



**Effects of seaweed suspensions on seed germination
and seedling growth of barley (*Hordeum vulgare* L.)
and lettuce (*Lactuca sativa* L.).**

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Abstract

Two seaweed suspensions, obtained from whole brown algae *Ascophyllum nodosum* (Linnaeus) Le Jolis (ANS), or lamina of *Laminaria hyperborea* (Gunn.) Foslie (LHS), were evaluated for their effects on seed germination and seedling growth of barley (*Hordeum vulgare* L.) and lettuce (*Lactuca sativa* L.).

The seaweed suspensions (SWS) tested had mineral compositions similar to that of fresh algae, and mineral concentrations at levels reflecting the dilution of the product during the manufacturing process. The activity of growth regulating substances found in the SWS were too low to be of physiological significance.

When pre-germinated seeds were exposed to SWS at 10 % or higher, radicle growth was inhibited both in barley and lettuce to a similar extent. The polyphenol content in ANS was found to be responsible for these effects in lettuce seedlings. For LHS unknown inhibitory compound(s) were present in the supernatant, active only in the light. The compound(s) was(were) organic, heat labile above 60°C, degradable by microbial activity and likely not to be a protein. A growth promoting compound, that increased lettuce cotyledon expansion in the light and hypocotyl elongation in the dark, was identified as potassium.

Priming barley or lettuce seeds in SWS was beneficial, and adhering algal material provided additional nutrients to the seeds, compensating for leakage losses. Priming for 12 hours with a 24 hour re-drying period did not reduce seed viability, or increase the number of dead seeds or abnormal seedlings. When germinated under optimum laboratory conditions, the mean germination time of primed seeds, irrespective of initial seed vigour, was generally reduced. Priming in SWS was not superior to water treatments. However, seedling emergence and growth of barley under greenhouse conditions was promoted by priming with ANS, and was better than priming in water.

In addition, priming of barley or lettuce seeds in ANS resulted in better performance than water primed seeds under simulated stress conditions: Germination of ANS primed lettuce seeds at higher temperatures was superior to water primed seeds, probably due to an intrinsic compound in ANS in the adhering algal cell wall debris. Barley seeds primed in ANS germinated better than seeds primed in water under conditions that cause oxygen deficiency. The hygroscopic property of ANS and the antibiotic property of ANS were responsible for this effect, and resulted in increased oxygen availability to the embryo.

The seaweed suspension prepared from *A. nodosum* exhibited a considerable antibiotic property, and was capable of reducing the microbial population on barley seeds by 86 %, and inhibited the proliferation of external microbial contamination in the algal preparation.

The application of SWS, especially ANS may provide an alternative priming treatment to the more traditional polyethylene glycol, but may be limited to more valuable seeds, or when seeds are expected to germinate under stress conditions often encountered under some commercial growing conditions.

Dedication

This work is dedicated to my son Jonas, born during the course of the PhD.

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List of abbreviations

Abbreviations are explained in the text when they are first mentioned. This list is provided to serve as a reference list for frequently used abbreviations in case an explanation cannot be located. Molecular formulae of chemicals used are not given here but are listed in Table 2.1.1. Internationally recognised units of measurements and their common abbreviations have been used throughout.

ABA	abscisic acid
ABAE	abscisic acid equivalents
ACC	1-amino-cyclopropane-1-carboxylic acid
ANS	<i>Ascophyllum nodosum</i> suspension
BSA	bovine serum albumin
CAL	Central Analytical Laboratory, Edinburgh
cfu	colony forming units
cv.	cultivar
dw	dry weight basis
EC	European Community
ELISA	enzyme-liked immunosorbent assay
fw	fresh weight basis
GA ₃	gibberellic acid
GAE	gibberellic acid equivalents
GC	gas chromatography
GC-MS	gas chromatography-mass spectrometry
GLC	gas-liquid chromatography
¹ H-NMR	proton magnetic resonance spectroscopy
HPLC	high performance liquid chromatography
IAA	indole-3-acetic acid
IAAE	indole-3-acetic acid equivalents
ICAEP	inductively coupled atomic emission spectrometry
[K ⁺]	concentration of K ⁺ in seedling tissue and SWS
KE	kinetin equivalents
LHS	<i>Laminaria hyperborea</i> suspension
MGT	mean germination time
MRET	mean radicle emergence time
MS	mass spectrometry
NA	nutrient agar
OP	osmotic potential
OWTC	Orkney Water Test Centre
PDA	potato dextrose agar
PE	phloroglucinol equivalents
PEG	polyethylene glycol
RH	relative humidity
RIA	radioimmunoassay
se	standard error
SMP	solid matrix priming
SWS	seaweed suspension, refers to both seaweed suspensions used in this study
TLC	thin layer chromatography

Chapter 1

Introduction, background and rationale

1.1 Introduction

In the Orient, seaweed harvesting for consumption has been known for almost 5000 years. For centuries, Chinese and Japanese people have used and still use algal material as a healthful, tasty supplement to their rice diet (Nisizawa *et al.* 1987; Surey-Gent and Morris, 1987). In Europe, the earliest record is given some 1400 years ago, in a Gaelic poem, attributed to St. Columba (Newton, 1951).

It is not only as a human food or in medicine, that algae are exploited. The application of seaweed in agricultural husbandry as an animal fodder or as a manure is a very old and widespread practice (Booth, 1965; Chapman, 1970). Using seaweeds as a plant fertiliser also has a long history. It has been practised since antiquity in the Far East but was not used until the XIIth century in Europe (Aitken and Senn, 1965; Hoppe, 1965; Chapman, 1970). Watson and More (1928) mentioned that seaweeds had been used as a manure for generations in the coastal districts of Great Britain and Ireland for improving the fertility of the soil, and was still popular in recent years (Aitken and Senn, 1965; Hoppe, 1965).

In 1950, a liquid fertiliser prepared from seaweed was first produced commercially in the United Kingdom (Stephenson, 1968). Since then a number of other products have been launched. They are widely used in a number of European countries including Norway, Denmark, France, Ireland, but also in the USA, South Africa and Australia (Abetz, 1980).

The application of seaweed products to improve the establishment, growth and development of plants is becoming accepted practice today. Especially in organic farming systems, where the use of chemical compounds are limited (The Soil Association, 1989) the use of seaweed extracts or suspensions and other seaweed products can be an alternative treatment.

1.2 The systematic classification of economically important seaweeds

Algae are photosynthetic, non-vascular plants, usually existing as single cells, as loosely organised clumps of cells, as flat leaflike sheets, or as intricately branched and intertwined filaments. Macroalgae, or kelps, become large and complex enough to differentiate their tissues into organs that resemble roots (holdfast), stems (stipe), and leaves (lamina).

Taxonomic grouping of the algae is highly artificial and based on colour. This 'artificial' taxonomic system is still in flux, even after decades of scientific study, because of the complexity of these variable organisms. The economically important species belong to the divisions (Lee, 1980):

- Chlorophyta (green algae),
- Cyanophyta (bluegreen algae),
- Chromophyta (Phaeophyceae) (brown algae),
- Rhodophyta (red algae).

Sometimes the Phaeophyceae are placed as a separate division Phaeophyta (Lobban and Wynne, 1981). Of the large number of algae known, just a small number are used in agriculture, mainly for feeding or as manure (Table 1.2.1). Only a few algal species have been used for the preparation of liquid seaweed products applied to plants, most of those belong to the group of brown seaweeds (Table 1.5.1).

1.3 The chemical composition of economically important brown seaweeds

There are numerous sources, articles, reviews and books on the use of algal polysaccharides; e.g. agar, alginate, carrageenan or furcellaran. These are of importance as natural resources for the industrial production of cosmetics, or as food additives as thickening or gelling agents, as emulsifier or stabiliser (MacLachlan, 1985; Yvin, 1987; Guist, 1990; Indergaard and Minsaas, 1991; Indergaard and Østgaard, 1991; Skjåk-Bræk and Martinsen, 1991), but probably of less significance for agricultural use.

Table 1.2.1: Systematic classification of algae used in agricultural and horticultural practices (compiled from: Chapman, 1970; Lobban and Wynne, 1981; Kain and Dawes, 1987)(classification after Lee, 1980).

Class	Order	Family	Genus	Species	Use	
Chlorophyceae	Ulotrichales	Ulvaceae	<i>Enteromorpha</i>	<i>intestinalis</i>	M	
			<i>Ulva</i>	<i>lactuca</i>	M	
Cyanophyceae	Nostocales		<i>Microcoleus</i>	<i>chthonoplastes</i>	G	
Phaeophyceae	Laminariales	Alariaceae	<i>Alaria</i>	<i>esculenta</i>	F	
				<i>fistulosa</i>	F,M	
		Laminariaceae	<i>Ecklonia</i>	<i>maxima</i>	E,M	
				<i>radiata</i>	M	
				<i>variegata</i>		
			<i>Laminaria</i>	<i>bongardiana</i>	F	
				<i>digitata</i>	F	
				<i>hyperborea</i>	E	
				<i>pallida</i>	M	
				<i>saccharina</i>	F	
				Lessoniaceae	<i>Lessonia</i>	<i>variegata</i>
		<i>Macrocystis</i>	<i>integrifolia</i>		M,E	
		<i>Nereocystis</i>	sp.		M	
		<i>Pelagophycus</i>	sp.		M	
		Chordaceae	<i>Chorda</i>	<i>filum</i>	F	
		Fucales	Fucaceae	<i>Ascophyllum</i>	<i>nodosum</i>	E,F
				<i>Fucus</i>	<i>evanescens</i>	F
					<i>serratus</i>	F,M
	<i>spiralis</i>					
	<i>vesiculosus</i>				E,F	
	<i>Pelvetia</i>			<i>canaliculata</i>	F	
	Durvilleaceae		<i>Durvillea</i>	<i>potatorum</i>	E,M	
	Himanthaliaceae		<i>Himanthalia</i>	<i>elongata</i>	M	
		<i>Sargassum</i>	sp.	F,M		
Rhodophyceae	Gigartinales	Gigartinaceae	<i>Chondrus</i>	<i>crispus</i>	F	
		Gracilariaceae	<i>Gracilaria</i>	<i>verrucosa</i>	M	
		Hypneaceae	<i>Hypnea</i>	<i>musciiformis</i>	M	
	Cryptonemiales	Corallinaceae	<i>Lithothamnion</i>	<i>coralloides</i>	Æ,M	
	Rhodymeniales		<i>Rhodymenia</i>	<i>palmata</i>	F	
			<i>Phymatolithon</i>	<i>calcareum</i>	Æ,M	
			<i>Furcelaria</i>	<i>lumbricalis</i>	F	

(F - animal feeding, M - manure, E - extract, Æ - mærl, G - algal guano)

1.3.1 Carbohydrates, proteins and fat

The major component of fresh brown seaweed material is water, which makes up about 70-85 % of the fresh weight. The dry matter consists of about 15-25 % and 21-38 % minerals in *Ascophyllum nodosum* and *Laminaria* sp. respectively, and a variable total carbohydrate amount of 40-70 % (Table 1.3.1). Alginic acid, laminaran, mannitol and fucoidan are the major carbohydrates of brown algae. However, considerable variability in carbohydrate partition is observed between analyses of the same algae. The protein content of brown algae varies between 5-10 % in *A. nodosum*, and 8-15 % in *Laminaria* sp. and the fat content between 2-7 % and 0.25-2 %, in *A. nodosum* and *Laminaria* sp. respectively (Table 1.3.1).

Table 1.3.1: Carbohydrates and proteins composition of some brown seaweeds (°Indergaard and Minsaas, 1991; *Stephenson, 1968)

Species	<i>Ascophyllum nodosum</i>		<i>Laminaria digitata</i>	<i>Laminaria hyperborea</i>	
Algal tissue Component	whole °	whole *	lamina °	lamina *	stipe *
Water %	70-85	89.7	73-90	n.d.	n.d.
	% of dry weight				
Minerals	15-25	15.4	21-35	21.8	37.6
Total carbohydrates	n.d.	n.d.	n.d.	60.2	44.1
Alginic acid	15-30	26.7	20-45	n.d.	n.d.
Xylan(pentose)	0	7.0	0	n.d.	n.d.
Laminaran	0-10	9.3	0-18	n.d.	n.d.
Mannitol	5-10	4.2	4-16	n.d.	n.d.
Fucoidan	4-10	n.d.	2-4	n.d.	n.d.
Floridoside	0	n.d.	0	n.d.	n.d.
Fibre	5	7.0	6-8	5.0	9.6
Other					
carbohydrates	ca.10	14.4	1-2	n.d.	n.d.
Protein	5-10	5.7	8-15	12.6	8.4
Fat	2- 7	2.6	1- 2	0.38	0.25
Tannins	2-10	n.d.	ca.1	n.d.	n.d.

(n.d. - not determined)

1.3.2 Amino acid content

The amino acid composition of brown seaweeds have been analysed by different researchers, and a summary of the amino acid composition of diverse algae is given by Indergaard and Minsaas (1991). Besides the essential amino acids, a number of non-essential amino acids can be detected, for example in the brown seaweed *Laminaria hyperborea*. This brown seaweed was found to be comparatively high in leucine, arginine and asparatic acid, whereas *Macrocystis pyrifera* had high asparatic acid and glutamic acid values (Table 1.3.2).

Table 1.3.2: Amino acid composition of crude protein from the brown algae *Laminaria hyperborea* (Indergaard and Minsaas, 1991), and *Macrocystis integrifolia* (Schmitz and Srivastava, 1979).

Species	<i>Laminaria hyperborea</i>	<i>Macrocystis integrifolia</i>
Amino acid	g amino acid nitrogen 100 g ⁻¹ crude protein	mg ml ⁻¹
Alanine	8.5	1.61
Arginine	12.3	n.d.
Asparatic acid	7.9	5.05
Cystine	traces	n.d.
Glutamic acid	3.4	3.94
Glycine	3.1	0.05
Histidine	0.0	n.d.
Isoleucine	0.7	0.09
Leucine	7.0	0.09
Lysine	3.7	n.d.
Methionine	0.9	0.07
Phenylalanine	1.1	0.16
Proline	3.9	n.d.
Serine	3.2	0.89
Threonine	3.1	1.03
Tryptophan	traces	n.d.
Tyrosine	0.9	0.17
Valine	3.5	0.19

(n.d. - not determined)

1.3.3 Mineral composition

Minerals are present in brown algae in varying amounts, differing significantly between algal species analysed. For example the potassium content of *A. nodosum* was measured to be around 2.0-3.0 % (dw), whereas *L. hyperborea* lamina and stipes contained 5.25 % and 8.15 % (dw) (Indergaard and Minsaas, 1991). Additionally, *L. hyperborea* contained very high amounts of chlorine and similar amounts of sodium and sulphur, compared to the mineral concentrations in *A. nodosum* (Table 1.3.3).

A wide range of trace elements are also present in brown algae. Besides important plant micro nutrients such as iron, manganese, zinc, copper and molybdenum, a number of other elements are present in larger amounts; e.g. iodine, fluorine, barium and arsenic (Table 1.3.3). Variation in the levels of heavy metals in algal material varies greatly, depending where the plants were collected. The concentration of heavy metals such as copper, zinc and cadmium in brown algae was examined by atomic absorption spectrophotometry by Cullinane and Whelan (1982). They found significantly different concentrations of zinc and copper concentration between sampling sites in *Fucus vesiculosus*, *F. spiralis* and *A. nodosum* from Ireland, while the levels of cadmium varied less (Table 1.3.4), and Cullinane *et al.* (1987) proposed to use the brown seaweeds as test specimens for the monitoring of water pollution. Thus, the location of seaweed harvest can have an influence on the trace element content of brown seaweeds, especially heavy metal concentration. This might partially explain the huge differences in trace element composition detected between the two samples of *A. nodosum* (Table 1.3.3).

Table 1.3.3: Mineral composition of diverse seaweeds (°Indergaard and Minsaas, 1991; *Stephenson, 1968)

Species	<i>Ascophyllum nodosum</i>		<i>Laminaria hyperborea</i>	
	°	*	lamina*	stipe*
Element	% of dry weight			
Calcium	1.0-3.0	1.90	1.04	1.80
Chlorine	3.1-4.4	3.68	5.92	12.48
Magnesium	0.5-0.9	0.21	0.58	0.73
Nitrogen	n.d.	0.06	n.d.	n.d.
Phosphorus	0.1-0.15	0.21	0.28	0.25
Potassium	2.0-3.0	1.28	5.25	8.15
Sodium	3.0-4.0	4.18	2.88	1.35
Sulphur	2.5-3.5	1.56	3.06	2.54
	ppm dry weight			
Antimony	0.19-0.53	1.42	n.d.	n.d.
Arsenic	22-44	n.d.	n.d.	n.d.
Barium	15-50	12.76	n.d.	n.d.
Chromium	n.d.	n.d.	n.d.	n.d.
Cobalt	0.4-0.7	12.27	n.d.	n.d.
Copper	18-35	6.35	n.d.	n.d.
Fluorine	25.5	326.50	n.d.	n.d.
Iodine	700-1200	624.00	n.d.	n.d.
Iron	101-176	895.60	n.d.	n.d.
Manganese	10-15	1235.00	n.d.	n.d.
Molybdenum	1-2	15.92	n.d.	n.d.
Nickel	2-5	35.00	n.d.	n.d.
Selenium	0.06-0.09	0.43	n.d.	n.d.
Vanadium	1.5-3.0	5.31	n.d.	n.d.
Zinc	70-240	35.16	n.d.	n.d.

(n.d. - not determined)

Table 1.3.4: Cadmium, copper and zinc concentrations in brown seaweeds sampled at different locations (Cullinane and Whelan, 1982).

Species	<i>Ascophyllum nodosum</i>	<i>Fucus spiralis</i>	<i>Fucus vesiculosus</i>
Heavy metal / Sample point	ppm dry weight		
Cadmium			
Carnsore Point	2.42	3.41	3.82
Monkstown	1.63	2.05	3.00
Copper			
Carnsore Point	3.49	4.21	6.91
Monkstown	10.87	6.15	14.21
Zinc			
Carnsore Point	61.92	50.83	47.92
Monkstown	176.20	215.19	318.99

1.3.4 Occurrence of vitamins

Numerous vitamins are present in brown seaweeds. Those found in *A. nodosum* and *Laminaria* sp. are listed in Table 1.3.5. *A. nodosum* is especially rich in ascorbic acid (vitamin C), and tocopherols (vitamin E), compared to *Laminaria* sp. These analyses of a variety of vitamins were undertaken early in the 1950's and 1960's, with the equipment available at that time. In a more recent investigation much higher vitamin B₁₂ levels have been detected in *A. nodosum* and *Laminaria* species using radioassay methods (van den Berg *et al.* 1988). Whether these differences reflect advances in separation and quantification technology, or were due to harvest location is not known.

Table 1.3.5: Vitamins in seaweeds (Indergaard and Minsaas, 1991).

Species		<i>Ascophyllum nodosum</i>	<i>Laminaria sp.</i>
Vitamin		ppm dry weight	
Carotene	(provitamin A)	35-80	n.d.
Ascorbic acid	(C)	550-1650	110.0
Thiamine	(B ₁)	1.0-5.0	0.8
Riboflavin	(B ₂)	5.0-10.0	3.2
Niacin	(B-group)	10.0-30.0	18.0
Folic acid	(B-group)	0.1-0.5	n.d.
Folinic acid	(B-group)	0.1-0.5	n.d.
Cobalamine	(B ₁₂)	0.0008-0.003	0.003
	(B ₁₂)	0.04*	0.028*
Tocopherols	(E)	260-450	n.d.
Biotin	(H)	0.1-0.4	n.d.
Menadione	(K ₃)	10.0	n.d.

(n.d. - not determined; * - van den Berg *et al.* 1988, *Ascophyllum* from Norway, *Laminaria* from Japan)

1.3.5 Polyphenols and their ecological significance

The most comprehensive review of brown algal polyphenols was given by Ragan and Glombitza (1986). Brown algae contain a range of low and high molecular weight phenolic compounds. Their quantification can be achieved by

different methods, including colorimetric methods, potentiometric titration, gravimetry, or ultraviolet spectrophotometry (Ragan and Glombitza, 1986). The results obtained with these methods differ widely (Table 1.3.6.), and comparison of results of phenol contents should include a reference to the method used (Pedersen, 1984).

The total quantity of phenolic compounds present in algae varied markedly among and between genera (Table 1.3.6). Thus, quantified with the identical method of analysis, *L. hyperborea* contained phenolics between 0.8-3.9 % (dw), whereas *A. nodosum* contained on average between 0.8-8.0 % (dw), increasing to 12-14 % (dw) during the winter (Ragan and Jensen, 1978). Investigation in Norway on *A. nodosum* revealed annual fluctuation in the phenol content, with the lowest values detected in April- May (Ragan and Glombitza, 1986). The distribution of phenols in algal tissue has been investigated by several researchers. Higher amounts of phenols were found in the lamina of *L. hyperborea*. In addition, the outer tissues of the stipes, contained higher levels than the inner tissue. Conversely, receptacles of *A. nodosum*, the swollen tips of branches carrying the reproductive organs, did not preferentially accumulate phenolics, and the majority of phenols was concentrated in the stipes (Pedersen, 1984). Another paper by Tugwell and Branch (1989), investigated the location of polyphenol distribution within organs of three kelps, *Ecklonia maxima*, *Laminaria pallida* and *Macrocystis angustifolia*, and revealed that polyphenols were almost entirely restricted to the thin outer meristoderm, as in *L. hyperborea*, to serve as a defence against herbivores. Additionally, they found high phenols in the meristoderm tissue of the holdfast and stipes, as found in *A. nodosum*.

Beside variation in different tissues, the algal phenolic content of *A. nodosum* was found to be affected by external factors. Its concentration within the tissue increased with age, with increase in salinity of the seawater (Pedersen, 1984), and depth of immersion in water (Ragan and Glombitza, 1986).

Polyphenols play a role in the defence system in algae, to prevent herbivore attack. Geiselman and McConnell (1981) found that polyphenols from two brown algae, *A. nodosum* and *F. vesiculosus* inhibited feeding of marine herbivorous snails by more than 50 % at concentrations as little as 1 %. The active compound were characterised as phloroglucinol polymers with a wide molecular weight range.

Table 1.3.6: Quantities of polyphenols in diverse whole brown seaweeds (Ragan and Glombitza, 1986).

Species	Polyphenol content	Location	Month	Method ¹⁾	Relative to ²⁾
	(% dry weight)				
<i>Ascophyllum nodosum</i>	8.7-12.8	Norway	1-12	FD	Phloro./G
	0.8- 8.0	Norway	n.g.	FD	Phlorogluc.
	9.4-12.2	Norway	2-10	Van.	Phloro./G
	9.4-14.4	Norway	1-12	Brent.	<i>F.v.poly</i> /G
	3.0- 6.0	Norway	1- 6	Brent.	<i>A.n.poly.</i>
	0.4- 9.0	Norway	1-12	Pot.T	EW=40
	1.1- 2.0	Norway	n.g.	Pot.T	EW=41.5
	0.1	Sweden	7- 8	DI/A	Ascorbate
<i>Laminaria hyperborea</i>	<0.1	Sweden	7- 8	DI/A	Ascorbate
	0.8-3.9	Norway	3	FD	Phlorogluc.
	0.1	Norway	3	Pot.T	Phlorogluc.
<i>Laminaria digitata</i>	0.4-1.3	Norway	3	FD	Phlorogluc.
<i>Laminaria saccharina</i>	0.8-2.4	Norway	3	FD	Phlorogluc.
<i>Fucus distichus</i>	3.0-6.8	Californ.	4-12	FD	Phlorogluc.
<i>Fucus vesiculosus</i>	8.2-14.4	Norway	1-12	FD	Phloro./G
	4.8-7.9	Norway	n.g.	FD	Phlorogluc.

(n.g. - data not given; ¹⁾ Method: Brent. - Brentamine; DI/A - dichlorophenol-indophenol titration after ascorbinase; FD - Folin-Denis; Pot.T - potentiometric-titration; Van - vanillin-H₂SO₄; ²⁾ Relative to: *A.n.poly.* - high-molecular weight phlorotannins from *A. nodosum*; *F.v.poly*/G - high-molecular weight phlorotannins from *F. vesiculosus* as colorimetric standard plus gravimetric correction; EW=40(41.5) - equivalent weight assumed to be 40 (41.5); Phloro./G - anhydrous phloroglucinol as colourimetric standard plus gravimetric correction; Phlorogluc. - anhydrous phloroglucinol)

1.3.6 Volatile halogenated organic compounds and defence strategy

Macroalgae are a source of volatile halogenated organic compounds, such as bromoform and dibromomethane. The mechanism by which these compounds are formed is unknown. The presence of bromoperoxidases in the brown seaweeds *A. nodosum*, *F. vesiculosus*, *Laminaria saccharina*, and *L. digitata* has been proven (Wever *et al.* 1991). Intact plants were able to brominate exogenous organic compounds when hydrogen peroxide and bromide were added to seawater. *A. nodosum*, contained two vanadium bromoperoxidases (Krenn *et al.* 1989), that exhibited brominating activity but only when the algae was exposed to light (Wever *et al.* 1991). This photochemical link was not detected in *Laminaria* species. The vanadium bromoperoxidases produced free hypobromous acid and bromine, and the authors suggested that bromination of seawater was due to the reaction with bromo-hydroxide, released by intact plants. The authors measured a rate of bromination of exogenous organic compounds in seawater by *A. nodosum* of 68 nmol (g of wet alga)⁻¹ h⁻¹, and for *L. digitata* of 123 nmol (g of wet alga)⁻¹ h⁻¹. As shown by Krenn *et al.* (1989), the vanadium bromoperoxidase was located on the thallus surface, the removal of which stopped bromination. Bromo-hydroxide is a strong biocidal agent and it was proposed that its formation by this seaweed or associated bacteria (Section 1.3.8.) is part of a host defence system that prevents certain bacterial and fungal infections.

1.3.7 Growth regulating substances and betaines

The occurrence of growth regulating substances in algae has been the subject of many studies (Table 1.3.7). The reason for this interest is the objective of using the algae as plant fertiliser.

The relevance of growth regulating substances on algal growth themselves has been reviewed by Evans and Trewavas (1991). The algae are regarded as a less

Table 1.3.7: Levels of endogenous growth regulating substances of diverse brown algae.

Growth regulating substance	Concentration (fresh weight)	Equiva-lent	Method / bioassay applied	Reference
Abscisic acid (ABA)				
<i>Ascophyllum nodosum</i>	3-15 µg kg ⁻¹	ABA	MS	Boyer and Dougherty, 1988
<i>Ascophyllum nodosum</i>	n.m.	ABA	lettuce-hyp.	Hussain and Boney, 1973
<i>Laminaria digitata</i>	n.m	ABA	and GLC	Hussain and Boney, 1973
Auxins				
<i>Caulerpa paspaloides</i>	1.0 mg kg ⁻¹	IAA	<i>Avena</i> col.s. HPLC, GC-MS	Jacobs <i>et al.</i> 1985
<i>Nereocystis</i> sp.	0.05-0.5 µg kg ⁻¹	IAA	<i>Avena</i> curv.	van Overbeck, 1940
<i>Ascophyllum nodosum</i>	<0.1 mg kg ⁻¹	IAA	TLC	Buggeln and Craigie, 1971
<i>Fucus vesiculosus</i>	<0.1 mg kg ⁻¹	IAA	TLC	Buggeln and Craigie, 1971
Cytokinins				
<i>Ecklonia maxima</i>	5-15 µg kg ⁻¹	K	HPLC	Featonby-Smith and van Staden, 1984a
<i>Ecklonia maxima</i>	10 µg kg ⁻¹ lamina 20 µg kg ⁻¹ stipe	K	soybean callus, ELISA, RIA	Hofman <i>et al.</i> 1986
<i>Laminaria digitata</i>	n.m.	K	radish leaf disk	Hussain and Boney, 1969
<i>Sargassum muticum</i>	0.9 µg kg ⁻¹ 0.2 µg kg ⁻¹	IPA <i>cis</i> -z. r.	GC-MS	Zhang <i>et al.</i> 1991
<i>Sargassum heterophyllum</i>	47 µg kg ⁻¹ laterals 50 µg kg ⁻¹ holdfast	K	soybean callus,	Mooney and van Staden, 1984
<i>Fucus vesiculosus</i>	n.m	K	<i>Amaranthus</i>	Kentzer <i>et al.</i> 1980
<i>Macrocystis pyrifera</i>	- ; 40 µg kg ⁻¹ 113 ; 148 µg kg ⁻¹ 23.4 ; - µg kg ⁻¹	Z ; ZR Z ; ZR Z ; ZR	<i>Amaranthus</i> HPLC GC-MS-SIM	de Nys <i>et al.</i> 1990
Gibberellic acid				
<i>Ecklonia radiata</i>	n.p.	GA	dw.-5 maize	Jennings, 1968
<i>Enteromorpha prolifera</i>	100 µg kg ⁻¹	GA	dw.-5 maize	Jennings, 1968
<i>Fucus vesiculosus</i>	0.76-12.0µg kg ⁻¹	GA	dw. pea s.	Radley, 1961
<i>Fucus vesiculosus</i>	n.m	GA	lettuce hyp.	Borowczak <i>et al.</i> 1977
<i>Fucus spiralis</i>	2.0-20 µg kg ⁻¹	GA		Mowat, 1965
<i>Macrocystis pyrifera</i>	n.p.	GA	dw-5 maize	Purves and Phinney, 1962

(Equivalent to: KE - kinetin equivalents; IPA - isopentenyl adenosine; Z - zeatin; ZR - zeatin riboside; *cis*-z. r. - *cis*-zeatin riboside; ABA - abscisic acid; GA - gibberellin acid. Method: ¹H-NMR - proton magnetic resonance spectroscopy; ELISA - enzyme-linked immunosorbent assay; GC-MS -gas chromatography-mass spectrometry; GC-MS-SIM - gas chromatography-mass spectrometry in selected-ion monitoring mode; GLC - gas-liquid chromatography; HPLC - high performance liquid chromatography; MS - mass spectrometry; RIA - radioimmunoassay; TLC - thin layer chromatography. Bioassay: *Amaranthus* - *Amaranthus* betacyanin bioassay; *Avena* col.s. - *Avena* coleoptile section bioassay; *Avena* curv. - *Avena* curvature bioassay; lettuce-hyp. - lettuce hypocotyl elongation bioassay; dw.-5 maize - dwarf-5 maize seedling bioassay; dw.pea s. - dwarf pea seedling bioassay; n.d. - not determined; n.m. - not mentioned; n.p. - not present.)

advanced group than higher plants, although many of their developmental processes are analogous. Exogenous application of growth hormones to algal explants or whole plants indicate that these substances may play a role in algal growth and development, inducing processes of cell division and elongation, organogenesis, apical dominance, respiration and photosynthesis (Jennings, 1968; Buggeln, 1976; Borowczak *et al.* 1977; Mooney and van Staden, 1986).

In 1958, Bentley postulated that algae have similar biochemical systems as higher plant species, as they contained substances with plant hormone-like activity. In early work on unicellular algae, auxins, cytokinins and gibberellin-like substances were detected using bioassay methods (Mowat, 1965; Bentley-Mowat and Reid, 1968). Pedersen (1973), and Kentzer *et al.* (1980) identified the cytokinin, 6-(3-methyl-2-butenylamino)purine (2iP), in seawater from the *Fucus-Ascophyllum* zone by bioassay, gas chromatography-mass spectrometry (GC-MS). A peak value for 2iP was found to be present when water was sampled near the bottom of the *Fucus-vesiculosus*-zone (Kentzer *et al.* 1980). It was considered that the exudation of some cytokinin-like substances by the algal cells into the surrounding water was possible.

Abscisic acid

Hussain and Boney (1973) using a lettuce hypocotyl growth bioassay and gas-liquid chromatography (GLC), detected compounds in *A. nodosum* and *L. digitata*, co-migrating with abscisic acid (ABA), and caused inhibition of lettuce hypocotyl growth. Identification of abscisic acid (ABA) in *A. nodosum* was achieved by Boyer and Dougherty (1988), and quantified as 0.03-0.15 µg ABA g⁻¹dry weight. The presence of ABA was also confirmed by mass spectrometry (MS).

Auxins

Since 1940 auxin-like substances have been investigated in algae. Van Overbeck (1940) detected the putative presence of indole-acetic acid in a range of marine algae, especially *Nereocystis*, using an *Avena*-curvature test. However, work by Buggeln and Craigie (1971) and Buggeln (1976) on diverse algae species, using thin layer chromatography (TLC), gave conflicting results as they were unable to detect endogenous indole-3-acetic acid (IAA). However, several auxin-like compounds were found in *Fucus* extracts. From their experiments on the recovery of exogenously added IAA from algal extracts, they calculated that the levels of IAA in the algae *A. nodosum*, if present, were below $0.1 \mu\text{g g}^{-1}$ fresh weight. Abe *et al.* (1972), using mass spectrometry, identified native auxins in *Undaria pinnatifida*. In addition, Jacobs *et al.* (1985) using an *Avena* coleoptile section bioassay, high performance liquid chromatography (HPLC) with an electrochemical detector and GC-MS, identified the active auxin, IAA in *Caulerpa paspaloides*.

Cytokinins

Hussain and Boney (1969) investigated cytokinins in *L. digitata* and detected kinetin-like substances by bioassay methods in the stipes and holdfast regions. The precise chemical nature of two peaks in an absorption spectra, however, was not further examined. Jennings (1969) detected cytokinin-like activity in *Ecklonia radiata* using a barley leaf segment assay. In 1980, Kentzer *et al.*, found cytokinins in *F. vesiculosus* using an *Amaranthus*-bioassay method, while Featonby-Smith and van Staden (1984a) investigated cytokinins of *E. maxima* using HPLC and a soybean bioassay. Featonby-Smith and van Staden (1984a) determined seasonal changes in cytokinin levels using paper chromatography, and found qualitative and quantitative variations over the year. During summer, zeatin, ribosylzeatin and their dihydroderivatives were responsible for most of the activity detected, while during

winter cytokinin glucosides increased above the levels of free cytokinins. Another highly sensitive technique, enzyme-linked immunosorbent assay (ELISA) has been used by Hofman *et al.* (1986) for the identification of isopentenyl adenosine (IPA) and a radioimmunoassay (RIA) for the identification of zeatin and ribosylzeatin in *E. maxima*. They found a close link between cytokinin levels and lunar periodicity. Mooney and van Staden (1984) investigated cytokinin-like activity in *Sargassum heterophyllum* and found interactions between seasonal changes and type of tissue examined. The highest cytokinin-like activity was found in vegetative laterals and holdfasts from June to August. In 1987, they identified the cytokinins by GC-MS in *S. heterophyllum* as *trans*-zeatin, *trans*-ribosylzeatin, *dihydro*-zeatin, and IPA. De Nys *et al.* (1990) using bioassays, immunoassays, and GC-MS, also analysed the distribution and seasonal variation of endogenous cytokinins of *M. pyrifera*. They found the highest cytokinin-like activity in the basal third of young rapidly growing blades, and at phases of maximum annual growth in April and October. The occurrence of cytokinins as free bases and ribosides correlated with the areas of maximum growth. In another work, Zhang *et al.* (1991) identified by GC-MS the cytokinins, IPA in *Porphyra perforata* and IPA and *cis*-zeatin in *Sargassum muticum*.

Gibberellins

In early work on brown algae, gibberellin-like activity was found in *F. vesiculosus* by Radley (1961), and in *Fucus spiralis* by Mowat (1965). However, Kato *et al.* (1962) were unable to detect such hormone-activity in extracts of *M. pyrifera*. The presence of gibberellin-like compounds in *F. vesiculosus* was confirmed in later work (Borowczak *et al.* 1977). Jennings and McComb (1967) investigated the red algae *Hypnea musciformis* and detected gibberellin-like activity. Jennings (1968),

investigated brown algae, and found gibberellin-like activity in extracts from *E. radiata*, detected by dwarf-5 maize mutant assays and paper chromatography.

Betaines

Betaines, quaternary ammonium compounds, are thought to have activities similar to cytokinins in growth tests (Wheeler, 1973). Certain claims for the growth effects of seaweed extracts could be explained by the presence of betaines, e.g. increased frost resistance of plants, or enhanced water utilisation efficiency (Bergmann and Eckert, 1984). Investigations on the betaine content of brown algae revealed their presence in most of the species used in the manufacture of seaweed extracts. Betaines such as γ -aminobutyric acid betaine, glycinebetaine, laminine and lysinebetaine have all been found in *L. digitata*, *L. hyperborea* and *L. saccharina* (Blunden *et al.* 1982).

Blunden *et al.* (1985) consistently found γ -aminobutyric acid betaine, δ -aminovaleric acid betaine and laminine in *A. nodosum*, and γ -aminobutyric acid betaine, glycinebetaine, and laminine in *Fucus* species. Identification and characterisation of betaines was performed using thin layer chromatography (TLC), mass spectrometry (MS) and proton magnetic resonance spectroscopy (^1H -NMR).

1.3.8 'Contaminating' symbionts of seaweeds

In a recent review Evans and Trewavas (1991) questioned the origin of growth regulating substances found in algae. Marine algae are known to live in close association with other organisms. For example, the thalli of all macroalgae are covered with bacteria, and Dreher *et al.* (1978) reported the presence of endophytic bacteria in the vacuole of *Caulerpa simpliciuscula*. Fries (1988) showed that axenic cultures of *A. nodosum* could not survive without exogenously applied growth regulators, but grew for 8 years in the presence of associated bacteria; *A. nodosum* was found to exist in symbiosis with the epiphytic bacteria and an endophytic fungus

Mycosphaerella ascophylli (Fries, 1988). The fungus, when growing within the alga seemed to have little effect on algal morphology. Apparently, in the mycophycobiosis between *A. nodosum* and *M. ascophylli* (Garbary and Gautam, 1989) the associated epiphytic bacteria kept the growth of the fungus in check, which otherwise would use the algae simply as a carbon source (Fries, 1988). These results indicated a closer dependence of *A. nodosum* on the bacteria rather than the fungus, which might be seen as a more parasitic organism. Based on these observations, the origin of growth substances detected in algal material is questionable. They may have originated, partially or in total, from associated or contaminating microorganisms. Previous work on algal growth regulators may have only checked for visible contamination, but none of them could be said to be bacteria free.

1.4 The biocidal activity of seaweed compounds

Marine algae are widely reported as a potential source of antibacterial and antifungal substances. Antibacterial activity in a marine planktonic algae was first reported in 1960 by Sieburth (Pesando, 1990) for an Antarctic species *Phaeocystis pouchetii*. It is only recently that macroalgae have been recognised as a possible reservoir of antimicrobial substances. Comprehensive summaries of work on antimicrobial activities in micro- and macroalgae have been published by Pesando (1990), and Sridhar and Vidyavathi (1991).

As most investigations were carried out on different algae material, such as homogenates of thalli, or whole thallus pieces or even extracts, which had been prepared using an array of different preparation methods, the effects of the resulting compound or compound mixture vary widely, because of the different characteristics of the extracted compound(s) (Rao and Parekh, 1981). A wide variety of different test pathogens have been used to investigate the biocidal effects of algae extracts, including different terrestrial plant pathogenic bacteria, fungi and viruses. In general,

biocidal activity has been detected in all classes of algae. However, seasonal variation in the expression of activity is also well documented. The brown seaweeds *A. nodosum*, *L. digitata*, and *L. saccharina* have been tested by Hornsey and Hide (1976). They found seasonal variation in *A. nodosum* from June to August, and for *L. saccharina* from November to April, whereas *L. digitata* produced antibiotic compounds throughout the year.

1.4.1 Antifungal activity

Some researchers have investigated the effectiveness of algal extracts to control *Phoma tracheiphila*, a destructive disease of citrus (*Citrus lemon*) trees in the Mediterranean. Lipid extracts from the brown algae, *Cystoseira balerica* totally inhibited germination of the pycnidiospores, and *Cystoseira ercegovicci* and *Zanardinia prototypus* partially inhibited the germination of spores (Caccamese *et al.* 1981). Moreau *et al.* (1988) used hexane extracts from the genera *Dictyota* and *Dilophus*. *Dictyota* sp. extracts were more active on hyphal growth inhibition of *P. tracheiphila*, as well as *Fusarium oxysporum albedinis*, *F. oxysporum dianthi*, *F. oxysporum lycopersici*, and *Colletotrichum acutatum*, but not *Phialophora cinerescens*. A recent publication by Becker *et al.* (1990), reported on a 95.5 % and 88.7 % reduced incidence of common bunt of wheat (*Triticum aestivum*) after a seed treatment with dried algae material of *L. saccharina*, and *L. digitata*.

1.4.2 Antibacterial activity

Extracts from brown seaweeds have not been tested on typical plant pathogenic bacteria, but against the more saprophytic *Bacillus subtilis*, a potential pathogen causing soybean seed decay (Grybauskas *et al.* 1979; Mengistu and Sinclair, 1979). In an early work on antibacterial activity of *A. nodosum*, Vacca and Walsh (1954) found no inhibitory effect of alcohol soluble compounds to *B. subtilis*.

Jing-wen and Wei-ci (1984) tested toluene-methanol extracts of diverse brown algae and found inhibiting activity only in the genus *Sargassum*, while Espeche *et al.* (1984) found antibiotic activity in the ethanol extracts of *M. pyrifera*. Caccamese *et al.* (1981, 1985) detected antibacterial activity in methanol-toluene extracts in *Cystoseira* species and *Z. prototypus*. Rosell and Srivastava (1987) testing acetone extracts of a range of diverse brown seaweeds on *B. subtilis* and found inhibitory activity in all the algae tested, including *Fucus distichus* and *L. saccharina*.

1.4.3 Antiviral activity

Caccamese *et al.* (1981) tested lipid extracts from brown seaweeds for their antiviral properties. The test solutions were mixed with purified tobacco mosaic virus (TMV), and consecutively tested by tobacco plant inoculation. The results indicated that the number of leaf lesions were reduced by up to 65 % compared to controls when *C. balearica* extract was tested at the highest concentration. In the same assay *Z. prototypus* extract reduced the number of lesions 47 %, while *C. ercegovicii* extract was less effective and reduced the number of lesions by a third.

1.4.4 Insecticidal effects

In screening experiments seaweeds were tested for their biocidal effect on larvae of the mosquito *Aedes aegypti* (Subramonia Thangam and Kathiresan, 1991). The LC₅₀ (LC = lethal concentration of vacuum evaporated concentrate) of the acetone extracts of the brown seaweeds *D. dichotoma* was found to be 61.6 mg l⁻¹, and a fractionation of the crude extract reduced the LC₅₀ to 25.7 mg l⁻¹. *Sargassum* species did not cause 50 % mortality of mosquito larvae, even at 100 mg l⁻¹. The authors did not investigate the active compound, but suggested the economical viability of the field use of these extracts in mosquito control programmes.

1.4.5 Antimicrobial compounds in seaweeds

It is only recently that biologically active secondary metabolites exhibiting antimicrobial properties from marine algae have been subjected to chemical analyses (Pesando, 1990). Halogenated terpenoids, which have antimicrobial properties are produced by many red algae, whereas green and brown algae are not known to produce these compounds (Pesando, 1990). The brown algae contain, however, substantial amounts of phlorotannins, amongst other active antibiotic compounds (Table 1.4.1).

Table 1.4.1: Antimicrobial substances isolated from marine brown algae (Pesando, 1990).

Species	Active compound	Activities	
		anti-bacterial	anti-fungal
<i>Cystoseira elegans</i>	eleganolone	+	+
<i>Dictyota</i> sp.	diterpene/dictyol	+	+
<i>Laminaria digitata</i>	acrylic acid	+	
<i>Laminaria hyperborea</i>	acrylic acid	+	
<i>Laminaria saccharina</i>	acrylic acid	+	
<i>Fucus serratus</i>	acrylic acid		+
<i>Fucus vesiculosus</i>	phlorotannins	+	
<i>Himanthalia elongata</i>	phlorotannins	+	
<i>Pelvetia canaliculata</i>	phlorotannins	+	

1.5 Diverse commercial seaweed extracts and suspensions

1.5.1 Manufacturing processes involved

In previous publications the application of different brown seaweed extracts or suspensions were tested on various crops. The algal products applied varied substantially in the method of preparation, and in the source material used (Table 1.5.1). With a view to the manufacturing process, the handling of the algal

Table 1.5.1: Commercial products from brown seaweeds and the algal source material used.

Product	Source algal species	Country of manufacture
Alginex	<i>Ascophyllum nodosum</i>	Iceland
Goëmill	<i>Ascophyllum nodosum</i>	France
Goëmar GA 14	<i>Ascophyllum nodosum</i>	France
GYFA 17	<i>Ascophyllum nodosum</i>	France
Marinure (Algifert)	<i>Ascophyllum nodosum</i>	Norway
Maxicrop	<i>Ascophyllum nodosum</i>	United Kingdom
Nitrozyme TM	<i>Ascophyllum nodosum</i>	USA
Seamac 600	<i>Ascophyllum nodosum</i>	United Kingdom
SM-3 (Sea Magic)	<i>Laminariaceae</i> and <i>Fucaceae</i>	United Kingdom
Seasol / Agrikelp	<i>Durvillea potatorum</i>	Australia
Cytex	<i>Ecklonia maxima</i>	USA
Kelpak 66	<i>Ecklonia maxima</i>	South Africa
Seaspray	<i>Macrocystis integrifolia</i>	Canada
Algistim	seaweeds	United Kingdom
Seaborn	green seaweeds	United Kingdom

material is also of importance, as it is well known that many seaweeds undergo enzymatic degradation upon removal from the sea (Hornsey and Hide, 1974), which may affect the final product. Preservation of beneficial seaweed properties must be ensured to obtain an effective product. In the following the production processes of diverse commercial seaweed extracts and suspensions are outlined.

Alkaline hydrolysis

Maxicrop, is prepared from Norwegian *A. nodosum* by alkaline hydrolysis. The subsequent extract is then dried by film evaporation and distributed in a soluble powder form, which is re-dissolved (20 % w/v) shortly prior application (Sanderson and Jameson 1986; Sanderson *et al.* 1987). The alkaline extracts are formulated as **Maxicrop Original**, **Maxicrop Triple** or **Maxicrop Concentrate**, which contain approximately 8 %, 16 % or 24 % (w/v) seaweed solids, respectively. **Maxicrop**

Triple is further enriched with trace elements, including magnesium, iron, boron, zinc, and copper (Whapham *et al.* 1994).

Seasol Powder and Seasol Liquid, are seaweed concentrates manufactured from Tasmanian bull kelp, *Durvillea potatorum*, by alkaline hydrolysis at elevated temperature and pressure. It contains a large number of degradation products and additives (Tay *et al.* 1985). It is claimed to possess natural growth stimulants and soil conditioners. One litre **Seasol** concentrate is the equivalent of ~1.5 kg wet weight of *D. potatorum* (Tay *et al.* 1987).

