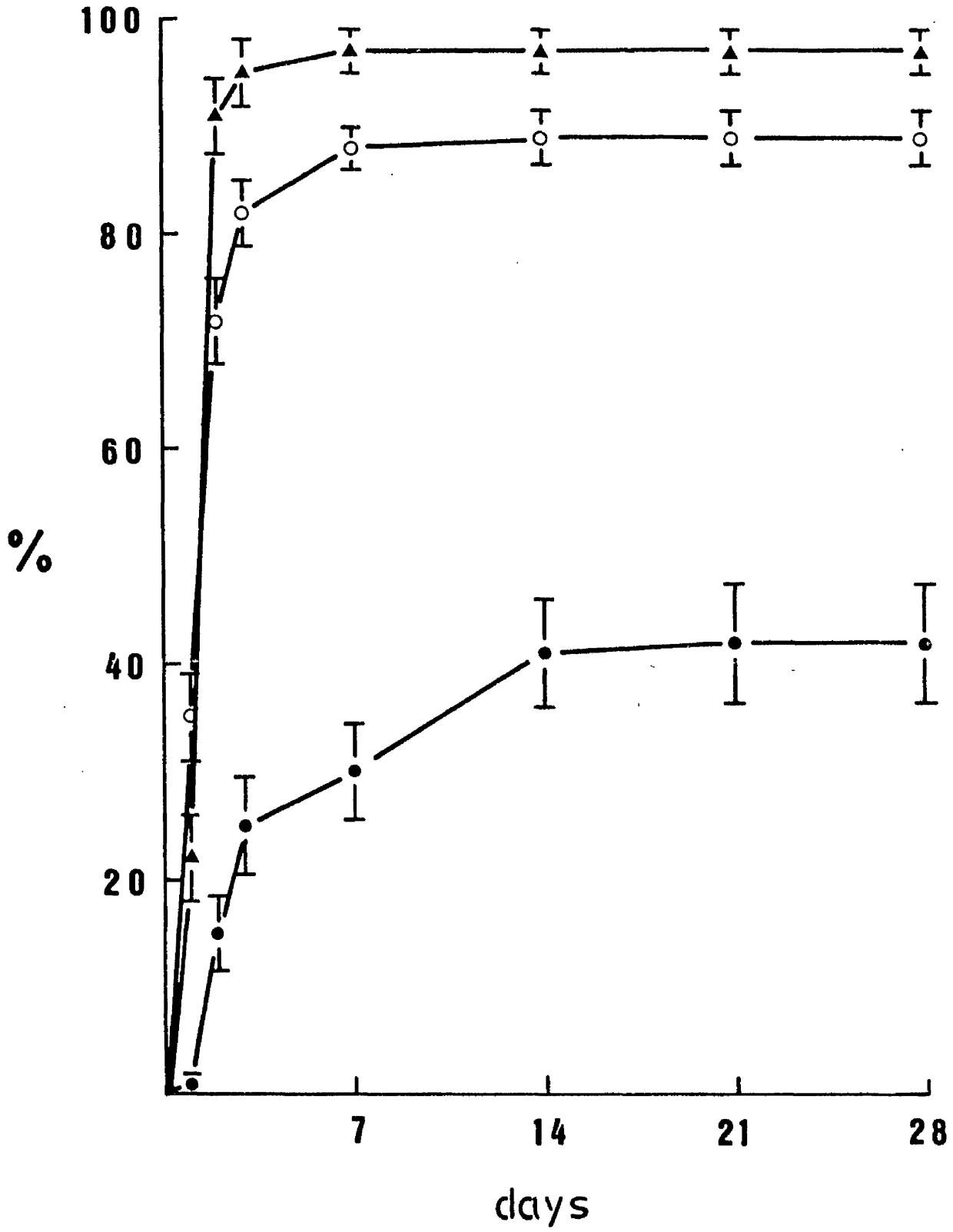
The effect of scarification for Lot 2 is even more noticeable than for Lot 1, not because sacrification produced higher germination percentages, which were within the same range than Lot 1, but because the figures of full germination for this batch of fruits were particularly low.

For all experiments described in Parts A, B and C it was found that sacrification caused a significant and consistent promotion of the germination, regardless of any differences between the batches of fruits and the particular scarification treatments applied to the fruits.

Scarification affected germination only when the fruit was scarified, scarification of the seed itself had no effect on germination. In addition, no major differences were noted between 'scarified fruit' and 'true seed'. These two fact suggest that the fundamental event in promoting germination

Figure 15.- Effect of scarification on sugar beet fruits and seeds from Lot 2 in the dark at 25 °C. Part C.
In the abscissa percentages of germination \pm standard error, in the ordinate time in days.

key: ● Intact fruit
 ○ Scarified fruit
 ▲ True seed



in Beta vulgaris through scarification is the disruption of the fruit material rather than the seed testas, an once the fruit has been scarified the presence of the fruit material does not delay germination provided that scarification is sufficiently extensive.

Attempts to explain the low germination in sugar beet have frequently invoked the presence of inhibitors in the fruit material (Tolman and Stout, 1940; Stout and Tolman, 1941; Duym et al., 1947; Evenari, 1949; De Kock and Hunter, 1950; De Kock et al., 1953; Rehm, 1953; Van Sumere, 1960; Abu-Shakra and Aqil, 1969; Battle and Whittington, 1969-b), and the action of these inhibitors of germination could be suppressed by scarification. Scarification may cause the release or removal of inhibitory substances, which may or may not be gaseous. If the inhibitor is a gas it will escape to the medium; if its is forming a soluble part of the inner tissues of the fruit, it will be leached during soaking. Scarification can also open a physical path to gases, and oxygen especially could inactivate inhibitory substances. Any or both of these two effects may alter the balance of inhibitors inside the fruit; if the extent of scarification is increased the release or inactivation of substances will be greater and consequently higher germination will be obtained, as in Fig. 11.

Scarification could increase oxygen availability to the seed and the promotion of germination will be a result of oxygen on seed metabolism. If the degree of scarification regulates the flow of gases, more extensive scarification will produce better germination, as shown in Fig. 11. In fact,

Heydecker et al. (1971) found that the removal of the ovary cap in sugar beet enhances oxygen uptake and improves germination, counteracting the effect of germination inhibitors present in the cluster. It can be argued that for Lot 2 where the fruits were easily contaminated by microorganisms an increase of oxygen available through scarification only eliminates the competition for oxygen between microorganisms and the seed (Heydecker and Chetram 1971). In this case the promotion will not be caused by oxygen itself, but it will be only the result of eliminating the cause of low germination. Although this explanation cannot be ruled out, it nevertheless does not completely satisfy the experimental results because the promotion was found for Lot 1 where contamination by microorganisms was not an important factor.

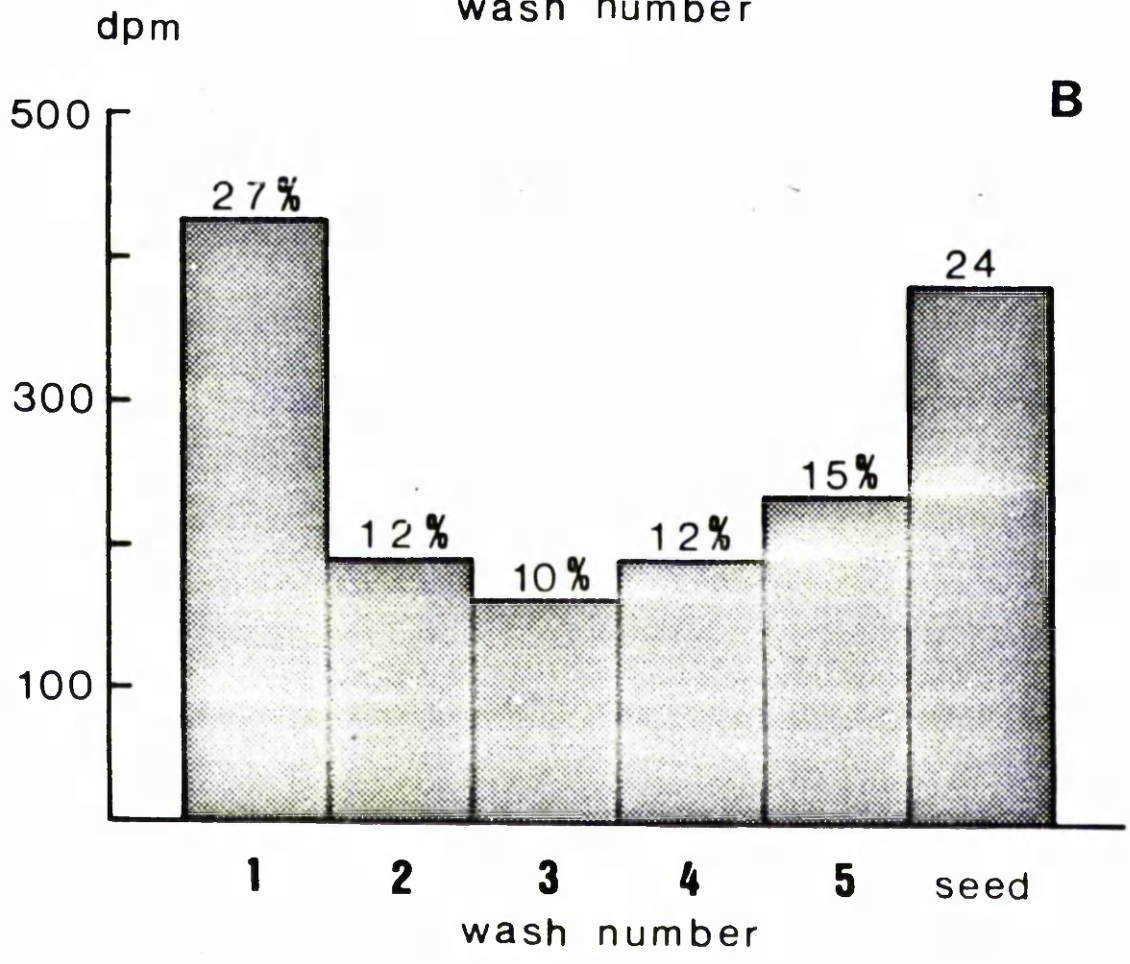
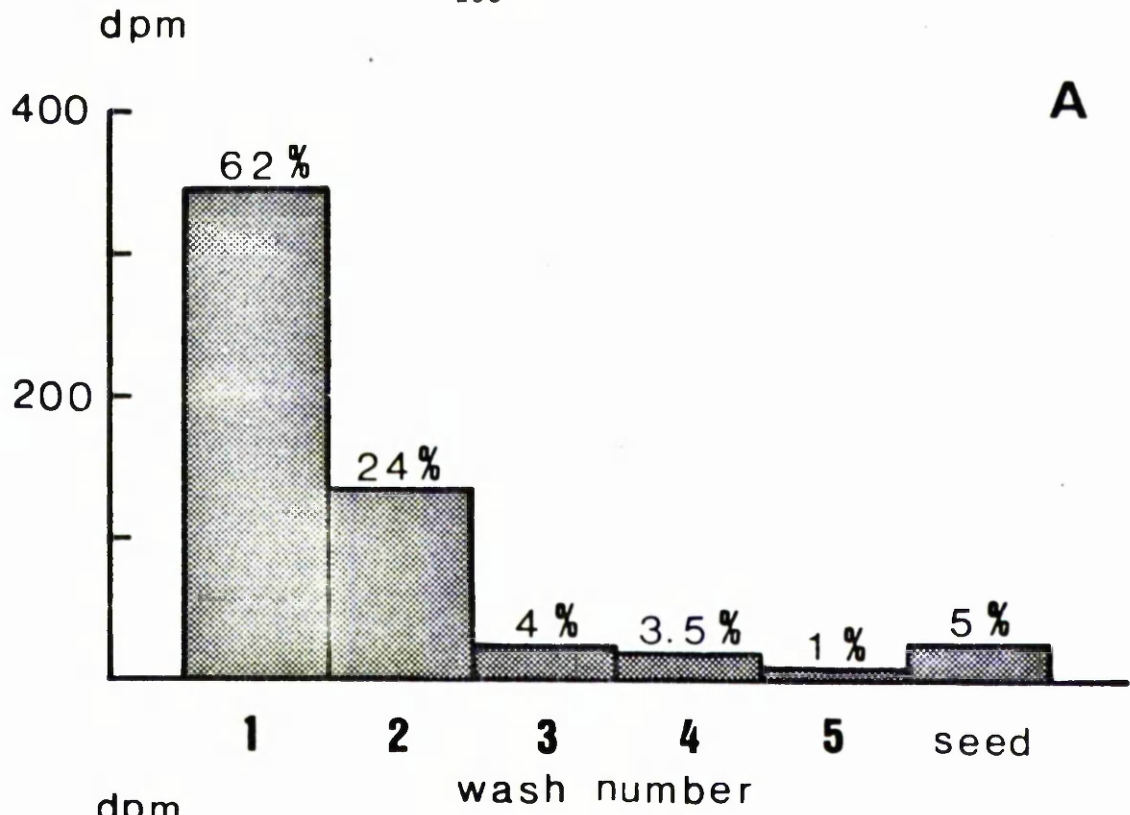
Uptake of Radioactive IAA

The experiments of page 180 showed that IAA at 10^{-5} M had no effect on germination when applied alone or in combination with gibberellic acid and kinetin to the intact fruit. One of the reasons given for this lack of action was that the hormones could not pass through the fruit or seed coat and reach the seed tissues to exert their effects. Other results concerning oxygen, ABA and stains tend to support this hypothesis. In an attempt to gain further knowledge on this aspect of uptake preliminary experiments were done on the uptake and leaching of radioactive IAA by the sugar beet fruit. Intact fruits were placed in a radioactive solution of 10^{-5} M IAA ; the fruits were kept in the dark at 25 °C for 24 hours, a period of time in which imbibition takes place. After this incubation period the intact fruits were washed five times and the true seed excised and extracted in ethanol. Samples of the washings of the fruits and

seeds were taken for the radioactivity assay. The results are shown in Fig. 16. The first five columns represent the amount of radioactivity in dpm found for the washings of the fruits and the sixth column represents the radioactivity detected in the seed. The results are also expressed as percentage of radioactivity, taking as 100% of radioactivity the total amount of count for all columns. In the same Figure, A, corresponds to the results found when the water was removed by drying and B the results obtained when the water was solubilized with alcohol.

Figure 16.- Radioactivity detected in the consecutive washes of the fruit and seed. The figures in each columns represent the percentages of radioactivity for each washing.

- A. Water removed by drying
- B. Water solubilised by alcohol



In A can be seen that after 5 washings very little radioactivity was removed from the fruits. Almost all the radioactivity taken up by the fruit was removed by the washings and 5% of it remained in the seed. If it assumed that the washings removed radiactivity mainly from the fruit coat, the fact that only 5% of it remained in the seed may lend support to the hypothesis that passage of IAA through the fruit coat is restricted.

A different result was found when the water from the washings was solubilized with alcohol (Fig. 16 - B). In this case 24% of radioactivity was now found in the seed. Although this finding could indicate some penetration of IAA through the fruit coat into the seed it could also be attributed to the inefficacy of the washings and subsequent contamination of the seed during dissection. It can be argued that there is no reason to suspect inefficiency of the washings, because 5 washings were enough in case A, but it has to be remembered that the fruit coat may vary in thickness and consequently in some cases it may imbibe more solution. This fact cannot be controlled, fruits of the same size were chosen for the experiment, but there is no convenient way of knowing beforehand the thickness of the fruit coat. Finally, other sources of error intrinsic to the method used might be involved.

In considering the washing profile of radioactivity the results of A would appear to be more reliable and they are more akin to that obtained for lettuce fruits (Mc Wha, 1973). These results would tend to give more support to the hypothesis of uptake imposed by the fruit coat, although it must be emphasized that they are only preliminary experiments.

Effect of Fruit Coat Extracts

Scarification treatments (page 84) showed that the presence of the fruit coat had a significant promotory effect on germination. One possible explanation of the scarification effect is that it results in the remove of inhibitory substances present in the fruit coat. This explanation has been examined in the following experiments.

Part A : Effect of the Intact Fruit Water Extract and the Fruit Coat Extract on Germination of True Seed

It has been reported that the inhibitory materials in aqueous extracts from Be1 vulgaris dispersal units are located in the perianth and pericarp tissue rather than the seed (Battle and Whittington, 1969-b; Abu-Shakra and Aqil, 1969). For this reason two of the media consisted of fruit coat powder and of empty (dissected) fruit coats, which were added to the petri dish in the assumption that if there are inhibitory substances in the fruit material they will be leached into the germination media. The results are presented in Figs. 17 and 18.

Fig. 17 shows the germination of true seed in the dark at 25 °C in the presence of intact fruit water extract, fruit coat powder and empty fruit coat. It can be seen that full germination percentage (90, 94, 88 respectively) of true seed was not significantly different in the three media. The results also show that the media tested had no effect on the germination of the true seed. The same full germination percentage is obtained in the media tested.

If true seed were placed in the same three media but under continuous irradiation (Fig. 18) the same results were obtained. It was again found that the extracts had no effect on germination as compared with distilled water under the same conditions (compare Fig. 18 with Fig. 14).

The results so far obtained indicate that neither the fruit coat nor the presence of the fruit coat per se have any effect on germination of the true seed in darkness or under continuous irradiation. In contrast with these results other authors have found an inhibition of the germination of sugar beet seeds in the presence of water extracts from Beta vulgaris fruits (Tolman and Stout, 1940; Stout and Tolman, 1941; Abu-Shakra and Aqil, 1969; Battle and Whittington, 1969-b, and also in the presence of the whole 'seed ball' (Froeschel, 1940; De Kock et al., 1953). However, it is relevant to point out, that apart from Tolman and Stout (1940) and Stout and Tolman (1941) who worked with the true sugar beet seed, the other authors previously examined the effect of the fruit coat extracts on the germination of the intact fruit of Beta vulgaris or on seeds from different species.

Part B : Effect of the Concentration of the Intact Fruit Water
Extract

Tables 3, 4 and 5 and Fig.19 show the results for intact fruit, scarified fruit and true seed respectively. It can be seen that there was no significant effect of the extract at any concentration on the intact fruit (Table 3, Fig. 19).

Figure 17.- Effect of the fruit coat on sugar beet germination. Part A
Time course of germination for the true seed in three media
in darkness at 25 °C. Both true seeds and germination media
from Lot 1. In the abscissa percentages of germination \pm
standard error, in the ordinate time in days.

Key: ● Intact fruit water extract
 ○ Fruit coat
 ▲ Fruit coat powder

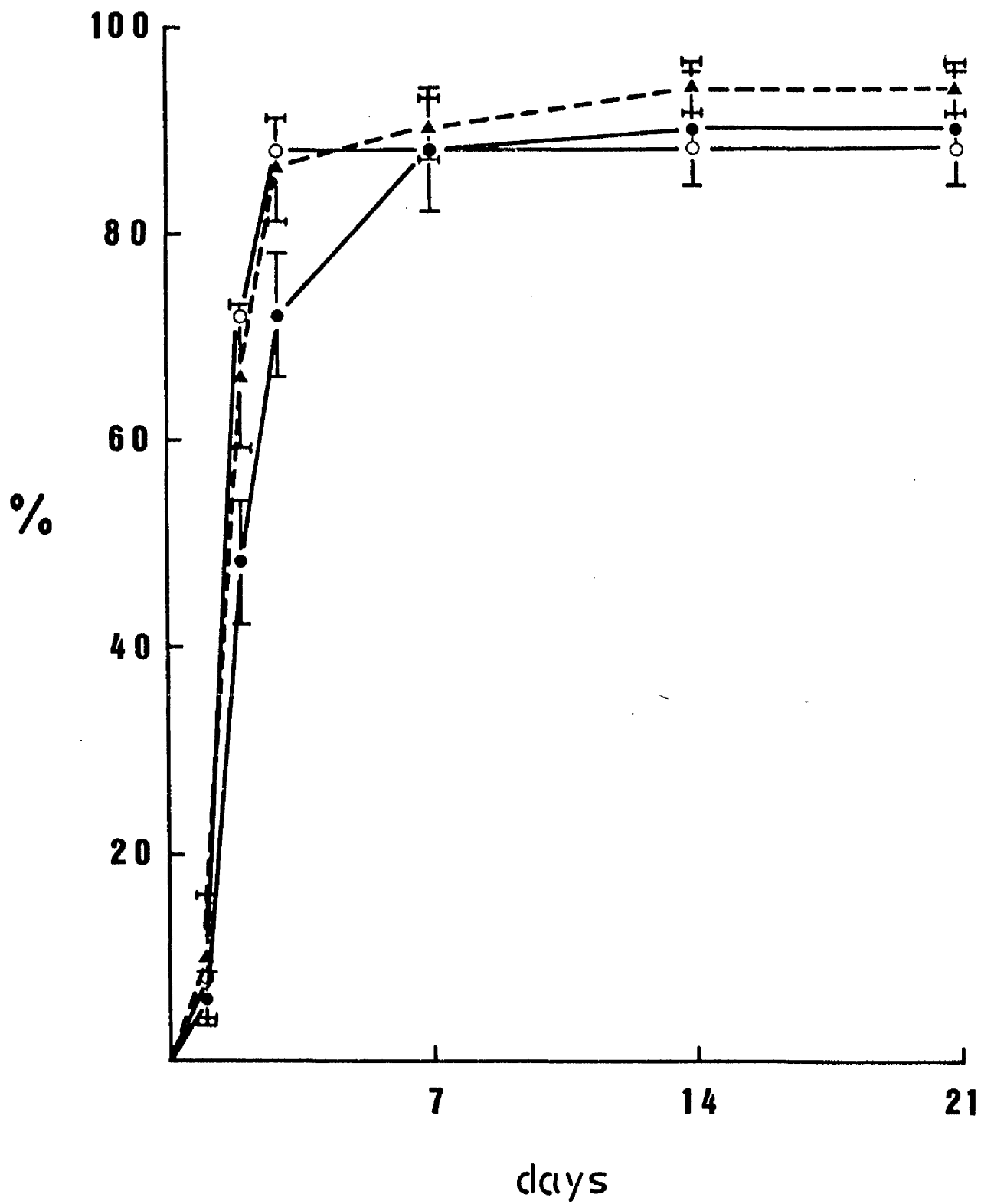
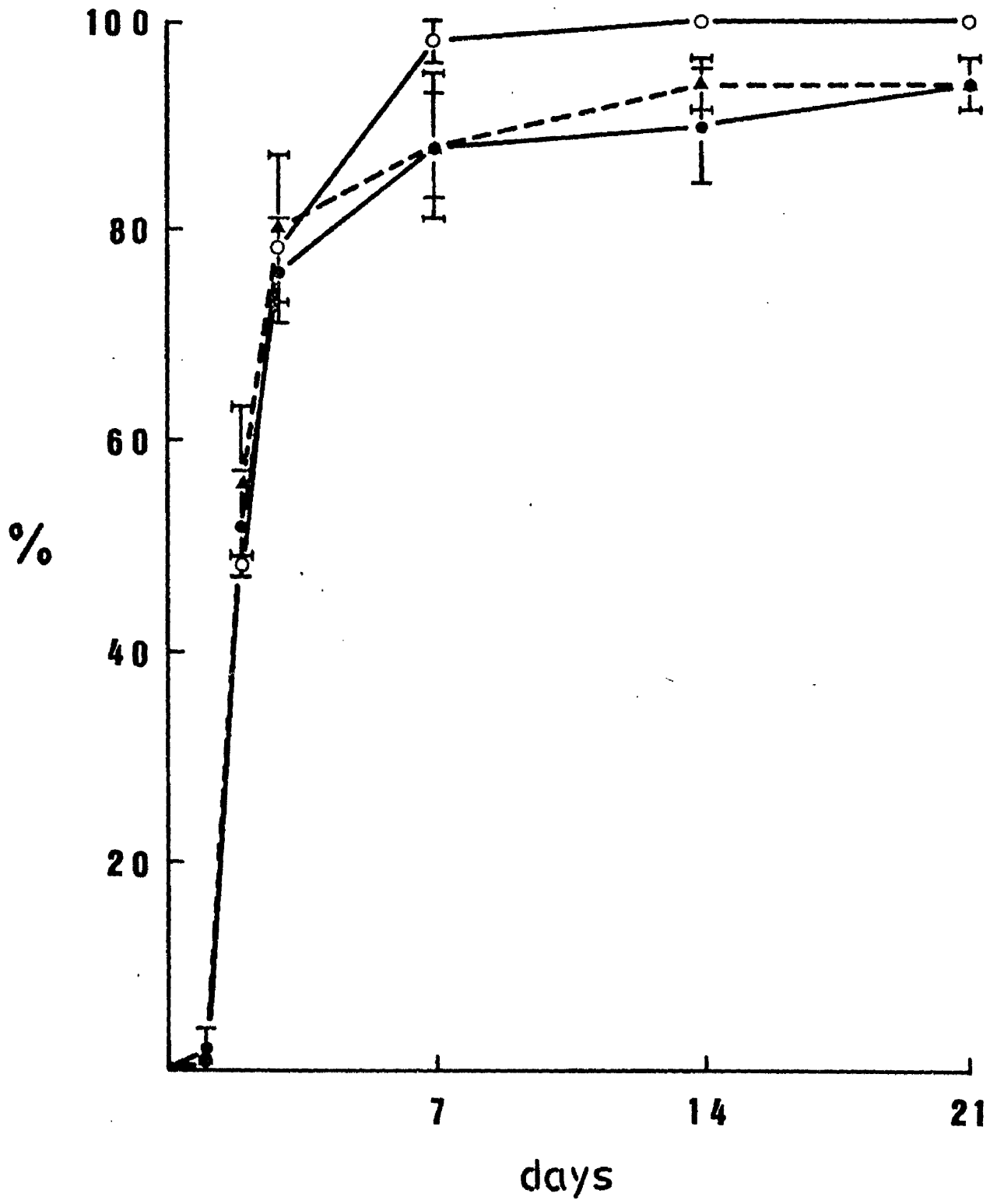


Figure 18.- Effect of the fruit coat on sugar beet germination. Part A.
Time course of germination for true seed in three media under
continuous irradiation at 25 °C. Both true seed and germination
media from Lot 1.
In the abscissa percentage of germination \pm standard error,
in the ordinate time in days.

