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A STUDY ON THE EFFECTS OF LOW TEMPERATURE PRE-SOWING TREATMENTS AND AGEING ON THE GERMINATION PERFORMANCE OF DIFFERENT TOMATO SEED LOTS

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ABSTRACT

Although the total germination capacities were not significantly different, ten different tomato seed lots differed in their vigour. LTPST caused significant improvement in germination rate both in high and low vigour seed lots of different cultivars of tomato seeds tested without altering their germination potential. The degree of improvement in germination rate was positively related to the initial germination rate of the seed lots and it was even possible to predict the effectiveness of the treatment based on the initial T50 of untreated seeds. Despite the treatment always causing a significant improvement in germination rate, the improvement in uniformity was lot-dependent and the treatment had little or no effect on initial seedling growth.

Increased SMC and temperature caused rapid loss of viability and germination rate during storage. The effectiveness of LTPST treatment before ageing differed with seed lot and subsequent ageing conditions used. The application of the LTPST after ageing restored the germination rate to that of unaged untreated seed but as expected had no effect on loss of viability. The survival pattern of tomato seeds under rapid ageing followed a normal distribution and parameters of survival curves were in agreement with the norms of the general viability model for orthodox seeds. On the basis of these results of LTPST on aged seeds, it is argued that factors affecting loss of seed vigour may be different from those causing loss of seed viability during storage.

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RAMAMURTHY RANGANARASIMHIAH

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CHAPTER 1
INTRODUCTION

Technological advancement has transformed traditionally low cost self—sustaining agriculture into an advanced capital intensive farming business aimed at maximizing net earnings. Todays management of farming aims at averting risks as much as possible. Getting timely, uniform and optimal stands of vigorous plants is a pre-requisite for this success, therefore the world farming community is demanding high quality seed suitable for sowing under all sowing conditions.

There is an enormous literature suggesting that pre-sowing treatments of various kinds could improve the planting quality of seeds. In particular, hydration treatments are reasonably successful in inducing early emergence with consequent improvement in plant growth, early harvesting and better yields although any improvement in relative growth rates of ensuing plants is debatable (Coolbear et al., 1987).

Some seed companies in USA and UK are already using pre-sowing seed treatments, particularly in vegetable crops. One major obstacle to the commercial application of pre-sowing treatments is the variability between cultivars and even between the seed lots of the same cultivar. There is very little literature demonstrating the repeatability and applicability of pre-sowing seed treatments to different seed lots and/or cultivars of the same species. Thus, one of main aims of this study was to investigate the effects of low temperature pre-sowing seed treatment (LTPST) on the germination performance of different cultivars and/or seed lots of tomato seeds and to determine whether treatment improves seed quality in different seed lots of tomato.

The commercial operation of seed production and marketing inevitably involve a transition stage of storage. The physical, physiological and biological factors affecting seed storability have been reasonably well established, seed moisture content and storage temperature being the most important factors in storage, exerting a great effect on the vigour and viability of the seed (Chin, 1988). Despite an enormous literature on seed storage there are only a few reports indicating the responses of different seed lots under the same

storage conditions. In tomato, the literature reviewed from 1978 suggests none. Pre-sowing treatments have also been used to retard seed deterioration during storage with mixed success and to improve the quality of stored seeds.

Tomato seeds was chosen for this investigation as it is economically the second most important vegetable crop in the world. Pre-sowing treatment LTPST of tomato seeds has already been shown to improve the seed quality (Coolbear et al., 1987) and also to protect or improve germination rate of stored seeds (Coolbear et al., 1984). LTPST was chosen to investigate its effects on different cultivars and seed lots of tomato seeds in storage as a continuation of this work.

Objectives of this study

With a view to exploring potential of LTPST as a pre-sowing and pre- and post- storage treatment, this study was designed with the following objectives:

a) To investigate the variation in response between cultivars and seed lots of tomato seeds to LTPST.

b) To investigate the variation in response between different cultivars and seed lots of tomato seeds to LTPST applied before and after ageing.

CHAPTER 2
REVIEW OF LITERATURE

Inevitably, seeds have to be stored for sometime from harvesting and several factors limit their ability to survive during storage. It is often considered that seeds are in store as soon as they reach maximum dry weight on the parent plant and are thus subject to deterioration from then on (Harrington, 1973). Any approach to tackling this problem should start from good production practice and the provision of proper storage conditions. Beyond this, seed treatments can be used to improve seed performance both in the field and in store. The literature on this topic is enormous.

This review highlights some established findings and developments in seed storage and the survival of seeds in store, followed by a discussion of the physiology of seed deterioration and finally the use of physiological seed treatments to protect and/or improve seed quality in the store and/or the field. Only the behaviour of orthodox seeds will be discussed.

2.1 SEED STORAGE

The provision on adequate storage facilities is an important aspect of any seed programme. Any loss in vigour and germinability is a reflection of seed deterioration in storage (Hampton, 1984) and has considerable economic impact. In fact the physical, physiological and biological factors affecting seed storability have been reasonably well established (Chin, 1988). It is now possible to store most orthodox seeds for several years without significant loss in viability. It is also possible to predict the seed survival of many species with some accuracy under constant storage conditions (Ellis and Roberts, 1981). Despite this, poor seed storage is still a major problem in both developed and developing seed industries because of economic constraints. Thus seed store management becomes a problem of risk assessment and cost-benefit analysis (Coolbear, 1989). To make appropriate decisions in this context, it is important to set clear objectives for seed storage operations and understand the factors affecting seed storage life.

2.1.1 Objectives of seed storage

Seed storage for further planting may be classified as follows:

a) Bulk Storage - Short/intermediate term (open storage)
b) Small scale storage - short term/intermediate term (packaging)
c) Small scale conditioned long term storage (genetic conservation).

In all these cases the choice of storage conditions is a compromise between optimum conditions and economic realities and requires good storage practice.

2.1.2 Factors affecting seed storability

Survival of seeds in store is the consequence of interactions between potential seed vigour determined genotypically and factors affecting storability. Several pre-storage and storage factors affect seed storability.

Pre-storage stresses include the following:

a) Parent plant stresses during development, e.g. mineral deficiencies, water and temperature stress may affect viability during seed development (Austin, 1972) and consequently affect seed storability.

b) Weathering after seed maturity (Woodstock et al., 1984).

c) Harvesting problems, e.g. poor harvest timing results in harvest of immature seed (Justice and Bass, 1978) and threshing damage (Moore, 1972).

d) Drying problems e.g. Escasinas (1986) showed reduced storability in maize due to rapid drying. Similarly damage during processing can also affect storability and is liable to insect

damage and attack by storage fungi (Moore, 1972; Hampton, 1984).

e) Effects of irrigation and use of plant growth regulators on seed quality also need to be examined.

Seed moisture content and storage temperature are crucial factors determining the life and death of seeds once in store (Chin, 1988). The higher these are, the more rapidly the seed deteriorates (Roberts, 1972a). Some exceptions to general principles are, low temperature may cause freezing damage (Roberts, 1972b), drying to very low levels of moisture content cause damage to seeds and also may cause soaking injury on rehydration (Powell and Matthews, 1978) and high hydration levels may trigger metabolic repair process, which may serve to limit the process of deterioration (Villers, 1974; Villiers and Edgcumbe, 1975).

Harrington's "Rules of Thumb" (Harrington, 1972) are useful indications of the relative effects of moisture and temperature on seed deterioration. Rule one states that for each 1% decrease in seed moisture content (SMC), the storage life of the seed is doubled between 5 and 14%. Rule two describes the effect of temperature: for every 5°C reduction in storage temperature the storage life of seed is doubled.

Although, these rules are not as exact as the formulae of Roberts and Abdulla (1968) and/or Ellis and Roberts (1981) (see section 2.2.3) they give a quick grasp of the importance of low SMC and temperature. Some other limitations of these rules include the restricted moisture range and the assumption of hygienic storage conditions (see section 2.1.4). In this latter context there may be severe interactions with seed processing methods, storage temperature and more particularly SMC. Biotic factors will be operative over 8% SMC and seed may even germinate in store over 40 - 60% SMC (Harrington, 1972).

Oxygen pressure and seed dressing chemicals are other important factors which affect seed storability. There is considerable scope for interaction between all storage factors and any pre-storage stress which the seed might have already experienced. Many deteriorative processes are oxidative, consequently increased levels of oxygen promote deterioration. Similarly many chemical treatments have an oxidising effect. Vacuum packaging may be of little or no use in improving the storage life of seeds (New, 1988). Increasing levels of carbon dioxide or Nitrogen around seeds may promote storability but are expensive. Some chemicals for example, organomercurials (in wheat and corn, Justice and Bass, 1978) can be toxic to general respiratory processes and their use should be avoided.

Good store management practice should aim at reducing pre-storage stresses, providing storage conditions which retard deterioration process and also take appropriate care in further handling. Suggested practical principles and key management decision areas are as follows:

1) Care at harvest and processing

2) Keep SMC low, but take care while drying

3) Keep store temperature low either by open ventilation or refridgeration.

4) Keep RH low in bulk storage, either by ventilation, or chemical or mechanical dehumidification.

5) Careful organisation of the store to maintain hygiene and good ventilation.

Key management decision areas include:

1) Assessing the situation: the design of the store facility should be based on the purpose of seed storage, the kind and amount of seeds to be stored and the availability of local materials.

2) Care in location of stores to maximise the advantage of natural variation in regional climates.

3) Care in day to day management of seed store to provide hygienic condition and also accurate monitoring of accessions and their location within the store.

4) Use of appropriate packaging materials in small scale storage (section 2.1.3) and prediction of seed survival particularly in long term seed storage (section 2.2) are important.

2.1.3 Seed packaging

The package is a storage container. It must protect the seed from physical damage, from losses or deterioration during storage, be useful for display and must be convenient to use (Southwick, 1974). Common packaging materials are cotton, jute, paper, aluminium foil, tinplate, plastic films, laminates, etc. The hazards incurred by packages in the tropics are more severe compared to temperate zones, because of poorer transport systems, increased risk of infection and, most importantly, climate (Warham, 1986). Often the choice of packing materials is based on cost effectiveness and availability of local materials. However, conditions under which the seeds are to be produced, processed and stored are also crucial. It is not a good policy to assume that temperate high-technology packing methods can be transferred directly to tropical situations.

Seed survival in packages depends on the ability of the packages to maintain low seed moisture content (Justice and Bass, 1978). The moisture resistance of the packaging materials is based on the water vapour transmission rate (WVTR) measured in $g/m^2/d$ and is the rate at which water vapour passes through a unit area of material per day at 37.8°C from 100% RH on one side to 0% on other. WVTR is a good physical criterion for the comparison of moisture protection by seed packaging materials of any

combination (Ching and Abushakra, 1965). Further moisture absorbance by packing materials is also crucial to prevent rotting.

2.1.4 Seed storage fungi

Fungi are classified into field and storage fungi (Christensen and Kaufmann, 1969). Field fungi and bacteria are of minor or no importance in storage, because the moisture content is too low for their proliferation. Christensen's studies on grain storage have demonstrated that storage fungi can be a major cause of quality losses in stored grain or seed (Christensen and Kaufmann, 1969). Storage fungi cannot proliferate on seed in equilibrium with RH below 65-70%, and maintaining storage RH at this level eliminates the storage fungi problem.

Aspergillus glaucus, A. versicolor, A. candidus and Pencillium Spp. are important storage fungi on tomato seeds and were found to accelerate the rate of deterioration (Kononkov et al., 1986). Seed treatments with thiram or captan were shown to protect the loss of germination in store in the tomato cultivar' Coimbatore-2' (Vaidivelu and Ramaswamy, 1983).

The main source of contamination of seed with storage fungi are the stores and seed handling procedures themselves. Little if any infection is of field origin. Insects and mites may be a major means of spreading infection and the increased moisture contents resulting from their activity may further promote fungal development. Seed treatment studies have shown that fungicides like carbendazion/maneb could be most effective in controlling storage fungi (Moreno-Martinez and Raminez, 1985) but some of the effective fungicides can also cause seed deterioration themselves (see 2.1.3).

2.1.5 Storage of tomato seeds

Based on a review of the literature from 1978, it appears that there has been no comprehensive study on the longevity of

tomato seeds under a wide range of storage conditions. Nevertheless, there is evidence to show that tomato seed have good storage potential, for example Zhuchenko et al., (1974) stored tomato seeds for 15 years.

The effects of storage environment on longevity of tomato seeds adopted from different studies are shown at Table 2.1. Rees (1970) work suggest that the frequency of seed death over time in tomato seeds under high temperature storage conditions is normally distributed. At the Plant Genetic Resource Unit of CATIE, Turrialba, Costa Rica, tomato seed are stored at -17°C for genetic preservation, and for short term storage at 5°C.

Storability of tomato seeds appears to differ with cultivars for example tomato Russian Cultivar 'XXIVA' lost viability in one year while cv. Moldavian Early lost its viability only after 10 years under the same storage conditions (Zhuchenko et al., 1979). Similarly Gill et al., (1983) noted 7 out of 10 cultivars had more than 70% germination after 3 years stored in paper bags under ambient conditions, but field emergence ranged from 32.2 to 85%.

Popovska et al., (1981) reported that tomato seed with an initial SMC of 8.23% stored well in cloth, glass polyethylene, nylon and tin containers for 8 years under ambient conditions retained over 85% germination. Even pregerminated seeds were successfully stored by placing them in 100 µm polyethylene bags either with vacuum or nitrogen at 7°C for 63 days (Ghate and Chinan, 1987).

Grout and Crisp's (1985) attempt to store cv. Ailsa Craig under fully imbibed conditions using 35% dimethyl sulphoxide (DMSO) as a cryoprotectant was only partially successful. The limitation of this technique were that rapidly cooled seeds failed to survive on transplanting into soil and slow cooled seeds grown up to maturity showed more abnormalities than controls. Lower concentrations of DMSO caused loss of germination. Effects of DMSO on orthodox seeds and cooling under low temperature are not clear.

TABLE 2.1 Effects of storage conditions on longevity of tomato seeds

Cultivar	Storage Conditions	Storage Period	Effect on Germination % Initial	Final	Reference
Down's Seedling	6% SMC 70°C	112 days	100	50	Rees (1970)
C44	6% SMC 80°C	25 days	100	50	
Minidown (GCR 39)	6% SMC 90°C	1 day	100	50	
Perfection	6.7% SMC 100% RH 42°C	10 days	79	36	Mitra & Basu (1979)
Perfection	6.7% SMC ambient condition [a]	4 months	79	62	
Pusa Ruby	6.7% SMC ambient condition	4 months	79	58	
CO -2	7% SMC ambient	30 months	NI	32-48%	Vaidivelu and Ramaswany (1983)
10 cultivars includes Pusa Ruby, Sioux & Reckruth	ambient condition in paper bags	3 years		>70 <70	Gill et al., (1983)
UC 204	6% SMC (dw) 10°C	6 months	94	91	Alvardo &
6203	6% SMC (dw) 20°C	6 months	93	93	Bradford
	30°C	6 months	91	90	(1988)
Moneymaker	70% SMC (dw) 45°C	7 days	100	0	Coolbear & Francis (1984)
Not indicated	95% RH 45°C	7 days	NI	50	Loubser (1989)
Not indicated	8.23% SMC ambient[b]	8 years	NI	>85	Popovska et al., (1981)
UC 2046	6% dwb 30°C	1 year	100	100	Argerich
	50°C	16 days	100	2	et al., (1989)
Alisa Craig	7.5% SMC sealed in air	15 years	100	90%	Harrison
	7.5% SMC sealed in CO_2	17 years	100	95%	(1966)

Note: NI - not indicated
[a] RU 75%, mean temp. 27°C; [b] temp. range 11 - 24°C, RH 46 - 86

2.2 ANALYSIS OF SEED SURVIVAL IN STORE

As already noted, there is considerable variation in the storability of different seed lots of even the same cultivar under identical conditions (Delouche and Baskin, 1973). Further, during short term storage, seeds can deteriorate substantially without apparent loss of germination. Thus, the assessment of relative storage potential, the evaluation and ranking of seed lots and the prediction of seed survival in storage are all important components of seed storage.

2.2.1 Seed vigour and storability

Seed vigour is a multiple concept (Perry, 1987) and an important seed quality component (Hampton and Coolbear, 1989). The definition of vigour (Perry, 1981) encompasses potential seed performance both in the field and in storage.

Generally, loss of viability is preceded by the loss of vigour (Heydecker, 1972; Delouche and Baskin, 1973; Ellis and Roberts, 1981). In the deterioration model proposed by Delouche and Baskin (1973), it is suggested that deterioration of several aspects of seed vigour precedes loss of germinability. Ellis and Roberts (1980) have shown a close correlation between initial viability and most other aspects of seed vigour. It has been pointed out that these important differences in seed quality are not detected by the standard germination test particularly at high viability values (Ellis and Roberts, 1980, 1981; Roberts, 1983).

Ellis and Roberts (1981) suggested that there is a continuity between loss of potential seed quality based on genotypes and ultimately, loss of seed viability during storage. This may be an oversimplification as all aspects of the seed deterioration process are not necessarily a sequential process or the part of the same continuum in which the loss of viability is merely an end point. The process of seed deterioration is a matrix of interrelated events (Priestley, 1986) (see Section 2.3). There is no evidence, for instance

that the storability of the seed inevitably reflects its ability to germinate under water or temperature stress conditions in the field.

2.2.2 Ageing techniques and prediction of storability

Artificial ageing techniques have been particularly used for the prediction of seed storability. All have been comprehensively described by Perry (1981) and/or AOSA (1983). However, ageing conditions for each species have yet to be standardised.

The promotion of seed deterioration following exposure to high temperature and moisture content forms the basis of these methods. In Accelerated Ageing (AA) seeds are held at high RH and allowed to take up moisture naturally while being held at adverse temperatures (Delouche and Baskin, 1973; Baskin, 1977). In Controlled Deterioration (CD) seeds are taken to a required moisture content before holding in moisture-tight containers at adverse temperatures (Matthews, 1980; Powell and Matthews, 1981). The comparative germination performance and ranking of seed lots after deterioration provides a vigour index for the seedlot which is known to correlate well with storage potential. The main assumption of these tests is those seeds that deteriorate rapidly under ageing conditions will also tend to perform poorly in open storage conditions (Delouche and Baskin, 1973; Matthews, 1980).

AA test results were shown to correlate well with the storability of seeds under open storage conditions (Delouche and Baskin, 1973; Baskin, 1977). One limitation is that the AA test results is confounded by the seed moisture status during the testing period. This parameter will not necessarily be related to seed vigour and may be affected by factors such as initial SMC, testa integrity, permeability and also by the position of the seed within the high humidity chamber used for the test. The CD test avoids some of these reproducibility problems because of its precise control over SMC. Some good correlations have been obtained with

commercial storage potential and also field emergence using the CD test on a range of species (Powell and Matthews, 1981, 1985; Matthews and Powell, 1987). The CD test has been shown to be reproducible for some species but not all (e.g. Powell et al., 1984; Matthews and Powell, 1987).

Ageing techniques have also been employed in studies of physiology and biochemistry of seed ageing. Two principal justifications have been advanced for its use in this context. Firstly, accelerated ageing has been used as a means to circumvent the need for experimental analysis that would otherwise extend over many years of storage (Priestly, 1986) and secondly some workers have attempted to investigate seed deterioration under simulated tropical conditions, which may sometimes approach those used in conventional accelerated ageing experiments (Minor and Paschal, 1982).

2.2.3 Viability equations and nomographs

The clear understanding of the relationship between seed viability decline and SMC and storage temperature has led to the suggestion that the rate and pattern of loss of viability will take place in a predictable manner in orthodox seeds and has been shown to be described by three basic viability equations (Roberts 1972a). The frequency of individual deaths in time in a population stored in constant hygienic conditions is described by the normal distribution

$$Y = [1/\sigma\sqrt{2\pi}] \exp [- (p-\bar{p})^2/2\sigma^2]$$

where Y is the relative frequency of deaths occuring at time p, $\bar{p}$ is the mean viability period, and σ is the standard deviation of the distribution of deaths in time. Consequently, the seed survival curve is a cumulative normal distributions of negative slope, which becomes a straight line if percentage viability values are transformed to probit. The spread of the distribution is directly proportional to the mean viability period, that is:

$$\sigma = K\sigma \bar{p}$$

where $K\sigma$ is a constant for the seed lot. Lastly, the relationship

between SMC (m) (Percentage on fresh wt basis) and storage temperature (t in °C) and mean viability period ($\bar{p}$) is described by the equation:

$$\text{Log } \bar{p} = Kv - C_1 m - C_2 t$$

where Kv, C_1 and C_2 are seed lot constants. In order to use these equations conveniently, Roberts and Roberts (1972) developed a series of nomographs based on them. The drawbacks of these equations were that the calculated constants were specific for individual seed lots, they assumed constant and hygienic storage conditions and they only worked accurately within a limited range of temperatures (5-35°C) and moisture contents (5-25%).

Based on observations of seed ageing in a wide variety of conditions of controlled temperature and humidity, Ellis and Roberts (1980a) developed a modified descriptive equation:

$$V = Ki - p/10\ [K_E - (C_W \times \log m) - (C_H \times t) - C_Q \times t^2)]$$

which predicts probit % viability (V) after any storage period (P in days), at any temperature (t in °C) and air dry moisture content (m in % fwt), where 'Ki' is the seed lot constant and K_E, C_W, C_H and C_Q are the species constants. The denominator in the above equation quantifies the standard deviation (σ) of the frequency distribution of seed death in time, which is species specific and constant irrespective of lots and cultivars under a given constant storage conditions (Ellis and Roberts, 1981a; Ellis et al., 1982). Thus seed storage conditions inevitably affect the slope of the survival curve ($1/\sigma$) but not the initial viability Ki before the seeds are stored, where as genotype and pre storage environment affect the value of Ki but not the slope ($1/\sigma$).

The practical application of this equation is that it is applicable to a wide range of conditions and it takes into account the intraspecific variations in seed quality. Nomographs and viability constants have been worked out in several crops including barley (Ellis and Roberts, 1980b), onion (Ellis and Roberts, 1981a) Soyabean, Cowpea and Chickpea (Ellis, 1988).

The improved viability equations were also applied by other workers, but in a modified form where the term: $(-C_H t - C_Q t^2)$ has been simplified to $-C_2 t$ in species the Araucaria columanaris (Forst). Hook (Tompsett, 1984), Lupinus polyphyllus (Dickie et al., 1985) and apple Malus domestica (Dickie and Bowyer, 1985).

2.3 PHYSIOLOGY OF SEED DETERIORATION

Deterioration is a common phenomenon in living things. This process results from a complex interaction of time, environmental factors, intrinsic constituents and mechanisms in the seed itself. However, seeds do have inbuilt repair and detoxification mechanisms to adapt themselves for survival in a wide range of unfavourable conditions. Despite an incomplete understanding of this process, the literature is extensive and fast growing. Some important physiological aspects of this process are discussed in this section.

2.3.1 Effects of ageing

As seed deteriorates, losing vigour (e.g. slower growth, abnormal growth, increased leachates, susceptability to stress) and viability in storage many subcellular changes occur, but it is still difficult to be certain which are the cause and consequence of deterioration (Roberts, 1983). The damage caused during deterioration could be pathological or physiological. The type of damage, site of deterioration and critical importance may vary from species to species, cultivar to cultivar and ageing conditions. It is more probable that several important cellular systems become significantly degraded with age, so that inefficiencies in any one function amplify imperfections in others. All the processes occurring during deterioration are not necessarily irrepairable or irreversible. Loss of vigour and viability is essentially the outcome of a race between deteriorative events and the repair processes designed to combat them.

Currently there are several arguments in the literature about the relative importance of these different deteriorative events which will now be discussed briefly in turn.

2.3.2 Membrane damage

Damage to the membrane is generally considered to be an important cause of seed deterioration because of its fundamental nature. Any breakdown or change in property of the seed membrane system is bound to have far reaching effects for the cell. The ability of the seed to reorganise its membrane rapidly as the dessicated tissue rehydrates is crucial for successful germination. Ageing may impair the capacity for rapid repair and subsequent maintenance of functional cellular and organellar membranes.

Free radical damage to cell components is the main candidate for the fundamental cause of seed deterioration. A free radical (FR) is an atom or group of atoms with an unpaired electron, which possesses the ability of donating or receiving an electron. The importance of FR reactions, formation and their consequences are well established (Tappel, 1973; Harman and Mattick, 1976; Stewart and Bewley, 1980: Buchvarov and Gantcheff, 1984). The hydroxyl (•OH) and superoxide (O_2-) are the two most important radicals believed to cause most damaging biological action. Main pathways of FR production are photolysis (Turro, 1965), enzymic processes (Yamazaki, 1971). Lipo-oxygenation of polyunsaturated fatty acids (PUFA's) (Pryor, 1976) and metal ion initiation. the FR chain reaction cycles may be terminated by reactions with another FR, natural scavengers (e.g. α-tocopherol, ascorbic acid, Haemoprotein, isoflavanoids), quenching with water or superoxide dismutase (SOD) activity.

The loss of structural integrity of cellular membranes, together with the deterioration of macromolecules, has been shown to be correlated with loss of viability (Villiers, 1980), although it is difficult to conclude, whether it is a cause or consequence of cellular debility.

Some symptoms are for example, the coalescence of lipid bodies (Anderson et al., 1970; Harman and Granett, 1972), the withdrawal of plasmalemma from the cellwall (Hallam et al., 1973), the unnatural distension of the outer mitochondrial membranes and irregularities in nuclear chromatin (Hallam et al., 1973) and damage to the nucleus, such as necrotic nuclei with condensed patches of chromatin (van Staden et al., 1981). There is evidence to show that some ultrastructural irregularities can be repaired within 48 hours of imbibition (Berjak and Villiers, 1972: maize).

Losses of lipid from membranes has often been associated with seed deterioration during ageing, e.g. in tomato seeds the loss of membrane lipid was associated with seed deterioration, although in artificially aged seed of the tomato cultivar Kingly Cross, this appeared to be a deteriorative event occuring after the loss of germinability (Francis and Coolbear, 1984). Moisture status, ageing regime or cultivar used may cause differences in this aspect of seed deterioration. Care must be taken in these studies to equate the viability or vigour of seed aged by different methods. For example, Priestley and Leopold (1983) noted little change in Phospholipids (PL) in naturally aged seeds where loss of viability was only 12%, but marked losses of PL (particularly phosphatidylcholine) associated with artificially aged seed where viability loss was 95% under a high humidity ageing regime (Priestley and Leopold, 1979).

The leakage from seed often increases with age as a result of membrane damage. For example, in soyabean (McDonald and Wilson, 1980) and sunflower (Halder and Gupta, 1980) the leakage of electrolytes during imbibition was highly correlated with ageing. This relationship between imbibitional leakage and ageing cannot be assumed to be the same for all species and/or ageing conditions. For example in tomato (Coolbear et al., 1984), in melon seeds (Pesis and Ng, 1983) there were no effects of ageing on the electrical conductivity of the leachates from these seeds.

2.3.3 Enzyme changes

Numerous attempts to correlate loss of viability and vigour with decreased activity of certain enzymes have been made. For example, decreased amylase activity has been noted in aged Rice (Rao et al., 1954), wheat (Fleming et al., 1960), Barley (Anderson, 1970), dehydrogenase enzyme (Moore, 1972).

Perl et al., (1978) categorised 3 types of enzyme activity in ageing sorghum seeds, viz.

a) Enzymes which increase early in the ageing process followed by a decrease, e.g. Amylase, glutamic Pyruvic transminase, Rnase and Glutamic decarboxylase.

b) Enzymes which show a continual decrease in activity throughout ageing, e.g. Acid phosphatase, tetrazolium reduction.

c) Enzymes which increase in activity, e.g. Proteinase.

The increase in first category of enzymes could be ignored on the basis that at high moisture in the initial stage of ageing process, seed will attempt to gear up for germination. The increased activity of protease may be important as lack of compartmentalisation or activation of protease may result in destruction of other enzymes in the seed. Sobieraj and Kulka (1983) showed that ageing caused a decrease in the level of a natural trypsin inhibitor which supports the results of Perl's group.

Loss of activity of repair and detoxification enzymes may also be an important component of seed ageing. Stewart and Bewley (1980) showed that non-viable soybean seeds failed to show any superoxide dismutase activity in early germination.

There are several complicating factors in the study of enzyme changes in seed deterioration, one obvious concern, particularly

relevant when seeds deteriorate at relatively high levels of hydration, is that some reported increases in hydrolytic activity may be due to fungal invasion. Uncertainties can also arise from the mechanics of the assay. Many enzymes exist in multiple forms or isozymes that vary slightly in composition and are coded for by different genes. Depletion of co-enzymes may be responsible for reduced enzymic activity within aged seed (Burton, 1982). Shifts in pH optima may also complicate the enzyme investigation (Kulka, 1971).

2.3.4 Respiration changes

Several aspects of respiratory metabolism are correlated with seed deterioration. For example, decline in O_2 uptake and increase in respiratory quotients (Woodstock and Grabe, 1967). Losses of ability to produce ATP were found to be highly correlated with loss of viability in cauliflower, rape and soybean seeds (Lunn and Madson, 1981). This correlation is not universal and, of course close correlation doesn't necessarily imply cause and effect. Several studies suggest that respiration rate doesn't always correlate with vigour (Abul-Baki, 1969; Anderson, 1970; Byrd and Delouche, 1971). Abdul-Baki and Anderson (1973) have suggested that biochemical and physiological studies of seed deterioration should be focussed on the embryonic axis rather than on the intact seed as a decrease in the metabolic activities of the embryo will be directly associated with a loss of vigour.

In soyabean seed, it has been observed that the respiration rate was decreased with ageing in both whole seed and the embryonic axis and an increased respiratory quotient was noted (Woodstock et al., 1984). It is suggested that imbalances in the components of respiratory metabolism, e.g. uncoupling of oxidative phosphorylation or loss of co-ordination between activity of glycolysis and the krebs cycle may be an early indication of ageing. Excess glycolysis may result in anaerobic respiration and the production of ethanol and aldehydes, both of which are toxic to seeds. Woodstock and Taylorson (1981b) have also shown that slowing imbibition of aged

seeds gave them a chance to repair some of their respiratory metabolism.

Takayanagi (1977) showed that Pentose-Phosphate pathway (PPP) activity was dramatically increased in aged rape seeds. The occurance of alternative respiration may have little significance except that it is less sensitive to ageing damage than normal respiration and thus can compete for oxygen more efficiently.

2.3.5. Impaired protein and RNA synthesis

Protein synthesis which is usually evident well within the first hour of imbibition is reduced in aged seed (Osborne et al., 1974). The location of ageing lesions that can lead to failure of protein synthesis at germination include damage to ribosomes, losses of enzyme activity and loss of DNA and/or mRNA (Osborne, 1980). Several or all of these factors probably serve to reduce the overall ability of aged seed to synthesize proteins. This deficiency in turn, has a severe consequence for the effectiveness of cellular repair mechanisms that operate during the early stages of imbibition. Usually protein synthesis is organised on a membrane surface. Therefore, membrane damage may be an important factor contributing to loss of activity.