Physical disintegration

Goëmill, is prepared from fresh *A. nodosum* seaweed which is quickly rinsed in water, and rapidly deep frozen at -25°C prior to cryo-micro-crushing. After various other procedures, including homogenisation and micro-partition, a liquid seaweed extract with a creamy consistency is obtained, containing tiny particles of about 6-10 µm in diameter. Numerous compounds may be blended with the cream to produce various formulations. **Goëmill** is one of these (Pellegrini *et al.* 1987).

GYFA 17, is produced from *A. nodosum*. The seaweeds are washed, stored at -25°C, and then crushed into microparticles about 100 µm in diameter while maintaining the temperature at -50°C with liquid nitrogen. Cellulose and alginate are then removed by centrifugation and the extract clarified by ultrafiltration using 20,000 Dalton MW cut-off mineral membranes (Patier *et al.* 1993).

Kelpak 66 Seaweed Concentrate Growth Stimulant, is a seaweed foliar stimulant derived from the stipes of the South African seaweed, *E. maxima*, by a cell burst process. This process does not involve heat, chemicals or dehydration that could affect some of the organic components of the concentrate (Beckett and van Staden, 1990a). The seaweed is progressively reduced in particle size, and the particles pass

under extremely high pressure into a low pressure chamber where they disintegrate, resulting in the liquid extract (Nelson and van Staden, 1984b).

Seaspray, is produced from *Macrocystis integrifolia*, harvested in British Columbia, frozen to -70°C until further use. It is then modified by an X-press procedure used to process the kelp into a concentrate by grinding shear forces without pressure, resulting in the disintegration of plant cell walls, membranes and reducing particle size (Temple and Bomke, 1989).

1.5.2 Composition of diverse seaweed extracts and suspensions

1.5.2.1 Mineral composition

Table 1.5.2 shows results of published mineral analyses of different seaweed products. For obvious reasons the mineral content initially depends on the source material used, as the mineral composition differs between algae species (Table 1.3.3). Differences in the elemental concentration measured for different products were most obvious for trace elements (Table 1.5.2). The amount and composition of heavy metals, especially, may reflect differences in the location of seaweed harvest (Table 1.3.4), or the season and year of harvest.

Basically, most extracts were high in chlorine and sodium, reflecting the high concentrations in fresh algae material (Table 1.3.3). Some were further enriched with nitrogen and potassium, due to the manufacturing process. For example, **Seasol Liquid** had elevated levels of nitrogen and potassium due to the urea and potassium hydroxide used in the manufacture (Abetz, 1980). Generally, the extracts prepared by alkaline hydrolysis, such as **Seasol Liquid**, exhibit a high pH, >9.0 (Abetz, 1980). Finally, the degree of concentration achieved during manufacture further increased the density of compounds in the product, as well as the addition of minerals or trace elements to the final product.

Table 1.5.2: Mineral composition and pH of diverse seaweed extracts.

Product	Algifert	Kelpak 66	Maxicrop dry meal	Maxicrop Triple	Maxicrop	Seasol Liquid
Reference	Verkleij (1992)	Metting <i>et al.</i> (1990)	Chapman (1970)	Abetz (1980)	Verkleij (1992)	Abetz (1980)
Preparation method	n.m.	physical disintegr.	alkaline hydrolysis	alkaline hydrolysis	alkaline hydrolysis	alkaline hydrolysis
Source material	brown seaweed	<i>Ecklonia maxima</i>	<i>Ascophyllum nodosum</i>			<i>Durvillea potatorum</i>
pH	n.d.	n.d.	n.d.	n.d.	n.d.	9.0-10.5
dry matter (%)	n.d.	n.d.	94.8	n.d.	8.0-8.2	>14.5
Element	g kg ⁻¹ dm	g l ⁻¹	g kg ⁻¹ ash	g l ⁻¹	g kg ⁻¹ dm	g l ⁻¹
Calcium	11.9	0.8	4.4	0.5	3.5	2.0
Chlorine	55.4	n.d.	67.0	n.d.	67.0	6.7
Magnesium	10.6	0.2	5.8	1.4	3.5	1.6
Nitrogen	8.7	3.6	7.2	3.5	7.2	18.0*
Phosphorus	1.4	8.2	20.0	1.2	9.0	1.8
Potassium	19.0	7.2	30.0	5.5	26.0	25.5*
Sodium	19.4	0.8	189.0	n.d.	70.0	0.48
Sulphur	49.6	0.6	61.0	1.4	23.0	1.4
	ppm	ppm	ppm	ppm	ppm	ppm
Aluminium	20.0	n.d.	2300.0	n.d.	60.0	n.d.
Boron	50.0	0.2	1.0	11.0	1.0	0.5
Bromine	0.6	n.d.	n.d.	n.d.	800.0	n.d.
Cobalt	2.0	0.3	4.0	14.0	4.0	n.d.
Copper	0.5	0.2	40.0	50.0	40.0	54.0
Iodine	200.0	8.6	9000.0	2.5	900.0	n.d.
Iron	60.0	13.6	3400.0	1100.0	2200.0	24.0
Manganese	24.0	8.4	40.0	44.0	40.0	3.0
Molybdenum	0.6	0.4	10.0	14.0	10.0	3.0
Nickel	5.0	0.3	24.0	n.d.	24.0	n.d.
Zinc	33.0	4.2	100.0	180.0	100.0	15.0

(n.d. - not determined; * - higher contents due to urea and potassium hydroxide used in the manufacture)

1.5.2.2 Growth regulating substances

Growth regulating substances have been identified in algae, and thus, much effort has focused on the identification of these substances in the commercial algal products. However, the detection of these algal metabolites created some problems when using unpurified samples, as inhibitors present in the extracts may prevent correct quantification in bioassays (Blunden, 1977; Temple and Bomke, 1989).

Therefore, most estimates are based on purified samples. The lack, or low concentration, of some growth regulating substances in certain algal products, despite their detection in the source material might be explained by a low concentration of the final extract, harsh extraction conditions, their instability in aqueous solutions (e.g. auxins), or instability over time (e.g. gibberellins) (Williams *et al.* 1981). A comprehensive review of growth regulating substances in algae and algae products has been compiled recently by Crouch and van Staden (1993a).

Abscissic acid

High amounts of abscissic acid (20 mg per gram dried extract) were measured in **Maxicrop** by GLC, but Kingman and Moore (1982) failed to identify the compound as ABA by GC-MS, as it was shown that compounds other than ABA present in algae co-elute on GLC with methyl ABA (Niemann and Dörffling, 1980). The unequivocal identification of ABA by GC-MS and a more conservative estimation of 0.10-0.46 $\mu\text{g ABA g}^{-1}$ dry weight (equal to 0.03-0.14 $\mu\text{g ml}^{-1}$ extract) in another extract, **Nitrozyme**[™] based on the same algal source material, was established by Boyer and Dougherty (1988).

Auxins

Some effects elicited by seaweed extracts have lead to analyses of several seaweed products for auxins, as it has been shown to be present in marine algae (Jacobs *et al.* 1985). Early work by Williams *et al.* (1981) using a bioassay failed to detect auxins in three seaweed extracts, but in 1982, Kingman and Moore detected IAA (50 mg g⁻¹ dry extract) in **Maxicrop** using GLC. Sanderson and Jameson (1986) identified IAA by GC-MS and established a lower concentration of IAA in **Maxicrop** of 6.63 $\mu\text{g g}^{-1}$ dry extract ($\sim 1.3 \mu\text{g ml}^{-1}$ freshly prepared suspension), after the application of [1-¹⁴C] IAA internal standards to enable correction for extraction losses (Sanderson *et al.*

1987). Crouch and van Staden (1991a) identified a rooting factor in **Kelpak** in a mungbean root bioassay, and later the presence of IAA, indole-3-carboxylic acid (ICA), N,N,-dimethyltryptamine, indole-3-aldehyde (IAld), and iso-indole,1,3-dione (N-hydroxyethyl phthalimide) in the extract was confirmed using GC-MS (Crouch *et al.* 1992).

Cytokinins

Cytokinin-like activity was first detected in **SM-3** using an *in vitro* callus growth assay with a cytokinin dependent strain of *Atropa belladonna*, and a leaf disc bioassay (Brain *et al.* 1973). Since then the cytokinin-activity of **SM-3**, **Maxicrop** and **Marinure** was estimated to be around between 15-250 mg l⁻¹, 25-200 mg l⁻¹, and 10-50 mg l⁻¹ by bioassay methods (Blunden and Wildgoose, 1977; Williams *et al.* 1981). In 1982, Kingman and Moore detected several cytokinin-like substances using TLC and GLC in **Maxicrop**. In **Kelpak** several other cytokinin-like substances were found using bioassay and paper chromatographic techniques, and zeatin and ribosyl-zeatin was estimated at 25.8 µg kg⁻¹ fresh weight (Featonby-Smith and van Staden, 1983a; Finnie and van Staden, 1985). A cytokinin-like activity in a soybean callus bioassay of 84-1680 µg l⁻¹ was found in **SeaSpray**. The huge variation in cytokinin activity of kelp samples in the bioassay was explained by the authors with the presence of a callus growth antagonist in the extract, whose inhibition disappeared disproportional to the growth promoter upon dilution of the extract (Temple *et al.* 1989). In another seaweed extract, **Seasol**, several cytokinins were identified by GC-MS (Tay *et al.* 1985; 1987).

Gibberellins

Although the presence of gibberellin-like substances in algae is documented, their occurrence in seaweed extract has been analysed only by a few workers. Williams *et*

al. (1981) detected gibberellin acid equivalents of 0.03, 2.20 and 18.40 mg l⁻¹ in freshly prepared **Marinure**, **SM-3** and **Maxicrop** respectively, using a lettuce hypocotyl elongation test. However, after 2 and 4 months storage the gibberellin activity in **Maxicrop** and **SM-3** was negligible.

Ethylene

The precursor of ethylene, 1-amino-cyclopropane-1-carboxylic acid (ACC) has been detected in **Kelpak** by TLC and gas-chromatography (GC). The level of the ethylene-releasing compounds was found to be 9.29 µmol l⁻¹ (Nelson and van Staden, 1985).

Betaines

Several betaines have been identified in seaweed extracts. Blunden *et al.* (1986) analysed four seaweed preparations by ¹H-NMR spectroscopy to their betaine content and measured glycinebetaine, γ-aminobutyric acid betaine and δ-aminovaleric acid betaine and found average levels of 25.2, 21.3 and 10.1 mg l⁻¹ in **SM-3**, 4.6, 11.1 and 6.6 mg l⁻¹ in **Seamac**, 7.8, 8.2 and 3.7 mg l⁻¹ in **Maxicrop**, and 16.9, 24.1 and 10.1 mg l⁻¹ in **Alginex**. They further identified laminine, lysinebetaine and ascophylline in the extracts. In a recent paper applying an off-line overpressured layer chromatographic procedure Tyihák *et al.* (1994) detected higher average levels of glycinebetaine, γ-aminobutyric acid betaine, and δ-aminovaleric acid betaine of 14.6, 39.0 and 20.4 mg l⁻¹ in **Maxicrop**. They further quantified laminine, carnitine, and trigonelline, with average levels of 1.2, 2.0, and 3.6 mg l⁻¹. The fact that the authors found comparable levels using ¹H-NMR spectroscopy to previous reported betaine levels, indicates again the problem of comparing results obtained using different techniques.

1.6 The application of seaweed extracts and suspensions

In commercial agricultural and horticultural practices seaweed extracts are mainly applied as foliar sprays to the growing crops with ordinary field nozzle sprayers. This is based on experimental work on the application and effectiveness of seaweed extracts on plant growth in field situations. However, in some cases, especially in horticultural crops, plants received soil flushes with seaweeds extracts, that resulted in similar effects (Featonby-Smith and van Staden, 1983b, 1984b; Nelson and van Staden, 1986; Beckett and van Staden, 1989). Nelson and van Staden (1984a), and Aldworth and van Staden (1987) tested the effectiveness of seaweed extracts as a root dip prior planting to alleviate transplanting shocks in diverse plant species, and obtained larger, earlier flowering plants. In other cases seed trays (Button and Noyes, 1964), or seeds have been treated directly by dipping or soaking in seaweed extracts, to minimise the extract volume needed (Wilczek and Ng, 1982; Miers and Perry, 1986; Nelson and van Staden, 1986; Taylor *et al.* 1990). The incorporation of seaweed extracts into carrier-gels for fluid drilling has been tested by Minero-Amador and Stewart (1987, 1994).

1.7 The effects of seaweed extracts and suspensions on plants

Numerous papers on the application and effects of seaweed extracts on plant developmental processes and disease development have been published following the availability of seaweed extracts. They have been reviewed periodically over the years (Abetz, 1980; Metting *et al.* 1990; Verkleij, 1992). In the past decade more scientific publications have been added to the literature, compared to earlier published advertising papers, with insufficient proof for manufacturers claims. The results of scientific experiments are sometimes contradictory and depended on the plants treated and extracts used.

1.7.1 Germination and seedling establishment

The application of diverse seaweed extracts to seeds to enhance germination, has been studied by only a few researchers. Button and Noyes (1964), investigated the seedling establishment of creeping red fescue (*Festuca rubra*). They flushed the germination media (sand with no addition of nutrients), with a seaweed preparation, **SM-3** and obtained enhanced seedling emergence over the control with 0.5 % and 1 % extract concentration. They also found, that the growth rate was faster for about one week for the seedlings. In a similar experiment using clover seeds sown in different soils or on filter paper, no difference in germination percentage and time was observed between treatments with or without a seaweed extract (Goh, 1971). Wilczek and Ng (1982) conducted a germination experiment in petri-dishes, using beetroot (*Beta vulgaris*) seeds, soaked for 12 hours in a 1 % (v/v) solution of **Cytex** (containing the equivalent of 10 mg l⁻¹ kinetin). They tested the germination of treated seeds without re-drying at temperatures between 10°C and 30°C. Treated seeds had higher germination to seeds soaked in water at all temperatures tested. The promotional effect of seaweed extracts on seed germination was attributed to the presence of cytokinin-like substances. Nelson and van Staden (1986) analysing data acquired from growth chamber experiments in which wheat (*T. aestivum*) seeds were soaked for 1 hour in 1 % **Kelpak** solution prior to planting. They reported less pronounced differences in yield increase between the control and seed treatments compared to soil drenches spread over a period of time. However, Miers and Perry (1986) found no significant increase in grain yield in several replicated field trials over a period of three years in Australia, when **Seasol Powder** or **Liquid** was applied to wheat (*T. aestivum*) seeds at various concentrations. The same result was recorded by Taylor *et al.* (1990) who compared yield and other parameters of barley (*Hordeum vulgare*) plants in field trials over two years. They treated seeds either with kinetin, or **Nitrozyme**[™], a seaweed preparation containing 0.04 % kinetin

equivalents (according to the manufacturers label). As a seed treatment, the extract was applied diluted (1:6; v/v) in water, at a rate of 1.3 litre t⁻¹. In neither case did the seaweed extract increase crop yield.

The effects of incorporation of **Cytex** into a carrier gel used for fluid drilling was investigated by Minero-Amador and Stewart (1987). This significantly reduced the pH and lowered the osmotic potential of the carrier gels. The incorporation of the seaweed extract reduced the emergence and establishment of pre-germinated tomato (*Lycopersicon lycopersicum*) seeds from soil, and also the number of marketable fruits (Minero-Amador and Stewart, 1994).

1.7.2 Plant growth and development

Plant metabolism

Analyses on the alkaloid production of a plantation of *Duboisia* hybrids sprayed with **Maxicrop** over 15 months revealed a change in the metabolism (Luanratana and Griffin, 1982). The usual hyoscine alkaloid drop between May and September did not occur in seaweed treated plants.

Physiological and metabolic changes in plants associated with tolerance to seaweed extract application to leaves have been studied by Pellegrini *et al.* (1987). In microscopic studies no major cell structural aberrations were found, thus indicating a non-toxicity of **Goëmill**. Various metabolic changes have also been reported after the application of the extract, such as enzyme conversions by the disappearance of starch granules, and observed accumulated osmophilic material within the vacuoles of cortical cells were suggested to be of phenolic nature. In a later paper, Patier *et al.* (1993) reported the effects of major components of the seaweed extract **GYFA 17** on raspberry (*Rubus fruticosus*) suspended-cell cultures. Several derivatives of laminaran, branched β -D-(1 $\rightarrow$ 3) glucans, were identified and were capable of eliciting laminaranase, and α -amylase activity in the cell cultures.

Phenol production as well as α -amylase activity have been associated with defence-related stimuli (Bowles, 1990).

Plant growth

Enhanced plant growth is probably the most frequently reported effect elicited after the application of seaweed extracts, and numerous papers have been published. In the following, a brief summary of results is given to illustrate the varying effects and the range of species treated.

In *in vitro* experiments, **Kelpak** incorporation into the culture media resulted in increased root growth of tomatoes (Finnie and van Staden, 1985). As ashing of the seaweed extract, but not autoclaving, resulted in loss of the stimulatory effect, the compound responsible was of organic nature. In another *in vitro* experiment the number of axillary shoots in potato (*Solanum tuberosum*) increased after the application of the same product (Crouch and van Staden, 1991b).

Root and shoot growth of hydroponically cultivated barley (*H. vulgare*) was found to be enhanced with **Maxicrop Triple** incorporated in the medium, whereas a trace element control solution was less effective. Spray treatments of both treatments showed a similar effect, but of a lower intensity (Steveni *et al.* 1992).

Plant growth under controlled conditions in phytotrons, has been shown to be enhanced by seaweed extracts. Wheat (*T. aestivum*) plants showed increased culm diameter after foliar **Kelpak** application (Nelson and van Staden, 1984b), as well as higher dry mass, grain size, number of grains, and grain nitrogen increased with increasing application of **Kelpak** (Nelson and van Staden, 1986). Jeannin *et al.* (1991) recorded increased root and stem fresh and dry weight in maize (*Zea mays*) seedlings, elicited by **Goëmar GA 14**. They did not, however, find differences in the gas exchange characteristics, transpiration rate and stomatal resistance. They concluded that the increased mass accumulation observed was not due to a

stimulation of the net carbon assimilation rate per unit of leaf area, but rather to an increased sink capacity of root and shoot. The causal factor in the seaweed extract had not been elucidated. Potato (*S. tuberosum*) plants, however, were not positively affected by **Kelpak** treatments (Kuisma, 1989).

Under optimal greenhouse conditions, growth of Swiss chard (Swiss mangel, *B. vulgaris* var. *flavescens*) and common bean (*Phaseolus vulgaris*) plants was enhanced by foliar **Kelpak** treatments, measured as increased dry mass production, but not the leaf area. A synergistic fertiliser effect was further found (Featonby-Smith and van Staden, 1983b; 1984b). However, only the leaf growth of Swiss chard was promoted after the seaweed extract was applied alone.

Under field conditions foliar applied **SM-3** increased tuber yield in one potato (*S. tuberosum*) variety, similar to a kinetin control. However, the response appeared to be variety specific, as a second variety showed no treatment response (Blunden and Wildgoose, 1977). This seaweed extract also increased root weight, root sugar content, and reduced amino-nitrogen and potassium content of the juice of sugar beet (*B. vulgaris* var. *altissima*) (Blunden *et al.* 1979). **Maxicrop** treatments reduced the number of plants failing to form hearts, and increased the weight of marketable lettuce (*Lactuca sativa*), and resulted in cauliflower (*Brassica oleracea* var. *botrytis*) with increased curd diameter (Abetz and Young, 1983). However, the application of **Seamac 600** to field grown onion (*Allium cepa*), immediately after commencement of bulbing had no effect on size, yield or maturity, contrary to manufacturer claims of yield improvement in this crop (McGeary and Birkenhead, 1984).

The application of seaweed extracts is not restricted to short season horticultural or agricultural crops alone. Longleaf pine (*Pinus palustris*) growth was found to be promoted synergistically by **Cytex** in combination with gibberellin acid (GA), and DPX (the triethanolamine salt of 3-(*p*-chlorophenyl)-6-methoxy-*s*-triazine-2,4(1H,3H)dione) (Hare, 1984).

Leaf chlorophyll content

The chlorophyll concentration of leaves of Swiss chard (*B. vulgaris* var. *flavescens*) and common bean (*P. vulgaris*) plants increased with **Kelpak** treatments by 25 % and 40 %, respectively, and an additive effect in combination with a fertiliser was found (Featonby-Smith and van Staden, 1983b, 1984b). Other reports also indicated increased chlorophyll accumulation after the application of seaweed extracts.

Whapham *et al.* (1992) treated tomato (*L. lycopersicum*) plants with either a foliar application or a root drench of **Maxicrop Triple**, and detected 20-50 % higher chlorophyll levels compared to untreated plants some 34 days later. Initially they attributed this effect to the cytokinins present in the extract. In a later work by Whapham *et al.* (1993) an increase in chlorophyll content in a bioassay was attributed to betaines, shown to be present in the extract (Blunden *et al.* 1984; 1986), as the cytokinin content of the extract was considered to be low (Blunden, 1991).

Nutrient uptake

Studies on the effects of seaweed extracts on nutrient uptake were initiated by the suggestion that the chelating property of the extracts should facilitate trace element uptake. The effects of the seaweed extracts **Kelpak** on the nutrient uptake of citrus (*C. lemon*) trees was investigated by Beckett and van Staden (1988). They found no enhanced uptake of foliar applied zinc after the addition of seaweed extract. In a further study Beckett and van Staden (1990a) investigated the effects of foliar applied **Kelpak** with and without copper, manganese, zinc, on the trace element uptake of tomato (*L. lycopersicum*) plants. The extract had little effect on the uptake of copper and manganese, but stimulated zinc uptake significantly in plants receiving additional macronutrients. They concluded that seaweed concentrates were unlikely to be useful in promoting the uptake of foliar-applied trace elements by the plants when grown under optimum conditions.

Alleviation of stress conditions

Several research groups investigated on the manufacturers claims of alleviation of adverse effects of stress conditions on plant development by seaweed extracts.

Cucumber (*Cucumis sativus*) seedlings grown in greenhouses under nutrient stress conditions were treated with root dips or foliar sprays of **Kelpak** after transplanting. Treated plants accumulated higher dry weight mass over the next ten weeks (Nelson and van Staden, 1984a). In a greenhouse pot experiment common bean (*P. vulgaris*) plants grown at different moisture regimes were sprayed with **Kelpak** and an experimental kelp extract, **Seaspray** (Temple *et al.* 1989), but the results obtained, were not conclusive.

Plant yield

Controversial and conflicting results have been observed for yield increase following seaweed extract treatment. Such studies have usually been achieved under controlled growth conditions or in artificial stress situations. According to Abetz (1980) yield increases after the use of seaweed extracts have often been observed in the field, particularly when adverse growth conditions have prevailed.

In growth chamber experiments under optimum conditions Featonby-Smith and van Staden (1987a) obtained higher yields in barley (*H. vulgare*) crops, irrespective the type of **Kelpak** application. The yield increase was attributed to an increase in number of fertile spikelets per ear. However, under normal field conditions Taylor *et al.* (1990) did not observe increased growth rates or yields of crops of barley (*H. vulgare*) treated with **Nitrozyme**TM. In field trials with peanuts (*Arachis hypogaea*), **Cytex** was applied over three seasons (Ketring and Schubert, 1981). In only one season a yield increase was observed, whereas Featonby-Smith and van Staden (1987b) obtained twofold yield increases in the same crop with **Kelpak** in a pot experiment. Under field conditions **Seaspray** and **Kelpak** were

tested in trials of common bean (*P. vulgaris*), and showed yield increases of 25 % (Temple and Bomke, 1989). Crouch and van Staden (1992), on the contrary, applied **Kelpak** as soil drench or foliar spray to tomato (*L. lycopersicum*) plants, and obtained inconsistent results. Under artificial drought conditions in a greenhouse experiment Mooney and van Staden (1985) obtained improved growth and up to 40 % higher yields of barley (*T. aestivum*) plants treated with **Kelpak**. Beckett and van Staden (1989, 1990b) did observe improved yields of nutrient or potassium stressed wheat (*T. aestivum*) plants treated with **Kelpak**, but not of plants receiving adequate K⁺, or for plants exposed to salt stress (Beckett and van Staden, 1991). As ashed extract had a similar effect, they concluded that the mineral content in **Kelpak** was mainly responsible for the effect on nutrient stressed plants.

1.7.3 Disease and pest development

The effect of foliar sprays of **SM-3** on lettuce (*L. sativa*) on the feeding preference of slugs was investigated by Pakarinen *et al.* (1990), but no consistent effect on the feeding behaviour of *Deroceras agreste* could be established.

Hankins and Hockey (1990) investigated the effect of **Maxicrop Triple** on the population dynamics of the two-spotted red spider mite (*Tetranychus urticae*). They found that seven applications over seven days significantly suppressed the population build-up on treated strawberry (*Fragaria x magna*) plants.

Soil flushes with seaweed extracts have been tested for the control of nematodes by several workers. Featonby-Smith and van Staden (1983a) achieved a 40 % reduction of root-knot nematodes (*Meloidogyne incognita*), in roots of tomato (*L. lycopersicum*) plants, but a 3.5 fold increase in the soil, when **Kelpak** soil flushes were applied. However, foliar application was not effective (Crouch and van Staden, 1993b). De Waele *et al.* (1988) found a 47-63 % reduction in the reproduction of root-lesion nematodes (*Pratylenchus zeae*) on excised maize (*Z. mays*) roots grown

in vitro, but no effect in a greenhouse experiment, when **Kelpak** was applied directly to the soil. In a recent paper, Whapham *et al.* (1994) reported a 42 % reduction in the reproductive capacity of root-knot nematodes (*Meloidogyne javanica*) on tomato (*L. lycopersicum*) plants, when four weekly soil flushes with diverse **Maxicrop** products were applied.

1.8 Seed priming

Although seaweed extracts have been applied to seeds (Section 1.7.1), only one paper reported on the application of a seaweed extract as a priming treatment (Wilczek and Ng, 1982). In this study, seaweed preparations were tested for their effect on seedling growth, as well as their effectiveness as priming media. For this reason a review on various aspects of seed priming is given in the following sections.

High quality seeds assure a high germination percentage and a rapid seedling establishment. Seed vigour, as one factor in seed quality, is the concept that describes the ability of seeds to establish in the field under adverse conditions. A broad definition was adopted by the ISTA Congress in 1977 (Perry, 1978): '*Seed vigour is the sum total of those properties of the seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence. Seeds which perform well are termed high vigour seeds, and those which perform poorly are called low vigour seeds*'.

Frequently it has been shown that seed vigour can be improved by pre-sowing priming treatments, observed by increased seed germination performance and improved seedling establishment.

1.8.1 Priming techniques

The improvement of seed germination has been attempted through various pre-sowing seed treatments, such as solid matrix priming (SMP) (Taylor *et al.* 1988 ; Rush, 1991; Hardegree and Emmerich, 1992; Aschermann-Koch *et al.* 1992), moisture pre-equilibration on wet filter paper in moisture saturated chambers (Perl and Feder, 1981; Pandey, 1988), plant growth regulator infusion (Persson, 1993), or soakings (Thomas, 1984; Taneja *et al.* 1992; Nayyar *et al.* 1995), or various osmotic 'priming' techniques (osmo-conditioning) in various solutions.

Seed priming has been tested by seed soaking in a range of different solutions including distilled water. This technique was termed 'hydro-priming' (Fujikura *et al.* 1993). Other, osmotic solutions tested include a range of inorganic salts, such as sodium chloride (Rush, 1991), monopotassium phosphate, potassium nitrate, and calcium sulphate (Alvarado *et al.* 1987; Nayyar *et al.* 1995), or tripotassium phosphate (Wurr and Fellows, 1984). In some species, however, dilute salt solutions were found to be detrimental to the anticipated germination enhancement (Manohar, 1966; Brocklehurst and Dearman, 1984; Akers and Holley, 1986; Bliss *et al.* 1986; Taylor *et al.* 1988). This resulted in delayed germination, compared to the effect caused by mannitol (Poljakoff-Mayber *et al.* 1994), or other neutral non-electrolytes, such as glycerol or sucrose at similar osmolarity. Betaine and L-proline, were also found to be detrimental to carrot germination when used as osmotica in priming experiments (Gray *et al.* 1991). Salts, and osmotica of lower molecular size, are capable of entering seed tissue (Manohar, 1966) and thus may not have the desired priming effect.

Polyethylene glycol (PEG) is most frequently used for osmotic priming treatments, because it is inert and is not phytotoxic. PEG with a molecular weight greater than 4000 was found to be excluded from plant cell walls and thus cannot be taken up by the seed (Carpita *et al.* 1979; Brocklehurst and Dearman, 1984). It is

usual for PEG 6000 to be used for priming purposes, although it does have some disadvantages; it is highly viscous and has a low oxygen solubility (Mexal *et al.* 1975).

1.8.2 General requirements for priming treatments

General requirements of osmo-conditioning have been summarised by Khan *et al.* (1980/81). The osmotic potentials usually applied are between -0.5 MPa to -2.0 MPa, at temperatures between 10°C and 20°C. The treatment duration usually is 4 to 35 days. Seeds exposed for longer would need protection from microbial attack, or the proliferation of seed borne pathogens, and need ample supply of oxygen. Seed with phytochrome control of germination must be irradiated daily during osmo-conditioning to obtain best results.

1.8.3 Species range tested for priming

Priming techniques have been widely used to achieve invigoration of tree seeds (Donald and Jacobs, 1993), seeds of wildflowers (Tallowin *et al.* 1994), flower seeds (Finch-Savage, 1991; Finch-Savage *et al.* 1991; Finnerty *et al.* 1992; Fay *et al.* 1994), vegetable seeds (Haigh and Barlow, 1987; Pill, 1988; Owen and Pill, 1994; Seale *et al.* 1994) and seeds of agricultural crops (Bodsworth and Bewley, 1981; Dell'Aquila and Tritto, 1990; Murray *et al.* 1993; Zheng *et al.* 1995).

1.8.4 Beneficial effects of priming

Priming has been used to alleviate pathogen attack (Rush, 1991; Maude *et al.* 1992). Under optimum germination conditions the final germination percentage of primed seeds was not necessarily improved over control treatments (Alvarado, *et al.* 1987; Dell'Aquila and Tritto, 1990). In general, the mean germination time of treated

samples was found to be shorter, and the spread of germination time reduced (Bodsworth and Bewley, 1981; Pill, 1988; Gray *et al.* 1991). Further benefits of seed priming observed include a higher germination percentage at conditions of sub-optimal temperatures (Pill *et al.* 1991; Fujikura *et al.* 1993), or supra-optimal temperatures (Wurr and Fellows, 1984; Valdes *et al.* 1985; Carpenter and Boucher, 1991). Increased field emergence (Rao *et al.* 1987; Muhyaddin and Wiebe, 1989; Seale *et al.* 1994), even under saline conditions (Pill *et al.* 1991), or low temperatures (Murray, 1990; Zheng *et al.* 1995) was also observed. However, Murray *et al.* (1993), found improved field emergence time for primed sugar beet (*B. vulgaris* var. *altissima*) seeds in only one out of three years, and Alvarado *et al.* (1987) found no increase in tomato (*L. lycopersicum*) yield despite improved seedling emergence of primed seeds. On the contrary, Chatterjee and Singh (1983) reported of a 37 % yield increase for primed barley seeds in a field experiment.

If osmo-conditioned seeds are not re-dried after priming treatments, part of the priming effect may be due to a simple reduction of the imbibition and lag phase (Brocklehurst and Dearman, 1983), and consequently upon re-drying of primed seeds, part of the beneficial effect was found to be reduced (Pill *et al.* 1991; Gray *et al.* 1991).

Although primed seeds were found to be more vigorous when sown shortly after the treatment, controverse results for the storability of treated seeds has been published. While primed and re-dried leek (*Allium porrum*) seeds did not loose viability within 12 months of storage, primed carrot (*Daucus carota*) seeds lost viability over the same storage period (Dearman *et al.* 1987). Furthermore, lettuce (*L. sativa*) and tomato (*L. lycopersicum*) seeds, were found to be highly susceptible to deterioration in storage after priming treatments (Argerich *et al.* 1989; Tarquis and Bradford, 1992).

1.8.5 Mode of action of priming techniques

The enhancement of seed germination and seedling emergence after osmotic priming has been variously explained by the occurrence of metabolic repair processes (Burgass and Powell, 1984; Bray *et al.* 1989), amelioration of ageing induced membrane disruption (Pandey, 1988), advanced DNA and RNA synthesis and nuclear replication (Clarke and James, 1991; Bino *et al.* 1992; Lanteri *et al.* 1994), reduction of ribosomal RNA or DNA degradation (Dell'Aquila *et al.* 1978), a build up of germination metabolites (Khan *et al.* 1978; Coolbear *et al.* 1980), and increased protein and enzyme synthesis (Sen and Osborne, 1974; Khan *et al.* 1980/81; Smith and Cobb, 1992).

1.9 The germination characteristic of barley and lettuce

In the following sections an overview on the germination characteristics of barley and lettuce is given, as seeds of these species were used throughout the study.

1.9.1 Germination of barley (*Hordeum vulgare*)

Germination of mature non dormant barley (*H. vulgare*) seeds (caryopses) proceeds without the need of light over a range of temperatures (10° to 25°C). However, freshly harvested seeds can exhibit various degrees of dormancy, and would germinate eventually at lower temperatures (0° to 15°C) (Côme *et al.* 1988), but for practical reasons the dormancy is expressed as the percentage of viable seeds which failed to germinate in a given set of conditions by the end of a given period of time (Cochrane, 1993). The depth of the initial dormancy depends on various factors, such as cultivar, year and geographic location of harvest (Côme *et al.* 1984; Aufhammer, 1985) and temperature during grain development (Rauber, 1986; Cochrane, 1993). Seeds recover from dormancy during storage more quickly at

higher temperatures. The rate of dormancy loss in relation to temperature was quantified and models fitted for the controlled, rapid and safe removal of dormancy by heat treatments (Favier, and Woods, 1993; Briggs *et al.* 1994; Woods *et al.* 1994). Several chemical substances, or growth regulating substances have been tried successfully in breaking seed dormancy, including sulphuric acid and GA₃ (Don, 1979; Doran and Briggs, 1993a). The underlying biochemical mechanisms involved in the expression of dormancy, are still unclear, although the involvement of the pentose phosphate pathway has been suggested (Côme *et al.* 1988). Knowledge on this subject has been summarised and discussed in a review by Côme *et al.* (1988).

In the presence of excess water some barley (*H. vulgare*) seed lots failed to germinate properly. This was first documented in 1954 by Essery *et al.*, who found that in germination experiments seeds germinated best in 4 ml water per petri dish, but failed to germinate properly when 8 ml water per petri dish was present. This phenomenon, termed water-sensitivity, is measured as the difference between percentage germination in the two water regimes. Currently this test is usually applied to seed lots intended for malting. If this test would be applied to field sown seed lots, it may have significant relevance for crops sown in regions of high rainfall during the sowing period, like Scotland, where failure in proper field emergence and plant establishment due to water sensitive seed lots could result in yield losses.

Similar to dormancy the biochemical mechanisms of water sensitivity are yet to be elucidated. Many chemical treatments and physical manipulations have been applied to seeds to reduce water sensitivity. A comprehensive summary is given by Briggs (1978). However, the main factor in the phenomenon of water sensitivity seems to be the availability of oxygen to and the oxygen sensitivity of the embryo, as even very water sensitive seeds germinated readily under high oxygen tensions, as indicated in the hydrogen peroxide viability test (Thunaeus, 1938), although with exceptions (Briggs *et al.* 1994).

1.9.2 Germination of lettuce (*Lactuca sativa*)

The germination of lettuce (*L. sativa*) seeds (achenes) is sensitive to light and temperature. The interaction between temperature and red light requirement was found to be variety and seed batch dependent (Smith, 1975). In certain varieties, usually not light-requiring under normal conditions, prolonged exposure to far red light, even of dry seeds (Kendrick and Russell, 1975), or a high germination temperature during the imbibition period may induce dormancy and a subsequent requirement for light or red light (Small *et al.* 1979). The phytochrome system is a water-soluble chromoprotein pigment acting as a photoreceptor. This exists in two spectrophotometrically distinguishable and photoconvertible forms, P_r and P_{fr} . P_{fr} is the active form of phytochrome. Germinability in the dark is dependent on the level of P_r . Germination of dormant lettuce (*L. sativa*) seeds is prevented by high levels of P_r which can be converted to P_{fr} upon irradiation with red light. This process was found to be reversible (Borthwick *et al.* 1952; Bewley and Black, 1994). Priming and red light irradiation artificially increases the endogenous P_{fr} levels, and seeds may maintain dark germinability for up to a year (Vidaver and Hsiao, 1972).

Lettuce (*L. sativa*) seeds germinate readily over a range of temperatures, from as low as 5°C. At temperatures above 25°C, however, germination is inhibited (Borthwick and Robbins, 1928), and prolonged exposure at elevated temperatures can induce a secondary dormancy (thermo-dormancy), and seed fail to germinate under conditions previously supporting their germination (Khan, 1980/81). The temperature limits to induce thermo-inhibition was found to be cultivar dependent (Gray, 1975). The inhibiting effects of high temperatures operated only during the early hours of imbibition (Borthwick and Robbins, 1928), and soaking for 3 hours at 15°C has been used to enable seeds to partly escape thermo-inhibition (Guedes and Cantliffe, 1980). Others have used chemicals such as thiourea and other sulphur containing compounds (Reynolds, 1974), application of single growth regulators

(Dunlap and Morgan, 1977; Gray and Steckel, 1977), different plant growth regulators in combination (Reynolds and Thompson, 1971, 1973; Saini *et al.* 1986), or sterilants such as sodium hypochlorite (Drew and Brocklehurst, 1984a). The activity of sodium hypochlorite in the release of seeds from thermo-inhibition was found to be due to the chlorine content, and the resulting weakening of the pericarp was suggested to cause the effect (Drew and Brocklehurst (1984b). Drew and Brocklehurst (1984a) further reported that the ability of treated seeds to germinate at temperatures around 35°C lasted for at least 18 months.

Various priming treatments have been tested and proved successful at overcoming thermo-dormancy (Guedes and Cantliffe, 1980; Cantliffe *et al.* 1981; Wurr and Fellows, 1984; Valdes *et al.* 1985; Weges *et al.* 1991).

1.10 Aims and objectives of the current study

With increasing pressure for environmentally conscious farming practices, the application of organic material, such as seaweed products, as a pre-sowing treatment or fertiliser could be an alternative to chemical compounds. Seed treatments generally aim to secure yield by achieving high field emergence of healthy seedlings. In practice, a high germination rate and a rapid seedling establishment especially under adverse conditions is essential, for which good quality seed of high vigour is essential.

Seed priming treatments to improve seed quality, with or without the addition of growth regulating substances or agro-chemicals, have been used successfully in the past (Section 1.8). Unlike foliar application of seaweed products, the application of seaweed products to improve seed germination and vigour, however, has been tested only sporadically. In scientific journals contradictory results for improved performance and yield of seaweed treated seeds and plants have been reported.

The present study is aimed to provide knowledge about the potential benefit of seaweed products on seedlings when applied in early growth stages, or when used as seed priming treatments to stimulate seed germination and seedling establishment.

Two seaweed suspensions, obtained from whole brown algae *Ascophyllum nodosum* (Linnaeus) Le Jolis or lamina of *Laminaria hyperborea* (Gunn.) Foslie, were extensively evaluated for their effects on seed germination, and seedling growth of barley (*Hordeum vulgare*) and lettuce (*Lactuca sativa*).

The first part of the study deals with the analysis of the physical and biochemical properties of the seaweed products, to enable characterisation of the products, and to establish differences between the two products.

In the second part the effect on seedling growth and physiology was investigated, where pre-germinated seedlings were exposed directly to the seaweed suspensions. At this stage of development plants are very sensitive to applied substances, and the expression of promotive or adverse effects of the seaweed products is most likely to be observed. Detailed analysis was performed to identify active compound(s), if any, in both seaweed products.

In the third part of the study pre-sowing seed soaking treatments, using both seaweed products as priming media, were performed, to investigate their effect on seed germination under optimum and stress conditions.

Chapter 2

Materials and Methods

2.1 Materials

2.1.1 Seed material

Seed lots of barley (*Hordeum vulgare*) cv's Blenheim(A) and Triumph(A) were obtained from Dods of Haddington Ltd., Haddington, UK, and seed lots of cv's Blenheim(B), Halcyon, Pastoral, and Triumph(B) were obtained from Sharpes International Seeds Ltd., Lincoln, UK. The seed lots were certified 2nd generation (C2) seed, harvested in 1992 or 1993. Seeds were stored in a cold store at 5°C, ~40 % RH, in paper bags until required. They did not show any residual dormancy, with the exception of cv. Pastoral (69 % germination after 3 days, 88 % after 7 days).

Two seed lots of lettuce (*Lactuca sativa*) cv. Reskia (B and C), and one of the cv's Columbus and Great Lakes were obtained from Wm.K. McNair, Edinburgh, UK. Seed was stored in a cold store at 5°C, ~40 % RH, in sealed paper / foil / aluminium composite bags.

All seed lots were tested for viability and germination. All complied with EC regulations. Prior to the experiments seed sub-lots were graded, and small or damaged seeds removed.

2.1.2 Seaweed suspensions

The seaweed products used in all experiments were produced by the Orkney Water Test Centre, Flotta, Stromness, Orkney Islands, UK: The source material was harvested around the northern area of the Orkney Islands; 'knobbed wrack', *Ascophyllum nodosum* (Linnaeus) Le Jolis (ANS) in the intertidal region, whereas 'tangle', *Laminaria hyperborea* (Gunn) Foslie (LHS) was harvested from depths of around 2-4 m. The seaweed suspensions have been prepared by a physical disintegration method, without the application of heat, acid or alkaline hydrolysis, gradually reducing the particle size. One batch of each seaweed suspension (SWS) was used throughout all experiments, stored frozen at -20°C in aliquots of 500 ml.

2.1.3 Chemicals

Table 2.1.1: List of chemicals used.

Chemical	Chemical formula	MW	Cat No.	Supplier*
acetone	CH ₃ COCH ₃	58.08	RG2001	Rathburn
amphotericin B	C ₄₇ H ₇₃ NO ₁₇	924.10	A 9528	Sigma
bovine serum albumin	standard solution	-	-	Pierce
calcium chloride	CaCl ₂	110.99	27587	BDH
calcium hypochlorite	Ca(OCl) ₂	142.99	21.138-9	Aldrich
gibberellic acid (GA ₃)	C ₁₉ H ₂₂ O ₆	346.40	G 3250	Sigma
hydrochloric acid	HCl	36.46	UN1789	BDH
hydrogen peroxide	H ₂ O ₂	34.01	UN2014	BDH
indole-3-acetic acid (IAA)	C ₁₀ H ₉ NO ₂	175.20	I 1250	Sigma
kinetin (6-furfurylaminopurine)	C ₁₀ H ₉ N ₅ O	215.20	K 3378	Sigma
magnesium chloride	MgCl ₂	95.22	26123	BDH
mannitol	C ₆ H ₁₄ O ₆	182.20	M 4125	Sigma
nutrient agar (NA)	-	-	CM 3	OXOID
nystatin	C ₄₇ H ₇₅ NO ₁₇	926.10	N 3503	Sigma
penicillin G (benzylpenicillin)	C ₁₆ H ₁₇ N ₂ O ₄ SNa	356.40	Pen-NA	Sigma
phloroglucinol (1,3,5, trihydroxybenzene)	C ₆ H ₆ O ₃	126.10	P 3502	Sigma
phosphomolybdic acid	12MoO ₃ •H ₃ PO ₄ •H ₂ O	3939.50	P 7390	Sigma
phosphoric acid	H ₃ PO ₄	98.00	29421	BDH
polyethylene glycol 8000	-	~8,000	P 2139	Sigma
polymyxin B sulphate	-	-	P 1004	Sigma
potassium chloride	KCl	74.55	10198	BDH
potato dextrose agar (PDA)	-	-	CM 139	OXOID
Ringer solution	-	-	BR 52	OXOID
sodium carbonate (anhydrous)	Na ₂ CO ₃	106.00	S 2127	Sigma
sodium carbonate (monohydrous)	Na ₂ CO ₃ •H ₂ O	124.00	S 4132	Sigma
sodium chloride	NaCl	58.44	10241	BDH
sodium hydroxide	NaOH	40.00	1823	Fisons
sodium hypochlorite	NaOCl	74.44	UN1791	Anderson
sodium nitrate	NaNO ₃	84.99	30187	BDH
sodium tungstate	Na ₂ WO ₄ •2H ₂ O	329.90	S 0765	Sigma
streptomycin sulphate	-	1457.40	S 6501	Sigma

(* - Aldrich Chemical Co., Dorset, UK; Anderson Gibb and Wilson, Edinburgh, UK; BDH Chemicals Ltd. Poole, UK; OXOID - Unipath Ltd., Basingstoke, Hampshire, UK; Pierce Europe, oud-Beijerland, Holland; Rathburn Chem. Ltd., Walkerburn, UK; Sigma Chemical Ltd., Dorset, UK)

2.1.4 Osmotic solutions

For the preparation of solutions of osmotic potentials (OP) of -0.25, -0.50 or -0.65 MPa, addition to distilled water of osmotica listed in Table 2.1.2 were made. Initial dose experiments with all osmotica allowed the calculation of the amount of each chemical needed for the individual OP levels. The OP of the osmotic solutions was checked with a Wescor 5100C vapour pressure osmometer (Wescor Inc., Logan, USA), using discs of Whatman No. 52, GF/C (glass fibre), or lens tissue, to check the concerns expressed by Hardegree and Emmerich (1990). No significant differences were found between OP measurements of the solutions using these filter paper discs, providing the calibration of the osmometer was done with the same filter type.

Table 2.1.2: The amounts of solutes added to obtain desired osmotic potentials of applied osmotica.

Osmotica	MW	Osmotic potential (MPa)		
		-0.25	-0.50	-0.65
		g l ⁻¹		
NaCl	58.44	3.56	6.36	8.41
KCl	74.55	4.67	8.18	10.76
Mannitol	182.20	21.91	38.59	50.85
PEG 8000	~8,000.00	143.37	212.90	264.05

2.1.5 Phloroglucinol solutions

Phloroglucinol (1,3,5, trihydroxybenzene) was dissolved in distilled water, and diluted to concentrations listed in Table 2.1.3 .

Table 2.1.3: The phloroglucinol concentration applied, and the equivalent SWS concentrations.