Key: ● Intact fruit water extract
 ○ Fruit coat
 ▲ Fruit coat powder



It is interesting to note that no inhibition was observed for intact fruit even at the extract concentration of 200%.

Abu-Shakra and Aqil (1969) worked with an aqueous extract equivalent to the extract concentration of 100% utilized in this study. These investigators found that their extract depressed the germination percentage of not only sugar beet but also of different crop seeds such as barley, alfalfa, sunflower and others. Tolman and Stout (1940) demonstrated that the toxic action of seed-ball extracts of sugar beet was not confined to the seed removed from the pericarp, but was also evident when intact fruit seed-balls were used. A possible explanation to the results presented here is that the fruit material acts as an impediment to inhibitors and does not allow them to reach the embryo.

TIME (DAYS)	CONCENTRATION OF THE WATER EXTRACT					
	200%		100%		75%	
	% G	+ s.e	% G	+ s.e	% G	+ s.e
1	0.0	-	0.0	-	0.0	-
2	12.0	1.3	16.0	4.9	7.0	2.6
3	16.0	2.2	28.0	6.9	16.0	3.7
7	22.0	3.6	33.0	7.0	31.0	4.6
14	27.0	3.0	38.0	7.8	42.0	4.6
21	34.0	3.4	39.0	8.2	46.0	3.0
28	38.0	4.1	39.0	8.2	48.0	2.9

TIME (DAYS)	CONCENTRATION OF THE WATER EXTRACT					
	50%		25%		WATER CONTROL	
	% G	+ s.e	% G	+ s.e.	% G	+ s.e
1	1.0	1.0	1.0	1.0	1.0	1.0
2	13.0	3.6	25.0	4.3	15.0	3.4
3	19.0	3.8	31.0	4.8	25.0	4.5
7	28.0	5.3	39.0	4.8	30.0	4.5
14	33.0	4.9	46.0	4.9	41.0	5.2
21	36.0	4.5	46.0	4.9	42.0	5.5
28	37.0	4.7	47.0	4.7	42.0	5.5

Table 3.- Effect of water extract on sugar beet germination. Part B. Percentages of germination of intact fruit placed to germinate in different concentrations of the water extract. Darkness at 25 °C. Extracts and fruits from Lot 2.

TIME (DAYS)	CONCENTRATION OF THE WATER EXTRACT					
	200%		100%		75%	
	% G	+ s.e	% G	+ s.e.	% G	+ s.e
1	5.0	2.2	14.0	3.0	19.0	2.8
2	28.0	5.0	50.0	6.3	51.0	5.5
3	33.0	5.6	62.0	6.8	61.0	5.8
7	42.0	5.7	74.0	5.2	69.0	4.8
14	52.0	4.4	77.0	4.5	71.0	4.3
21	52.0	4.4	77.0	4.5	71.0	4.3
28	52.0	4.4	77.0	4.5	72.0	4.4

TIME (DAYS)	CONCENTRATION OF THE WATER EXTRACT					
	50%		25%		WATER CONTROL	
	% G	+ s.e	% G	+ s.e	% G	+ s.e
1	4.0	2.2	6.0	2.2	15.0	2.7
2	41.0	3.8	41.0	3.8	54.0	5.8
3	51.0	4.6	48.0	4.1	62.0	6.1
7	70.0	4.7	67.0	3.3	74.0	4.9
14	71.0	4.3	70.0	3.3	77.0	3.9
21	71.0	4.3	70.0	3.3	77.0	3.9
28	71.0	4.3	70.0	3.3	77.0	3.9

Table 4.- Effect of water extract on sugar beet germination. Part B. Percentages of germination of scarified fruit placed to germinate in different concentrations of the water extract. Darkness at 25 °C. Extracts and fruits from Lot 2.

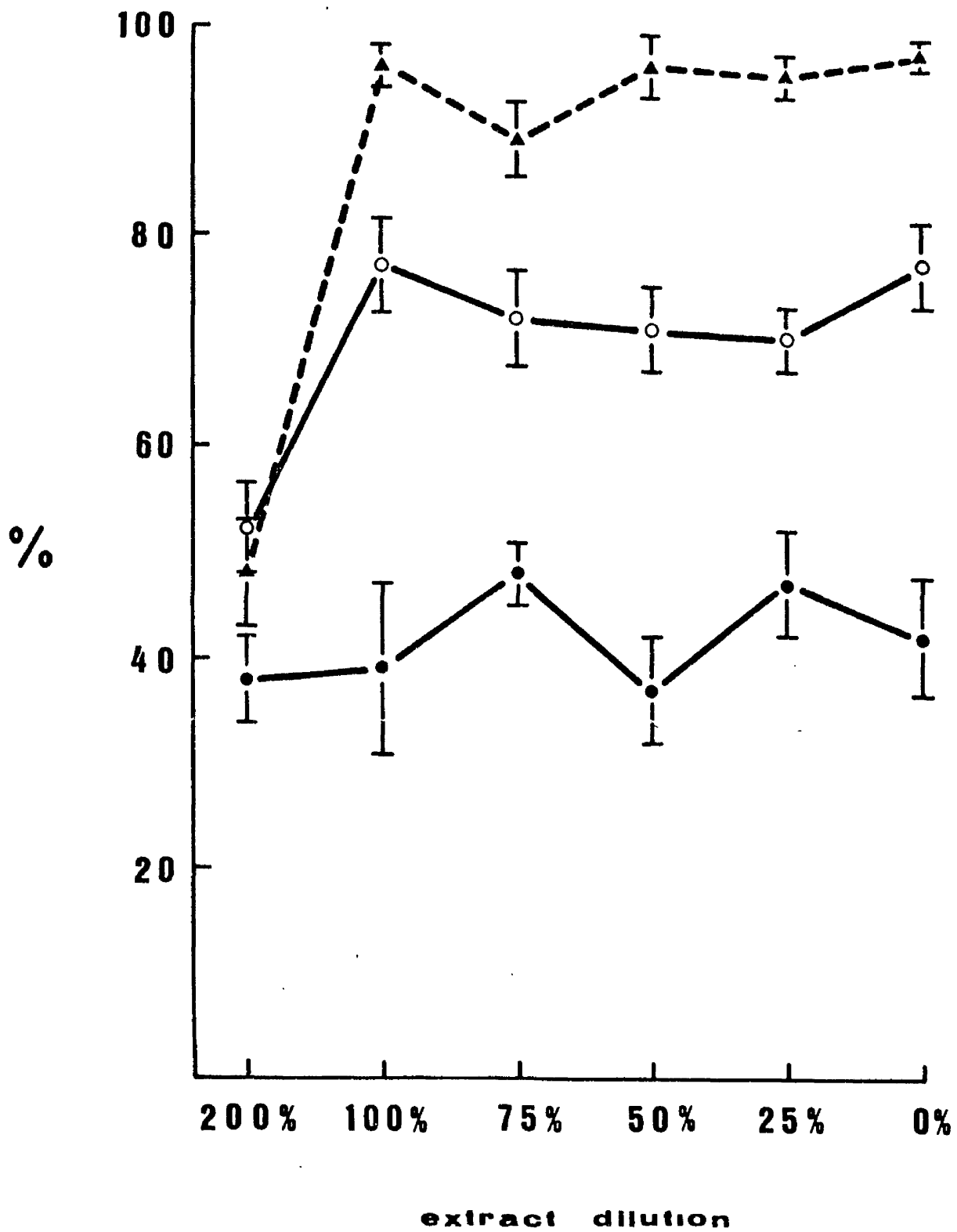
TIME (DAYS)	CONCENTRATION OF THE WATER EXTRACT					
	200%		100%		75%	
	% G	<u>±</u> s.e	% G	<u>±</u> s.e.	% G	<u>±</u> s.e
1	3.0	2.1	8.0	2.5	3.0	2.1
2	33.0	5.7	66.0	5.2	60.0	5.9
3	44.0	4.5	92.0	4.2	79.0	4.0
7	48.0	5.1	96.0	2.2	89.0	3.5
14	48.0	5.1	96.0	2.2	89.0	3.5
21	48.0	5.1	96.0	2.2	89.0	3.5
28	48.0	5.1	96.0	2.2	89.0	3.5

TIME (DAYS)	CONCENTRATION OF THE WATER EXTRACT					
	50%		25%		WATER CONTROL	
	% G	<u>±</u> s.e	% G	<u>±</u> s.e	% G	<u>±</u> s.e
1	4.0	2.2	5.0	1.6	15.0	2.7
2	75.0	3.7	81.0	3.5	82.0	3.8
3	90.0	3.3	89.0	2.3	95.0	2.7
7	96.0	3.0	94.0	2.6	97.0	1.5
14	96.0	3.0	95.0	2.2	97.0	1.5
21	96.0	3.0	95.0	2.2	97.0	1.5
28	96.0	3.0	95.0	2.2	97.0	1.5

Table 5.- Effect of water extract on sugar beet germination. Part B. Percentages of germination of true seed placed to germinate in different concentrations of the water extract. Darkness at 25 °C. Extracts and seeds from Lot 2.

Figure 19.- Effect of the water extract on sugar beet germination. Part B. Percentages of germination for 28 days for intact fruit, scarified fruit and true seed as a function of the concentration of the extract. Darkness at 25 °C. Extracts, fruits and seeds from Lot 2. In the abscissa percentages of germination \pm standard error, in the ordinate the extract dilution.

Key: ● Intact fruit
 ○ Scarified Fruit
 ▲ True seed



The extract concentrations between 100% and 25% had no significant effect on the germination of scarified fruit and true seed as compared to the water control (Tables 4, 5; Fig. 19). The higher extract concentration however, markedly depressed germination, the 200% concentration caused a reduction of germination of 25% for scarified fruit and of 49% for true seed, compared to the water control. This depression of germination could be due to the presence of a level of inhibitory substances high enough to inhibit germination. On dilution of the extract, however, the concentrations of inhibitors decreases below the necessary level to inhibit germination.

Another explanation is that this extract provides an osmotic pressure high enough to impose a restriction to germination. Duym et al., (1947) claimed that the osmotic effect is more important in delaying sugar beet germination than the effect of inhibitory substances. On the other hand, Stout and Tolman (1941) found that naked beet seeds (true seeds) are tolerant to fairly high osmotic pressure. De Kock et al., (1953) also affirmed that the inhibition of germination on sugar beet cannot be explained only on the basis of osmotic pressure and is due rather to inhibitory substances in the water extracts.

Part C : Effect of the Sterilization of the Fruit Coat Water

Extract

The results for intact fruit, scarified fruit and true seed in sterile and non-sterile extracts, are presented in Figures 20, 21 and 22 respectively.

Fig. 20 indicates that for intact fruit the same germination is obtained in the 'sterile extract' and in the 'non-sterile extract', with a figure of full germination of 57% for 'sterile extract' and of 50% for 'non-sterile extract'. Also, the extract itself did not significantly affect germination as can be seen by comparing the two curves of Fig. 20 with the curve of 'basic germination behaviour' of Fig. 5.

Fig. 21 shows the germination of scarified fruit in the 'sterile extract' and 'non-sterile extract'. In this case there was a difference in germination depending on the extract used as the germination medium. When the extract had been sterilized the germination curve was practically the same as obtained with distilled water (compare curve a of Fig. 21 with the curve for scarified fruit of Fig. 15). But when the extract had not been sterilized there was a noticeable effect on germination. The shape and trend of 'non-sterile extract' curve is the same as 'sterile extract' curve, but the percentage of germination for each point of 'non-sterile extract' curve was consistently lower than those for 'sterile extract' curve. At 28 days the 'sterile extract' resulted in 84% germination, and the 'non-sterile extract' in 64% germination; these data being highly statistically significant.

Figure 20.- Effect of fruit coat powder extract on sugar beet germination. Part C. Time course of germination of intact fruit in two substrata in darkness at 25 °C. Intact fruits and crude water extracts from Lot 2. In the abscissa percentages of germination \pm standard error, in the ordinate time in days.

Key: —●— Sterile extract
---○--- Non-sterile extract

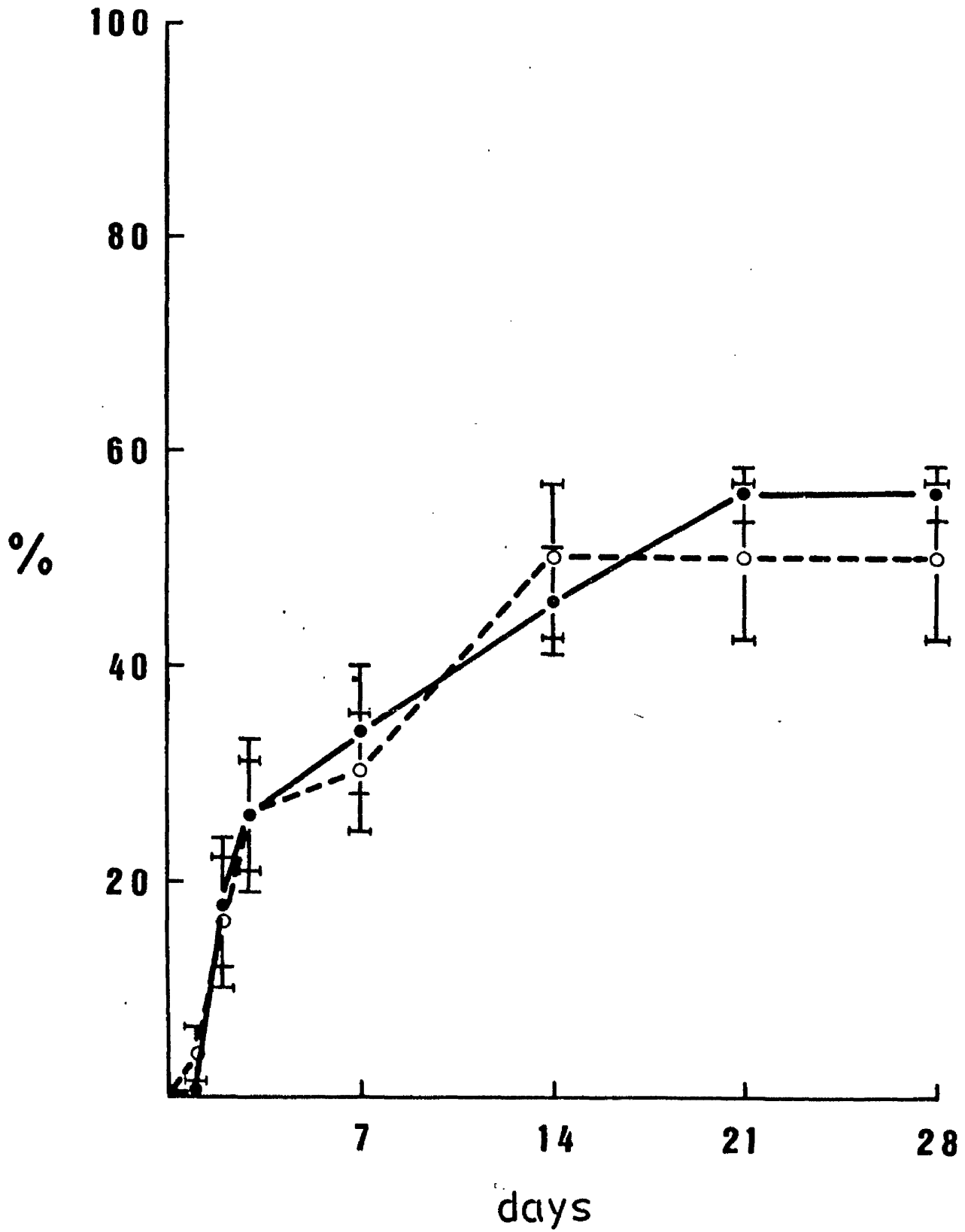


Figure 21.-

Effect of water extract on sugar beet germination.
Part C. Time course of germination of scarified
fruit in two substrates in darkness at 25 °C.
Scarified fruits and crude water extracts from Lot 2.
In the abscissa percentages of germination \pm
standard error, in the ordinate time in days.

Key: —●— Sterile extract
----○---- Non-sterile extract

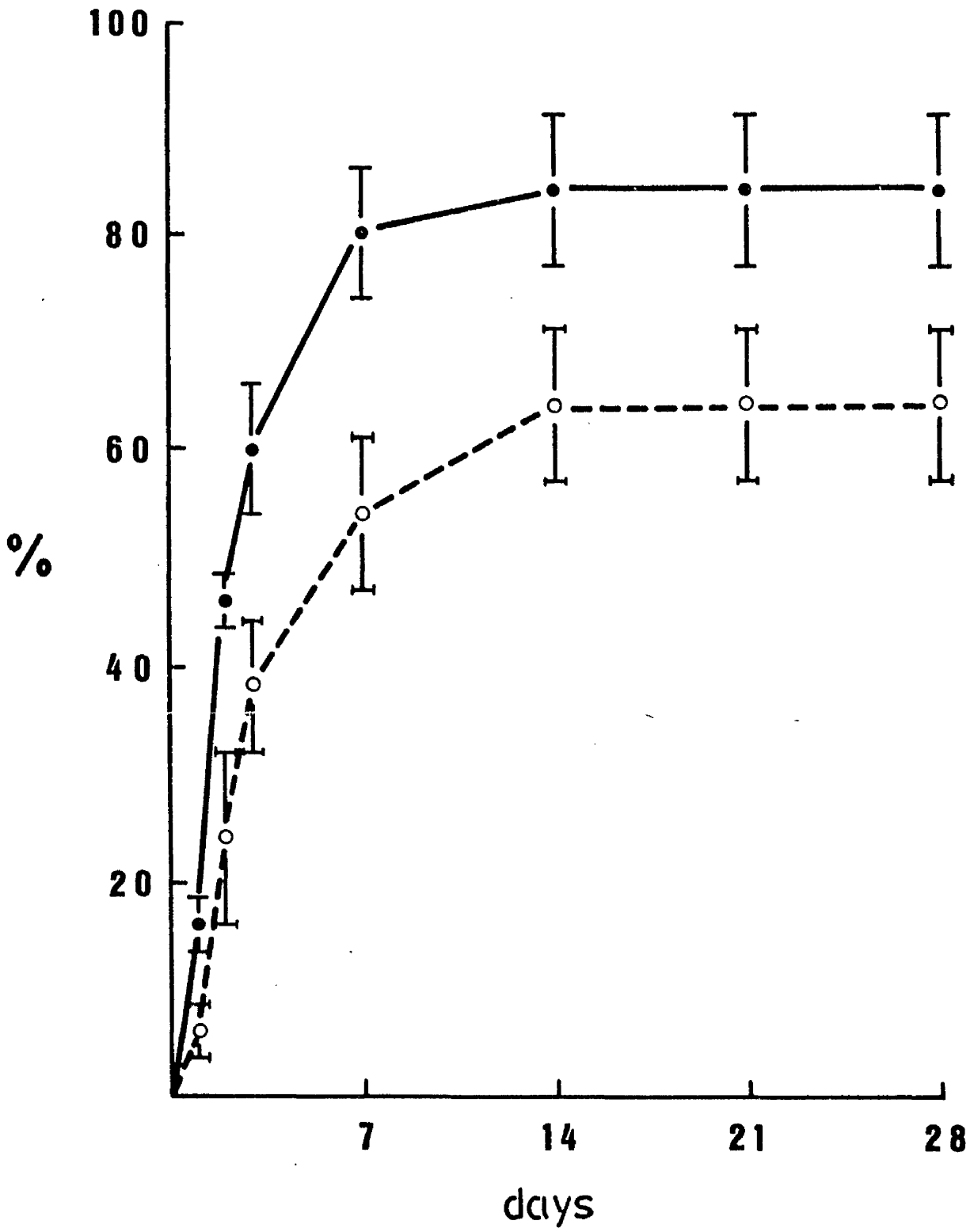
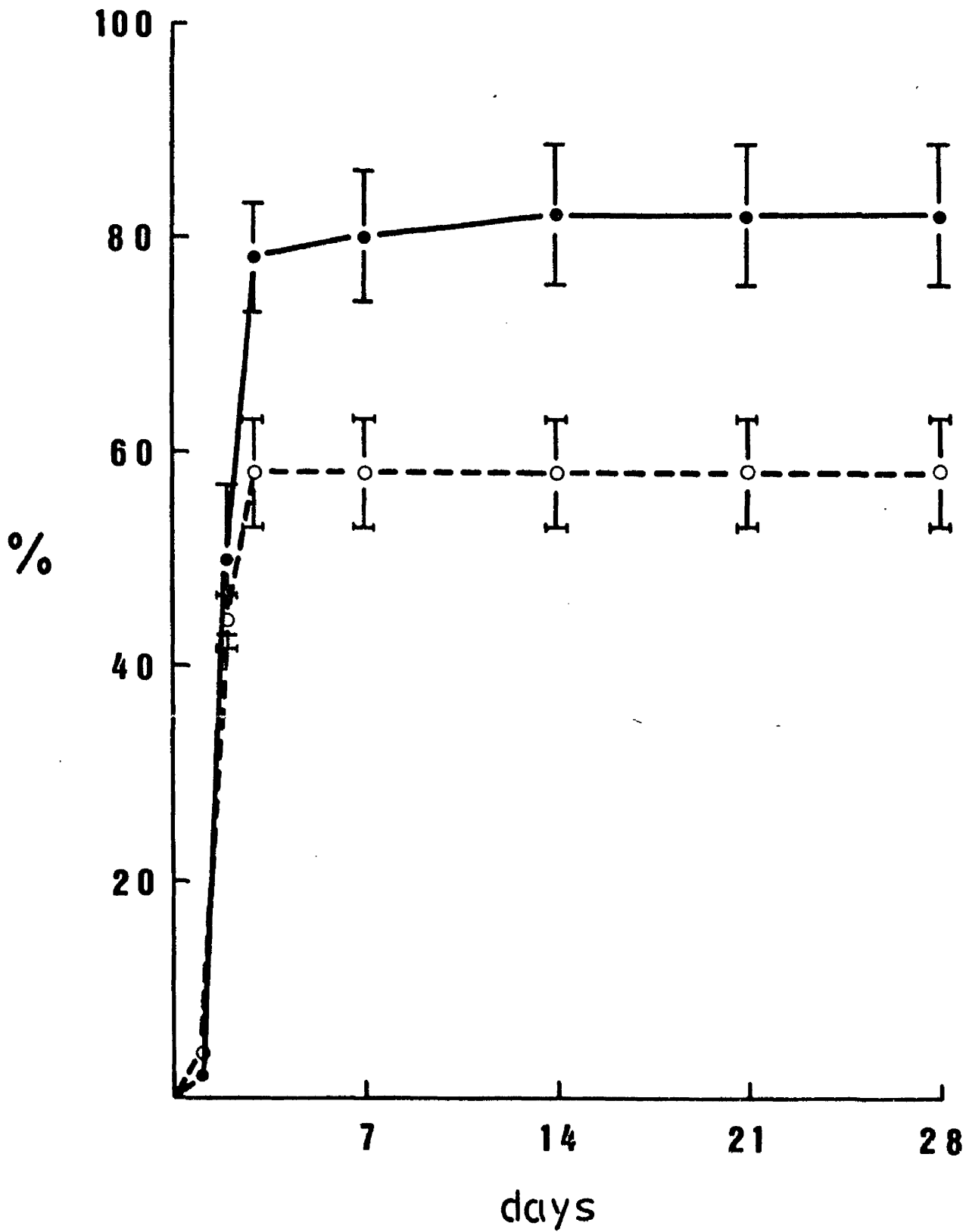


Figure 22.-

Effect of water extract on sugar beet germination.
Part C. Time course of germination of true seed in
two substrates darkness at 25 °C. True seed and crude
water extract from Lot 2. In the abscissa percentages
of germination \pm standard error, in the ordinate time
in days.