2.3.6 Genetic damage

Although total DNA may not change in aged seeds, it has been shown that certain qualitative change may take place, e.g. the amount of spoolable DNA from non-viable rye embryos was reduced (Osborne et al., 1980). This is a crude index of fragmentation and was confirmed by separation of total nuclear DNA on a sucrose density gradient which revealed that randomly sized fragments of low molecular weight DNA were accumulated in stored seeds. Normally repair enzymes (e.g. ligases) may repair the breaks in DNA, for example, DNA synthesis is more intense in partially deteriorated seed (Osborne, 1983). Dourado and Roberts (1984b)

showed that specific point mutations caused by ageing were heritable e.g. albinism in barley and pea seeds. Many of the chromosomal aberrations noted in germinating seed may be lethal to the cells and it has been suggested that loss of a critical number of cells can compromise viability (Roberts et al., 1967).

2.3.7 Hormonal changes

Changes in hormone sensitivity (Aspinall and Paleg, 1971) or a reduced ability to produce hormones (Harrington, 1973) might be expected to be involved in seed deterioration. Evidence for this is sketchy. Petruzzelli and Taranto (1985) showed that preapplication of GA_3 in acetone increased the resistance of wheat seed to ageing. Huber and McDonald (1982) found little or no effect of applied GA on aged barley seeds. Puls and Lambeth (1974) found that application of kinetin and KNO_3 improved the performance of naturally aged tomato seeds, while GA had no effect on germination performance (see Section 2.5.3).

2.3.8 Accumulation of toxic metabolites

Toxic metabolites are the cause of many secondary events in the deterioration of seeds. Ethanol, aldehydes (see Section 2.3.4), short chain fatty acids and phenolics are found in aged seeds. Whether the effects of these compounds are more deleterious than the damage that produced them in the first place is still a matter for debate.

2.3.9 Role of microflora in seed deterioration

Production of toxins and extra cellular hydrolytic exzymes such as lipase are main means by which microflora damage the seeds (Halloin, 1986). There has been very little attempt to integrate studies on the physiology of seed deterioration with studies on the pathology associated with it (Halloin, 1986).

2.4 PRE-SOWING SEED TREATMENTS (PST)

2.4.1 General principles

All seed treatments are designed to improve or protect seed quality. Essentially there are 4 types: dormancy breaking, chemical, energy and hydration treatments (Coolbear, 1988). Such treatments have been reviewed in detail by Heydecker and Coolbear (1977) and the possible basis for their effects by Khan et al., (1980). It is not the objective here to discuss all the many and varied published methods. Discussion is limited to pre-sowing hydration treatments aimed at enhancement of germination performance of seeds. Firstly, the need for and the types of hydration treatments will be outlined and then possible mechanisms for their effects will be highlighted with particular emphasis on tomato seeds.

The time from planting to seedling establishment is a crucial phase in the production cycle. The period of imbibition is extremely sensitive to changes in the environment, and slight or sudden changes appear to profoundly affect seedling emergence (Khan, 1977). Percent emergence and uniformity of direct seeded crops can have a major impact on stand establishment, yield and quality (Bradford, 1986). Any treatment which aims to reduce the period between sowing and emergence or allows seed to complete various stress-sensitive parts of the germination process will mean that seeds are more likely to escape from a hostile environment and consequently result in improved emergence and crop establishment (Coolbear et al., 1987; 1988). Treatments which increase rates of germination often (but not always) increase uniformity of germination too. These treatments could also be aimed at allowing seed to germinate in conditions (e.g. sub-optimal temperature and saline conditions) which would normally be marginal. As a result of early emergence, seedlings from treated seed may maintain greater mean plant dry weight, leaf areas, ground cover percentages and also early maturity compared to those from untreated seeds. There is little evidence for improved seedling relative growth rates

(RGR) as a result of seed treatment, nevertheless Ely and Heydecker (1981), for example, found that priming of parsley seeds did give a significant improvement on subsequent plant growth which was just not due to the advantage gained by early emergence.

Coolbear (1988) suggested that the following questions be asked to evaluate the usefulness of the reported technique, viz.;

(i) can the seed be dried-back successfully and sown conventionally after treatment?

(ii) if additions have been made to the soak solution, have adequate controls been done to demonstate real effects for the added chemicals over and above the water or osmotic soak?

(iii) have the physiological effects been clearly identified? (e.g. are there any changes in seedling growth rates or altered physiology or simply stress avoidance as a result of earlier emergence?).

(iv) does the treatment have commercial potential?

Often, considerable germination advantage is obtained by many seeds after drying back (Coolbear, 1988), but reports are not unanimous. Dehydration of primed seed to their initial moisture content without embryo damage or loss of the metabolic advancement induced during imbibition have been well documented (Brocklehurst and Dearman, 1983; Dell'Aquilla et al., 1978; Coolbear et al., 1980). Detrimental effects have also been recorded in celery (Gibbins and Heydecker, 1977; Biddington et al., 1982a; Toledo, 1988), pepper (O'Sullivan and Bouw, 1984), muskmelon (Nerson et al., 1984) and onion (Haigh et al., 1986). Cultivar variations in response to drying have also been noted e.g. the germination rate, uniformity and total emergence percentage were reduced after drying in onion cv. creamgold (Haigh et al., 1986) but not in cv. cima (Furutani et al., 1986).

In general, seeds can't be dried back without injury after the onset of radicle emergence and DNA replication (Berrie and Drennan, 1971). This suggests that during treatment water uptake by the seeds should be monitored in such a way that germination doesn't proceed beyond the lag phase, by limiting either the imbibition time or the quantity of water made available to the seeds. The deteriorative effects of drying in losing the advantage of treatment have been attributed to build up of germination inhibitors (Gibbins and Heydecker, 1977); embryo damage as a result of cell expansion (Biddington et al., 1982a); induction of secondary dormancy (Toledo, 1988) or apparent damage to newly synthesised mRNA (Chen et al., 1968).

2.4.2 Methods of pre-sowing treatments

Most common pre-sowing treatments involve initiation of germinative metabolism and normally result in dry seed which can be sown by conventional techniques. The main types are:

(i) Soaking and drying sometime referred to as 'hardening' (May et al., 1962) or advancing or wetting and drying.

(ii) Osmotic treatments with the use of PEG or salt solutions often termed priming (Heydecker, et al., 1973) or osmoconditioning (Khan et al., 1978).

(iii) Hydration treatments at low temperature often referred to as Psychro-priming (Heydecker, 1974) or low temperature pre-sowing treatment (LTPST) (Coolbear et al., 1984).

In soaking and drying treatment, seeds are allowed to take up water and are then dried back to near their original moisture content, a process which may be repeated one or more times. Soak time is the key to limit water uptake and also a variable between reported studies. It was suggested that this treatment induces drought hardening to subsequent plants (May et al., 1962)

and the phase of dehydration was responsible for a hardening effect (Henckel, 1964), but Hanson (1973) showed that the effective invigoration of seed occurs in the imbibition phase and is subsequently fixed by drying.

Priming is a technique accomplished by imbibing the seed in an osmotic solution that allows the seed to imbibe water to a level that permits some of the initial steps of germination to proceed but prevents radicle emergence occurring (Heydecker, 1975). Consequently, each seed in a seed lot is ready to germinate and emerge faster and more uniformly with no adverse effects on the seedlings (Heydecker and Gibbins, 1978). In general, priming variables in reported studies are;

(i) Osmotic concentration of soak solution
(ii) Soak duration
(iii) Temperature
(iv) Aeration
(v) Light
(vi) Drying procedure (Surface/air dry-slow/rapid)
(vii) Storage
(viii) Initial seed quality
(ix) Crop species, cultivars and even seed lots used
(x) Protection from microbial infection (hygienic condition)
(xi) Type of osmoticum used.

Heydecker and his associates have extensively used priming treatments with the inert osmoticum, polyethyelene glycol (PEG '6000' or '8000') and have reported highly significant and interesting results with vegetables and flower seeds. However, other osmotica such as glycerol, KH_2PO_4, KNO_3, K_3PO_4 and Nacl have also been used successfully in a wide variety of crop species (Heydecker and Coolbear, 1977). When the effectiveness of different osmotica are compared, the relative advantage of salt over PEG has been shown in some crop spcies for example, tomato (Bussell and Gray, 1976; Haigh and Barlow, 1987; Alvarado et al., 1987), carrot (Haigh

and Barlow, 1987a), pepper (Rivas et al., 1984), while the reverse was documented for carrot, celery, leek and onion (Brocklehurst and Dearmann, 1984), beet (Khan et al., 1985). The advantages of using PEG are that it is a chemically inert, readily dissolves in water and is a true osmoticum in that it does not penetrate the cell membranes, but associated problems are high viscosity, poor oxygen diffusity and possible toxicity (Heydecker and Coolbear, 1977; Haigh and Barlow, 1987). However, the use of certain salts (such as Nacl) and their accumulation to deleterious concentrations within the cells is an undesirable risk (Brocklehurst and Dearman, 1984; Haigh and Barlow, 1987).

The concept of using low temperature alone as a pre-sowing technique to improve germination performance is relatively recent although, it has been used in priming to delay the germination in various crops by several workers. the idea of allowing the seeds to imbibe water at a temperature too low for radicle emergence but sufficient for commencing initial germinative metabolism was suggested by Heydecker (1974) due to its promotive effect on the germination of capsicum seeds upon transfer to 30°C. This proposition was tested by Bussell and Gray (1976) who imbibed tomato seeds at 8°C for 24 h, finding improved uniformity of germination at 25°C. Parsnip seeds imbibed at 1°C for 6 to 10 d then transferred to 20°C, showed reduced mean germination times and spread of germination without affecting final germination percentage (Finch-Savage and Cox, 1982). The LTPST technique has been applied only on tomato (see Section 2.4.3) and celery seeds (Toledo, 1988).

Finally, the role of initial seed quality in all these treatments discussed above is debatable. Brocklehurst and Dearman (1983) suggested that priming is most effective in low vigour seed lots of celery, onion and carrot, while Perkins, Veazie and Cantliffe (1983) found that only seeds of highest quality could be effectively primed to overcome thermodormancy.

TABLE 2.2 The effects of some priming treatments on tomato seeds

TREATMENT	PRIMING Temp °C	PRIMING Days	DRYING	RESULTS	REFERENCE
-1.07 MPa KNO_3	24	5	DB	Accelerated germination and emergence rate at 8-10°C	Bussel and Gray, 1976
-1.25 MPa KNO_3	20	7	DB	Accelerated germination and emergence rates; improved uniformity; increased mean plant dry weight; No effect on yield.	Alvardo et al., 1987
-1.25 MPa KNO_3	15	12	DB	Accelerated emergence rate; improved uniformity; increased dry weight; increased ripe fruits and yield; early harvest	Barlow and Haigh, 1987
KNO_3 (0.15 M)+K_3PO_4 (0.07 M)	24	5	DB	Accelerated germination at low temperature	Rumpel and Szudyga, 1978
-1.2 MPa KNO_3+KH_2PO_4	25	2	DB	Accelerated germination rate at 10°C	Maluf and Tigchelaar, 1980
-1.0 MPa KNO_3+K_3PO_4	15	18	DB	Accelerated germination and emergence rate; improved uniformity and germination.	Haigh et al., 1986
-1.0 MPa KNO_3+KH_2PO4	25	7	DB	Accelerated germination rate; improved germination % and uniformity	Globerson and Feder, 1987
1.0% KNO_3+1.5% K_3PO_4	25	6	DB	Accelerated emergence under supra optimal temperature; No effect on total emergence	Odell, 1987

-1.25 MPa KNO_3+KH_2PO_4	20	5	DB	Accelerated emergence at all temperatures	Argerich et al., 1989
2% K_2PO_4	NI	1	DB	Accelerated emergence at low temperature	Gerber, 1981
-0.75 MPa PEG	20	7	DB	Accelerated germination at low temperature	Rumpel and Szudyga, 1978
-0.625 MPa PEG	25	9	DB	Accelerated germination rate; improved uniformity	Coolbear et al., 1980
-0.5 MPa PEG	15	7	DB	Accelerated germination rate; improved final stand; increased emergence percentage	Wolfe and Sims, 1982
-1.0 MPa KNO_3+K_2HPO_4	15	24	Moist	Accelerated germination rate; improved uniformity	Haigh and Barlow, 1987a
KH_2PO_4 (0.11 M) +$(NH_4)_2HPO_4$ (0.11 M)	27-29	3	NI	No effect on emergence	Ghate et al., 1983
Mannitol (0.1 - 1.6 M)	25	7	NI	Accelerated germination	Georghiou et al., 1983
-1.2 MPa PEG	16	8	NI	Accelerated emergence rate; increased % emergence under salinity	Wiebe and Muhyaddin, 1987
29% PEG	4°C	12	Moist	Increased percent of germination seedling length, fresh and dry weight	Saxena and Gita Singh, 1987

1. MPa = megapascals; PEG = polyethylene glycol '8000'
2. DB = Driedback; NI = Not indicated

2.4.3 Presowing seed treatments in tomato seeds

A variety of techniques have been used with some success for tomatoes including soaking and drying, short pre-soaks in NAA or GA, salt solution treatments, the use of inert osmotica and low temperature pre-sowing treatments. The effects of some priming treatments on tomato seed are shown at Table 2.3.

The soaking and drying treatment has been used successfully (Berrie and Drennan, 1971), with improved plant growth and yield of tomatoes (Gafarov, 1973). The most spectacular results was reported by Mart'Janova et al.,(1961) who reported a 105% increase in yield in water stressed tomatoes as a result of one cycle wetting and drying treatment.

Coolbear et al.,(1987) showed that LTPST can substantially enhance germination rates and was much more effective at improving uniformity of germination than equivalent osmotic treatments in tomato seeds. The emergence rate as a result of LTPST was much greater at lower sowing temperatures. They have also noted that as a result of pretreatment the initial axis growth may be temporarily reduced, probably as a consequence of depletion of reserves during the treatment period. LTPST has also been shown to significantly improve the germination capacity of tomato seeds apart from emergence rate under severe moisture stress conditions (30°C, -0.5 MPa), but uniformity varied with seed lot (Coolbear and McGill, 1989).

2.4.4 Commercial relevance of pre-sowing seed treatments

These pre-sowing seed treatments would interest growers or agronomic and horticultural crops and could find application in where early emergence was required. Giving, for example, a competitive advantage to the seed over weeds or allowing the seed to perform better under stress conditions. Seed treatments could also be used to allow extension of the planting and/or growing season.

Another important benefit is the potential for increased crop uniformity, both for planting on seedlings and for mechanical harvesting.

2.4.5 Mechanism of action and events associated with PST

The key basis of all pre-sowing seed treatments is to hydrate the seed under controlled conditions, so that they became physiologically active. Thus they are able to initiate repair and detoxification systems and also to complete certain initial steps of germination without radicle emergence. Limited uptake of water appears to be the key to success in all treatments. According to Heydecker (1974) priming allows 'slow' or 'fast' seeds in a population to attain the same stage of readiness, a property of considerable significance in obtaining a rapid and uniform population of seedlings.

2.4.5.1 **Rate limiting factors in germinative metabolism**

The physiology of incompletely hydrated seed is not fully understood (Hegarty, 1978). Bradford (1986) proposed a hypothesis suggesting that effectiveness of priming is related to the osmotic adjustment process. The benefits gained from priming is due to a change in cell osmotic potential may be preserved during dehydration, so that primed seed have a lower osmotic potential ($\Psi\pi$) and higher turgor pressure (Ψp) upon rehydration. This idea seems to work well for lettuce but has still to be demonstrated in tomato, although it was supported by Akers et al., (1987).

However, the osmotic adjustment hypothesis has been questioned by other workers. Haigh and Barlow (1987b) found that $\Psi\pi$ of embryos never rose above -1.6 MPa in untreated seeds placed in water until the end of lag phase of imbibition. Similar results have been found in carrot and broccoli (Hegarty, 1977) and lettuce (Cantliffe et al., 1984). Haigh and Barlow (1987b) concluded that the rate limiting step of germination lies in the mechanism which leads to the weakening of the enclosing tissue that restricts embryo water uptake and embryo expansion. This is supported by the

observation of Groot and Karssen (1987), who showed that germination of tomato seed was absolutely dependent on the presence of either endogenous or exogenous gibberellins which facilitated germination by stimulating enzyme activity to weaken the mechanical restraint of the surrounding endosperm cells. Nevertheless, although this may be the rate limiting step in the GA defficient ga-1 mutants it is not necessarily so in normal seed. Liptay and Schopfer (1983) compared cold tolerant and osmotic stress resistant tomato seeds with sensitive ones. Their seed cutting experiments showed that resistance of endosperm did not account for the differences between cultivars in germination rate, but they have not looked at treated seed.

2.4.5.2 Biochemical changes

A limited number of studies have been conducted on the biochemical basis of pre-sowing treatments. Some observed changes are:

(i) DNA metabolism. Coolbear and Grierson (1979) showed that the priming did not cause any change in DNA content of tomato seeds during treatment. Similarly, there was little DNA synthesis prior to radicle protrusion in treated lettuce seeds (Khan, 1980). These results indicate that DNA synthesis may not coincide with radicle protrusion and that priming may be associated with processes related to cell elongation and expansion.

(ii) RNA Synthesis. It has been shown that priming not only increased the accumulation of RNA in tomato (Koehler, 1967; Coolbear and Grierson, 1979) but also advanced the time of synthesis (e.g. in lettuce seeds, Khan et al., 1978). However, the presence of Cordycepin (CP), an inhibitor of RNA synthesis, during treatment or subsequent germination failed to inhibit radicle protrusion of treated seed. From studies on C. bonus-henricus seeds, it was suggested that germination as well as promotion of germination by priming may be modulated by stored mRNA which might be functionally different from the newly synthesised poly $A^{(+)}$ RNA (Khan, 1980). The possibility that synthesis of some RNA, not inhibited by CP

might be involved in germination of these seeds cannot however, be ruled out (Khan, 1980).

(iii) Changes in proteins, enzymes and hormones. Koehler (1967) showed 140% increase in proteins during the priming treatment of tomato seeds. Both the rate and quantity of protein synthesized were found to be significantly higher in primed lettuce seed than control (Khan et al., 1978). Presence of Cycloheximide (CH) strongly inhibited the rate of protein synthesis and germination in both treated and untreated seeds prior to radicle protrusion and also reduced the advancement effect of the treatment in lettuce seeds, indicating a requirement for protein synthesis for this process (Khan et al.,1980). This indicates that protein synthesis is involved in regulation of germination and germination advancement by priming.

Activation and/or synthesis of a number of enzymes have been reported during priming of lettuce seeds (Khan et al., 1978). Increased α-amylase activity is noted in oats (Berrie and Drennan, 1971) and in wheat (Hanson, 1973). It was suggested that priming might affect key metabolic processes related to breakdown of protein and lipid reserves and turnover of phosphate esters (Khan et al., 1980).

Priming has also been shown to cause the disappearance of both free and hydrolyzable ABA in lettuce seed (Khan et al., 1978). However, these workers also found that the time taken for germination of lettuce seeds at 25°C was independent of the ABA content of the seed. Thus, not only the disappearance of ABA in primed seed, but also sensitivity of the tissue to the hormone may be related to improved seed metabolism and rate of germination.

2.5 REPAIR AND PROTECTION OF SEEDS IN STORAGE

Pre-sowing seed treatment can improve the storability of seed even under less than ideal conditions (Basu, 1976; Basu and Rudrapal, 1980; Savino et al., 1979) and also the germination perfor-

mance of stored seeds (Brocklehurst and Dearman, 1984). Although numerous papers have been published on this topic no coherent body of information has emerged. Pre-sowing treatments designed for improving storability can be classified as follows:

a) Antioxidant treatments
b) Hydration treatments and
c) Hormone treatments.

2.5.1 Antioxidant treatments

Pammenter et al's (1974) demonstration that cathodic protection can protect maize seed in high temperature storage conditions (13.6% SMC, 40°C) supports the hypothesis that free radicals and/or lipid peroxidation could be the major cause of seed deterioration. Consequently, the use of antioxidants may have the potential to become a cost-effective mechanism for prolonging storage life and also as an effective research tool (Wilson and McDonald, 1986).

The different responses to antioxidants by a particular species may be influenced by concentration effects, toxicity, tissue permeability, application technique and possibly the ageing and evaluation conditions. With the use of mercaptoethanol and sodium thiosulphate in aqueous solution , protective effects have been noted in both natural and accelerated ageing conditions in a variety of species including wheat, rice, pulses, sunflower (Basu et al., 1975), in tomato (Francis, 1985) and carrot (Kundu and Basu, 1981), although Barnes and Berjak (1978) found no protective effect on maize in store. The beneficial effects of iodine vapour as a pre-storage treatment have been noted in mungbean (Rudrapal and Basu, 1980), soyabean and sunflower (Dey and Mukherjee, 1984). Gorecki and Harman (1987) found no effect of water soluble antioxidants on pea seeds stored under high humidity storage conditions (92% RH, 30°C) and only Butylated hydroxytoluene (BHT) and α-tocopherol (Vitamin E) impregnated via acetone gave some measure of protection. Woodstock et al.,(1983) showed that BHT and Vitamin E appli-

cation improved the storability of Parsley seeds while in pepper these antioxidants accelerated the rate of deterioration.

2.5.2 Hydration treatments

Pre-sowing hydration treatments have been found to be effective in a range of species of seed for protecting and/or improving germination performance of aged seeds, e.g. wheat, rice, sunflower pulses (Basu, 1976), soyabean (Woodstock and Tao, 1981; Tilden and West, 1985), pea, tomato and carrot (Savino et al., 1979). Methods including incubation at high RH, soaking or dipping in water, priming in solution with PEG or salt, LTPST have all been used with mixed success.

It has been well established that imbibed dormant seeds possess a highly developed metabolism. For example, respiration is evident (Powell et al., 1983), membrane lipids and proteins are subject to turnover (Cuming and Osborne, 1978a, 1978b), RNA is synthesized (Hecker and Bernhardt, 1976) and active polysomes are present (Fountain and Belwey, 1973). It is presumably the existence of such metabolic functions in the hydrated seed that permits extensive repair to take place. Villiers (1974) has shown that storage of lettuce in imbibed dormant conditions reduces chromosomal aberrations, which provided evidence for the existence of a continuous repair system in fully imbibed seeds. Further, subjecting lettuce seeds to occassional brief interludes of wetting and drying during conventional dry storage has largely eliminated the usual age-induced accumulation of chromosomal injury (Villiers and Edgecumbe, 1975). It has been shown that longevity is promoted when SMC is increased above 15 to 20% in lettuce (Ibrahim and Roberts, 1983). An increase in hydration of onion seeds above 15% has similar beneficial effects on their longevity (Ward and Powell, 1983), although responses varied between the cultivars.

Relatively short hydration and dehydration treatments (2-24 h imbibition) or incubation at high RH followed by redrying have

been reported to increase the vigour of stored seed and/or extend the longevity both under natural and adverse storage conditions in several crop species, e.g. Tomato (Mitra and Basu, 1979: Perl, 1979; Savino et al., 1979), maize, mustard (Dey and Mukherjee, 1986), wheat (Goldsworthy et al., 1982; Hanson, 1973; Rudrapal and Basu, 1982), rice jute, sunflower, pulses and vegetable seeds (Basu, 1976). The problem of soaking injury in some seeds could also be overcome by moisture equilibriation retaining the advantage of hydration treatment, e.g. soyabean (Saha and Basu, 1984). The beneficial effects of treatments have been attributed to reduced lipase activity, lowered fatty acids (e.g. Dey and Mukherjee, 1986), lowered lipid peroxidation (e.g. Rudrapal and Basu, 1982) in treated seeds compared to untreated seeds. Often it has been suggested that free radical chain reaction in the aged seeds were terminated by the treatment through some quenching or scavenging mechanisms (e.g. Basu, 1976; Mitra and Basu, 1979). The improved vigour and increase in the longevity associated with priming or long hydration treatments have been interpreted as indications that repair of accumulated damage occurs during treatment. (e.g. onion, Dearman et al., 1986; Ellis and Butcher, 1988; wheat, Dell'aquila, 1987; soyabean, Tilden and West, 1985). Often the beneficial effects were greatest in low vigour seeds, e.g. carrot, celery, and onion (Brocklehurst and Dearman, 1983).

Although hydration-dehydration treatments increase and/or protect seed vigour measured by criteria such as rates of germination and field emergence,. Coolbear et al., (1984) found that LTPST protected and/or improved germination rate but not seed viability in tomato seed and suggested that the damage causing decreased rate of germination was different to that ultimately causing loss of viability while Dearman et al., (1986) found priming protected both viability and vigour and suggested that loss of viability in onion is merely a consequence of more of the same type of damage which has already impaired seed vigour. However, the same authors, Dearman et al. (1987) found that loss of viability in leek and carrot seeds were dramatically faster in primed seeds compared to control.

A reduction in storage life of lettuce (Kabbabe, 1987), leek (Jones, 1977), wheat and ryegrass (Lush et al., 1981) and tomato (Alvarado and Bradford, 1988a; Odell and Cantliffe, 1986; Argerich et al., 1989) seeds after treatment have been reported.

2.5.3 Hormone treatment

Success with hormone treatments may provide evidence for the loss of hormones (Harrington, 1973) and/or sensitivity during ageing (Aspinall and Paleg, 1971). Exogenous application of GA promoted the germination rate in wheat (Micrzwinska, 1977) or, in combination with ethephon protected the seeds in storage (Petruzzelli and Taranto, 1985) while it had little or no effect on aged barley seed (Huber and McDonald, 1982). In tomato, kinetin + KNO_3 application improved the germination performance of 10 years naturally aged seeds, while GA had no effect despite effecting various aspects of metabolism such as soluble sugars and RNase activity (Puls and Lambeth, 1974).

The process of deterioration could be different under different ageing conditions. For example, Petruzzelli et al., (1982) showed that fusiccocin application promoted the viability of wheat (80%) under ambient laboratory storage conditions compared to control (50%) while had no beneficial effect under artificial ageing conditions (Petruzzelli and Carella, 1983).

2.6 CONCLUSION

Undoubtedly, several pre-storage and storage factors affect seed quality, of which moisture and temperature play significant role. Survival of seed and its quality is determined by the interaction between these factors and potential seed quality determined by genotype (see Section 2.1.2). There is some evidence to show that different seedlots of a given species, cultivar, chronological age

and germination do not perform equally well in storage and/or field under identical conditions (e.g. Delouche and Baskin, 1973; Ellis and Roberts, 1981). Although, the primary cause of seed deterioration is not known, several changes and/or types of damage take place during storage which may differ with cultivar/seed lot and ageing conditions (Coolbear, 1988). The survival pattern of tomato seed under different ageing conditions and of different seed lots under the same ageing condition is less well understood and is being examined in this project.

Presowing seed treatments have been successfully used in a range of species to improve germination performance and/or emergence even under less than ideal conditions (e.g. in tomato Coolbear et al., 1987, 1989; Argerich and Bradford, 1989) (see Section 2.4). Similarly, PST have also been shown to protect and/or improve the performance of stored seeds, for example, in tomato (Savino et al., 1979; Coolbear et al., 1984). However, there are reports suggesting that although PST may improve the vigour according to criteria such as rate of germination and/or field emergence (Coolbear et al., 1987; Argerich and Bradford, 1989), it may cause reduction in storage life of treated seeds (Argerich et al., 1989; Alvardo et al., 1987) (see Section 2.5). In these treatments one additional problem is variability between seed lots (Haigh and Barlow, 1987). The germination responses of different tomato seed lots to LTPST and its application before and after ageing are being examined in this project.

CHAPTER 3
MATERIALS AND METHODS

3.1 SEED MATERIALS

Three different cultivars of tomato seeds (Lycopersicon esculentum Mill.) were used during the course of this study. Each cultivar was represented by three or four different seedlots, obtained from a variety of sources. Details are presented in Table 3.1.

3.2 LOW TEMPERATURE PRE-SOWING TREATMENTS (LTPST)

Seeds were allowed to imbibe distilled water at a constant 10°C in darkness for several days depending on the treatment duration as described by Coolbear et al. (1987). On completion of the pretreatment period, germinants were removed and the remaining seeds were dried at 20°C in the light for 2 d on filter paper in open petri dishes. After drying, treated seeds were used for setting up the germination trials or stored at 5°C in heat sealed pouches until required. Moisture contents were determined in each seedlot according to ISTA rules (1 h at 130°C; ISTA, 1985) using 4 replications and calculated on a fresh weight basis. Treated, dried-back seeds from different experiments had average mean moisture contents ranging from 10.30 (± 0.7) to 10.71 (± 0.14)% compared to those of dried back untreated controls ranging from 8.07 (± 0.10) to 8.50 (± 0.19)%.

Pretreatment durations examined were 7 d, 10 d, 14 d, 17 d and 21 d. Unless otherwise stated, 14 d LTPST was chosen to compare the effects of treatment on normal germination, initial seedling growth and soil emergence of different seed lots (see Chapter 4) and also to examine the potential for protective effects in ageing experiments involving various seed lots (Chapter 5). No aseptic conditions were imposed during the treatment period.

TABLE 3.1 Tomato seed lots used in the experiments.

CULTIVAR	STOCK NO.	LABELLED GERMINATION %	CODE USED IN RESULT AND DISCUSSION
Moneymaker	78021[a]	90	M-1
	CDW021[a]	90	M-2
	AC81[b]	92	M-3
	17310[c]	-	M-4
Scoresby dwarf Ky-1	17400[c]	-	S-1
	XK_3[b]	96	S-2
	38912N902R[d]	94	S-3
Grosse lisse improved	CCV254[a]	92	G-1
	AB73[b]	94	G-2
	42922/N881H[d]	93	G-3

SOURCE

a) Corson Grain Ltd, P.O. Box 1046, Gisborne, New Zealand

b) Webling and Stewart Ltd, 90 - 92, Sydney Street, Petone, New Zealand

c) Ocean View Seeds Ltd, P.O. Box 468, New Plymouth, New Zealand

d) Arthur Yates & Co., 270 Neilson Street, Onehunga, Auckland, New Zealand

3.3 GERMINATION TRIALS

Petridish germination trials were conducted in 3 ml distilled water at a constant 20°C in darkness (except while setting up the germination trials and taking counts). Fifty seeds were used per 9 cm dish on 2 layers of 7 cm Whatman No. 1 filter paper. Germinants were counted and removed at least thrice daily during peak periods of germination activity. Counting was continued until no further germination occurred for a continuous period of 5 days. All seeds were surface sterilised prior to germination trials by a method modified from Abdul-Baki (1974) (15 minutes in about 1% NaOCl-Janola solution, 10 min in 0.01 M HCl, followed by 3 washes in distilled water). Except for this, no asceptic conditions were imposed during the trials.

The median germination time (T50) and uniformity (T90-T10) were calculated for germinated seeds only, for each replicate according to the formulae described by Coolbear (1988). T85-T50 (late) and T50 - T15 (early) were also computed in some cases to examine the distribution pattern of germination rate. In these trials germination refers to radicle emergence.

3.4 NORMAL GERMINATION AND SEEDLING GROWTH TRIALS

Normal germination tests were conducted as per ISTA (1985) rules on the top of the paper. In brief, moist towelling was placed on a tray, covered by sheet of white Whatman No. 1 filter paper and germination blotters were placed on top of this. After surface sterilisation, seeds were placed on each pad and then a plastic cover placed over the pad. These were incubated in germinator cabinets in the dark at 20°C. Germinants were counted as previously described. Normal seedlings were evaluated as per ISTA rules and removed as and when they could be identified. Total emergence percentage and normal seedling percentage were recorded and T50 and uniformity were computed.