Phloroglucinol concentration applied (µg ml ⁻¹)	SWS Equivalent	
	ANS Original (%)	LHS Original (%)
5.6	0.1	1.4
22.5	0.4	5.6
90.0	1.6	22.6
360.0	6.3	90.0
1440.0	25.0	-
5760.0	100.0	-

2.1.6 Mineral solutions

Solutions of a number of different minerals, abundantly present in the two different SWS, were prepared, to allow the evaluation of possible nutritional effects of SWS on seedling growth. Solutions of calcium (as CaCl_2), potassium (as KCl), magnesium (as MgCl_2), and nitrogen (as NaNO_3) were prepared at concentrations ranging from 0.63 % to 100 % of the amounts of these minerals present in the LHS (200 % in the case of Mg^{2+}) (Table 2.1.4).

Table 2.1.4: The concentration of diverse minerals applied, and the equivalent SWS concentrations.

Mineral solution applied ($\mu\text{g ml}^{-1}$)	SWS Equivalent	
	ANS Original	LHS Original
Ca^{2+}	(%)	(%)
5	1.0	0.9
50	10.4	8.9
100	20.8	17.9
200	41.7	35.7
300	62.5	53.5
600	125.0	107.0
N		
10	2.6	1.3
50	13.1	6.3
100	26.3	12.5
200	52.5	25.0
400	105.0	50.0
800	210.0	100.0
Mg^{2+}		
5	1.4	2.8
20	5.7	11.1
45	12.9	25.0
90	25.8	50.0
180	51.5	100.0
360	103.0	200.0
K^{+}		
10	1.2	0.6
100	12.4	6.3
200	24.8	12.5
400	49.5	25.0
800	99.0	50.0
1600	198.0	100.0

2.2 General methods

2.2.1 Preparation of original and modified seaweed suspensions

Original SWS

Aliquots of SWS were thawed shortly before use. Prior the use or dilution, both SWS were homogenised for 10 seconds at 5°C to disintegrate aggregated cell wall material that appeared after thawing. Dilutions of SWS were prepared with distilled water to various concentrations depending on the experiment.

SWS supernatant

The preparation of this fraction was achieved by centrifugation of original SWS for 1 hour at 12,000g at 5°C. The supernatant was decanted and regarded as the 100 % concentration and not adjusted to the initial volume. However, the concentration of dissolved particles in the aqueous phase was identical to that of original SWS, and the cell wall debris was regarded as solid material not contributing to the concentration of dissolved substances in the aqueous solution immediately after thawing. This method was chosen to avoid the introduction of a dilution factor, and to allow a comparison between original SWS (aqueous plus solid fraction) and the supernatant fraction (aqueous fraction) at identical compound(s) concentration in the liquid phase. A re-adjustment of the supernatant to the original volume would introduce a dilution factor of 1.2 (LHS) or 1.3 (ANS). The comparable concentration to 10 % original LHS or 10 % re-adjusted LHS supernatant would be 8.2 % not re-adjusted LHS supernatant.

SWS solid fraction

The residual solids obtained after centrifugation were re-suspended in the same amount of distilled water as had been removed. This fraction was regarded as the 100 % SWS solid fraction. However, this meant in effect that a dilution had occurred, that may have affected the activity of this fraction in experiments. This was borne in mind, when results were interpreted.

Ashed SWS

Ashed SWS was prepared by drying the SWS at 105°C for 16 hours, ashing at 500°C for 5 hours, and re-dissolving of the residual ash in the minimum of 1M hydrochloric acid, with a final dilution with distilled water to the appropriate concentrations.

Before application the solution was filtered through Whatman No1 filter paper.

The pH of all seaweed preparations was adjusted to 5.6 or 7.4, according to the values of unaltered original ANS or LHS, respectively.

2.2.2 Determination of initial seed moisture content

The seed moisture content of barley and lettuce seeds was determined by the hot oven method (ISTA, 1976), at 130°C for 1 hour, and expressed as percentage of the dry weight. Prior to drying, the barley seeds were crushed.

2.2.3 Calculation of seed moisture and uptake rate during imbibition

The seed moisture content during imbibition or priming was determined as weight difference compared to the initial sample weight, considering the initial seed moisture content, and calculated as seed moisture content on the dry weight basis.

The seed moisture uptake rates were calculated from the weight gain over the initial sample weight, divided by the time elapsed from the beginning of the imbibition treatment.

2.2.4 Standard germination test

The germination of barley and lettuce cultivars was tested according to recommended ISTA conditions (ISTA, 1976).

Seeds were placed in 9 cm petri-dishes, on top of 2 layers Whatman No. 181 filter paper, wetted with 5 ml distilled water. The dishes were kept at 20°C in the

dark. Usually, one hundred seeds of barley and fifty seeds of lettuce were placed per petri-dish, unless otherwise mentioned elsewhere.

2.2.5 Determination of seed viability

a) *Barley*

The initial viability of barley seeds was determined using a modified hydrogen peroxide viability test (Thunaeus, 1938; Anon., 1986). Two hundred seeds were submerged in 200 ml of a 0.75 % hydrogen peroxide solution in 500 ml flasks. The flasks were placed in an incubator at 20°C in the dark for two days after which the solution was replaced by fresh hydrogen peroxide solution. After a further 24 hours, all the seed which had started to germinate (coleorhiza or radicle emergence) were counted as viable.

b) *Lettuce*

Lettuce seed viability was determined in the standard germination test as described in Section 2.2.4 after 7 days incubation, according to ISTA standard rules (ISTA, 1976). Germinated seeds and ungerminated seeds when firm were classified as viable. Ungerminated seeds were recorded as dead when soft.

The results of the viability tests were expressed as percentage viable seeds.

2.2.6 Water sensitivity test for barley

Water sensitivity of seed lots was tested in germination experiments, based on recommended methods of analysis of the Institute of Brewing (Anon., 1986), in heavy 10 cm glass petri-dishes (British Standard BS 611), lined with two layers of Whatman No.1 filter paper, wetted with 8 ml distilled water (Essery *et al.* 1954). In each dish 100 seeds were placed with the ventral side down (the embryo facing upwards). The dishes were kept in the dark at 20°C. Germinated seeds were counted daily, usually over a three day period, unless otherwise mentioned elsewhere. Water

sensitivity, however, was indicated by the percentage 'chitted' seeds after 3 days at 8 ml water level (Kelly and Briggs, 1993). 'Chitting' describes the event in the germination of barley seeds where the pericarp and the testa is ruptured by extension growth of the coleorhiza. A high germination rate at 8 ml water per dish indicated a low water sensitivity and *vice versa*.

2.2.7 Test for thermo-inhibition of lettuce seeds

Fifty seeds were spread on top of 2 layers of Whatman No.181 filter papers in 9 cm petri dishes wetted with 5 ml distilled water. The dishes were incubated at 26°C either in the dark, or light (80 $\mu\text{mol m}^{-2} \text{s}^{-1}$) in Fisons Fi-totron 600 H growth cabinets, as described under the specific experiments.

The germination (radicle emergence) of seeds sown under dark conditions was evaluated under safe green light, achieved by using an incandescent light source covered with two filter sheets 'primary green' P40374/5 (Philip Harris, Lichfield, Staffordshire, UK).

2.2.8 Preparation of pre-germinated seeds

a) *Barley*

Seeds of barley were surface sterilised by immersion for 5 minutes in 10 % sodium hypochlorite (1.4 % available chlorines), rinsed three times in sterile distilled water and pre-germinated on two layers of Whatman No.181 filter paper in 9 cm petri-dishes soaked with 5 ml distilled water at 20°C in the dark for 48 hours.

b) *Lettuce*

Seeds of lettuce were surface sterilised in 10 % sodium hypochlorite for 5 minutes, rinsed three times in sterile distilled water and pre-germinated at 20°C in the dark in 9 cm petri-dishes on two layer of Whatman No.181 filter paper soaked with 5 ml distilled water for 36 hours.

2.2.9 Modified slant test for pre-germinated seeds

A modified slant test (Wurr and Fellows, 1985) was used for the evaluation of lettuce seedling growth. Ten pre-germinated seeds, prepared as described in Section 2.2.8, were selected for uniform root length and placed on top of two layers of Whatman No.181 filter paper wetted with 5 ml of distilled water or test solution / suspension, in two rows in the upper third in each 9 cm petri-dish. The petri-dishes were placed at random in plastic bags and arranged at a 45° angle (Plate 2.2.1), in Fisons Fi-totron 600 H growth cabinets at 20°C, either in the dark or under continuous fluorescent light (250 $\mu\text{mol m}^{-2} \text{s}^{-1}$).

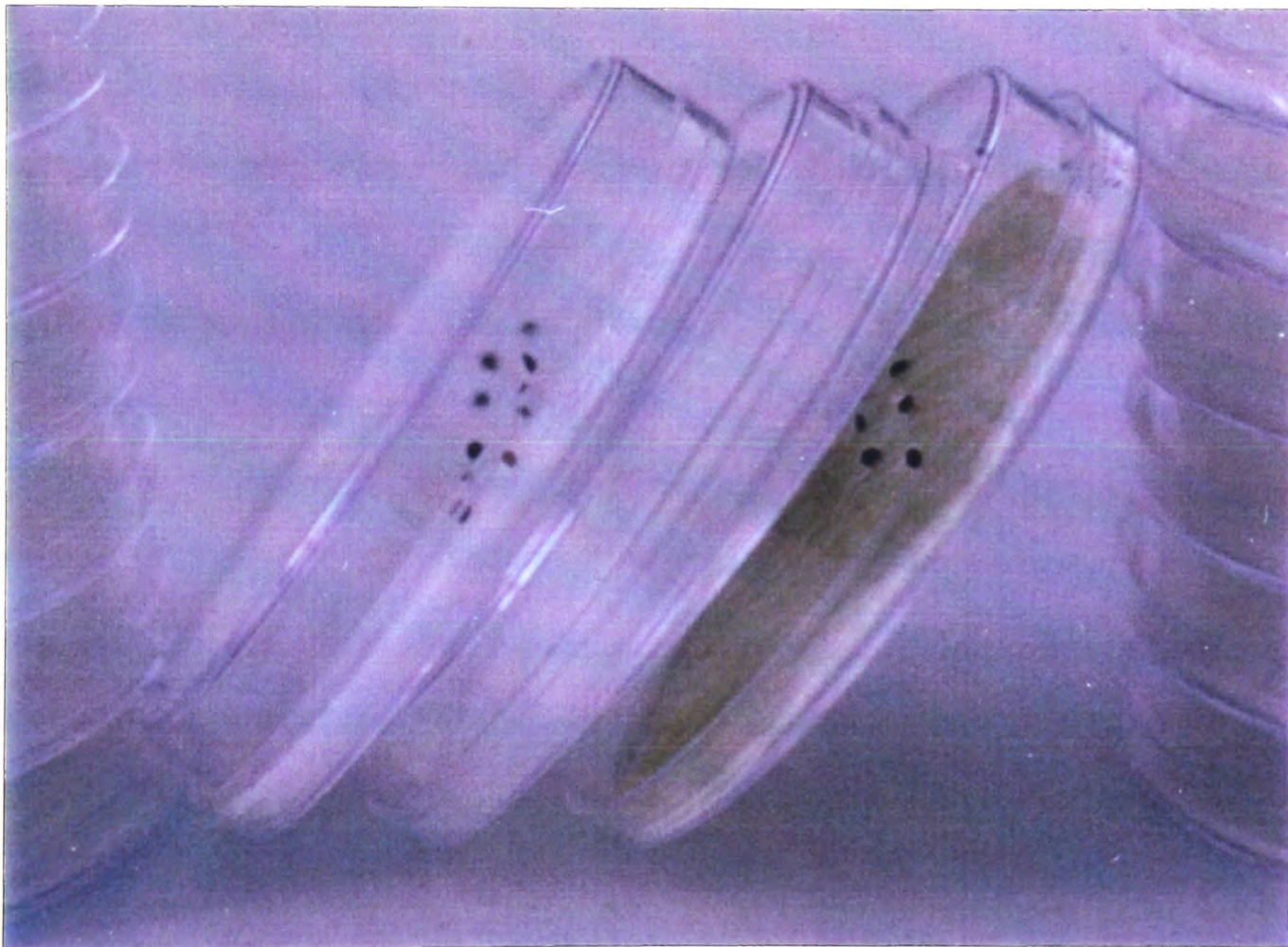


Plate 2.2.1: Position of petri-dishes in the modified slant test.

2.2.10 Seed priming treatments

Barley or lettuce seed sub-lots were soaked in priming media of depths not exceeding 1 cm, at 20°C in the light. Priming media to seed ratio was 1: 4 or 1: 20 (w/v) for barley or lettuce seeds, respectively. After the priming treatment the seeds were removed from the beaker and excess priming media removed. The seeds were spread between two dry sheets Kimberley Hi-Dri filter paper and allowed to re-dry under laboratory conditions at ~20°C and ~30 % RH.

Seeds soaked prior to the germination test are referred to as *primed*, *dry control* refers to untreated seeds, and *water control* refers to seeds soaked in water instead of SWS or osmotic solutions.

2.2.11 Plumule growth test for barley seedlings

Barley seedling growth was tested using a modified plumule growth test (Perry, 1977), where the seeds were not glued to the paper towels, as this interfered with the adhering seaweed material. Samples of 50 seeds were spread evenly on the lower half of a double sheet of Kimberley Clarke Hi-Dri 7390 rolled paper towel (220 x 380 mm) wetted with distilled water, and covered with a third towel, and loosely rolled to a diameter of 6 cm. An elastic band near the base held the roll together. The rolls were placed at random in an upright position in wire baskets, which were enclosed and in a plastic bag to prevent water loss. The baskets were placed into an incubator at 20°C in the dark. After 7 days the shoots of normal seedlings were measured to the nearest millimetre.

2.2.12 Modified slant test for primed lettuce seeds

The test was carried out basically as described in Section 2.2.9, except that 10 seeds were placed in two rows in the upper third of a 9 cm petri-dish, on top of two layers of Whatman No.181 filter paper and wetted with 5 ml distilled water.

2.2.13 Evaluation of germination experiments

Barley samples were evaluated daily, while lettuce seeds were checked at 8 hour time intervals, as lettuce germination is more rapid than that of barley seeds. Germination was observed usually over three days, unless mentioned otherwise elsewhere. The results of germination experiments were expressed as percentage germinated seeds, in relation to the initial number of seeds sown. Where appropriate, remaining ungerminated seeds were classified as fresh ungerminated seeds or dead seeds after seven days, according to ISTA rules (ISTA, 1976).

2.2.14 Calculation of the mean germination time and mean emergence time

The mean germination time (MGT) for barley, and the mean emergence time (MET) for both species was calculated according to a modified formula after Ellis and Roberts (1981) and expressed in hours:

$$\text{MGT or MET} = \frac{\sum Dn}{\sum n} * 24$$

where n is the number of seeds which germinated or with radicle emerging on day D, and D is the number of days counted from the start of the germination test.

For the calculation of the MGT of lettuce samples a modified formula was used to account for the difference in the evaluation intervals, and expressed in hours:

$$\text{MGT} = \frac{\sum Hn}{\sum n}$$

where n is the number of seeds which germinated after H hours, and H is the time elapsed from the start of the germination test.

2.2.15 Abnormal seedlings

Seedlings were evaluated at the end of the germination tests, and scored as normal or abnormal according to ISTA rules (ISTA, 1976). Barley seedlings with no

or short roots, short or split coleoptile, and lettuce seedlings with stunted radicle, swollen hypocotyls, or necrotic cotyledons were regarded as abnormal.

2.2.16 Viability of non-germinated barley seeds

The viability of seeds which had not germinated after 7 days in the water sensitivity test, was tested where appropriate, with a modified hydrogen peroxide test (Thunaeus, 1938; Anon., 1986). Seeds that had not germinated after 7 days were submerged in 100 ml 0.75 % hydrogen peroxide solution in 250 ml flasks. The flasks were placed in an incubator at 20°C for two days, after which germinated (chitted = viable) seeds were counted.

The total viability of seed samples was calculated as the sum of the percentage viable seeds in the hydrogen peroxide test added to the percentage germinated seeds in the water sensitivity test.

2.2.17 Evaluation of seedling growth

After four days on the filter paper the root, shoot or coleoptile length of barley seedlings, and the root, hypocotyl length or cotyledon area of lettuce seedlings were measured for 8 seedlings to the nearest millimetre. The outer left and right seedling per dish were not measured to exclude margin effects. The area of the cotyledon was calculated, according to the formula for elliptical shapes, matching the form of lettuce cotyledon:

$$\text{cotyledon area (mm}^2\text{)} = \frac{1}{4}\pi * \text{length(mm)} * \text{width(mm)}.$$

2.2.18 Root hair measurements

Root hair length (12 individual measurements) and number (five individual counts) of barley and lettuce seedlings was measured under a light microscope at 40x magnification, at a standard position for both species, 1 cm from the tip.

2.2.19 Quantification of the potassium content of lettuce seedlings

For K⁺ determinations eight seedlings were separated into radicle, hypocotyl and cotyledon, weighed, dried at 105°C for 3 hours, and ashed at 500°C for 5 hours (MAFF, 1986). The ash was re-dissolved in 50 µl concentrated hydrochloric acid, and diluted with distilled water to a final volume of 1 ml. The K⁺ concentration was determined with a Corning 400 flame photometer (Corning Ltd., Halstead, Essex, UK).

2.2.20 Removal of microbes from solutions

Microbes were removed from solutions by filter-sterilisation using autoclaved Nalgene vacuum sterilisation filter units (Nalge Company, Rochester, NY, USA) fitted with a Whatman cellulose nitrate membrane filter, pore size 0.45 µm. All glassware and disposable pipette tips used were, where possible, sterilised prior to use by autoclaving at 121°C at 1.1 bar pressure for 15 minutes.

2.2.21 Quantification of the protein content in LHS supernatant

Proteins were quantified using the Pierce Coomassie® protein assay reagent (Pierce, Rockford, IL, USA). Bovine serum albumin (BSA) was used as protein standard in two-fold dilutions from 10 µg l⁻¹ to 1.25 µg l⁻¹. The LHS supernatant preparations were diluted to 10 % prior to total protein determination.

Aliquots (1 ml) of 10 % LHS supernatant or distilled water as a blank were pipetted into reagent tubes. One ml protein assay reagent was added and the solution well mixed. The absorbance was read at 595 nm with a Beckman DU®65 Spectrophotometer within 90 minutes. The total protein content was expressed as µg BSA equivalents per ml LHS.

2.3 Experimental design

All experiments were conducted in completely randomised design. In general, the analyses were carried out, or treatments were applied, in three replications, unless otherwise stated.

2.4 Analysis of physical and chemical properties of SWS and a commercial product

2.4.1 Determination of the physical properties of SWS

2.4.1.1 Experiment 3.1: Osmolarity

The osmotic potential of the SWS was determined using a Wescor 5100C vapour pressure osmometer (Wescor Inc., Logan, USA). Diluted samples of 1 %, 10 %, 50 % and 100 % of ANS, LHS and the modified fractions were analysed. Undiluted original and the corresponding solid fractions of both SWS were not measured at 100 %, as the solid matter in the suspensions at this concentration did not allow proper sample loading.

2.4.1.2 Experiment 3.2: Conductivity

Conductivity was measured with a WPA CMD 750 Conductivity Meter (WPA, Linton, Cambridge, UK). Dilutions to 10 %, 20 %, 30 %, 40 %, 50 %, 75 % and 100 % of ANS and LHS were tested.

2.4.1.3 Experiment 3.3: Suspension pH

The pH of both SWS was determined and monitored using a WPA CD 620 Digital pH Meter (WPA, Linton, Cambridge, UK), on undiluted samples.

2.4.2 Experiment 3.4: Mineral composition of SWS and modified SWS

The mineral composition of the SWS and modified SWS fractions was determined by the Central Analytical Laboratory (SAC, Edinburgh, UK); original SWS, or SWS solid fraction samples were dried overnight at 100°C, while the ashed SWS were subjected directly to a standard perchloric-nitric acid digestion method (MAFF, 1986). The mineral composition of the SWS supernatant fractions were determined directly after dilution to 1 %. The minerals were quantified by inductively coupled atomic emission spectrometry (ICAEP) using a Thermo-Electron ICAEP 61-E (Thermo Jarrell Ash Corporation, Warrington, UK). The nitrogen content of samples was determined by a standard Kjeldahl method (MAFF, 1986).

2.4.3 Quantification of growth regulating-like substances in SWS

2.4.3.1 Experiment 3.5: Absciscic acid

The detection and quantification of absciscic acid (ABA) in the SWS was performed by the Institute of Environmental and Biological Sciences, Lancaster University, according to the radioimmunoassay method using monoclonal-antibodies to (σ)-absciscic acid (Quarrie *et al.* 1988).

2.4.3.2 Experiment 3.6: Auxin-like activity

Auxin-like activity was tested in a modified root growth inhibition assay (Swanson, 1946; Moewus and Moewus, 1952).

Materials: Seeds of barley (*H. vulgare* cv. Triumph(A)) were pre-germinated as described in Section 2.2.8.a.

Standard solutions: A stock solution of indole-3-acetic acid (IAA) was prepared by dissolving IAA in 1 N sodium hydroxide. Treatment concentrations ranged from 17.5 mg l⁻¹ (10⁻⁴ M) to 1.7 µg l⁻¹ (10⁻⁸ M) and were prepared by 10-fold dilutions in

distilled water, and a distilled water treatment was added as control. The pH of all solutions was adjusted to 6.5.

SWS samples: Both original SWS were prepared as described in Section 2.2.1, and applied undiluted, and in 10-fold dilutions to 0.001 %.

Methods: The IAA solutions, SWS samples, or distilled water were applied at 5 ml aliquots to 9 cm petri-dishes lined with 2 layers of Whatman No.181 filter paper. Ten pre-germinated seeds, selected for uniform root length (approximately 5 mm), were placed with the ventral side onto the filter paper (embryo-side up) in two rows into each petri-dish. Four petri-dishes per treatment were prepared and the dishes placed at random in plastic bags, positioned at a 45° angle in a Fisons Fi-totron 600 H growth cabinets. After four days at 20°C in the dark the length of the roots of eight seedlings were measured to the nearest millimetre, discarding the outer left and right seedling per dish. Result were expressed as µg IAA equivalents (IAAE) per litre SWS.

2.4.3.3 Experiment 3.7: Cytokinin-like activity

Cytokinin-like activity was measured according to a modified method of Fletcher *et al.* (1982), using etiolated detached cucumber cotyledons.

Materials: Cucumber seed (*Cucumis sativus* cv. Bedfordshire Prize) were surface-sterilised in 10 % sodium hypochlorite for 10 minutes, rinsed three times with sterile distilled water and placed on two layers of Whatman No.181 filter paper wetted with 5 ml distilled water, for germination at 28°C in the dark.

Standard solutions: Kinetin (6-furfurylaminopurine) was chosen as a cytokinin standard. A stock solution was prepared by dissolving kinetin with 1 N sodium hydroxide in distilled water containing 40 mM KCl. Concentrations for the standard curve ranged from 0.1 µg l⁻¹ (0.5x10⁻¹⁰ M) to 100 µg l⁻¹ (0.5x10⁻⁷ M) and were prepared by 10-fold dilutions in 40 mM KCl solution. A distilled water treatment

and a 40 mM KCl treatment, without kinetin, were added as controls. The pH of all solutions was adjusted to 6.5.

SWS samples: Both original SWS were prepared as described in Section 2.2.1, except that the diluent contained 40 mM KCl solution, and applied undiluted, and in 10-fold dilutions to 0.001 %. The addition of KCl was used to eliminate a K⁺ effect at lower SWS concentrations, as it was shown by Arnold and Fletcher (1986) that KCl concentration above 40 mM up to 100 mM did not affect chlorophyll synthesis in this bioassay.

Methods: The cotyledons from 6 day old seedlings were excised under safe green light. Safe green light was achieved as described in Section 2.2.7. Ten cotyledons, were floated with the abaxial surface down per 5 cm glass petri-dish containing 3 ml distilled water, kinetin or diluted SWS samples, and the dishes incubated in the dark at 28°C. After 20 hours they were exposed to fluorescent light (80 µmol m⁻² s⁻¹). After 3.5 hours the cotyledons were immediately homogenised for 15 seconds at 24,000 rpm using an Ultra-Turrax T25 (Janke & Kunkel GmbH & Co. KG, IKA®-Labortechnik, Staufen i. Br., Germany), and the chlorophyll extracted in 5 ml 80 % cold acetone. The homogenate was centrifuged at 3000g for 10 minutes and the absorbance of the supernatant measured with a Beckman DU®-65 Spectrophotometer at a wavelength of 663 nm at 20°C. The cytokinin activity of the suspensions was expressed as µg kinetin equivalents (KE) per litre SWS.

2.4.3.4 Experiment 3.8: Gibberellin-like activity

Gibberellin-like activity was quantified using a modified lettuce hypocotyl elongation bioassay (Frankland and Wareing, 1960).

Materials: Seeds of lettuce (*L. sativa* cv. Reskia(B)) were pre-germinated as described in Section 2.2.8.b.

Standard solutions: A stock solution of gibberellic acid (GA_3) was prepared by dissolving GA_3 in 1 N sodium hydroxide. A dilution series of concentrations ranging from 34.6 mg l^{-1} (10^{-4} M) to $34.6 \text{ } \mu\text{g l}^{-1}$ (10^{-7} M) were prepared by 10-fold dilution with distilled water. A distilled water treatment was added as control. The pH of all solutions was adjusted to 6.5.

SWS samples: Both original SWS were prepared as described in Section 2.2.1, and applied undiluted, and in 10-fold dilutions to 0.001 %.

Methods: Aliquots of 5 ml of GA_3 solution, SWS samples, or distilled water were applied to 9 cm petri-dishes lined with 2 layers of Whatman No.181 filter paper. Pre-germinated seeds, selected for uniform root length ($\sim 2 \text{ mm}$), were placed on top of the filter paper in two rows of five in each petri-dish. Four petri-dishes per treatment were prepared, and dishes placed at random in plastic bags at a 45° angle, in a Fisons Fi-totron 600 H growth cabinet under constant illumination ($250 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$). After four days at 20°C the hypocotyls of eight seedlings were measured to the nearest millimetre, discarding the outer two seedlings per dish. Results were expressed as $\mu\text{g GA}_3$ equivalent (GAE) per litre SWS.

2.4.4 Experiment 3.9: Quantification of phenolic compounds

Total polyphenols in crude SWS samples and partitioned extracts were quantified without sample purification by the colorimetric Folin-Denis method (AOAC, 1990), without gravimetric correction, and without correction for interfering compounds (Ragan and Jensen, 1977).

Materials: Folin-Denis reagent was prepared by the addition of 100 g sodium tungstate, 20 g phosphomolybdic acid, and 50 ml phosphoric acid, to 750 ml of distilled water, refluxed for 2 hours, cooled and diluted to 1 litre with distilled water. Saturated sodium carbonate solution was prepared by addition of 35 g anhydrous

sodium carbonate to each 100 ml distilled water, dissolved at 70-80°C and cooled overnight. The supersaturated solution was seeded with crystals of monohydrous sodium carbonate. After crystallisation, the saturated solution was filtered through glasswool.

Standard solutions: Phloroglucinol (1,3,5, trihydroxybenzene) was used as standard in two-fold dilutions from 62.5 mg l⁻¹ to 1.95 mg l⁻¹. A distilled water treatment was added as blank control.

SWS samples: The two original SWS, the corresponding supernatants and the solid fractions were prepared as described in Section 2.2.1, and diluted to 1 % (ANS), or 10 % (LHS), respectively, prior determination of phenols.

Methods: Aliquots of 1 ml of the standard solutions, original SWS, SWS fractions, or distilled water (control), were added to 7.5 ml distilled water. Folin-Denis reagent (0.5 ml) was added and the solution mixed. Three minutes later 1 ml saturated sodium carbonate, was added and the whole preparation mix thoroughly. After 30 minutes the samples were centrifuged at 3000g for 5 minutes, and the absorbance determined using a Beckman DU®65 Spectrophotometer at a wave length of 725 nm. The total polyphenol content was expressed as mg phloroglucinol equivalents (PE) per litre SWS.

2.4.5 Experiment 3.10: Physical and mineral analysis of a commercial seaweed product

To allow a comparison of the physical and chemical properties of products prepared according to different manufacturing processes, a commercial product, **Maxicrop Triple**, was analysed as described for the SWS in Sections 2.4.1 and 2.4.2.

2.5 Experiment 3.11: Quantification of initial microbial contamination in SWS and over time

Materials: Ringer solution ($\frac{1}{4}$ strength) was prepared by dissolving 2 tablets OXOID Ringer solution in 1 litre distilled water, and was sterilised by autoclaving at 1.1 bar pressure at 121°C for 15 minutes. Agar, selective for bacteria was prepared by dissolving 28 g OXOID nutrient agar (NA) (pH 7.4), and for yeasts and fungi by dissolving 39 g OXOID potato dextrose agar (PDA) (pH 5.6) (both Unipath Ltd., Basinstoke, Hampshire, UK), in 1 litre distilled water, and sterilised by autoclaving at 1.1 bar pressure at 121°C for 15 minutes. Penicillin G ($50 \mu\text{g ml}^{-1}$) and streptomycin sulphate ($100 \mu\text{g ml}^{-1}$) was added to PDA to suppress bacterial growth on these plates.

Test suspensions: SWS samples were thawed at 5°C shortly prior to microbe extraction, used undiluted and stored in 25 ml aliquots.

Methods: The inherent microbial contamination of SWS was estimated and monitored over a period of 5 days, starting immediately after thawing and then after incubation times of 12, 24, 48 and 120 hours at 20°C.

The microbes were extracted by adding 25 ml aliquots SWS to 225 ml Ringer solution, followed by a 2 minute treatment with a Colworth Stomacher 400 (A.J. Seward, London, UK), extracting the microbes into solution (Sharpe and Jackson, 1972). To allow an accurate estimation of the viable microbial 'load' of SWS, serial dilutions of the solutions were plated on NA and PDA plates, and incubated at 20°C in the dark for 5 days, after which the plates were evaluated. Plates where 30 to 300 colonies were present were counted (Postgate, 1969). The results were expressed as total number of viable colony forming units (cfu) per ml SWS suspension.

2.6 Experimental procedures Chapter 4: Effects on barley and lettuce seeds and seedlings in the presence of SWS

2.6.1 Experiment 4.1: The germination characteristics of diverse barley and lettuce cultivars

a) Barley

Materials: The germination characteristic of two seed lots of barley (*H. vulgare*) cv's Blenheim and Triumph, as well as one seed lot of cv. Halcyon and cv. Pastoral was tested.

Methods: The initial viability of the seed lots was determined (Section 2.2.5.a). Seeds were tested for germination (Section 2.2.4) (radicle emergence) and evaluated over seven days (Section 2.2.13), and the MGT calculated (Section 2.2.14).

b) Lettuce

Materials: Two seed lots of lettuce (*L. sativa*) cv. Reskia, and one seed lot of cv. Columbus and cv. Great Lakes were tested.

Methods: The germination characteristics of the seed lots was established (Section 2.2.5.b), and the viability tested (Section 2.6.1.a).

2.6.2 Experiment 4.2: The germination of dry untreated seeds on SWS

Materials: Four replicates of 25 dry untreated seeds of barley (*H. vulgare* cv. Triumph(A)), or 50 dry untreated seeds of lettuce (*L. sativa* cv. Reskia(B)) were used. Original ANS and LHS were prepared as described in Section 2.2.1, and applied at 0.01 %, 0.1 %, 1 %, 10 % or 50 %. A distilled water treatment was used as a control.

Methods: The seeds were placed on top of 2 layers of Whatman No.181 filter paper soaked with 5 ml distilled water, or 5 ml SWS suspension. The dishes were placed at random in a Fisons Fi-totron 600 H growth cabinet at 20°C under 16 hours fluorescent light supplement (250 $\mu\text{mol m}^{-2} \text{s}^{-1}$) daily.

In this experiment seed germination was recorded as per ISTA rules for normal seedlings (ISTA, 1976). Seeds were regarded as germinated when radicles were present, and the coleoptile longer than the seed, while complete cotyledon unfolding was taken as marker for lettuce seed germination.

The percentage germination, was recorded over seven days (Section 2.2.13), and the MGT was calculated (Section 2.2.14).

2.6.3 Experiment 4.3: The effect of LHS and osmotica on germination

Materials: One seed lot each of barley (*H. vulgare* cv. Triumph(A)) or lettuce (*L. sativa* cv Reskia(B)) were used. Solutions of sodium chloride (NaCl) or polyethylene glycol (PEG) were prepared to obtain osmotic potentials of -0.25 MPa (Table 2.1.2). Original LHS at 50 % dilution (Section 2.2.1) resulted in an OP of -0.25 MPa. A distilled water treatment was used as an additional control.

Methods: Dry untreated seeds were placed on top of 2 layers of Whatman No.181 filter paper soaked with 5 ml of either distilled water, NaCl, PEG or LHS, in an experimental set-up as described in Section 2.6.2. In this experiment and all following experiments radicle emergence was chosen as germination indicator. The percentage germination (radicle emergence) was recorded (Section 2.2.13), and the MGT was calculated (Section 2.2.14).

2.6.4 Experiment 4.4: The comparative effect of SWS and osmotica on seed moisture content during imbibition

Materials: One seed lot each of barley (*H. vulgare* cv. Triumph(A)) and lettuce (*L. sativa* cv. Reskia(B)) were used. Both original SWS were prepared as described in Section 2.2.1, and applied at concentrations of 50 % or 100 %. Solutions of sodium chloride (NaCl) or polyethylene glycol (PEG) were both prepared to obtain osmotic

potentials of -0.25 MPa (Table 2.1.2). A distilled water treatment was used as a control.

Methods: The initial seed moisture content of the seed lots was determined (Section 2.2.2). Sub-samples of barley (~2.5 g) or lettuce (~0.1 g) were imbibed on two layers of Whatman No. 181 filter paper soaked with 5 ml of either distilled water, NaCl, PEG, or both concentrations of ANS, or LHS. The sub-samples were weighed at the start of the experiment and after varying time intervals over a 48 hours, or 72 hours period for lettuce or barley, respectively. The seed moisture content during imbibition was calculated (Section 2.2.3).

2.6.5 Experiment 4.5: The effect of SWS on the seedling growth of pre-germinated seeds

Materials: Seeds of one seed lot each of barley (*H. vulgare* cv. Triumph(A)) or lettuce (*L. sativa* cv. Reskia(B)) were pre-germinated (Section 2.2.8). Original ANS and LHS were prepared (Section 2.2.1), and applied undiluted, or with 10-fold dilutions to 10 %, 1 %, 0.1 %, 0.01 % or 0.001 %. A distilled water treatment was used as a control.

Methods: Pre-germinated seeds were prepared in four replicates in a modified slant test (Section 2.2.9) on two layers of Whatman No. 181 filter paper soaked with 5 ml of either distilled water or both SWS suspensions at all concentrations, either in the light or dark. Seedling root growth was evaluated under light and dark conditions (Section 2.2.17). Additionally, the hypocotyl length was measured in dark, and cotyledon growth determined for light grown seedlings (Section 2.2.17).

Root hair number and the length of root hairs of seedlings of the light treatments were evaluated (Section 2.2.18), omitting treatment concentrations less than 1 % of either SWS, due to the ineffectiveness of these treatments on root growth.

2.6.6 Experiment 4.6: The effect of osmotica on lettuce root growth

Materials: Seeds of one seed lot of lettuce (*L. sativa* cv. Reskia(B)) were pre-germinated (Section 2.2.8). Sodium chloride (NaCl), potassium chloride (KCl), mannitol, or polyethylene glycol (PEG) solutions were prepared at OP of -0.25, -0.50, or 0.65 MPa (Table 2.1.2), and distilled water was used as a control.

Methods: Pre-germinated seeds were arranged (Section 2.2.9) on filter paper soaked with distilled water or osmotic solutions of three strengths, in four replicates, in the light or dark. The root length was measured (Section 2.2.17), as was the number and length of root hairs (Section 2.2.18).

2.6.7 Experiment 4.7: The effect of phloroglucinol on lettuce root growth

Materials: Seeds of one lettuce (*L. sativa* cv. Reskia(B)) seed lot were pre-germinated (Section 2.2.8). Phloroglucinol solutions were prepared at concentrations described in Section 2.1.5 (Table 2.1.3). A distilled water treatment was used as a control.

Methods: Four replicates of pre-germinated seeds were prepared and exposed to the phloroglucinol solutions and root growth and root hairs evaluated (Section 2.6.6).

2.6.8 Experiment 4.8: The effect of modified SWS on lettuce root growth

Materials: Seeds of one lot of lettuce (*L. sativa* cv. Reskia(B)) were pre-germinated (Section 2.2.8). Original and modified solutions / suspensions of both SWS were prepared (Section 2.2.1), and all diluted to 10 %. A distilled water treatment was used as a control.

Methods: Pre-germinated seeds were exposed to distilled water or SWS preparations in the light and dark (Section 2.2.9). Root growth was evaluated under light and dark conditions, while additionally hypocotyl length was measured in the dark, and the cotyledon growth determined for light grown seedlings (Section 2.2.17).

2.6.9 Experiment 4.9: Dose response of lettuce roots to LHS supernatant

Materials: Seeds of one lot of lettuce (*L. sativa* cv. Reskia(B)) were pre-germinated (Section 2.2.8). The supernatant fraction of LHS was prepared (Section 2.2.1), and diluted to 10 %, 8.2 %, 7.5 %, 5.0 %, 2.5 %, 1.25 % or 0.63 %. Original LHS was prepared (Section 2.2.1), diluted to 10 % and included for comparative purposes. A distilled water treatment was used as a further control.

Methods: Pre-germinated seeds were exposed to distilled water or LHS test solutions in the light (Section 2.2.9), and root growth (Section 2.2.17), and root hairs measured (Section 2.2.18).

2.6.10 Experiment 4.10: The effect of LHS supernatant on root growth of lettuce cultivars

Materials: Seeds of each lot of lettuce (*L. sativa*) cultivar Columbus, Great Lakes, Reskia(B) or Reskia(C) were pre-germinated (Section 2.2.8). Original LHS and the supernatant fraction of LHS were prepared (Section 2.2.1), and diluted to 10 %. A distilled water treatment was used as a control.

Methods: Pre-germinated seeds were exposed to distilled water, original or modified LHS in the light (Section 2.2.9), and root growth evaluated (Section 2.2.17).

2.6.11 Experiment 4.11: The significance of light exclusion on the inhibitory effect of LHS supernatant on lettuce root growth

Materials: Seeds of one lot of lettuce (*L. sativa* cv Reskia(B)) were pre-germinated (Section 2.2.8). The supernatant fraction of LHS was prepared (Section 2.2.1), and diluted to 10 %. Distilled water was used as a control treatment.

Methods: Pre-germinated seeds were exposed to distilled water or LHS supernatant (Section 2.2.9). One set of petri dishes, of both test solutions was kept in the dark, while three sets were exposed to light ($250 \mu\text{mol m}^{-2} \text{s}^{-1}$). In one set of the light

treatment, the whole seedling was exposed to the light, whereas in the remaining two sets either only the roots or the hypocotyls plus cotyledons were illuminated. Light exclusion from the roots was achieved by covering the wetted filter paper with aluminium foil, and holes punctured in two rows in the upper third of the petri-dish, through which radicles of pre-germinated seedlings were positioned (Plate 2.6.1). Light exclusion from the upper seedling part was achieved by covering the seed in an aluminium foil bag with holes through which the radicle were placed in good contact with the filter paper to avoid drying out (Plate 2.6.2). Root length was measured (Section 2.2.17).

2.6.12 Experiment 4.12: Heat stability of inhibitor(s) in LHS supernatant

Materials: Seeds of one lot of lettuce (*L. sativa* cv Reskia(B)) were pre-germinated (Section 2.2.8). The original and supernatant fraction of LHS was prepared (Section 2.2.1), and diluted to 10 %. Original LHS (10 %) and LHS supernatant (10 %), both non-heated, and distilled water treatments were used as controls.

Methods: Aliquots of LHS supernatant were heated for 10 minutes in sealed 25 ml Erlenmeyer flasks in a water bath at temperatures of 40, 60, 80 or 100°C, or autoclaved at 1.1 bar pressure at 121°C for 10 minutes. After the heat treatment the samples were cooled to room temperature. Pre-germinated seeds were exposed to distilled water, non-heated original LHS or LHS supernatant, or to heated LHS samples in the light (Section 2.2.9), and root growth evaluated (Section 2.2.17).

2.6.13 Experiment 4.13: The significance of microbial contamination in LHS supernatant on the inhibitory effect on lettuce root growth

Materials: One seed lot of lettuce (*L. sativa* cv Reskia(B)) was used and seeds were pre-germinated (Section 2.2.8). The supernatant fraction of LHS was prepared (Section 2.2.1) and used diluted to 10 %. Distilled water was used as a control.



Plate 2.6.1: Arrangement of seedlings of lettuce (*L. sativa* cv. Reskia) in petri-dishes in light treatments (right), or in order to exclude light from roots (left).

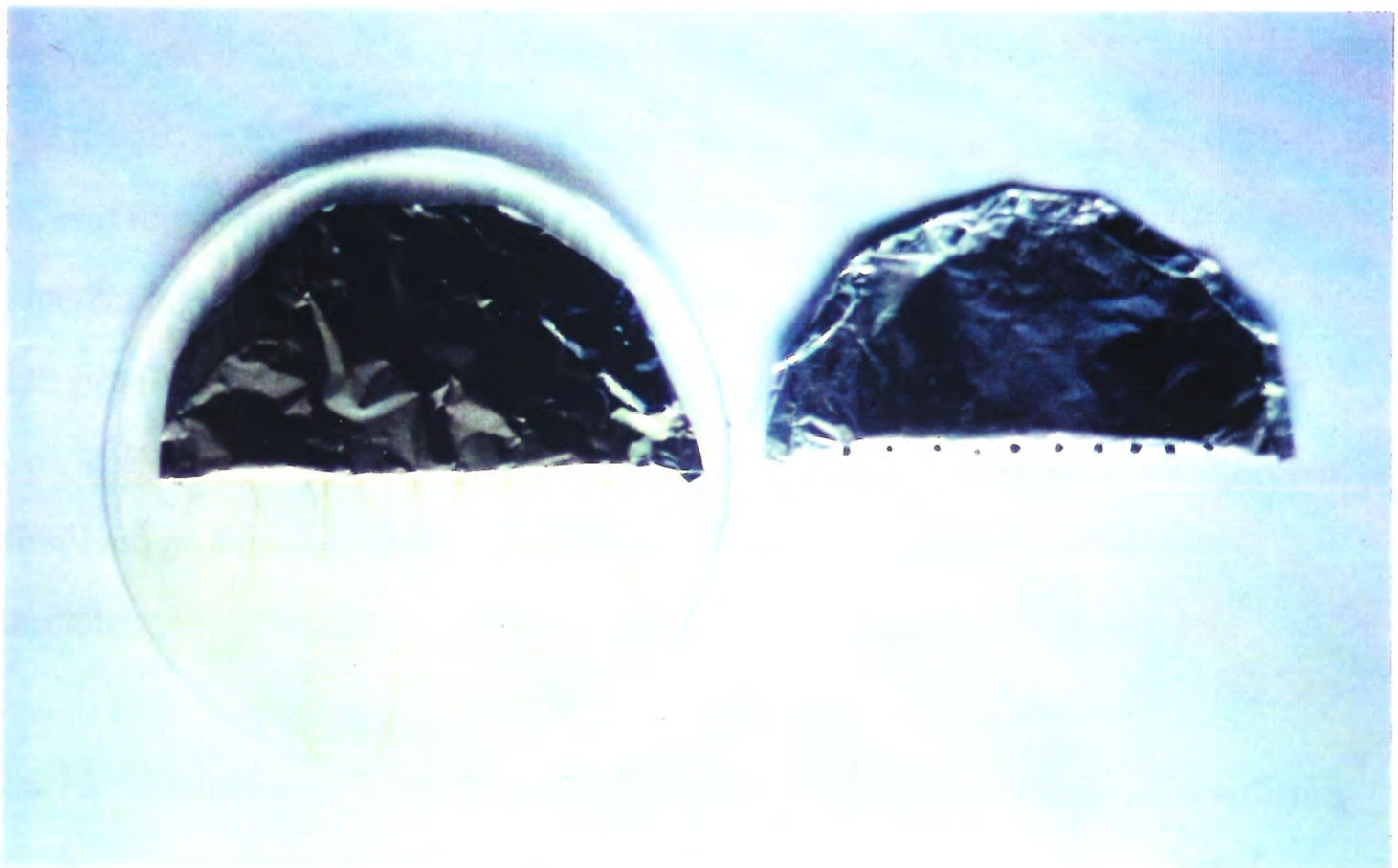


Plate 2.6.2: Arrangement of seedlings of lettuce (*L. sativa* cv. Reskia) in petri-dishes in order to exclude light from hypocotyls and cotyledons. View of lower side of the aluminium bag (right).

Methods: Microbes were removed from LHS supernatant (Section 2.2.20). Six aliquots of each non-sterilised or filter-sterilised solutions were stored in 50 ml flasks either at 5°C or 20°C in the dark for up to one week. Distilled water or the LHS supernatant preparations were tested for activity in the light, either immediately after thawing, or after storage for 1, 2, 3, 4, or 7 days (Section 2.2.9), and root growth was observed (Section 2.2.17).

2.6.14 Experiment 4.14: The role of proteins in LHS supernatant with respect to the inhibitory effect on lettuce root growth

Materials: Seeds of one lot of lettuce (*L. sativa* cv Reskia(B)) were pre-germinated (Section 2.2.8). The supernatant fraction of LHS was prepared (Section 2.2.1). Distilled water was used as a control.

Methods: Total proteins were removed from the LHS supernatant by organic solvent precipitation (Scopes, 1982). To 5 ml of undiluted SWS supernatant, 95 ml ice-cold acetone was added and the mixture shaken. After 15 minutes the mixture was centrifuged at 3000g for a further 15 minutes, the supernatant decanted, and the solvent removed by vacuum rotary evaporation. The protein content of the LHS supernatant prior and after protein precipitation was determined (Section 2.2.21). The protein precipitate pellet was re-dissolved in 5 ml distilled water.

Unaltered LHS supernatant, protein-deprived LHS supernatant and the re-dissolved protein precipitate were diluted to 10 %, and applied to pre-germinated seeds in the light (Section 2.2.9), and root growth evaluated (Section 2.2.17).

2.6.15 Experiment 4.15: The effect of diverse mineral solutions on cotyledon expansion and hypocotyl elongation of lettuce

Materials: Seeds of one seed lot of lettuce (*L. sativa* cv. Reskia(B)) were pre-germinated (Section 2.2.8). Mineral solutions were prepared (Table 2.1.4). Distilled

water was used as a control.