Key: —●— Sterile extract

---○--- Non-Sterile extract



Similar results are obtained with true seed (Fig. 22). When the extract was sterilized the germination is similar to that in distilled water (see curve of true seed of Fig. 15). Germination of true seed, however, was depressed by the 'non-sterile extract', and at 28 days there was 82% germination for the 'sterile extract' and only 58% for the 'non-sterile extract' which gives a statistically significant difference of 24%.

It should be noted that in both Figs. 21 and 22 the 'non-sterile extract' produces a curve shifted to lower values in relation to the curve of 'sterile extract'. This is of some importance because it suggests a real inhibition and not merely a delay in germination.

The inhibitory effect of the 'non-sterile extract', must be attributed to the microorganisms in the extract. The mode of action of the microorganisms in lowering the germination, however, cannot be deduced from these experiments. One possibility is that the microorganisms attack the seed and injure the embryo, so that the seeds lose viability. Alternatively there could be a competition between bacteria and seeds for oxygen, as has been claimed by Heydecker and Chetram (1971) and Heydecker et al. (1971). Rehms (1953) suggested that the growth of bacteria on germinating seeds and their metabolism, may be responsible for many irregularities observed in germination tests.

The results of these experiments enlighten some aspects concerning the effect of the fruit material on sugar beet germination. Whether the fruit material itself or water extract of this material was used, no inhibition of germination of true seed was found (Part A). If the extract, however, is used at higher concentration (Part B) or the microbial factor is not excluded from the extract (Part C) an inhibition of germination can be demonstrated, but only in the case of scarified fruit or true seed. An inhibition of germination of intact fruit was not observed in any experiment. This suggests that the fruit wall protects the seed or embryo against the inhibitory effect of the extract, whether the cause of the inhibitory action is inhibitory substances or microbial factors.

Effect of Oxygen and a Respiratory Inhibitor

In several species, especially where the seed embryo is enclosed within covering structures (testa, pericarp, etc.) it has been suggested that the inability to germinate is due to a resistance to the diffusion of oxygen through the covering structures. Among such seeds are Xanthium (Schull, 1911, 1914; Thornton, 1935; Wareing and Foda, 1957) Avena fatua (Atwood, 1914; Johnson, 1935; Hay, 1962; Hart and Berrie, 1966) Sinapsis arvensis (Bibbey, 1948; Edwards, 1968) and Pyrus (Come, 1967; Visser, 1954). Furthermore, an increase in oxygen tensions improves the germination of dormant wild oats (Black, 1959), Betula (Black and Wareing, 1959), wheat (Harrington, 1923) and rice (Roberts, 1962).

The seed-covering structures of many other species have been suggested to function as rate-limiting layers. The rupture or removal of the covering layers enhances germination (Durham and Wellington, 1961; Villiers and Wareing, 1965). However, in other seeds such as Acer pseudoplatanus (Webb and Wareing, 1972) the dormancy is not exclusively due to and oxygen impermeability of the covering structures. Nevertheless, these structures do play an important role in the dormancy mechanism.

The dispersal units of Beta vulgaris possess a thick and hard fruit wall enclosing the seed, and this structure could function by limiting oxygen supply to the embryo. To investigate the role of gas exchange on sugar beet germination, experiments involving the use of oxygen, hydrogen peroxide and potassium cyanide were performed.

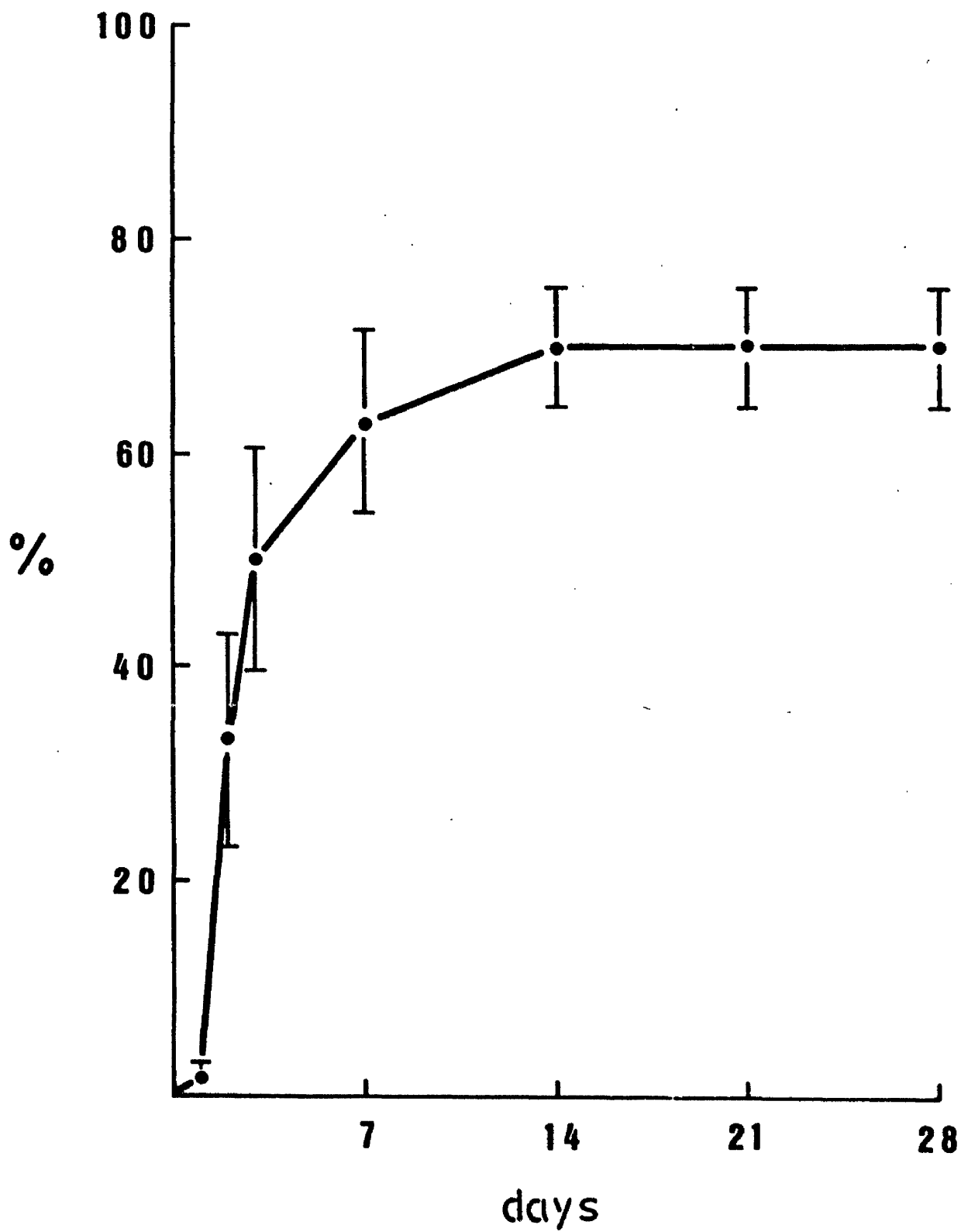
Effect of Oxygen

1. The percentage of seed germination in 100% of O₂ is shown in Fig. 23.

There was little germination during the first 24 hours, but this increased rapidly in the next 72 hours, at which time germination reached a value of 50%. From this point the rate of germination was slower, reaching a plateau of 70% (full germination) at 14 days. After 14 days in 100% oxygen, the plateau of the germination curve was reached, while in air this took 21 days and the plateau value was only 39% (Fig. 5). This enhancement of germination, however, in 100% oxygen was not as effective as scarification of the fruit. Thus comparing Figs. 15 and 23 it can be seen that for Fig. 15

Figure 23.-

Time course of germination for intact fruit in 100% oxygen. Darkness, 25 °C. Lot 2. In the abscissa percentages of germination \pm standard error, in the ordinate time in days.



the values of germination are higher and the plateau of germination is reached earlier for both scarified fruit and true seed.

The experiment of Fig. 23 was repeated under conditions of continuous irradiation. As can be seen from Fig. 24 light had little effect on germination. The curve obtained is similar to that in Fig. 23. The slightly lower value of full germination obtained is not significant.

2. The results are shown in Fig. 25. The germination curve obtained for 100% oxygen is similar to that of Fig. 23, while fruits germination in nitrogen or under vacuum showed a marked reduction in their germination. Fruits germinating in nitrogen exhibited a reduction of 31% in relation to fruits in air (see Fig. 5), and 63% in relation to fruits in 100% oxygen. Similar results have been reported for other seeds, such as Acer pseudo-platanus (Webb and Wareing, 1972).

3. The results are presented in Tables 6, 7 and 8 for intact fruit, scarified fruit and true seed, respectively, and in Fig. 26. This Figure shows the percentage of germination for 28 days of incubation for intact fruit, scarified fruit and true seed as a function of oxygen percentages.

Figure 24.- Time course of germination for intact fruit in 100% oxygen. Continuous irradiation, 25 °C. Lot 2. In the abscissa percentages of germination \pm standard error, in the ordinate time in days.

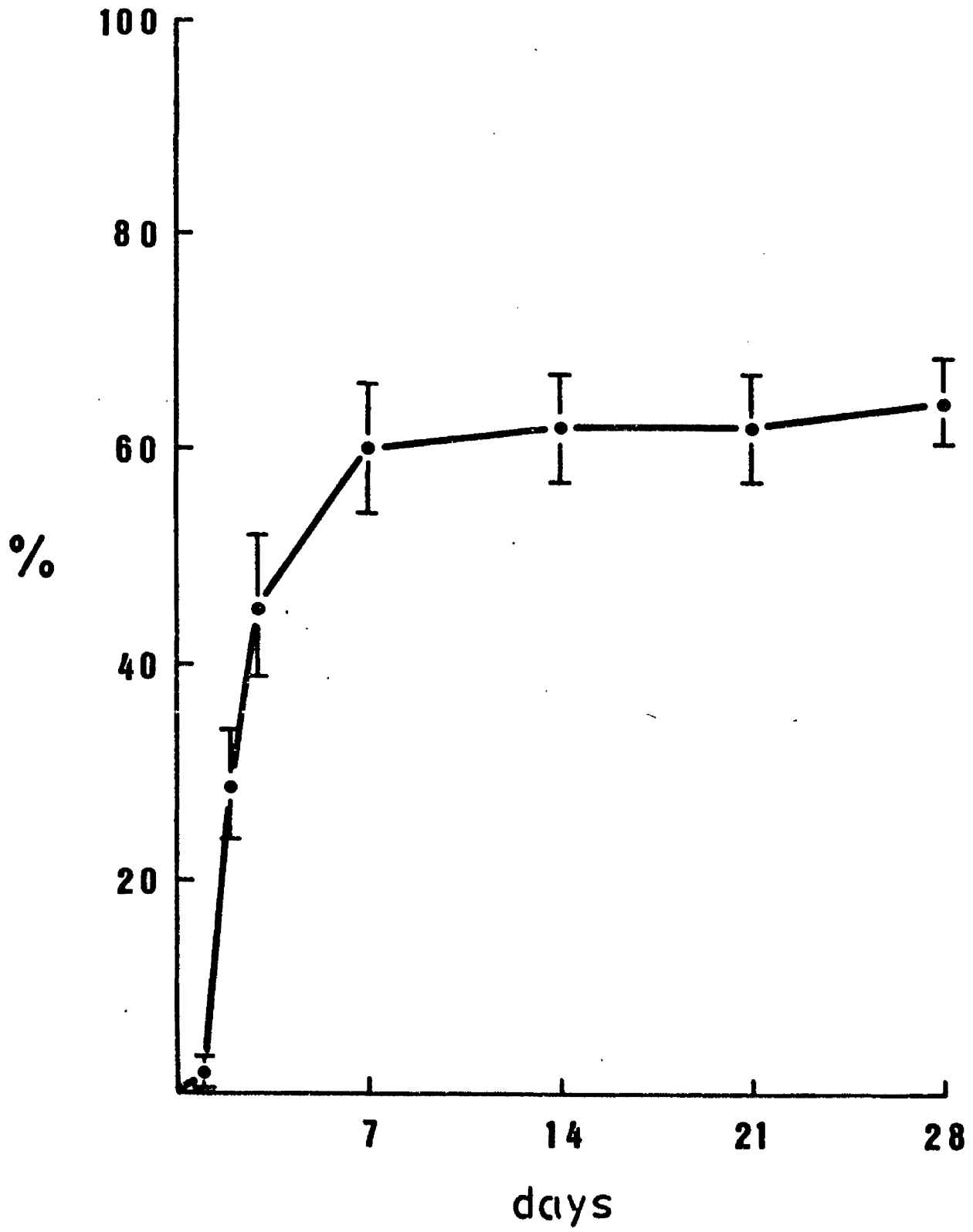
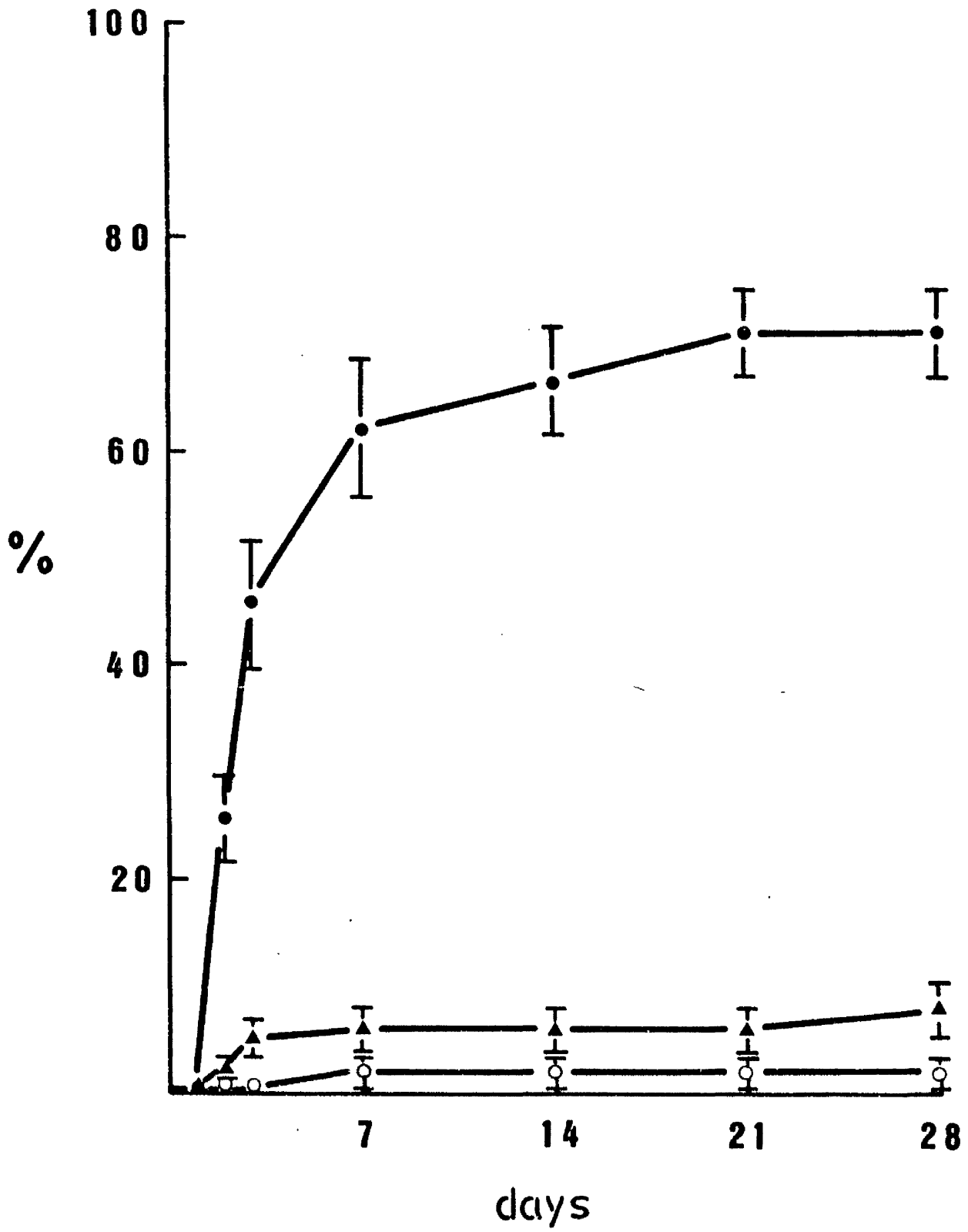


Figure 25.- Time course of germination for intact fruit in 100% oxygen, 100% nitrogen and vacuum. Darkness, 25 °C. Lot 2. In the abscissa percentages of germination \pm standard error, in the ordinate time in days.

Key: ● 100% O₂
 ▲ 100% N₂
 ○ Vacuum



TIME (DAYS)	PERCENTAGES OF OXYGEN					
	100% (0% N ₂)		50% O ₂ (50% N ₂)		20% O ₂ (80%N ₂)	
	% G	+ s.e.	% G	+ s.e.	% G	+ s.e.
1	0.0	-	0.0	-	2.2	1.5
2	25.5	4.4	13.3	2.8	3.3	1.6
3	45.5	5.8	26.2	4.2	10.0	2.8
7	62.2	6.4	50.0	7.5	17.7	3.2
14	66.6	5.2	52.5	7.5	23.3	3.3
21	71.1	4.2	53.7	7.5	24.4	2.9
28	71.1	4.2	53.7	7.5	24.4	2.9

TIME (DAYS)	PERCENTAGES OF OXYGEN					
	10 % O ₂ (90% N ₂)		5% O ₂ (95% N ₂)		0% O ₂ (100% N ₂)	
	% G	+ s.e.	% G	+ s.e.	% G	+ s.e.
1	0.0	-	1.6	1.6	0.0	-
2	6.6	3.3	3.3	2.1	2.5	1.3
3	8.3	4.0	5.0	2.2	5.0	2.0
7	21.6	7.0	10.0	4.4	5.8	2.0
14	23.3	7.1	10.0	4.4	5.8	2.0
21	23.3	7.1	10.0	4.4	5.8	2.0
28	23.3	7.1	10.0	4.4	8.3	2.4

Table 6.- Percentages of germination for intact fruit in different percentages of oxygen. Darkness, 25 °C.
Lot 2.

TIME (Days)	PERCENTAGES OF OXYGEN					
	100% O ₂ (0% N ₂)		50% O ₂ (50% N ₂)		20% O ₂ (80% N ₂)	
	% G	± s.e.	% G	± s.e.	% G	± s.e.
1	20.0	4.3	5.7	2.9	28.5	7.0
2	92.8	2.8	92.8	2.8	64.3	4.3
3	97.1	1.8	97.1	1.8	82.8	5.2
7	98.5	1.4	100.0	-	87.1	4.2
14	98.5	1.4	100.0	-	90.0	3.0
21	98.5	1.4	100.0	-	90.0	3.0
28	98.5	1.4	100.0	-	90.0	3.0

TIME (DAYS)	PERCENTAGES OF OXYGEN					
	10% O ₂ (90% N ₂)		5% O ₂ (95% N ₂)		0% O ₂ (100% N ₂)	
	% G	± s.e.	% G	± s.e.	% G	± s.e.
1	2.8	1.8	4.3	4.3	0.0	-
2	48.6	5.5	25.7	7.5	0.0	-
3	78.5	4.6	54.3	6.5	5.7	-
7	87.1	4.2	85.7	3.7	5.7	2.0
14	88.5	3.4	87.1	2.8	5.7	2.0
21	88.5	3.4	87.1	2.8	5.7	2.0
28	88.5	3.4	87.1	2.8	5.7	2.0

Table 7.- Percentages of germination for scarified fruit in different percentages of oxygen. Darkness, 25 °C. Lot 2.

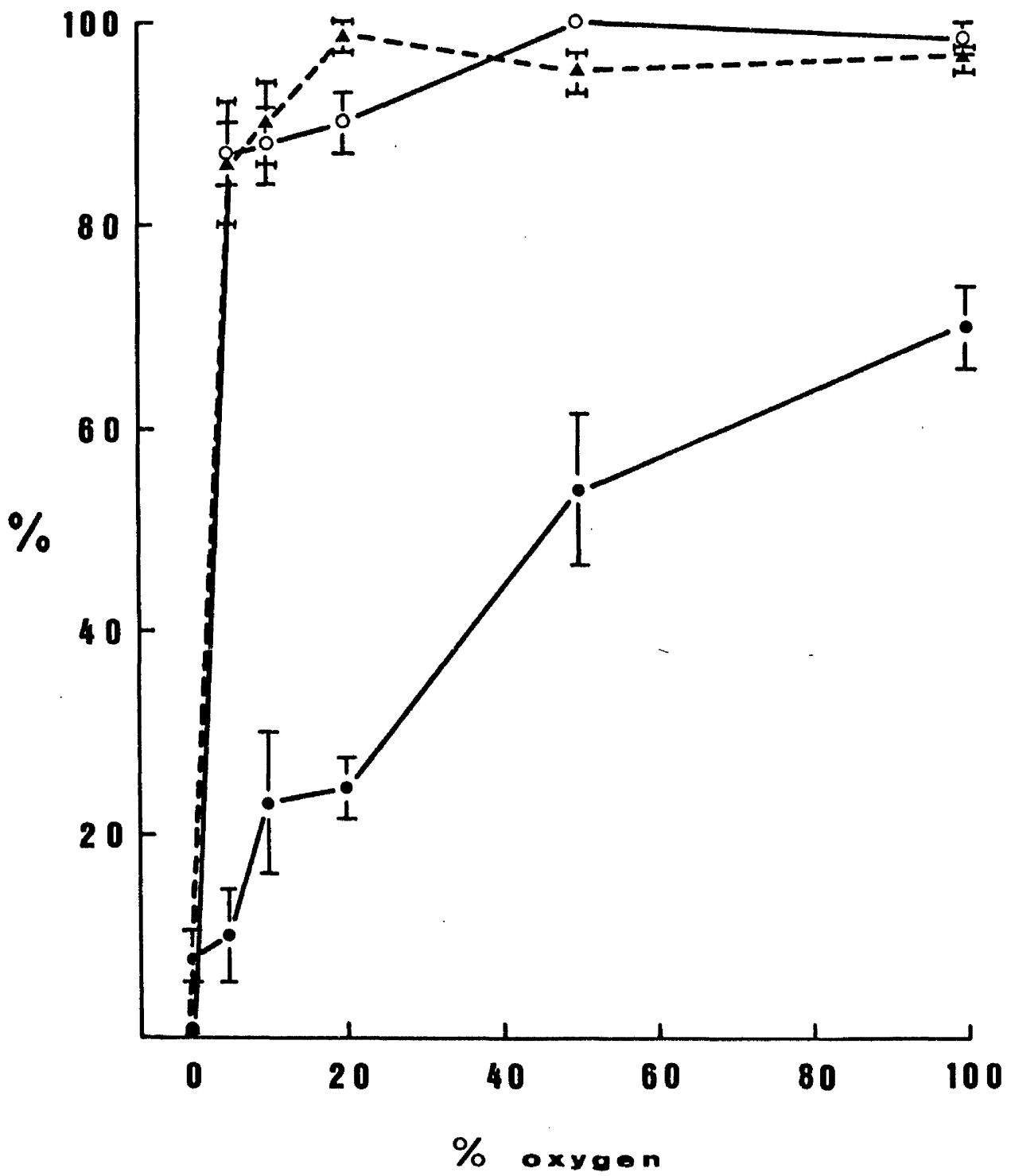
TIME (DAYS)	PERCENTAGES OF OXYGEN					
	100% O ₂ (0% N ₂)		50% O ₂ (50% N ₂)		20% O ₂ (80% N ₂)	
	% G	± s.e.	% G	± s.e.	% G	± s.e.
1	20.0	3.0	15.0	5.0	11.4	2.6
2	90.0	2.2	90.0	2.6	90.0	2.2
3	94.0	2.0	95.0	2.2	95.7	2.0
7	97.1	1.8	95.0	2.2	98.6	1.4
14	97.1	1.8	95.0	2.2	98.6	1.4
21	97.1	1.8	95.0	2.2	98.6	1.4
28	97.1	1.8	95.0	2.2	98.6	1.4

Time (Days)	PERCENTAGES OF OXYGEN					
	10% O ₂ (90% N ₂)		5% O ₂ (95% N ₂)		0% O ₂ (100% N ₂)	
	% G	± s.e.	% G	± s.e.	% G	± s.e.
1	7.1	2.8	4.3	2.0	0.0	-
2	77.1	3.6	24.3	4.8	0.0	-
3	84.3	5.7	64.3	7.5	0.0	-
7	90.0	4.3	85.7	5.7	0.0	-
14	90.0	4.3	85.7	5.7	0.0	-
21	90.0	4.3	85.7	5.7	0.0	-
28	90.0	4.3	85.7	5.7	0.0	-

Table 8.- Percentages of germination for true seed in different percentages of oxygen. Darkness, 25 °C. Lot 2.