In the seedling growth test normal seedlings were harvested on the 12th day after sowing on top of the paper and seedling fresh weight was recorded for each replicate. The dry weight was obtained by drying the plant material at 65°C for 4 d (Coolbear et al., 1987).

Soil emergence trials

Blooms potting mix was placed in seed trays and watered until the potting mix was well moistened. Seeds were placed on them and covered with potting mix to a depth of approximately 10 mm. Until the initiation of visible germination, the trays were covered with moist brown paper and then watered daily. Subsequently watering was done every two days.

Fifty seeds were sown in 4 replications. Seedling emergence (appearance of a visible hypocotyl hook) and normal seedling development (expansion of cotyledons) were counted at least thrice a day during peak periods and then once a day until no germination occurred continuously for 5 days. The pretreated seeds used in this experiment were stored in sealed pouches at 5°C for more than eleven weeks. This experiment was carried out in a green house where the temperature was regulated to approximately 20°C.

3.5 AGEING TREATMENTS

Controlled deterioration conditions used were a seed moisture content of 40%, 14% or 8% and a temperature of 40° or 45°C. The duration of ageing treatment was 0 to 28 d and/or 0 - 15 d depending on the experiment. The seed moisture content was increased by the following methods;

(a) The SMC was increased by imbibing the seed for 2 h at 20°C in light on 9 cm petri dishes lined with 2 layers of 7 cm Whatman No. 1 filter paper with 3 ml distilled water. They were then surface blotted to remove the excess surface

TABLE 3.2 Seed moisture content of different tomato seed lots in untreated and treated before and after ageing after 2 h imbibition.

CULTIVAR	SEED LOT	UNTREATED (%)	TREATED BEFORE AGEING (%)	TREATED AFTER AGEING (%)
Moneymaker	M-2	37.8 ± 0.17	40.0 ± 0.26	38.8 ± 0.32
	M-3	38.1 ± 0.15	38.4 ± 0.32	38.3 ± 0.14
Scores by dwarf	S-2	40.9 ± 0.41	42.3 ± 0.31	41.8 ± 0.19
	S-3	40.3 ± 0.13	42.1 ± 0.34	41.1 ± 0.10
Grosse lisse	G-1	37.3 ± 0.44	37.7 ± 0.20	37.3 ± 0.20
improved	G-2	36.9 ± 0.22	37.5 ± 0.02	37.5 ± 0.26
	G-3	37.0 ± 0.11	37.7 ± 0.09	37.7 ± 0.16

TABLE 3.3 Seed Coat embryo water relationship.

Seed components	Moisture Contents of the seed components after 2 h imbibition (Percentage on fresh weight basis)*					
	Before Ageing			After 10 d Ageing		
	Fresh weight (of 25 seeds) (grams)	Moisture content (grams)	Moisture %	Fresh weight (of 25 seeds) (grams)	Moisture content (grams)	Moisture %
Actual moisture content						
Entire seed	0.0754 ±0.0046	0.0155 ±0.0016	20.6	0.0803 ±0.0010	0.0227 ±0.0008	28.3
Seed coat (including endosperm)	0.0609 ±0.0018	0.0121 ±0.0009	19.7	0.0640 ±0.0019	0.0186 ±0.0005	29.1
Embryo	0.0247 ±0.0013	0.0024 ±0.0002	9.7	0.0295 ±0.0008	0.0047 ±0.0002	15.9
Estimated moisture content						
Entire seed	0.1185	0.0474	40.0	0.1170	0.0468	40.0
Seed coat	0.0843	0.0396	47.0	0.0801	0.0374	46.7
embryo	0.0342	0.0078	22.8	0.0369	0.0094	25.5

NOTE: 1.* After 2 h imbibition initial SMC of the entire seed was around 40% and the moisture content indicated were determined after separating the embryo's from seed at lab condition (25°C) which took about 90 minutes time for each replication hence loss of moisture.

2. Moisture contents are mean of 6 replications.

TABLE 3.4 Seed moisture content (SMC) during moisture equilibration at different concentrations of Glycerol water solutions at 20°C.

Ratio of Glycerol:Water	Period of incubation			
	1 d (%)	2 d (%)	4 d (%)	7 d (%)
75: 25	9.4 ± 0.05	9.2 ± 0.06	9.3 ± 0.09	9.3 ± 0.09
50: 50	12.2 ± 0.14	12.0 ± 0.14	12.4 ± 0.14	12.5 ± 0.20
40: 60	14.3 ± 0.45	14.3 ± 0.69	13.9 ± 0.48	14.5 ± 0.50
30: 70	16.2 ± 0.52	16.5 ± 0.19	16.0 ± 0.07	16.3 ± 0.08
25: 75	17.4 ± 0.38	17.8 ± 0.20	18.3 ± 0.26	18.7 ± 0.21
0:100	23.6 ± 0.17	27.8 ± 0.32	33.5 ± 0.21	36.9 ± 0.14

NOTE: Data is mean of 4 replications.

moisture. This 2 h imbibition period was chosen to conform with the imbibition period used by Coolbear et al. (1984) to increase the SMC. The SMC with this method on a fresh weight basis was approximately 40% with a range of ± 3%, depending on the seed lot and the age of the seeds (Table 3.2).

After 2 h imbibition the moisture content of embryo, seed coat (including endosperm) and the entire seed (after cutting) were determined separately. Table 3.3 shows that the moisture content of the seed coat plus perpherial seed tissue (endosperm) was higher than the centrally located embryo both before and after ageing (10 d). The estimated moisture content of the embryo at 40% SMC was about 23 - 25% on fresh weight basis. The embryo is surrounded by endosperm and hairy seed coat and without any cellular connection between them, thus the difference in the moisture content between embryo and surrounding tissue may be due to lack of cellular capillary movement between embryo and endosperm. Also to the hairness of the seed coat, which adsorb and desorbs the moisture very quickly, may contribute to the differential moisture content within the seed.

(b) Based on preliminary trials (Table 3.4) the moisture content of seed was increased to 14% by incubating the seed for 2 d at about 80% RH with the use of 40:60 Glycerol and water solution.

(c) The initial SMC of the seed was 8% on fresh weight basis.

After increasing the SMC to the required level, seeds were put into heat-sealed polyester-aluminium foil-polyethylene laminated Corson pouches and then incubated at different temperatures for varying periods as decided by the experiment. Corson pouches only were used in all ageing experiments during the course of this study, as the SMC of seeds stored in these pouches were not

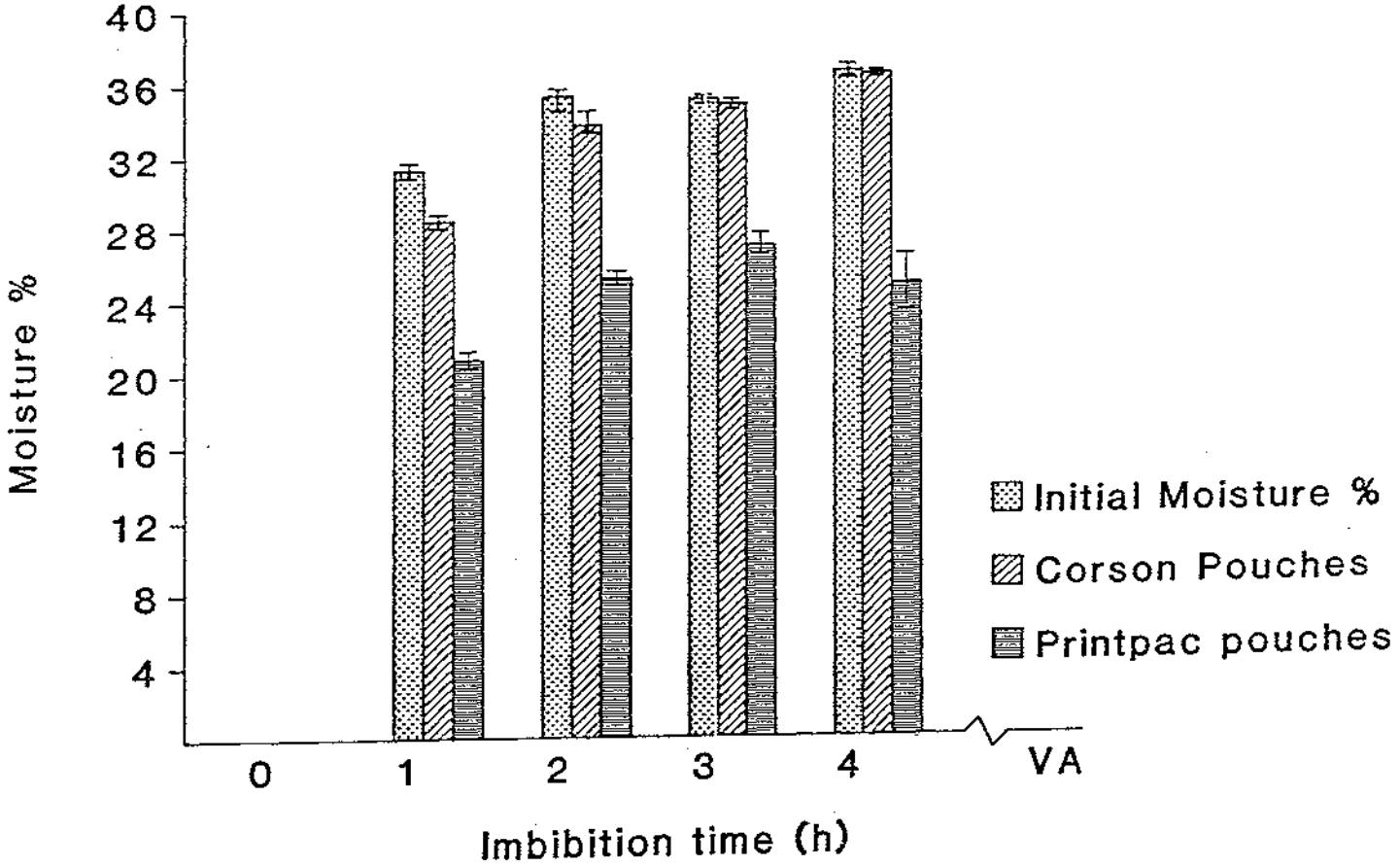

Figure 3.1 Loss of moisture in tomato seed cv. Moneymaker lot M-1 packed in Corson pouches vs Printpack pouches. Individually calculated standard errors are shown for each mean of four replications.

significantly different before and after ageing. Other pouches proved less effective in preliminary trials (Fig. 3.1). After ageing treatments seeds were dried back as previously described.

3.6 EXPERIMENTS AND DATA ANALYSIS

The experiments carried out in this study were grouped into 2 sets. Firstly, the germination and/or growth responses of different tomato seed lots to LTPST were assessed. Secondly, the effects of ageing and the protective or invigorative effects of LTPST on germination and germination rates of different seed lots of tomato seeds were examined.

A split plot design was used in this experiment retaining control and LTPST treatment as a set unless otherwise stated. Data were analysed stastically without any transformation unless otherwise stated. The probit analysis (Finney, 1971) was employed for the purpose of computing theoretical initial viability value (Ki) and slopes of deterioration.

CHAPTER 4
RESPONSES OF TOMATO SEED LOTS TO LOW TEMPERATURE PRE-SOWING TREATMENTS (LTPST)

4.1 INITIAL SEED QUALITY

Table 4.1 shows the results of preliminary germination trials on the seedlots used during the course of this study. Analysis of variance of untransformed data indicated that there were no significant differences in germination capacity between seedlots or cultivars. However, Lot M-3 and S-1 were shown to be different from Lot M-1 using the Duncan's multiple range test.

There were considerable differences between seed lots in respect of their median germination time (T-50), uniformity (T90--T10) of germination and thousand seed dry weight ($P < 0.01$). Although there were obvious differences between cultivars, it can be seen from Table 4.1 that variation between lots within a cultivar were just as great. There was a highly significant positive correlation between T50 and uniformity ($r = 0.86$, $P < 0.01$). Except for this, there was no other significant correlation between parameters tested within the species. In only cv. Grosse lisse improved was there a significant correlation between seed dry weight and T50 of untreated seeds ($r = -0.998$ and $r = -0.988$) in two out of 3 experiments conducted during the course of this study. It was also noted, however, that the T50's of untreated seeds differed between experiments. For example, variation in Grosse lisse improved is shown in Table 4.2.

4.2 EFFECTS OF LTPST DURATION

The effects of treatment duration on mean percentages of pregermination, radicle emergence, T50 and Uniformity for each seed lot are shown in Figures 4.1 to 4.10. The degree of pregermination varied significantly between seed lots ($P < 0.001$) and was

TABLE 4.1 The initial germination peformance of tomato seed lots

CULTIVAR	SEED LOT	GERMINATION (%)	MEDIAN GERMINATION TIME (T50) (h)	UNIFORMITY (T90 - T10) (h)	1000 SEED DRY WEIGHT (g)
Moneymaker	M-1	100 a	89 e	65 b	2.444 cd
	M-2	95 ab	140 bcd	71 b	2.526 c
	M-3	91 b	144 bc	113 a	1.872 f
	M-4	95 ab	126 d	110 a	2.788 b
Scoresby Dwarf	S-1	93 b	80 e	53 b	2.932 a
	S-2	95 ab	95 e	61 b	2.416 d
	S-3	96 ab	96 e	66 b	2.388 d
Grosse Lisse	G-1	96 ab	176 a	127 a	2.256 e
Improved	G-2	98 ab	150 b	102 a	1.814 f
	G-3	96 ab	131 cd	108 a	2.440 cd

NOTE: Duncan's multiple range test: Means with the same letters are not significantly different at 0.05 level.

TABLE 4.2 The variations in T50 between germination tests for untreated Grosse lisse Improved tomato seeds

Seed Lot	Median germination time (T50) (h) of untreated seeds		
	Expt. A (4.1)	Expt. B (4.2)	Expt. C (4.3)
G-1	176 ± 10.4	121 ± 3.0	109 ± 1.9
G-2	150 ± 6.5	132 ± 2.9	121 ± 4.2
G-3	131 ± 2.8	115 ± 5.8	107 ± 2.2

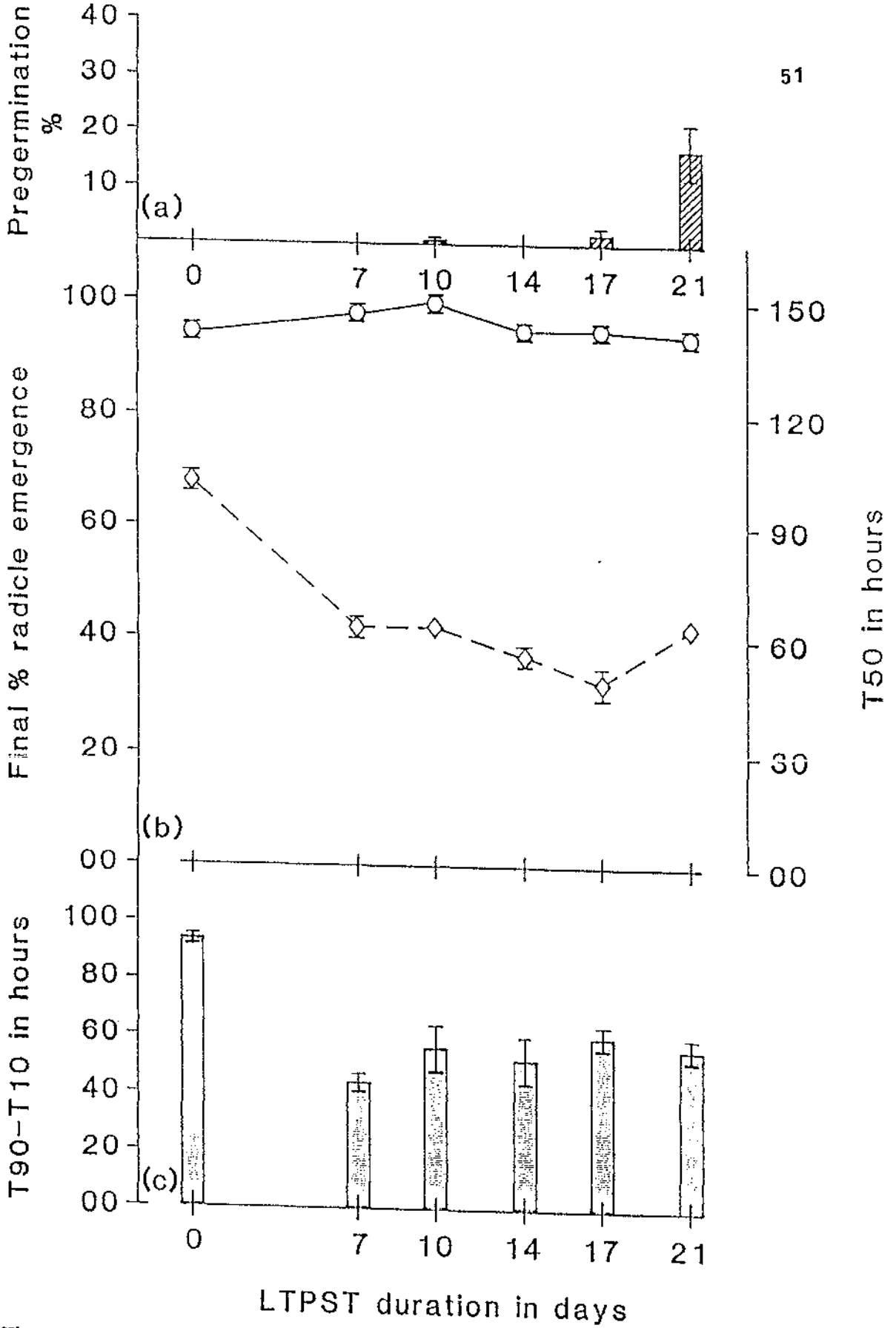

Figure 4.1 Effects of different durations of LTPST on the germination behaviour of tomato seed lot M-1.
(a) Pregermination percentage [histogram with diagonal lines].
(b) Final radicle emergence percentage [O] and T50 [◇].
(c) Uniformity as T90 - T10 [histo gram with horizontal lines].
Data presented are means of four replications; vertical bars represent SE's calculated for individual means and are shown where larger than the symbols used.

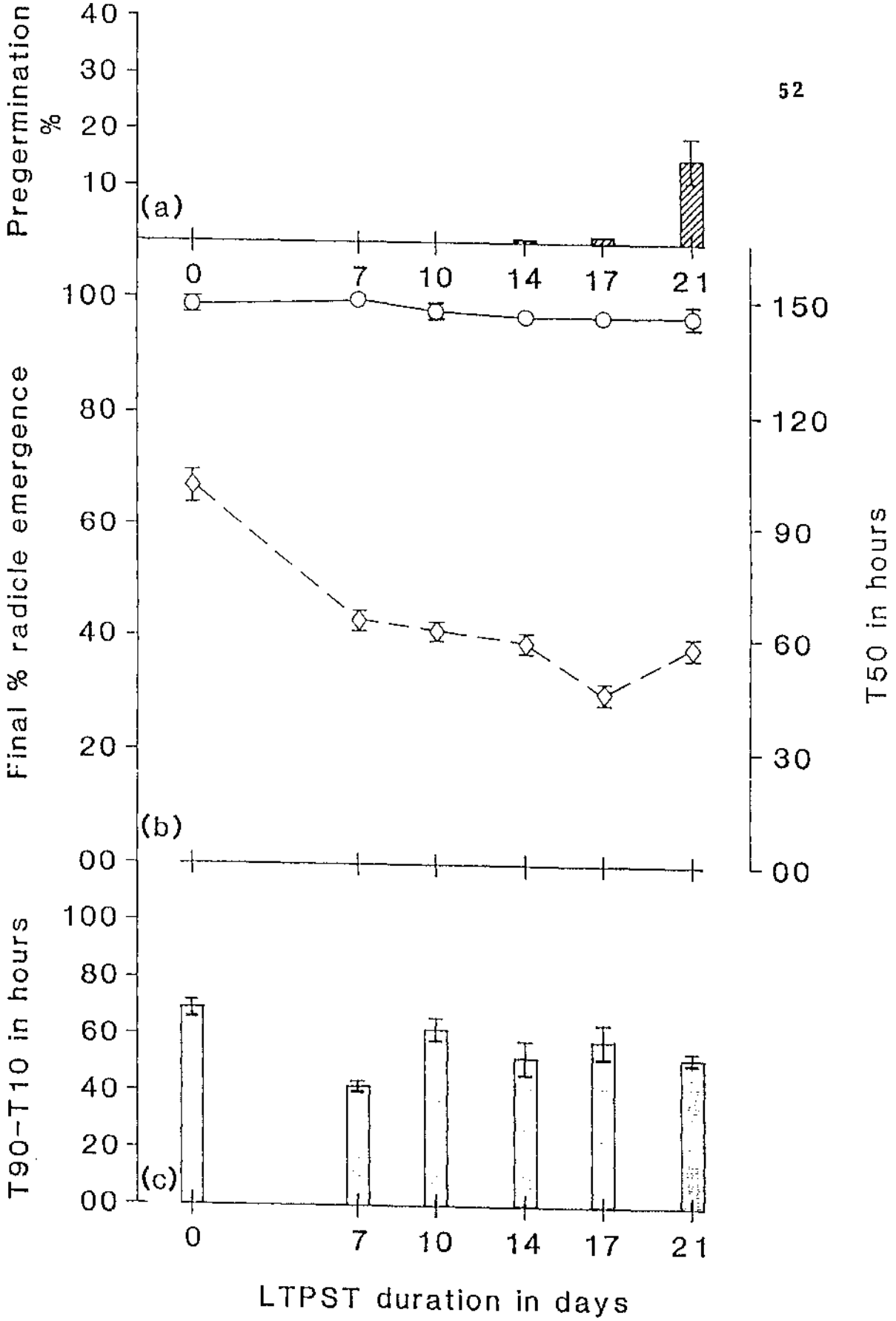

Figure 4.2 Effects of different durations of LTPST on the germination behaviour of tomato seed lot M-2.
(a) Pregermination percentage [histogram with diagonal lines].
(b) Final radicle emergence percentage [**O**] and T50 [◊].
(c) Uniformity as T90 - T10 [historgram with horizontal lines].
Data presented are means of four replications; vertical bars represent SE's calculated for individual means and are shown where larger than the symbols used.

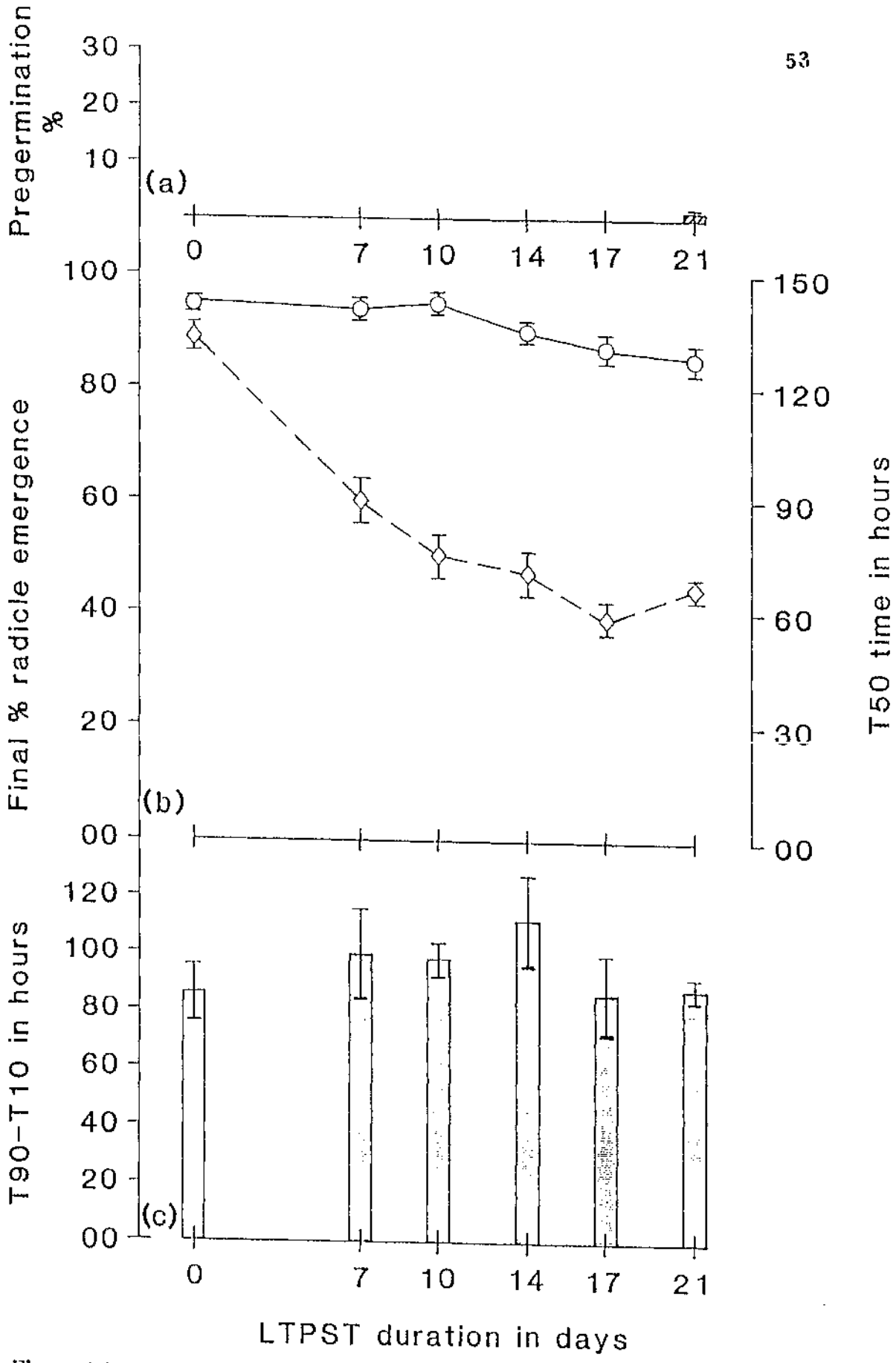

Figure 4.3 Effects of different durations of LTPST on the germination behaviour of tomato seed lot M-3.
(a) Pregermination percentage [histogram with diagonal lines].
(b) Final radicle emergence percentage [O] and T50 [◊].
(c) Uniformity as T90 - T10 [histogram with horizontal lines].
Data presented are means of four replications; vertical bars represent SE's calculated for individual means and are shown where larger than the symbols used.

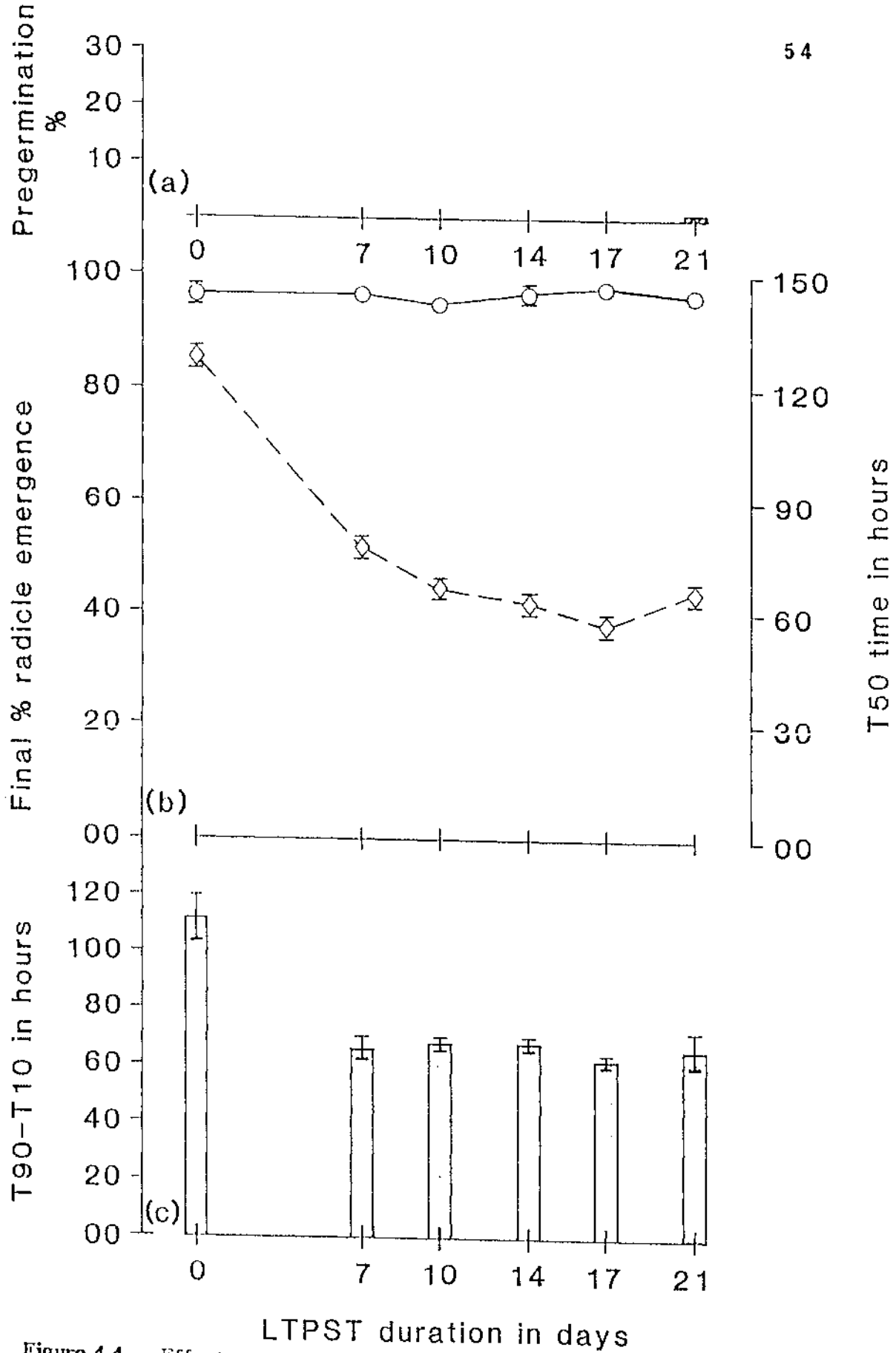

Figure 4.4 Effects of different durations of LTPST on the germination behaviour of tomato seed lot M-4.
(a) Pregermination percentage [histogram with diagonal lines].
(b) Final radicle emergence percentage [O] and T50 [◊].
(c) Uniformity as T90 - T10 [histogram with horizontal lines].
Data presented are means of four replications; vertical bars represent SE's calculated for individual means and are shown where larger than the symbols used.