Methods: Pre-germinated seeds were treated with distilled water or all mineral solutions in the light and dark (Section 2.2.9). The hypocotyl length of dark treatments, and the cotyledon area of light treated seedlings was measured (Section 2.2.17).

Additionally, the K^+ content of seedlings receiving potassium chloride solutions was quantified (Section 2.2.19).

2.6.16 Experiment 4.16: The effect of original, modified SWS or KCl on seedling potassium and cotyledon chlorophyll content of lettuce

Materials: Seeds of one lettuce (*L. sativa* cv. Reskia(B)) seed lot were pre-germinated (Section 2.2.8). Both original and modified SWS were prepared (Section 2.2.1), and diluted to 10 %. Potassium chloride (KCl) solutions were prepared to give concentrations of 80 mg l⁻¹ or 160 mg l⁻¹ K⁺, equivalent to the K⁺ concentration of 10 % ANS or LHS, respectively.

Methods: Six replicates of pre-germinated seeds were treated with either of each SWS preparations, or the corresponding potassium chloride solution, in the light or dark (Section 2.2.9). Three replicates were analysed for the K⁺ content (Section 2.2.19). Figures for expected K⁺ content per seedling, per mg cotyledon fresh weight, were calculated from fitted regression curves to K⁺ values obtained in Experiment 4.15 for potassium chloride treatments. The same method was used to calculate expectancy values for the cotyledon area in the light, and the hypocotyl length in the dark.

The three remaining replicates and distilled water controls were processed for the cotyledon chlorophyll determination. Chlorophyll was determined according to the method by Arnon (1948), with some modifications. Cotyledons of eight seedlings were weighed, homogenised for 15 seconds at 24,000 rpm using an Ultra

Turrax T25 (Janke & Kunkel GmbH & Co. KG, IKA®-Labortechnik, Staufen i. Br., Germany) and chlorophyll extracted in 5 ml 80 % cold acetone. The homogenate was centrifuged at 3000g for 10 minutes and the absorbance measured with a Beckman DU®-65 Spectrophotometer at wavelengths of 663_A and 645_B nm (MacKinney, 1941) at 20°C. The total chlorophyll content in µg ml⁻¹ solution was calculated according to the formula (Arnon, 1948):

$$\mu\text{g ml}^{-1} = (A_{663A} * 8.02) + (A_{645B} * 20.2)$$

The results were expressed as µg total chlorophyll per seedling, per mg cotyledon fresh weight, or per mm² cotyledon area.

2.7 Experimental procedures Chapter 5: Effects of seed priming in SWS on lettuce and barley seed germination and seedling growth

2.7.1 Experiment 5.1: The SWS priming effect on water relations, the adhesion of SWS cell wall debris to seeds and the mineral composition of seeds

Materials: One seed lot of each barley (*H. vulgare* cv. Triumph(A)) and lettuce (*L. sativa* cv. Reskia(B)) were used in this experiment. Original ANS and LHS were prepared (Section 2.2.1), and used undiluted. A distilled water treatment was used as a control.

Methods: The initial seed moisture of the seed lots was determined (Section 2.2.2). Twelve sub-samples each were submerged in distilled water, ANS or LHS (Section 2.2.10). All the sub-samples (~0.5 g) were weighed at the beginning of the experiment, removed from the priming media at varying time intervals, over a 48 hours period for lettuce, or 72 hours period for barley, respectively. They were rinsed

to remove adhering SWS, blotted dry and weighed to determine fresh weight change over time.

At an intermediate period of 12 hours soaking, six sub-samples each of water, ANS or LHS soaked seeds were taken out of the imbibition experiment, and allowed to re-dry under conditions described in Section 2.2.10, three with and three without adhering algal material. The removal of adhering algal material was achieved by rinsing the seed sub-samples under running distilled water. The fresh weight of all re-drying sub-samples was recorded by weighing at the beginning of the re-drying period, and at varying time intervals during a 24 hour (lettuce) or 36 hour (barley) re-drying period, and the seed moisture content calculated as described in Section 2.2.3. The final dry matter content was calculated as described in Section 2.2.2.

The mineral content of those sub-samples soaked for 12 hours in either water, ANS or LHS and re-dried either with or without adhering algal material, and of dry untreated seeds was determined by the Central Analytical Laboratory (CAL, Edinburgh, UK); re-dried sub-samples (~0.5 g) were ground, dried for 6 hours at 100°C (MAFF, 1986), and the mineral content determined according to the method described under Section 2.4.2, used for original SWS.

2.7.2 Experiment 5.2: The effect of SWS priming duration on germination

Materials: Seeds of one seed lot each of barley (*H. vulgare* cv. Triumph(A)) and lettuce (*L. sativa* cv. Reskia(B)) were used. Both original SWS were prepared (Section 2.2.1), and applied undiluted. Distilled water was used as a control.

Methods: Dry seeds were either dipped in ANS or LHS, or primed in ANS or LHS for 6, 12, 24 or 48 hours, and all seeds were re-dried under conditions described in Section 2.2.10 for 24 hours, to determine the optimal priming duration.

Dry control, water control, and ANS or LHS treated seeds were sown as described in Section 2.2.4.

Germination (radicle emergence) was evaluated (Section 2.2.13), and the MGT calculated (Section 2.2.14). Also abnormal seedlings (Section 2.2.15), and the number of dead seeds were scored after seven days (Section 2.2.13).

2.7.3 Experiment 5.3: The effect of re-drying of SWS primed seeds on germination

Materials: Seed material and priming media were the same as in Experiment 5.2.

Methods: All seeds except for controls were primed in distilled water, ANS or LHS for 12 hours (Section 2.2.10). They were sown either without any re-drying, after 24 hours, or after one week re-drying (Section 2.2.10), to investigate the long term effect of SWS priming on seed germination.

Dry seeds, the water, ANS or LHS primed seeds were sown as described in Experiment 5.2, and germination (radicle emergence) (Section 2.2.13) and MGT were calculated (Section 2.2.14).

2.7.4 Experiment 5.4: Effect of removing adhering SWS on germination

Materials: The seed material and priming media used were identical to that in Experiment 5.2.

Methods: The seed priming treatments were identical to that described in Experiment 5.3, followed by a 24 hours re-drying period under conditions described in Section 2.2.10. ANS and LHS primed seed samples were re-dried either rinsed or non-rinsed prior to re-drying, to investigate the effect of algal cell wall material adhering to the seed surface on seed germination.

Dry seeds, the water control, or all treated seeds were sown as described in Experiment 5.2, germination (radicle emergence) evaluated (Section 2.2.13), and the MGT calculated (Section 2.2.14).

2.7.5 Experiment 5.5: The effect of controlled deterioration on seed germination and seedling growth

Sub-samples of barley or lettuce seed lots were artificially aged by controlled deterioration (CD) (Powell and Matthews, 1981). The controlled deterioration parameter range was predicted using a computer program (CPRO-DLO, 1990) based on the viability equation of Ellis and Roberts (1980), to achieve a maximum deterioration effect, without markedly compromising the viability of the seeds.

Materials: Seeds of one seed lot of barley (*H. vulgare* cv. Triumph(B)) and lettuce (*L. sativa* cv. Reskia(C)) were used.

Methods: Adjustment to the desired seed moisture level of lettuce seeds was achieved by allowing seeds of known moisture content to imbibe to elevated seed moisture contents, on 2 layers of Whatman No. 1 filter paper moistened with 5 ml distilled water. Frequent weighing of the samples assured the attainment of the desired fresh weight (*DFW*) calculated according to the formula:

$$DFW = \frac{(100-OSM)}{(100-DSM)} * IFW$$

where *OSM* is the original seed moisture content (on a fresh weight basis), and *DSM* is the desired seed moisture content (fw), and *IFW* is the initial fresh weight of the sample. All sub-samples of barley and lettuce were sealed in polythene bags and stored at 5°C overnight, to allow moisture equilibration to occur between seeds.

Barley seed samples were deteriorated in a water bath at 50°C, at the initial seed moisture content of 11.4 % (fw = 12.8 % dry weight basis). The CD conditions for lettuce seeds were 45°C and a moisture level of 11.0 % (fw = 12.3 % dw). The duration of the controlled deterioration was either 1, 2 or 3 days.

The degree of seed deterioration was evaluated in germination tests as outlined in Experiment 5.2. Barley shoot length (Sections 2.2.11), and lettuce root and hypocotyl length (Section 2.2.12) were measured.

2.7.6 Experiment 5.6: The significance of seed vigour prior to SWS priming on germination and seedling growth

Materials: Seeds of one seed lot of barley (*H. vulgare* cv. Triumph(B)) and lettuce (*L. sativa* cv. Reskia(C)) were used either non-deteriorated (high vigour), or deteriorated for 2 days as described in Experiment 5.5 (low vigour). Both original SWS were processed and used undiluted (Section 2.2.1). A polyethylene glycol (PEG) solution at a concentration to give an OP of -0.5 MPa (Table 2.1.2), and distilled water were used as controls.

Methods: High and low vigour seeds were primed in either distilled water, ANS, LHS or PEG for 12 hours and re-dried for 24 hours under conditions described in Section 2.2.10.

Dry control and water controls, or seeds primed in ANS, LHS or PEG were sown and evaluated as in Experiment 5.2. Seedling growth performance was assessed as in Experiment 5.5.

2.7.7 Experiment 5.7: The significance of seed vigour prior to SWS priming on seedling emergence, establishment and growth

Materials: Seed material and priming treatments used in this experiment was identical to that of Experiment 5.6.

Methods: Dry control, water control seeds and seeds primed in ANS, LHS or PEG were sown in screened sterilised loam-based John Innes Seed Compost, at depths of 5.0 cm or 1.5 cm, for barley or lettuce, respectively. For both species, for each treatment four replicates of 50 seeds were sown in 16.5 cm plastic pots, and placed at random on tables in a greenhouse. The average night and day temperatures were 13.1°C (± 0.6) and 23.3°C (± 0.3), respectively. A light supplement to give a 16 hour daylength was provided by Philips high pressure sodium tubular lamps, fitted to

Philips SGR 200 luminaires. The pots were watered with tap water, and the compost held at moisture levels between 65 % - 75 % of the field capacity.

Seedling emergence was counted daily up to day 21, and the mean emergence time (MET) was calculated as described under Section 2.2.14.

After 21 days, the shoot length of all emerged barley seedlings was measured to the nearest millimetre. For the assessment of lettuce growth, plants were cut at the base, just above the soil surface. The fresh weight of the plants was determined per pot, and average single plant values calculated by dividing the fresh weight by the total number of plants harvested.

2.7.8 Experiment 5.8: The determination of thermo-inhibition of lettuce cv. Reskia

Materials: Seeds of lettuce (*L. sativa* cv. Reskia(B)) were used.

Methods: Dry untreated seeds were prepared for germination either in the dark or light (Section 2.2.7), except that the dishes were incubated at 10, 15, 20, 26 or 30°C.

The germination (radicle emergence) was evaluated (Section 2.2.13), and the MGT calculated (Section 2.2.14).

2.7.9 Experiment 5.9: The thermo-sensitivity of lettuce cultivars

Materials: Two seed lots of lettuce (*L. sativa*) cultivar Reskia, and one seed lot of each cultivar of Columbus and Great Lakes were tested for thermo-sensitivity.

Methods: Seeds of all varieties were sown at 26°C in the dark as previously described (Section 2.2.7).

Germination (radicle emergence) was evaluated (Section 2.2.13), and the MGT calculated (Section 2.2.14).

2.7.10 Experiment 5.10: The effect of SWS priming on thermo-inhibition of lettuce

Materials: One seed lot of lettuce (*L. sativa* cv. Reskia(B)) was used. Both original SWS were prepared (Section 2.2.1), and applied undiluted. A distilled water soak treatment was added as a control.

Methods: Seeds were primed for 12 hours and re-dried for 24 hours as described in Section 2.2.10. Dry control, water control seeds and seeds primed in ANS or LHS were sown (Experiment 5.9), and germination (radicle emergence), fresh and dead seeds were recorded and evaluated (Section 2.2.13), and the MGT calculated (Section 2.2.14).

2.7.11 Experiment 5.11: The effect of priming in modified ANS on thermo-inhibition of lettuce

Materials: One seed lot of lettuce (*L. sativa* cv. Reskia(B)) was used. Original and modified ANS were prepared (Section 2.2.1), and used undiluted. A PEG solution at an osmotic potential of -0.25 MPa (Table 2.1.2), and a distilled water treatment were added as controls.

Methods: Seeds were primed for 12 hours in water, PEG, ANS, and modified ANS, and re-dried for 24 hours as described in Section 2.2.10. Dry seeds, water and PEG primed seeds and ANS primed seeds were sown and evaluated as per Experiment 5.9.

2.7.12 Experiment 5.12: The effect of storage after ANS priming on thermo-inhibition of lettuce

Materials: Seeds of lettuce (*L. sativa* cv. Reskia(B)) were used. Original ANS was prepared (Section 2.2.1), and used undiluted.

Methods: Seeds were primed in ANS as described in Experiment 5.10. They were sown either without re-drying, or re-dried (stored) for 1 day, or 1, 4 or 20 weeks

under conditions described in Section 2.2.10, before they were sown alongside dry control seeds, as described and evaluated as in Experiment 5.9.

2.7.13 Experiment 5.13: The determination of water sensitivity of barley cultivars

Materials: Two seed lots of each barley (*H. vulgare*) cultivars Blenheim and Triumph, and one seed lot of each cultivar Halcyon and Pastoral were tested for water sensitivity.

Methods: Water sensitivity of seeds was tested as per Section 2.2.6. The MGT was calculated (Section 2.2.14), and the total viability of the seeds determined (Section 2.2.16).

2.7.14 Experiment 5.14: The effect of SWS priming on water sensitivity of barley

Materials: Seeds of one seed lot of barley (*H. vulgare* cv Triumph(A)) were used in this experiment. Both original SWS were prepared (Section 2.2.1), and applied at concentrations of 1 %, 10 % or undiluted. Distilled water was used as a control.

Methods: Seed sub-samples were primed either in distilled water, ANS or LHS (Section 2.2.10), for 6, 12, or 24 hours, and either sown without re-drying, re-dried to 50 % or re-dried to 100 % loss of moisture gained during the priming treatment (Table 2.7.1).

Dry control seeds, water control and ANS or LHS primed seeds were tested for water sensitivity (Section 2.2.6). Due to the scale of the experiment, the treatments were carried out with two replications of 100 seeds (Anon. 1986), thus on a population of 200 seeds, in accordance with recommendations of the Official Seed Testing Station for Scotland (OSTS, 1974). Water sensitivity was tested as described in Experiment 5.13.

Table 2.7.1: Re-drying periods to reach 50 % or 100 % loss of moisture gained during SWS priming of barley (*H. vulgare* cv. Triumph(A)) seeds.

	Priming duration (hours)		
Moisture loss (% of uptake)	6	12	24
	Re-drying period (hours)		
0	0	0	0
50	6	8	10
100	15	24	36

2.7.15 Experiment 5.15: The significance of ANS application method on water sensitivity of barley

Materials: One seed lot of barley (*H. vulgare* cv Triumph(A)) was used. Original ANS was prepared as in Section 2.2.1, and used for priming undiluted. A distilled water treatment was added as control.

Methods: Treatments of seeds included a control, or a priming in water or ANS for 12 hours (Section 2.2.10), or a dip in ANS. Of those seeds primed in ANS half were rinsed under distilled water to remove adhering ANS material after the priming period, while the rest was not treated further. All treated seeds were re-dried for 24 hours before sowing.

Dry control seeds, water control seeds, and ANS treated seeds were tested for water sensitivity and evaluated as described in Experiment 5.13.

2.7.16 Experiment 5.16: The effect of priming in modified ANS on water sensitivity of barley

Materials: One seed lot of barley (*H. vulgare* cv Triumph(A)) was used. Original and modified ANS were prepared (Section 2.2.1), and used undiluted for priming. A PEG solution at an osmotic potential of -0.25 MPa (Table 2.1.2), and distilled water served as controls.

Methods: Seeds were either soaked in distilled water or primed in original or modified ANS for 12 hours and re-dried for 24 hours, as described in Section 2.2.10.

Dry control, water control seeds, and ANS treated seeds were tested for water sensitivity and evaluated as described in Experiment 5.13.

2.7.17 Experiment 5.17: The effect of seed to ANS ratio on water sensitivity of barley

Materials: Seeds of one lot of barley (*H. vulgare* cv Triumph(A)) was used. Original ANS (Section 2.2.1) was used undiluted. Dry seeds and seeds soaked in distilled water served as controls.

Methods: Seeds were primed in original ANS. Seed to ANS ratios of either 1 : 4 or 1 : 1 were used in conditions described in Section 2.2.10, for 12 hours and re-dried for 24 hours. Dry control, water control seeds, and ANS primed seeds were tested for water sensitivity and evaluated as described in Experiment 5.13.

2.7.18 Experiment 5.18: The significance of seed storage prior ANS priming on water sensitivity of barley

Materials: Seed of one seed lot of barley (*H. vulgare* cv Triumph(A)) was used. Original ANS was prepared (Section 2.2.1), and used undiluted. A distilled water soak treatment was added as control.

Methods: For the evaluation of the effect of storage prior to priming on the release of seeds from water sensitivity, dry untreated seeds were stored in paper bags in a cold store at 5°C (~40% RH). Samples were taken on week 78, 82, 86 or 95. These were either primed in distilled water or ANS, for 12 hours and re-dried for 24 hours, as described in Section 2.2.10.

Dry control seeds, water control seeds, and ANS primed seeds were tested for water sensitivity (Section 2.2.6). The MGT was also calculated (Section 2.2.14).

2.7.19 Experiment 5.19: The effect of seed storage conditions after ANS priming on water sensitivity of barley

Materials: One seed lot of barley (*H. vulgare* cv Triumph(A)) was used. Original ANS was prepared (Section 2.2.1), and used undiluted. A distilled water soak treatment was used as a control.

Methods: Sub-samples were placed in paper bags, and stored either in a cold store at 5°C (~40 % RH), or at 20°C (~30 % RH), for 1 day, or 1, 2, 8, 16, or 20 weeks.

Parallel to the storage of dry untreated seeds at 20°C, seed samples were primed for 12 hours in distilled water or original ANS (Section 2.2.10). These were stored for the same periods of time at 20°C (~30 % RH) to investigate the significance of the storage period after priming on the release from water sensitivity at elevated temperatures.

All samples were subjected to the water sensitivity tests at the end of the assigned storage periods and evaluated as described in Experiment 5.18.

2.7.20 Experiment 5.20: The effect of seed surface sterilisation and suppression of microbial proliferation on water sensitivity of barley

Materials: One seed lot of barley (*H. vulgare* cv Triumph(A)) was used. Filtered calcium hypochlorite solution ($\text{Ca}(\text{OCl})_2$), was used as a seed surface sterilant. The suppression of the seed micro flora was achieved by an antibiotic solution / suspension, containing, 800 mg l⁻¹ penicillin G, 800 mg l⁻¹ streptomycin sulphate, 200 mg l⁻¹ nystatin, 50 mg l⁻¹ amphotericin B, and 50 mg l⁻¹ polymyxin.

Methods: Seeds were either soaked for 5 minutes in sterile distilled water, or surface sterilised by immersion for 5 minutes in calcium hypochlorite. After the soak the seeds were rinsed three times in sterile distilled water, and re-dried between two dry sheets Kimberley Hi-Dri filter paper for 24 hours.

Dry untreated control seeds, water soaked or surface sterilised seeds were tested for water sensitivity as described in Experiment 5.13, either on filter paper soaked in 8 ml distilled water or 8 ml antibiotic solution / suspension, as described in Section 2.2.6.

2.7.21 Experiment 5.21: The effect of SWS on seed microbes of barley and the fate of microbial contaminants in priming media

Materials: Seeds of one seed lot of barley (*H. vulgare* cv Triumph(A)) were used. Filtered calcium hypochlorite solution ($\text{Ca}(\text{OCl})_2$), was used as seed surface sterilant. Original SWS were prepared (Section 2.2.1), and used undiluted for priming. A polyethylene glycol (PEG) solution of an OP of -0.5 MPa (Table 2.1.2), and a distilled water treatment were used as controls. Both solutions were filter sterilised prior application as described under Section 2.2.20.

Methods: The seeds were either surface sterilised as described in Experiment 5.19, or primed in distilled water, PEG, ANS or LHS for 12 hours, and re-dried for 24 hours as described in Section 2.2.10.

The microbial population on the seed surface of dry untreated control seeds, water, PEG, ANS or LHS primed seeds and surface sterilised seeds was quantified (Section 2.5), by adding 25 g seeds to 225 ml Ringer solution. To prevent fluid losses due to penetration of seeds through the stomacher bags, two stomacher bags were used.

Quantification of microbes in priming media after 12 hours was achieved following procedures described in Section 2.5, by adding 25 ml distilled water, PEG, ANS or LHS, sampled from used priming media after the 12 hours priming treatment, to 225 ml Ringer solution.

2.8 Statistical analyses

For results displayed in Chapter 3 (Section 3.3.1), concerning the mineral data, statistical analysis was restricted to the calculation of standard errors.

Biochemical assays reported in Chapter 3 (Section 3.3.2) were statistically analysed by fitting regression curves, and empirical relationships between the standard solution and the dose related response were determined. Using these equations, quantification of activity for the SWS or their fractions in the corresponding assay was obtained.

Standard errors are either listed in tables, or represented as bars on each data point (mean) in figures, where they were larger than the symbols.

ANOVA were performed on all other data, using GENSTAT 5 Release 2.2 (Lawes Agricultural Trust, Rothamsted Experimental Station, UK), testing for normal distribution of data sets prior analyses. All data sets were found to fit normal distribution. The mean comparison and separation was based on the least significant differences (LSD), calculated for probabilities of $p=0.05$, $p=0.01$, or $p=0.001$. In figures the LSD calculated for $p=0.05$ are displayed.

Confidence intervals ($p=0.05$) displayed in diverse graphs in Section 4.7 were added using the graphic package Fig-P for Windows Version 2.1.A.

Chapter 3

Properties of the seaweed suspensions

3.1 Introduction

As outlined in Section 1.5, the physical and chemical properties of seaweed products depend on the manufacturing process and the algal material used. As the seaweed suspensions used in this study are not commercially available products, and confidentiality about the preparation method had to be acknowledged, only limited information was available. In general, the process could be described as a physical disintegration method, without heat, alkaline or acid hydrolysis, and a final 1:3 dilution of the resulting mash with distilled water. The physical properties of such an extract is important with a view to treatments, such as priming, as the properties may limit the duration of soak treatments, or may indicate the necessity of dilution or pH adjustment prior to seed treatment. Emphasis was placed on the determination of the mineral content and the level of plant growth regulators probably present in the SWS. These are of prime importance in an evaluation of effects of seaweed extracts on plant physiology and growth as reported for other extracts in the literature. The inclusion of modified SWS was for complementary reasons to facilitate investigations on the nature of possible active components.

Contents of reports by other authors on the mycophytobiotic relationship between brown algae, bacteria and fungi (Section 1.3.8.), and the reported antimicrobial properties of algae and algal products (Sections 1.4. and 1.7.3) lead to investigations to estimate the initial microbial contamination of SWS, and the proliferation of the microbes over time in SWS. However, no attempt was undertaken to identify the organisms present in the SWS, as this would have been outside the scope of this study.

3.2 The physical properties of the seaweed suspensions

3.2.1 Experiment 3.1: Osmolarity

Figure 3.2.1 illustrates the osmotic potentials (OP) of the unaltered SWS and modified SWS. Undiluted original and solid preparations of both SWS were not measured. However, values for original SWS at lower concentrations were similar to SWS supernatant levels. Re-dissolved SWS solid fraction diluted to 10 % or 50 % had an OP of -0.05 MPa or -0.06 MPa, indicating a low osmotic strength of the undiluted fraction. Undiluted supernatants of ANS and LHS had OP of -0.25 MPa and -0.50 MPa, respectively. However, following ashing and re-dissolving, undiluted ashed ANS and ashed LHS samples had higher negative OP of -0.78 MPa and -0.79 MPa, than corresponding supernatants. This was due to the increased number of particles dissolved in the solutions, after the destruction of organic macromolecules during ashing.

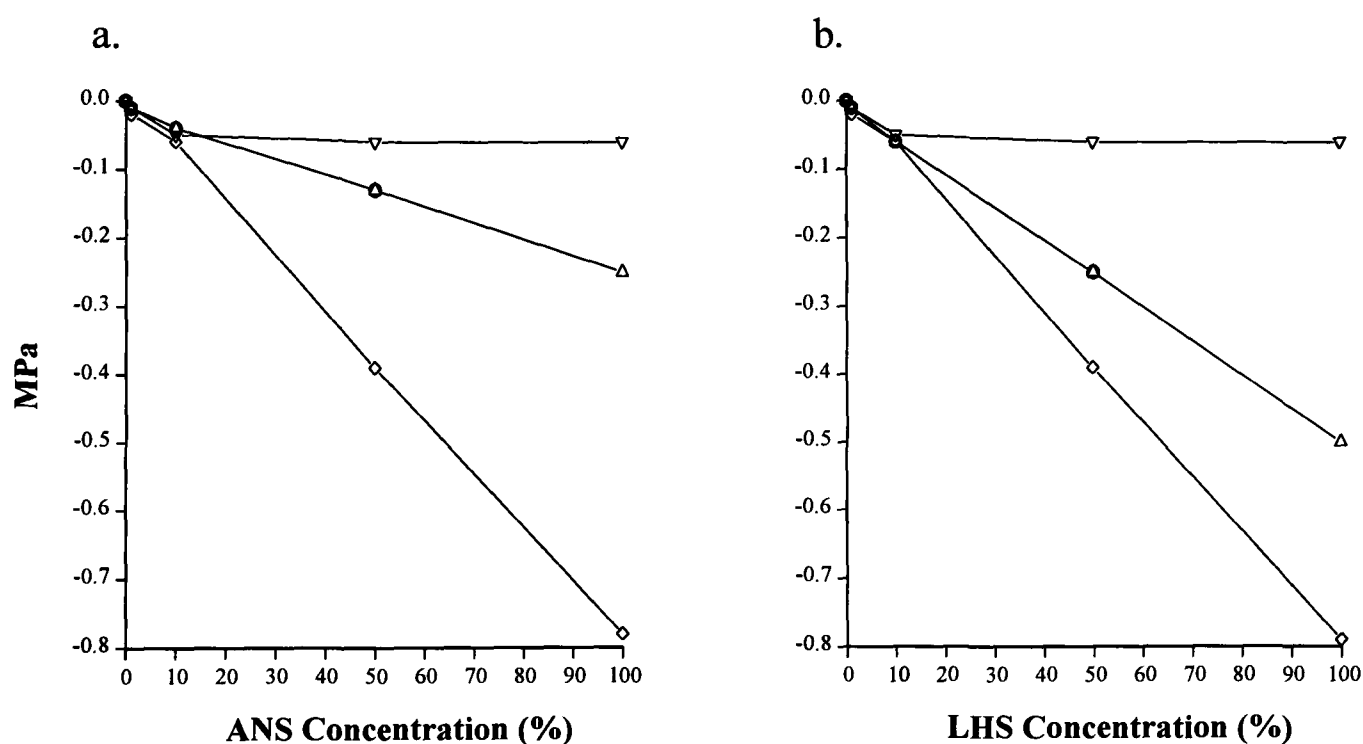


Figure 3.2.1: The osmotic potential of ANS (a.), and LHS (b.) at different dilutions, and modifications, original (○), supernatant (Δ), solid fraction (▽), and ashed (◇).

3.2.2 Experiment 3.2: Conductivity

The conductivity of undiluted suspensions was 5.00 mS ml^{-1} and 7.42 mS ml^{-1} , for ANS and LHS, respectively. This decreased linearly as concentrations decreased (Figure 3.2.2).

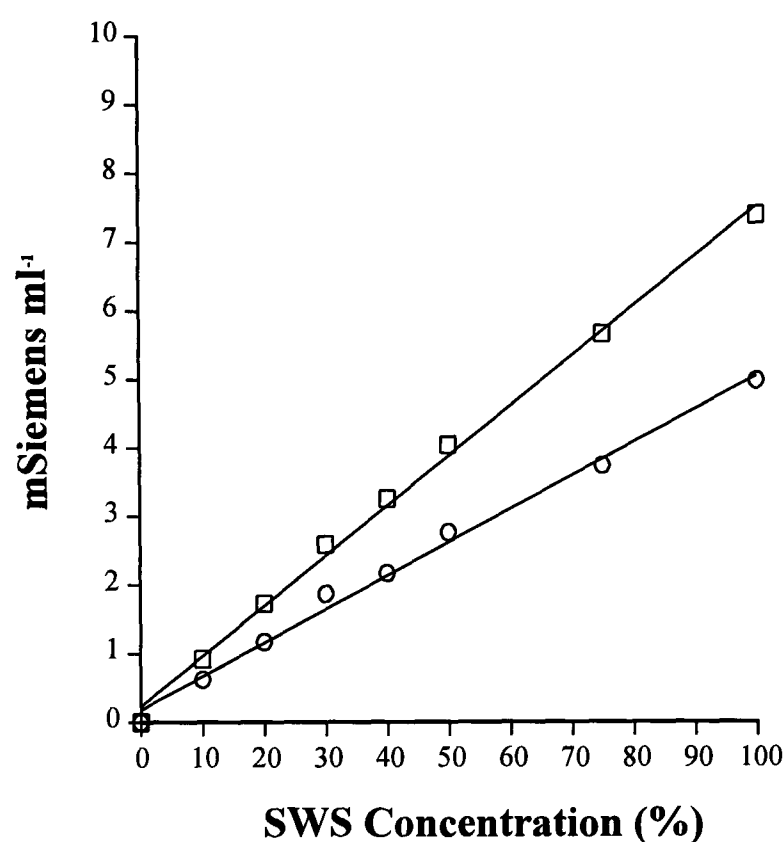


Figure 3.2.2: The conductivity of ANS (○), and LHS (□) at different dilutions.

3.2.3 Experiment 3.3: Suspension pH, and change over time

For undiluted ANS or LHS a pH of 5.6 and 7.4, respectively, was measured. To monitor possible chemical changes in the suspensions, the pH was monitored over time. The pH of original ANS declined slowly, but linearly from 5.6 to 5.3 after 96 hours, indicating a chemical stability of the suspension (Figure 3.2.3). For original LHS, on the other hand, a marked drop in pH was observed after 6 hours from pH 7.4 to pH 6.0 after 16 hours, and the pH declined further to 5.3 after 72 hours (Figure 3.2.4).

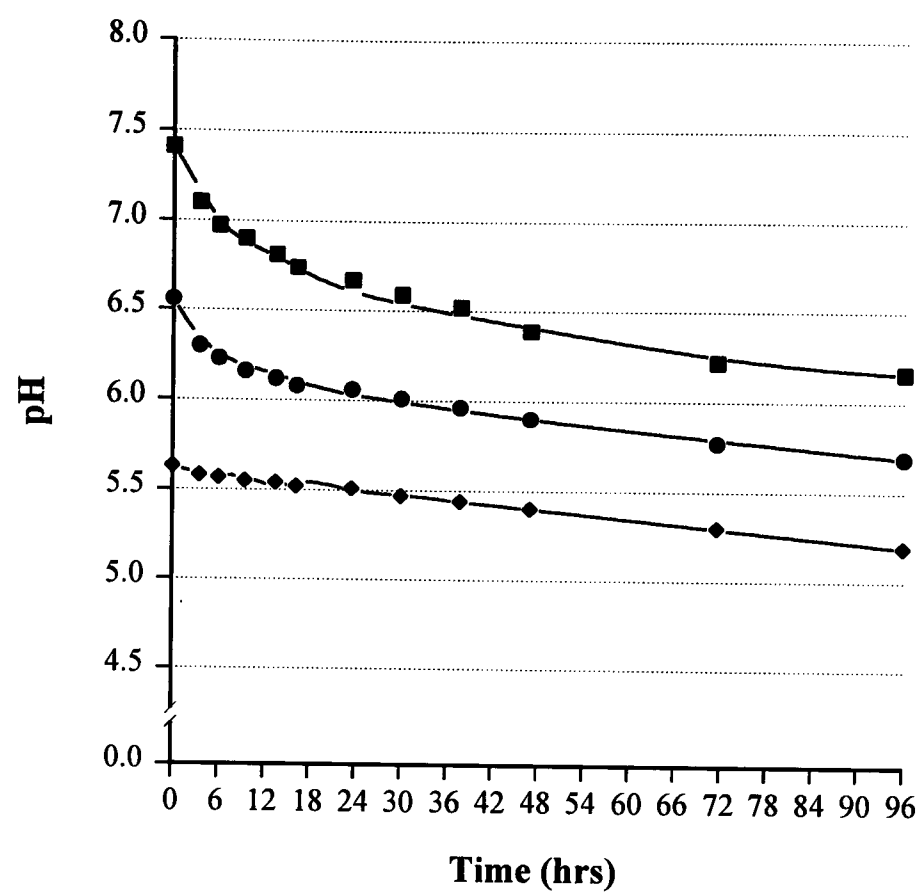


Figure 3.2.3: The effect of storage period on the change of the pH of undiluted suspensions of ANS, at the inherent pH levels of 5.6 (♦), or at elevated pH levels of 6.5 (●) or 7.4 (■). Storage at 20°C. n=3.

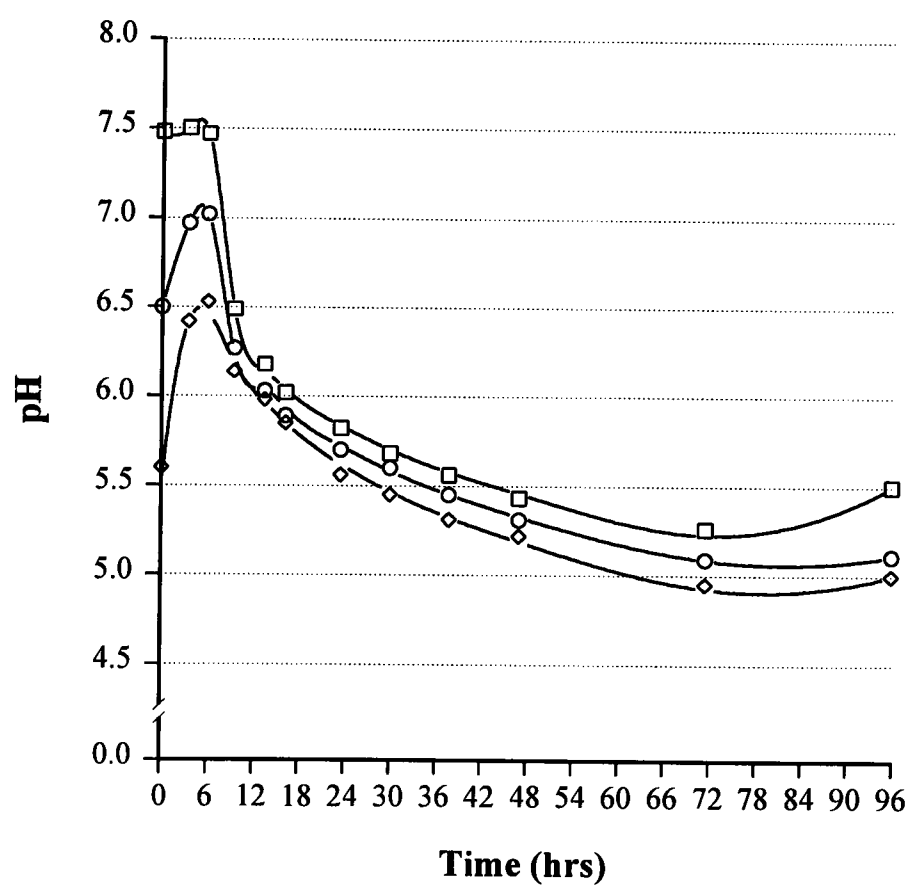


Figure 3.2.4: The effect of storage period on the change of pH of undiluted suspensions of LHS, at the inherent pH level of 7.4 (□), or at lowered pH levels of 6.5 (○) or 5.4 (◇). Storage at 20°C. n=3.

The buffering capacity of the SWS suspensions was observed over 96 hours. After artificial elevation of ANS samples to a pH of 7.4, only a gradual decrease was observed, and a constant difference between the levels of pH was present (Figure 3.2.3). The LHS suspension, on the other hand, had a significant H^+ buffering potential, as samples adjusted to pH 5.6 regained one pH point within 6 hours (Figure 3.2.4).

3.3 The chemical composition of the seaweed suspensions

3.3.1 Experiment 3.4: Mineral composition of SWS and modified SWS

Due to the method of preparation, the SWS possessed mineral levels, on the dry weight basis, comparable to fresh algae material (Table 1.3.3, and 3.3.1). In general, the algal preparations had moderate sodium and high chlorine and iodine levels, compared to other minerals (Table 3.3.1). Based on the fresh weight, the chlorine levels of ANS and LHS were 5.03 g l^{-1} , and 3.70 g l^{-1} , respectively. Potassium levels were high in LHS with 1.59 g l^{-1} , compared to ANS. Both SWS were rich in iron (ANS: 5.20 mg l^{-1} ; LHS 3.07 mg l^{-1}) and boron (ANS: 3.60 mg l^{-1} ; LHS 4.02 mg l^{-1}). LHS also had high levels of aluminium (4.16 mg l^{-1}) and silicon (7.00 mg l^{-1}), compared to ANS (Table 3.3.1).

After SWS partitioning and ashing, the resulting fractions of the seaweed suspensions were analysed for major minerals and selected trace elements. As expected, in the ashed fraction all major elements were found to be present at identical levels compared to the original SWS, except for sulphur, that was 30 % less in both suspensions (Table 3.3.2). Of the trace elements analysed only boron levels in both ashed SWS, and zinc levels in ashed ANS were unchanged. Iron, manganese and copper levels in both ashed SWS and zinc in ashed LHS were reduced by about 50 % of the original values.

Table 3.3.1: Dry matter content and mineral composition of the SWS used.
(analyses by the Central Analytical Laboratory, SAC, Edinburgh, 1992;
or ¹ OWTC, Orkney, 1992). Means ±se, n=3.

Suspension	<i>Ascophyllum nodosum</i>		<i>Laminaria hyperborea</i>	
% dry matter	4.47		3.77	
Element	g l ⁻¹ (fw)	% (dw)	g l ⁻¹ (fw)	% (dw)
Nitrogen	0.38 ±0.02	0.85 ±0.05	0.79 ±0.01	2.09 ±0.03
Phosphorus	0.03 ±0.01	0.07 ±0.01	0.11 ±0.00	0.30 ±0.01
Potassium	0.81 ±0.01	1.81 ±0.02	1.59 ±0.02	4.21 ±0.06
Calcium	0.48 ±0.04	1.07 ±0.08	0.56 ±0.03	1.48 ±0.09
Magnesium	0.35 ±0.01	0.77 ±0.03	0.18 ±0.01	0.48 ±0.02
Sulphur	0.92 ±0.03	2.07 ±0.07	0.42 ±0.02	1.11 ±0.04
Sodium	1.21 ±0.05	2.70 ±0.11	0.75 ±0.02	1.99 ±0.05
¹ Chlorine	5.03	11.26	3.70	9.81
	mg l ⁻¹ (fw)	mg kg ⁻¹ (dw)	mg l ⁻¹ (fw)	mg kg ⁻¹ (dw)
Iron	5.20 ±0.87	116.32 ±19.5	3.07 ±0.12	81.33 ±3.18
Manganese	0.47 ±0.04	10.43 ±0.98	0.14 ±0.01	3.58 ±0.32
Copper	0.40 ±0.04	8.91 ±0.94	0.41 ±0.06	10.87 ±1.67
Zinc	1.26 ±0.04	28.13 ±0.87	1.61 ±0.14	42.77 ±3.69
Boron	3.60 ±0.29	80.51 ±6.40	4.02 ±0.33	106.60 ±8.70
Nickel	0.09 ±0.00	1.95 ±0.09	0.08 ±0.00	2.07 ±0.03
Barium	0.28 ±0.01	6.20 ±0.27	0.27 ±0.00	7.03 ±0.08
Cobalt	0.05 ±0.01	1.21 ±0.11	0.05 ±0.01	1.19 ±0.32
Aluminium	1.61 ±0.01	36.03 ±0.27	4.16 ±0.03	110.24 ±0.88
¹ Iodine	32.00	716.20	47.00	1246.35
¹ Molybdenum	<0.30	<7.00	<0.50	<14.00
¹ Silicon	<0.30	<7.00	7.00	185.63
Lead	0.20 ±0.05	4.36 ±1.03	0.15 ±0.10	4.03 ±2.60
Selenium	<0.70	<16.00	<0.70	<19.00
Arsenic	<0.40	<9.00	<0.40	<11.00
Cadmium	0.04 ±0.01	0.81 ±0.11	0.03 ±0.00	0.90 ±0.11
Chromium	0.06 ±0.03	1.32 ±0.65	0.11 ±0.02	2.97 ±0.48

(< - below detection limit, figure indicates limit)

Fractionation of SWS resulted in a shift of mineral concentration: The ANS solid fraction contained higher calcium, magnesium, sulphur, iron, manganese and copper, whereas nitrogen, potassium, and sodium were present to equal amounts in both 'fractions', and the phosphorus and boron levels were higher in the supernatant (Table 3.3.2).

Table 3.3.2: Mineral composition of ANS and modified ANS. (analyses by Central Analytical Laboratory, SAC, Edinburgh, 1993; 1994). Means $\pm$ se, n=3.

Suspension	ANS					
Modification	Supernatant		Solid		Ashed	
Element	g l ⁻¹	se	% of Original	g l ⁻¹	se	% of Original
Nitrogen	0.155	± 0.057	41	0.206	± 0.002	54
Phosphorus	0.034	± 0.002	103	0.018	± 0.002	54
Potassium	0.468	± 0.031	58	0.543	± 0.022	67
Calcium	0.084	± 0.003	18	0.472	± 0.008	98
Magnesium	0.119	± 0.005	35	0.292	± 0.004	85
Sulphur	0.277	± 0.022	30	0.752	± 0.013	81
Sodium	0.823	± 0.018	68	0.747	± 0.005	62
	mg l ⁻¹			mg l ⁻¹		
Iron	2.342	± 0.116	45	6.207	± 1.907	119
Manganese	0.175	± 0.019	38	0.422	± 0.065	91
Copper	0.079	± 0.028	20	0.369	± 0.055	93
Zinc	1.656	± 0.559	132	0.754	± 0.084	60
Boron	4.255	± 0.143	118	1.245	± 0.048	35

(n.d. - not determined)

In the LHS solid fraction, disproportionately high iron and zinc levels occurred. These were higher than those in the original suspension. Otherwise the partition of trace elements followed that described for ANS. However, differences in the concentration of macro-elements between LHS fractions were found. Calcium and sulphur were concentrated in the solid fraction, magnesium evenly distributed between the supernatant and the solid fraction, whereas the majority of phosphorus, potassium, and sodium were in the supernatant (Table 3.3.3).

The potassium concentrations of the original and modified SWS are illustrated in Table 3.3.4. Original LHS contained 1587 mg l⁻¹ and original ANS, 808 mg l⁻¹ K⁺. The concentration of K⁺ in the ANS supernatant after centrifugation was 468 mg l⁻¹ (~58 % of original ANS), and 1474 mg l⁻¹ for the LHS supernatant (~93 % of

Table 3.3.3: Mineral composition of LHS and modified LHS. (analyses by Central Analytical Laboratory / SAC / Edinburgh, 1993; 1994). Means ±se, n=3.

Suspension	LHS								
Modification	Supernatant		Solid		Ashed				
Mineral	g l ⁻¹	se	% of Original	g l ⁻¹	se	% of Original	g l ⁻¹	se	% of Original
Nitrogen	0.275	±0.068	35	0.394	±0.003	50	n.d.		
Phosphorus	0.131	±0.048	115	0.042	±0.001	37	0.110	±0.018	97
Potassium	1.474	±0.028	93	0.465	±0.021	29	1.642	±0.068	103
Calcium	0.112	±0.007	20	0.381	±0.038	68	0.508	±0.048	91
Magnesium	0.110	±0.006	61	0.095	±0.001	53	0.166	±0.013	92
Sulphur	0.140	±0.033	34	0.234	±0.005	56	0.269	±0.034	64
Sodium	0.696	±0.031	93	0.302	±0.049	40	0.801	±0.149	107
	mg l ⁻¹			mg l ⁻¹			mg l ⁻¹		
Iron	0.314	±0.049	10	4.874	±1.023	159	2.865	±0.339	93
Manganese	0.046	±0.005	34	0.096	±0.011	71	0.068	±0.027	51
Copper	0.076	±0.034	19	0.339	±0.044	83	0.212	±0.120	52
Zinc	0.944	±0.253	59	3.330	±1.391	207	0.694	±0.351	43
Boron	4.199	±0.237	104	1.783	±0.187	44	4.224	±0.399	105

(n.d. - not determined)

original LHS). This indicated that for LHS, K⁺ was not bound to the cell debris to a large degree, unlike ANS. In the concentrated solid pellet of LHS and ANS, K⁺ levels were 2576 mg l⁻¹ (~162 % of original LHS) and 1769 mg l⁻¹ (~219 % of original ANS), thus between 1.5 - 2-fold increased over unaltered SWS. Calculations between the K⁺ concentration in the solid matter after centrifugation and the K⁺ levels in the supernatant revealed that the solid matter in LHS and ANS accumulated around 2 - 4 times higher amounts of K⁺. However, re-dissolving the solids to the initial volume diluted the K⁺ content and the final K⁺ concentration was 543 mg l⁻¹ in the ANS solid fraction (~67 % of original ANS), and 465 mg l⁻¹ in the LHS solid fraction (~29 % of original LHS) (Table 3.3.4.).

Table 3.3.4: Effects of suspension modification on the potassium concentration of original and modified SWS. Means $\pm$ se, n=3.

		K⁺ concentration (mg l⁻¹)		K⁺		SWS
SWS	Modification	at 100% application rate	in SWS solid fraction after centrifugation	adsorption ratio	as % of dry matter	% volume after centri- fugation
		$\pm$ se	$\pm$ se			
ANS	Original	808 11			1.81	100.0
	Supernatant	468 6		1		67.2
	Solid	543 7	1769 22	3.8	0.38	32.8
	Ashed	791 11				-
LHS	Original	1587 17			4.23	100.0
	Supernatant	1474 15		1		80.8
	Solid	465 7	2576 30	1.8	0.25	19.2
	Ashed	1642 15				-

3.3.2 Quantification of growth regulating-like substances in SWS

3.3.2.1 Experiment 3.5: Absciscic acid

The amount of absciscic acid was found to be $2.58 \mu\text{g kg}^{-1}$ ($\sim 10 \text{ nM kg}^{-1}$) and $18.83 \mu\text{g kg}^{-1}$ ($\sim 76 \text{ nM kg}^{-1}$) dry weight, for ANS and LHS, respectively. Calculated on the fresh weight basis, undiluted ANS and LHS contained about $0.12 \mu\text{g l}^{-1}$ ($\sim 0.4 \text{ nM l}^{-1}$) and $0.71 \mu\text{g l}^{-1}$ ($\sim 2.7 \text{ nM l}^{-1}$).