Figure 26.- Percentages of germination for 28 days for intact fruit, scarified fruit and true seed as a function of percentages of oxygen. Darkness, 25 °C. Lot 2. In the abscissa percentages of germination \pm standard error, in the ordinate percentages of oxygen.

Key: —●— Intact fruit
—○— Scarified fruit
---▲--- True seed



Intact fruit exhibited a clear enhancement of germination with an increase of oxygen concentrations, although, as expected from the previous results (Figs. 23, 24 and 25), 100% oxygen was not able to induce total germination. Scarified fruit and true seed responded with a noticeable promotion of germination when the oxygen concentration was increased. This promotion was greater than that observed for the intact fruit, and low concentrations of oxygen of 5% produced in both scarified fruit and true seed a high percentage of germination of 86%. Above this concentration both curves stabilized with no further increase in germination values.

An atmosphere of 100% oxygen increased the germination after 28 days to 71%. Nevertheless, this value was obtained by scarified fruit or true seed in only 5% oxygen. This indicates that the fruit coat is having a very significant effect on the availability of O₂ to the developing embryo.

There is little information in the literature about the effect of oxygen on sugar beet germination. The results in this section, however, are in agreement with those obtained for other seeds (Crocker, 1948; Black and Wareing, 1959; Major and Roberts, 1968; Come and Tissaoui, 1973). Several reasons can be ascribed to the promotion of germination by oxygen. An increase of the oxygen tensions may allow a better oxygen supply to the embryo, leading to an activation of the metabolic events of germination.

An additional explanation for this observed oxygen effect involves the presence in the seed of inhibitors, and has been put forward by several authors. Chetram and Heydecker (1967) have suggested an interaction between oxygen and inhibitors present in sugar beet seeds, and that the restriction of oxygen supply renders the embryo sensitive to the inhibitor. A different but related approach has been postulated by Black and Wareing (1959). They suggested an interaction between inhibitors and oxygen, where the inhibitor increases the oxygen requirement of the embryo or, alternatively that oxygen at certain concentrations is required to overcome the inhibitor.

For Acer pseudoplatanus (Webb and Wareing, 1972) it has been suggested that dormancy is the result of the restriction by the testa of an outward - diffusion of a germination inhibitor present in the embryo. Once this block of germination has been overcome, any further delays in germination may be due to a restriction of oxygen uptake by the covering structures.

Evidence is presented in other sections of the impermeability of the sugar beet fruit coat to certain stains (see page 207) and to smaller molecules such as ABA (see page 200). Furthermore it has been reported by Abu-Shakra and Aqil (1969) and Battle and Whittington (1969) that the inhibitors in sugar beet are located in the internal wall of the fruit material.

If this is the case, when intact fruit is scarified or the true seed insulated, a mechanical pathway of leaching of inhibitors is produced, and simultaneously a pathway to O₂ entry is opened. Thus low concentrations of

O₂ in the atmosphere around the seed will be sufficient to promote the high levels of germination observed in Fig. 26.

A different idea has been postulated by Heydecker and Chetram (1971) . These authors suggested that microbial contamination of the fruit coat may result in competition for scarce O₂. In true seed there will be no competition with microorganisms because they have been eliminated by discarding the fruit material and germination will be high. In scarified fruit a physical pathway to oxygen has been opened and oxygen can reach the embryo. But in intact fruit the seed is surrounded by microorganisms, the microatmosphere will be low in oxygen, and germination will be low.

These experiments thus reinforce the previous conclusions that the fruit coat may be a very significant barrier to either O₂ or inhibitors in the germination process. It also seems likely from these results that in sugar beet there is more than one mechanism involved in seed dormancy.

Effect of Hydrogen Peroxide

The results are shown in Table 9, and in Fig. 27, where germination is expressed as a function of hydrogen peroxide concentration. It can be seen - that a hydrogen peroxide concentration between 10^{-3} M and 10^{-1} M had no effect on germination in relation to the water control, but 1 M produced a promotion of germination of 30%. Higher hydrogen peroxide concentrations resulted in a sharp decrease in germination, possibly due to a toxic effect of H_2O_2 . It was observed that fruits germinating in 2 M H_2O_2 showed signs of injury; at 28 days of incubation the radicle had a dark brown coloration and less radicular growth than that observed in the water control.

The results give no indication as to whether the hydrogen peroxide - itself is active or if it acts because it is enzymatically decomposed to oxygen within the tissues. The hydrogen peroxide may also increase the oxygen tensions in the fruit periphery, thus increasing the availability of oxygen to the - embryo. The effect of greater availability of oxygen and its possible interaction with inhibitors and seed covering structures, has been already discussed.

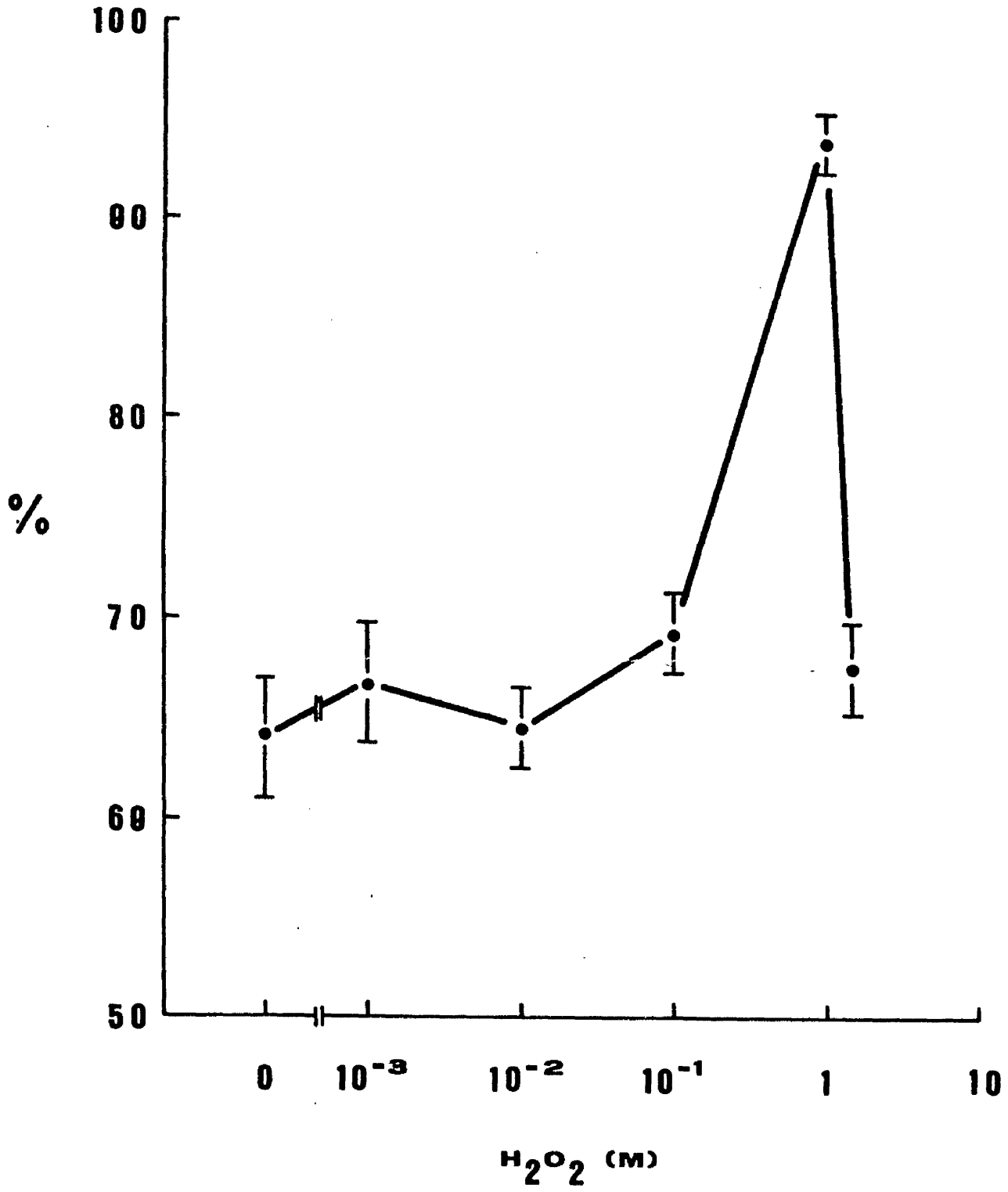
This appears to be the first report of the effect of H_2O_2 on sugar beet germination, but the promotion of germination by this chemical has been shown for other seeds (Hay and Cumming 1959; Roberts, 1962, 1964-a; Pollock and - Essery, 1955) .

TIME (DAYS)	CONCENTRATION OF H ₂ O ₂ (M)					
	2		1		10 ⁻¹	
	% G	± s.e	% G	± s.e	% G	± s.e
1	0.0	-	0.4	0.3	0.0	-
2	4.2	0.8	38.8	2.1	15.2	1.9
3	32.8	2.0	65.8	1.8	26.6	2.7
7	62.8	2.0	87.6	1.7	56.6	2.1
14	66.8	2.3	92.4	1.6	67.8	2.0
21	67.4	2.3	93.6	1.6	68.8	2.0
28	67.4	2.3	93.8	1.6	69.2	1.8

TIME (DAYS)	CONCENTRATION OF H ₂ O ₂ (M)					
	10 ⁻²		10 ⁻³		WATER CONTROL	
	% G	± s.e	% G	± s.e	% G	± s.e
1	0.0	-	0.0	-	0.2	0.2
2	10.6	1.1	11.6	1.8	8.2	1.4
3	18.4	1.2	20.0	2.5	14.2	1.9
7	48.0	2.9	48.2	2.8	39.4	2.9
14	60.4	3.0	61.2	2.6	59.4	3.0
21	63.8	2.4	66.2	2.7	63.4	3.3
28	64.6	2.2	66.8	2.7	64.0	3.3

Table 9.- Percentages of germination for intact fruit at different concentrations of hydrogen peroxide. Darkness, 25 °C. Lot 1.

Figure 27. - Percentages of germination for 28 days for -
intact fruit as a function of hydrogen peroxide
concentrations. Darkness, 25 °C. Lot 1. In -
the abscissa percentages of germination \pm -
standard error, in the ordinate hydrogen peroxide
concentrations.



Effect of Potassium Cyanide

The dispersal unit of sugar beet has certain characteristics in common with that of rice and it is possible that they share similar germination mechanisms (Major and Roberts, 1968; Roberts, 1964-a, 1964-b, 1973). These characteristics are: i) the sensibility of sugar beet seeds to low oxygen concentration (see section Effect of Oxygen); ii) scarification of the fruit coat results in an increase of germination (section Scarification); iii) application of hydrogen peroxide results in an enhancement of germination of intact fruit (section Effect of Hydrogen Peroxide).

One important difference in the seed, however, is that rice seeds germinate in anaerobic conditions, while sugar beet seeds do not (section Effect of Oxygen).

The effect of different concentrations of KCN was thus tested on intact fruit, scarified fruit and true seed. The results are shown in Tables 10, 11 and 12 and in Fig. 28, where the percentages of germination for 28 days are expressed as a function of potassium cyanide concentrations. The germination figures obtained for intact fruit were not significantly different from the water control for this lot of fruits. Scarified fruit showed a scatter of values for the effect of different KCN concentrations; there is no correlation between the decrease of germination and the increase of KCN concentrations. The highest observed inhibition of germination

TIME (DAYS)	CONCENTRATION OF KCN (M)					
	10^{-3}		10^{-4}		10^{-5}	
	% G	\pm s.e	% G	\pm s.e	% G	\pm s.e
1	0.0	-	1.0	1.0	0.0	-
2	15.0	2.7	12.0	2.5	18.0	2.5
3	31.0	5.6	21.0	3.1	23.0	3.0
7	39.0	5.2	27.0	3.6	29.0	4.6
14	40.0	5.4	35.0	4.3	34.0	5.8
21	42.0	5.3	43.0	5.0	36.0	5.6
28	43.0	5.2	46.0	5.2	37.0	5.4

TIME (DAYS)	CONCENTRATION OF KCN (M)					
	10^{-6}		10^{-7}		WATER CONTROL	
	% G	\pm s.e	% G	+ s.e	% G	+ s.e
1	1.0	-	0.0	-	1.0	1.0
2	16.0	3.4	21.0	5.0	15.0	3.4
3	20.0	3.3	23.0	4.7	25.0	4.5
7	26.0	4.0	29.0	4.8	30.0	4.5
14	30.0	3.3	37.0	6.1	41.0	5.2
21	38.0	5.5	49.0	6.5	42.0	5.5
28	38.0	5.5	50.0	6.1	42.0	5.5

Table 10.- Percentages of germination for intact fruit at different concentrations of potassium cyanide. Darkness, 25 °C. Lot 2. The values of germination for water control are those obtained in "Effect of scarification on sugar beet fruits and seeds", - Figure 15.

TIME (DAYS)	CONCENTRATION OF KCN (M)					
	10^{-3}		10^{-4}		10^{-5}	
	% G	\pm s.e	% G	\pm s.e	% G	\pm s.e
1	13.0	3.6	12.0	3.2	8.0	2.9
2	59.0	4.8	46.0	4.3	50.0	3.3
3	64.0	6.3	53.0	3.9	61.0	4.0
7	71.0	6.9	57.0	3.6	73.0	3.3
14	73.0	6.1	58.0	3.6	74.0	3.4
21	74.0	6.2	58.0	3.6	75.0	3.7
28	74.0	6.2	58.0	3.6	75.0	3.7

TIME (DAYS)	CONCENTRATION OF KCN (M)					
	10^{-6}		10^{-7}		WATER CONTROL	
	% G	\pm s.e	% G	\pm s.e	% G	\pm s.e
1	6.0	2.2	14.0	4.5	35.0	3.7
2	44.0	4.3	49.0	5.2	72.0	4.1
3	48.0	5.0	57.0	4.7	82.0	3.2
7	57.0	4.5	66.0	4.7	82.0	2.0
14	61.0	4.3	72.0	5.1	89.0	2.3
21	61.0	4.3	72.0	5.1	89.0	2.3
28	61.0	4.3	72.0	5.1	89.0	2.3

Table 11.- Percentages of germination for scarified fruit at different concentrations of potassium cyanide. Darkness, 25 °C. Lot 2. The values of germination for water control are those obtained in "Effect of Scarification on sugar beet fruits and seeds", Figure 15.

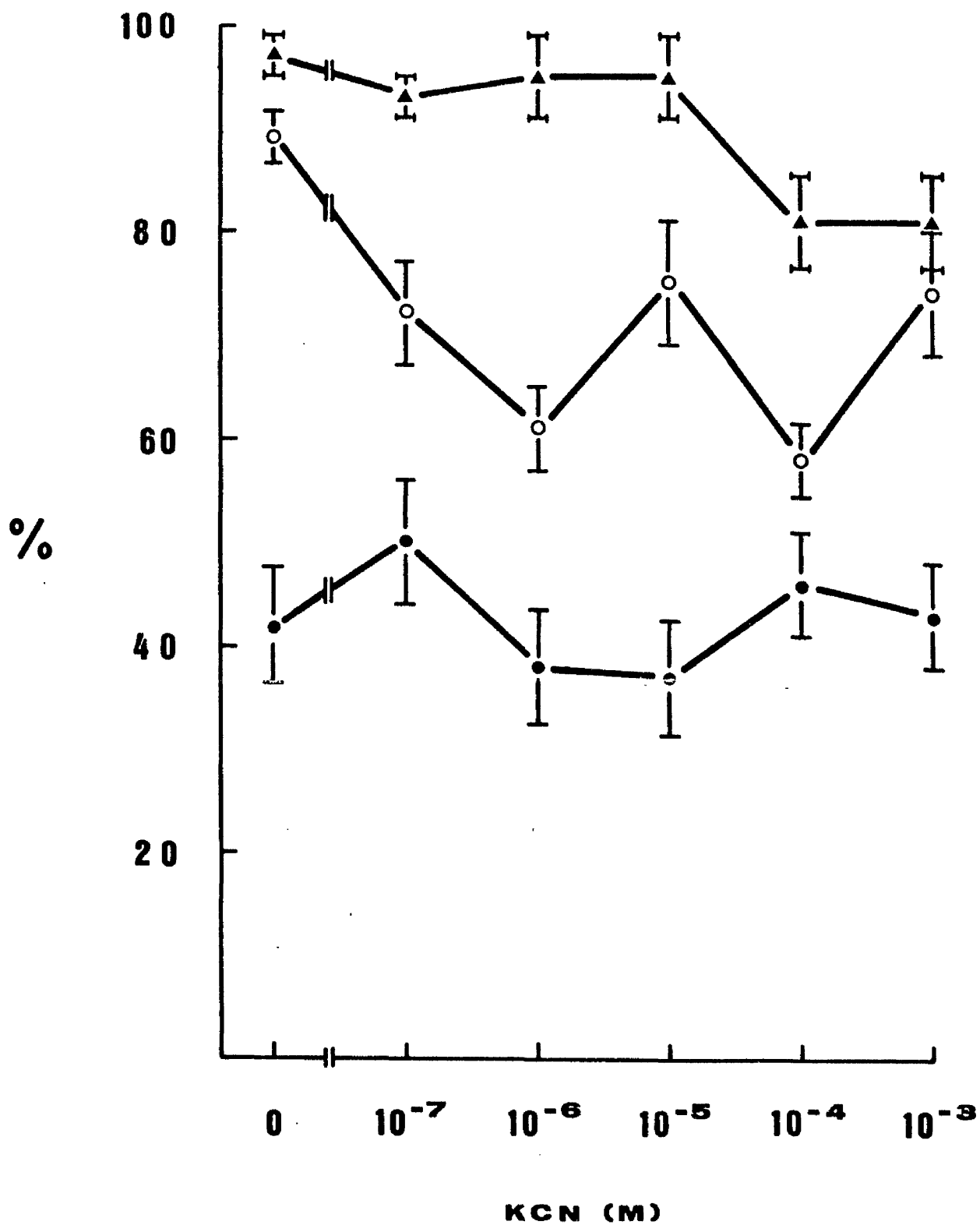
TIME (DAYS)	CONCENTRATION OF KCN (M)					
	10^{-3}		10^{-4}		10^{-5}	
	% G	+ s.e	% G	+ s.e	% G	+ s.e
1	3.0	1.5	20.0	3.9	9.0	4.3
2	52.0	6.1	63.0	4.2	83.0	3.0
3	72.0	5.0	76.0	3.4	90.0	1.5
7	81.0	4.6	81.0	3.1	95.0	2.2
14	81.0	4.6	81.0	3.1	95.0	2.2
21	81.0	4.6	81.0	3.1	95.0	2.2
28	81.0	4.6	81.0	3.1	95.0	2.2

TIME (DAYS)	CONCENTRATION OF KCN (M)					
	10^{-6}		10^{-7}		WATER CONTROL	
	% G	+ s.e	% G	+ s.e	% G	+ s.e
1	16.0	3.4	11.0	2.7	22.0	3.8
2	79.0	4.0	83.0	3.3	91.0	3.5
3	90.0	1.5	90.0	2.1	95.0	3.0
7	95.0	2.2	92.0	2.0	97.0	2.1
14	95.0	2.2	93.0	2.1	97.0	2.1
21	95.0	2.2	93.0	2.1	97.0	2.1
28	95.0	2.2	93.0	2.1	97.0	2.1

Table 12.- Percentages of germination for true seed at different concentrations of potassium cyanide. Darkness, 25 °C. Lot 2. The values of germination for water control are those obtained in "Effect of scarification on sugar beet fruits and seeds", Figure 15.

Figure 28 . - Percentages of germination for 28 days for intact fruit , scarified fruit and true seed as a function of concentration of potassium cyanide. Darkness, - 25 °C. Lot 2. In the abscissa percentages of germination \pm standard error, in the ordinate potassium - cyanide concentrations.

Key: ● Intact fruit
 ○ Scarified fruit
 ▲ True seed



(30%) was produced by 10^{-4} M of KCN. These oscillations may be the result of irregular uptake of the chemical. Although the fruits have been scarified, - some fruit material is left and could hinder the uptake of KCN.

For true seed only 10^{-4} M and 10^{-3} M was noted to exert an inhibitory effect. For these concentrations a decrease of 16% was observed in relation to water control. Taking into account the results of scarified fruit, greater inhibition was expected for true seed, because in this case a lack of inhibition cannot be due to hindrance of the uptake of the chemical by the fruit - coat, because this material had been discarded.

The range of KCN concentrations used in these experiments are known to inhibit the respiration of certain living tissues (James, 1953), but it had little effect on sugar beet germination. It is interesting to note that the higher observed inhibition (Fig. 28, intact fruit) for 10^{-4} M was 30% in relation to water control. This inhibition is not very great, or at least is not the inhibition expected from a potent respiratory inhibitor such as KCN.

The lack of inhibition may be due to ineffective uptake of the chemical through the covering embryo structures, specially in the case of intact and - scarified fruit. This is unlikely, because KCN had little effect on true seed, where the fruit coat has been removed. If there is any uptake problem, the site ought to be located at the level of the testas or the tissues surrounding the embryo. It is difficult, however, to assume this, because KCN is a small

molecule (m.w. = 65,12) and larger molecule such as ABA (m.w. = 264) can exert an effect on true seed (see Fig. 42). Alternative explanations is that KCN is degraded within the seed tissues of that the KCN concentrations were not high enough for sugar beet.

Nevertheless, the metabolic pathway postulated by Roberts remains a possibility. This author (1964-b) found that not all the respiratory inhibitors are aqually effective in promoting germination, and to confirm if the mechanism proposed by Roberts exists in sugar beet, it will be necessary to repeat the experiment using several other respiratory inhibitors.

Water Supply

Part A: Hydration - Dehydration

For some crop seeds, germination can be enhanced by the process termed 'hardening', in which seeds are moistened and dried back as a preliminary - treatment to germination (Austin et al., 1969; Berrie and Drennan, 1971 ; - Hanson, 1973; Hegarty, 1970). This treatment has been applied to sugar beet fruits with positive results (Heydecker and Chetram, 1967; Longden 1971). - The effect or hardening could not be the same for all varieties of Beta vul- garis, and may also be different within batches of fruits. An investigation of hardening is important for two reason: a) it is a realatively simple - process that is easily applicable in agriculture to obtain better field germi- nation; and b) it may give valuable insights into the physiological processes involved in germination. Another question that has been examined here is the effect on germination of washing the fruits. It has been claimed for some - time (Tolman and Stout, 1940) that inhibitors may be removed from sugar beet fruits by washing, wich promotes germination.

In order to investigate these two aspects intact fruits from Lot 1 - were hydrated for different times. After this period, one group of fruits - was dried and germination tests then carried out. On the second group of fruits the germination test was carried out without this drying step. The - results are presented in Table 13 and 14. In Fig. 29 the values of germina- tion for 28 days area expressed as a function of hydration periods.

For fruits hydrated for different times followed by dehydration (Fig. 29, curve a) there was a tendency for better germination with longer hydration periods. A two hour hydration period produced an enhancement of germination of 20% over the 'basic germination behaviour' (54%, Fig. 3). Longer hydration periods than 2 hours caused a decrease of germination, but the values increased again from 8 hours, until a maximum figure of 84% was reached after 48 hours of hydration. In the controls (curve b) fruits hydrated for the same time, but without dehydration, showed no correlation between hydration times and germination.

The increase of germination with longer hydration periods might be due to leaching of inhibitors from the fruit, as has been claimed by Tolman and Stout (1940), but the results indicate that the dehydration period prior to the germination test is also important.

It is most likely that the process of hydration and dehydration is having a mechanical effect on the fruit coat. The fruit coat will swell on hydration and will shrink on dehydration. This may cause cracking in the coat and a scarification is produced, which will consequently enhance germination (see Scarification, page 84).

TIME (DAYS)	TIME OF HYDRATION (HOURS)					
	2 h		4 h		8 h	
	% G	+ s.e	% G	+ s.e	% G	+ s.e
1	6.2	1.3	4.0	0.8	4.0	0.6
2	19.4	1.8	15.2	1.6	19.2	1.9
3	32.8	1.8	23.6	2.0	29.6	2.1
7	57.2	1.9	45.0	2.5	51.4	2.5
14	72.2	1.6	56.8	2.5	58.2	2.3
21	73.8	1.9	59.8	2.6	61.2	2.3
28	74.1	1.9	60.6	2.8	61.2	2.3

TIME (DAYS)	TIME OF HYDRATION (HOURS)					
	12 h		24 h		48 h	
	% G	+ s.e	% G	+ s.e	% G	+ s.e
1	5.2	0.9	35.4	1.8	39.0	1.5
2	18.4	2.1	55.2	2.9	70.6	1.6
3	31.6	2.0	65.8	2.7	78.8	1.4
7	55.4	2.3	74.4	2.5	83.4	1.7
14	65.6	1.7	79.2	2.0	84.2	1.7
21	68.2	1.5	79.4	2.0	84.2	1.7
28	69.4	1.5	79.4	2.0	84.2	1.7

Table 13.- Water supply. Part. A. Hydration-Dehydration. Effect of - different times of hydration and a single period of dehydration on sugar beet germination. Fruits from Lot 1. Germination test in darkness at 25 °C .