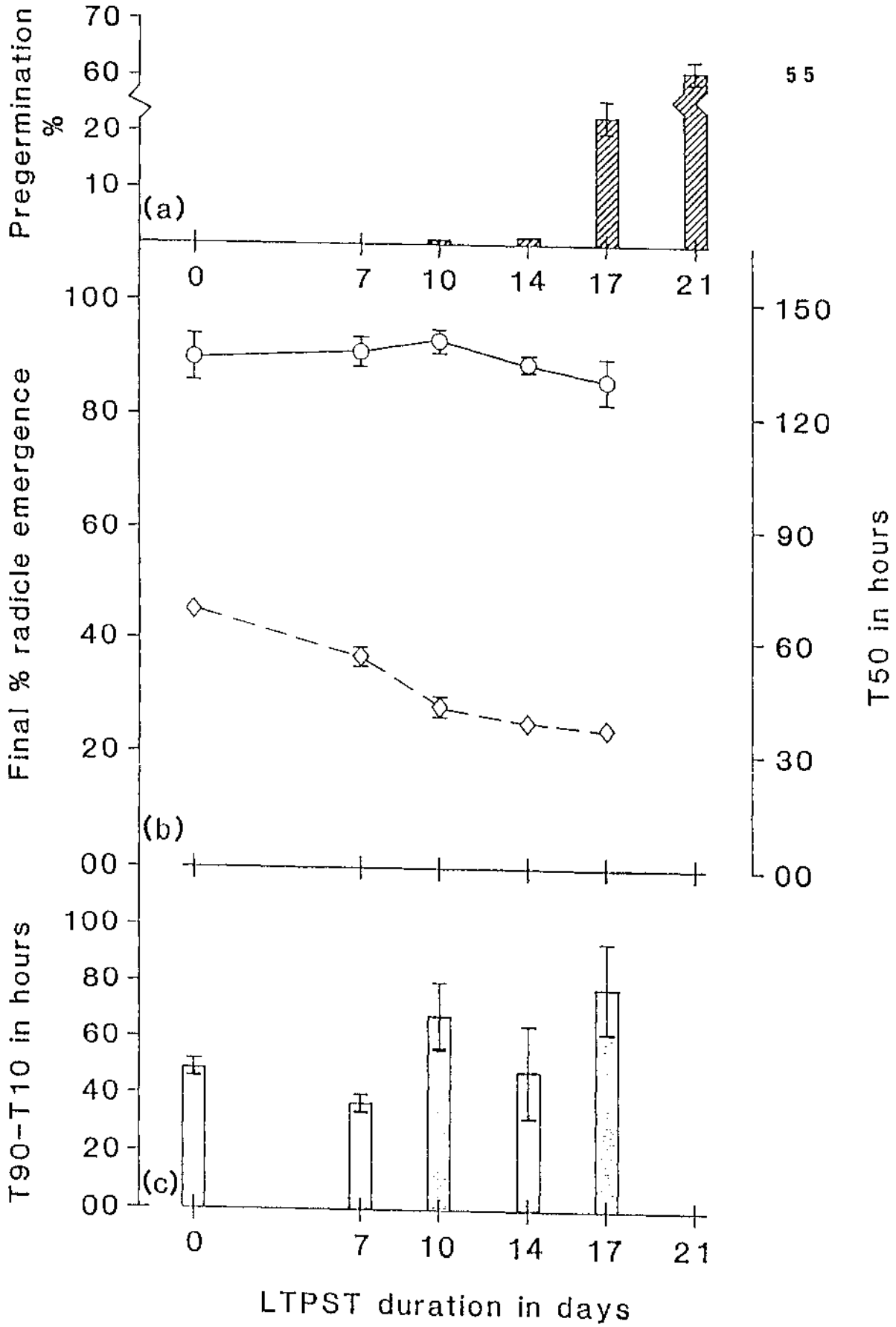

Figure 4.5 Effects of different durations of LTPST on the germination behaviour of tomato seed lot S-1.
(a) Pregermination percentage [histogram with diagonal lines].
(b) Final radicle emergence percentage [O] and T50 [◊].
(c) Uniformity as T90 - T10 [histo gram with horizontal lines].
Data presented are means of four replications; vertical bars represent SE's calculated for individual means and are shown where larger than the symbols used.

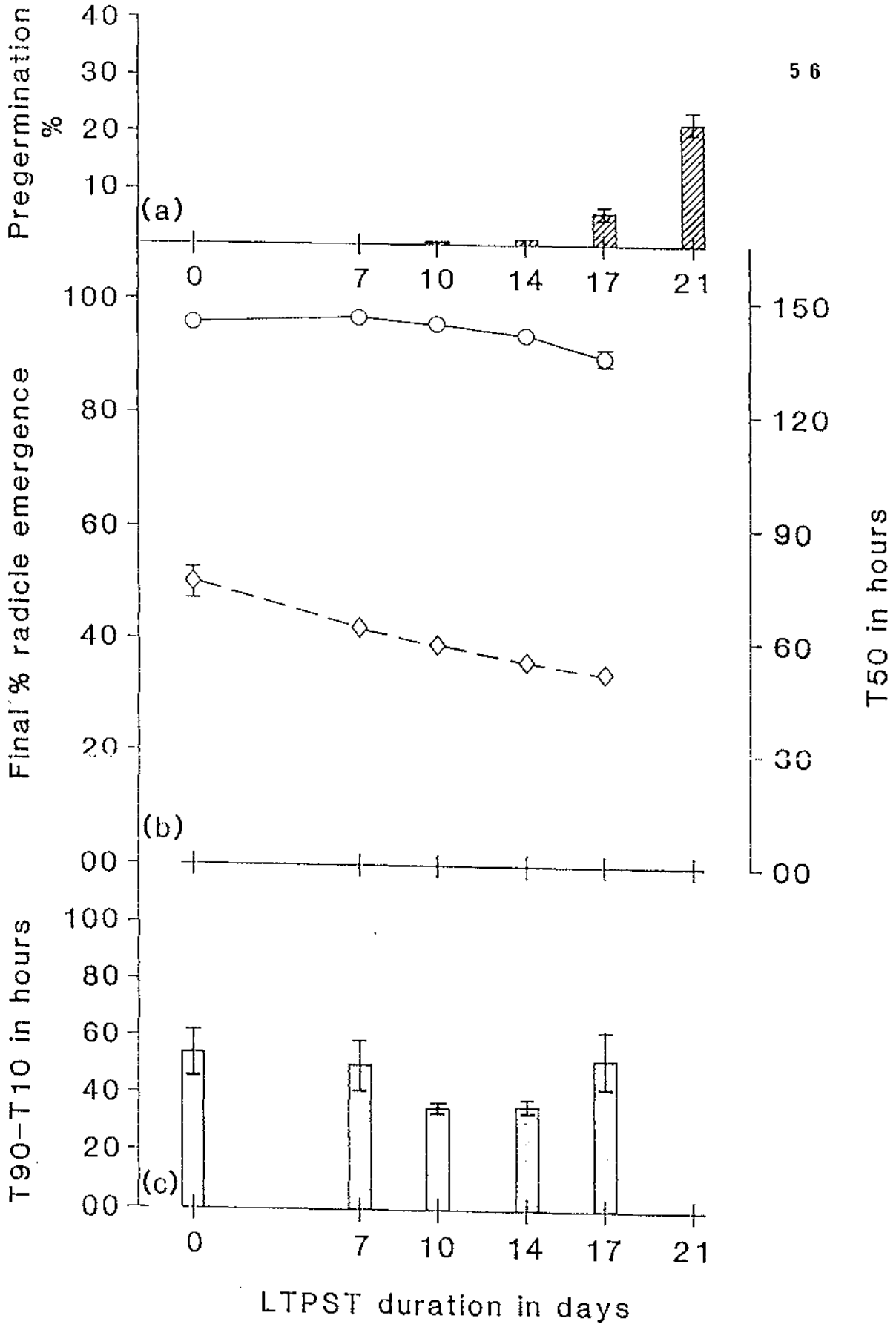

Figure 4.6 Effects of different durations of LTPST on the germination behaviour of tomato seed lot S-2.
(a) Pregermination percentage [histogram with diagonal lines].
(b) Final radicle emergence percentage [o] and T50 [◇].
(c) Uniformity as T90 - T10 [historgram with horizontal lines].
Data presented are means of four replications; vertical bars represent SE's calculated for individual means and are shown where larger than the symbols used.

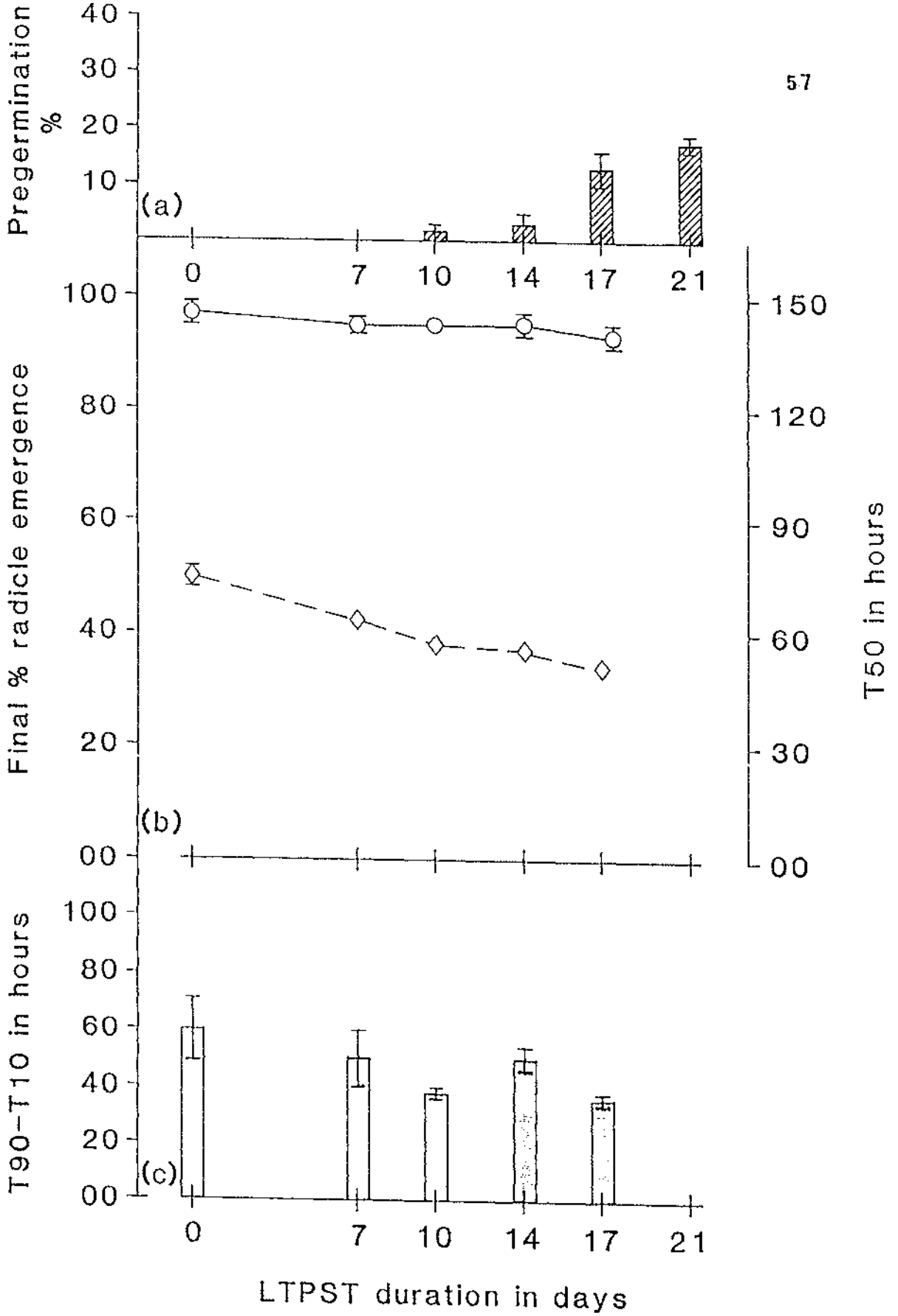

Figure 4.7 Effects of different durations of LTPST on the germination behaviour of tomato seed lot S-3.
(a) Pregermination percentage [histogram with diagonal lines].
(b) Final radicle emergence percentage [o] and T50 [◊].
(c) Uniformity as T90 - T10 [histogram with horizontal lines].
Data presented are means of four replications; vertical bars represent SE's calculated for individual means and are shown where larger than the symbols used.

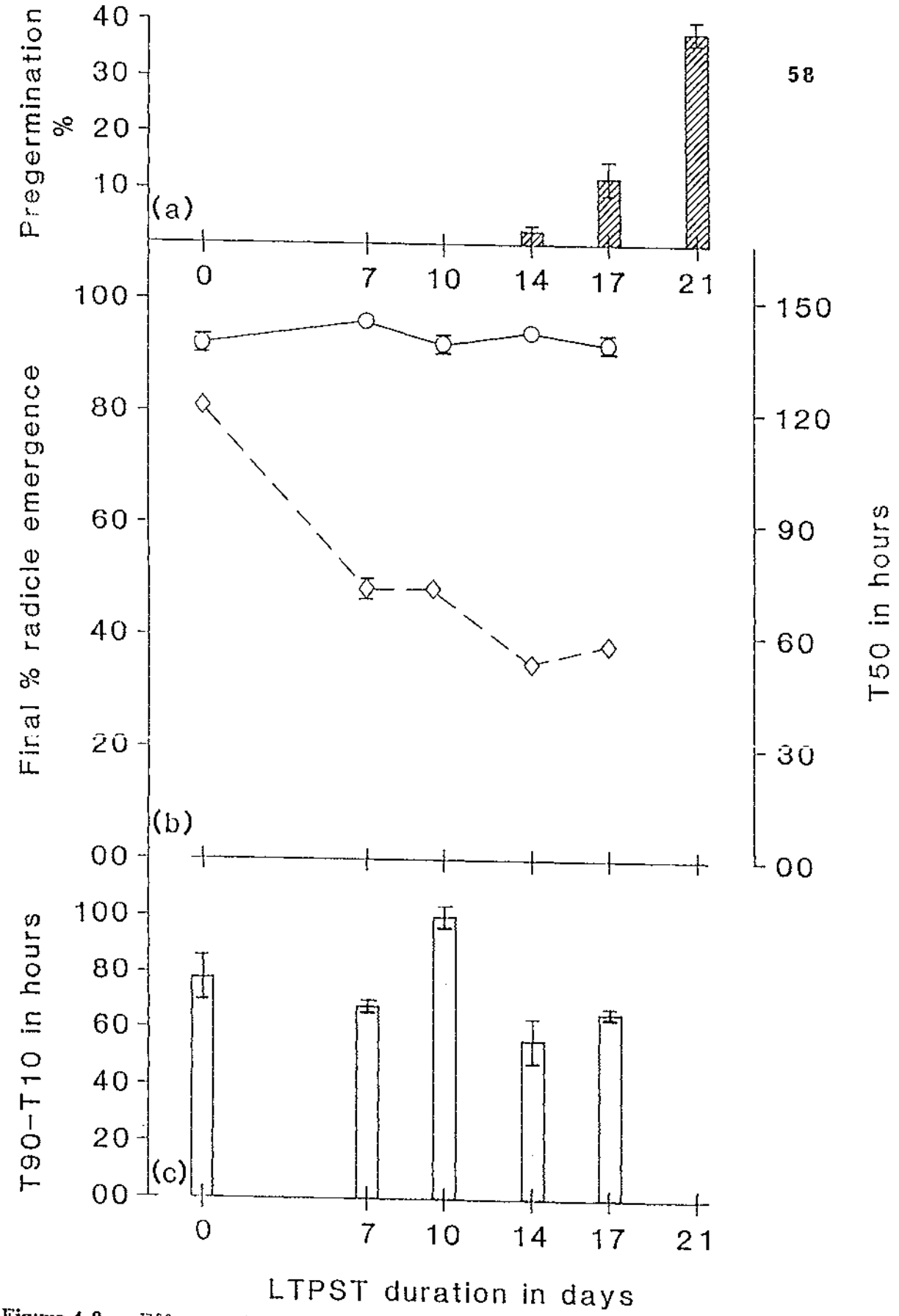

Figure 4.8 Effects of different durations of LTPST on the germination behaviour of tomato seed lot G-1.
(a) Pregermination percentage [histogram with diagonal lines].
(b) Final radicle emergence percentage [O] and T50 [◇].
(c) Uniformity as T90 - T10 [histogram with horizontal lines].
Data presented are means of four replications; vertical bars represent SE's calculated for individual means and are shown where larger than the symbols used.

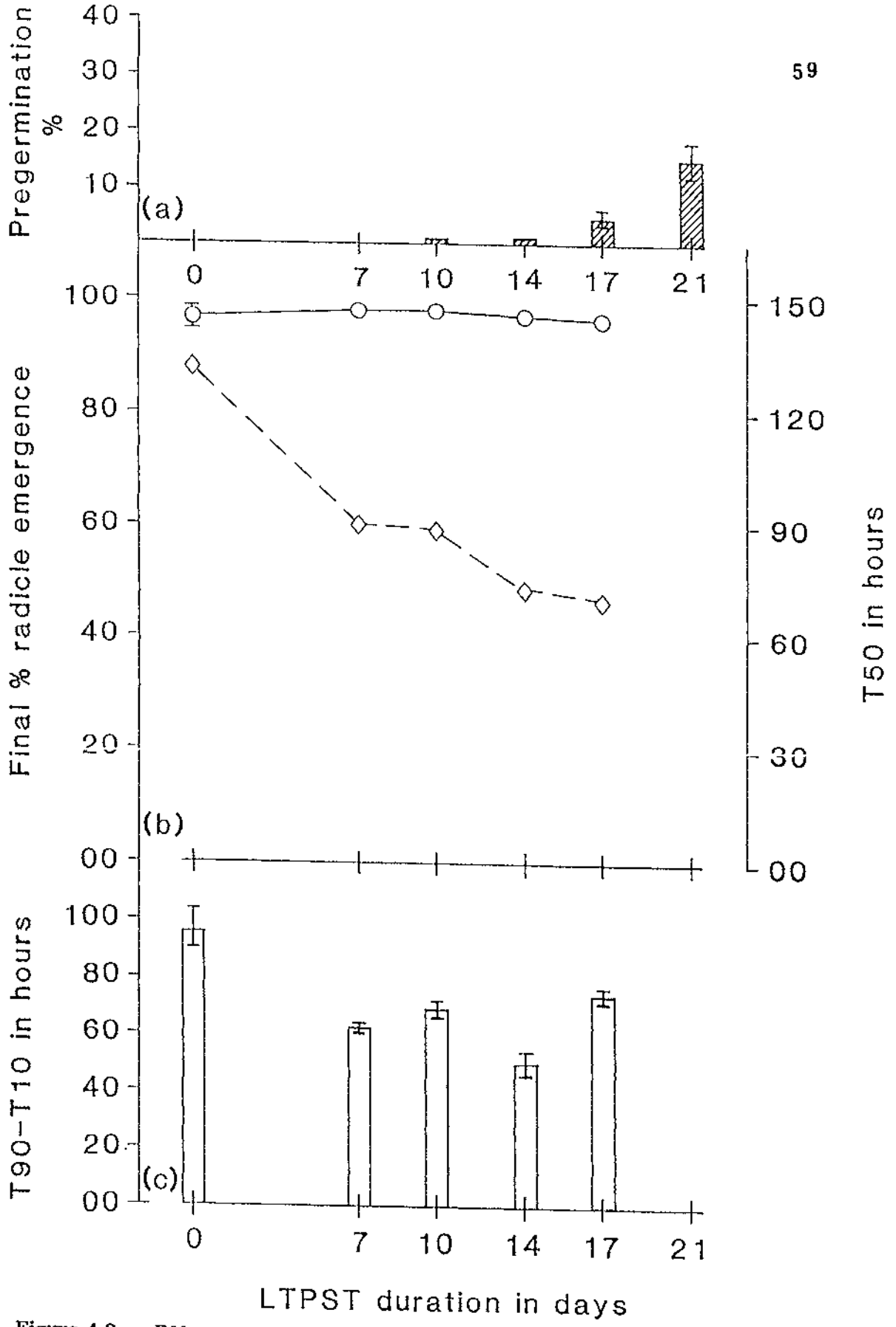

Figure 4.9 Effects of different durations of LTPST on the germination behaviour of tomato seed lot G-2.
(a) Pregermination percentage [histogram with diagonal lines].
(b) Final radicle emergence percentage [O] and T50 [◊].
(c) Uniformity as T90 - T10 [histogram with horizontal lines].
Data presented are means of four replications; vertical bars represent SE's calculated for individual means and are shown where larger than the symbols used.

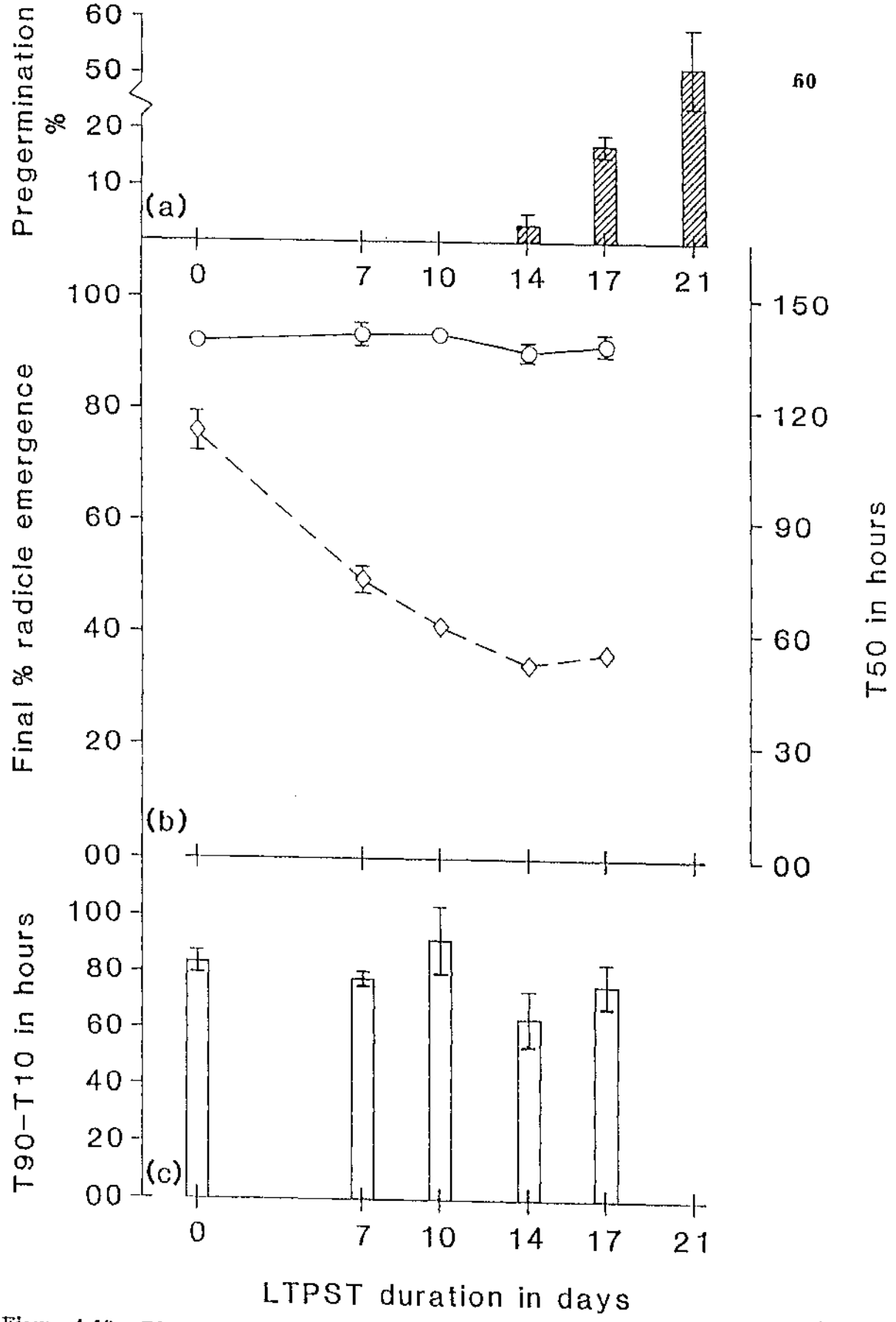

Figure 4.10 Effects of different durations of LTPST on the germination behaviour of tomato seed lot G-3.
(a) Pregermination percentage [histogram with diagonal lines].
(b) Final radicle emergence percentage [O] and T50 [◇].
(c) Uniformity as T90 - T10 [histogram with horizontal lines].
Data presented are means of four replications; vertical bars represent SE's calculated for individual means and are shown where larger than the symbols used.

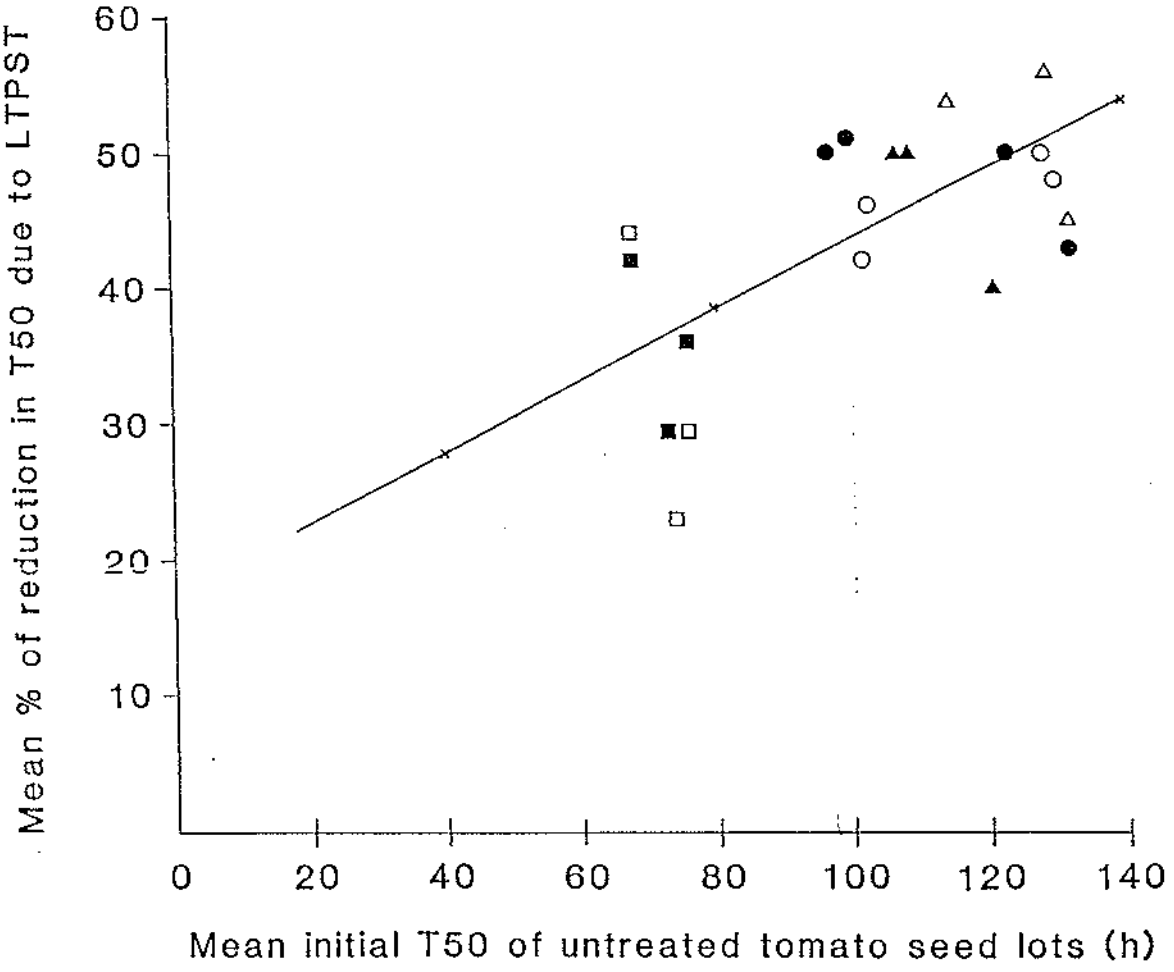

Figure 4.11 The relationship between the reduction in mean T50 resulting from 14 d LTPST and the initial mean T50 of untreated seed. Data from two experiments are presented of tomato seed cv. Moneymaker (o ●), Scoresby dwarf. (□ ■), Grosse lisse Improved (Δ ▲), Correlation r = 0.672, $p < 0.01$, n = 20. [each data point is mean of four replications].

correlated with T50 at 20°C ($P < 0.01$). After 14 d incubation, germination during pretreatment was less than 1% in 7 seedlots and 2 to 3% in the remainder. After 21 d it ranged from less than 1% e.g. M-4 (Fig. 4.4) to about 62% in Lot S-1 (Fig. 4.5).

Pretreatment upto 14 days had no significant effect on germination capacity compared to untreated seed, but longer periods may cause a reduction in radicle emergence in some seedlots for example, M-3 (Fig. 4.3) and S-2 (Fig. 4.6).

LTPST significantly reduced the median germination time (T50) in all seedlots at all treatment durations, being evident by 7 days which was the shortest duration tested (Fig's 4.1 - 4.10). With the exception of lot M-2, taking 17 d, the maximum reduction in T50 was obtained following 14 days pretreatment. Thereafter further reduction in T50 was not significant. The magnitude of reduction in T50 varied significantly between seedlots ranging from 23% in S-3 (Fig. 4.7) to 56% in G-1 (Fig. 4.8) following pretreatment for 14 days. The correlation between the percentage of reduction in T50 following 14 days pretreatment and the initial T50 of untreated seeds was highly significant ($r = 0.72$ $P < 0.01$). This means that the largest reduction in T50 was obtained by the slowest germinating seed (Fig. 4.11). In general, seedlots showed increased T50 where there was pregermination during treatment, with the exception of the seedlots of scoresby dwarf (Fig's 4.5 - 4.7).

Assessment of uniformity of radicle emergence as (T90-T10) showed that different seed lots reacted very differently to treatment in terms of this parameter (interaction between treatment and seed lot significant: $P < 0.01$). Pretreatment for 7 d caused significant improvement in uniformity in 5 seed lots (M-1, M-2, M-4, G-2 and G-3) while in others the uniformity were not significantly different from untreated seeds (Fig. 4.1 - 4.10). After 14 d pretreatment significant reductions in uniformity times were noted only in seed lots of M-1, M-4 and G-2.