3.3.2.2 Experiment 3.6: Auxin-like activity

In the present study, barley root growth inhibition in the dark was found to be correlated with indole-3-acetic-acid (IAA) concentration ($r=-0.988$) (Figure 3.3.1). Auxin-like activity, expressed as indole-3-acetic acid equivalents (IAAE), was found to be $175.2 (\pm 6.7) \mu\text{g l}^{-1}$ ($\sim 1 \mu\text{M l}^{-1}$) and $98.7 (\pm 3.4) \mu\text{g l}^{-1}$ ($\sim 0.6 \mu\text{M l}^{-1}$) for undiluted ANS and LHS, respectively (Figure 3.3.2).

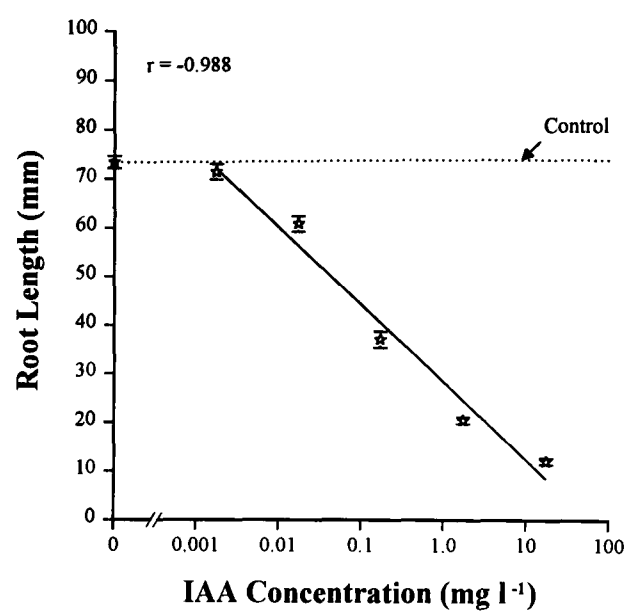


Figure 3.3.1: The effect of varying IAA concentrations on the root length of barley cv. Triumph seedlings in the dark. Means $\pm$ se (standard errors represented as bar on each point of the figure), n=32.

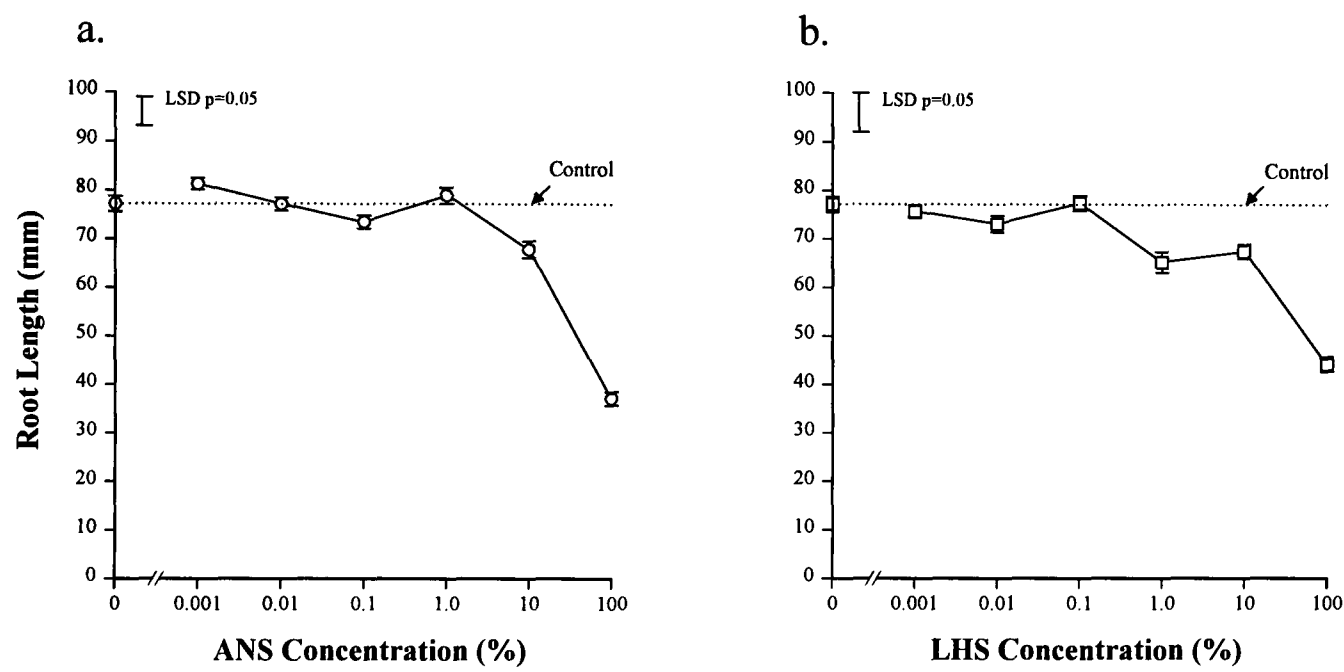


Figure 3.3.2: The effect of ANS (a.) or LHS (b.) at varying concentrations on the root length of barley cv. Triumph seedlings in the dark. Means $\pm$ se, n=32.

3.3.2.3 Experiment 3.7: Cytokinin-like activity

Figure 3.3.3 shows the standard curve for the cytokinin, kinetin, illustrating the positive relationship between cytokinin concentration and chlorophyll content of cucumber cotyledons ($r=0.984$). Unpurified SWS were tested for cytokinin activity in the bioassay, but the resulting response curves were not linear (Figure 3.3.4). In

the case of ANS highest cytokinin-like activity was found to be 2.93 (± 0.29) μg kinetin equivalents (KE) l^{-1} in the undiluted suspension, whereas for LHS no consistent response was obtained. Further, the increased chlorophyll levels for LHS treatments were not significantly different from the KCl control treatment.

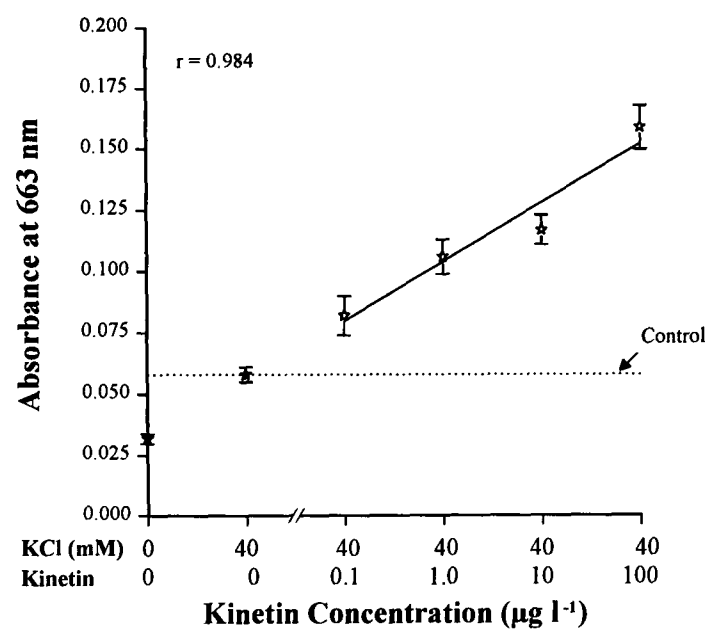


Figure 3.3.3: The effect of varying kinetin concentrations on the chlorophyll content of detached cotyledon of cucumber cv. Bedfordshire Prize. Means $\pm$ se, $n=3$.

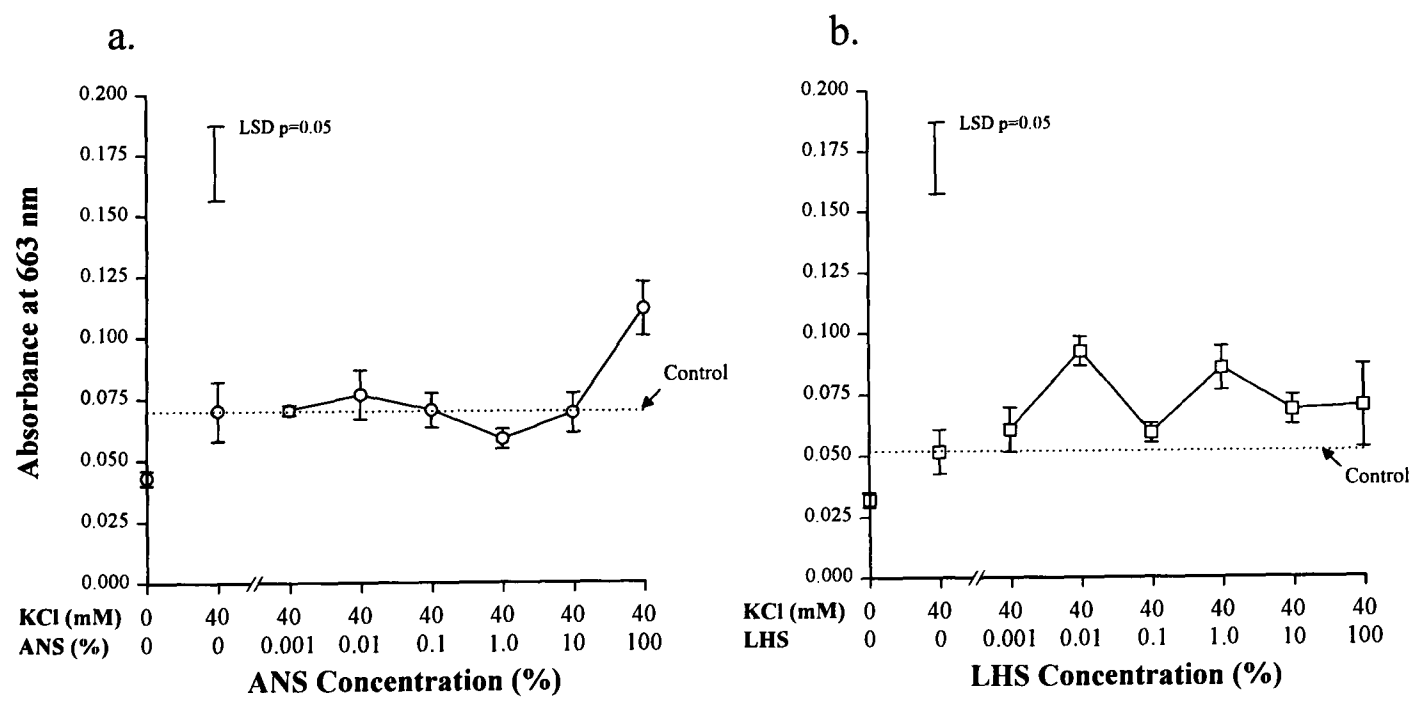


Figure 3.3.4: The effect of ANS (a.) or LHS (b.) at varying concentrations on the chlorophyll content of detached cotyledon of cucumber cv. Bedfordshire Prize. Means $\pm$ se, $n=3$.

3.3.2.4 Experiment 3.8: Gibberellin-like activity

A standard curve was produced with lettuce hypocotyl elongation in the light against varying known concentrations of gibberellic acid (GA₃) (r=0.989) (Figure 3.3.5). Promotion of hypocotyl elongation measured in the presence of SWS samples was negligible (Figure 3.3.6).

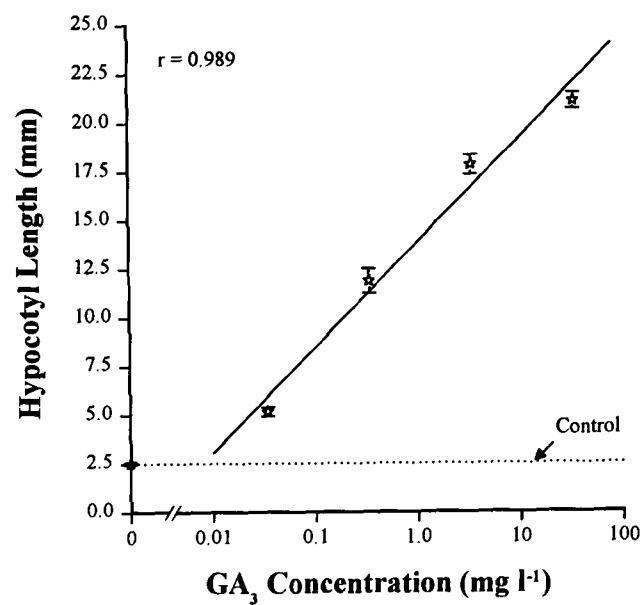


Figure 3.3.5: The effect of varying GA₃ concentrations on the hypocotyl length of lettuce cv. Reskia seedlings in the light. Means ±se, n=32.

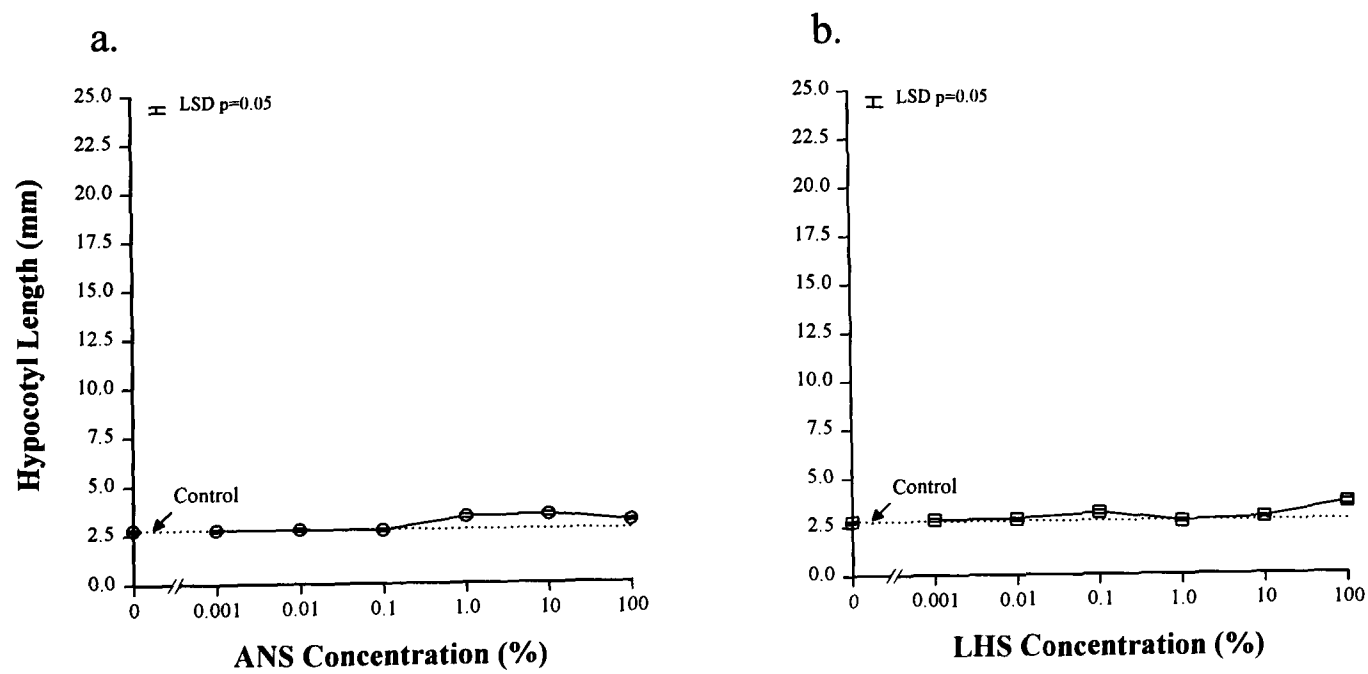


Figure 3.3.6: The effect of ANS (a.) or LHS (b.) at varying concentrations on the hypocotyl length of lettuce cv. Reskia seedlings in the light. Means ±se, n=32.

Table 3.3.5: Growth regulating substance-like activity of SWS and diverse terrestrial plants.

Plant growth substance		ABA	Auxin	Cytokinin	GA	Reference
Seaweed suspension	Tissue	$\mu\text{g l}^{-1}$ fw	$\mu\text{g l}^{-1}$ fw	$\mu\text{g l}^{-1}$ fw	$\mu\text{g l}^{-1}$ fw	
ANS	fresh	0.12	175.2	2.93	n.dt. ²	this study
LHS	suspension	0.71	98.7	n.dt.	n.dt.	this study
<i>plant species</i>		$\mu\text{g kg}^{-1}$ fw	$\mu\text{g kg}^{-1}$ fw	$\mu\text{g kg}^{-1}$ fw	$\mu\text{g kg}^{-1}$ fw	
<i>Oryza sativa</i>	shoot	243-973				Lee <i>et al.</i> 1993
<i>Zea mays</i>	seed	100-150				Cheikh and Jones, 1994
<i>Hordeum vulgare</i>	leaf	660				Afitlhile <i>et al.</i> 1993
<i>Triticum aestivum</i>	shoot		155.40		2.68	Grossmann <i>et al.</i> 1994
<i>Cucurbita pepo</i>	cotyledon		99.86			Grossmann <i>et al.</i> 1991
<i>Solanum tuberosum</i>	tuber			52.9-84.8		Sukhova <i>et al.</i> 1993
<i>Solanum tuberosum</i>	shoot			117.4-231.5		Catsky <i>et al.</i> 1993
<i>Pisum sativum</i>	seed				2.4-31.3	Swain <i>et al.</i> 1993

(² - n.dt. - no activity detected)

3.3.3 Experiment 3.9: Quantification of phenolic compounds

The concentration of phenolic compounds, expressed as phloroglucinol equivalents, was found to be 5760 mg l⁻¹ and 399 mg l⁻¹ for undiluted ANS and LHS, respectively. This was equivalent to 13.0 % and 1.1 % of the dry weight (Table 3.3.6).

Partitioning of the SWS suspension reduced the concentration of phenols in the ANS supernatant and ANS solid fraction to 71 % and 52 %, respectively, of the values of original ANS (Table 3.3.6). Calculations based on the adsorption of phenols after centrifugation showed that the ANS solid fraction contained about twice the phenolic compounds as were present in the ANS supernatant. The

polyphenol concentration in LHS supernatant and LHS solid fraction was 17 % and 86 %, respectively, of the level present in original LHS. Most of the phenols were probably bound to the algae cell wall debris, as the solid phase exhibited a 27 times higher concentration, compared to the supernatant.

Table 3.3.6: Polyphenol content of original and modified SWS, and partition ratio, expressed as phloroglucinol equivalents. Means ±se, n=3.

		Polyphenol concentration (mg l ⁻¹)					
SWS	Modification	at 100% application rate ±se	%	in SWS solid fraction after centrifugation ±se	absorption ratio	% of dry matter	
ANS	Original	5760 39	100			13.03	
	Supernatant	4090 11	71		1		
	Solid	3012 37	52	9183 99	2.2		
LHS	Original	399 4	100			1.06	
	Supernatant	66 1	17		1		
	Solid	345 7	86	1797 37	27.2		

3.4 Experiment 3.10: Physical and mineral analysis of a commercial seaweed product

For comparative purposes, the physical properties of **Maxicrop Triple**, a commercial seaweed extract, was determined. This extract exhibited an OP of -0.14 MPa at 1 % and of -0.76 MPa at 10 %, and of -6.5 MPa undiluted. The conductivities at the same concentrations were found to be 1.3 mS ml⁻¹, 9.5 mS ml⁻¹, and 59.0 mS ml⁻¹. The pH of undiluted extract was 8.9, while in solutions diluted to 1 % concentration the pH measured was still 8.2.

Expressed on the basis of the dry weight, the mineral analysis indicated lower levels of calcium and magnesium and high levels of phosphorus, potassium and trace element levels in **Maxicrop Triple**, compared to ANS (Table 3.3.7). These

differences were also to observe when comparing the concentrations of those minerals in the different preparations on the fresh weight basis, and may reflected differences in the degree of extract concentration. However, compared to fresh algae both preparations contained lower levels of calcium and sodium (Table 3.3.7).

Table 3.3.7: Mineral composition and pH of diverse seaweed suspensions prepared from the brown algae *Ascophyllum nodosum*. (Values modified after ² - means of data from Stephenson, 1968, and Indergaard and Minsaas, 1991; or ^o - Central Analytical Laboratory, SAC, Edinburgh, 1992).

Product	Fresh Material	ANS	Maxicrop Triple	Fresh Material	ANS	Maxicrop Triple
Reference	²	^o	^o	²	^o	^o
Preparation method	fresh material	physical dis-integration	alkaline hydrolysis	fresh	physical dis-integration	alkaline hydrolysis
pH	n.d.	5.6	9.0-9.5			
dry matter %	~20	4.47	22.7			
	fresh weight basis			dry weight basis		
Element	g kg ⁻¹ fw	g l ⁻¹	g l ⁻¹	g kg ⁻¹	g kg ⁻¹	g kg ⁻¹
Calcium	3.90	0.48	0.23	19.50	10.74	1.00
Chlorine	7.43	5.03	n.d.	37.15	112.58	n.d.
Magnesium	0.91	0.35	0.10	4.55	7.72	0.20
Nitrogen	0.06	0.38	1.97	0.30	8.51	8.67
Phosphorus	0.34	0.03	5.50	1.68	0.74	24.30
Potassium	3.78	0.81	20.40	18.90	18.08	89.80
Sodium	7.68	1.21	4.40	38.40	26.99	19.40
Sulphur	4.56	0.92	5.00	22.80	20.68	22.00
	mg kg ⁻¹	mg l ⁻¹	mg l ⁻¹	mg kg ⁻¹	mg kg ⁻¹	mg kg ⁻¹
Aluminium	n.d.	1.61	90.0	n.d.	36.03	395.0
Boron	n.d.	3.60	39.0	n.d.	80.51	172.0
Bromine	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cobalt	1.28	0.054	0.3	6.41	1.21	1.2
Copper	3.29	0.40	44.0	16.43	8.91	195.0
Iodine	157.40	32000	n.d.	787.00	716.20	n.d.
Iron	103.41	5.20	180.0	517.05	116.32	793.0
Manganese	124.75	0.47	64.0	623.75	10.43	282.0
Molybdenum	1.74	n.d.	n.d.	8.71	n.d.	n.d.
Nickel	3.85	0.087	0.7	19.25	1.95	3.2
Zinc	19.02	1.26	34.0	95.08	28.13	150.0

(n.d. - not determined)

3.5 Experiment 3.11: Quantification of initial microbial contamination in SWS and over time

The manufacture of the suspensions was not carried out aseptically, and as no preservatives were added to the product, the initial suspension microbial inoculum could be fairly high (see also Section 1.3.8.). Conversely, certain algae are capable of producing antibiotic substances and these may have a direct effect on contaminants.

After thawing of the SWS at 5°C, ANS contained 1.40×10^3 colony forming units (cfu) ml^{-1} . After 120 hours at 20°C, the number of cfu dropped by 96 % (Figure 3.5.1). LHS contained 10.13×10^3 cfu ml^{-1} , seven times higher initial microbial contamination compared to ANS. After 12 hours at 20°C the number of microbes increased by 255 %, and was 72 times higher after 120 hours (Figure 3.5.1).

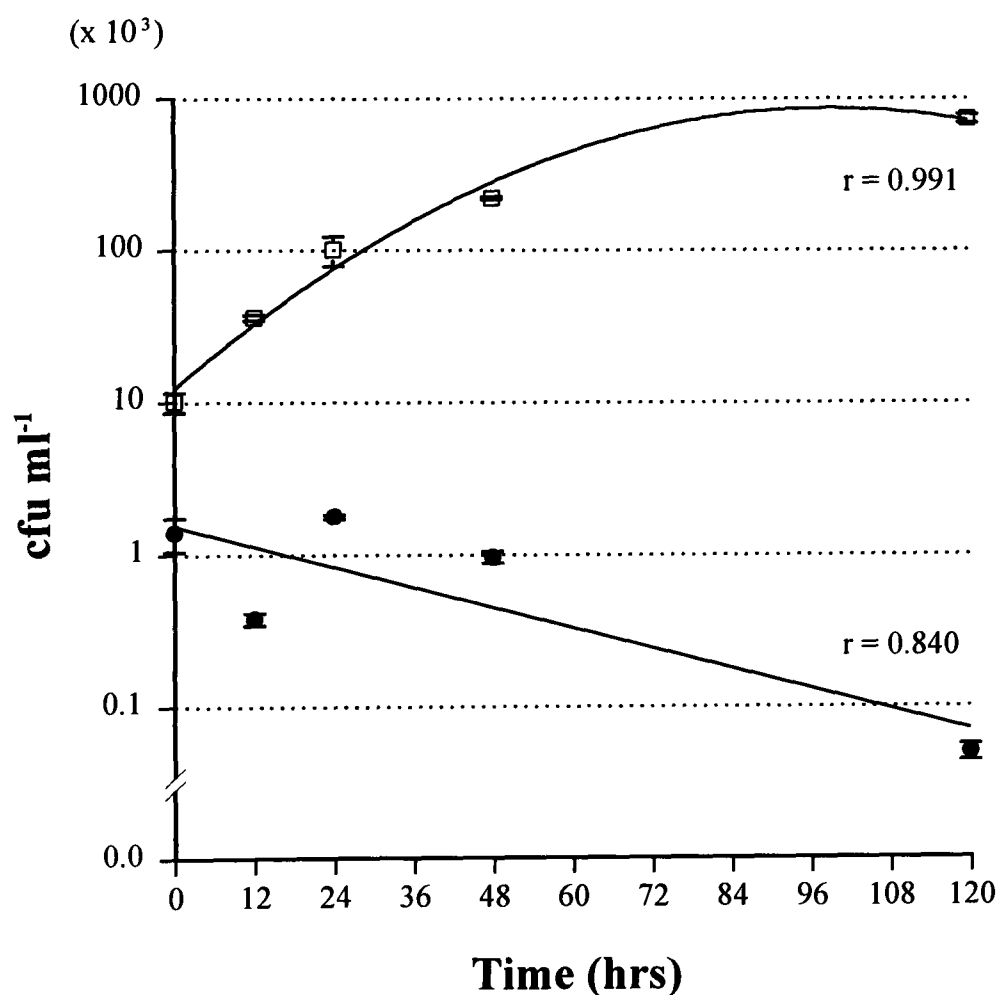


Figure 3.5.1: The proliferation of the total microbial contamination (cfu - colony forming units) in ANS (●), or LHS (□) over storage time at 20°C. Means $\pm$ se, n=3.

3.6 Discussion

The two seaweed preparations from the brown algae *Ascophyllum nodosum* (ANS), and *Laminaria hyperborea* (LHS), possessed considerable osmotic potential in the undiluted state. The cytoplasm of brown algae contains high amounts of organic compounds, and the sieve tube sap has OP values of -2.6 to -3.3 MPa, that are higher than sea water (Kirst and Bisson, 1979). The production process of the seaweed suspensions in this study resulted in maximum rupturing of cell components, releasing cytoplasm, disrupting the large vacuoles that contain high amounts of electrolytes, especially Cl^- , K^+ , and Na^+ , necessary for turgor pressure regulation in algae (Kirst and Bisson, 1979). LHS had a higher negative OP than ANS, reflected in higher conductivity measurements. Thus LHS had a higher electrolyte concentration based on more dissolved salts than ANS. Upon ashing similar values of OP were found for both suspensions, but these were substantially higher than the original suspensions. This was due to the destruction of organic molecules and the liberation of minerals during the ashing and re-dissolving process.

Mineral and trace element dry matter composition was similar between the SWS and their algal source material (Tables 1.3.3, and 3.3.7), further indicating a conservative production method. This is in contrast to other, harsher, extraction methods used for the production of other seaweed products, that may result in excessive accumulation of elements, such as chlorine, iodine, sodium, sulphur or potassium, unbalancing the product (Tables 1.5.2, and 3.3.7). Calculated on a fresh weight basis, the SWS contained lower mineral concentrations than fresh algae, reflecting the dilution step in the production process.

Comparisons between the mineral compositions of unaltered and modified SWS indicated differences in the distribution of elements in the fractions. This distribution was affected by the grade of mineral adsorption to solid particles or their molecular incorporation, and was different between SWS due to differences in the

degree of adsorption of minerals in relation to minerals dissolved. The disproportionately high levels of iron and zinc in the LHS solid fraction were most likely due to experimental errors, as indicated by the high standard errors. The reduction of certain trace elements in the ashed SWS fractions (Tables 3.3.2, and 3.3.3) could be explained by insoluble complex formation during re-dissolving the ash with concentrated HCl, pH adjustment and removal following filtration of the solutions.

Seaweed extracts produced by alkaline hydrolysis can have high pH levels (Abetz, 1980), even when diluted (Section 3.4). The original SWS used in this study possessed moderate pH values, within the range for optimal growth of most terrestrial plants (Mengel, 1991). Analyses of the pH-buffer potential of the SWS indicated a low buffer capacity for ANS, as shown by the slow re-adjustment of artificially increased pH, and a constant pH differences between the pH levels. A higher buffer capacity was found for LHS, as indicated by the rapid re-adjustment of the pH after artificial pH reduction (Figure 3.2.4). However, due to microbial activity detected in LHS after thawing (Figure 3.5.1), and further microbial degradation processes, initially aerobic but later anaerobic as the suspensions were not aerated, the pH decreased. In contrast, ANS had a biocidal effect, that resulted in a marked decline in the initial microbial population over time (Figure 3.5.1).

This may be due to higher concentrations of phenolic compounds in this SWS. Although no correction factor for interfering compounds was used, the polyphenol concentration established for the SWS were within the range of values found in previous analyses for the relevant algae species (Ragan and Jensen, 1977; 1978). Thus the method was reliable with reasonable accuracy for the determination of the polyphenol content. Ragan and Jensen (1978), who investigated the seasonal variation in phenols in *Ascophyllum nodosum*, found peak levels of 12.8 % (dw) in October. It is during this month that the source material for the two SWS under

investigation in this project was harvested. The polyphenol levels calculated for *Laminaria hyperborea* were between 0.3 % and 5.3 % (dw) (Ragan and Jensen, 1977), 0.8 % and 3.9 % (dw) (Ragan and Glombitza, 1986), and 1.1 % (dw) in this study which might not be high enough to keep microbial growth in LHS in check.

Most literature on algae and algal products was found to be concerned with growth regulating substances apparently present in various extracts. All growth regulating substances have been unequivocally identified by physico-chemical methods in brown algae (Section 1.3.7), but not all substances have been identified in *A. nodosum*, and *L. hyperborea* (Table 1.3.7). However, regardless of the chemical similarity to plant growth substances, the final decision that a substance is of a certain hormone type is made on the basis of its physiological activity. Therefore, bioassays are the ultimate basis for identifying plant growth substances. In some bioassays used in the past the presence of inhibitors in seaweed products has been observed (Blunden, 1977; Temple *et al.* 1989). The probable presence of inhibitors of hormonal activity interfering with growth promoting substances in SWS could have an effect on bioassay results. The interacting and sometimes inhibiting activity of hormones on each other also has to be taken into account (Thimann, 1992). However, from a practical point of view, the determination and measurement of the net activity on plant growth, including possible effects of inhibitors that may have been present in unmodified SWS was considered more important than exact hormone quantification. The SWS were thought to be applied to plants as unaltered product and not as purified fractions. Thus, the presence of growth regulating substances in the SWS was tested on unpurified samples.

In the present study, the estimation of growth regulating activity of the suspensions was attempted by various bioassays, with the exception of abscisic acid (ABA) determination which was performed using a radioimmunoassay method (Section 2.3.4). Results showed the presence of very low levels of ABA in both

SWS. In comparison, terrestrial plants contain 2 to 3 thousand times higher values (Table 3.3.5). On a dry weight basis, ANS contained one tenth of the ABA obtained by Boyer and Dougherty (1988) for the same algal species. This might reflect differences in sensitivity of the methods applied.

Auxin-like activity was found in SWS at levels similar to that of higher plant tissue (Table 3.3.5), or one green algae, *Caulerpa paspaloides* (Jacobs *et al.* 1985) and a commercial seaweed extract, **Maxicrop** (Sanderson *et al.* 1987), where the determination and identification of IAA was based on a physico-chemical method, GC-MS. In the present study, unpurified samples were tested, and the inhibition of root growth could have been induced by other factors, such as a high negative osmotic value, salt concentration, or the polyphenol content of the SWS (see Chapter 4, Section 4.5).

Cytokinin-like activity in ANS were found to be within the range of concentrations generally found in fresh algae material (Table 1.3.7), taking into account the dilution effect during the manufacturing of the SWS, but were lower than generally found in higher plant material (Table 3.3.5). It would be wrong, however, to assume that chlorophyll increase in cucumber cotyledons in this bioassay were entirely due to the cytokinins present, as it was shown in a recent paper by Whapham *et al.* (1993) that betaines were active in this particular bioassay. It is known that betaines are present in *A. nodosum* and *L. hyperborea* (Blunden *et al.* 1982, 1985). Although lower concentrations of LHS increased chlorophyll accumulation in the cucumber bioassay, no response was observed at higher concentrations. This may indicate the presence of inhibitors acting antagonistically to the presumed cytokinin-like compound in LHS. A similar result was found by Temple *et al.* (1989). Other commercial seaweed extracts contain much higher cytokinin concentrations (Section 1.5.2.2), which might variously reflect their degree of concentration; illustrate the

artificial addition of cytokinins; or simply the inaccuracy of the bioassays employed to detect this plant growth substance.

Gibberellin-like activity was not found in the SWS. The observed promotion of hypocotyl elongation elicited by both SWS was negligible, and a GA content, if present in the SWS was below the detection limit of the bioassay chosen (Figure 3.3.5). As unpurified samples were tested, the possibility of antagonistic effects due to other compounds, such as polyphenols was also possible (Corcoran *et al.* 1972).

Chapter 4

**Results of investigations on the effects
on barley and lettuce germination and
seedlings growth in the presence of SWS**

4.1 Introduction

In the literature, a major part of research reported on growth promotion responses after the application of seaweed extracts to plants (Section 1.7.2). The promotion of plant growth was attributed to the growth hormones present in the products. However, as levels of plant growth substances in the SWS investigated in this study are low, emphasis was focused on other compounds present in the SWS. To test physiological effect on plant growth, a monocotyledonous and a dicotyledonous plant species were investigated. Lettuce (*Lactuca sativa*), and barley (*Hordeum vulgare*), were used with both SWS under controlled conditions.

The initial experiment investigated the germination characteristics of the barley and lettuce varieties used in this study. To investigate the possible effects of the high osmotic potential (OP) of both SWS during seed imbibition, two different osmotic solutions, sodium chloride (NaCl) and polyethylene glycol (PEG), were applied to dry untreated seeds in a further experiment. Consecutive experiments were carried out on pre-germinated seeds, to exclude possible osmotic effects on seeds in the imbibition phase.

4.2 Experiment 4.1: The germination characteristics of diverse barley and lettuce cultivars

The germination characteristics of the two species tested were analysed. The purpose of this examination was to check on the seed quality and to enable the selection of a suitable germination parameter for the description of the performance of seed lots adequately, without the need to display numerous data sets.

a) Barley

After three days, germination ranged from 92 % to 97 % for the cultivars tested,

except for cv. Pastoral, that germinated 69 % ($p<0.001$) (Figure 4.2.1.a). However, after seven days all cultivars had a percentage germination of 94 % or above.

The MGT of the seed lots tested ranged from 36 to 51 hours, calculated over three days. Both seed lots of cv. Triumph exhibited the shortest MGT. The seed lot from 1993 germinated faster than the older seed lots ($p<0.001$) (Figure 4.2.1.b). The MGT calculated over a period of seven days changed for cv. Pastoral, as a significant number of seeds germinated after the third day (Figure 4.2.1.b).

The viability test indicated a high viability of the seed lots, with all varieties possessing between 97 % and 99 % viable seeds (Figure 4.2.1.c).

b) Lettuce

Lettuce germination ranged between 94 % to 99 % after three days. This was also the final germination result after seven days (Figure 4.2.2.a).

The MGT for the individual cultivars was between 24 to 28 hours (Figure 4.2.2.b), and the viability was between 97 % to 99 % (Figure 4.2.2.c).

4.3 Imbibition and early germination

4.3.1 Experiment 4.2: The germination of dry untreated seeds on SWS

Control seed germination of barley and lettuce was 92 % or 93 % after 7 days. In both species the germination was not affected by any SWS treatment applied (Figure 4.3.1).

With 50 % LHS, MGT for barley lengthened 51 % ($p<0.001$), compared to controls. For lettuce seeds 50 % ANS and LHS lengthened MGT by 29 % ($p<0.001$) or 100 % ($p<0.001$), respectively (Figure 4.3.2).

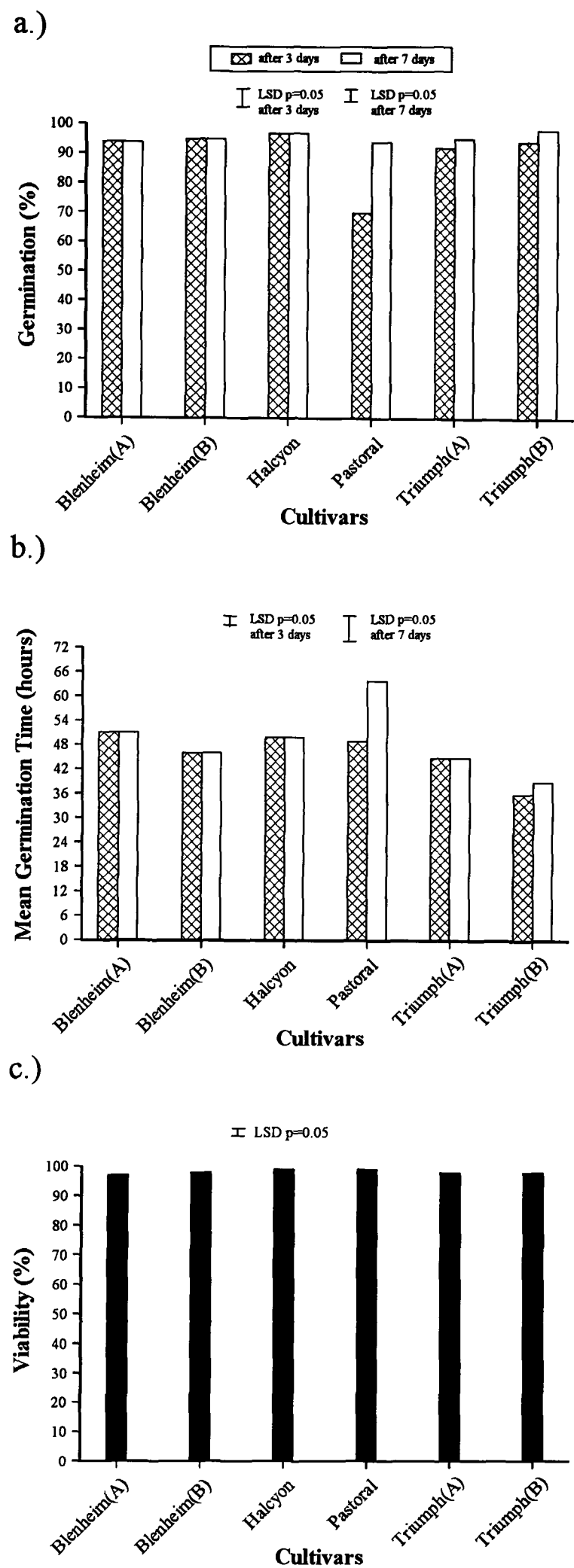


Figure 4.2.1: The germination characteristic of barley cultivars at 20°C in the dark. a.) Percentage germination after 3 or 7 days, b.) mean germination time over 3 or 7 days, and c.) viability (H₂O₂ test). n=3.

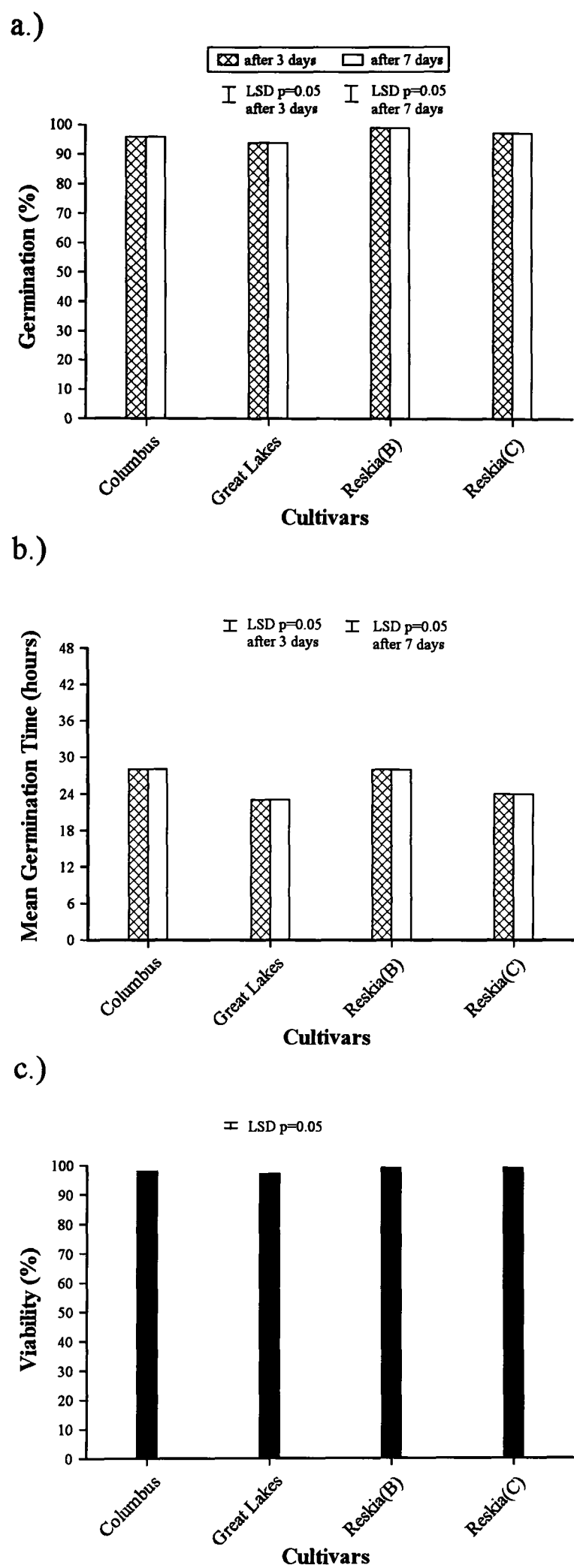
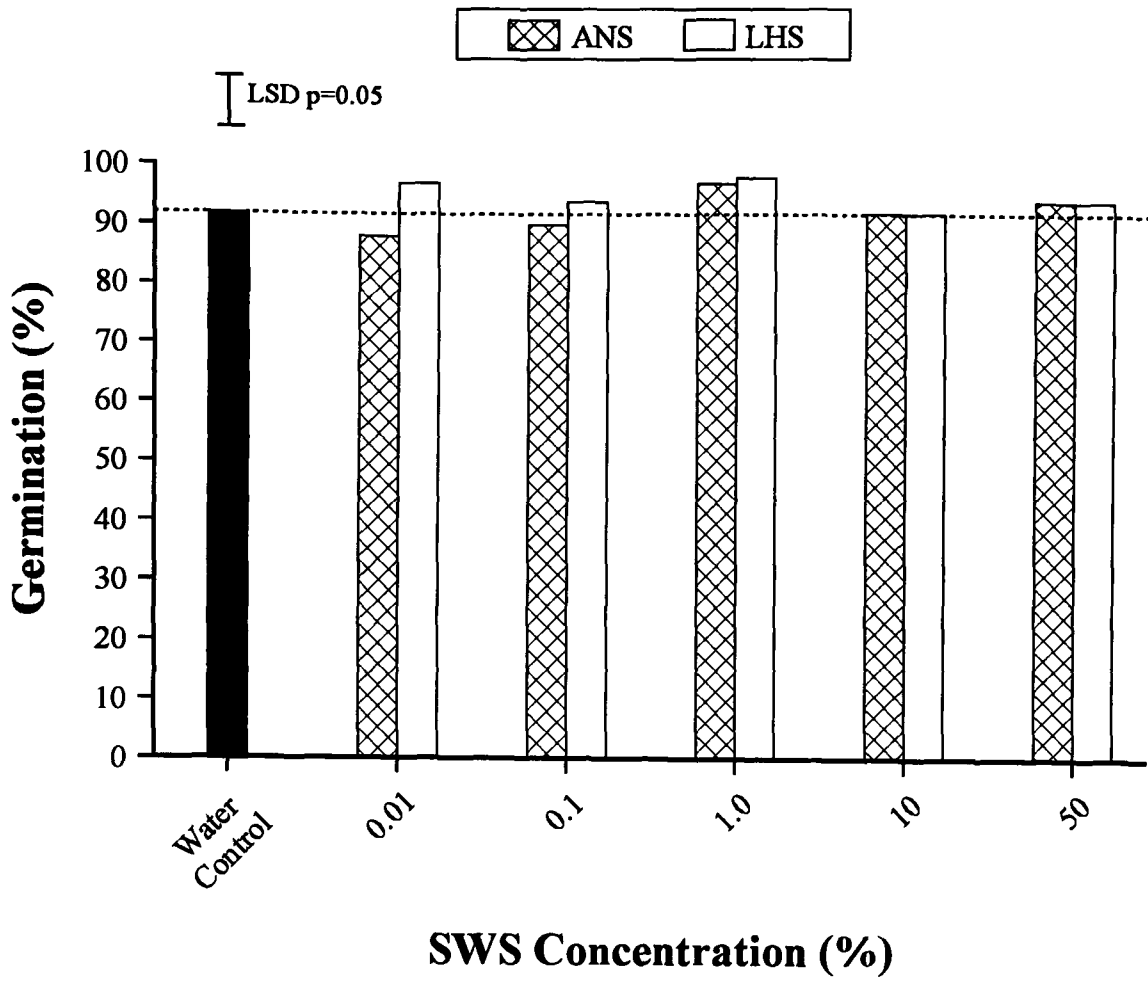


Figure 4.2.2: The germination characteristic of lettuce cultivars at 20°C in the dark. a.) Percentage germination after 3 or 7 days, b.) mean germination time over 3 or 7 days, and c.) viability. n=3.

a.)



b.)