TIME (DAYS)	TIME OF HYDRATION (HOURS)					
	2 h		4 h		8 h	
	% G	\pm s.e	% G	\pm s.e	% G	\pm s.e
1	0.2	0.2	0.4	0.3	0.4	0.3
2	6.4	0.8	5.0	0.8	6.2	1.1
3	11.4	1.3	11.2	0.9	15.4	1.8
7	28.4	2.3	27.8	2.3	40.0	2.9
14	39.6	3.0	39.8	2.3	54.2	3.3
21	43.8	2.9	42.8	2.3	59.2	2.9
28	54.4	2.8	43.8	2.2	60.4	2.9

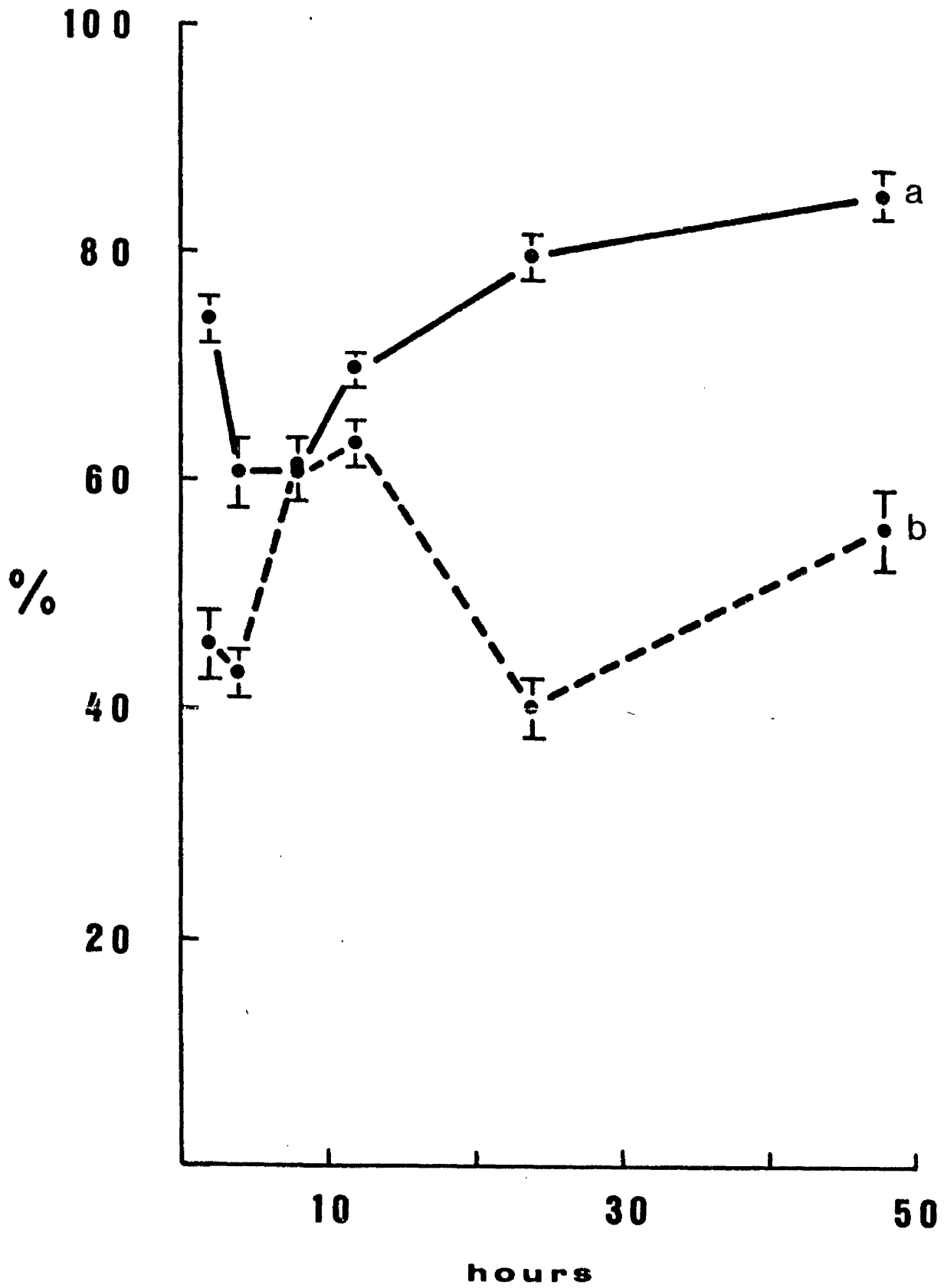
TIME (DAYS)	TIME OF HYDRATION (HOURS)					
	12 h		24 h		48 h	
	% G	\pm s.e	% G	\pm s.e	% G	\pm s.e
1	1.0	0.4	1.8	0.5	2.2	0.6
2	6.2	0.9	4.6	1.1	9.4	1.1
3	13.6	0.9	8.5	1.5	13.0	1.3
7	37.8	2.2	20.8	1.8	27.2	2.5
14	54.8	2.2	32.2	1.8	49.4	3.2
21	60.8	2.0	39.8	2.5	54.0	3.3
28	62.8	2.2	40.2	2.5	55.4	3.5

Table 14 .- Water supply. Part A. Hydration-Dehydration
 CONTROL: Effect of different times of hydration without
 dehydration on sugar beet germination. Fruits from Lot 1.
 Germination test in darkness at 25 °C.

Figure 29 . - Water supply. Part A. Hydration-Dehydration
Effect of different time of hydration on -
sugar beet germination. Darkness, 25 °C.
Lot 1. In the abscissa percentages of germi
nation \pm standard error, in the ordinate time
of hydration in hours.

Key: — ● — Dehydration treatment prior to
germination (a)

--- ● --- Hydration treatment prior to
germination (b)



With longer hydration periods the softening of the tissues will be greater , resulting in more extensive scarification. Curve a of Fig. 29 shows a decrease of germination for hydration periods from 2 to 8 hours with a further increase of germination for longer hydration periods. One explanation for this decrease could be that short hydration periods not only remove inhibitors, but growth - promoters as well. For short times it is possible that there is a more - vigorous leaching of promoters than inhibitors , causing a reduced germination. For longer hydration periods, however, there is a more vigorous leaching of inhibitors than promoters thus increasing germination.

The process of hydration-dehydration may also allow the initiation of metabolic events leading to germination as has been suggested by Berrie and - Drennan (1971) and Hanson (1973). It has been suggested by these authors that the hydration-dehydration treatment involves the completion of early metabolic steps in germination which may be quite stable to gentle drying treatments. If these processes also occur in sugar beet they may bring about germination in a shorter time or produce higher germination percentages. Increasing the hydration period will increase the number of these metabolic events and better - germination will be obtained.

For sugar beet, the beneficial effect of drying the fruits prior to - germination test has been reported by Heydecker and Chetram (1967) These - workers suggested that this may be due to interference with respiration by - excess moisture.

Heydecker et.al. further reported(1971) that an excessive water supply caused a thin layer of mucilage to arise all over the fruit material, thus limiting oxygen diffusion to the seed.

This interference with respiratory processes by excess moisture could explain the lack of tendency according to the length of hydration, the production of mucilage around the fruit and the ovary cap could be of different thickness, causing different oxygen tensions around each fruit or originating an irregular oxygen uptake.

Part B: Accumulative Effect of Hydration-Dehydration Cycles

The effect of accumulative cycles of hydration and deshydration (H-D cycles) was studied on intact fruits from Lot 1. The results are shown in Tables 15 and 16 and in Figs. 30 and 31.

Fig. 30 expresses the germination percentage after 28 days for fruits subjected to different numbers of H-D cycles and placed to germinate in either water or water crude extract. The hydration was carried out by continuous leaching with running water. However, when the fruits were hydrated by leaving then to soak (Fig. 31) the results are similar to those of Fig. 30 . This indicates that the method of hydration has no effect on germination and the

a)

TIME (DAYS)	NUMBER OF CYCLES							
	1		2		3		4	
	% G	+ s.e	%G	+ s.e	% G	+ s.e	% G	+ s.e
1	0.0	-	0.4	0.3	12.4	1.3	11.6	1.1
2	2.2	0.7	7.0	0.9	36.2	2.4	45.4	2.9
3	7.0	1.1	17.6	1.8	54.6	2.8	66.4	2.7
7	32.0	3.4	47.2	1.9	74.2	2.6	82.8	1.9
14	51.6	3.0	58.0	2.3	78.4	2.3	84.6	1.7
21	55.2	2.9	61.0	2.5	78.8	2.3	85.2	1.7
28	56.6	2.7	61.6	2.5	79.0	2.3	85.4	1.7

b)

TIME (DAYS)	NUMBER OF CYCLES							
	1		2		3		4	
	% G	+ s.e	% G	+ s.e	% G	+ s.e	% G	+ s.e
1	0.0	-	0.2	0.2	4.4	0.9	5.8	0.9
2	2.4	0.6	6.0	1.0	18.6	2.3	35.4	2.3
3	6.4	1.2	15.0	1.2	28.6	2.1	56.0	2.0
7	34.2	3.6	45.4	3.3	48.6	2.7	75.0	2.1
14	50.4	3.3	61.4	2.8	63.2	2.7	78.8	1.4
21	53.8	3.6	63.6	2.7	64.8	2.7	79.0	1.3
28	56.4	3.6	64.0	2.7	65.4	2.6	79.4	1.3

Table 15.- Water supply. Part B. Accumulative effect of hydration and deshydration cycles. Fruits in continuous leaching - with running water.

a) last treatment Hydration, sowed in water

b) last treatment Hydration, sowed in water crude extract.

c)

TIME (DAYS)	NUMBER OF CYCLES							
	1		2		3		4	
	% G	\pm s.e	% G	\pm s.e	% G	\pm s.e	% G	\pm s.e
1	0.8	0.3	5.4	0.9	6.8	0.7	21.2	1.6
2	18.6	1.8	34.2	2.6	38.6	2.2	60.2	1.5
3	29.2	2.0	50.6	2.7	62.2	1.7	72.2	1.8
7	62.0	2.6	75.0	2.4	81.2	1.6	81.0	1.5
14	76.0	2.4	79.8	1.5	83.2	1.7	82.4	1.6
21	77.0	2.4	80.2	1.5	83.4	1.7	83.0	1.5
28	77.4	2.4	80.2	1.5	83.4	1.7	83.2	1.5

d)

TIME (DAYS)	NUMBER OF CYCLES							
	1		2		3		4	
	% G	\pm s.e	% G	\pm s.e	% G	\pm s.e	% G	\pm s.e
1	0.0	-	0.4	0.3	3.6	0.7	11.8	1.2
2	9.2	1.2	7.2	1.2	38.2	1.6	48.4	2.4
3	10.2	1.1	14.4	1.5	60.4	2.2	60.8	2.0
7	14.4	1.5	39.6	3.3	76.4	2.0	72.8	2.1
14	19.4	2.0	46.2	3.4	78.4	2.0	75.8	1.9
21	23.2	1.9	48.6	3.4	78.4	2.0	76.4	2.0
28	26.6	2.3	49.6	3.3	78.4	2.0	77.0	1.9

Table 15.- (Continuation)

c) last treatment Dehydration, sowed in water

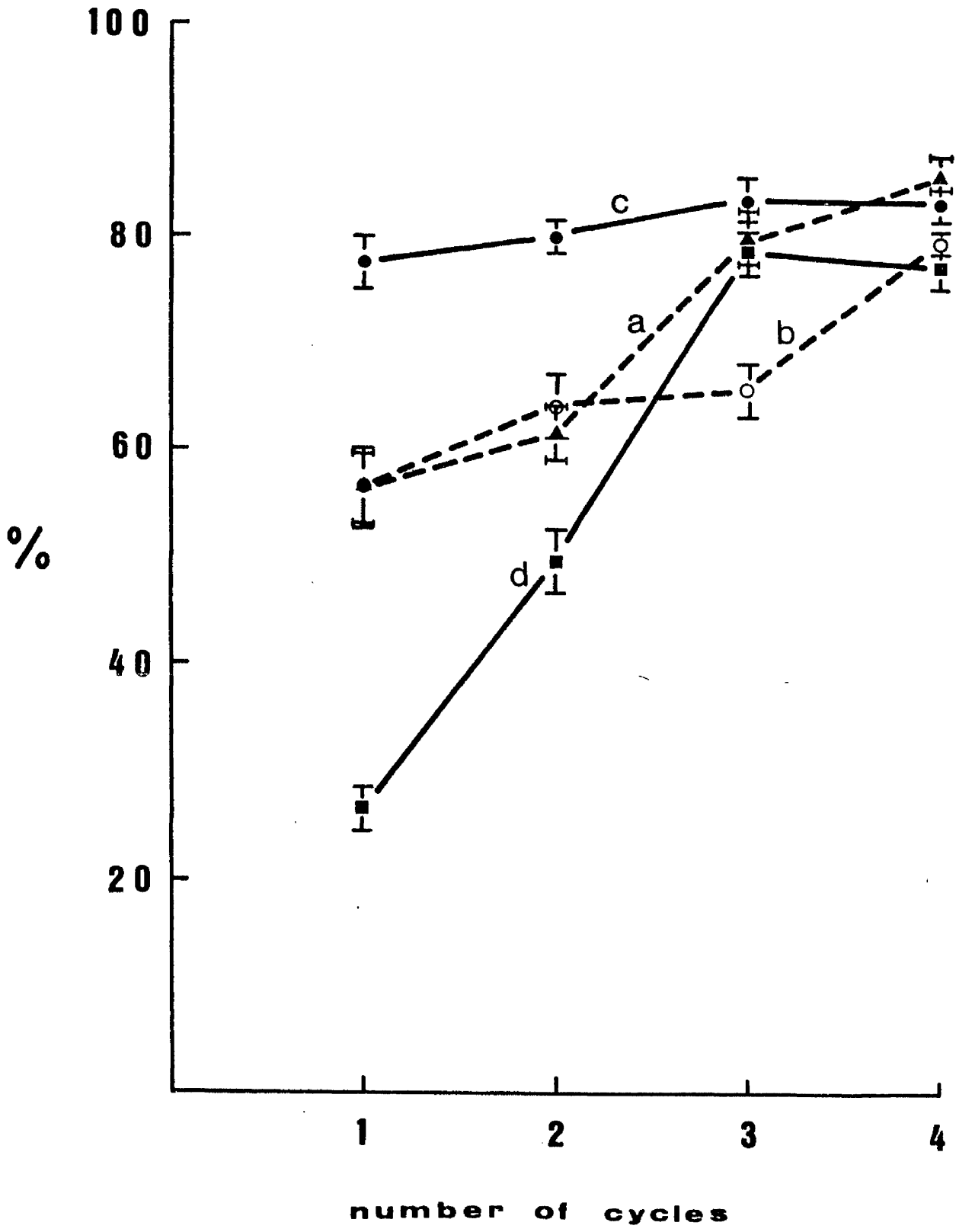
d) last treatment Dehydration, sowed in water crude extract.

Lot 1. Germination test in darkness at 25 °C.

More details in the text.

Figure 30 . - Water supply. Part B. Accumulative effect of Hydration-Dehydration cycles. Percentages of germination for 28 days as a function of number of H-D cycles. Fruits in continuous leaching with running water. Darkness, 25 °C Lot 1. In the abscissa percentages of germination \pm standard error, in the ordinate number of H-D cycles.

- Key: \blacktriangle Last treatment hydration, sowed in water. (a)
- \circ Last treatment hydration, sowed in water crude extract. (b)
- \bullet Last treatment dehydration, sowed in water.
- \blacksquare Last treatment dehydration, sowed in water crude extract (d).



a)

TIME (DAYS)	NUMBER OF CYCLES							
	1		2		3		4	
	% G	+ s.e	% G	+ s.e	% G	+ s.e	% G	+ s.e
1	0.0	-	0.0	-	8.8	0.9	40.8	1.8
2	4.2	0.9	8.4	1.1	27.0	2.0	65.2	2.5
3	11.2	1.5	19.8	1.7	48.2	1.9	74.0	2.3
7	32.2	2.3	47.8	2.8	65.2	1.9	82.2	1.8
14	49.8	3.1	60.0	2.7	71.4	2.2	83.8	1.7
21	52.6	3.3	63.2	2.8	72.0	2.1	83.8	1.7
28	54.8	2.9	64.2	2.8	72.6	2.0	84.0	1.7

b)

TIME (DAYS)	NUMBER OF CYCLES							
	1		2		3		4	
	% G	+ s.e	% G	+ s.e	% G	+ s.e	% G	+ s.e
1	0.0	-	0.4	0.3	3.6	0.8	39.2	1.8
2	2.2	0.5	8.2	1.5	13.0	1.4	61.4	2.1
3	5.0	0.8	19.2	1.9	21.0	1.5	69.4	1.5
7	22.2	1.9	46.0	2.4	45.6	2.5	80.4	1.6
14	48.2	2.2	61.4	2.8	61.6	2.5	81.4	1.7
21	52.0	1.9	66.4	2.4	64.2	2.4	81.6	1.7
28	54.2	1.9	67.4	2.5	64.4	2.5	81.6	1.7

Table 16.- Water supply. Part B. Accumulative effect of hydration and dehydration cycles. Fruits left to soaked.
 a) last treatment Hydration, sowed in water
 b) last treatment Hydration, sowed in water crude extract.

c)

TIME (DAYS)	NUMBER OF CYCLES							
	1		2		3		4	
	% G	<u>±</u> s.e	% G	<u>±</u> s.e	% G	<u>±</u> s.e	% G	<u>±</u> s.e
1	7.4	1.2	19.8	1.5	19.0	2.0	23.6	1.8
2	33.2	2.1	54.0	2.0	53.2	2.4	55.0	2.3
3	45.6	2.3	66.4	1.8	64.2	2.2	70.0	1.7
7	69.2	2.1	80.6	1.3	78.2	1.8	81.2	1.5
14	77.0	1.8	82.6	1.3	82.2	1.4	82.6	1.5
21	78.2	1.8	82.6	1.3	82.4	1.4	82.8	1.4
28	78.2	1.8	82.6	1.3	82.4	1.4	82.8	1.4

d)

TIME (DAYS)	NUMBER OF CYCLES							
	1		2		3		4	
	% G	<u>±</u> s.e	% G	<u>±</u> s.e	% G	<u>±</u> s.e	% G	<u>±</u> s.e
1	0.0	-	1.4	0.5	7.2	1.2	19.0	2.0
2	8.6	1.4	7.6	1.1	15.6	1.5	56.0	2.3
3	8.8	1.3	13.8	1.8	22.8	2.0	71.4	2.3
7	12.2	1.5	38.0	2.5	40.0	2.2	82.0	2.2
14	24.0	2.5	49.4	3.1	52.0	2.3	83.7	2.1
21	27.6	2.5	53.0	2.8	54.4	2.4	83.7	2.1
28	30.2	2.3	53.6	2.8	54.8	2.4	83.7	2.1

Table 16.- (Continuation)

c) last treatment Dehydration, sowed in water

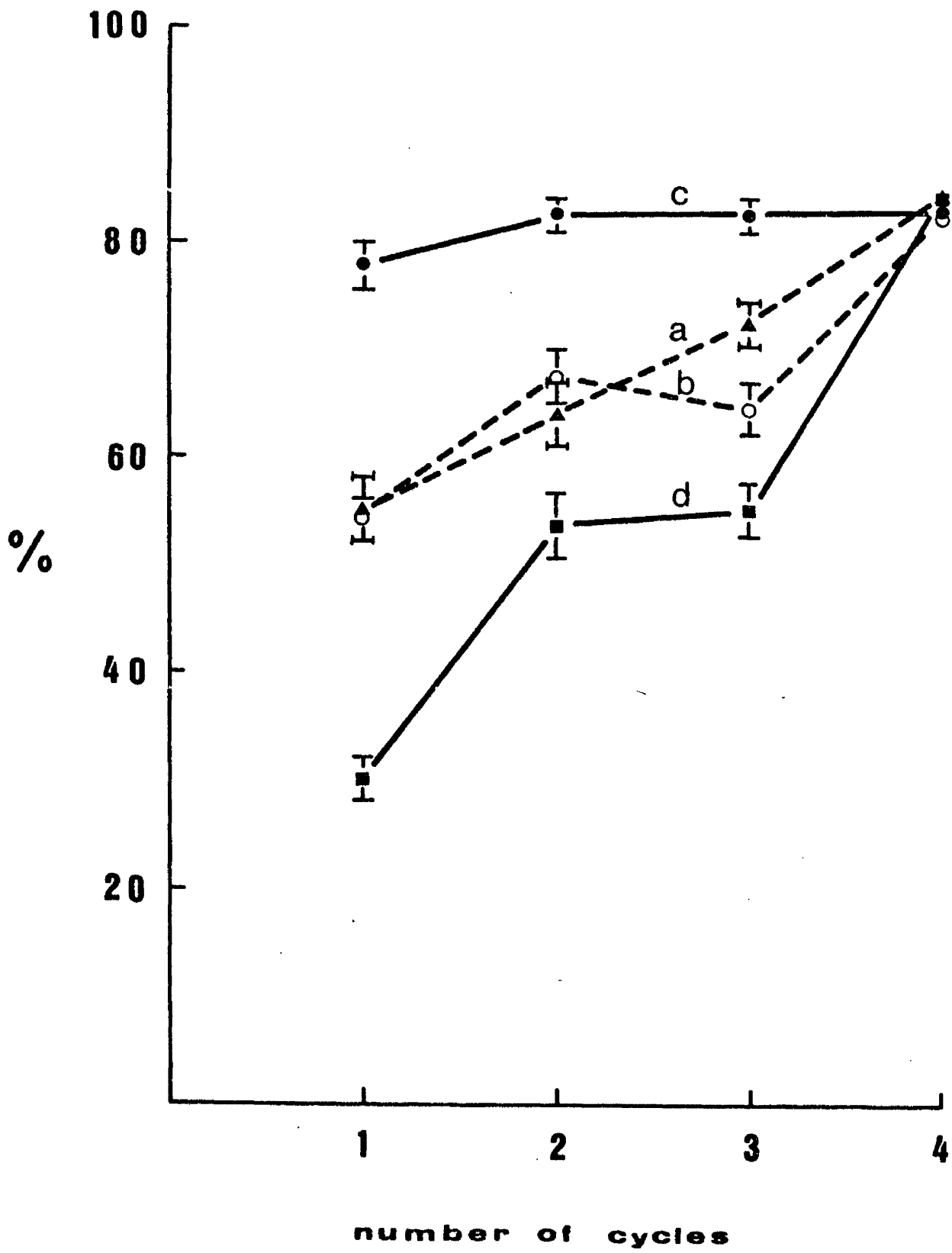
d) last treatment Dehydration, sowed in water crude extract.

Lot 1. Germination test darkness at 25 °C.

More details in the text.

Figure 31 . - Water supply. Part B. Accumulative effect of Hydration-Dehydration cycles. Percentages of germination for 28 days as a function of number of H-D cycles. Fruits left to soak. Darkness, 25 °C. Lot 1. In the abscissa - percentages of germination \pm standard error, in the ordinate number of H-D cycles.

- Key: ▲ Last treatment hydration, sowed in water (a).
○ Last treatment hydration, sowed in water crude extract. (b).
⊙ Last treatment dehydration, sowed in water. (c).
■ Last treatment dehydration, sowed in water crude extract.(d).



analysis of the results can be applied to both Figs. 30 and 31.

Curves a, b, c and d of Fig. 30 show an increase of germination with the number of cycles. Two effects can be distinguished:

- 1) Influence of the last treatment prior to germination (hydration or dehydration)

- 2) The medium of the germination test (water or extract)

- 1) When the last treatment prior to germination was hydration, the germination response was similar, regardless of the germination medium. - Curves a and b show the same pattern; 1 cycle did not promote germination - compared to the basic germination behaviour (54%, Fig. 3) and only at cycles 3 and 4 was an enhancement of germination obtained. 2) When last treatment prior to germination was dehydration there was a significant influence of the germination medium: 2-i). When the medium was water (curve c) high germination percentages were obtained from the first cycles while further cycles - had no significant effect on germination; at cycle 4 a maximum value of germination of 83% was reached. This contrasts with the work of Longden (1971) - where the optimum H-D treatment was obtained after four cycles. Moreover - the hydration and dehydration periods used in this work are different from those of Longden.

If the germination medium is extract (curve d) there an inhibition of germination is noted for the 2 first cycles. Further cycles, however, were very effective in promoting germination. By the fourth cycle levels of germination equivalent to those in curves a, b and c were obtained.

The first point to emerge from the data is that the processes of germination are not adversely affected by the cycle of hydration-dehydration. Each cycle enhanced the germination of the fruits in the various treatments. As expected from the experiment described in the previous section, one cycle of H-D (curve c) resulted in a greatly increased germination and possible reasons for this have been discussed.