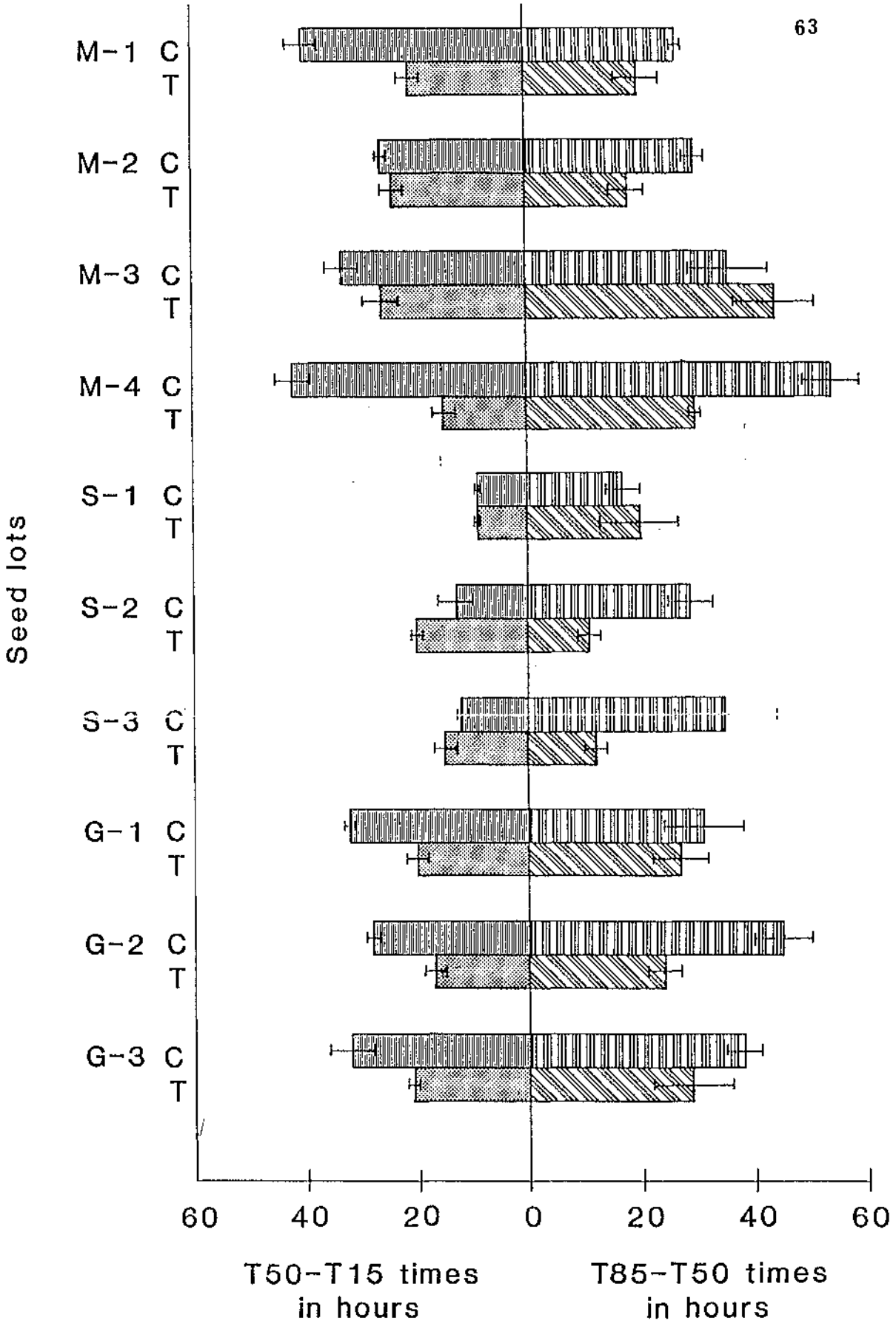

Figure 4.12 Spread of germination times in untreated and treated seeds of different seed lots of tomato. Each block represents the mean time taken for germination by a population covered by one standard deviation. Data presented are means of four replications with horizontal bars showing SE's calculated individually for each mean [T = treated, C = untreated control].

TABLE 4.3 The effect of LTPST on seed dry weight

CULTIVAR	SEED LOT	THOUSAND SEED DRY WEIGHT	
		Untreated (grams)	Treated (grams)
Moneymaker	M-2	2.44 ± 0.02	2.37 ± 0.03 *
	M-3	1.92 ± 0.04	1.91 ± 0.02
Scoresby dwarf	S-2	2.39 ± 0.01	2.35 ± 0.04
	S-3	2.41 ± 0.02	2.37 ± 0.02
Grosse lisse improved	G-1	2.19 ± 0.02	2.18 ± 0.02
	G-2	1.80 ± 0.03	1.78 ± 0.02
	G-3	2.29 ± 0.02	2.25 ± 0.02

NOTE 1.* Significant at 0.10 level

2. The data shown is means of 20 replications of about 50 seed each.

The analysis of spread of radicle emergence as (T85 - T15) and a normality test between early germinants, as (T50 - T15) and late germinants, as (T85 - T50) within the population indicated that the germination times were normally distributed in 6 seed lots, but not in M-1, S_2, S_3 and G-2 (Fig. 4.12). After treatment, the distribution became normal in M-1. S-3 and G-2 with improved uniformity, while it became skewed in M-4 with improved uniformity and unaffected in M-3 with no improvement in uniformity of germination.

Table 4.3 shows that pretreatment for 14 d tended to cause a reduction in seed dry weight in several seed lots although this was generally not significant.

4.3 EFFECTS OF LTPST

LTPST for 14 d was chosen for further investigation of its effects on normal germination, seedling growth and protection/invigoration of stored seeds. In future discussion LTPST refers to pretreatment for 14 d unless otherwise indicated. Pregermination was generally less than 1% and did not exceed 4% (S_1, S_3, G-1 and G-3).

4.3.1 Germination and germination performance

Figure 4.13 shows the effect of LTPST on radicle emergence and normal germination. Analysis of arc-sin transformed data showed no significant changes in radicle emergence were induced by LTPST except for Lot S_1. Similarly, LTPST had no significant effect on normal germination with the exception of Lot S-1. In seed lot S-1, and also a few replicates of S-3 and G-3, treated seedlings were affected by damping off disease.

Reductions in T50 were similar to the previous experiment under the same pretreatment conditions (Fig. 4.14). The relationship between the initial T50 of untreated seed and T50 of treated seed caused by LTPST was highly significant ($P < 0.01$) (Fig. 4.11).

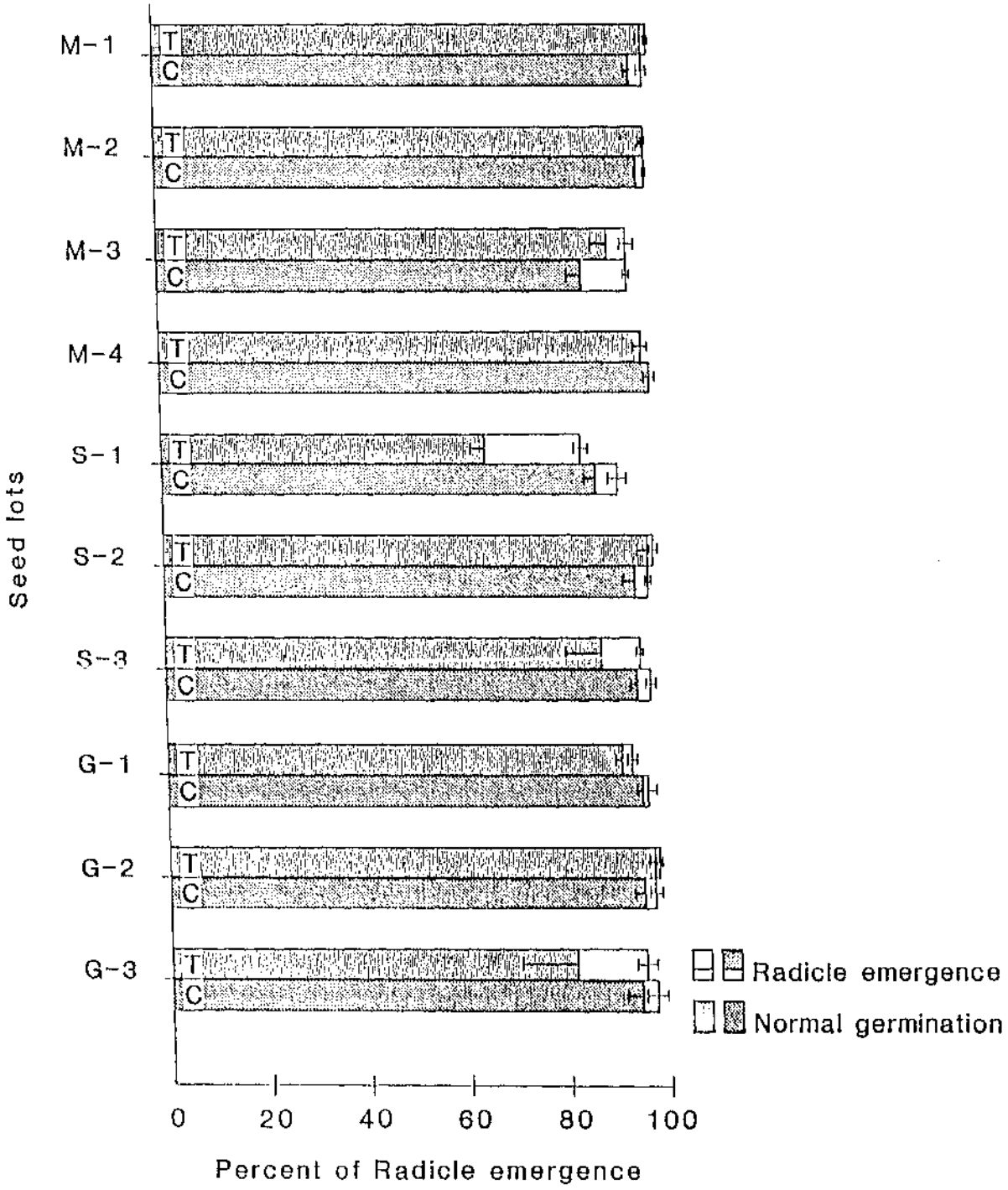

Figure 4.13 The effect of 14 d LTPST on total radicle emergence capacity and on normal germination of different seed lots of tomato seeds. Data presented are means of four replications with horizontal bars showing SE's calculated individually for each mean [T = treated, C = untreated control].

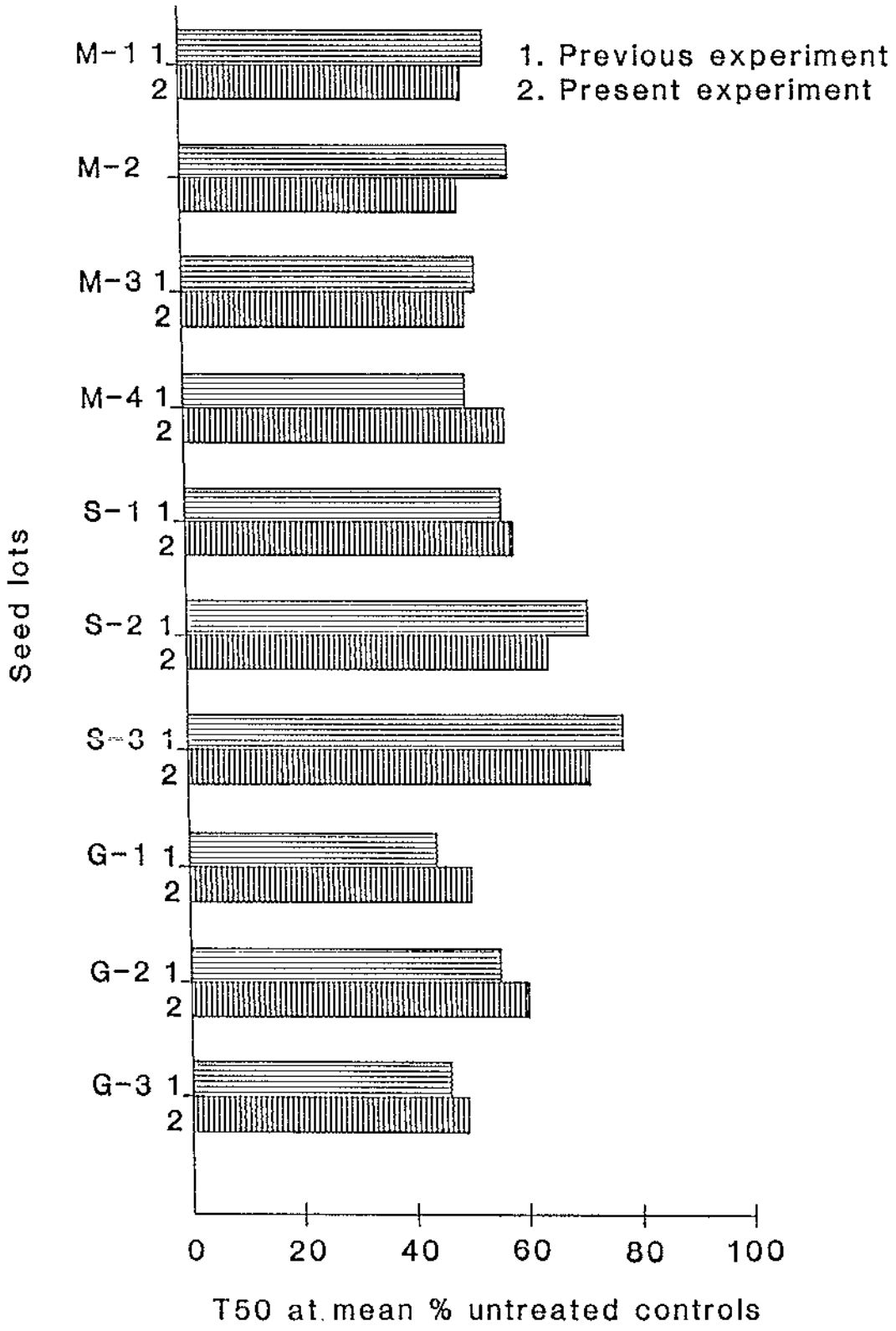

Figure 4.14 The effect of 14 d LTPST on median germination time presented as percentage of means of untreated control in two repeated experiments of different seed lots of tomato seeds. Data presented are means of four replications.

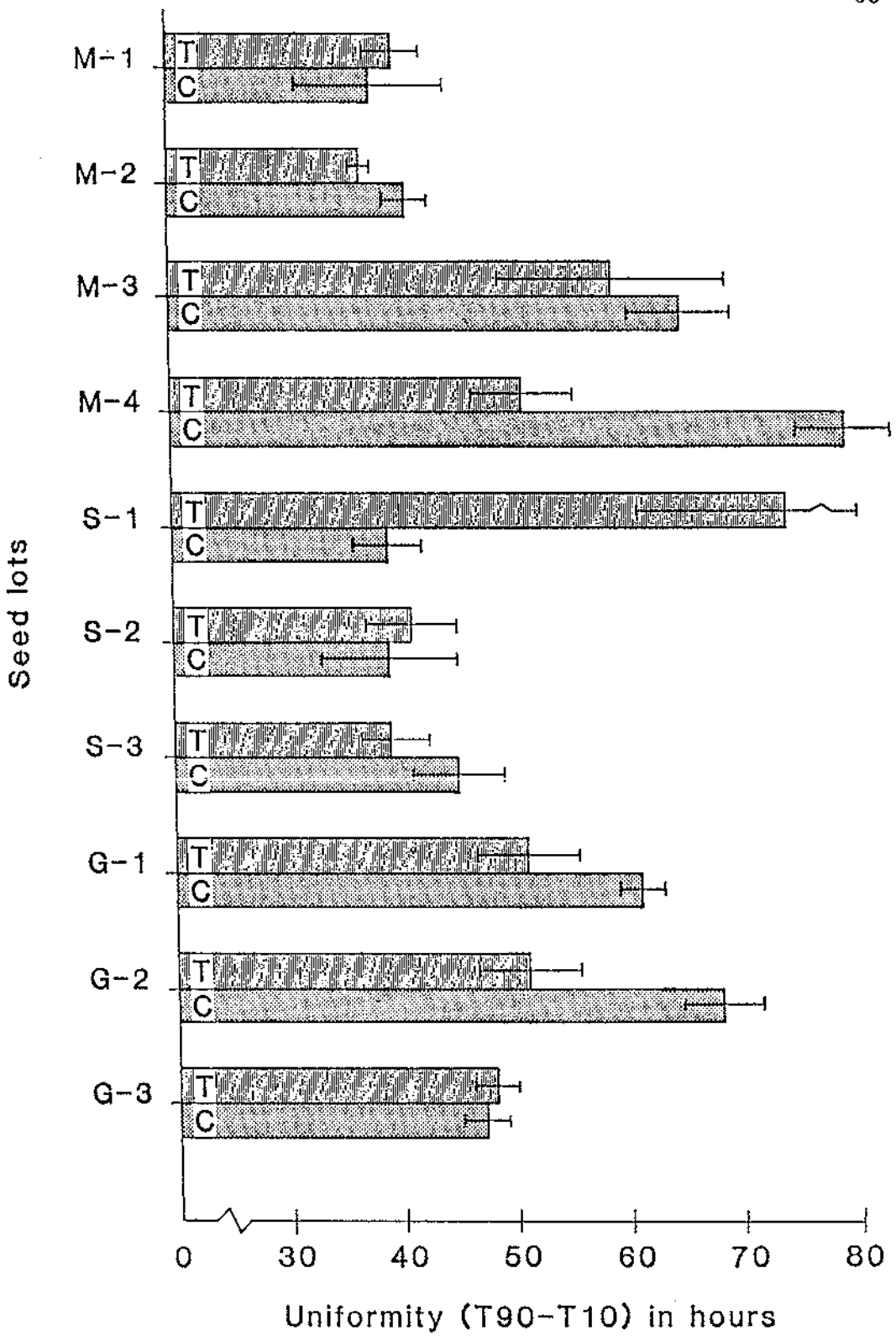

Figure 4.15 The effect of 14 d LTPST on uniformity (T90 - T10) of different seed lots of tomato seeds. Data presented are means of four replications with horizontal bars showing SE's calculated individually for each mean [T = treated, C = untreated control].

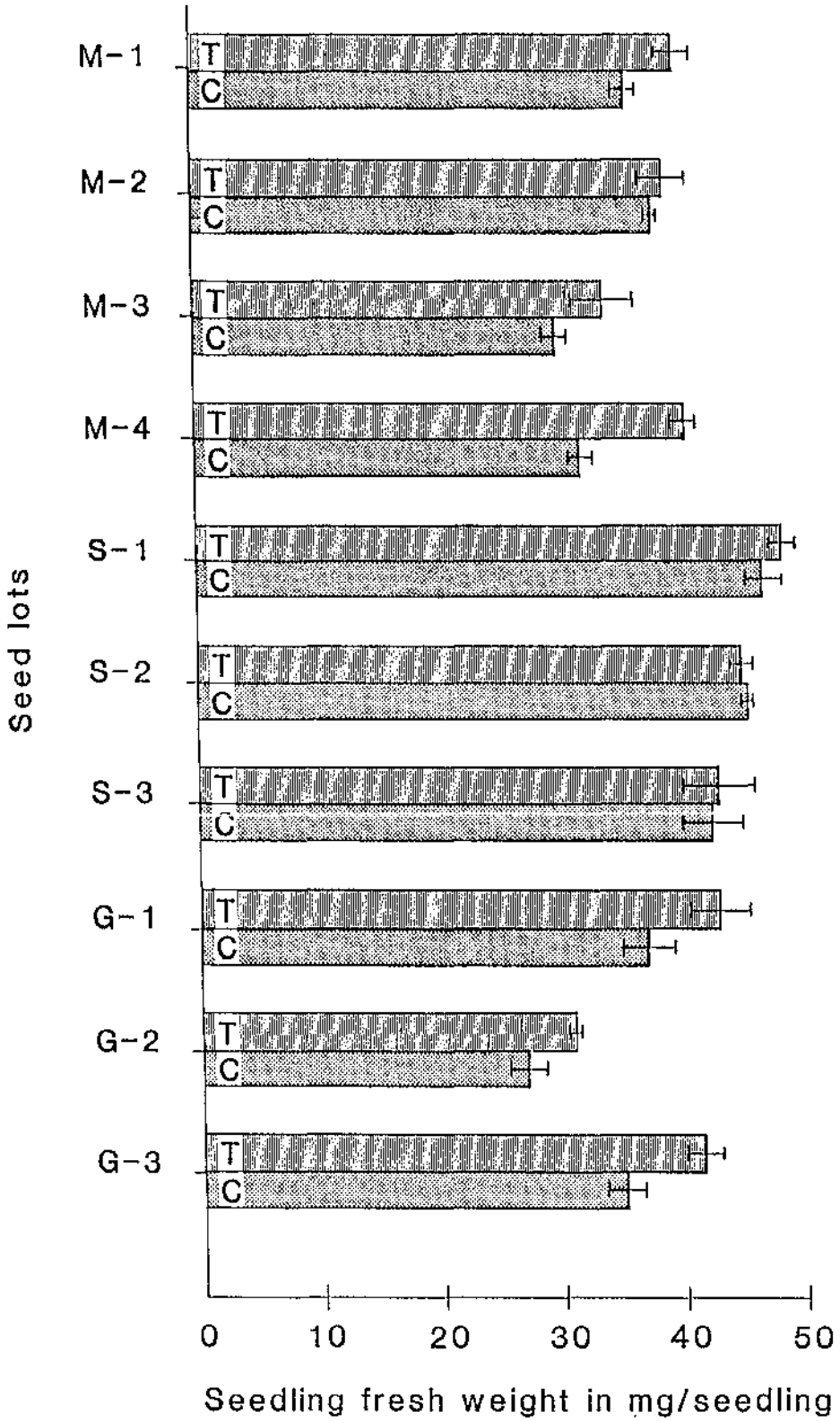

Figure 4.16 The effect of 14 d LTPST on seedling fresh weight of different seed lots of tomato seeds. Data presented are means of four replications with horizontal bars showing SE's calculated individually for each mean (T = treated, C = untreated control).

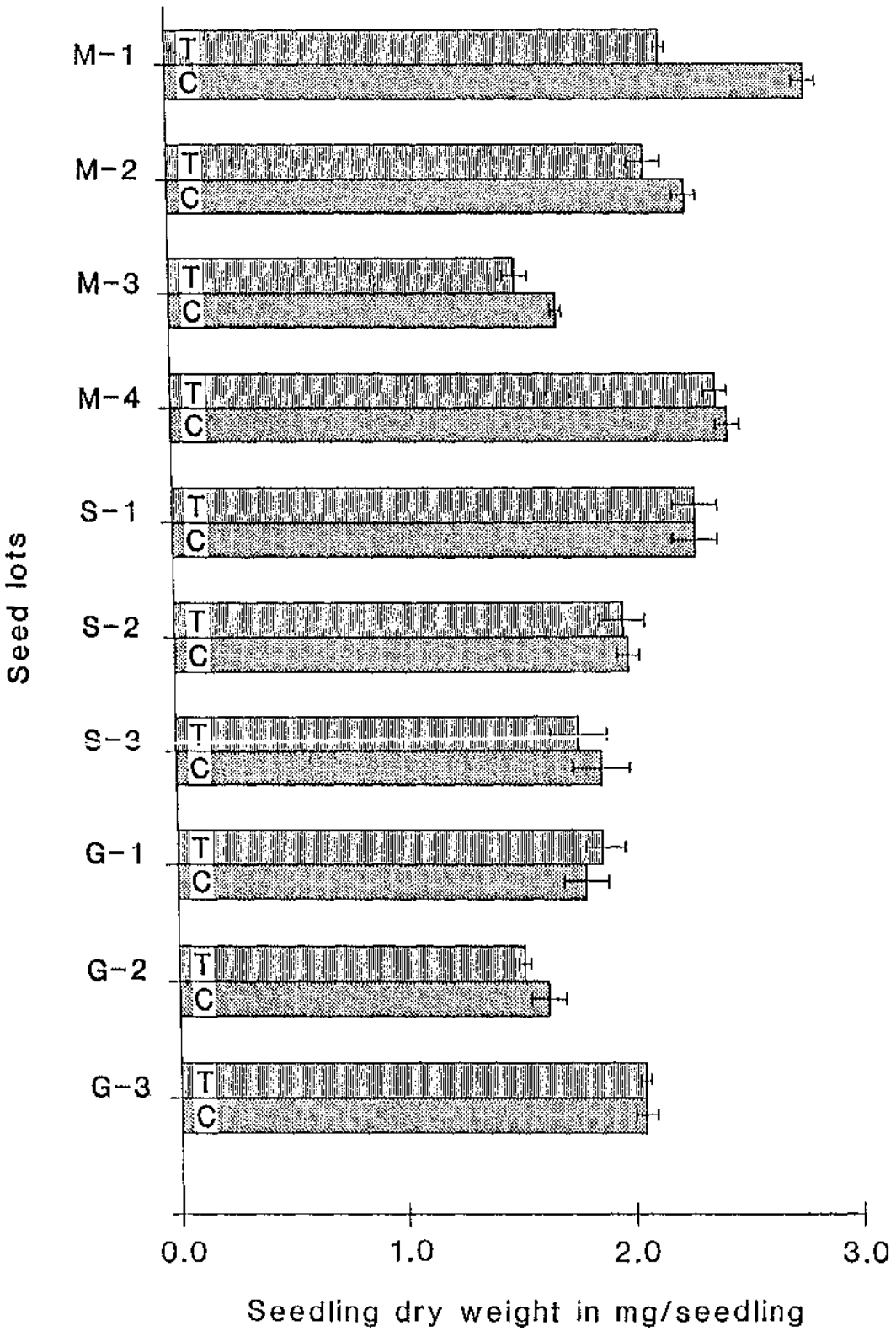

Figure 4.17 The effect of 14 d LTPST on seedling dry weight of different seed lots of tomato seeds. Data presented are means of four replications with horizontal bars showing SE's calculated individually for each mean (T = treated, C = untreated control].

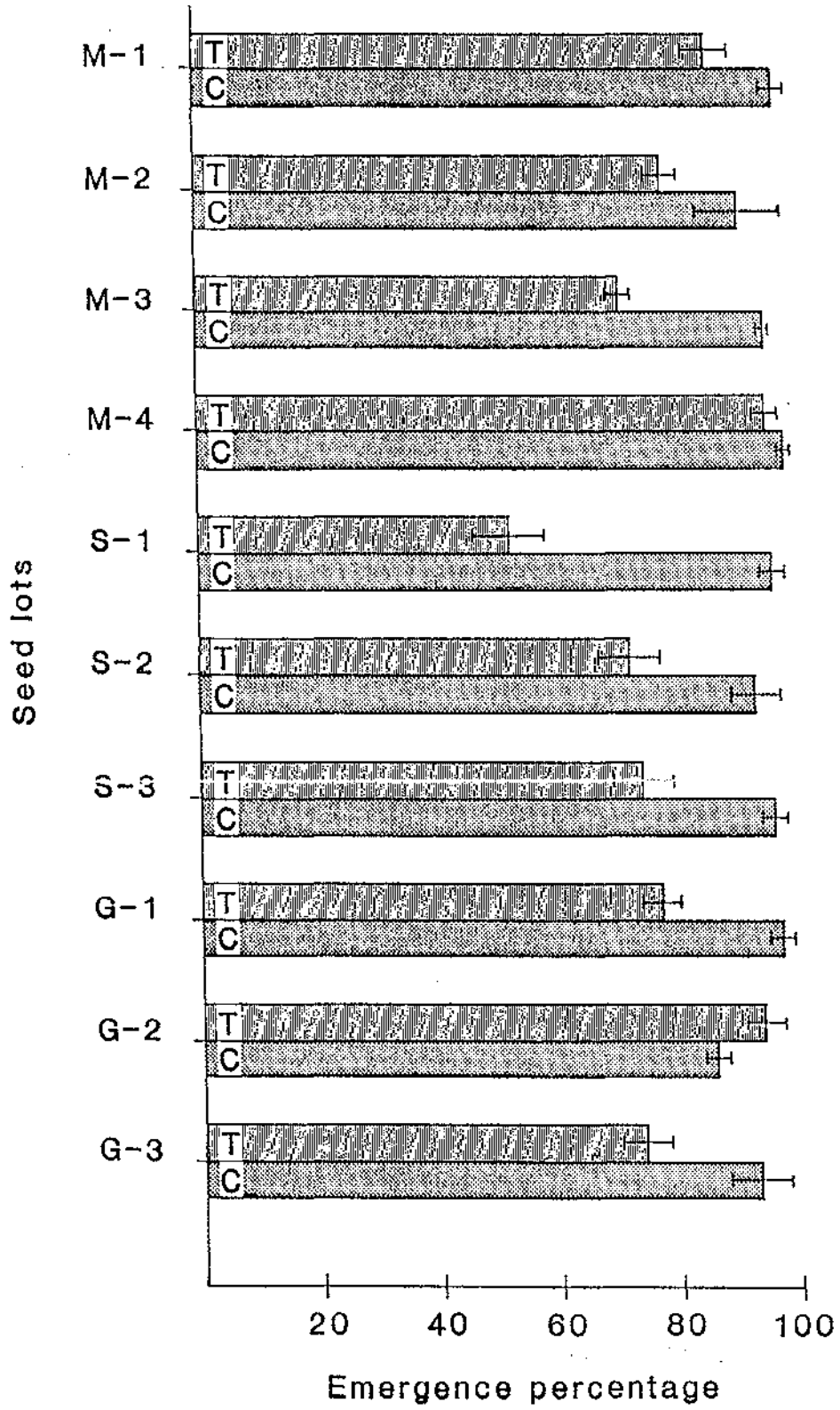

Figure 4.18 The effect of 14 d LTPST on total seedling emergence percentage of different seed lots of tomato seeds in soil. Data presented are means of four replications with horizontal bars showing SE's calculated individually for each mean (T = treated, C = untreated control].

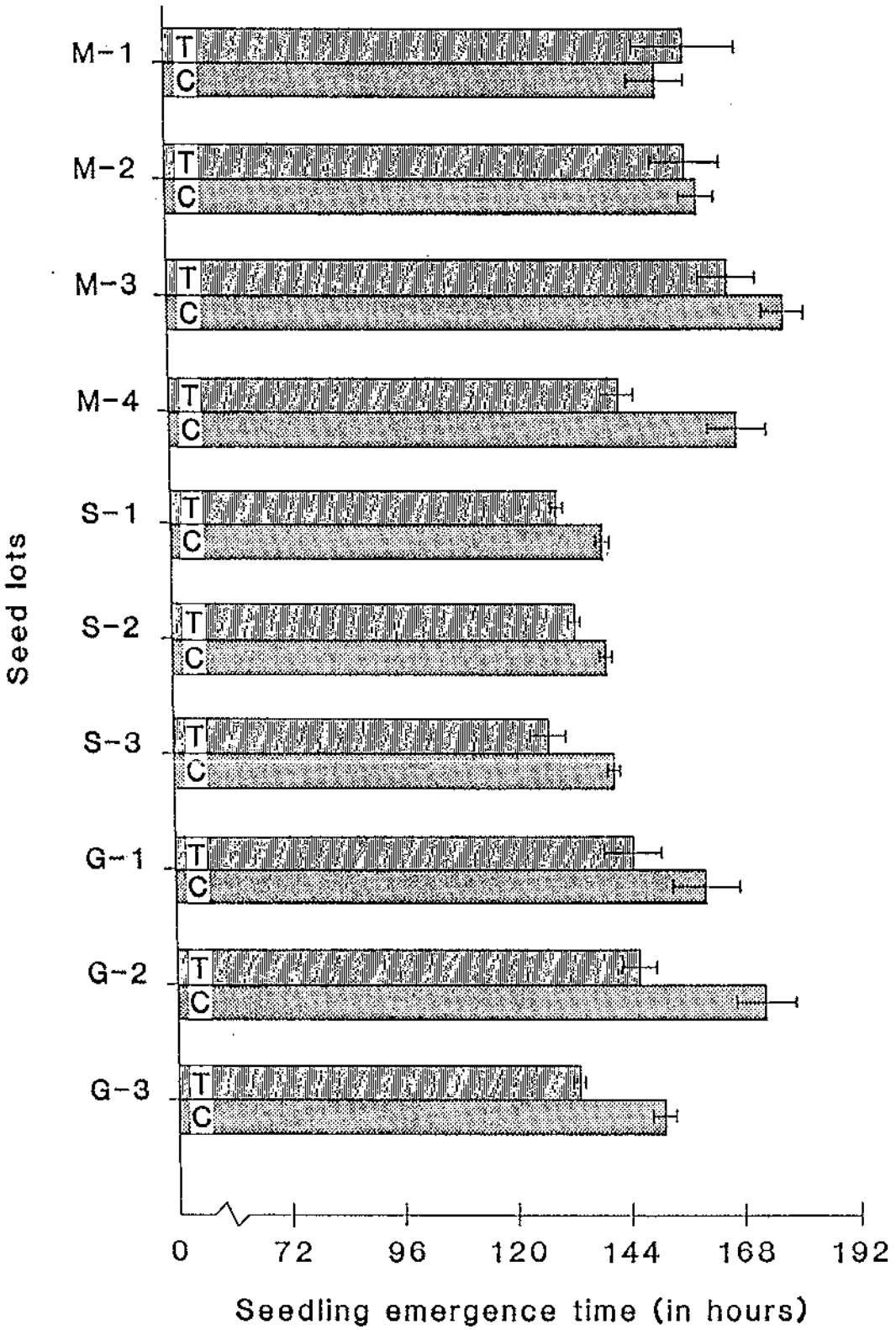

Figure 4.19 The effect of LTPST on mean seedling emergence time of different seed lots of tomato seeds in soil. Data presented are means of four replications with horizontal bars showing SE's calculated individually for each mean [T = treated, C = untreated control].