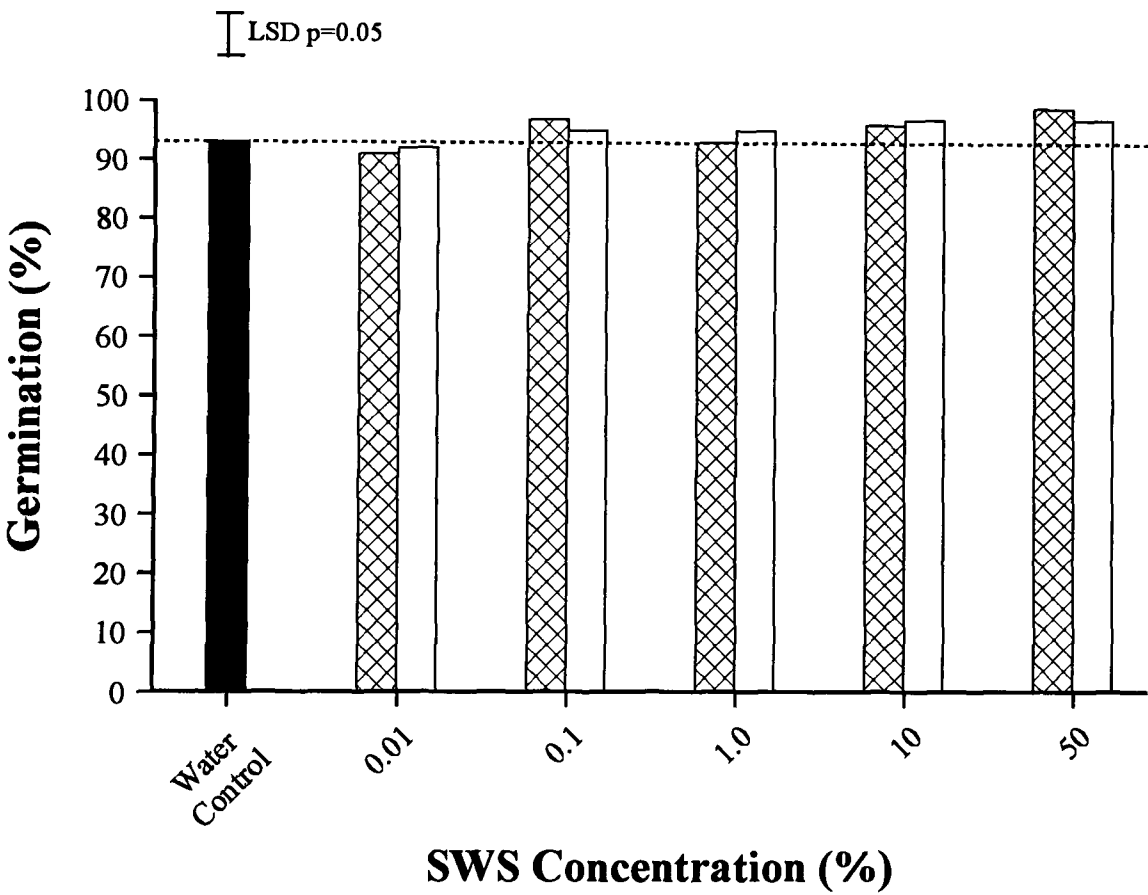


Figure 4.3.1: The effect of ANS and LHS at various concentrations on the percentage germination of a.) barley cv. Triumph(A), and b.) lettuce cv. Reskia(B), at 20°C after 7 days. n=4.

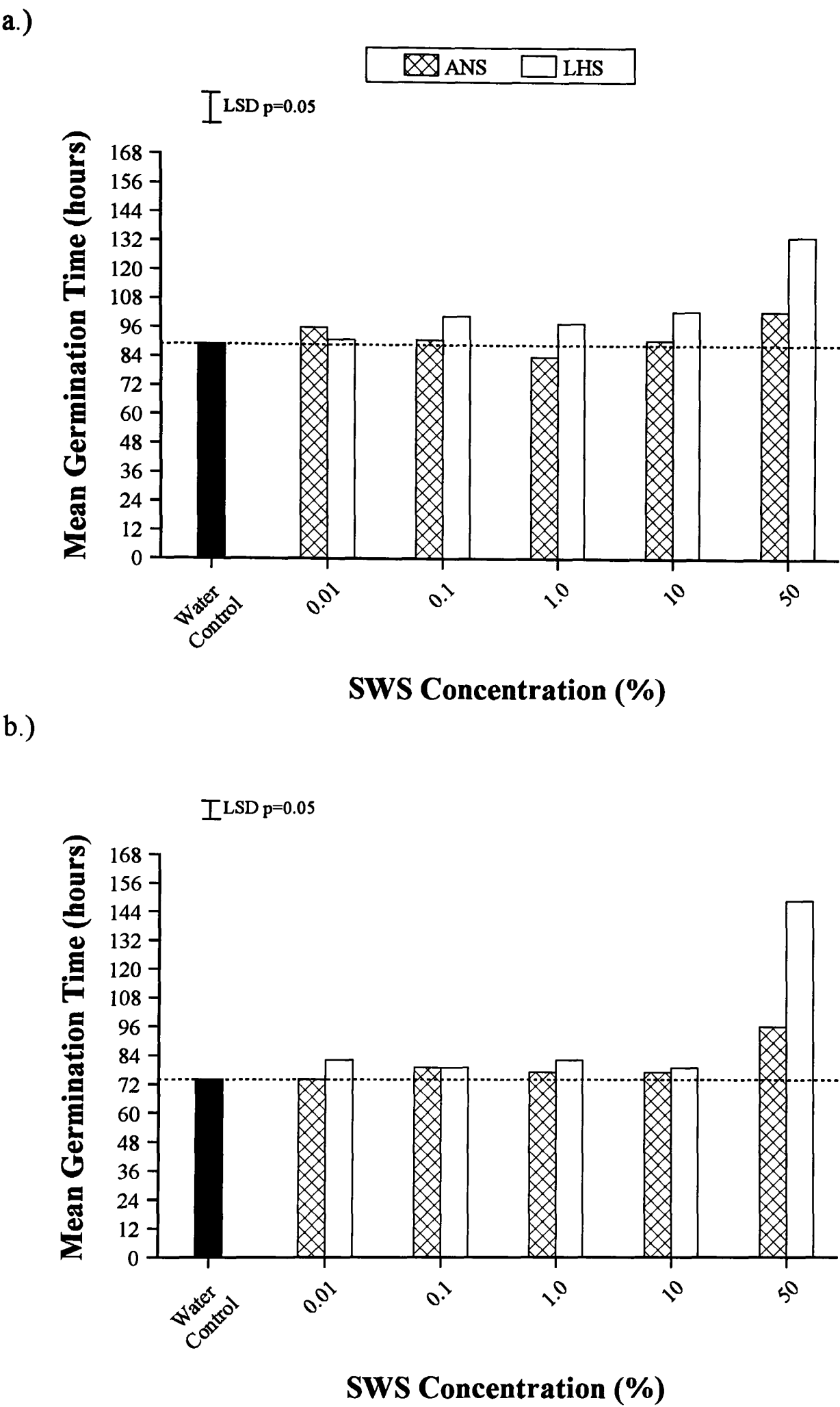


Figure 4.3.2: The effect of ANS and LHS at various concentrations on the mean germination time of a.) barley cv. Triumph(A) and b.) lettuce cv. Reskia(B), at 20°C over 7 days. n=4.

4.3.2 Experiment 4.3: The effect of LHS and osmotica on germination

Lettuce seed germination was not affected by any treatment (Figure 4.3.3.a). For barley seeds, however, germination percentage after three days was reduced 31 % ($p < 0.001$) when 50 % LHS was applied (Figure 4.3.3.a). No other treatment had any effect. Germination percentage after seven days was not increased over controls by any treatment.

The MGT increased, compared to the control, 56 % with LHS, and 45 % with PEG ($p < 0.001$) for barley seeds, and 100 % with LHS and 60 % with PEG ($p < 0.001$) for lettuce seeds (Figure 4.3.3.b). The MGT increased for barley seeds 17 % ($p < 0.05$), and lettuce 30 % ($p < 0.001$) with salt treatments (Figure 4.3.3.b).

4.3.3 Experiment 4.4: The comparative effect of SWS and osmotica on seed moisture content during imbibition

Water uptake by lettuce seeds exhibited an s-shaped curve (Figure 4.3.4), with a rapid uptake of water in the first 6 hours, from initial 6.5 % (dw) up to 59.9 % moisture content (dw), followed by a lag-phase of about 12 hours, during which the rate of water uptake slowed. After the lag-phase the rate of moisture increased (coinciding with the MGT), caused by rapid radicle expansion which ruptured the testa.

During the first phase of imbibition, water uptake by lettuce seeds in the presence of SWS at an OP of -0.5 MPa was not inhibited. After 6 hours, however, the moisture content of seeds imbibed on 100 % ANS (-0.25 MPa) and 100 % LHS (-0.5 MPa) were 2.1 % ($p < 0.01$) and 10.9 % ($p < 0.001$) less than seeds that had been imbibed in water (Figure 4.3.4). This difference increased over the lag-phase (18 hours) to 8.3 % and 14.0 % ($p < 0.001$). The difference in seed moisture between water and SWS soaked seeds, however, increased rapidly after 24 hours imbibition, at which point radicle protrusion of seeds imbibed on water soaked filter papers

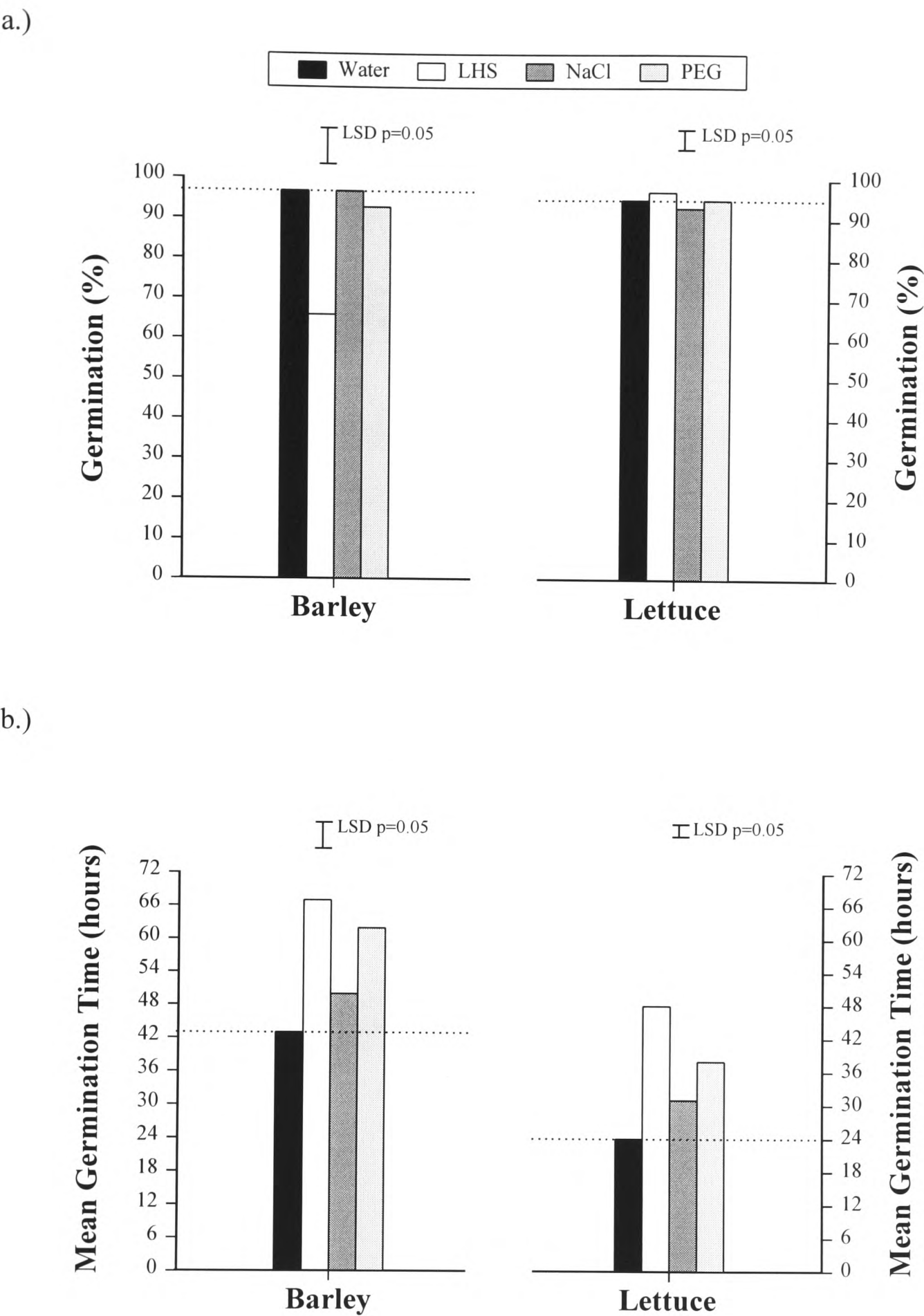
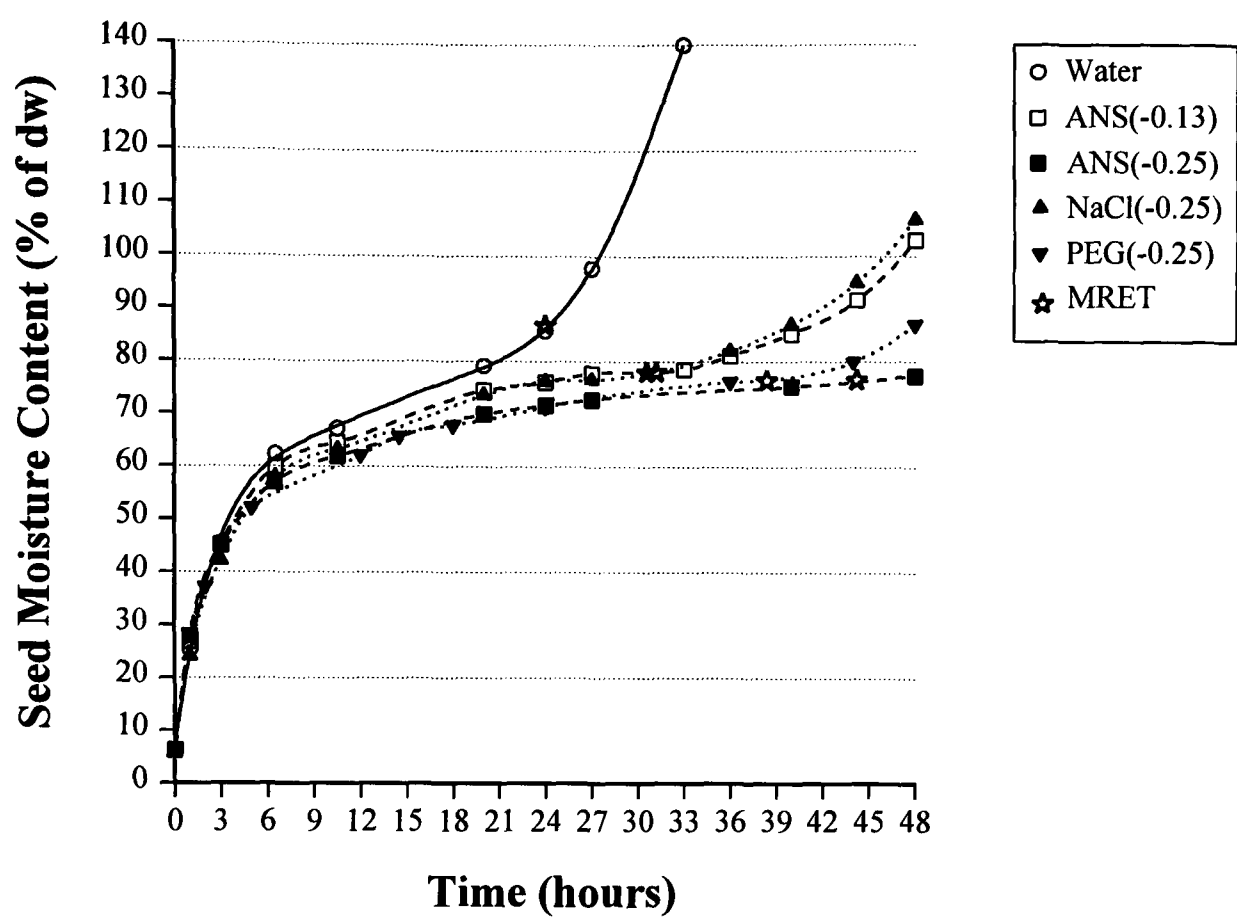


Figure 4.3.3: The effect of water, different osmotica and LHS (50 %) at OP of -0.25 MPa on a.) germination percentage, and b.) mean germination time, of barley cv. Triumph(A) and lettuce cv. Reskia(B), at 20°C over 3 days. n=3.

a.)



b.)

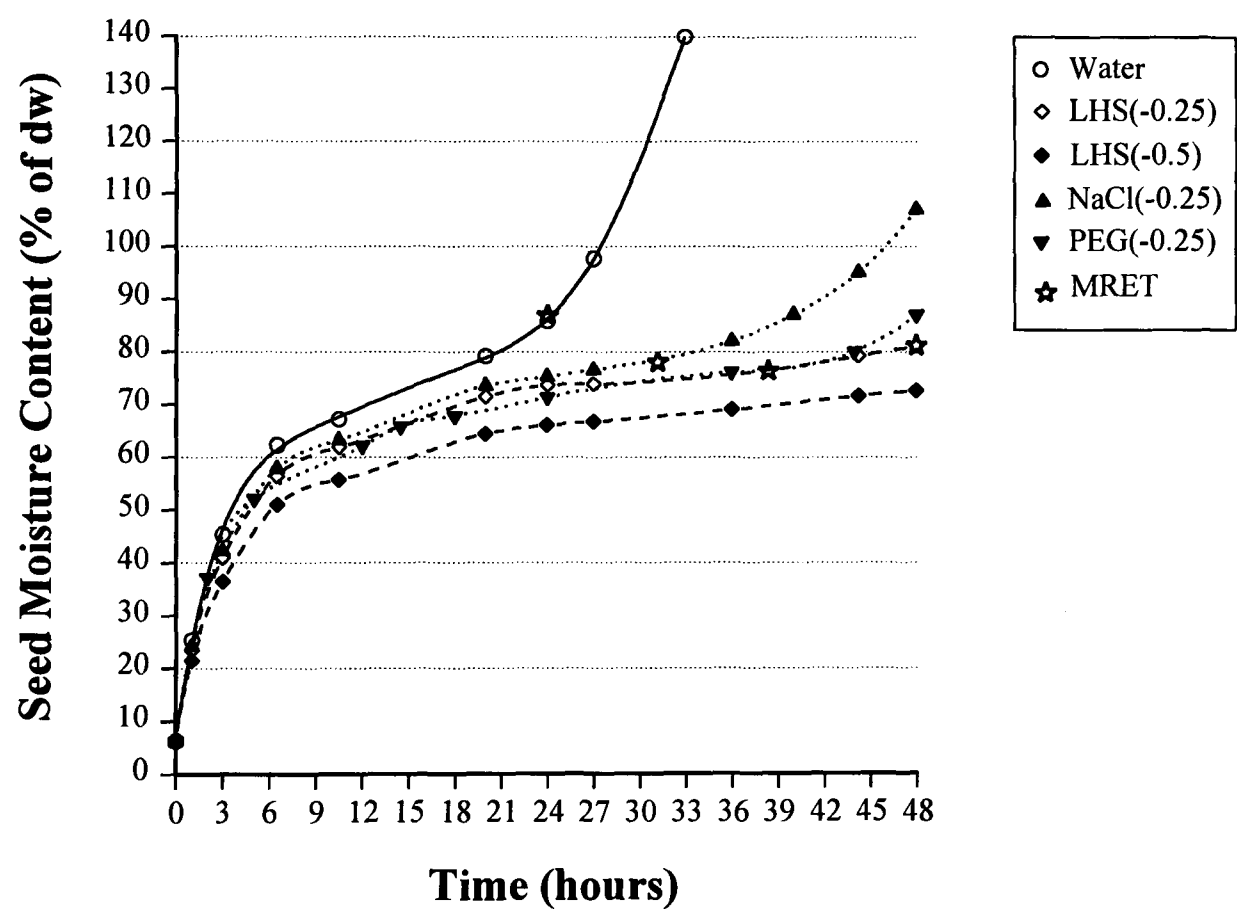


Figure 4.3.4: The effect of various osmotica or a.) ANS, or b.) LHS, on moisture uptake of lettuce cv. Reskia(B) seeds, imbibed on top of filter paper. Figures in brackets indicate the osmotic potential. n=3. MRET - mean radicle emergence time.

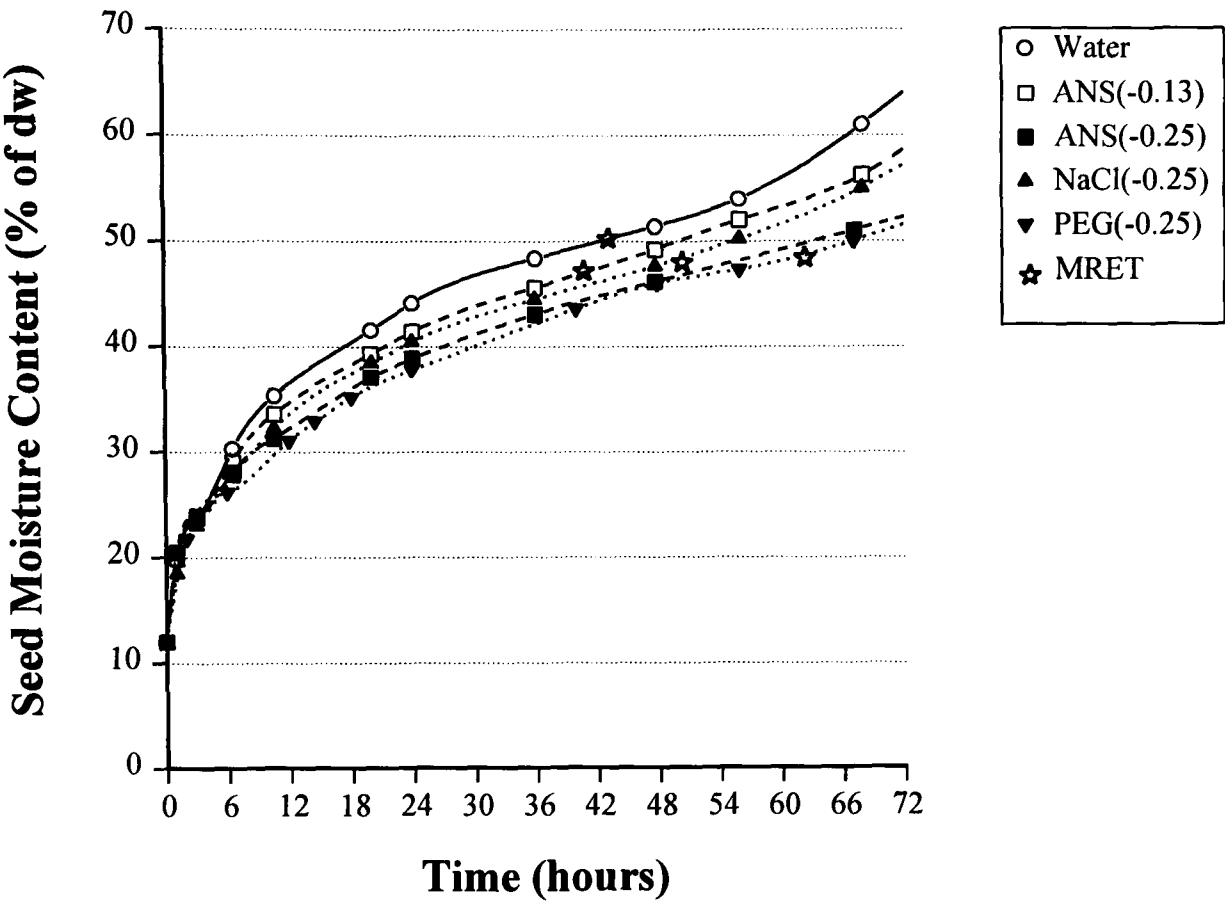
occurred (Figure 4.3.4). There was no difference in the moisture uptake of seeds treated with either SWS at equivalent OP (-0.25 MPa = ANS 100 %, LHS 50 %).

The imbibition rate of seeds germinated on osmotica was similar between the two SWS and PEG at equivalent OP's. Salt (NaCl) treated seeds imbibed faster than seeds treated with SWS or PEG over the whole imbibition period (Figure 4.3.4). Radicle protrusion of lettuce seeds imbibed in water occurred after 24 hours, at a seed moisture content of 85.5 % (dw). In the presence of NaCl and PEG at -0.25 MPa germination was delayed by 7 or 14 hours, respectively. Germination in these cases proceeded at seed moisture contents of 78.0 % and 76.0 %. When LHS was applied at -0.25 MPa (50 %) radicle protrusion was delayed by 24 hours, 10 hours beyond the values for PEG imbibed seeds, however the seed moisture content was 82.0 % (dw).

In contrast to lettuce seeds, the moisture content of water imbibed barley seeds, increased continuously but with a gradual decrease in the uptake rate, from 12.8 % (dw) up to a seed water content of 50.6 % (dw), after 43 hours imbibition, at which radicle protrusion occurred, and the uptake rate increased (Figure 4.3.5). The water uptake by barley seeds was inhibited according to the OP of the SWS or osmotica present. After 48 hours imbibition on ANS or LHS at -0.25 MPa (ANS 50 %; LHS 100 %) seed moisture content was 5.3 % or 3.8 % ($p < 0.001$) lower than water imbibed seeds (Figure 4.3.5). Seeds imbibed on osmotica had similar figures, but PEG tended to inhibit water uptake more severely than salt.

Radicle emergence of barley seeds imbibed in water occurred after 43 hours, at a seed moisture content of 50.3 % (dw). Seeds imbibed on NaCl or PEG exhibited a delayed radicle emergence of 7 hours ($p < 0.05$) or 19 hours ($p < 0.001$), at seed moisture contents of 48.0 % and 49.0 % (dw). While radicle protrusion of seeds on 50 % ANS was not inhibited, radicle protrusion of seeds imbibed on 50 % LHS was delayed by 24 hours, at a seed moisture content of 53.0 % (dw) (Figure 4.3.5).

a.)



b.)

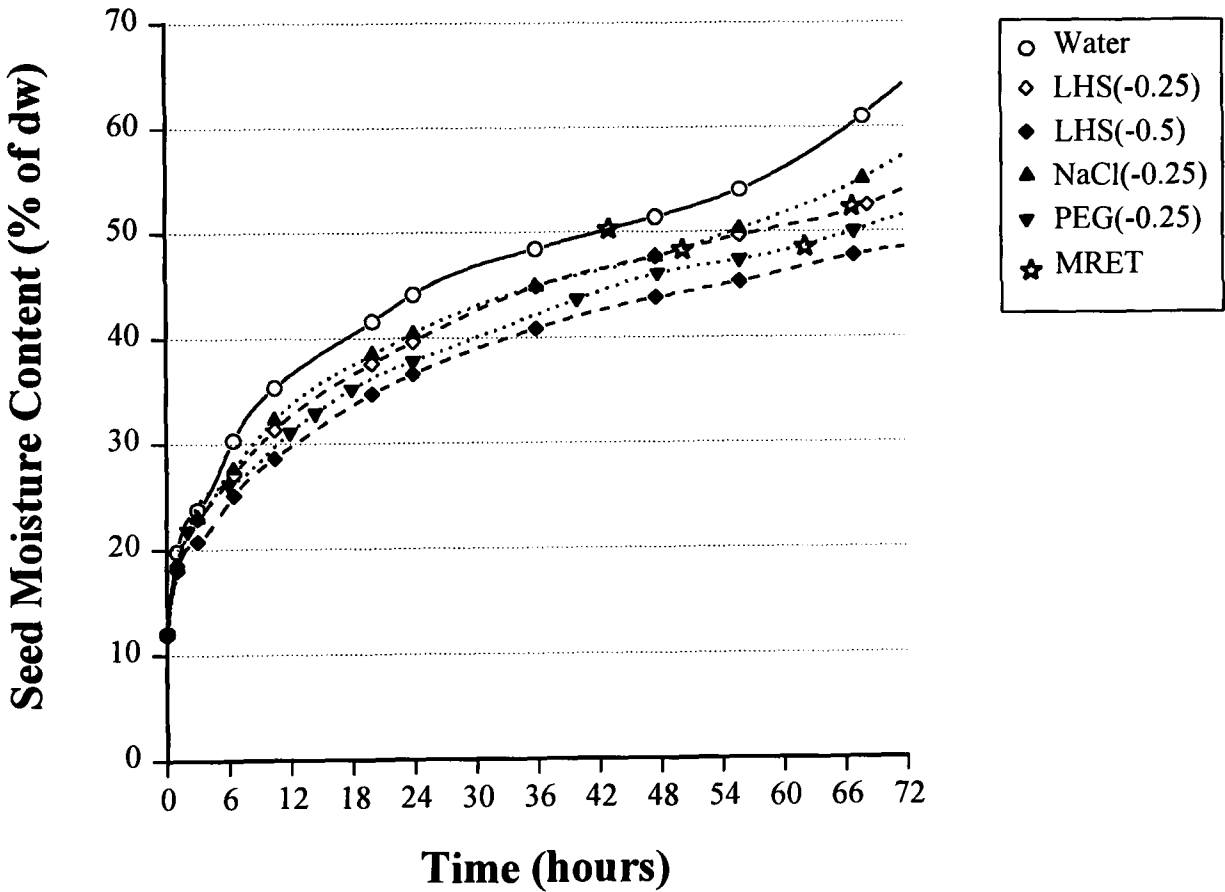


Figure 4.3.5: The effect of various osmotica or a.) ANS, or b.) LHS, on moisture uptake of barley cv. Triumph(A) seeds, imbibed on top of filter paper. Figures in brackets indicate the osmotic potential. n=3. MRET - mean radicle emergence time.

4.4 Experiment 4.5: The effects of SWS on the seedling growth of pre-germinated seeds

Introduction

The previous experiments indicated the possible presence of germination inhibiting compounds in both SWS. In Experiment 4.4 it was shown that the osmotic potential of both SWS had an inhibitory effect on seed imbibition, and delayed radicle emergence. To eliminate this inhibitory effect of SWS in the imbibition phase, seeds were pre-imbibed in distilled water and the seedlings exposed to the SWS. This procedure allowed the analysis of physiological effects of SWS on seedling growth exclusively, without interfering effects of the SWS in the imbibition phase.

Root length of barley

In the light, ANS between 0.001 % to 0.1 % did not affect root growth. At 1 % dilution of ANS, root growth was promoted 13 % ($p<0.01$). LHS between 0.001 % to 10 % had no effect. At 50 % or 100 % root growth was inhibited up to 57 % ($p<0.001$) by both SWS, compared to controls (Figure 4.4.1.a).

In the dark ANS up to 1 % and LHS up to 0.1 % did not alter root growth. Undiluted LHS inhibited root growth 43 % ($p<0.01$), and ANS 52 % ($p<0.01$) (Figure 4.4.1.b).

Coleoptile length of barley

Generally SWS inhibited coleoptile length. ANS at 10 %, 50 % or 100 % inhibited coleoptile growth 7 % ($p<0.01$), 68 %, and 73 % ($p<0.001$) in the light, or 22 % ($p<0.01$), 60 %, and 73 % ($p<0.001$) in the dark (Figure 4.4.2.a). LHS was less inhibitory in the light, and only undiluted suspensions reduced coleoptile length 21 % ($p<0.001$). In the dark inhibition with LHS at 10 %, 50 % and 100 % was 27 % ($p<0.01$), 17 % ($p<0.05$), and 42 % ($p<0.001$), respectively (Figure 4.4.2.b).

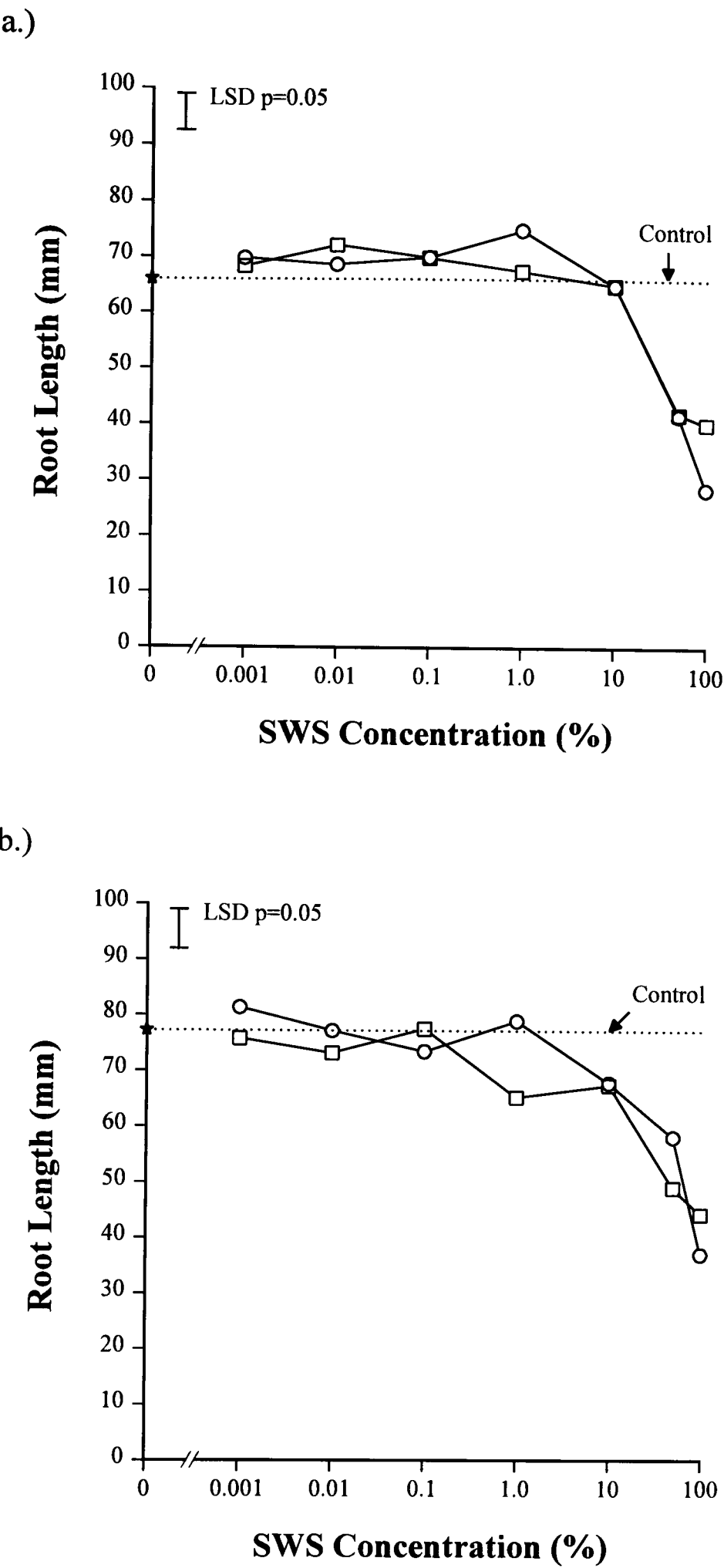


Figure 4.4.1: The effect of SWS at various concentrations on the root growth of pre-germinated seeds of barley cv. Triumph(A), on filter paper soaked with water (★), ANS (○), or LHS (□), after 4 days in the a.) light or b.) dark. n=40.

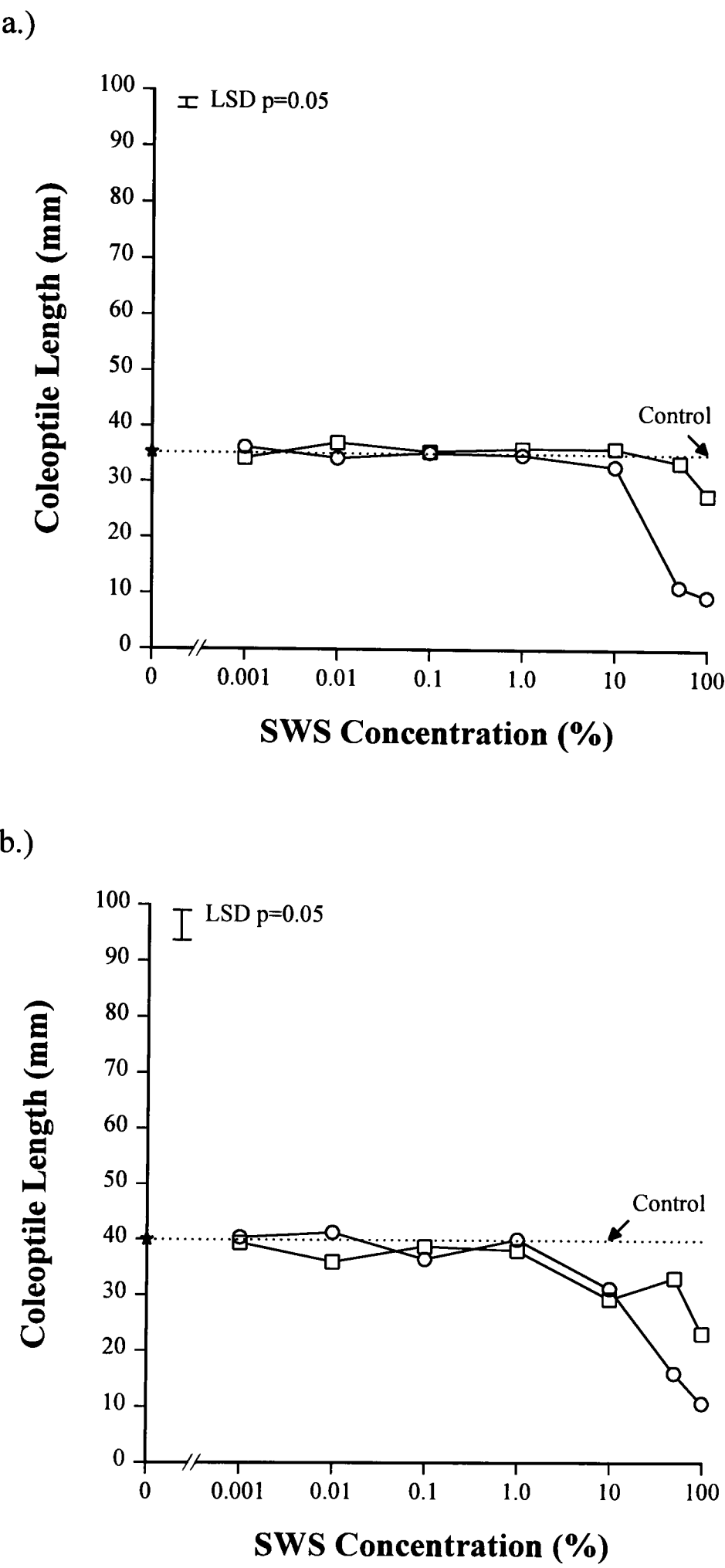


Figure 4.4.2: The effect of SWS at various concentrations on the coleoptile length of pre-germinated seeds of barley cv. Triumph(A), on filter paper soaked with water (★), ANS (○), or LHS (□), after 4 days in the a.) light, or b.) dark. n=40.

Shoot length of barley

With SWS applications up to 1 % shoot length was unaffected. ANS at 10 %, 50 %, or 100 % inhibited shoot growth 17 %, 79 %, or 82 % ($p<0.001$). LHS at 50 % or 100 % reduced shoot length 24 % and 45 % ($p<0.001$) (Figure 4.4.3).

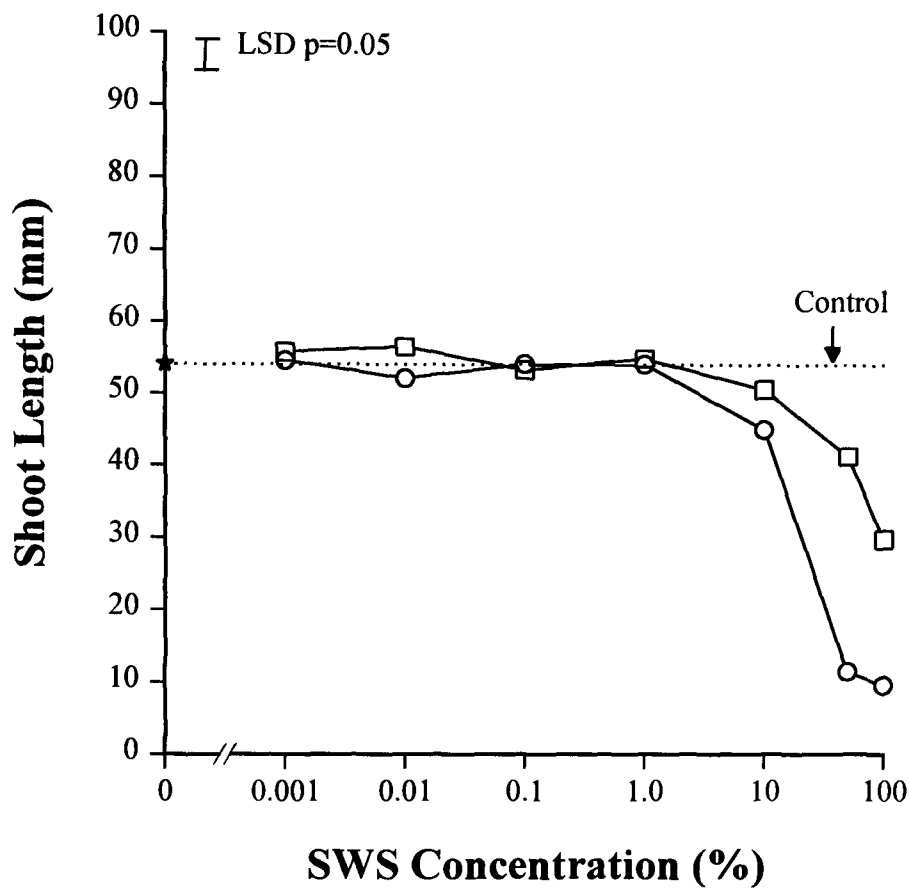


Figure 4.4.3: The effect of SWS at various concentrations on the shoot length of pre-germinated seeds of barley cv. Triumph(A), on filter paper soaked with water (★), ANS (○), or LHS (□), after 4 days in the light. n=40.

Root length of lettuce

In the light, root growth was promoted 6 % ($p<0.05$) due to applications of ANS at dilutions of 0.01 % (Figure 4.4.4.a). At higher concentrations root growth was unaffected or inhibited. Root length was progressively inhibited when either SWS was applied at or above 10 % in the light or dark (Figure 4.4.4).

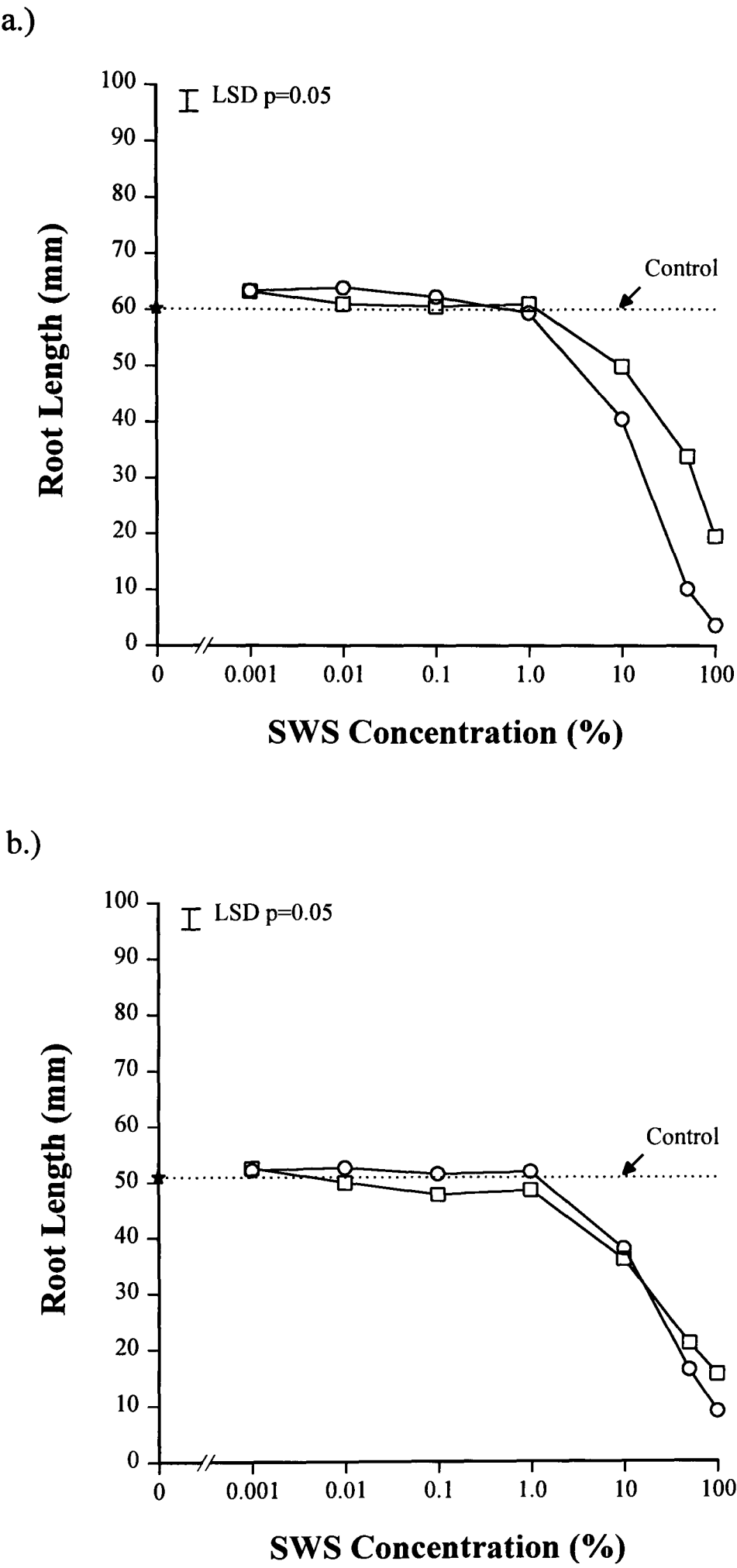


Figure 4.4.4: The effect of SWS at various concentrations on the root growth of pre-germinated seeds of lettuce cv. Reskia(B), on filter paper soaked with water (★), ANS (○), or LHS (□), after 4 days in the a.) light, or b.) dark. n=32.