The second point about the data is the inhibitory effect of the water extract on seed germination. This can be clearly seen by comparison of the first points of curves c and d, where more than 50% reduction in germination is observed; the reasons for this are not clear.

It is interesting, however, that increasing cycles of H-D overcome this inhibitory effect of the extract, and several possibilities for this can be suggested, along similar lines to the previous sections.

Thus, washings in the H-D cycles may be slowly leaching out inhibitors present in the fruit. During early cycles this results in a significant increase of germination if the medium is water (curve c), but the inhibitory effect of the extract counteracts this (curve d). This inhibitory effect is not enough to prevent germination and the values approach those of curve c with

further cycles.

Repeated H-D cycles will also cause a greater extent of cracking in the fruit coat. This may have a beneficial effect on germination because a better leaching of inhibitors of better gaseous exchange, as previously described, would therefore counteract the inhibitory effect of the extract. Alternatively, if the extract is preventing early metabolic processes during the germination, the repeated cycle may allow these events to be completed, thus overcoming the inhibitory effect.

This inhibitory effect, however, is not apparent if the last treatment prior to germination is hydration (curve a and b). Reasons for this are not clear. It is possible that the hydration step allows the formation of a mucilage around the fruit that impedes the inhibitory effect of the extract.

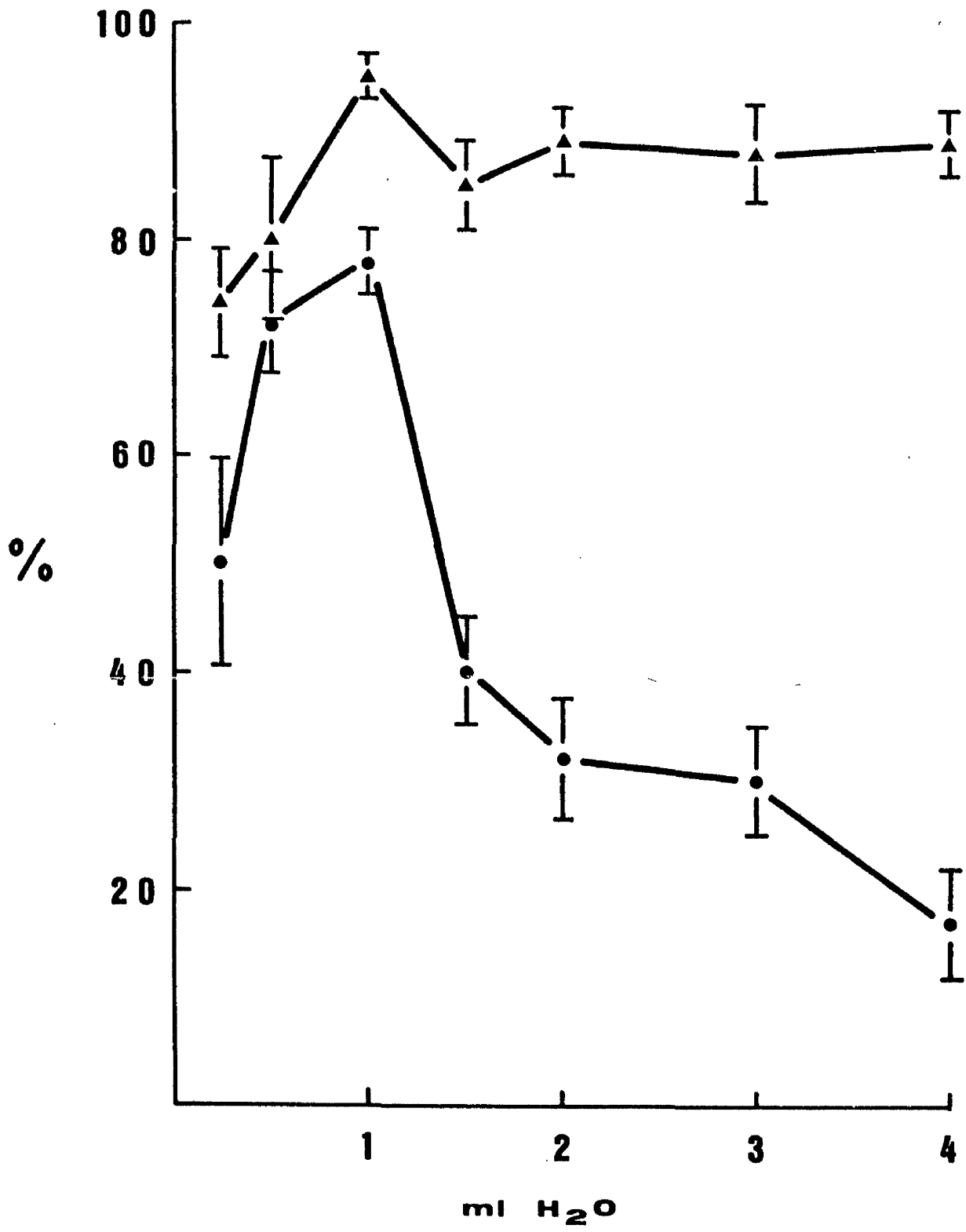
Part C : Water Sensitivity

The effect of delayed germination with an increase of water supply has been previously observed by Perry (1973) in field experiments, where excessive soil moisture resulted in low emergence of beet seedlings.

The effect of different quantities of water on sugar beet germination were tested here. Intact fruits and true seed from Lot 1 were placed in petri dishes, to which different quantities of water were added. The results are shown in Fig. 32 in which the values of germination percentage are expressed as a function of the water volume.

Figure 32.- Water supply. Part C. Water sensitivity. Percentages of germination for 28 days for intact fruit and true seed placed to germinate in different volumes of water. Darkness, 25 °C. Lot 1. In the abscissa percentages of germination \pm standard error, in the ordinate ml of water.

Key : ● Intact fruit
 ▲ True seed



For true seed, a small volume of water of 0.25 ml produced the lowest germination percentage (74%). Germination increased with a greater water supply, until the maximum percentage (94%) was reached at 1 ml of water. Increasing the quantity of water to 1.5 ml caused slight decreases of germination and the curve stabilized at this point. For intact fruit small volumes of water (0.25 ml) also produced poor germination. Germination increased with increase of water volume, reaching the value of 78% for 1 ml. From this point germination was reduced and 4 ml of water produced a low figure of 17%, a reduction of 60% from the maximum.

For both the true seed and the intact fruit it could be expected that an optimum ratio of water volume: seed number, will exist.

Less than this amount slows the imbibition of colloids and other processes leading to germination. This trend is observed both for the true and the intact fruit in Fig. 32. Small increases in the volume of water added have large effects on the percentage of germination, up to the optimum value.

It would also be expected that larger volumes of water may reduce germination for several reasons which have been discussed in the previous section, such as water injury or the formation of a mucilage coat.

A further explanation is suggested by the work of Adebona and Odu (1972) on Vigna unguiculata. These workers reported that during long imbibition periods materials are leached from the seed, such as reducing sugars, amino acids and protein. This leaching is accompanied by a considerable decrease in

germination of the seeds. Such a decrease in germination is observed in Fig.32 for intact fruit, but increasing the water volume had little or no effect on true seed germination. This is puzzling because leaching of low molecular weight substances could be expected to occur mainly from true seed, and not from intact fruit where the thick fruit material would prevent this loss. This indicates effects other than the leaching described by Adebona and Odu may be occurring.

Another possibility has been suggested by the work of Heydecker and Chetram (1971). They have reported that increasing the volume of water in the petri dish is related to an increase in the bacterial population around the seeds. This would explain the lack of effect observed here for the true seed, since such bacterial contamination has already been removed.

Although the reasons for water sensitivity are still obscure, it is important to take this effect into account when performing sugar beet germination studies. The selection of the volume of water to be added to the petri dish will influence the results. In addition the water content of the dishes must be rigorously controlled throughout the experiment, especially when using batches of fruits with long germination periods. If this is not done the results obtained may not be reliable.

Chemicals

Promotors

There is much evidence that endogenous hormones are involved in the regulation of many aspects of plant growth and development. It has been known for a considerable time that the application of exogenous growth substances such as gibberellins, kinetin and indole acetic acid, overcomes the dormancy of many seeds (Mayer and Poljakoff - Mayber, 1963; Evenari, 1965; Lang, 1965; Stokes, 1965; Cleland, 1969; Fox, 1969). In spite of the extensive bibliography on the effect of exogenous application of these hormones on the germination of many seeds, there are relatively few reports with sugar beet seeds (Peterson, 1958; Snyder, 1959; Hoover and Goodin, 1966; Coumans, 1973).

In this section the effect of these hormones on sugar beet fruits and seeds will be analysed. Figures 33, 34, 35, 36, 37, and 38 show the time course of germination for intact fruit, scarified fruit and true seed germinating in 10^{-4} M and 10^{-5} M gibberellic acid (GA_3), IAA and kinetin respectively. All the curves show no significant difference in germination at 10^{-4} and 10^{-5} M for all three hormones. In all cases the curve of 'basic germination behaviour' was found.

The effects of combining two hormones was also tested. Fig. 39 shows the time course of germination for intact fruit, scarified fruit and true seed in a solution of 10^{-5} M gibberellic acid and IAA; Fig. 40 in a solution of 10^{-5} M kinetin and IAA; and Fig. 41 in a solution of 10^{-5} M gibberellic acid and kinetin. None of the combinations of hormones tested showed any effect on sugar beet germination, and again it was found that all the curves are similar to those of 'basic germination behaviour'. There is one exception to these negative results, which is a small promotion of germination for intact fruit by the gibbe-

rellic acid/IAA combination (Fig. 39). This showed an increase of 15% in relation to the value obtained for 21 days for 'basic germination behaviour'. This promotion, although small, is statistically significant and it is interesting to note that it was not observed for scarified fruit and true seed.

These results are in agreement with those reported by Peterson (1958), Snyder (1959), Hoover and Goodin (1966) and recently by Coumans (1973). In general, experiments with hormones in sugar beet have been carried out using intact fruits. Coumans examined the germination of monogerm sugar beet fruits in the presence of indole acetic acid, kinetin and gibberellic acid and obtained negative results with these hormones. He suggested that the lack of action of the hormones may be due to problems of uptake of the chemical due to the presence of the fruit coat. The results presented here, however, suggest that the lack of action must be due to reasons other than problems of uptake of the chemical at the fruit coat, because the hormones have no effect on scarified fruit and true seed. However, a barrier to hormone entry at the true seed level can not be ruled out.

It is known that there are many endogenous forms of gibberellin (Steward and Krikorian, 1971), but not all of these are effective in promoting germination. The experiments shown in Figs. 33 and 34 demonstrate the ineffectiveness of GA_3 in promoting germination, but it is possible that other gibberellins may be effective in this respect. This reason, however, may not apply in the case of the auxin, because IAA has been shown to be the only endogenous auxin, at least in Zea mays coleoptile tips (Greenwood et al. 1972). An alternative explanation for the lack of action of the hormones in true seed is that there is

Figure 33.- Chemicals. Germination promotors.
Time course of germination for intact fruit,
scarified fruit and true seed at 10^{-4} M of
gibberellic acid. Darkness, 25°C. Lot 1.
In the abscissa percentages of germination
 \pm standard error, in the ordinate time in
days.

Key: ● Intact fruit
 ○ Scarified fruit
 ▲ True seed

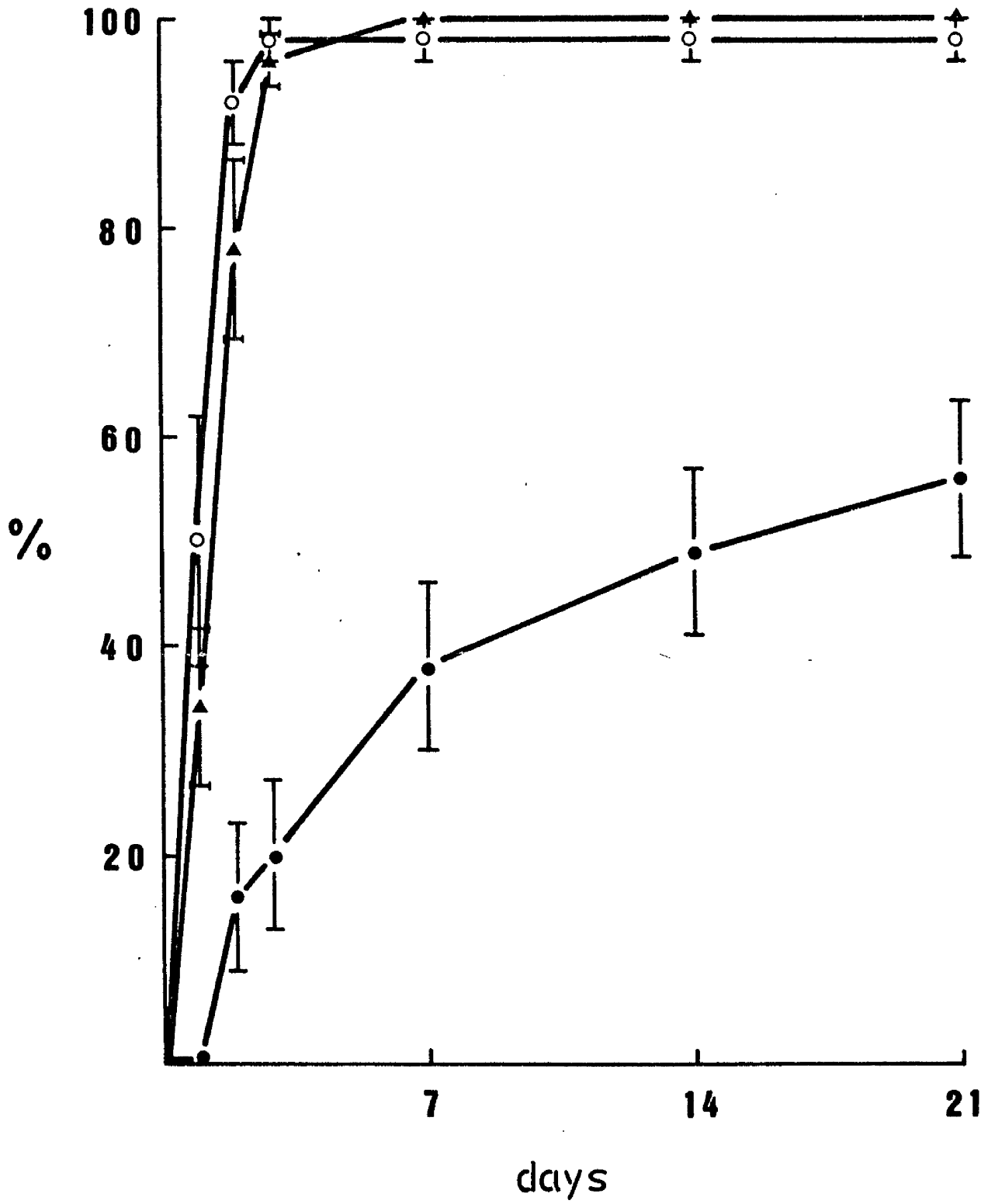


Figure 34.-

Chemicals. Germination promoters.

Time course of germination for intact fruit, scarified fruit and true seed at 10^{-5} M of gibberellic acid. Darkness, 25 °C. Lot 1.

In the abscissa percentages of germination \pm standard error, in the ordinate time in days.

Key: ● Intact fruit
 ○ Scarified fruit
 ▲ True seed

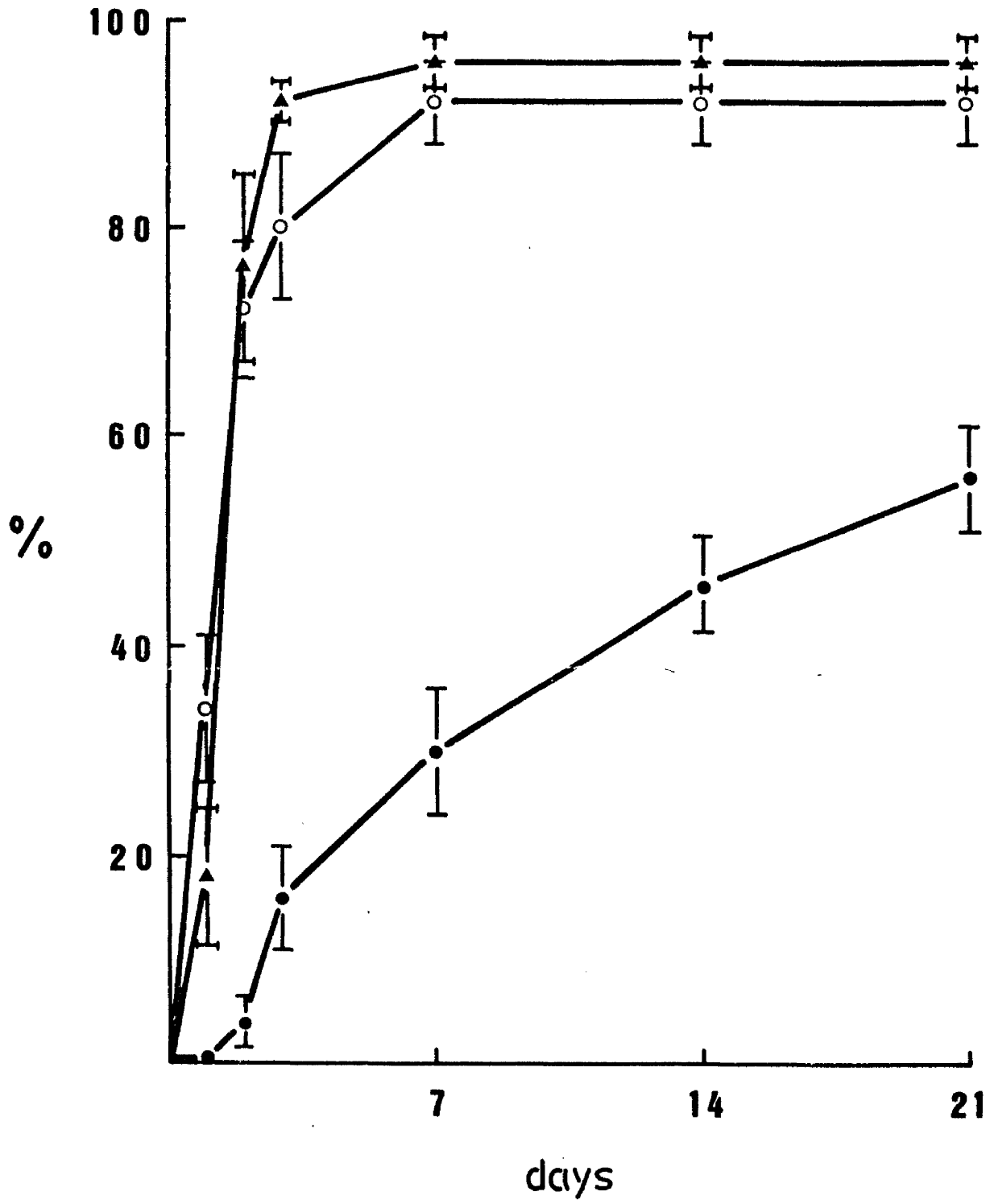


Figure 35.-

Chemicals. Germination promoters.

Time course of germination for intact fruit, scarified fruit and true seed at 10^{-4} M of indole acetic acid. Darkness, 25 °C. Lot 1.

In the abscissa percentages of germination \pm standard error, in the ordinate time in days.

Key: ● Intact fruit
 ○ Scarified fruit
 ▲ True seed

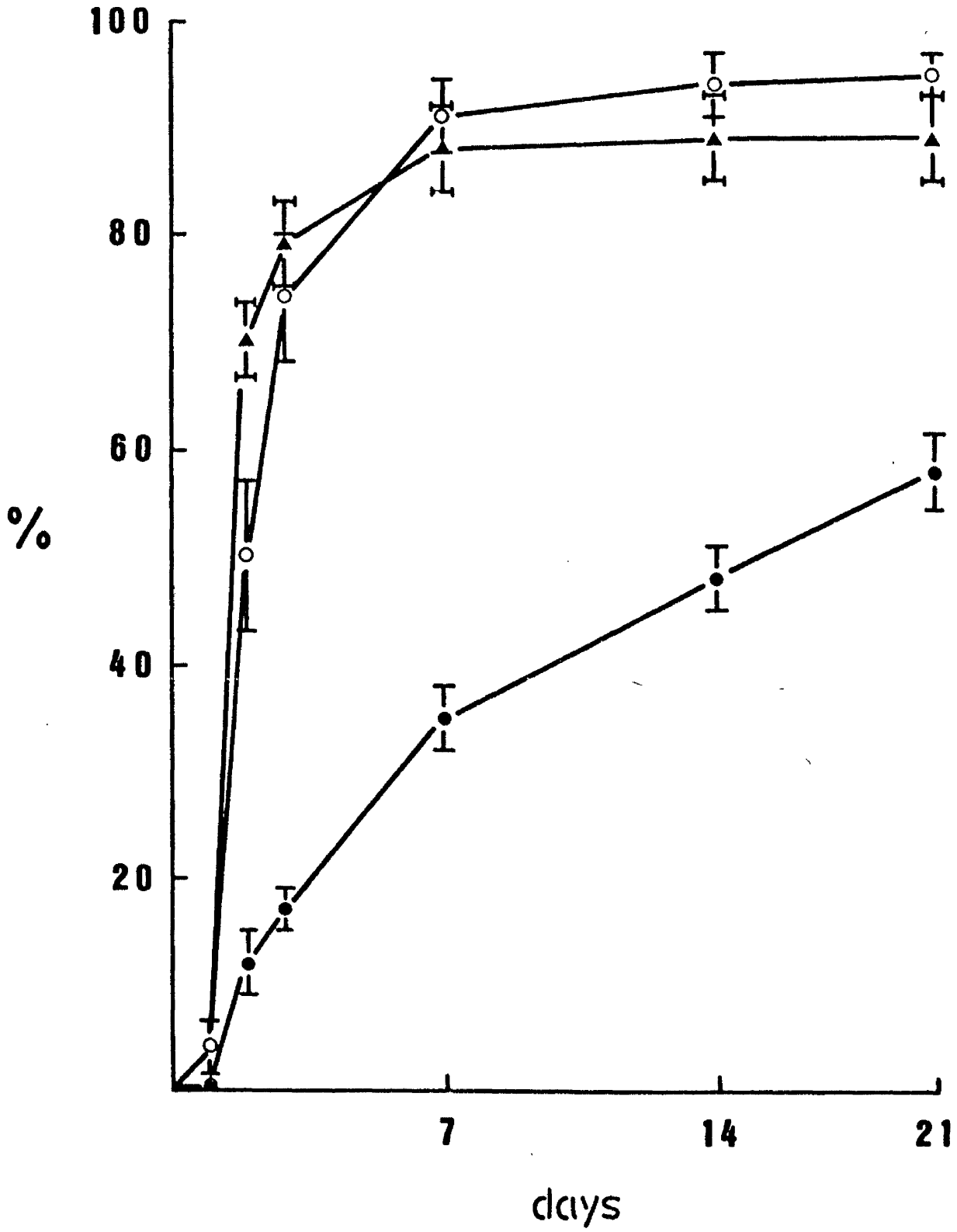


Figure 36.-

Chemicals. Germination promoters.

Time course of germination for intact fruit, scarified fruit and true seed at 10^{-5} M of indole acetic acid. Darkness, 25 °C. Lot 1.

In the abscissa percentages of germination \pm standard error, in the ordinate time in days.