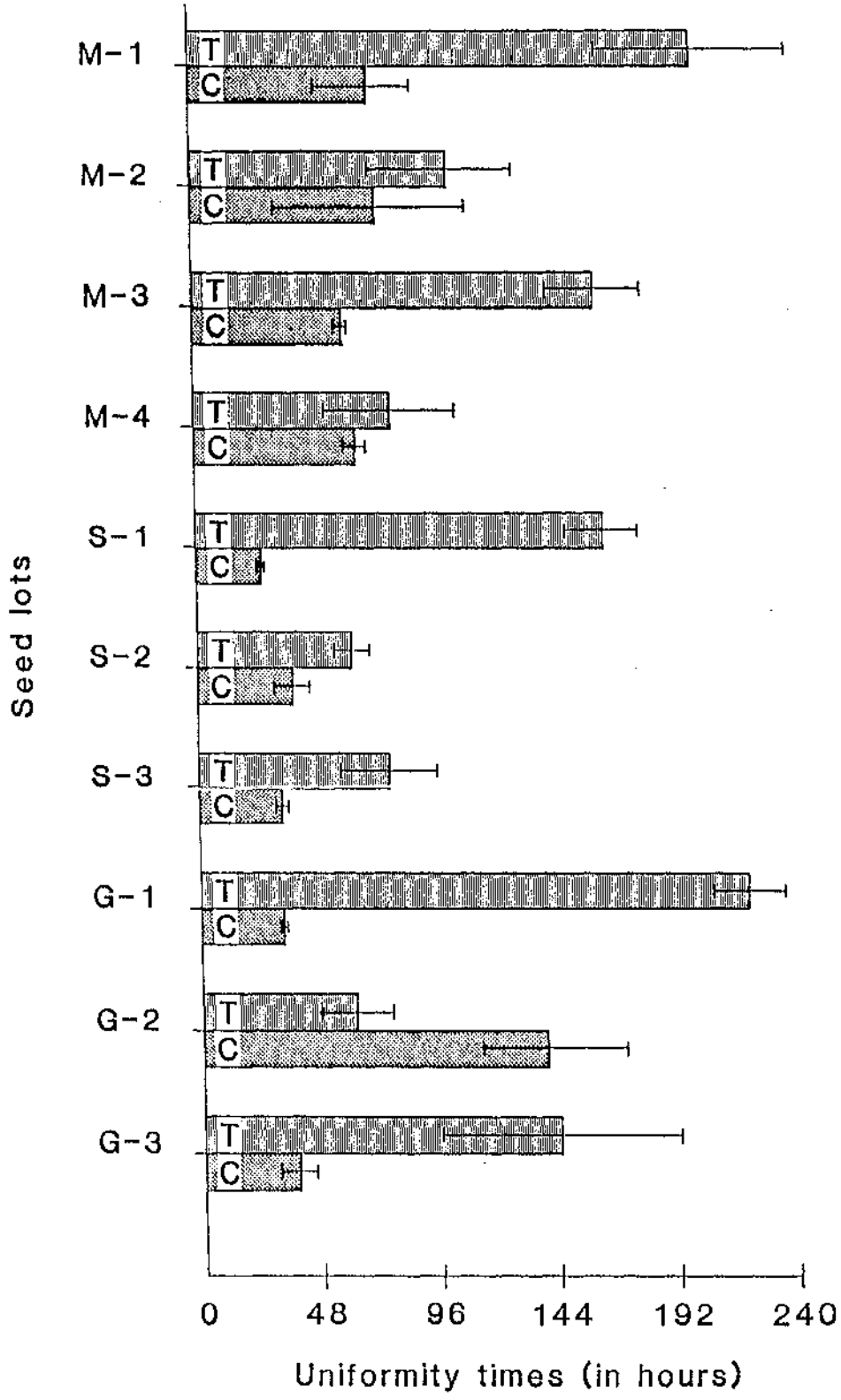

Figure 4.20 The effect of 14 d LTPST on uniformity of seedling emergence of different seed lots of tomato seeds in soil. Data presented are means of four replications with horizontal bars showing SE's calculated individually. [T = treated, C = untreated control].

Assessment of uniformity of radicle emergence indicated that LTPST had significantly improved the uniformity in seed lot M-4, G-1 and G-2 while in previous experiment under same pretreatment conditions uniformity were improved in 3 seed lots (M-1, M-4, and G-2). In other seed lots with exception seed lot S-1 the uniformity was not significantly different from untreated seeds (Fig. 4.15).

4.3.2 LTPST effects on seedling growth

There were no significant differences between treated and untreated seeds in seedling fresh weight 12 d after sowing except for seed lot M-4 which showed a significant improvement due to treatment (Fig. 4.16).

There were no significant differences in seedling dry weight caused by treatment except for seed lot M-1, where the dry weight was signficantly reduced (Fig. 4.17).

4.3.3 Soil emergence

Pretreated seeds stored from 16/4/89 to 5/7/89 were used in this experiment. With the exception of Lots M-4 and G-2, treated seed showed significant reductions in total emergence percentage (Fig. 4.18) compare to untreated seeds. Significant reductions in T50 was retained by 7 seed lots but in lots M-1, M-2 and M-3 T50's of treated seeds were not different from controls (Fig. 4.19). Improved uniformity of emergence due to LTPST was noted only in lot G-2, it was unchanged in Lots M_2, M-4 and significantly greater in other lots (Fig. 4.20).

CHAPTER 5
RESPONSES OF DIFFERENT TOMATO SEEDLOTS TO AGEING AND LTPST APPLIED BEFORE AND AFTER AGEING

5.1 EFFECTS OF THE DIFFERENT STORAGE REGIMES

Viability - It is quite evident from Figure 5.1 that increased SMC and storage temperature caused a rapid loss of viability in seedlot M-1, for example, although there was a significant reduction in total radicle emergence capacity by 28 d under the storage regime of 8% SMC, 45°C, 14% SMC and 40% SMC, at this temperature caused a significant reduction in viability by 3 d. At 40°C significant reductions were noted after 14 d and 7 d, for 14 and 40% moisture, respectively. As can be seen, the variability between replications increased with the ageing duration (Fig. 5.1a and b).

The percentages of radicle emergence were transformed to probits, adding the value 5 to all probits to avoid negative values (Finney, 1971). In this case the value 5 is the mean of the distribution where the proportion is 50%. The same convention is used to describe Ki (initial viability of seeds before storage and/or seed lot constant) throughout this study.

Plotting these transformed values against storage time resulted in survival curves which were straight lines (Fig. 5.2 and 5.3). Linear plots on a probit scale indicates a normal Gaussian distribution of the population and the slopes are equal to the inverse of the standard deviation (σ) of the population $1/\sigma$ (Finney, 1971). This implies that even under adverse storage environments where death is rapid, the seed population behaves as a normal one.

The correlations between storage periods and probit viability values were highly significant (Table 5.1) except for the two mildest storage environments (8% SMC, 40°C and 45°C), losses in viability were insufficient to construct survival curves and thus determine the values of their parameters. Figures 5.2 and 5.3 shows that

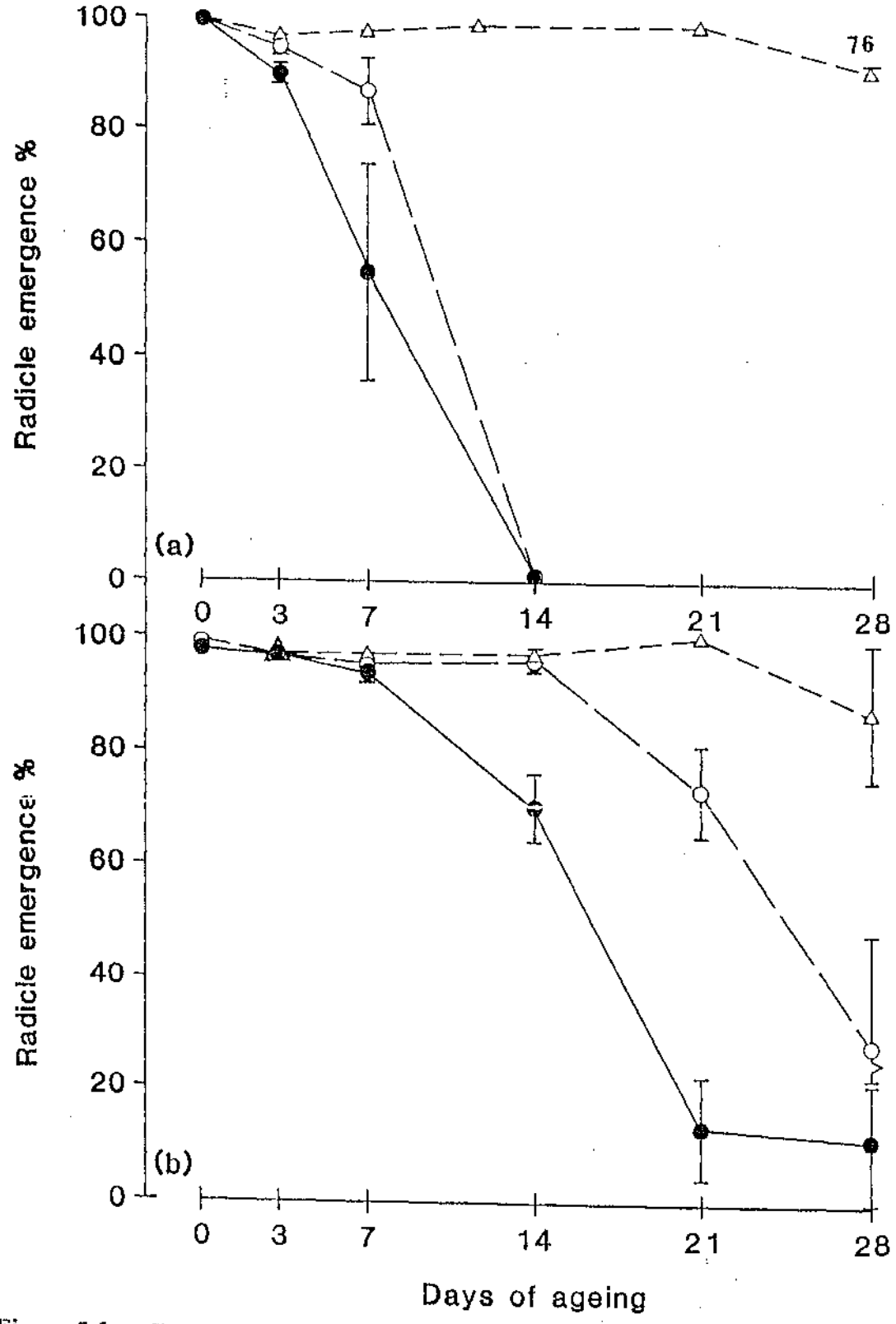

Figure 5.1 Effects of different storage regimes on total radicle emergence of seed lot M-1 of cv. Moneymaker tomato seeds. (a) at 45°C (b) at 40°C at different SMC of 40% [●], 14% [O] and 8% [Δ]. Data presented are means of four replications; vertical bars represent SE's calculated for individual means and are shown where larger than the symbols used.

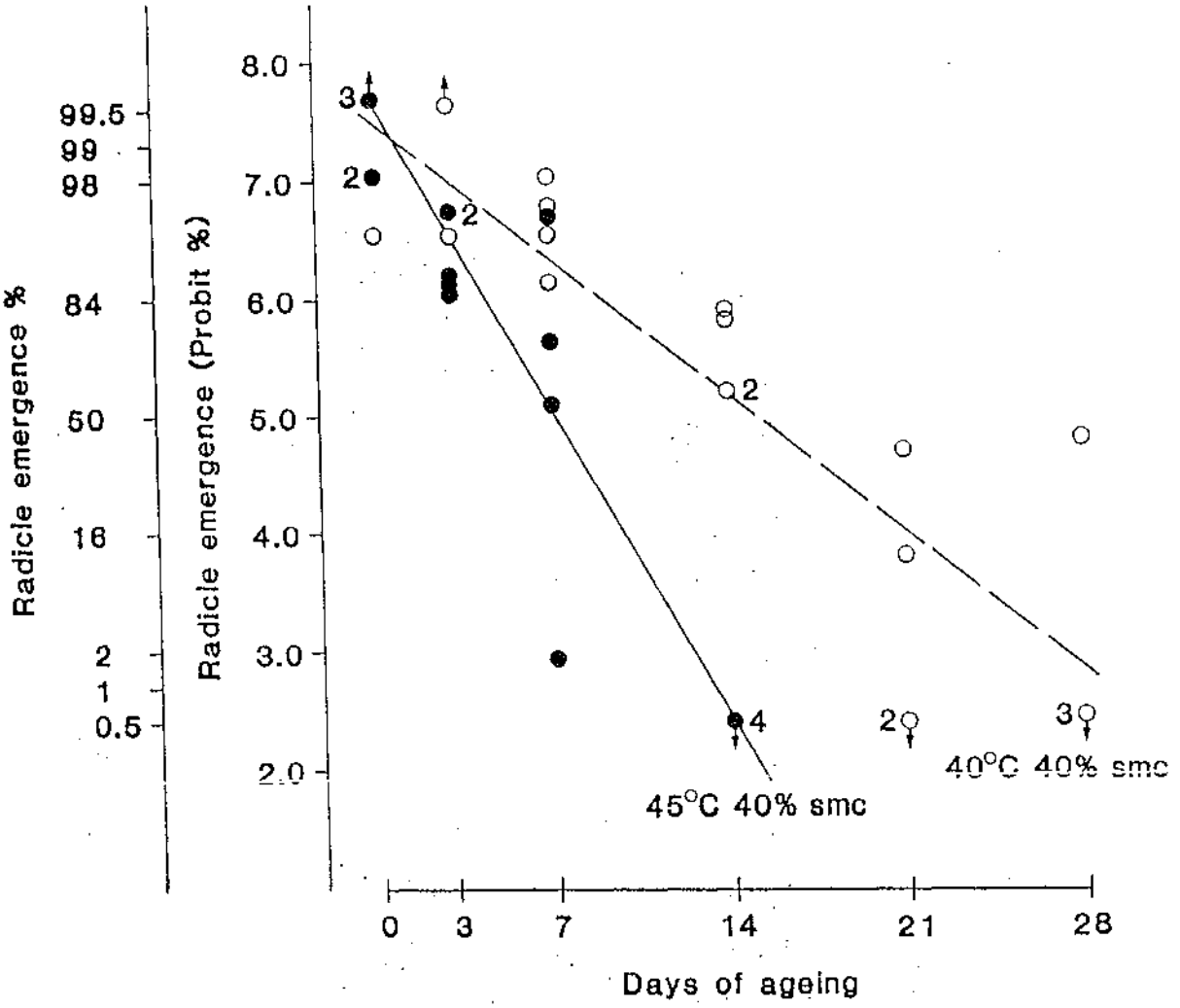

Figure 5.2 The effects of different storage temperature on the survival of tomato cv. Moneymaker seed lot M-1 at 40% SMC (40°C [O] 45°C [●]). Percent viability of each replication is plotted on a probit scale. Since values of 100 and 0% cannot be plotted on a probit scale, where such values occured experimentally they are indicated by points at 99.5 and 0.5% with arrows pointing up and down respectively. The resultant straight line seed survival curve was fitted by probit analysis. The data points with the number indicate the number of data points with same viability percent.

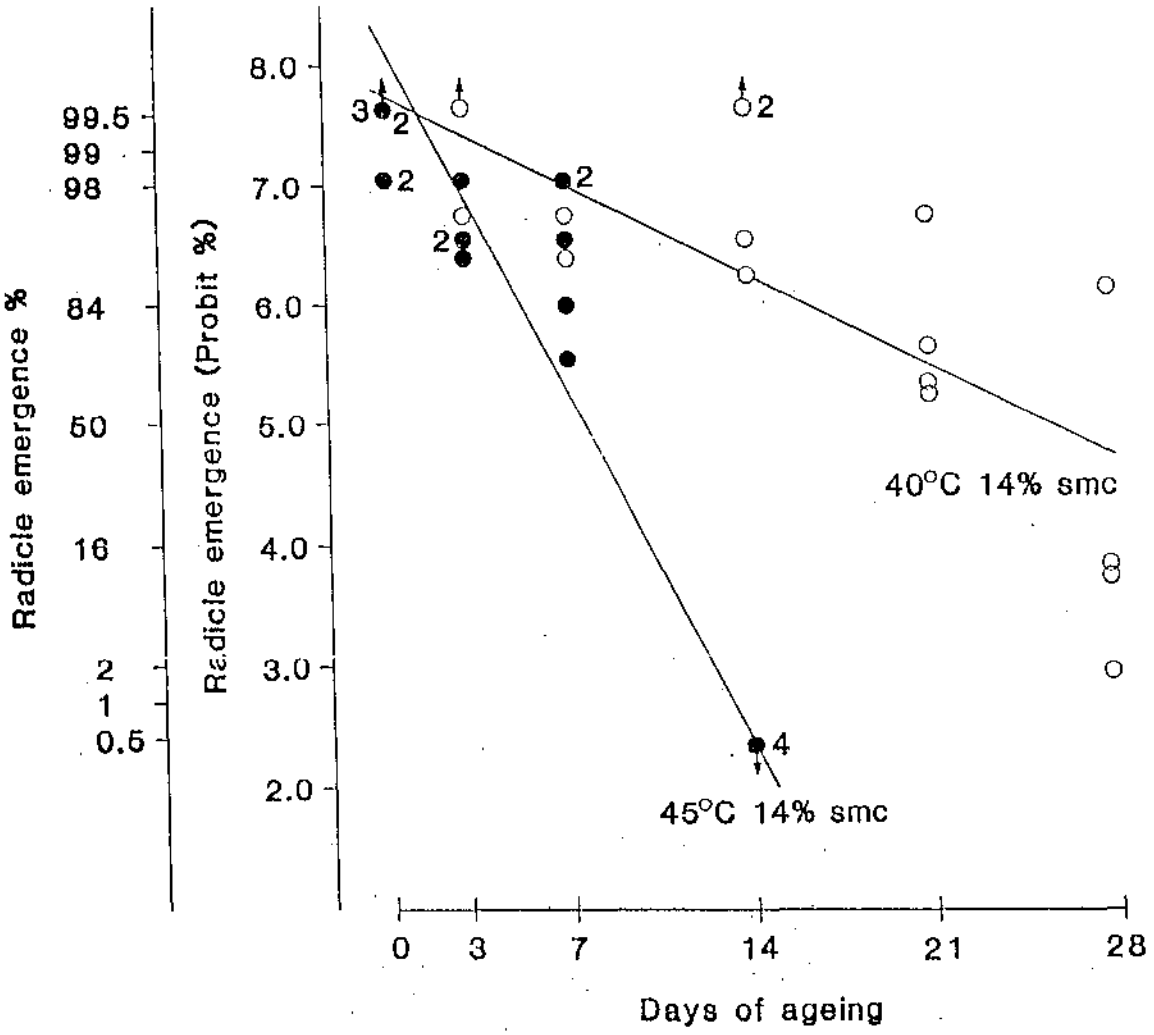

Figure 5.3 The effects of different storage temperature on the survival of tomato cv. Moneymaker seed lot M-1 at 14% SMC (40°C [O] 45°C [●]). Percent viability of each replication is plotted on a probit scale. Since values of 100 and 0% cannot be plotted on a probit scale, where such values occured experimentally they are indicated by points at 99.5 and 0.5% with arrows pointing up and down respectively. The resultant straight line seed survival curve was fitted by probit analysis. The data points with the number indicate the number of data points with same viability percent.

TABLE 5.1 The effects of different storage regimes on the viability decline pattern of tomato seeds cv. moneymaker seed lot M-1.

Ageing Conditions					
Temperature °C	Moisture % (FWB)	Correlation (r)	Probit initial Viability (Ki)	Slope $1/\sigma$	P50 (days)
45	40	-0.935***	7.68 ± 0.31[a]	-0.377 ± 0.038[a]	7.11[a]
45	14	-0.950***	8.14 ± 0.29[a]	-0.412 ± 0. 36[a]	7.62[a]
45	8	NS	7.42 ± 0.23[a]	NS	
40	40	-0.904***	7.50 ± 0.26[a]	-0.167 ± 0.017[b]	14.97[b]
40	14	-0.763***	7.73 ± 0.30[a]	-0.106 ± 0.019[c]	25.75[c]
40	8	NS	7.36 ± 0.27[a]	NS	

Note:
- *** - Highly significant at 0.001 level
- NS - not significant at 0.05 level
- Ki - is the intercept value of survival curve of the seed lot expressed in probit percentage of viability at the beginning of storage period and also referred to seed lot constant.
- r - correlation between probit percentage of viability and storage periods.
- P50 - the time taken for viability to fall to 50%.
- Values with the same letters are not significantly different at 0.05 level.

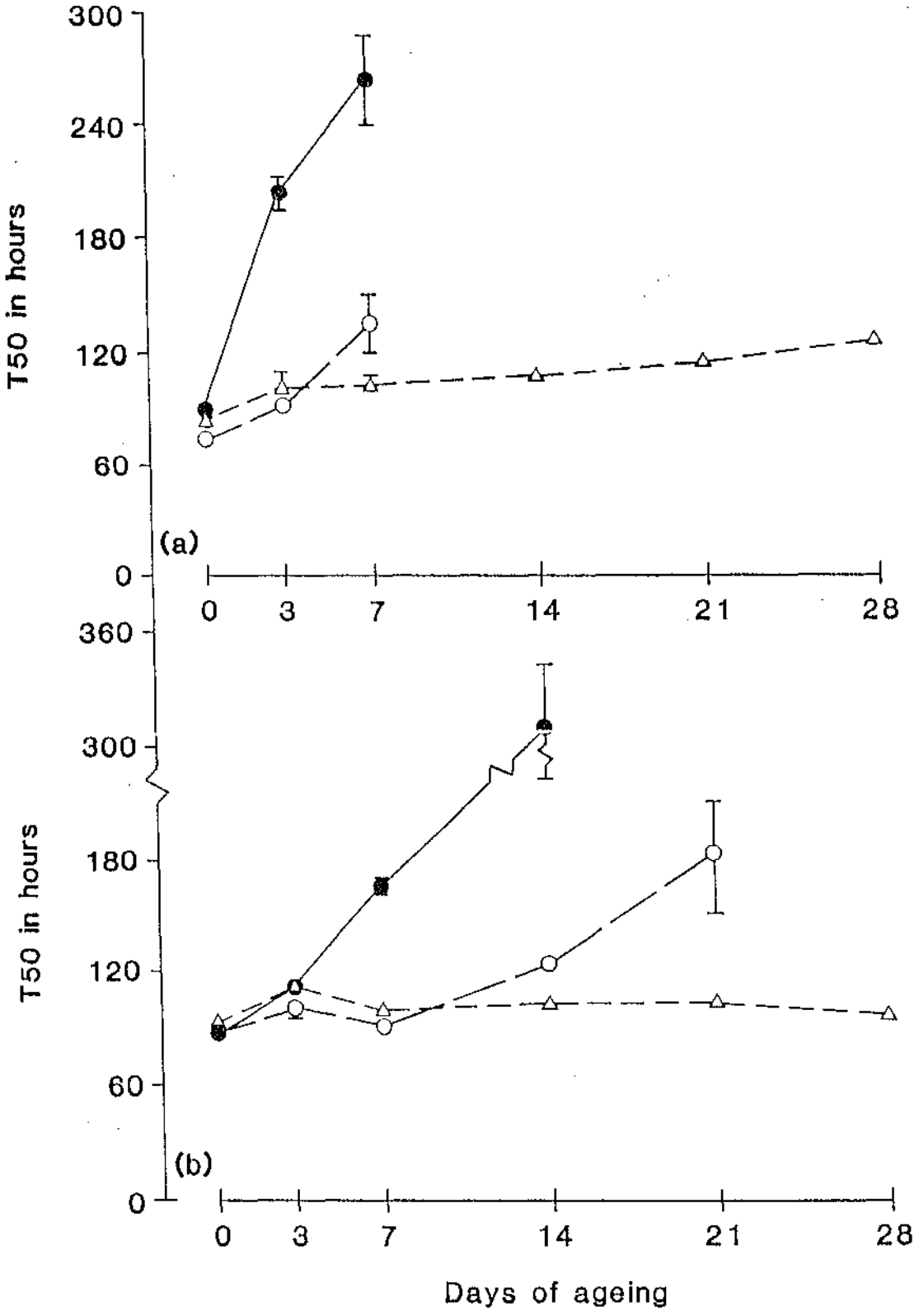

Figure 5.4 Effects of different storage regimes on median germination time of seed lot M-1 of cv. Moneymaker tomato seeds. (a) at 45°C (b) at 40°C at different SMC of 40% [●], 14% [O] and 8% [Δ]. Data presented are means of four replications; vertical bars represent SE's calculated for individual means and are shown where larger than the symbols used.

TABLE 5.2 The effect of storage temperature on the loss of seed vigour as measured by the increased T50 in tomato cv. Moneymaker seed lot M-1.

Days of Ageing at 8% SMC	Median germination time (h)	
	at 45°C	at 40°C
0 d	86 ± 2.2	91 ± 2.3^{NS}
3 d	99 ± 6.4	110 ± 7.0^{NS}
7 d	100 ± 4.3	97 ± 0.0^{NS}
14 d	109 ± 4.6	104 ± 1.9^{NS}
21 d	115 ± 3.8	104 ± 4.9^{NS}

NOTE: NS - Not significantly different between 40°C and 45°C at 0.05 level.

the slope of survival curves and the half viability period, P50 (the time taken for viability to fall to 50%) were obviously different between the storage environments, except for 14% and 40% SMC at 45°C. Differences in slope mean that the standard deviation (σ) of the distribution of death in time were significantly altered by the differences in storage environments (Table 5.1). Possibly the lack of difference between 14% and 40% SMC at 45°C isbecause the germination testing intervals was too large to identify the difference in effects of two storage regimes. Seeds under these two storage regimes had both lost their complete viability by 14 d (Fig. 5.1a). Table 5.1 shows that the calculated Ki, the probit of the intercept percentage germination value of the survival curves of the seed lot M-2 at zero storage time were not significantly different. This means that the Ki value was not affected by the ageing treatments and will remain as a constant for the seed lot.

Vigour - Figure 5.4 shows that ageing treatment caused a significant decrease in vigour as measured by increased T50. Comparison of Figures 5.1 and 5.4 shows that although there was no significant changes in radicle emergence capacity under many short or less severe ageing treatments, the mean T50's were increased significantly. For example, there was an increase in T50 from 88 h (± 2.1) of unaged seed to 165 h (± 5.4) and 124 h (± 1.3) for seeds aged for 7 d and 14 d under the storage regime of 40% and 14% SMC, 40°C respectively. Similarly, for the seed aged under 8% SMC, 45°C the T50 was increased from an 86 h (± 2.2) in unaged to 115 h (± 3.8) for seed aged for 21 d. Table 5.2 shows that the T50's of seeds aged for different durations at the same SMC were not significantly different between the two storage temperature tested. This means that simply increasing the temperature from 40°C to 45°C had no significant effect on the rate of loss of vigour provided the total REC of the seeds are not affected on contrast increased temperature caused rapid loss of viability. On the other hand, the increased SMC under the same storage temperature caused rapid loss of vigour. For example, seed aged for 7 d at 40% SMC, 40°C had a mean T50 of 165 h (± 5.4) compared to 90 h (± 3.5) of

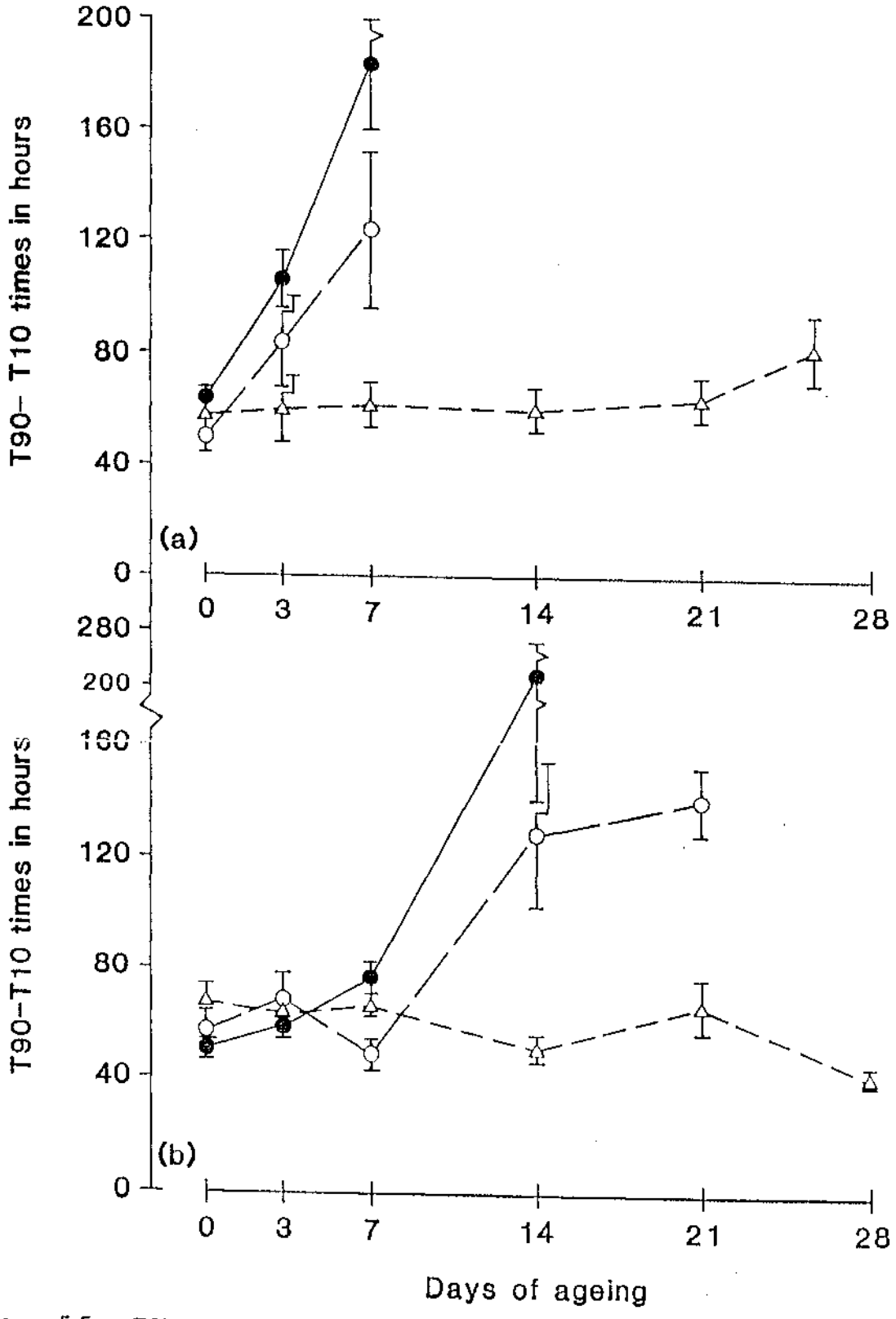

Figure 5.5 Effects of different storage regimes on uniformity (T90 - T10) of seed lot M-1 of cv. Moneymaker tomato seeds. (a) at 45°C (b) at 40°C at different SMC of 40% [●], 14% [○] and 8% [△]. Data presented are means of four replications; vertical bars represent SE's calculated for individual means and are shown where larger than the symbols used.