Cotyledon size of lettuce

Cotyledon expansion in the light was promoted by all ANS treatments, except for the 0.001 % dilution. The highest effect with ANS was obtained with 1 % that caused an increase in cotyledon area of 67 % ($p<0.001$). ANS at or above 50 % concentration inhibited cotyledon expansion 37 % ($p<0.001$). LHS at 0.001 % to 0.1 % did not increase cotyledon size. LHS at or above 1 % promoted cotyledon expansion, with 50 % LHS giving an increase in size of 179 % ($p<0.001$) (Figure 4.4.5.a).

Hypocotyl length of lettuce

In the dark, dilutions of 1 % ANS promoted hypocotyl elongation 15 % ($p<0.01$), and at 10 % ANS 51 % ($p<0.001$). Whereas ANS applied at 50 % inhibited hypocotyl length 17 %, and at 100 % by 29 % ($p<0.001$). All LHS treatments, except 0.1 % and 0.01 %, promoted elongation, with the best treatment being 1 % LHS that induced a 29 % increase ($p<0.001$) over control seedlings (Figure 4.4.5.b).

Root morphology of barley and lettuce seedlings

In the presence of SWS, barley and lettuce seedlings not only developed shorter roots, but also root morphology was affected. Barley roots were greyish in colour with SWS treatments at 50 % and above. ANS at 50 % or above, caused curled roots. This effect was not observed with LHS. Lettuce seedling roots subjected to ANS applications above 50 % were curled, brownish with necrotic tips. Seedling roots that resulted from LHS applications above 50 % were yellowish necrotic and spindly, with a few adventitious roots emerging along the upper part of the root.

Root hair number and length of barley seedlings

The application of unaltered SWS also had effects on root hair length and number (Figure 4.4.6). Treatment with SWS above 10 % dilution caused an inhibition

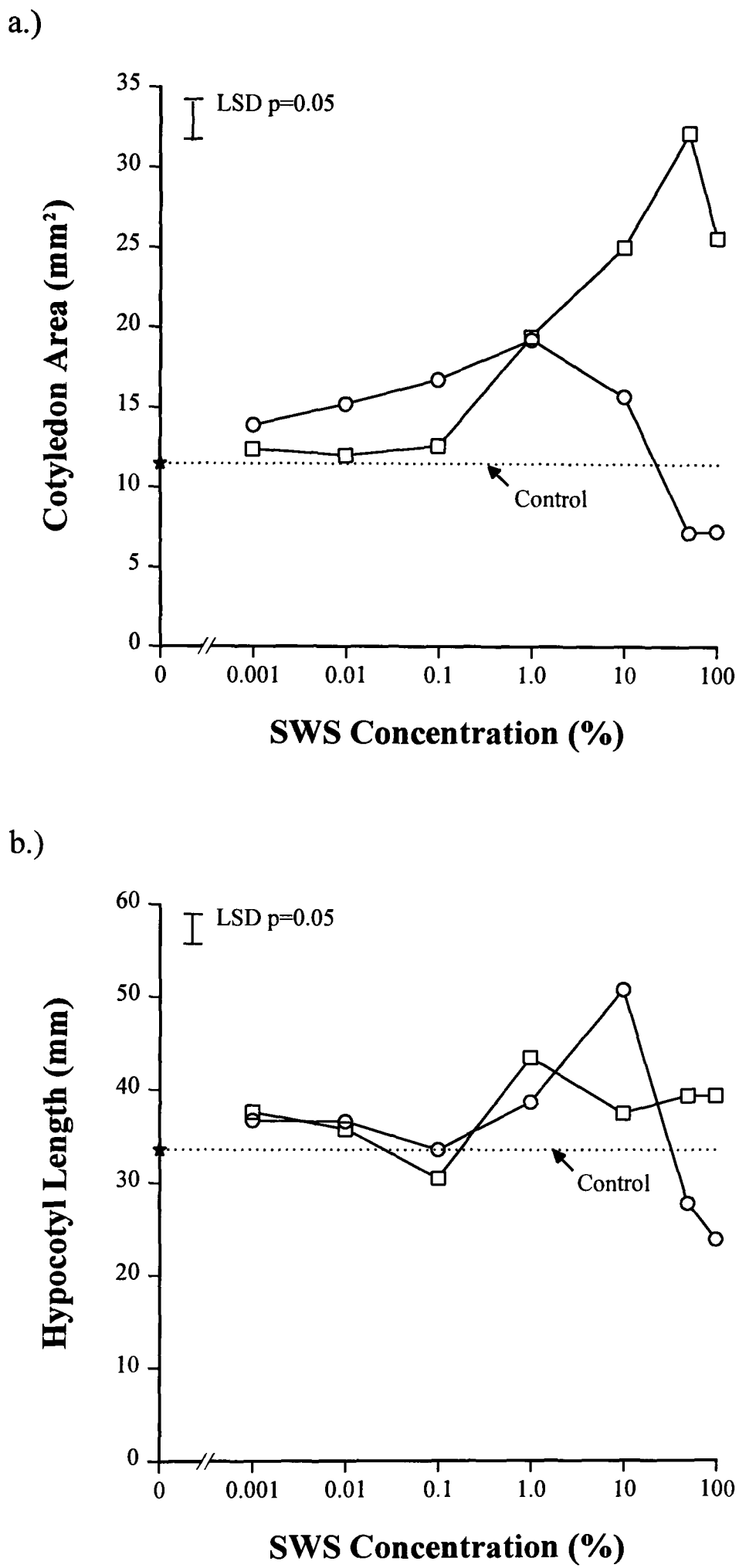


Figure 4.4.5: The effect of SWS at various concentrations on a.) the cotyledon expansion in the light, and b.) hypocotyl elongation in the dark, of pre-germinated seeds of lettuce cv. Reskia(B), on filter paper soaked with water (★), ANS (○), or LHS (□), after 4 days. n=40.

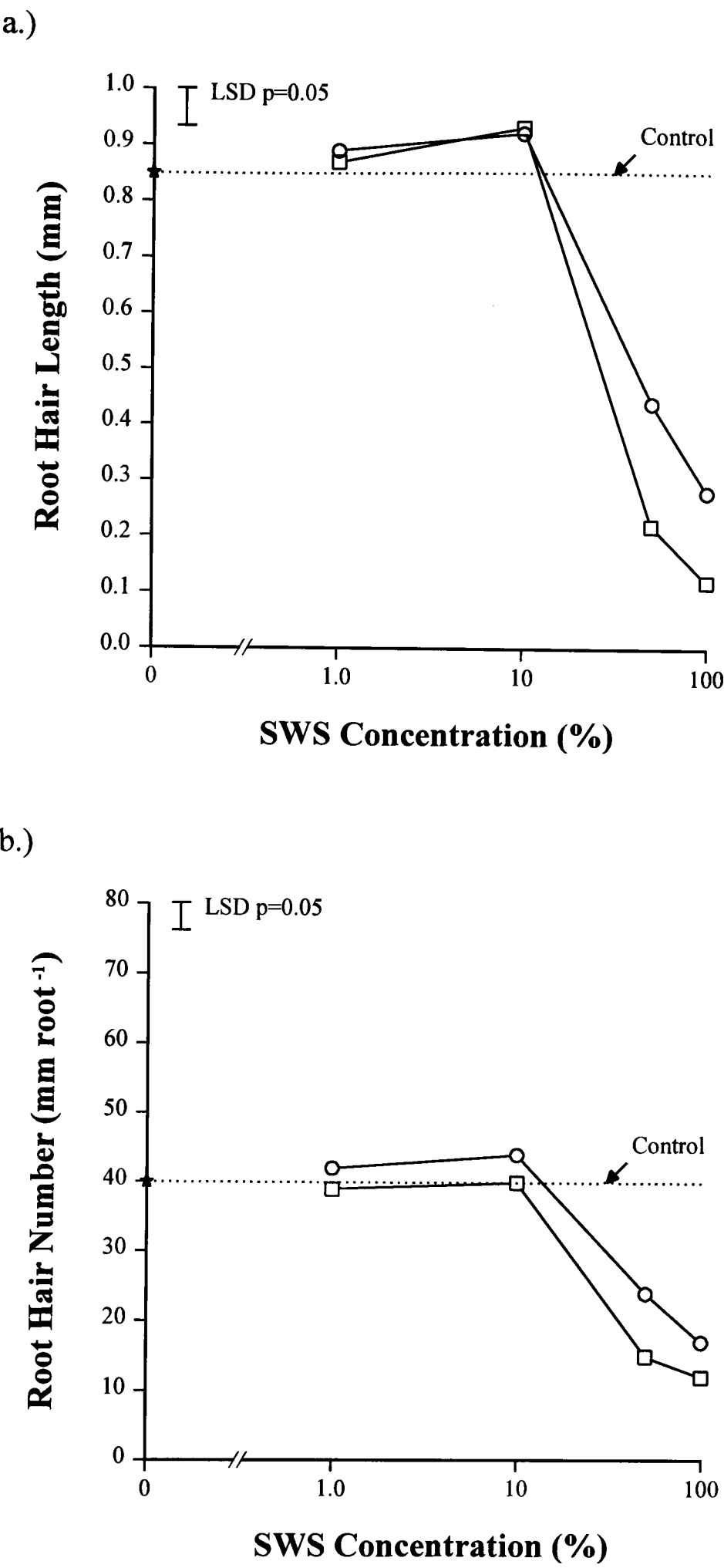


Figure 4.4.6: The effect of SWS at various concentrations on a.) the root hair length ($n=12$), and b.) root hair number ($n=5$), on roots of pre-germinated seeds of barley cv. Triumph(A), after 4 days in the light. ANS ($\circ$), LHS ($\square$), water control ($\star$).

of the root hair length by up to 67 % or 86 % ($p<0.001$) with undiluted ANS or LHS, and a reduction in root hair number by up to 57 % or 79 % ($p<0.001$) by applications of undiluted ANS or LHS (Figure 4.4.6).

Root hair number and length of lettuce seedlings

Application of SWS above 10 % progressively reduced root hair number, and 72 % or 62 % ($p<0.001$) fewer root hairs were present compared to control seedlings when 50 % ANS or LHS was applied, and furthermore the length of those root hairs was reduced 96 % or 94 % with the same treatments (Figure 4.4.7) (Plate 4.4.1). The application of undiluted SWS resulted in the total suppression of root hairs.

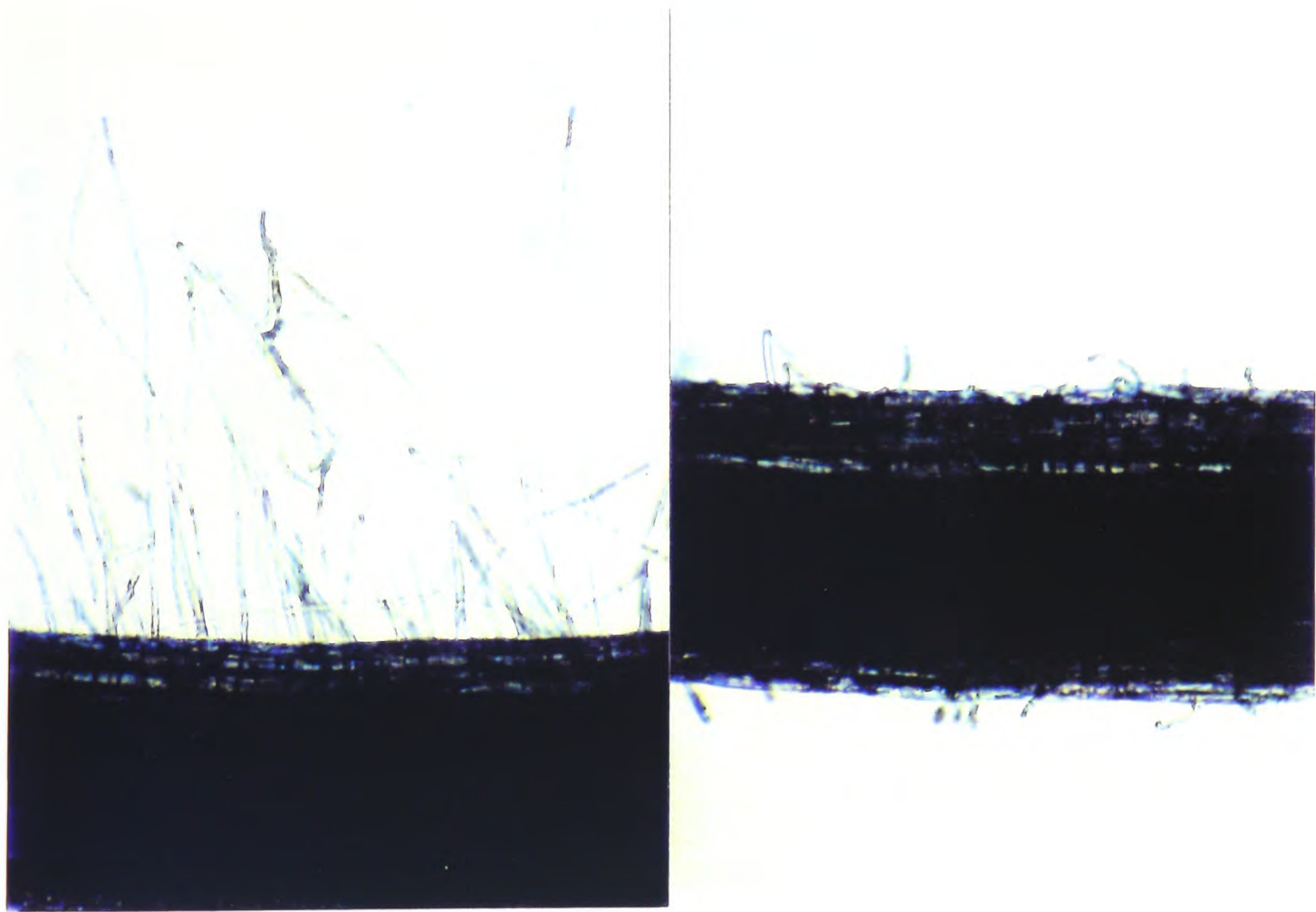


Plate 4.4.1: The appearance of root hairs on lettuce cv. Reskia(B) roots in the presence of distilled water (left), or 50 % LHS (right), after 4 days in the light.

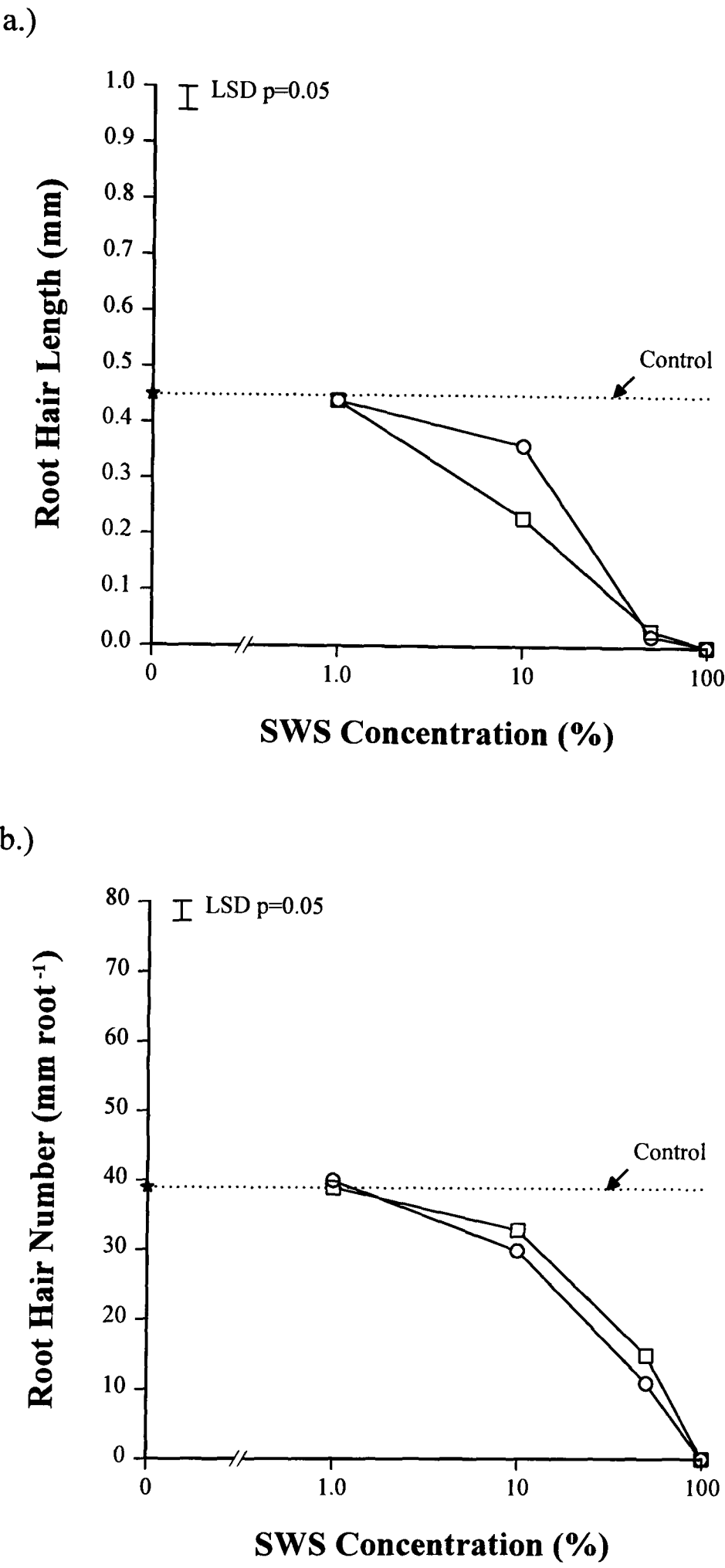


Figure 4.4.7: The effect of SWS at various concentrations on a.) the root hair length (n=12), and b.) root hair number (n=5), on roots of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days, in the light. ANS (○), LHS (□), water control (★).

4.5 Factors affecting root growth of lettuce seedlings

Introduction

In previous experiments (Section 4.3.3), it was shown that a high negative OP could affect imbibition, and subsequent germination. The following experiments were designed to analyse the effect of osmotic solutions alone on the inhibition of root growth of pre-germinated seedlings. For these experiments diverse osmotica were tested and compared with the effect of SWS for their degree of root growth inhibition.

In addition, the presence of phenolic compounds in the SWS (Ragan and Glombitza, 1986) may also affect root development. Thus, phloroglucinol treatments were compared with the effect of SWS on pre-germinated lettuce seeds.

Finally, the application of modified SWS may produce further evidence on the nature of the root growth inhibiting caused by SWS.

4.5.1 Experiment 4.6: The effects of osmotica on lettuce root growth

Root length

All osmotic treatments, whether conducted in the light or dark, progressively reduced root length, except for PEG at -0.25 MPa in the dark (Figure 4.5.1.b). At all osmotic potentials PEG exhibited the least and NaCl the strongest growth inhibition. At -0.25 MPa, (equivalent to ANS 100 %), -0.5 MPa (equivalent to LHS 100 %), and -0.65 MPa, PEG inhibited root growth in the light 10 %, 48 %, and 60 % ($p < 0.001$), respectively. In the dark -0.5 MPa and -0.65 MPa decreased root length 19 %, and 73 % ($p < 0.001$). For OP's at -0.25 MPa, -0.5 MPa and -0.65 MPa, NaCl inhibited root growth in the light 53 %, 71 %, and 73 % ($p < 0.001$), and in the dark 44 %, 52 %, and 60 % ($p < 0.001$) (Figure 4.5.1). Although the root length of lettuce seedlings was reduced in the presence of osmotica, the roots appeared white, with no signs of necrotic tissue.

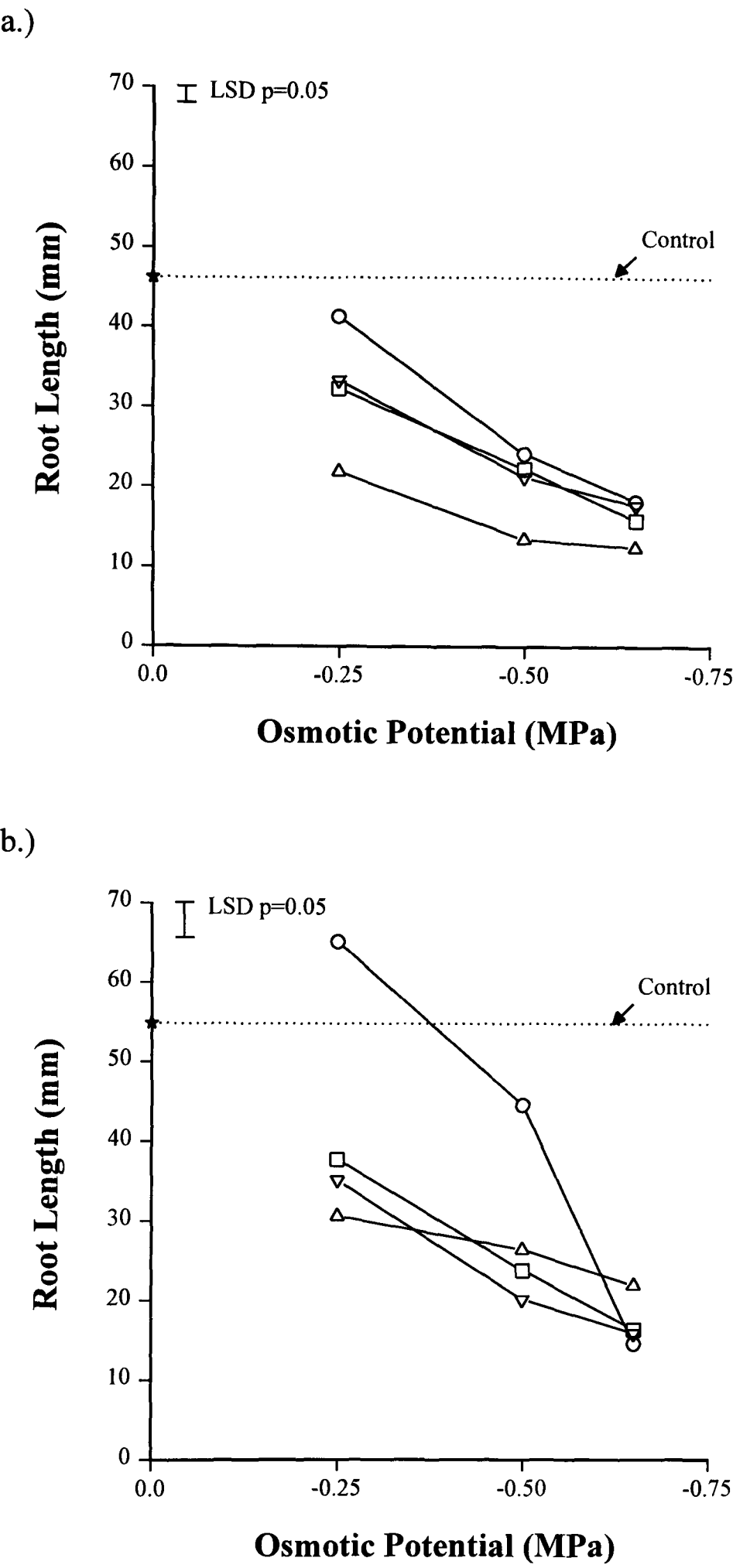


Figure 4.5.1: The effect of osmotica at various osmotic potentials on the root length of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days in a.) light, or b.) dark. Mannitol (□), PEG (○), NaCl (Δ), KCl (▽), water control (★). n=32.

Root hairs

Root hair number was reduced by 16 % ($p<0.05$) by mannitol in the light at an OP of -0.65 MPa. PEG treatments reduced the number of root hairs at all OP's by up to 44 % ($p<0.001$) in the light, and by up to 24 % in the dark. Conversely, use of NaCl or KCl increased root hair number progressively with increasing OP by up to 44 % or 42 % ($p<0.001$) in the light, and 62 % or 45 % ($p<0.001$) in the dark, respectively (Figure 4.5.2).

Mannitol had no effect on root hair length in the light, and KCl had no effect in either the light or dark. PEG progressively reduced root hair length up to 87 % ($p<0.001$) with increasing OP, irrespective of growth conditions. At an OP of -0.65 MPa NaCl reduced root hair length by around 19 % ($p<0.01$) in the light or dark, respectively. On the contrary, at an OP of -0.25 MPa NaCl increased root hair length by 14 % ($p<0.05$) under both conditions (Figure 4.5.3).

4.5.2 Experiment 4.7: The effect of phloroglucinol on lettuce root growth***Root length***

Root growth was promoted 17 % ($p<0.001$) in the light with phloroglucinol at 5.6 $\mu\text{g ml}^{-1}$, and 20 % ($p<0.001$) at 22.5 $\mu\text{g ml}^{-1}$ and 90 $\mu\text{g ml}^{-1}$ compared to the control. At 360 $\mu\text{g ml}^{-1}$ (equivalent approximately to 6.3 % of the phenol content in ANS, or 90 % of the phenol content in LHS) growth was inhibited 39 % ($p<0.001$). When seedlings were grown in the dark, the inhibition of root growth was more severe. At 90 $\mu\text{g ml}^{-1}$ the inhibition was 47 % ($p<0.001$) which increased further with higher phloroglucinol concentrations (Figure 4.5.4). Phloroglucinol at 360 $\mu\text{g ml}^{-1}$ or greater resulted in brown, partially necrotic roots under both growth conditions.

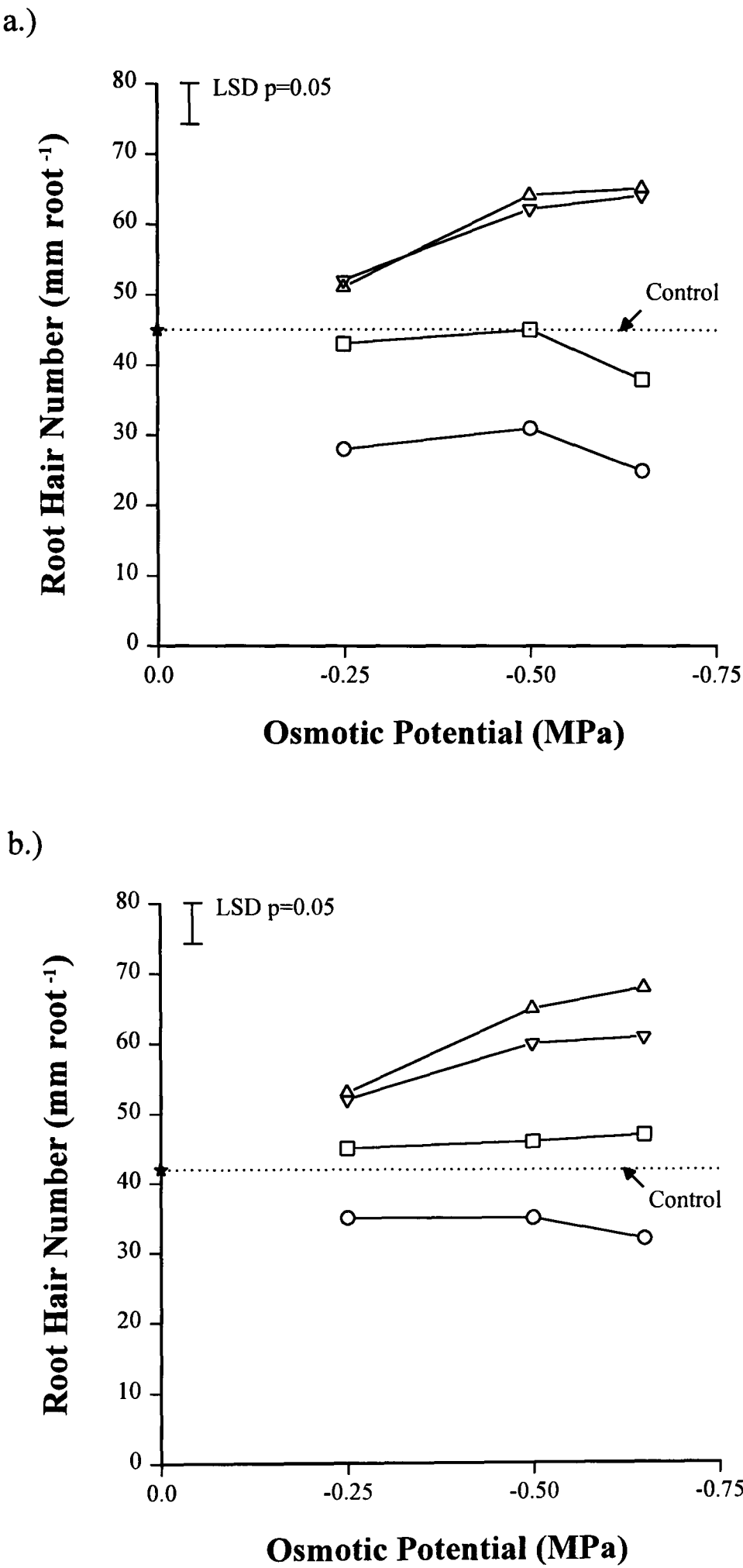


Figure 4.5.2: The effect of osmotica at various osmotic potentials on the root hair number per mm root of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days, in a.) the light, or b.) dark. Mannitol (□), PEG (○), NaCl (Δ), KCl (▽), water control (★). n=5.

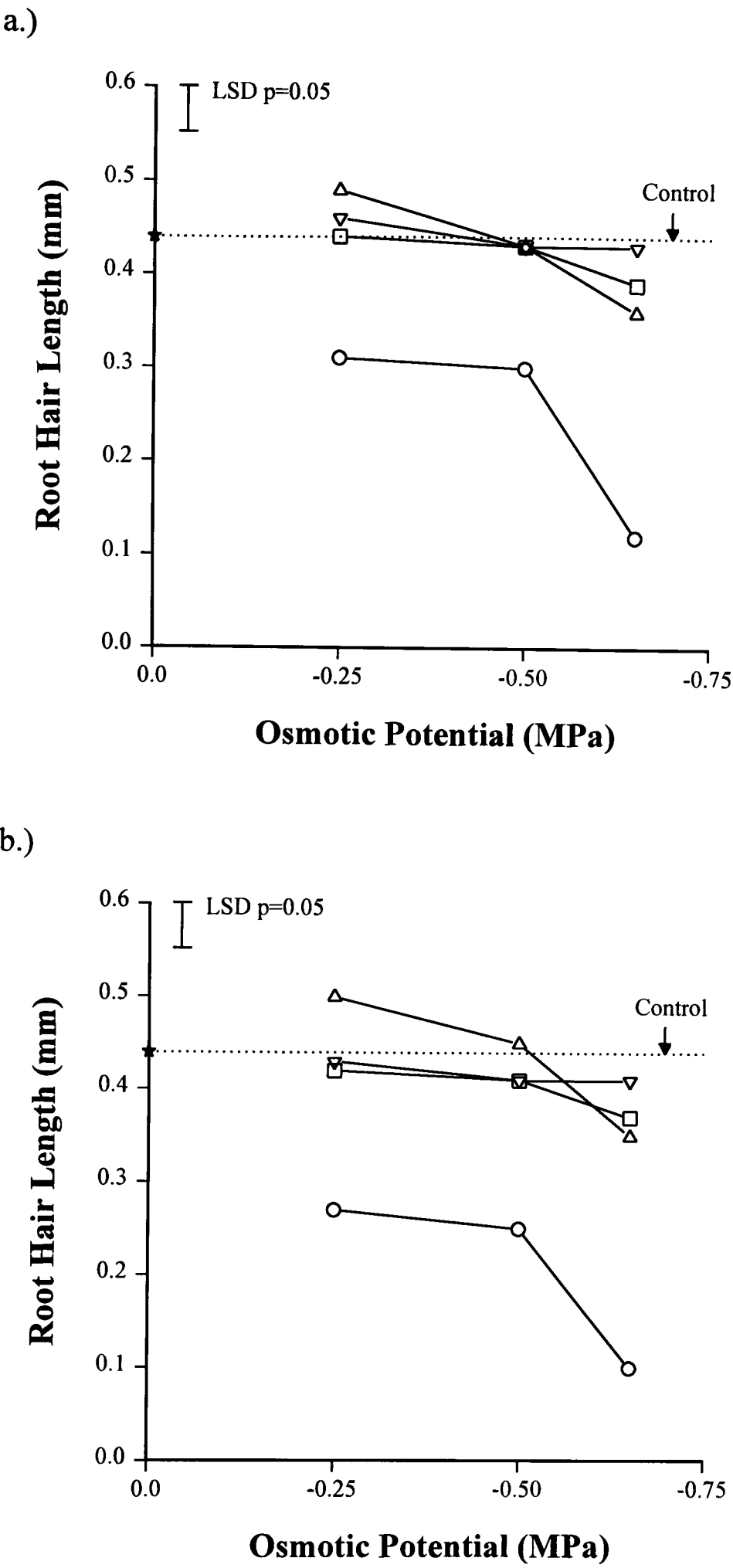


Figure 4.5.3: The effect of osmotica at various osmotic potentials on the root hair length of roots of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days, in a.) the light, or b.) dark. Mannitol (□), PEG (○), NaCl (Δ), KCl (▽), water control (★). n=12.

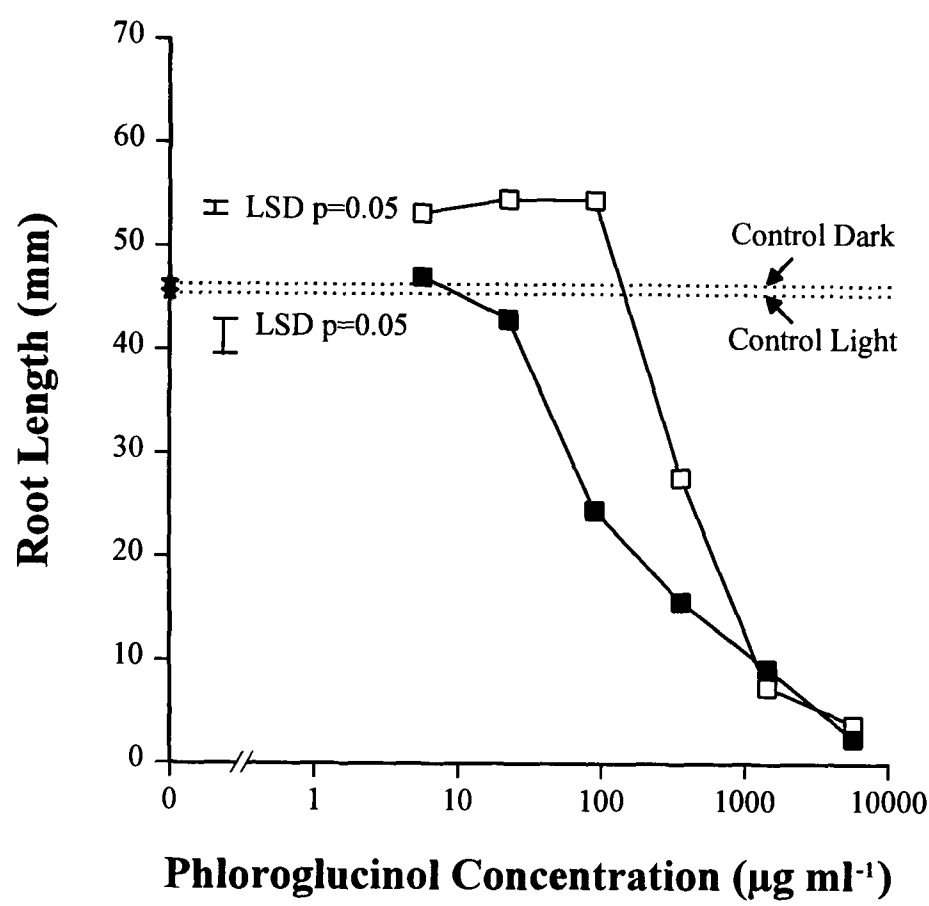


Figure 4.5.4: The effect of phloroglucinol at various concentrations on the root length of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days in the light (□), or dark (■). Water control (★). n=32.

Root hairs

No reduction in root hair number with phloroglucinol concentrations up to 90 µg ml⁻¹ was observed under both growth conditions. At a concentration of 360 µg ml⁻¹ root hair number was reduced 68 % (p<0.001), and root hair length inhibited 77 % (p<0.001). Higher concentrations totally inhibited root hair formation. At 360 µg ml⁻¹ in the light root hair number increased 26 % (p<0.01). However, at 1440 µg ml⁻¹ root hair number was reduced 26 % (p<0.01) and root hair length reduced 46 % (p<0.001). At 5760 µg ml⁻¹ no root hairs were found on the roots (Figure 4.5.5).

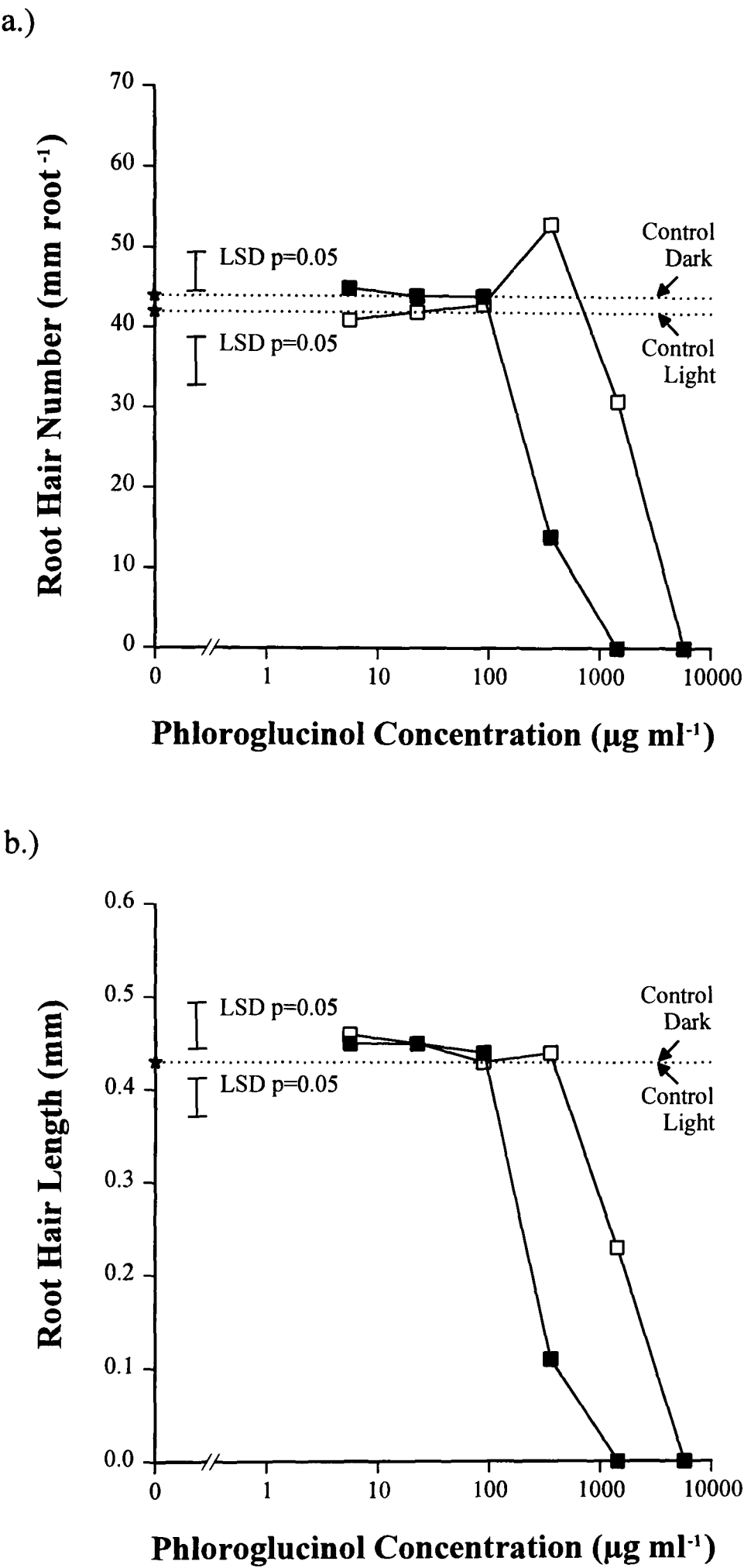


Figure 4.5.5: The effect of phloroglucinol at various concentrations on the a.) root hair number (n=5), and b.) root hair length (n=12), on roots of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days in the light (□), or dark (■), water control (★).

4.5.3 Summary of effects of individual factors on lettuce root growth

Table 4.5.1 summarises the results of experiments reported in Sections 4.4, 4.5.1 and 4.5.2. The table shows that there was a close correlation between root growth inhibition and phenol concentration for ANS in the light and LHS in the dark. Application of osmotica had a less inhibitory effect on root growth compared to the other treatments. In the dark ANS caused less inhibition than corresponding phloroglucinol treatments. There was less correlation between inhibition caused by LHS compared with phloroglucinol in the light (Table 4.5.1). This was because LHS contained only 7 % of the phenol concentrations found in ANS. At such a low concentration root growth was promoted by phloroglucinol (Figure 4.5.4.).

Table 4.5.1: Summary of results of diverse experiments mimicking SWS properties on the growth inhibition of roots of pre-germinated lettuce cv. Reskia(B) seedlings. Values in % inhibition compared to the appropriate control treatments.

Condition	Light			Dark		
Concentration (%)	Seaweed suspension	Osmotic potential (mean of osmotica) ²	Poly-phenols	Seaweed suspension	Osmotic potential (mean of osmotica) ²	Poly-phenols
ANS						
10	-33	n.d.	-54	-25	n.d.	-70
50	-83	n.d.	-87	-68	n.d.	-89
100	-94	-30	-91	-82	-23	-95
LHS						
10	-17	n.d.	+20	-29	n.d.	-20
50	-44	-30	-10	-59	-23	-59
100	-68	-56	-45	-69	-47	-68

(n.d. - not determined; ² - adjusted to appropriate OP's at respective SWS concentration)

4.5.4 Experiment 4.8: The effect of modified SWS on lettuce root growth

For this experiment 10 % was chosen as a standard concentration as root growth of lettuce seedlings was distorted at SWS concentrations at or above 50 % (Section 4.4).

The application of ashed ANS in the light increased root length 14 % (p<0.01) (Table 4.5.2). In the dark this treatment reduced root length 19 % (p<0.001). Treatments with the ANS solid fraction inhibited root growth 35 % (p<0.001) in the light or dark. Original ANS inhibited root growth 35 % (p<0.001) in the light, or 38 % (p<0.001) in the dark. The supernatant fraction of ANS inhibited root growth 16 % (p<0.001) in the dark

Ashed LHS inhibited root growth 13 % (p<0.001); original LHS 22 % (p<0.001) in the light. This increased to 32 % or 40 % (p<0.001) in the dark. The LHS solid fraction inhibited root growth 19 % (p<0.001) only in the dark. The greatest root growth inhibition was caused by LHS supernatant (86 %, p<0.001 in the light, but only 32 %, p<0.001 in the dark) (Table 4.5.2). The roots of seedlings grown in the light on LHS supernatant were brown and necrotic, of thin spindly appearance and with no visible root hairs present (Plate 4.5.1).

Table 4.5.2: The effects of SWS or modified SWS at 10 % concentration to seedling root growth of pre-germinated seeds of lettuce cv. Reskia(B) after 4 days in the light or dark. n=32.

Condition	Light				Dark			
SWS	ANS		LHS		ANS		LHS	
Modification	root length (mm)	% of Control	root length (mm)	% of Control	root length (mm)	% of Control	root length (mm)	% of Control
Control	55.2	100	49.0	100	56.8	100	52.1	100
Ashed	62.9 **	114	42.6 **	87	46.0 ***	81	35.6 ***	68
Original	36.1 ***	65	38.5 ***	78	35.0 ***	62	31.3 ***	60
Solids	35.8 ***	65	51.4 NS	105	36.7 ***	65	42.5 ***	81
Supernatant	52.8 NS	96	6.7 ***	14	42.2 ***	74	35.5 ***	68
LSD (DF 10)								
p=0.05	4.3		4.3		3.9		3.2	
p=0.01	6.1		6.1		5.5		4.6	
p=0.001	8.8		8.8		8.0		6.6	

(² - different from the control at * = 5%, ** = 1%, and *** = 0.1% probability level, NS = not significant)



Plate 4.5.1: The appearance of roots of lettuce cv. Reskia(B) in the presence of distilled water (top) or 10 % LHS supernatant (bottom) after 4 days in the light.

4.6 An investigation of the behaviour of the inhibitory compound(s) identified in LHS supernatant on lettuce root growth

Introduction

The following experiments were undertaken to further elucidate the characteristics of the possible compound(s) identified in LHS that caused root growth inhibition of lettuce seedlings in the light. As the inhibitory effect of LHS supernatant was only observed under illumination, the experiments were all carried out in the light, except for experiments where light was excluded from individual seedling parts.

4.6.1 Experiment 4.9: Dose response of lettuce roots to LHS supernatant

Root length

Original LHS at 10 % (equivalent to 8.2 % LHS supernatant, see Section 2.2.1) reduced root length 41 % ($p<0.001$). With 5 % LHS supernatant the root length was reduced 17 % ($p<0.001$), and was progressively reduced further with increased concentrations applied (Figure 4.6.1). In LHS supernatant concentrations up to 8.2 %, roots were white without necroses, while at 10 % roots were brown and necrotic.

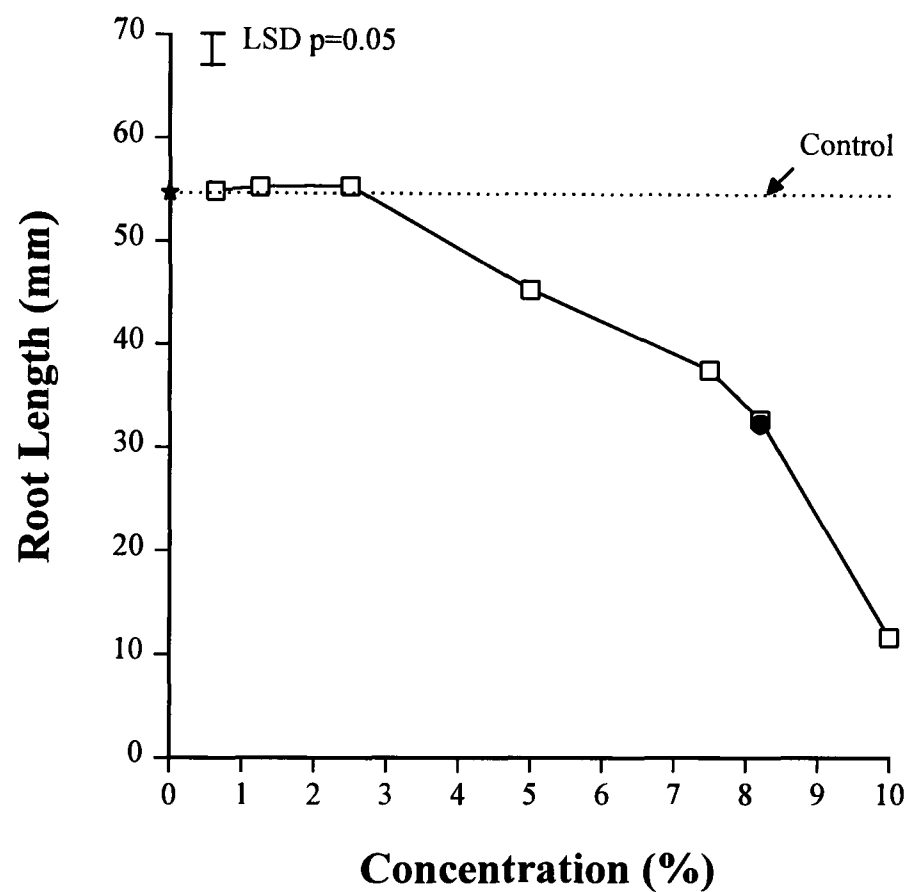


Figure 4.6.1: The effect of LHS supernatant at various concentrations (□), or 10 % original LHS (●), on the root length of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days in the light. Water control (★). $n=24$.