Key: ● Intact fruit
 ○ Scarified fruit
 ▲ True seed

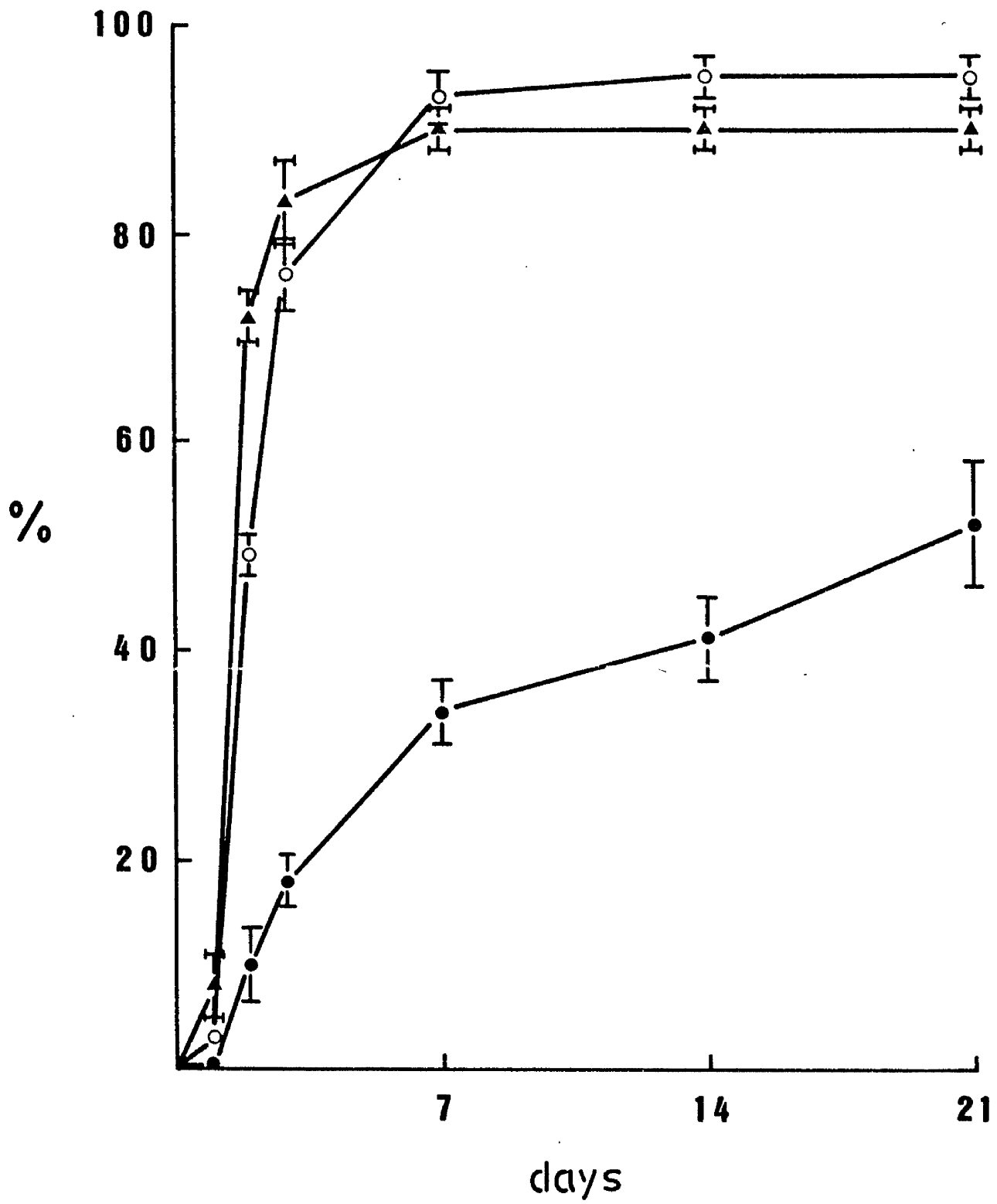


Figure 37.-

Chemicals. Germination promotors.

Time course of germination for intact fruit, scarified fruit and true seed at 10^{-4} M of kinetin. Darkness, 25 °C. Lot 1.

In the abscisa percentages of germination \pm standard error, in the ordinate time in days.

Key: ● Intact fruit
 ○ Scarified fruit
 ▲ True seed

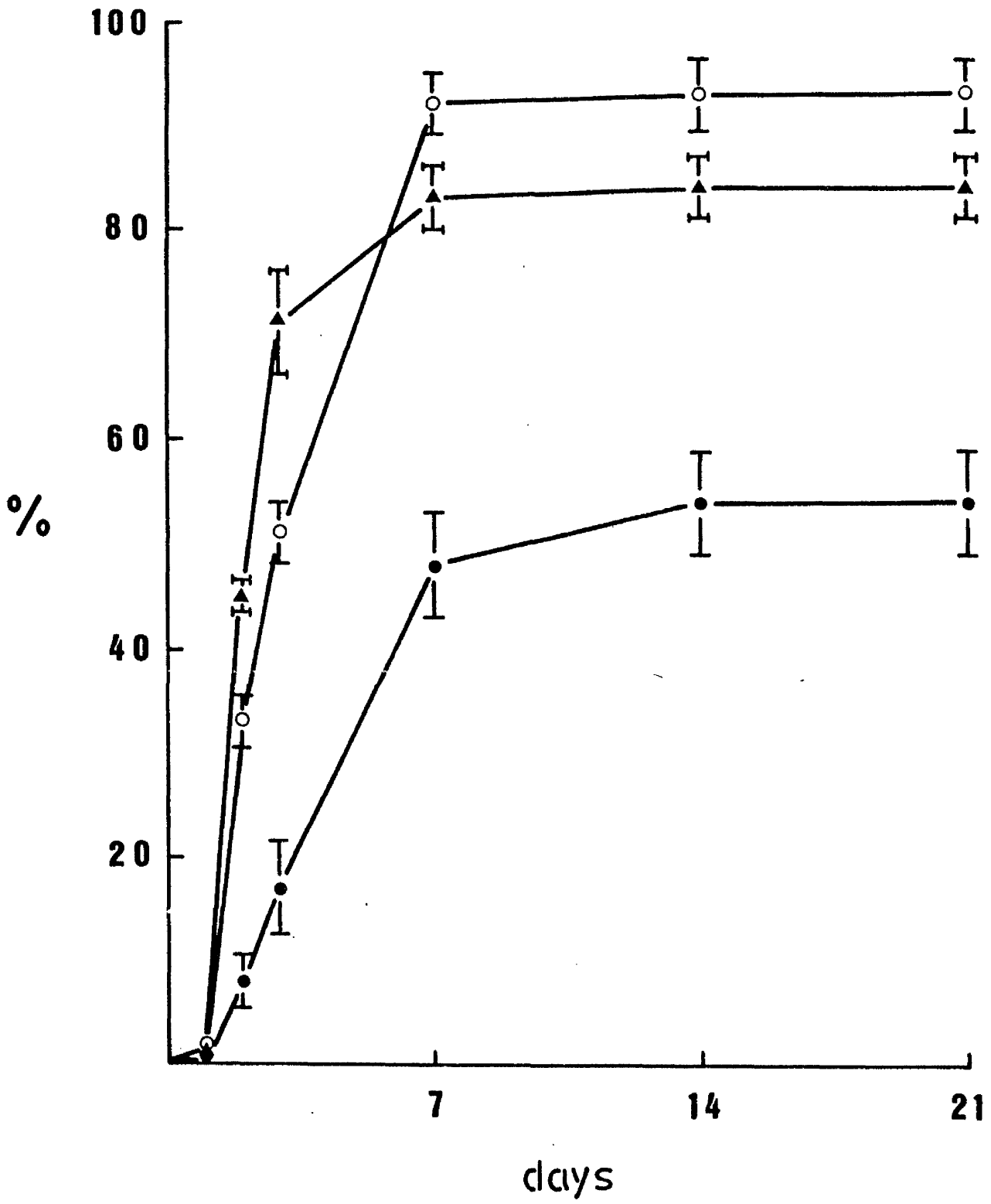


Figure 38.- Chemicals. Germination promoters.
Time course of germination for intact fruit,
scarified fruit and true seed at 10^{-5} M of
kinetin. Darkness, 25 °C. Lot 1.
In the abscissa percentages of germination
 \pm standard error, in the ordinate time in
days.

Key: ● Intact fruit
 ○ Scarified fruit
 ▲ True seed

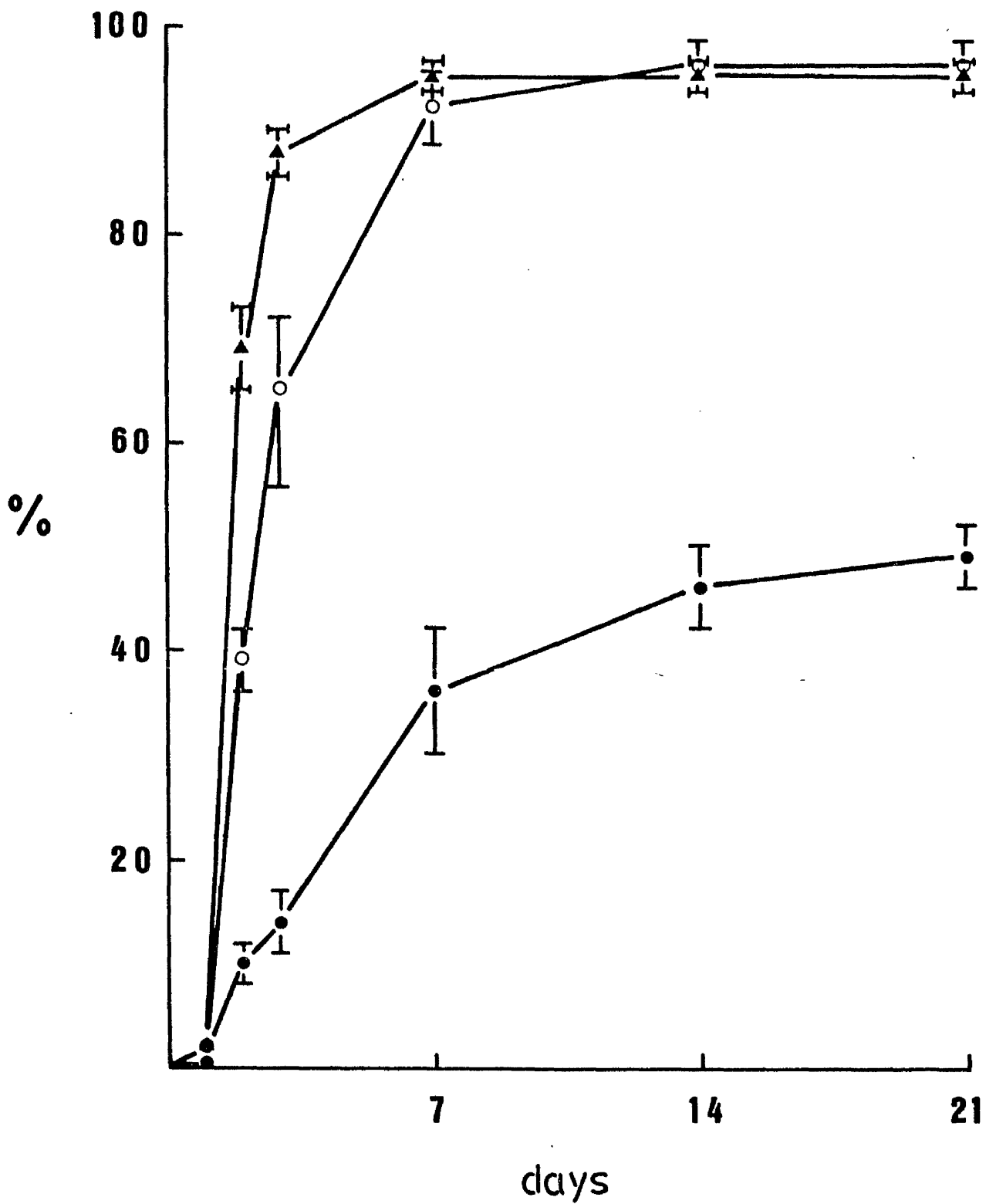


Figure 39.-

Chemicals. Germination promoters.

Time course of germination for intact fruit, scarified fruit and true seed at 10^{-5} M of gibberellic acid/indole acetic acid. Darkness, 25 °C. Lot 1.

In the abscissa percentages of germination \pm standard error, in the ordinate time in days.

Key: ⊙ Intact fruit
 ○ Scarified fruit
 ▲ True seed

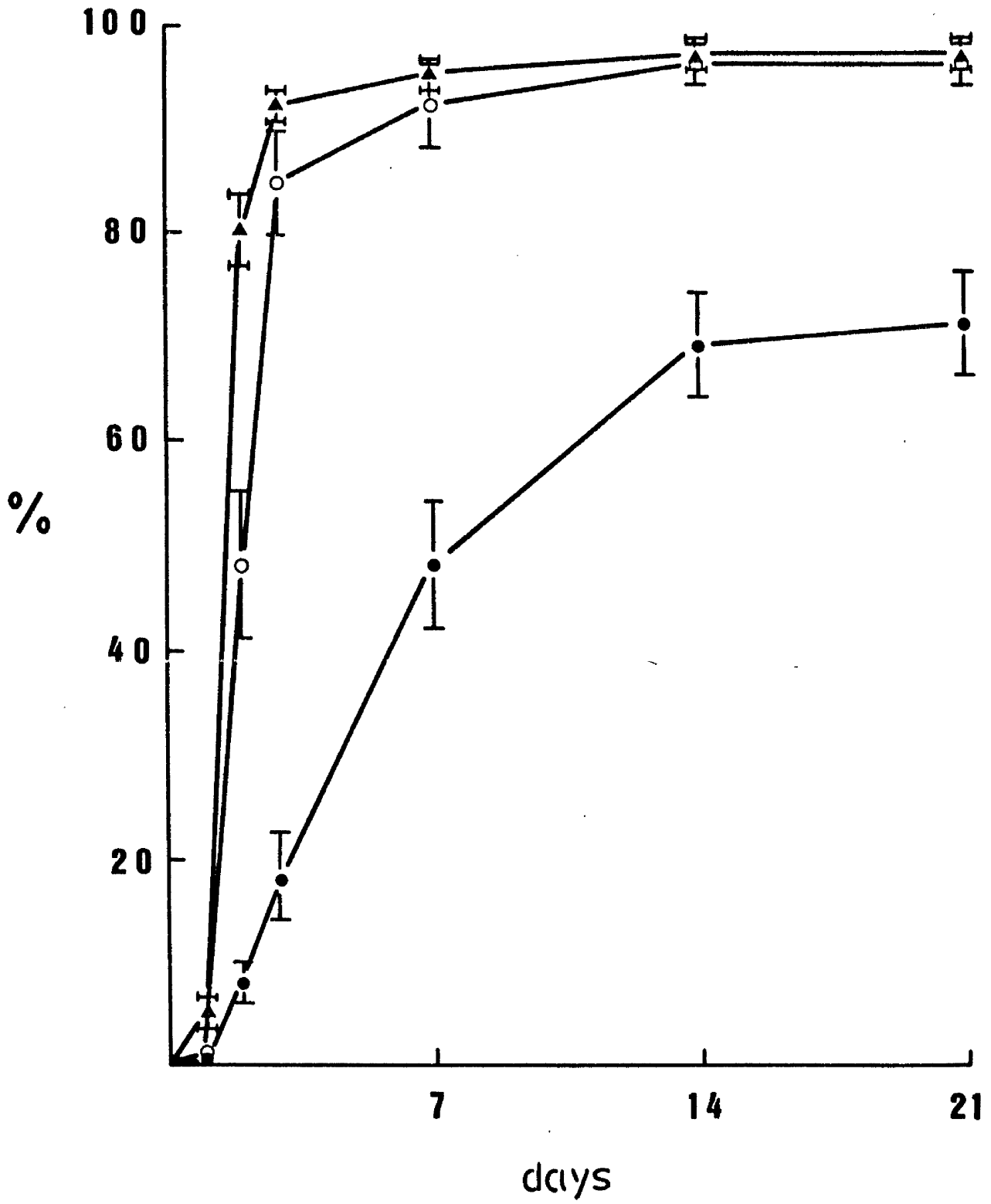


Figure 40.- Chemicals. Germination promotors.

Time course of germination for intact fruit, scarified fruit and true seed at 10^{-5} M of kinetin/indole acetic acid. Darkness, 25 °C. Lot 1.

In the abscissa percentages of germination \pm standard error, in the ordinate time in days.

Key: ● Intact fruit
 ○ Scarified fruit
 ▲ True seed

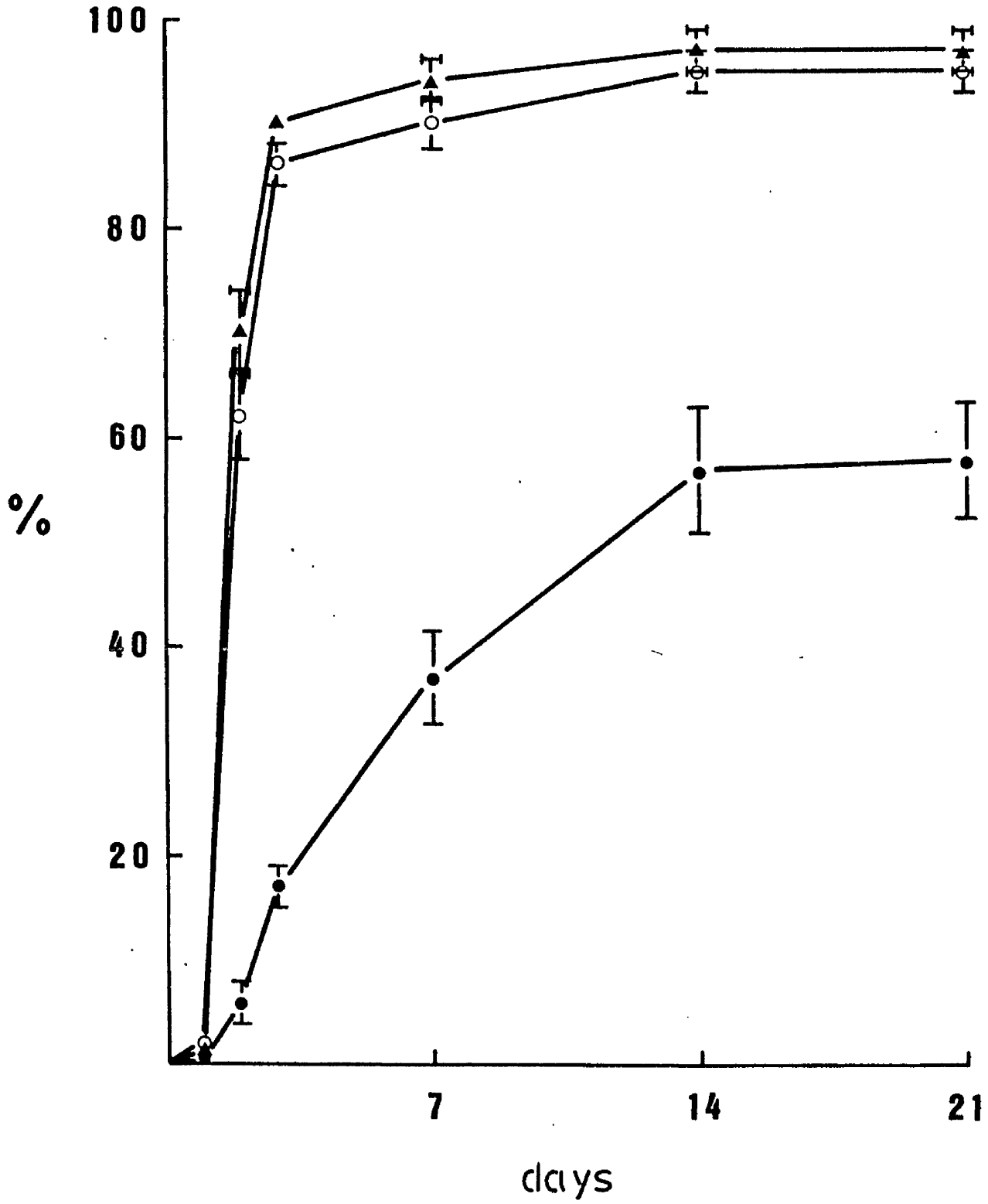
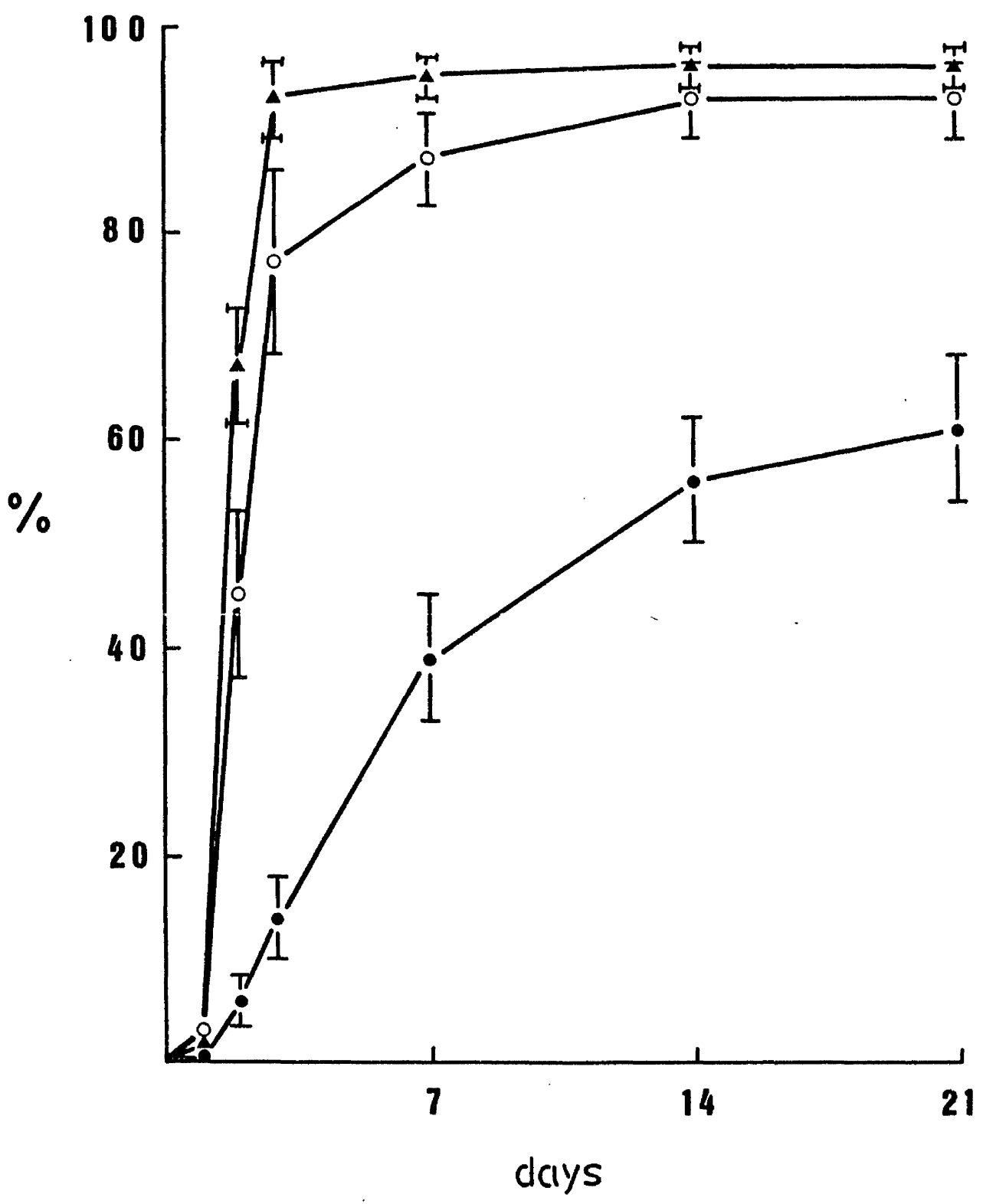


Figure 41.- Chemicals. Germination promoters.
Time course of germination for intact fruit,
scarified fruit and true seed at 10^{-5} M of
gibberellic acid/kinetin. Darkness,
25 °C. Lot 1.
In the abscissa percentages of germination
± standard error, in the ordinate time in
days.

Key: ● Intact fruit
 ○ Scarified fruit
 ▲ True seed



enzymic breakdown of the hormones by these tissues, although there is no evidence in support of this idea.

Inhibition

Of the many endogenous and exogenous growth and germination inhibitors reported, abscisic acid (ABA) is one of the most widely distributed in the plant kingdom (Addicott and Lyon, 1969; Wareing and Ryback, 1970). ABA was thus chosen as the inhibitor to be tested on sugar beet germination. In order to do so, intact fruits, scarified fruits and true seed from Lot 2 were allowed to germinate in different concentrations of ABA in darkness at 25 °C. The results are presented in Tables 17, 18 and 19. In Fig. 42 the percentages of germination after 28 days for both fruits and seeds are shown as a function of ABA concentrations.

It can be seen from Fig. 42 that ABA had no effect on the germination of intact fruit. The germination of true seed and scarified fruit, on the contrary, were affected by ABA. Concentrations of 10^{-6} M and 10^{-5} M did not inhibit germination, but a higher concentration of 2×10^{-4} M produced an inhibition of 14% for scarified fruit and 56% for true seed in relation to the values obtained for the water control. These results are additional evidence that the fruit coat may act as a barrier to the uptake of some substances. The true seed itself is clearly affected by ABA, but when it is completely covered by the fruit coat the chemical cannot exert its effect. Also, if the seed is partially covered by the fruit coat there will be a partial protection and ABA will have a reduced effect.