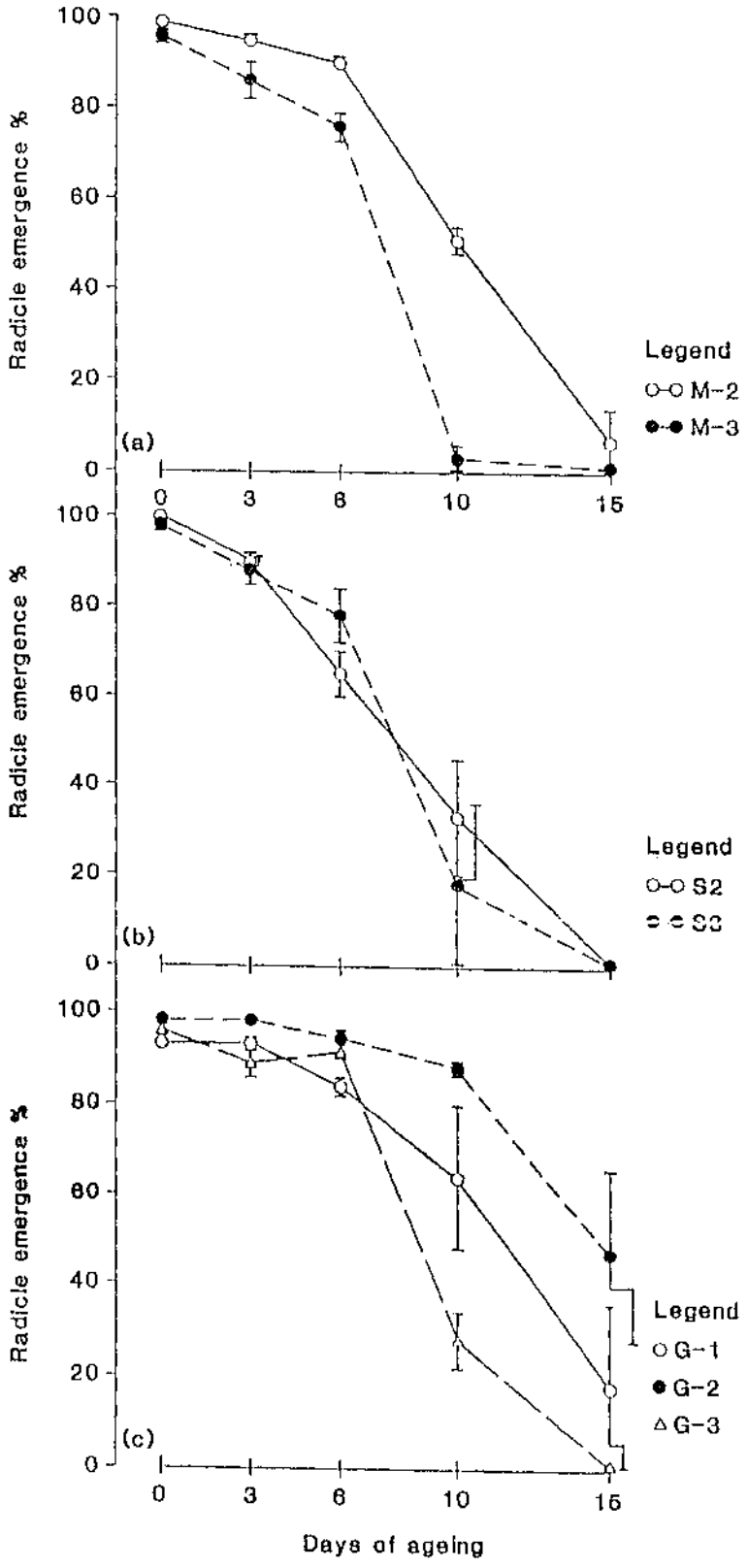

Figure 5.6 Effects of a storage regime (40°C, 40% SMC) on radicle emergence capacity of different seed lots of cultivars. (a) Moneymaker (b) Scoresby dwarf and (c) Grosse lisse Improved. Data presented are means of four replications; vertical bars represent SE's calculated for individual means and are shown where larger than the symbols used.

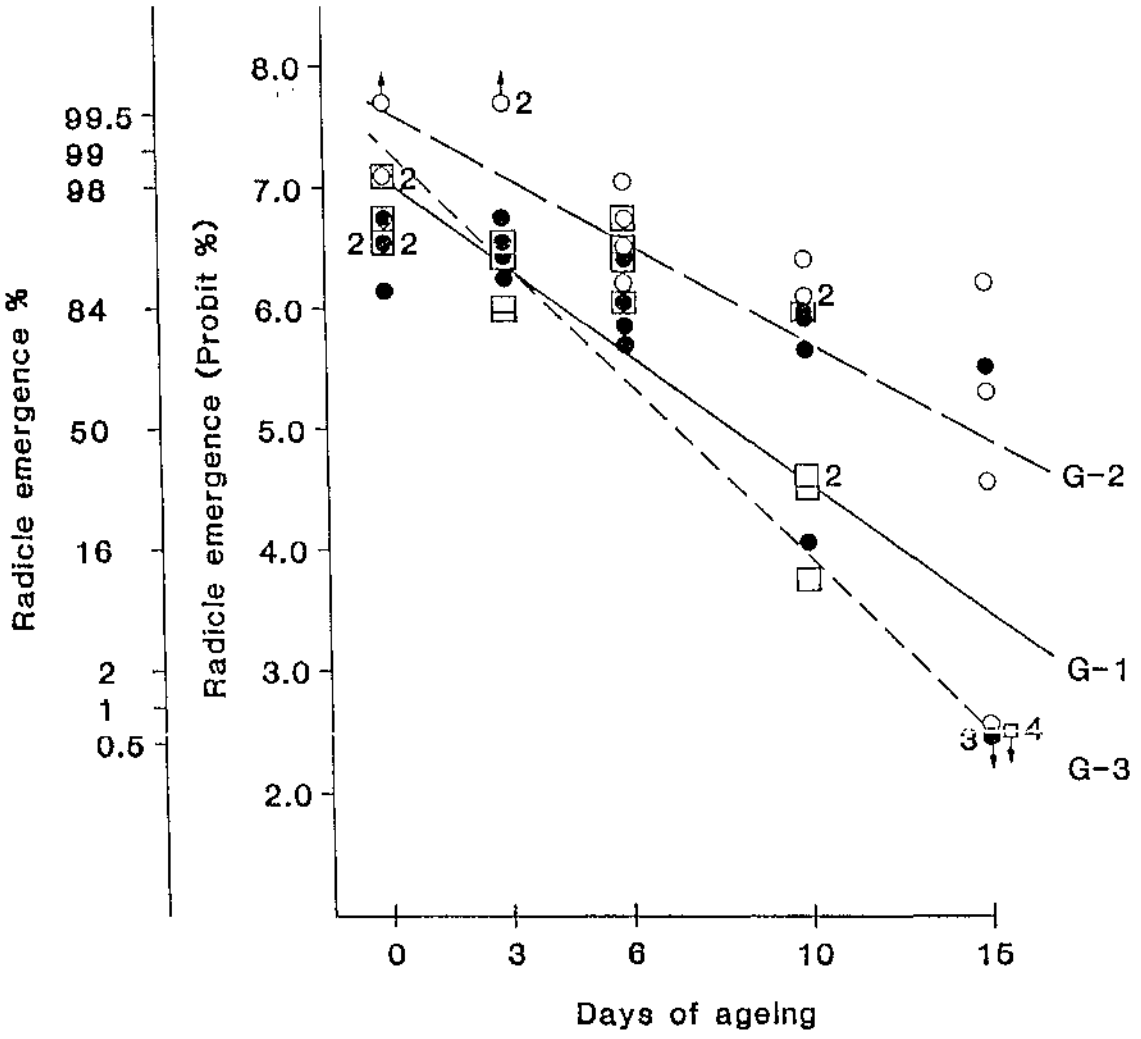

Figure 5.7 Seed survival curves of different seed lots of Grosse lisse Improved under same storage conditions (40°C, 40% SMC) G-1 [□], G-2 [O] and G-3 [●]. Percent viability of each replication is plotted on a probit scale. Since values of 100 and 0% cannot be plotted on a probit scale, where such values occured experimentally they are indicated by points at 99.5 and 0.5% with arrows pointing up and down respectively. The resultant straight line seed survival curve was fitted by probit analysis. The data points with the number indicate the number of data points with same viability percent.

TABLE 5.3 The effect of a storage regime (40% SMC, 40°C) on different seed lots of tomato seeds.

Cultivar	Seed Lot	Germination Percentage			Seed Lot Constant (Ki)	Slope ($1/\sigma$)	Half Viability Period (P50) in days
		Zero day	3 d	10 d			
Moneymaker	M-2	99 ±0.57	95 ±1.0	51 ± 3.7	7.82[a] ±0.25	-0.324[a] ±0.029	8.70[a]
	M-3	96 ±1.15	86 ±4.4	3 ± 2.5	7.07[a] ±0.29	-0.367[a] ±0.034	5.64[a]
Scoresby dwarf	S-2	100 ±0.5	90 ±1.5	33 ±13.2	7.68[a] ±0.25	-0.379[a] ±0.029	7.07[a]
	S-3	98 ±1.41	88 ±3.09	18 ±17.5	7.35[a] ±0.35	-0.382[a] ±0.041	6.15[a]
Grosse Iisse Improved	G-1	93 ±1.7	93 ±1.29	64 ±16.1	7.08[a] ±0.37	-0.241[ab] ±0.043	8.63[a]
	G-2	98 ±0.81	98 ±1.5	88 ± 1.6	7.64[a] ±0.34	-0.185[b] ±0.040	14.27[b]
	G-3	96 ±0.95	89 ±2.3	28 ± 6.0	7.36[a] ±0.26	-0.325[a] ±0.030	7.26[a]

Note: Numbers with the same letter are not significant at 0.05 level.

the seed aged at 14% SMC, 40°C. Similarly T50 of 14 d aged seed at 14% SMC, 40°C was 124 h (± 1.3) compared to 104 h (± 1.9) of the seed aged at 8% SMC, 40°C (Fig. 5.4b).

Fig. 5.5 shows that ageing treatments caused a decrease in uniformity measured as (T90 - T10). The comparison of Figures 5.1 and 5.5 indicated that the loss of uniformity was more evident in the seeds where the total REC was significantly reduced. For example, there was an increase in (T90 - T10) from 65 h (± 4.8) of unaged seeds to 106 h (± 10.8) in seed aged for 3 d under the storage regime of 40% SMC, 45°C.

5.1.1 Effects of a storage regime on different seed lots

Although different seed lots showed different mean survival periods under identical storage conditions (40% SMC, 40°C) (Fig. 5.6), the standard deviations of the probit survival curve or slope ($1/\sigma$) were not significantly different (Table 5.3) with the exception of seed lot G-2 and therefore the form of the survival curves were also similar for different seed lots. Plotting probit values against storage time resulted in straight line survival curves, confirming that the frequency distribution of seed death in time is a normal one in all the seed lots. For example, figure 5.7 shows the survival curves of the seed lots of cv. Grosse lisse improved. Table 5.3 also shows that there were no significant differences in the Ki values (seed lot constant) and half viability period (P50) between the seed lots with an exception to the seed lot G-2. The P50 which indicates the storage potential or the seed lot was not significantly correlated with zero day REC of seed lots, but was significantly correlated with REC of seeds aged for 3 d or 10 d ($r = 0.915$, $p < 0.01$) ($r = 0.926$ $p < 0.01$), respectively, indicating the usefulness of controlled deterioration treatment for identifying the storage potential of the tomato seed lots (Table 5.3).

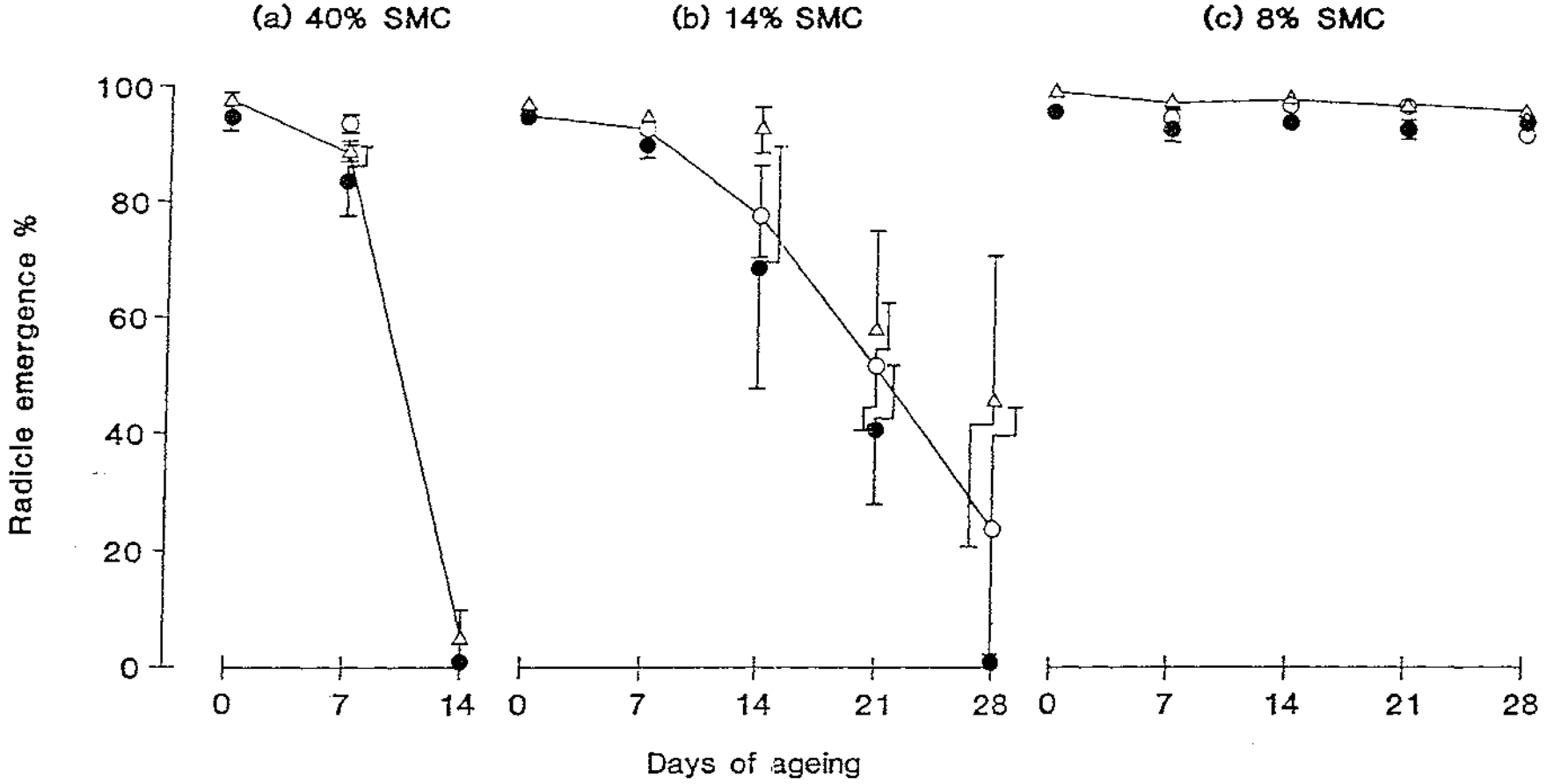

Figure 5.8 The effect of 14 d LTPST on total radicle emergence capacity of tomato cv. Moneymaker seed lot M-1 applied before [●] and after [○] ageing [Δ] under three different storage regimes. (a) 40°C, 40% SMC (b) 40°C, 14% SMC (c) 40°C, 8% SMC. Data presented are means of four replications; vertical bars represent SE's calculated for individual means and are shown where larger than the symbols used.

TABLE 5.4 The effects of LTPST applied before and after ageing on seed lot M-1 under different storage regimes

Treatment	Ageing Condition SMC (%)	Temp. °C	Initial Probit Viability (Ki)	Slope $1/\sigma$	P50 in days
AAT	40	40	7.94⊕ ± 0.48[a]	-0.338 ± 0.053[a]	8.70[a]
BAT	40	40	7.49 ± 0.45[a]	-0.360 ± 0.050[a]	6.92[a]
Untreated	40	40	7.84 ± 0.43[a]	-0.338 ± 0.047[a]	8.40[a]
AAT	14	40	7.22 ± 0.40[a]	-0.121 ± 0.024[b]	18.35[b]
BAT	14	40	7.10 ± 0.44[a]	-0.147 ± 0.026[b]	14.29[b]
Untreated	14	40	7.29 ± 0.49[a]	-0.100 ± 0.029[b]	22.90[b]

Note:
1. ⊕: Od germination percent of AAT was assumed to be equivalent to control. The CD treatment of increased SMC to 40% and drying back before LTPST caused increased pre-germination percentage during the treatment and hence the germination trial were discontinued.
2. Means with same letters are not significantly different at 0.05 level.
3. AAT: LTPST application after ageing
   BAT: LTPST application before ageing
4. 100 and 0% of germination was considered an 99.9 and 0. respectively for analysis purposes.
5. P50 Half viability period.

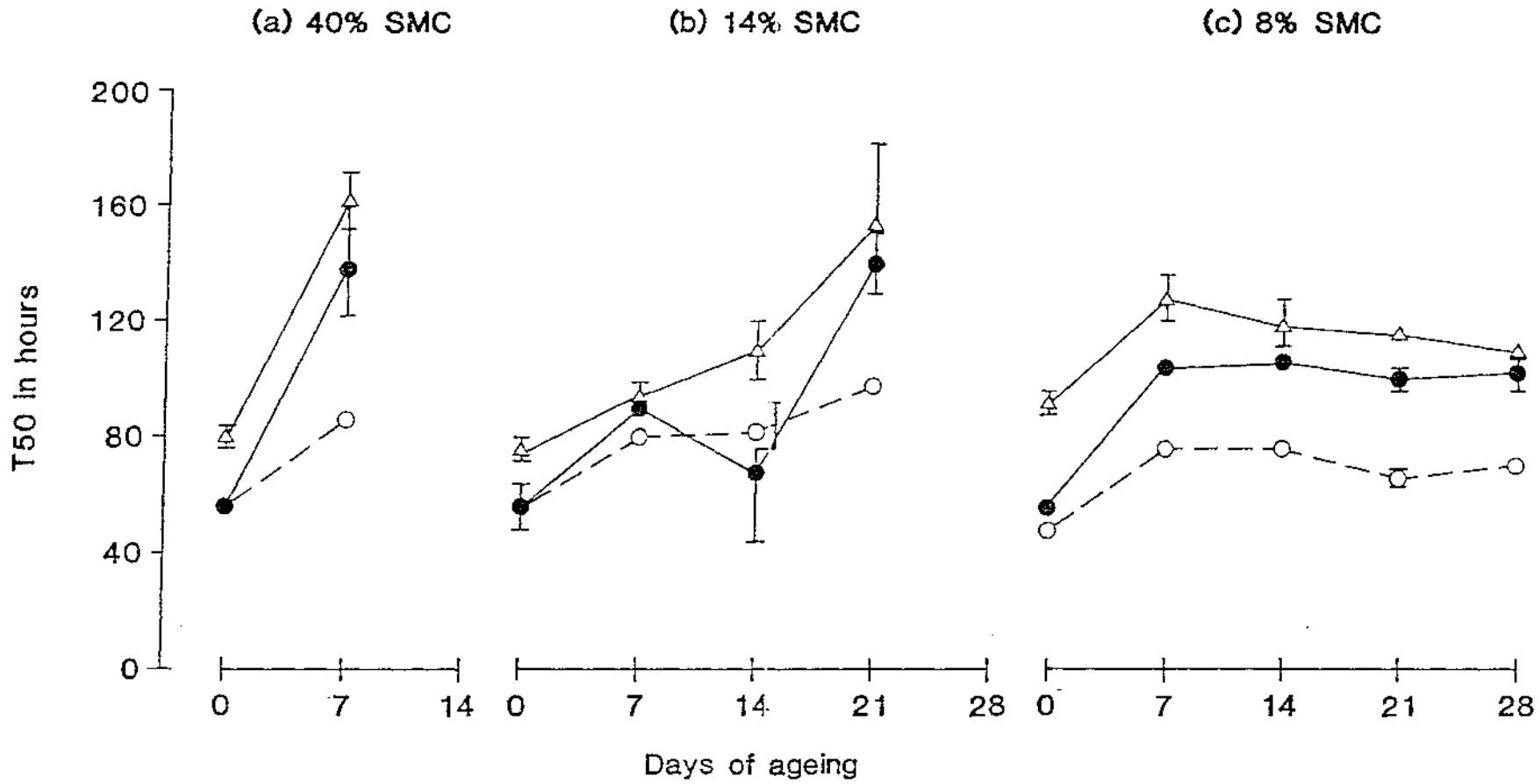

Figure 5.9 The effect of 14 d LTPST on median germination time (T50's) of tomato cv. Moneymaker seed lot M-1 applied before [●] and after [O] ageing [Δ] under three different storage regimes. (a) 40°C, 40% SMC (b) 40°C, 14% SMC (c) 40°C, 8% SMC. Data presented are means of four replications; vertical bars represent SE's calculated for individual means and are shown where larger than the symbols used.

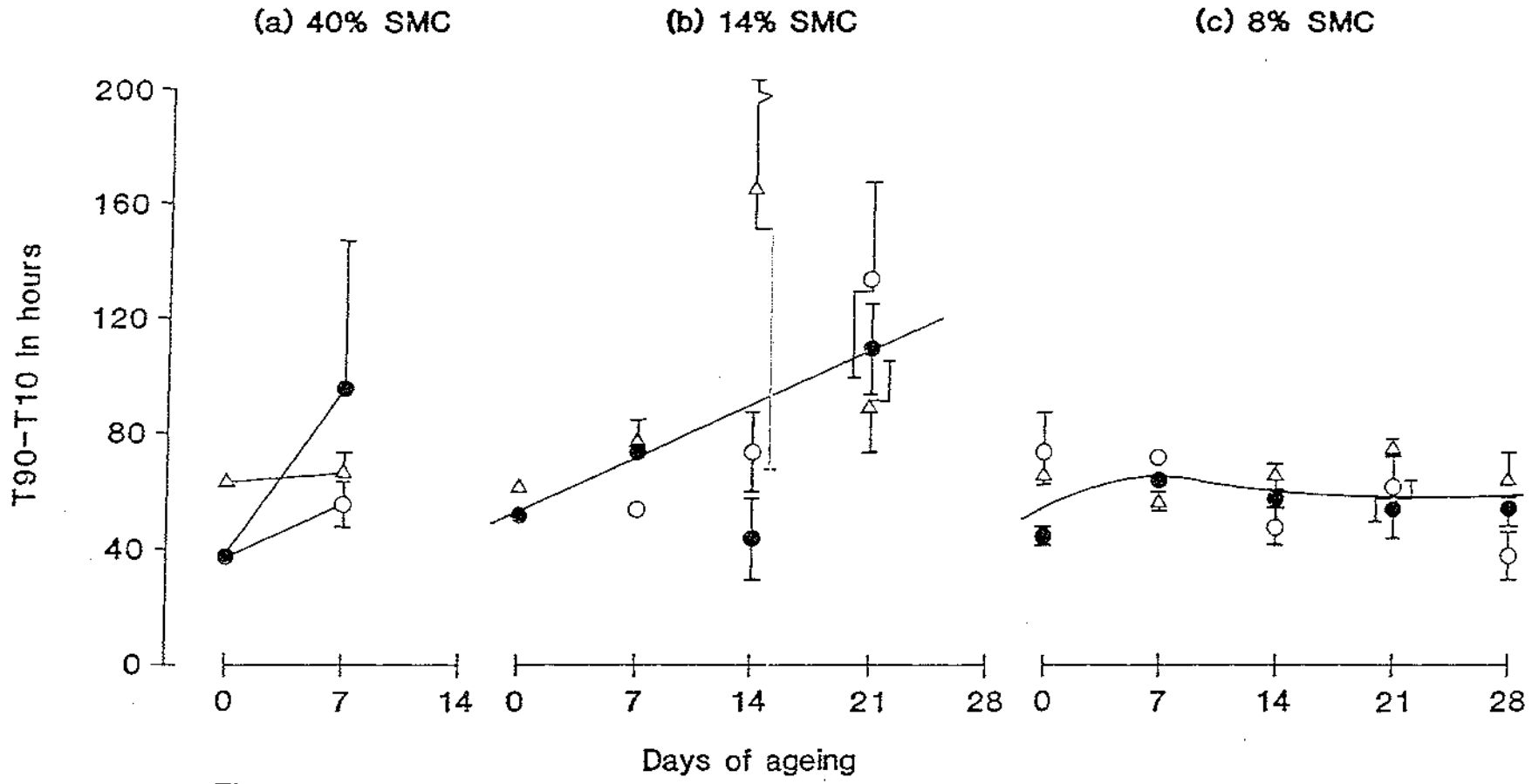

Figure 5.10 The effect of 14 d LTPST on uniformity of (T90 - T10) tomato cv. Moneymaker seed lot M-1 applied before [●] and after [○] ageing [△] under three different storage regimes. (a) 40°C, 40% SMC (b) 40°C, 14% SMC (c) 40°C, 8% SMC. Data presented are means of four replications; vertical bars represent SE's calculated for individual means and are shown where larger

5.2 EFFECTS OF LTPST APPLIED BEFORE AND AFTER AGEING

The effects of LTPST on unaged seed of different seed lots have already been discussed (see Chapter 4).

5.2.1 Effects of LTPST applied before and after ageing on seed lot M-1 under different storage regimes

Figure 5.8 shows that LTPST application before and after storage had no significant effect on final percent of radicle emergence of seed lot M-1 under all storage regimes. It is obviously clear from the Table 5.4 that the slopes were different between the storage environment, but Ki value was not significiantly different between storage regimes and neither slopes nor Ki values were significantly different between treated and untreated seeds. The parameters Ki, slope and P50 for the seeds stored at 8% SMC, 40°C are not shown as the loss of viability was insignificant to construct the survival curves.

Figure 5.9 shows that LTPST application after ageing in seed lot M-1 caused a significant improvement in germination rate in all the aged seeds under all ageing regimes, while before ageing treatment protected the germination rate only under the storage regime 8% SMC, 40°C up to 21 d (Fig. 5.9c), otherwise the germination rate was not significantly different from unaged seed. Figure 5.10 shows that LTPST application before and after ageing had no significant effect on uniformity.

5.2.2 Effects of LTPST applied before and after ageing on different seed lots of tomato seeds under identical storage conditions

5.2.2.1 Effect of LTPST applied before ageing

Table 5.5 shows that the response of seed lots to LTPST applied before ageing under identical conditions differed between the seed-lots. Treatment before ageing had no significant effect on total

TABLE 5.5 The effects of LTPST applied before and after ageing on different cultivars/seed lots of tomato seeds. ①

Cultivar	Seed Lot	Treatment before ageing			Treatment after ageing		
		Final Radicle emergence % and Normal germination	Median germination time (T50)	Uniformity (T90 - T10)	Final radicle emergence % and Normal germination	Median germination time (T50)	Uniformity (T90 - T10)
Moneymaker	M-1②	No significant difference	No significant difference by 7 d	No significant difference	No significant difference	Significant reduction in T50	No significant difference
	M-2	No significant difference up to 6 d Rapid loss by 10 d	Significant reduction up to 3 d	- do -	- do -	- do -	- do -
	M-3	Significant reduction by 3 d	- do -	- do -	- do -	- do -	Significant reduction only at 3 d ageing
Scoresby dwarf	S-2	No significant difference up to 3 d Rapid loss by 6 d	No significant difference by 3 d	Not significant upto 3 d	- do -	- do -	No significant difference
	S-3	No significant difference upto 6 d Rapid loss by 10 d	Significant difference up to 6 d Rapid loss by 10 d	No significant difference	- do -	- do -	- do -
Grosse lisse Improved	G-1	No significant difference up to 3 d Rapid loss by 6 d	Significant reduction up to 6 d	- do -	- do -	Significant reduction up to 6 d	- do -
	G-2	No significant difference up to 3 d Rapid loss by 6 d	- do -	Not significant by 10 d	- do -	- do -	Significant reduction only at 6 d ageing
	G-3	Significant reduction by 3 d	Significant reduction up to 3 d	No significant difference	- do -	Significant reduction in T50	Significant reduction only at 3 d ageing

NOTE:
1. Ageing condition; 40°C at 40% SMC FWB, Ageing duration, 0, 3, 6, 10 and 15 days; LTPST 14 d.
2. Ageing duration was 0, 7, 14, 21 and 28 days.

TABLE 5.6 The effects of LTPST applied before ageing on the viability decline pattern of different tomato seed lots.

Cultivar	Seed Lot	Category	Correlation [0] (r)	Probit initial Viability (Ki)	Slope [0] ($1/\sigma$) (-ve values)	Half Viability Period (P50) in days
Treatment before ageing						
Moneymaker	M-2	LTPST	-0.919	7.92 ± 0.32^{NS}	0.368 ± 0.037^{NS}	7.94^{NS}
		Cont	-0.836	7.83 ± 0.35	0.260 ± 0.040	10.89
	M-3	LTPST	-0.923	6.27 ± 0.26*	0.311 ± 0.031^{NS}	4.08*
		Cont	-0.932	7.27 ± 0.25	0.310 ± 0.028	7.32
Scoresby dwarf	S-2	LTPST	-0.924	6.97 ± 0.30^{NS}	0.358 ± 0.035^{NS}	5.50^{NS}
		Cont	-0.914	7.22 ± 0.30	0.329 ± 0.034	6.75
	S-3	LTPST	-0.934	7.10 ± 0.30^{NS}	0.383 ± 0.034^{NS}	5.48^{NS}
		Cont	-0.891	7.48 ± 0.33	0.317 ± 0.038	7.82
Grosse lisse Improved	G-1	LTPST	-0.918	7.17 ± 0.25^{NS}	0.307 ± 0.031^{NS}	7.07^{NS}
		Cont	-0.879	7.66 ± 0.35	0.314 ± 0.040	8.47
	G-2	LTPST	-0.843	7.75 ± 0.30^{NS}	0.231 ± 0.035^{NS}	11.91^{NS}
		Cont	-0.719	7.88 ± 0.36	0.184 ± 0.042	15.65
	G-3	LTPST	-0.905	6.98 ± 0.31^{NS}	0.320 ± 0.035^{NS}	6.19^{NS}
		Cont	-0.760	7.61 ± 0.39	0.226 ± 0.045	11.55

Note:

[0] Correlation between probit viability and storage period was highly significant at $p < 0.001$ level.

[0] The data indicated for the slope were all -ve values.

NS Not significant between treated and untreated at 0.05 level..

* Significantly different at 0.05 level.

TABLE 5.7 The effects of LTPST applied after ageing on the viability decline pattern of different tomato seed lots.

Cultivar	Seed Lot	Category	Correlation [0] (r)	Probit initial Viability (Ki)	Slope [β] (1/σ) (-ve values)	Half Viability Period (P50) in days
Treatment after ageing						
Moneymaker	M-2	LTPST	-0.923	7.77 ± 0.27^{NS}	0.319 ± 0.031^{NS}	8.68^{NS}
		Cont	-0.934	7.82 ± 0.25	0.324 ± 0.029	8.70
	M-3	LTPST	-0.911	7.04 ± 0.33^{NS}	0.356 ± 0.038^{NS}	5.73^{NS}
		Cont	-0.910	7.07 ± 0.29	0.367 ± 0.034	5.64
Scoresby dwarf	S-2	*LTPST	-0.951	7.73 ± 0.25^{NS}	0.386 ± 0.030^{NS}	7.07^{NS}
		Cont	-0.950	7.68 ± 0.25	0.379 ± 0.029	7.07
	S-3	*LTPST	-0.910	7.35 ± 0.35^{NS}	0.382 ± 0.041^{NS}	6.15^{NS}
		Cont	-0.910	7.35 ± 0.35	0.382 ± 0.041	6.15
Grosse lisse	G-1	LTPST	-0.869	7.78 ± 0.36^{NS}	0.312 ± 0.042^{NS}	8.91^{NS}
Improved		Cont	-0.795	7.08 ± 0.37	0.241 ± 0.043^{NS}	8.63
	G-2	LTPST	-0.747	7.47 ± 0.35^{NS}	0.191 ± 0.040^{NS}	12.93^{NS}
		Cont	-0.734	7.67 ± 0.35	0.185 ± 0.040	14.43
	G-3	LTPST	-0.955	7.94 ± 0.25^{NS}	0.390 ± 0.029	7.54^{NS}
		Cont	-0.932	7.36 ± 0.26	0.325 ± 0.030	7.26

Note:

0 The data indicated in slope column are all -ve values.

β Correlation r between probit viability and storage period were highly significant at 0.001 level.

NS Not significant between treated and untreated seeds at 0.05 level. 100 and 0% of germination were considered to be 99.9 and 0.1% respectively for analysis purpose.

* Od germination of treated were considered to be equivalent to control (see Table 5.4 for explanation).

REC and normal germination of seed aged for 6 d in seed lot M-2, S-3 and for 3 d in seed lot S2, G-1 and G-2. Thereafter the treated seed lost their viability more rapidly than untreated seeds (Table 5.5). In lots M3 and G3, deterioration was more rapid from the start. Table 5.6 shows LTPST application before ageing had no significant effect on Ki values of the seed lots except for lot M-3 where treatment caused a significant reduction in Ki value. It is also clear from the table that the slopes were not significantly different between treated and untreated seed. The slopes of all seed lots irrespective of treated or untreated under identical storage condition were not significantly different except for that of seed lot G-2, untreated seed, compared to treated lots of M2, S2 and S3.

Table 5.5 shows that LTPST application before ageing protected the germination rate (inverse of T50) only upto 3 d ageing in seed lot M-2, M-3, S-3 and G-3 and upto 6 d ageing in seed lot G-1 and G-2. There after and also in other seed lots the germination rate was not significantly different between treated and untreated seed. In general LTPST application before ageing had no significant effect on uniformity or germination of seed lots and in a few cases the ageing of treated seed caused significant loss of uniformity compared to untreated seed (for example in seed lots S-2 aged for 3 d).

5.2.2.2 Effects of LTPST applied after ageing

Table 5.5 shows that LTPST application after ageing had no significant effect on total REC and normal germination of different seed lots of tomato seeds aged under identical storage regime (40% SMC, 40°C). Table 5.7 gives the data on the values of Ki, slope and P50 of survival curves for treated and untreated seed confirming that treatment had no significant effect. The slopes of all seed lots irrespective of application of treatment or not were not significantly different under identical storage condition with exception to the seed lot G-2 (Table 5.7).

Table 5.5 shows that LTPST application after ageing caused significant reduction in T50 or improved the germination rate in all the seed lots under all ageing durations with the exception of seed lots G-1 and G-3 where the reduction in T50 was significant only up to 6 d. Although, the treatment after ageing caused significant improvement in germination rate a component of seed vigour, it had no significant effect on uniformity of germination with exception of the seed lot M-3, G-3 and G-2 where the uniformity were significantly improved only in seeds aged for 3 d and 6 d respectively (Table 5.5).

CHAPTER 6
DISCUSSION

6.1 DIFFERENCES IN QUALITY OF TOMATO SEEDS

The standard germination test remains the accepted criterion for seed viability measurement and is carried out under optimum conditions primarily for reasons of repeatability and reproducibility (AOSA, 1983). The percentages of seedling emergence of untreated seed in the soil (Fig. 4.18) under favourable conditions were not significantly different from laboratory germination test results (Fig. 4.13) with the exception of the seed lot G-2 which recorded comparatively lower emergence percentages in the soil. The laboratory germination rate was also closely correlated with the rate of seedling emergence in the soil (Fig. 4.19, $r = 0.919$, $p < 0.01$). These results are in accordance with the general observation that laboratory test results correlate well with field performance under favourable conditions e.g. Perry (1987).

Although the germination capacity of the cultivars and seed lots were not significantly different, differences in seed dry weight and vigour as measured by the median germination time (T50), uniformity, seedling fresh and dry weights (Table 4.1; Fig's 4.16 and 4.17) were obvious between the seed lots. These results are in agreement with the general notion that vigour test can differentiate the quality attributes of seed better than the germination test (AOSA, 1983; ISTA, 1987). The existence of vigour difference between seed lots were further confirmed by differences in pregermination magnitude at 10°C during pretreatment (Fig's 4.1 to 4.10).

There are several other reports indicating the existence of vigour difference between lines or cultivar of tomato species (Liptay and Schopfer, 1983; Abdul-Baki and Stoner, 1978; Scott and Jones, 1985; Whittington and Fierlinger, 1972). These differences may be related to genotype (Abdul-Baki and Stoner, 1978;

Hassan, 1978; Andreeva, 1977) and/or to the extent of seed deterioration before sowing (Ellis and Roberts, 1980).

The significant correlations between T50 and uniformity ($r = 0.838$, $p < 0.01$) and T50 and seedling fresh weight ($r = -0.865$, $p < 0.01$) in untreated seed may be an indication that the improved plant growth and yield observed in high vigour seed are due to earlier emergence (cf. Coolbear et al., 1987).

A positive correlation between germination time and seed weight was reported by Whittington and Fierlinger (1972). The delaying of germination in tomato with increased seed size was attributed to the thickness of the endosperm which the radicle has to penetrate before emergence, which may be the rate limiting step of radicle protrusion (Haigh and Barlow, 1978b; Groot and Karssen, 1987). In contrast, this investigation showed that, firstly there is no significant overall correlation between seed weight and germination time and secondly there was a significant negative correlation noted within cv. Grosse lisse Improved. This is in agreement with the general observation that increased seed size is correlated with vigour in several species (Priestly, 1986; Heydecker, 1972). Liptay and Schopfer (1983) have also shown that the resistance of endosperm may not account for the differences in germination rate in different cultivars.

6.2 EFFECTS OF LTPST ON THE GERMINATION PERFORMANCE OF DIFFERENT TOMATO SEED CULTIVARS AND/OR SEED LOTS.

The beneficial effects of LTPST in improving the germination performance of seed is achieved by allowing the seed to initiate some facets of germinative metabolism while radicle emergence is prevented (Coolbear et al., 1987). In this pretreatment, seed germination is merely delayed because of low temperature. In order to get the best advantage of the treatment certain factors

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should be taken into account while selecting the pretreatment conditions.

6.2.1 Selection of pretreatment conditions

Although an increased pretreatment duration induces greater germination responses up to 14 d or 17 d depending on the seed lot (Fig's 4.1 to 4.10), this has to be balanced against the occurrence of an unacceptable degree of pregermination (cf. Coolbear et al., 1980). Similar linear relationships between treatment advantage and treatment duration have been reported in tomato with the use of priming (Koehler, 1967; Coolbear et al., 1980). The pregermination data shown in Fig's 4.1 to 4.10 demonstrate the heterogeneous responses of seed lots to this type of treatment. The significant correlation between initial T50 and levels of pregermination suggest that initial seed quality should be considered while determining the duration of the treatment. Extending the pretreatment period beyond the appearance of pregermination may cause reduction in final REC of the seed lot e.g. S-2 (Fig. 4.6), probably because seeds not discarded as pregerminated, but on the point of radicle emergence or cell expansion may have been irreversibly damaged by drying (Berrie and Drennan, 1971; Biddington et al., 1982a).

In general (and for probably similar reasons), treated seedlots showed an increased T50 when they encountered pregermination during treatment (results from Coolbear et al., 1987 shows a similar trend) with the exception of seed lots Scoresby dwarf (Fig's 4.5 to 4.7). This suggests that deteriorative effects of drying back may differ with cultivars. Cultivar variations in response to drying have been noted in onion (Haigh et al., 1986; Furutani et al., 1986). An economically viable pretreatment involving drying back must therefore be one where there is a very low percentage of pregermination, not just from the point of view of the proportional value of the seed lost, but because the lost seed may be of the

highest vigour and thus some of the most phenotypically desirable (Coolbear et al., 1980).

Although selection of treatment conditions for each seed lot may give the best treatment advantage, for commercial application a treatment method suitable for all seed lots and cultivars of a species much preferable for convenience. Thus 14 d LTPST have been used in all the subsequent investigations in this study, a duration shorter than that used in tomato by Coolbear and his co-workers in previous papers.

6.2.2 Treatment duration and seed lot performance

All the tested durations caused significant reductions in T50 (Fig. 4.1 to 4.10) and this was evident by 7 days, the maximum reduction being obtained by 14 d in all the seed lots except M-2 which took 17 d. This does not conform with the results of Coolbear et al. (1987) who found that 7 d LTPST caused no significant reduction in T50 and maximum reduction was noted by 21 d with less than 1% pregermination in their study. The possible explanation could be differences in the seed lines/strains used.

Heydecker and Gibbins (1970) suggested that priming induces a state of readiness during treatment and consequently the treated seed give rapid and uniform germination on sowing. Although LTPST caused significant improvement in germination rate, improvement in uniformity of germination was only in 5 and 3 seed lots out of 10 seed lots tested following 7 d and 14 d pretreatment, respectively (Fig's 4.1 to 4.10, section 4.2).

There was a significant correlation between T50 and uniformity in untreated seed but the nature of this relationship changed following LTPST, suggesting that uniformity is not a simple function of the rate of germination. This also suggests that improvement in uniformity is seed lot dependent (cf. Coolbear and McGill, 1989) and the optimum pretreatment conditions required for the improve-

ment in uniformity may be different from that required for improvement of germination rate. Supporting this view, Coolbear et al. (1980) noted that the improvement in uniformity caused by PEG treatment was not consistent and depended on duration and concentration of PEG solution used. From Fig. 4.12 it could be said that 14 d LTPST caused improvement in uniformity mostly in seed lots where there was initially greater variance in germination time between the individual seeds within the population (e.g. seed lots M-4, M-1 and G-2). Fig. 4.12 also shows that germination time in tomato seed lots is not always normally distributed. This is in agreement with the findings of Ellis and Roberts (1980) in other species.

6.2.3 14 d LTPST effects on germination and seedling growth performance

Low temperature presowing treatment of 14 d (LTPST) significantly improved the germination rates in all the cultivars and seed lots tested without altering the potential performance of the seed lots measured in terms of REC and also normal germination capacity (Fig's 4.14 and 4.15). The data in Fig. 4.15 clearly demonstrate a high repeatability and reproducibility of the LTPST technique in causing improved germination rate. This confirms that the success obtained by Coolbear and his co-workers with seed lots of cv. Moneymaker (Coolbear et al., 1987, 1985; Francis, 1985; Coolbear and McGill, 1989) could be extended to different cultivars and/or seed lots. The differences in response to LTPST by various seed lots and/or cultivars were shown here to be clearly related ($p < 0.01$) to the T50 of the untreated seed in each lot (Fig. 4.11). This suggests that both high and low vigour seed responds well to the treatment, however, low vigour seed lots get the highest benefit. Similarly, Brocklehurst and Dearman (1983) noted that the largest reduction in mean germination time due to priming of carrot, celery and onion was with the slowest germinating seed lots.

Considering the high reproducibility of the treatment, the following prediction equation was developed by using regression analysis:

$$(T50)_T = 20 + 0.353\ (T50)_U$$

where $(T50)_T$ is the T50 of treated seed, $(T50)_U$ that for untreated seed. The appropriateness of this equation was tested in 3 cultivars comprising 7 seed lots with 3 ageing durations. Table 6.1 clearly demonstrates that the actual T50's of treated seed were not different from that predicted provided germination did not fall below 80%. Furthermore, the application of this equation to the previous results obtained by Coolbear and his co-workers (Table 6.2) shows that improvement in germination rate obtained by their use of LTPST were almost equivalent to the predicted value. The small differences noted may be attributed to variation in treatment duration. This undoubtedly confirms the reproducibility of the treatment and its applicability to other seed lots.

The relationship noted between untreated T50 and treated T50 (Fig. 4.11) is similar to the results of Coolbear et al., (1984) in aged tomato seed, while Brocklehurst and Dearman (1983) found a similar relationship between reduction in mean germination time due to priming and the mean time of germination of untreated seeds for lots of differing vigour of carrots, onion and celery. These results support the hypothesis that the events affecting the germination rate may be associated with a single rate limiting process (Coolbear et al., 1984) and amenable for manipulation by ageing and treatments.

The 14 d LTPST effect on uniformity reconfirmed that improvement in uniformity of germination is seed lot dependent (Coolbear and McGill, 1989). Despite improving germination rate, no improvement in uniformity was also noted by Argerich et al. (1989) using priming in tomato, while Brocklehurst and Dearman (1983) reported cultivar variations in other species.

TABLE 6.1 Mean actual vs predicted median germination time (T50) of treated seed in an experiment of applying LTPST on stored seeds.

Cultivar	Lot	Ageing days	Radicle emergence of untreated seed %	Initial T50 (h)	Actual T50 of treated seeds (h)	Predicted T50 of seeds (h)
Moneymaker	M-2	0 d	99 ± 0.6	86 ± 6.5	49 ± 3.4	50
		3 d	95 ± 1.0	127 ± 4.2	72 ± 3.3	65
		6 d	90 ± 1.0	168 ± 2.0	90 ± 5.6	79
	M-3	0 d	96 ± 1.2	107 ± 9.9	55 ± 1.9	58
		3 d	86 ± 4.4	175 ± 3.9	86 ± 5.9	82
		6 d	76 ± 3.2	252 ± 12.6	183 ± 6.9	109
Scorsby dwarf	S-2	0 d	99 ± 0.5	67 ± 2.2	48 ± 2.2	44
		3 d	90 ± 1.5	104 ± 3.7	57 ± 2.7	57
		6 d	66 ± 5.1	162 ± 9.8	89 ± 13.2	78
	S-3	0 d	98 ± 1.4	69 ± 3.1	47 ± 1.7	45
		3 d	88 ± 3.1	110 ± 5.0	57 ± 1.0	59
		6 d	78 ± 6.2	157 ± 17.1	85 ± 19.9	75
Grosse lisse Improved	G-1	0 d	93 ± 1.7	98 ± 1.1	52 ± 1.4	54
		3 d	93 ± 1.3	138 ± 5.8	75 ± 4.8	69
		6 d	84 ± 3.5	171 ± 9.8	90 ± 4.6	81
	G-2	0 d	98 ± 0.8	117 ± 2.0	67 ± 3.8	61
		3 d	98 ± 1.5	155 ± 3.9	85 ± 5.7	75
		6 d	94 ± 1.8	188 ± 6.8	94 ± 7.3	86
	G-3	0 d	96 ± 1.0	94 ± 5.4	51 ± 3.2	53
		3 d	89 ± 2.4	139 ± 3.7	73 ± 4.7	69
		6 d	93 ± 1.7	151 ± 6.6	83 ± 5.4	73

NOTE 1*. Prediction equation:-

Treated Seed T50 = 20 + 0.353 x Untreated T50

Constant Tc = 20 ± 1.49

Uc = 0.353 ± 0.014

TABLE 6.2 The actual vs the predicted effect of LTPST (21 d) on median germination time obtained by different workers in tomato seed.

Seed Lot	Mean Untreated T50 (h)	Mean Treated T50 Actual (h)	Predicted T50 of treated seed (h)	Reference
Moneymaker - Harrison reselected strain	152	90	74	Coolbear et al., 1987
Moneymaker lot 8415.1	135	67	68	Coolbear et al., 1984
Kingley Cross 1974 stock	155	53	75	Francis, 1985

Primed seed is metabolically highly active (Coolbear et al., 1980; Khan et al., 1980; Koehler, 1967) and due to the depletion of the seed in food reserve during the pretreatment period, a reduction in seed dry weight in treated seed may be anticipated (Coolbear et al., 1980; 1987). Table 4.3 shows a general reduction in seed dry weight of treated seed but this was not significantly different from untreated material. This is in agreement with the results of Argerich and Bradford (1989) for primed tomato seeds.

Assessment of seedling growth in response to LTPST on 12 d old normal seedlings generally indicated no significant effect of the treatment both on fresh weight (Fig. 4.17) and dry weight (Fig. 4.18). The exceptions were seed lot M-4 and M-1 which showed increased fresh weight and decreased dry weight respectively. These indications that pre-sowing treatment has little effect on subsequent seedling growth are in accordance with studies on tomato seeds using LTPST (Coolbear et al., 1987; Francis, 1985) and priming (Argerich and Bradford, 1989). Similarly Odeil and Cantliffe (1986) found that priming had no effect on seedling growth under controlled environmental conditions. This supports the hypothesis proposed by Scott and Jones (1985a) who indicated that the metabolic processes determining seedling growth rate are distinct from those controlling germination rate. Several reports suggesting improvement in subsequent plant growth and/or yield are likely to be due to the retained advantage of early emergence by treated seeds (Coolbear et al., 1987).

The results of preliminary soil emergence trials under controlled conditions were unexpected. Two out of ten seed lots showed a reduction in total radicle emergence (Fig. 4.19) but early emergence was retained by 7 seed lots (Fig. 4.20). One possible explanation is that these treated seeds were stored for about 10 weeks before the trials which may be the cause. Also any deteriorative effects may have been aggrevated by over watering. However before any commercial application of LTPST, more extensive field trials are needed in this area.

6.3 EFFECTS OF AGEING AND LTPST ON DIFFERENT SEED LOTS OF TOMATO SEEDS

6.3.1 Viability

The pattern of seed deterioration in tomato seeds under differernt storage regimes (Fig. 5.1) are in accordance with the pattern described for various orthodox seeds indicating that increased SMC and storage temperature accelerate seed deterioration (Roberts, 1972 and, in tomato, Rees, 1970). Table 5.3 shows that there were significant differences in the viability of seed lots stored under identical storage conditions after 3 or 10 d, despite their initial germination being not significantly different. These results conform with the reports suggesting that there is a considerable variation in the storability of different cultivars e.g. tomato (Zhuchenko et al., 1979; Gill et al., 1983) and also between the seed lots of the same cultivar even under the same storage conditions (e.g. corn, onion and watermelon: Delouche and Baskin, 1973).

One special feature of controlled deterioration in tomato is that the increased SMC of whole seed is not a true representation of the embryo moisture content at which the seed is aged and even after 10 d storage embryo moisture content had not equilibriated with the surrounding tissue (Table 3.3). A similar observation was made by Loubser (1989).

LTPST application either had no significant effect (Fig. 5.8) on REC or caused rapid deterioration depending on seed lot (Table 5.5). In accordance with this, other reports have also suggested no effect of LTPST on viability (Coolbear et al., 1984) or rapid deterioration following priming in tomato (Argerich et al., 1989; Alvarado and Bradford, 1988a; Odell and Cantliffe, 1986) and also in other species like carrot and leek (Dearman et al., 1987), wheat and ryegrass (Lush et al., 1981). The possible explanation for the rapid loss of viability under high moisture

rapid ageing would be that increased SMC may cause further advancement in the germination processes of treated seed leading to a stage where seed do not tolerate any more dessication and may have been irreversibly damaged by drying back (cf. Berrie and Drennan, 1971; Biddington et al., 1982a). Any damage to the repaired and restructured (Dearman et al., 1986; Ellis and Butcher, 1988) machinery in treated seed which are set to carry out highly developed metabolism (Coolbear et al., 1980; Khan et al., 1980) may cause irreversible damage and consequently result in rapid loss of viability. This is supported by the observation that the effectiveness of repriming to restore the germination performance of aged primed seed depends on the degree of deterioration present and on the initial advantage gained due to the first priming treatment (Alvarado and Bradford, 1988). This may also explain the reasons for the variation observed between seed lots (Table 5.5). In contrast, extended longevity in onion following priming (Dearman et al., 1986) has also been noted. This may have been an artefact resulting from a shorter test duration (4 d).

Application of LTPST to a single seed lot aged under different ageing regimes or to different cultivars and seed lots aged under the same storage regime had no effect on the loss of viability. This was expected and confirms the results of Coolbear et al. (1984) with tomato, and Dearman et al., (1986) and Jones (1977) with onion and leek, respectively.

6.3.2 The seed viability model

This study shows that the frequency distribution of seed deaths in time is normal even in rapid ageing conditions where death is rapid (Fig. 5.2 and 5.3) and this is in agreement with the report of Ellis and Roberts (1980b) in barley seed. However, the results do not support the suggestion that under rapid ageing conditions the distribution of death in time becomes skewed (Roberts and Abdalla, 1968; Moore and Roos, 1982). The skewness reported may have been an artefact resulting from a a shorter germination

test where slow germinating seed may have been treated as dead (Ellis and Roberts, 1980). Alternatively, the testing intervals may have been large to clearly identify the pattern of seed death in this study.

In the model developed by Ellis and Roberts (1980) based on a probit analysis of the loss of viability as a function of time under constant storage conditions, it has been shown that genotype and pre-storage environment affect the value of Ki (initial viability before storage or seed lot constant). The slope of the survival curves which is a reciprocal function of the standard deviation (σ^{-}) of the distribution of death in time is not affected by the above factors, but by storage environment. Accordingly it was proposed that the slope of survival curve will be different between storage environments while the intercept (Ki) reflecting seed quality before storage remains unaffected for a given seed lot. Under constant storage conditions the slope of the survival curves should be a constant for a given species while the intercept (Ki) will reflect the differences in seed quality (Roberts and Ellis, 1984) between seed lots. When this model was applied for a single tomato seed lot stored under different storage regimes the slopes did significantly differ between conditions, but Ki was not significantly different (Table 5.1). However comparing different seed lots under the same storage environment, neither the slopes of the survival curves nor the Ki values were significantly different, with one exception to the seed lot G-2 (Table 5.3). Nevertheless the model appears to be valid for tomato seed deterioration.

Application of LTPST before and after storage for a single seed lot under different storage regime (Table 5.4) and also to different seed lots under same storage regime (Table 5.6 and 5.7) had no effect either on Ki or slope with an exception to the seed lot M-2, where before ageing application reduced the value of Ki (Table 5.6). This suggests that LTPST has no significant effect on the rate of seed deterioration when measured in terms of loss of viability.

6.3.3 Viability model and vigour

The Ki value of each lot represents the initial quality of a seed lot (Ellis and Roberts, 1981). The improvement in germination rate by LTPST both in unaged and aged seeds (Table 6.3) and the differences in initial vigour as measured by uniformity, germination rate (Table 4.1), seedling fresh and dry weight (Fig's 4.16 and 4.17) had no effect on Ki and slope of the survival curves of different seed lots (Table 5.6 and 5.7). Similarly Ellis and Roberts (1980) have also found that pre-storage factors such as mineral nutrition, repeated handling and premature harvesting in barley had no effect on seed lot constant and did not affect longevity of the seed, but all these factors are known to cause significant differences in vigour (Coolbear 1989; Priestley, 1986). These results suggest that firstly the above components of vigour of the seed lot have no relationship with storability of seeds. This apparently contradicts the concept that vigorous seed store better under adverse storage conditions (Matthews, 1980; Powell and Matthews, 1981). Secondly it supports the hypothesis that loss of viability and germination rate are controlled by two different independent process, of which only the process responsible for loss of germination rate could be reversed (Coolbear et al., 1984). The problem raised by the first suggestion may be resolved if we recognise that processes controlling storability may be different from those controlling other vigour parameters in tomato seed.

6.3.4 Vigour

Germination rate is a more sensitive measure of seed deterioration than the loss of viability (Priestley, 1986). These results (Section 5.1) are in agreement with the general observation that germination rate declines before the viability begins to decline (Priestley, 1986). This also supports the notion that germination rate increases substantially with only a slight decrease in viability e.g. in cabbage and onion (Ellis and Roberts, 1980). Germination rate could be used as a vigour test to determine the deterioration

status of a seed lot but standardization of germination conditions is a prerequisite (Ellis and Roberts, 1980) because this parameter is sensitive to temperature and moisture conditions (Coolbear and McGill, 1989). Table 5.2 shows that simply increasing the storage temperature had no significant effect on rate of loss of germination rate (cf. Alvardo and Bradford, 1988) but an increase in SMC caused a rapid loss of germination rate (Fig. 5.4). The close correlation between germination rate after 6 d ageing with initial germination rate may suggest that the deterioration caused by a storage regime is additive (Priestly, 1986).

The protection offered by seed treatment against loss of germination rate under mild storage regimes (Fig. 5.9c) and for a short period of storage for 3 to 6 d under adverse storage condition depends on seed lot (Table 5.5) and may also depend on ageing conditions. In concurrence with this idea, Coolbear et al. (1984) noted LTPST application before ageing protected the germination rate for 3 d while Alvardo and Bradford (1988b) and Argerich et al., (1989) both noted rapid loss of germination rate in treated seeds before ageing with priming in tomato seeds. Dearman et al. ,(1986) noted priming protected the germination rate in onion during controlled deterioration, but not in lettuce, leek or carrot (Dearman et al., 1987).

As expected LTPST application after ageing of seed stored under different storage regimes (Fig. 5.9) and also of different seed lots stored under same storage regime (Table 5.5) caused significant reduction in germination rate which agrees with the results of Coolbear et al., 1984 and also data for other crops, e.g. onion, carrot and celery (Brocklehurst and Dearman, 1983) and wheat (Deli'aquilla, 1987). Table 6.3 shows that LTPST completely restored the germination rate in 6 d aged seed lot to that of the original unaged untreated seed in all the seed lot tested, with an exception to the seed lot M-3 where the restoration was limited to 3 d consecutive ageing. This supports the hypothesis that pre-sowing treatment have the capacity to induce mechanisms to repair previously sustained damage in the seed (Burgass and Powell, 1984).

CHAPTER 7
CONCLUSIONS AND SCOPE FOR FUTURE WORK

7.1 CONCLUSIONS

The following inferences could be drawn from the results and discussion of this study:

1. It is obvious from the results of the initial quality analysis that seed cultivars and/or seed lots differ in their vigour parameters despite not being significantly different in their germination capacity. Consequently, differences could be anticipated in performance, response to pre-sowing treatments or storage conditions (section 4.1).

2. With the first objective (Chapter 1) inview; the results of this study undoubtedly demonstrate that LTPST causes a significant reduction in T50 in all the cultivar and/or seed lots of tomato seeds. The degree of response by each seed lot do differ but is highly correlated with its initial T50.

3. Pretreatment for 14 d is generally applicable to all the seed lots tested, irrespective of their vigour but for best advantage the choice of treatment duration should be based on a compromise between the benefits of treatment and occurrence of undesirable pregermination.

4. The high reproducibility and repeatability of the treatment is of great importance either for commercial application or to use as a manipulative tool in physiological studies under laboratory conditions.

5. The result of this study strongly supports the hypothesis that germination rate is associated with a single, manipulable rate limiting process.

6. Although LTPST significantly improves germination rate the effect of the treatment on uniformity is seed lot dependent, suggesting that uniformity is not a simple function of germination rate.

7. Treatment has little or no effect on further seedling growth.

8. The results of this study with the second objective inview (Chapter 1) confirms that increased SMC and storage temperature causes rapid loss in viability and vigour over a period of time in tomato seeds, but that the SMC plays a very important role. The applicability of the viability model developed by Roberts and his co-workers in tomato seeds under rapid ageing condition has been demonstrated in this study. The results support the hypothesis that the slope of the survival curves of seed lot differ significantly between storage conditions but not between seed lots under the same storage conditions irrespective of their initial vigour status. The results also support the hypothesis that the seed lot constant and/or probit initial viability value is not affected by the differences in storage conditions of the seed.

9. The results suggest that events associated with loss of storability in seed are different from those causing loss of other vigour parameters, for example germination rate.

10. LTPST restores the loss of germination rate in aged viable seed but had no effect on the germination capacity of the aged seed. During pre-storage treatment increased germination rate had no effect on loss of germination capacity during ageing. This supports the hypothesis that events controlling loss of germination rate and germination capacity may be different but could occur simultaneously.

7.2 SCOPE FOR FUTURE WORK

Mechanisms

One possible candidate for advancement of germination rate would be that treatment may cause loosening and softening of cell walls which is the pre-requisite for cell wall elongation (which is the first stage of germination). There appears to be no work on seeds in this area, although this process has been shown in growing tissue. This investigation would facilitate our understanding of physiological aspects of vigour and also the germination process.

One of the possible cause of loss of vigour could be damage to protein synthesing machinery and its metabolism. Therefore, the effect of ageing and LTPST on this process might be examined.

Commercial implications

The effects of treatment on seed germination and crop establishment in the field under sowing conditions needs to be carefully investigated before commercial application of LTPST.

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