TIME (DAYS)	CONCENTRATION OF ABA (M)					
	2×10^{-4}		10^{-4}		10^{-5}	
	% G	+ s.e.	% G	+ s.e.	% G	+ s.e.
1	0.0	-	0.0	-	1.0	1.0
2	3.0	1.5	1.0	1.0	11.0	5.0
3	15.0	2.7	14.0	2.6	19.0	6.6
7	20.0	4.2	17.0	3.3	22.0	6.5
14	27.0	3.6	24.0	4.2	27.0	6.5
21	31.0	4.3	27.0	4.7	31.0	6.9
28	31.0	4.3	27.0	4.7	31.0	6.9

TIME (DAYS)	CONCENTRATION OF ABA (M)			
	10^{-6}		H ₂ O CONTROL	
	% G	+ s.e.	% G	+ s.e.
1	0.0	-	0.0	-
2	12.0	2.5	17.0	3.3
3	17.0	3.0	19.0	3.1
7	23.0	4.7	22.0	2.5
14	31.0	3.5	26.0	3.7
21	34.0	4.0	31.0	3.5
28	35.0	4.0	32.0	3.9

Table 17.-Chemicals. Effect of ABA. Percentages of germination for intact fruit at different concentrations of ABA. Lot 2. Darkness at 25 °C.

TIME (DAYS)	CONCENTRATION OF ABA (M)					
	2×10^{-4}		10^{-4}		10^{-5}	
	% G	\pm s.e.	% G	\pm s.e.	% G	\pm s.e.
1	5.0	2.7	6.0	3.0	8.0	2.5
2	11.0	4.6	19.0	4.8	51.0	4.0
3	33.0	4.7	41.0	4.3	69.0	4.6
7	47.0	5.2	52.0	3.6	76.0	4.9
14	55.0	5.2	60.0	5.1	79.0	4.8
21	55.0	5.2	60.0	5.1	79.0	4.8
28	56.0	5.4	60.0	5.1	79.0	4.8

TIME (DAYS)	CONCENTRATION OF ABA (M)			
	10^{-6}		H ₂ O CONTROL	
	% G	\pm s.e.	% G	\pm s.e.
1	6.0	2.2	15.0	4.5
2	51.0	4.8	56.0	5.1
3	62.0	5.7	66.0	4.3
7	67.0	6.5	70.0	4.2
14	72.0	7.7	70.0	4.2
21	72.0	7.7	70.0	4.2
28	72.0	7.7	70.0	4.2

Table 18.-Chemicals. Effect of ABA. Percentages of germination for scarified fruit at different concentrations of ABA. Lot 2. Darkness at 25 °C.

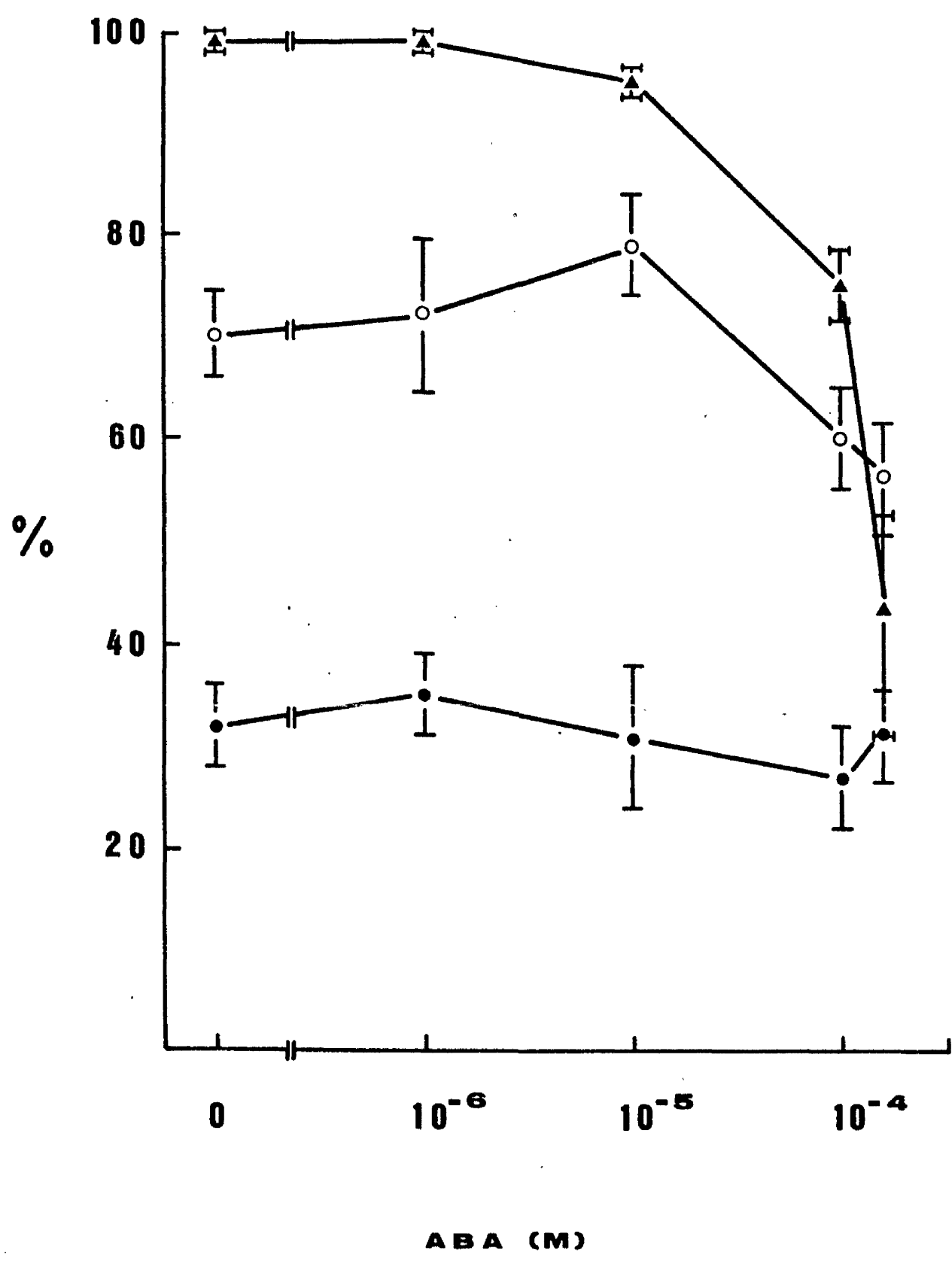
TIME (DAYS)	CONCENTRATION OF ABA (M)					
	2×10^{-4}		10^{-4}		10^{-5}	
	% G	\pm s.e.	% G	\pm s.e.	% G	\pm s.e.
1	0.0	-	0.0	-	2.0	1.3
2	4.0	1.6	10.0	2.6	65.0	6.4
3	14.0	3.0	32.0	4.5	86.0	3.4
7	28.0	5.9	42.0	4.1	94.0	2.2
14	37.0	9.1	66.0	5.0	95.0	1.6
21	41.0	9.9	74.0	3.0	95.0	1.6
28	43.0	9.4	75.0	3.7	95.0	1.6

TIME (DAYS)	CONCENTRATION OF ABA (M)			
	10^{-6}		H ₂ O	CONTROL
	% G	\pm s.e.	% G	\pm s.e.
1	5.0	2.2	19.0	4.6
2	86.0	3.4	91.0	3.1
3	96.0	1.6	95.0	1.6
7	97.0	1.5	99.0	1.0
14	99.0	1.0	99.0	1.0
21	99.0	1.0	99.0	1.0
28	99.0	1.0	99.0	1.0

Table 19.- Chemicals. Effect of ABA. Percentages of germination for true seed at different concentrations of ABA. Lot 2. Darkness at 25 °C.

Figure 42.- Chemicals. Effect of ABA.
Percentages of germination for 28 days for
intact fruits, scarified fruit and true seed as a function
of concentration of ABA. Darkness, 25 °C. Lot 2. In the
abscissa percentages of germination \pm standard error, in
the ordinate ABA concentrations.

Key: ● Intact fruit
 ○ Scarified fruit
 ▲ True seed



It is difficult to determine how ABA may be exerting this effect on germination. A wide variety of effects on many plants tissues have been reported, although the mechanism of such action is not clear at present. This is particularly true for the seed system and germination process. It is known that, in general, ABA behaves as a growth inhibitor when it is applied to actively growing systems and also as an inhibitor of anabolic processes, such as protein synthesis in non-growing tissues (Wareing and Phillips, 1970). During seed germination events inherent to germination as well as cell division and tissue growth are occurring. These events are intimately related and the experiments described here cannot distinguish which of the processes ABA may be affecting to produce the observed inhibition of germination.

Stains

In some of the of the experiments performed in this work it is frequently assumed that the fruit coat is impermeable to large molecules. Therefore some observations with stains were made, to see if large molecules are capable of passing through the fruit coat during the process of imbibition. The results are qualitative and give an indication of the capability of the stains to pass through the fruit material.

The results obtained with the three stains, methylene blue, neutral red and hematoxylin, were the same. After 72 hours the external fruit tissues showed a very intense coloration, but when the fruits were sectioned it was observed that the seed tissues remained unstained. All the seed structures - embryo and storage material - remained completely white although they were thoroughly imbibed. Neither of the testas were stained. A certain degree of penetration of the stains in the fruit material was also observed. The external part of the fruit was heavily stained but the coloration became less intense towards the internal part of the fruit. When the stain solution was added directly to the seed tissue of a sectioned fruit, it was observed that the tissue became intensely stained within a few minutes. This demonstrates that the seed tissue per se is able to take up the stain. Similar results were obtained with sectioned dry fruits. These observations were repeated at 96 hours and similar results were obtained.

These findings indicate that methylene blue, neutral red and hematoxylin molecules cannot pass through the fruit coat, and it is suggested that fruit coat is also impermeable to other large molecules. The mode of action of this barrier could be merely mechanical, or it could be physiochemical; it is unlikely to be a biological mechanism such as active transport since the fruit wall material is a non-living tissue.

The experiment thus gives further qualitative evidence in favor of the hypothesis that uptake of substances into the seed is prevented by an impediment or barrier.

GENERAL DISCUSSION

In all germination experiments using sugar beet it is of great importance to appreciate differences which may occur between batches of fruits from the same cultivar. These differences may not extend to differences in assessing viability but can be expressed in terms of their pattern of dormancy (Garner and Sanders, 1932; Price, 1938; Smith, 1952; Byford, 1963; Sneddon, 1963; Snyder, 1963-a, -b; Battle and Whittington, 1969-a, -b; Heydecker and Chetram, 1971). Indeed in the two batches used in this study viability assays showed that in excess of 90% of the fruits were capable of germinating. Using standard germination conditions at 25 °C sugar beet fruits will germinate very slowly with maximum germination of 67% for Lot 1 and 39% for Lot 2. Light does not appear to exert any effect and it may be significant that there are few descriptions dealing with light on sugar beet germination in the literature. The description presented in this thesis for the pattern of germination would appear to be one of the most detailed descriptions given for sugar beet. Data referring to percentages of germination for various incubation times and germination conditions are found in the literature, not only for monogerm varieties but for polygerm varieties as well (Tolman and Stout, 1940; Rehm, 1953; Hoover and Goodin, 1966; Francois and Gooding, 1972).

The range of constant temperatures within which germination takes place was found to be 0 °C - 40 °C, with a maximum response between 5 °C - 15 °C, a value below the optimum found by other investigators. Constant temperatures of 20 °C and 25 °C have been reported to produce maximum

germination for monogerm sugar beet (Hoover and Goodin, 1966; Francois and Goodin, 1972), and in some instances an alternating temperature of 20 °C for 16 hours and 30 °C for 8 hours have been reported as optimum (DeKock and Hunter, 1950; DeKock, Hunter and MacDonald, 1953).

Because germination under apparently optimum conditions does not approach the figure of viability it can be assumed that a dormancy mechanism is involved in the suppression of germination. The simplest dormancy mechanism would be the hindrance to water uptake by the fruit or seed coat, especially in those cases of a dispersal unit possessing a thick coat (See review of Wareing, 1969), but there is no evidence of a restricted imbibition in sugar beet seed. A study of imbibition has shown that the seeds imbibe fairly rapidly, completing their imbibition period within 24 hours, in addition, the presence of the fruit or seed coat does not delay this period. The results are in agreement with those found by Battle and Whittington (1969-b) who measured the rate of imbibition for clusters and seeds, finding no differences in their imbibition rates and that the imbibition period was completed in less than 24 hours.

It can be argued that because there is evidence that water is able to pass through the fruit coat to hydrate the seed tissues, most gases will also have the same facility of reaching the embryo: thus, in such a case any dormancy mechanism based on a restriction of gaseous interchange ought to be regarded with caution. The role of the fruit coat was investigated in those experiments dealing with scarification treatments and

complete removal of the coat. Removal of the fruit coat led to total germination, indicating that the seed itself is not innately dormant and that the presence of the fruit coat is responsible for the delayed germination. This is an important fact and it has been recently stressed by Heydecker et al. (1971), who claimed that the fruit material may act as a physical barrier to oxygen supply to the embryo. Peto (1964) has also shown that the presence of the ovary cap depresses germination, but he suggested a possible mechanical blockage of the emergence of the radicle.

It can be assumed that the presence of the fruit coat controls the germination behaviour in sugar beet, but the nature of this control may be due to several causes. Evidence that inhibitors are involved has been previously shown by Froschell (1939, 1940), Tolman and Stout (1940), Stout and Tolman (1941), DeKock et al. (1953), Massart (1957), De Roubaix and Lazar (1960), Van Sumere (1960), Abu-Shakra and Aqil (1969), Battle and Whittington (1969-b).

Nevertheless, no inhibition activity was noted in the crude water extracts described in this work. Failure to detect inhibition may be attributable to at least four causes. Firstly there may have been inadequate preparation of the extract, although they were prepared following the same procedure of Tolman and Stout (1940) and Abu-Shakra and Aqil (1969) who reported inhibition when their extracts were tested on sugar beet and other species. Moreover, DeKock et al. (1953) have used a crude water extract prepared in the same way and isolating and unsaturated yellow oil. They

found that the water extract strongly inhibited the germination of sugar beet and other crop seeds. Secondly, a low concentration of the extract can also be the cause of lack of imbibition, although the concentrations used were in the same range of those used by others who reported the prevention of germination. Thirdly, the batch of fruits could be a reason because the extracts prepared in this work were made using fruits which have been exposed to the mechanical treatment of rubbing, and this process may remove sources of germination inhibitors, e.g. perianth material, thereby decreasing the possibility of obtaining water extracts with a high concentration of inhibitors. Fourthly, there is the possibility that despite having an extract with an adequate concentration of inhibitors, there could have been inactivation during preparation perhaps by the action of light or oxygen.

On the other hand, different dormancy mechanisms might be involved. The scarification treatments on the fruit coat showed that germination increased with an increase in the extent of scarification. This suggests the existence of a mechanism involving the restriction of gaseous exchange. On the basis of two pieces of evidence, the hypothesis that removal of parts of the fruit coat leads only to a decrease in the levels of inhibitors must be discarded. viz a) the extracts obtained possessed no inhibitory properties, and b) some of the scarification experiments were done without excluding any part of the fruit material from the petri dishes and this latter point would tend to argue against a role of leachable inhibitors, because the some amount of leachable tissue as in intact fruit is still present. Nevertheless, the objection about oxidation of sensitive inhibitors within the ovary locule

cannot be eliminated. In Xanthium, for instance, it has been found by Wareing and Foda (1956) that an inhibitor in embryos of this species is enzymatically inactivated by oxygen. These authors consider that the beneficial effect of the seed coat removal in dormant seed is due to an increase in oxygen supply to the embryo, this bringing about the destruction of inhibitors.

The role of gas exchange is readily shown by the experiments using various oxygen tensions. Low oxygen concentrations are capable of promoting germination of scarified fruit and true seed, and 20% (equivalent to the normal oxygen concentration in the atmosphere) produces total germination. Intact fruit are markedly affected by oxygen concentrations, higher germination percentages are obtained increasing the concentrations above 20%. The results suggest that the fruit coat is an impediment to oxygen uptake; when this barrier is eliminated (scarified fruit, true seed) any concentration above the normal concentration in the atmosphere has no effect because the levels of oxygen required for the processes of germination have been fulfilled. It is interesting to note that in the intact fruit a maximum oxygen concentration of 100% is not capable of producing a germination percentage equivalent to scarified fruit and true seed. This indicates that the supposed barrier to gaseous exchange is very effective: in its absence only 20% oxygen is required to produce total germination, but when present a concentration of 100% in the environment is insufficient to allow the equivalent of 20% to reach the embryo.

The actual effect of oxygen on sugar beet germination can be due to an increase of metabolic events such as respiration, or, as it has been already pointed out, it may cause inactivation of inhibitors. Experiments with various H_2O_2 concentrations also showed that for intact fruits an increase in oxygen supply produces an enhancement of germination. There appears to be little information concerning to the role of oxygen, but DeKock et al. (1953) have found that the respiration of sugar beet tissues was prevented by the inhibitor obtained from sugar beet clusters. Chetram and Heydecker (1967) have suggested an interaction of inhibiting principles and oxygen, that is, that the restriction of oxygen supply renders the embryo sensitive to the inhibitor in the cluster material, or vice versa. Subsequently, Heydecker and Chetram (1971) claimed that contamination by microorganisms was largely responsible for oxygen consumption in sugar beet leading to the depression of germination by competition for oxygen.

The interesting work of Roberts (1964-a,-b, 1969) and Major and Roberts (1968) on respiratory inhibitors was briefly considered in this work by the use of potassium cyanide at "physiological concentrations", including those concentrations found by Roberts to promote germination in rice seeds. The germination of intact fruits was not affected by KCN. Scarified fruit and true seed were slightly inhibited by KCN, but the degree of this inhibition is very small, even for the higher concentrations used (10^{-4} M, 10^{-3} M) which are known to inhibit the respiration of certain living tissues (James, 1953). These findings can be taken as mild confirmation of Robert's hypothesis of the existence of an oxidation reaction

needed for germination not directly connected but competing for oxygen with the cytochrome oxidase system. Thus, any poisoning of the cytochrome oxidase system would not directly affect germination. This suggestion is also supported by the sensitivity of sugar beet seeds to oxygen. Beet seeds however, differ from rice in the sense that they will not germinate under anaerobic conditions, although there is some evidence that as in rice, the conditions around the embryo are relatively anaerobic because the fruit structures may act as a barrier to the inward diffusion of oxygen. In addition, any treatment that leads to an increase of oxygen tensions results in an increase of germination, e.g., scarification of the fruit coat, application of hydrogen peroxide and oxygen.

The lack of inhibition of KCN can be also due to problems of uptake of the chemical. This could be specially true for intact fruit. It is difficult, however, to assume the same for the true seed. KCN is a small molecule (m.w.=65.12) and molecules larger than KCN (e.g. ABA) can exert an effect on true seed. Alternative explanations for failure to inhibit germination could be that the substance is degraded within the seed tissues or that the concentrations of KCN were not high enough for this species.

The effects of washing and drying the fruits prior to germination are shown in the experiments on the hydration and dehydration sequences. When the fruits are subjected to a single hydration period of various lengths followed by a dehydration period, then a clear enhancement of germination is obtained. The promotion is greater with longer periods

of hydration. Now, if the fruits are not dried prior to germination, the dehydration treatment does not promote germination and in some cases radicle emergence is inhibited. There would appear to be no clear relationship between hydration time and germination. Washing the fruits has been shown to increase germination in sugar beet, an effect attributed to the leaching of inhibitors (Tolman and Stout, 1940). The results presented in this thesis can be explained on this basis, but they also suggest that a dehydration period is necessary to bring about the beneficial effect of the washing. Waterlogging might interfere with respiratory processes of the germinating seed, interference that may depend on an irregular water uptake by the fruits, thereby causing different oxygen tensions around the individual fruits. In fact, it has been reported that an excessive water supply causes the formation of mucilage around the fruit, thus preventing gaseous interchange (Heydecker et al., 1971). The dehydration process may cause cracks in the fruit wall by shrinking the fruit coat; with longer hydration periods it may be expected that the softening of the tissues will be greater, and consequently greater scarification by cracking will be obtained. This would result in improved germination. The beneficial effect of hydration - dehydration sequences may also involve the completion of early metabolic steps which prepare the seed for germination; these events may take place to a greater extent during longer treatments periods, and may be quite stable to gentle drying periods, as suggested for other seeds (Berrie and Drennan, 1971; Hanson, 1973).

The accumulative effect of hydration and dehydration cycles was also studied. There would appear to be two effects: a) The significant influence of the last treatment prior to germination (hydration or dehydration), and b) The substrate of the germination test (water or extract). When the treatment prior to germination is dehydration and the substrate water then an increase in the number of cycles above one had no effect in promoting germination. If, on the other hand, the substrate is extract then germination is inhibited for the first 3 cycles of H-D when compared to the water treatment. This pattern may be due to a supply of germination inhibitors in the extract in addition to those already present in the fruit; with successive cycles leaching of the fruit inhibitors takes place and the extract cannot supply the required levels of inhibitors to counteract the beneficial effect of the H-D cycles. The H-D cycles could also induce metabolic events prior to and necessary for germination, when these events have reached a certain level through successive cycles, the inhibitors of the extract would not be capable of suppressing germination. Yet another explanation for the effect of the extract is to assume cracking of the fruit coat, a process which would be greater with increasing numbers of cycles. It is feasible that when applied during the first three cycles the extract might impede gaseous exchange, but with scarification enough oxygen is able to reach the seed.

When the treatment prior to germination is hydration the results show that in general both water and extract are less effective than when the final treatment is hydration. By four cycles, however, the percentage germination data are similar regardless of substrate or cycle treatment.

An interesting aspect of this work is the effect of water sensitivity of the intact fruit. Small changes in the amount of water in the petri-dish (e.g. from 1 to 1.5 ml) markedly modifies the germination behaviour. This effect has been previously noted by Heydecker et al. (1971). The reason of this sensitivity is obscure, but it might relate to an increase in microbial population. This sensitivity shows how important it is to check carefully the conditions in germination studies of sugar beet.

Three germination promoters were tested on fruits and seeds. Treatment with gibberellins, indole acetic acid and kinetin is known to promote germination in many species (Mayer and Poljakoff-Mayber, 1963; Evenari, 1965; Stokes, 1965; Cleland, 1969; Fox, 1969) but they were found to have no effect on sugar beet. Several reasons can be advanced for this failure. Hormones such as the gibberellins and auxins have diverse structural forms, and not all of them are effective in all cases. Therefore, the promotion of germination may depend on the type of hormone. The substances used could also have been broken down in the seed tissues, or their uptake could have been impeded by the embryo coverings. Concentration is another factor that cause lack of promotion, especially when only two concentrations were tested for each substance, although they were within the range of concentrations used by other investigators.

The results obtained are in agreement with those found by Peterson (1958), Snyder (1959) and Hoover and Goodin (1966) and Coumans (1973), who observed little or no effect of gibberellins, KNO_3 and thiourea on sugar

beet germination, but obviously more experimentation is required on this aspect.

Further studies on hormonal effects were carried out with abscisic acid, a substance known to induce or retard dormancy and inhibit germination in many species (Addicott and Lyon, 1969). The results showed conclusively that the application of ABA modifies the germination pattern of sugar beet. Concentrations of 2×10^{-4} M inhibited the germination of true seed by 50% in relation to water controls, whereas intact fruit were not affected by ABA even at the highest concentration tested. Scarified fruit germination was only slightly affected by ABA treatment. These results could be taken as additional evidence in favor of the hypothesis of resistance to uptake imposed by the fruit coat; true seed is notably inhibited by ABA, but this hormone cannot exert any effect when the fruit coat is present.

The mode of action of this barrier can be merely mechanical by acting as a sieve to large molecules, but it can also be due to a more physiochemical effect and be controlled by forces similar to those of ionic exchange resins. The possibility of a mechanism of selectivity due to active transport ought to be ruled out, because it is difficult to assume such a mechanism in a non-living tissue like the fruit coat.

The aspect of resistance to uptake imposed by the fruit coat was considered further in two brief preliminary experiments concerning the use of stains and uptake of radioactive IAA. Intact fruits were placed in solutions

of methylene blue, neutral red and hematoxylin. Sections of the dispersal unit revealed that the fruit material was externally stained with an intense coloration, but the seed tissues remained unstained, although they were hydrated. Sections of the true seed placed in the stains showed that seed tissues can be stained. Although these experiments are only qualitative they indicate that large molecules such as stains cannot pass through the fruit coat.

The results on the uptake of radioactive IAA are not conclusive and different results were obtained depending on the experimental procedure. In one case it was found that of all the radioactivity detected 24% of it was detected in the seed but in another case only 5% of the radioactivity was found in the seed. The latter results appear to be more reliable when it is considered that the washing profile of radioactivity is more like that obtained for lettuce fruits (MacWha, 1973). It has to be emphasized, however, that these are only preliminary experiments and more research is required.

It has not proved possible to resolve the question of role of inhibitors in sugar beet. On the other hand this study does highlight a number of serious problems which need to be resolved.

Among the most important problems are: i) the chemical analysis of solutions found within the ovary locule; ii) uptake, metabolism and distribution of plant growth hormones; iii) investigation of the effect of hydration and dehydration cycles; iv) the role of oxygen and scarification,

especially the determination of usable levels of oxygen that reach the embryo through the fruit wall; v) the role of respiratory inhibitors to investigate the possibility of non-metabolic oxidation reactions needed for germination and finally, vi) careful consideration of the fruit batches and conditions for germination.

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