Root hairs

In the presence of 5 %, 8.2 %, or 10 % LHS supernatant the number of root hairs was reduced 33 %, 28 %, or 59 % ($p<0.001$), while at 7.5 % the number of root hairs

was almost doubled (Figure 4.6.2.a). Original LHS (10 %) produced 15 % ($p<0.001$) fewer root hairs.

The length of root hairs after treatment with 5 % LHS supernatant were reduced 29 % ($p<0.001$). At 8.2 % or 10 % the root hair length was reduced 96 % ($p<0.001$). Treatment with 10 % original LHS, only reduced root hair length 49 % ($p<0.001$) (Figure 4.6.2.b).

4.6.2. Experiment 4.10: The effect of LHS supernatant on root growth of lettuce cultivars

The average root lengths of control seedlings of cv. Columbus, Great Lakes, Reskia(B), or Reskia(C), were 38.1, 45.4, 54.2, or 55.3 mm (Table 4.6.1). When seedlings were treated with 10 % original LHS, the root length was inhibited 18 %, 26 %, 35 % or 31 % ($p<0.001$), respectively. In the presence of 10 % LHS supernatant, the root growth was further reduced to between 79 % to 88 % ($p<0.001$) (Table 4.6.1).

Table 4.6.1: The effect of 10 % LHS supernatant on the effect of root growth inhibition of pre-germinated seeds of diverse lettuce cultivars in the light. n=24.

	Root length						
SWS modification	Control		Original		Supernatant		
Variety	(mm)	% of Control	(mm)	% of Control	(mm)	% of Control	<i>LSD (DF 6) p= 0.05/0.01/0.001</i>
Columbus	38.1	100	31.0	81	8.0	21	2.9 / 4.4 / 7.0
Great Lakes	45.4	100	33.8	74	9.7	21	2.2 / 3.3 / 5.4
Reskia(B)	54.2	100	35.2	65	6.5	12	2.1 / 2.6 / 4.2
Reskia(C)	55.3	100	38.3	69	9.8	18	5.4 / 8.2 / 13.2
<i>LSD (DF 8)</i>							
<i>p=0.05</i>	2.1		<i>NS</i>		0.6		
<i>p=0.01</i>	3.1				0.9		
<i>p=0.001</i>	4.7				1.3		

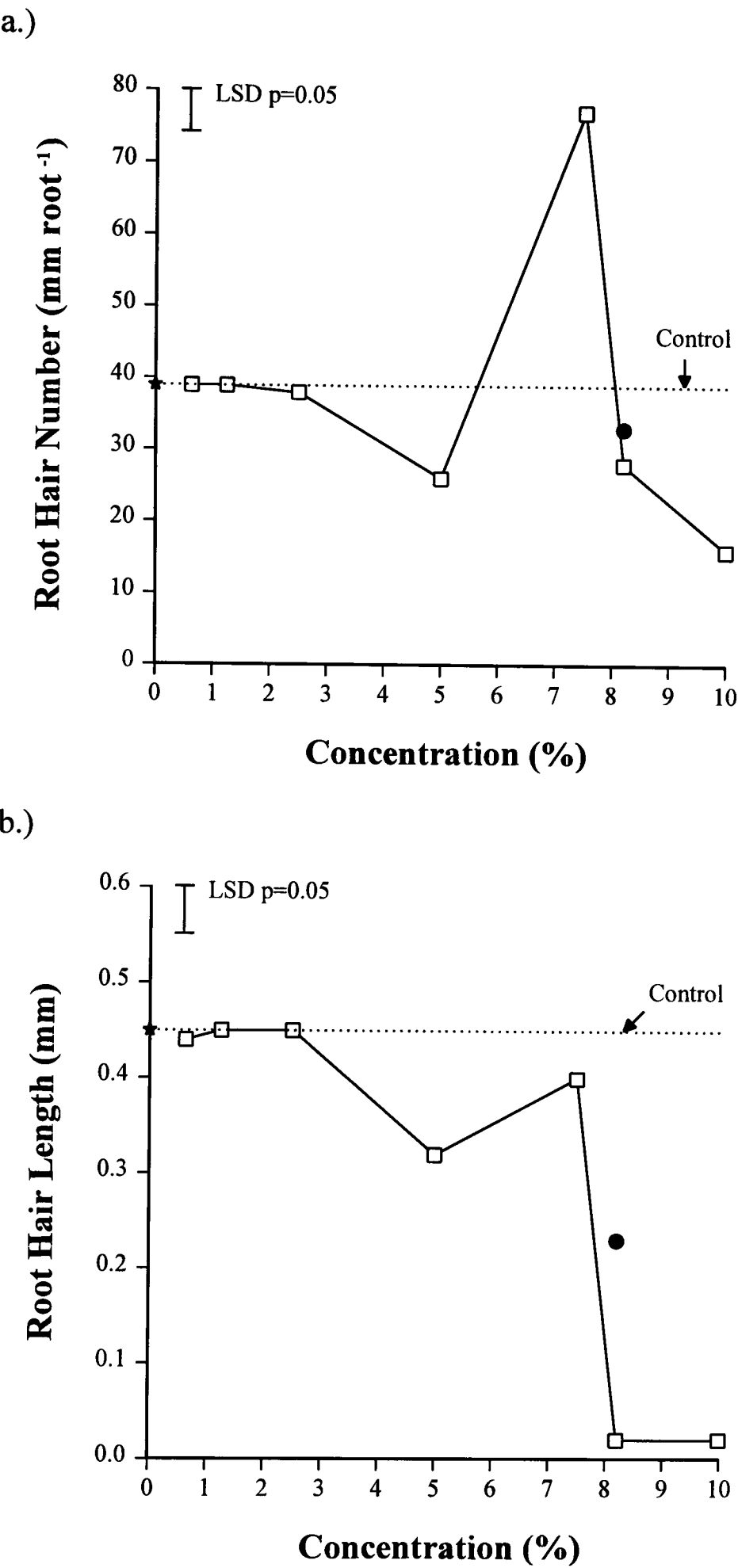


Figure 4.6.2: The effect of LHS supernatant at various concentrations (□), or 10 % original LHS (●), on a.) the root hair number (n=5), and b.) root hair length (n=12) on roots of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days, in the light. Water control (★).

4.6.3 Experiment 4.11: The significance of light exclusion on the inhibitory effect of LHS supernatant on lettuce root growth

When the whole seedlings were grown in the light, the roots of control seedlings were on average 54.6 mm long (Table 4.6.2). When only the roots were exposed to light, growth was inhibited 23 % ($p<0.001$). If hypocotyls plus cotyledons were exposed to light no effects on root growth was observed. The same was observed for seedlings grown in the dark (Table 4.6.2). In the presence of 10 % LHS supernatant, the root growth of seedlings grown in the light was inhibited 80 % ($p<0.001$). A similar inhibition of root growth was observed for seedlings with only their roots exposed to light. Exposure of the hypocotyl and cotyledon to the light caused 20 % ($p<0.01$) inhibition. Seedlings grown on LHS supernatant in the dark had 28 % ($p<0.001$) inhibited root length (Table 4.6.2).

Table 4.6.2: The relation between growth condition and 10 % LHS supernatant on the effect of root growth inhibition of pre-germinated seeds of lettuce cv. Reskia(B). n=24.

			Root length					LSD (DF 4) p= 0.05/0.01/0.001
Solution			Water Control		LHS Supernatant			
Exposure of seedling part to			(mm)	% of	(mm)	% of	% of	
Root	Cotyledon + hypocotyl	Code		H ₂ O L/L		H ₂ O L/L	corres. H ₂ O	
Light	Light	L/L	54.6	100	11.1	20	20	
Light	Dark	L/D	41.8	77	7.3	13	17	
Dark	Light	D/L	53.8	98	43.3	79	80	
Dark	Dark	D/D	53.0	97	38.0	70	72	
LSD (DF 8)								
p=0.05			2.1		4.2			
p=0.01			3.1		6.2			
p=0.001			4.6		9.3			

4.6.4 Experiment 4.12: Heat stability of inhibitor(s) in LHS supernatant

Not heated original LHS caused 22 % ($p<0.01$) root length reduction, whereas not heated LHS supernatant inhibited root growth 80 % ($p<0.001$) (Figure 4.6.3). A ten minute heat treatment at 40°C or at 60°C made little difference on the root growth inhibition observed. However, at 80°C only a 30 % ($p<0.001$) reduction in root length occurred and at 100°C, or after autoclaving the inhibitory effect disappeared (Figure 4.6.3).

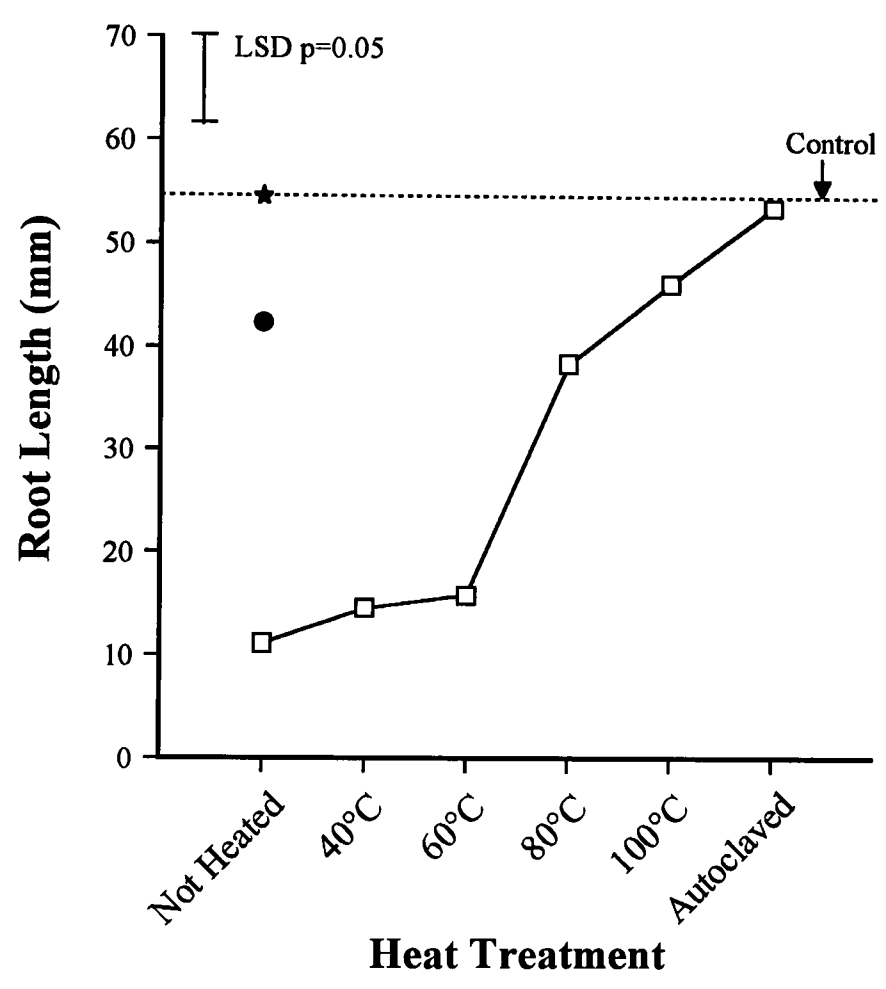


Figure 4.6.3: The effect of a 10 minute heat treatment at various temperatures, or autoclaving at 121°C, of 10 % LHS supernatant, on the effect of root growth inhibition of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days in the light. Water control (★), original LHS 10 % (●), LHS supernatant (□). n=24.

4.6.5 Experiment 4.13: The significance of microbial contamination in LHS supernatant on the inhibitory effect on lettuce root growth

In the absence of microbes, LHS supernatant was inhibitory after one week storage, irrespective of previous storage temperature. Root growth was reduced by more than 82 % ($p<0.001$) compared to control seedlings (Figure 4.6.4). In the presence of microbes and at a storage temperature of 5°C, LHS supernatant lost some inhibitory properties after 2 days, and roots were three times longer ($p<0.001$) than those of seedlings of the corresponding LHS treatment without microbes (Figure 4.6.4). Inhibition progressively reduced over time, disappearing altogether after 4 days. At 20°C, observed inhibition was reduced after one day with average root length 98 % ($p<0.001$) longer than roots subjected to the corresponding LHS supernatant treatment without microbes. After 2 days at 20°C root growth inhibition had completely disappeared.

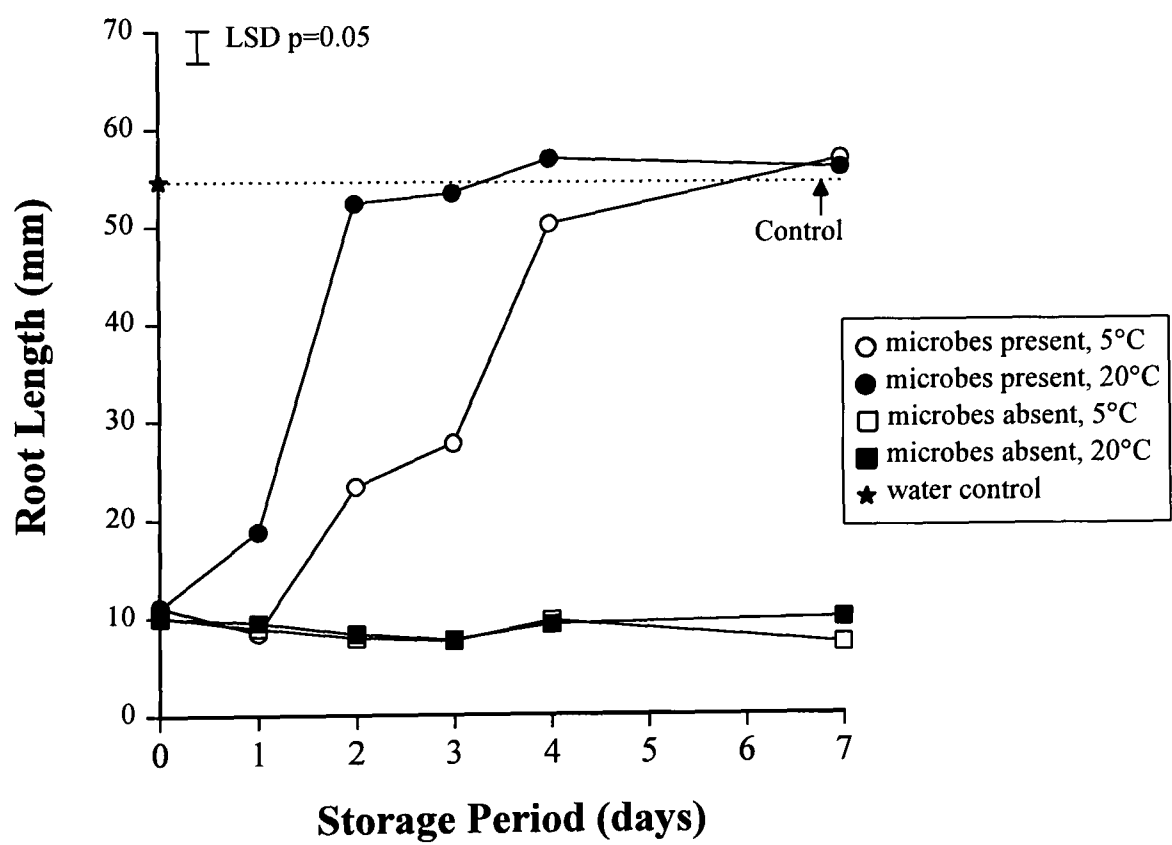


Figure 4.6.4: The significance of the presence or absence of microbes, and storage temperature on the effect of root growth inhibition of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days, in the light. n=24.

4.6.6 Experiment 4.14: The role of proteins in LHS supernatant with respect to the inhibitory effect on lettuce root growth

The total amount of protein present in the crude supernatant of LHS was 32.5 $\mu\text{g ml}^{-1}$. After acetone precipitation 83 % of the total protein fraction was removed. In the lettuce bioassay, control lettuce seedling root length was 54.7 mm (Table 4.6.3). Unaltered LHS supernatant inhibited root growth 79 % ($p<0.001$) compared to control seedlings. The protein reduced fraction of LHS supernatant reduced root length only 50 % ($p<0.001$). However, the re-dissolved protein fraction did not alter root growth.

Table 4.6.3: The significance of the protein fraction in LHS supernatant on the effect of root growth inhibition of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days. n=24.

Fraction	Protein concentration		Root length	
	BSA equivalents* ($\mu\text{g ml}^{-1}$)	% of unaltered	(mm)	% of Control
Control			54.7	100
LHS unaltered supernatant	32.5	100	11.7 *** ²	21
LHS fraction after protein precipitation	5.5	17	27.3 ***	50
LHS protein fraction re-dissolved	n.d.		57.1 NS	104
LSD (DF 8)				
$p=0.05$			4.6	
$p=0.01$			6.6	
$p=0.001$			10.0	

(² - different from the control at * = 5%, ** = 1%, and *** = 0.1% probability level, NS = not significant)
(n.d. - not determined; * - bovine serum albumin equivalents)

4.7 The effects of diverse treatments on lettuce cotyledon and hypocotyl growth

Experiments in the following sections focused on the evaluation of promoting effects on cotyledon expansion and hypocotyl elongation of lettuce seedlings under the influence of SWS, as first observed in Experiment 4.8.

4.7.1 Experiment 4.8: The effect of modified SWS on cotyledon expansion and hypocotyl elongation

Cotyledon area

Cotyledon expansion was promoted 158 % ($p<0.001$) over the control with ashed ANS, and 119 % ($p<0.001$) with ANS supernatant. Application of ashed LHS, LHS solid fraction, and original LHS resulted in a promotion of cotyledon expansion of 131 %, 70 % and 86 % ($p<0.001$), respectively. In contrast, cotyledon growth was inhibited 39 % ($p<0.01$) when LHS supernatant was applied (Table 4.7.1).

Hypocotyl length

All treatments promoted hypocotyl extension in the dark (Table 4.7.1). Ashed ANS, ANS supernatant, ANS solid fraction, and original ANS increased hypocotyl length 64 %, 43 %, 27 %, and 53 % ($p<0.001$), over the control. The same fractions of LHS increased hypocotyl length 79 % ($p<0.001$), 25 % ($p<0.01$), 60 %, and 43 % ($p<0.001$), compared to control seedlings.

Table 4.7.1: The effects of SWS and modified SWS at 10% concentration to cotyledon expansion in the light, and hypocotyl elongation in the dark of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days. n=24.

Condition	Light				Dark			
SWS	ANS		LHS		ANS		LHS	
'modification'	cotyledon area (mm ²)	% of Control	cotyledon area (mm ²)	% of Control	hypocotyl length (mm)	% of Control	hypocotyl length (mm)	% of Control
Control	10.0	100	10.3	100	29.7	100	27.6	100
Ashed	25.8 *** ²	258	23.8 ***	231	48.7 ***	164	49.5 ***	179
Original	11.3 NS	113	19.1 ***	186	45.5 ***	153	39.6 ***	143
Solids	12.8 NS	128	17.5 ***	170	37.7 ***	127	44.0 ***	160
Supernatant	21.9 ***	219	6.3 **	61	42.5 ***	143	34.6 **	125
LSD (DF 10)								
p=0.05	3.1		2.2		3.5		4.4	
p=0.01	4.3		3.1		4.9		6.3	
p=0.001	6.3		4.5		7.1		9.2	

(² - different from the control at * = 5%, ** = 1%, and *** = 0.1% probability level, NS = not significant)

4.7.2 Experiment 4.15: The effect of diverse mineral solutions on cotyledon expansion and hypocotyl elongation of lettuce

Cotyledon area

Cotyledon size was reduced with various CaCl_2 concentrations (Figure 4.7.1.a). The application of NaNO_3 at 50 % LHS equivalents (400 mg N l^{-1}) increased cotyledon size 24 % ($p < 0.05$) (Figure 4.7.1.a). Various concentrations of Mg^{++} inhibited cotyledon growth up to 28 % ($p < 0.01$) (Figure 4.7.1.a). The expansion was greatly promoted by KCl solutions in the light. At K^+ concentrations of 800 mg l^{-1} (50 % LHS equivalents) cotyledon growth was 98 % ($p < 0.001$) greater than controls.

Hypocotyl length

Hypocotyl extension growth was inhibited 17 % ($p < 0.05$) with CaCl_2 at 0.9 % LHS equivalents ($5 \text{ mg Ca}^{++} \text{ l}^{-1}$) and was 15 % ($p < 0.05$) greater with 107 % LHS equivalents ($600 \text{ mg Ca}^{++} \text{ l}^{-1}$) (Figure 4.7.1.b). Treatments at 9 % to 54 % LHS equivalents (50 to $300 \text{ mg Ca}^{++} \text{ l}^{-1}$) Ca^{++} had no effect on hypocotyl length. A NaNO_3 treatment at 25 % LHS equivalents (200 mg N l^{-1}) increased length 18 % ($p < 0.01$), but 100 % LHS equivalents (800 mg N l^{-1}) reduced length 17 % ($p < 0.05$) compared to the control. Other concentrations applied had no effect on hypocotyl length. MgCl_2 treatments had no effect on hypocotyl length at levels applied from 2.8 % to 200 % LHS equivalents (5 to $360 \text{ mg Mg}^{++} \text{ l}^{-1}$) Mg^{++} . KCl solutions promoted elongation from 6.3 % LHS equivalents ($100 \text{ mg K}^+ \text{ l}^{-1}$) K^+ concentration upwards. A maximum promotion was reached at 50 % LHS equivalents ($800 \text{ mg K}^+ \text{ l}^{-1}$) K^+ , with 93 % ($p < 0.001$) longer hypocotyls than control seedlings.

4.7.3 Experiment 4.15: Effects of KCl solutions on seedling K^+ content

The concentration of K^+ ($[\text{K}^+]$) in seedlings treated with KCl in the light or dark increased with K^+ application in a logarithmic manner (Figure 4.7.2).

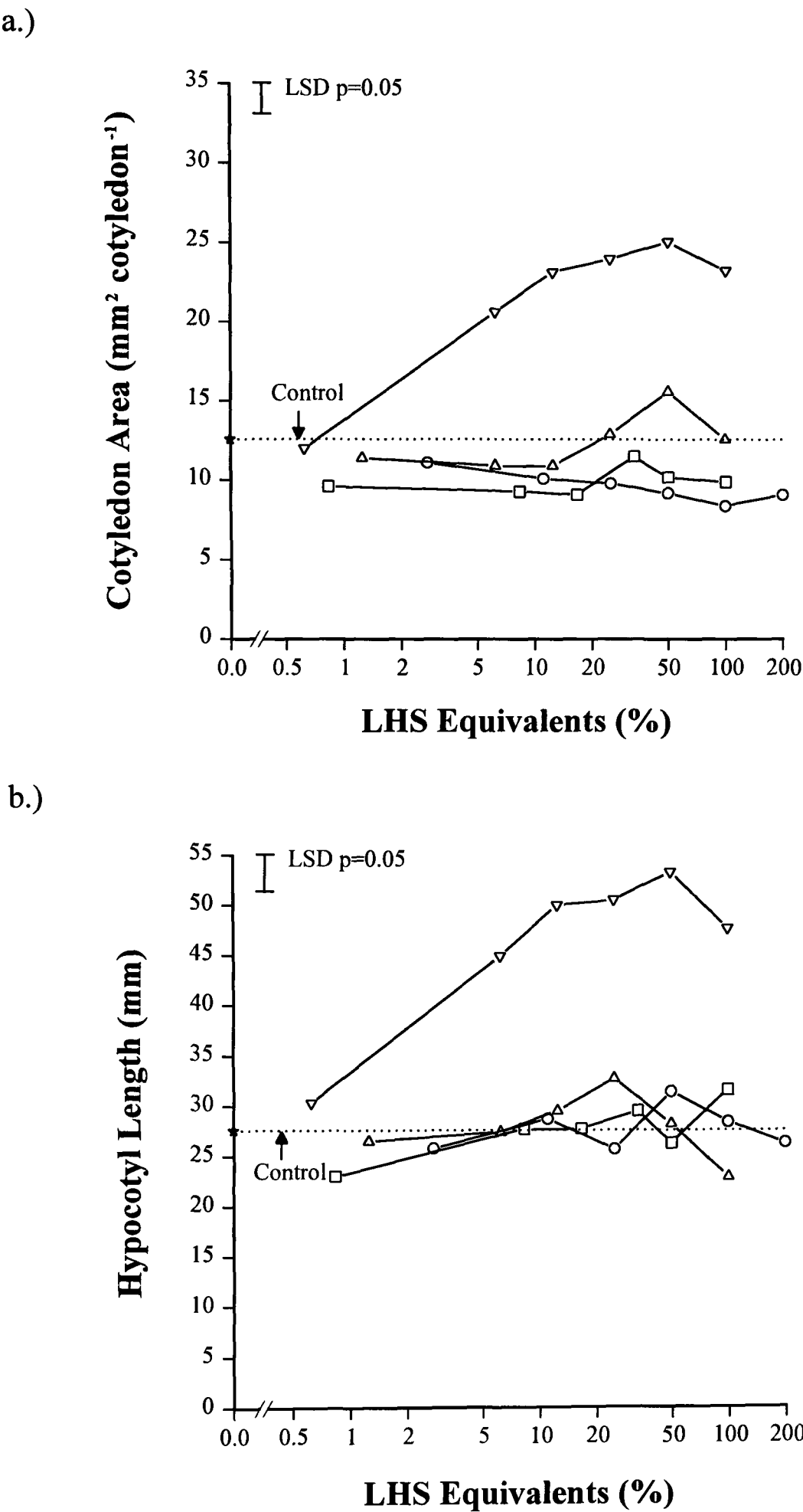


Figure 4.7.1: The effect of diverse mineral solutions on a.) the cotyledon expansion in the light and b.) the hypocotyl elongation in the dark of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days in the light. Mineral solutions: CaCl_2 ($\square$), NaNO_3 (Δ), MgCl_2 ($\circ$), KCl (∇), water control ($\star$). $n=24$.

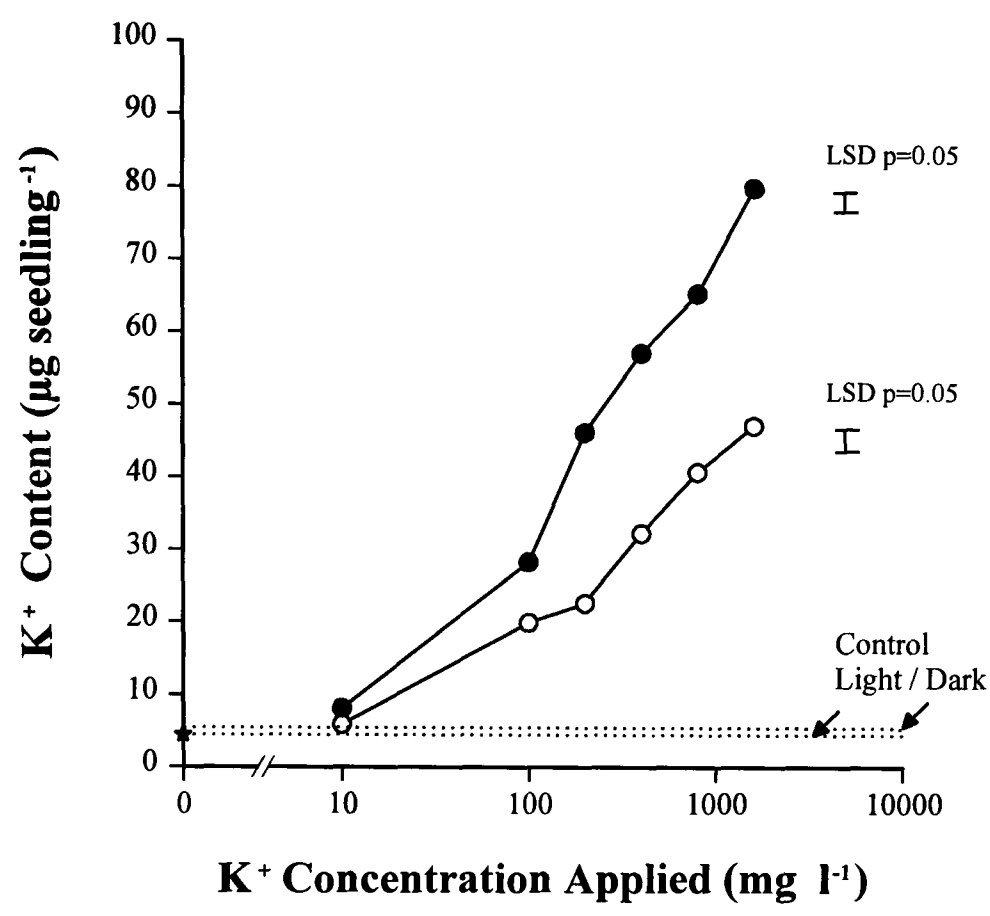


Figure 4.7.2: The effects of KCl solutions on seedling K⁺ content of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days in the light (○), or dark (●). Water control (★). n=3.

Control seedlings contained 4.5 µg K⁺ in the light, and 5.5 µg K⁺ in the dark. At 1600 mg l⁻¹ KCl, [K⁺] in seedlings increased to 47.2 µg (p<0.001) in the light, and 79.9 µg (p<0.001) in the dark (Figure 4.7.2). The K⁺ uptake efficiency for seedlings treated with 100 mg l⁻¹ K⁺, expressed as a percentage of K⁺ taken up by the plant, was 31 % in the light and 46 % in the dark. This efficiency decreased with increasing K⁺ concentration applied (Figure 4.7.3).

The total K⁺ accumulation in the hypocotyl of dark grown seedlings was almost twice as high, compared to the K⁺ accumulation in cotyledons of seedlings grown in continuous light (Figure 4.7.4.a). However, expressed in fresh weight terms, [K⁺] was similar in both tissues (Figure 4.7.4.b).

The increase in seedling [K⁺] with increasing KCl application was not proportional to the growth response. Cotyledon size increased by a maximum of

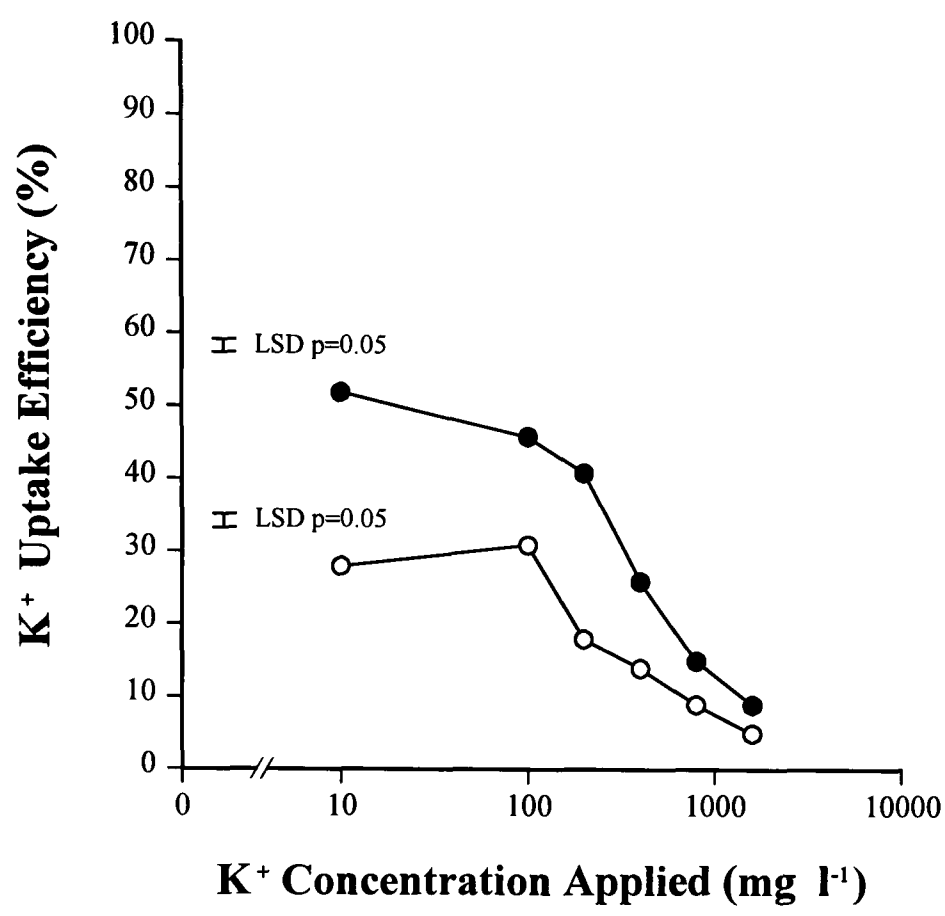


Figure 4.7.3: The effects of KCl concentrations on the K⁺ uptake efficiency of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days in the light (○), or dark (●). n=3.

98 % (Figure 4.7.1.a), but the K⁺ content per mg fresh weight of tissue increased by 560 % (Figure 4.7.4.b). Similar figures were obtained for hypocotyl tissue (Figure 4.7.4.b). This was due to a 'luxury' uptake of K⁺ which was not accompanied by a growth promotion at [K⁺] above 2.5 µg mg⁻¹ fresh weight of tissue (Figure 4.7.5).

The correlation coefficient between application and total seedling [K⁺] was r=0.988 (p<0.01) in the light, and r=0.979 (p<0.01) in the dark (Figure 4.7.6), and between application and [K⁺] per mg fresh weight of cotyledon tissue in the light was r=0.981 (p<0.01), and per mg fresh weight hypocotyl tissue in the dark was r=0.990 (p<0.01) (Figure 4.7.7). The correlation coefficient for the binomial relation between [K⁺] per mg fresh weight and cotyledon expansion in the light was r=0.985, and hypocotyl elongation in the dark r=0.991 (Figure 4.7.8).

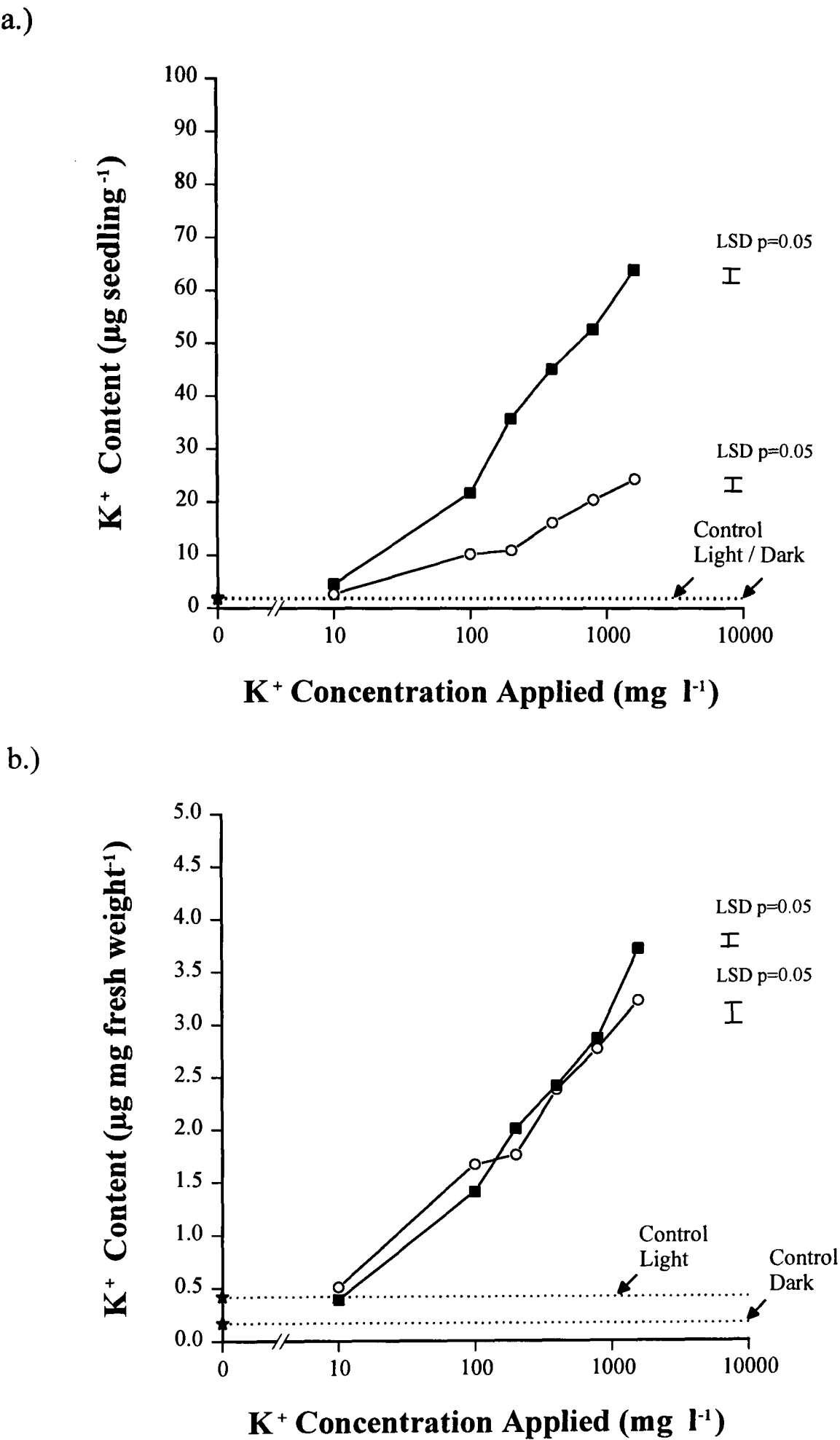


Figure 4.7.4: The effect of KCl solution on the [K⁺] of cotyledons in the light (○), or hypocotyls in the dark (■) of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days, a.) per seedling or b.) per mg tissue fresh weight. Water control (★). n=3.

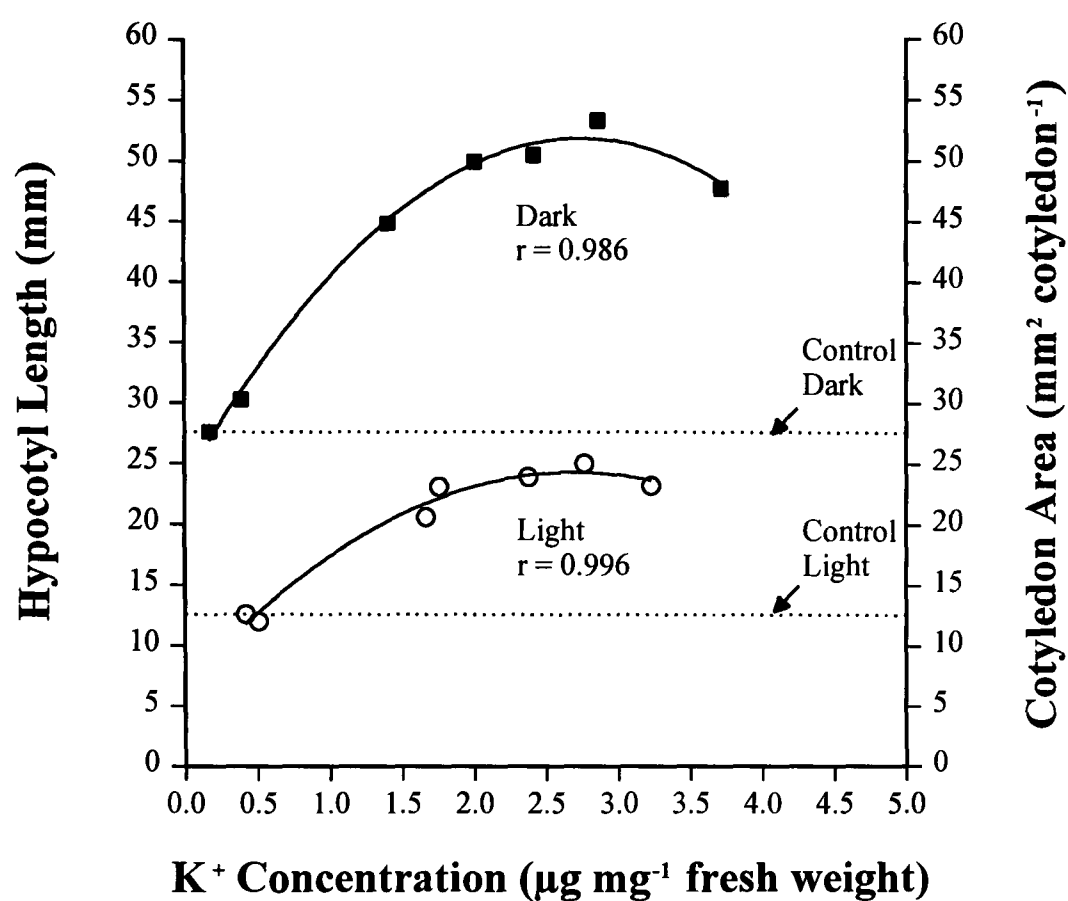


Figure 4.7.5: The correlation between [K⁺] per mg tissue fresh weight and growth response of cotyledons in the light (○), or hypocotyls in the dark (■) of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days.

4.7.4 Experiment 4.16: The effect of original, modified SWS or KCl on seedling potassium content

As reported in Section 3.3.1, original ANS contained only half the amount K⁺ compared to original LHS, and the total K⁺ content of seedlings under both, light and dark conditions reflected this difference. Original ANS treated seedlings in the light contained 9.8 µg K⁺ while LHS treated seedlings contained 17.6 µg K⁺ (Figure 4.7.6.a). In the dark, this difference was not reflected in the uptake rates (Figure 4.7.6.b). Because of different K⁺ adsorption capacities of the organic cell debris of the SWS (see Section 3.3.1), seedlings treated with modified SWS accumulated different amounts of K⁺, as varying [K⁺] were applied. The efficiency of uptake and accumulation was best described by comparison of SWS treatments with KCl treatments. By fitting a logarithmic equation and 95 % confidence intervals to the

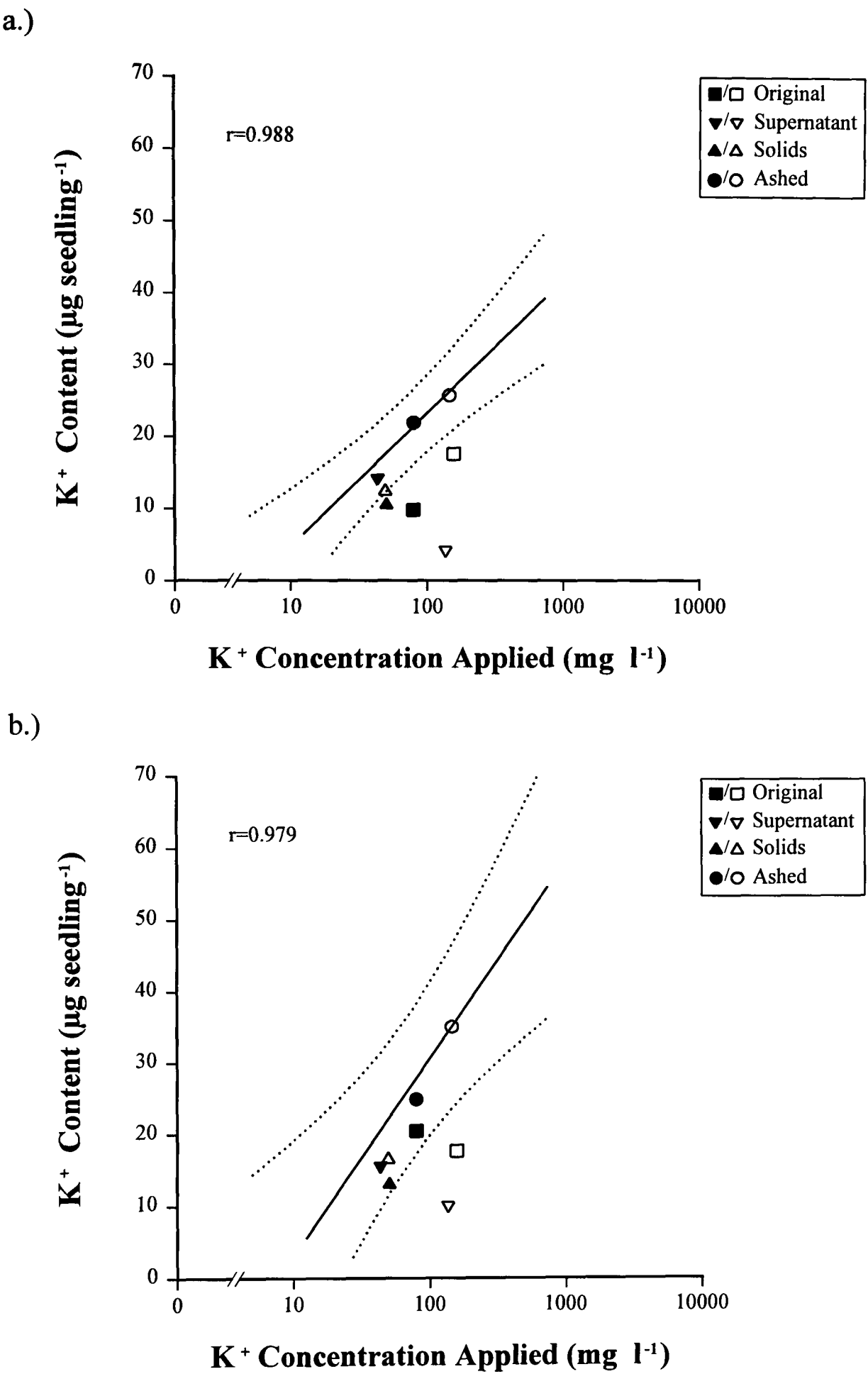
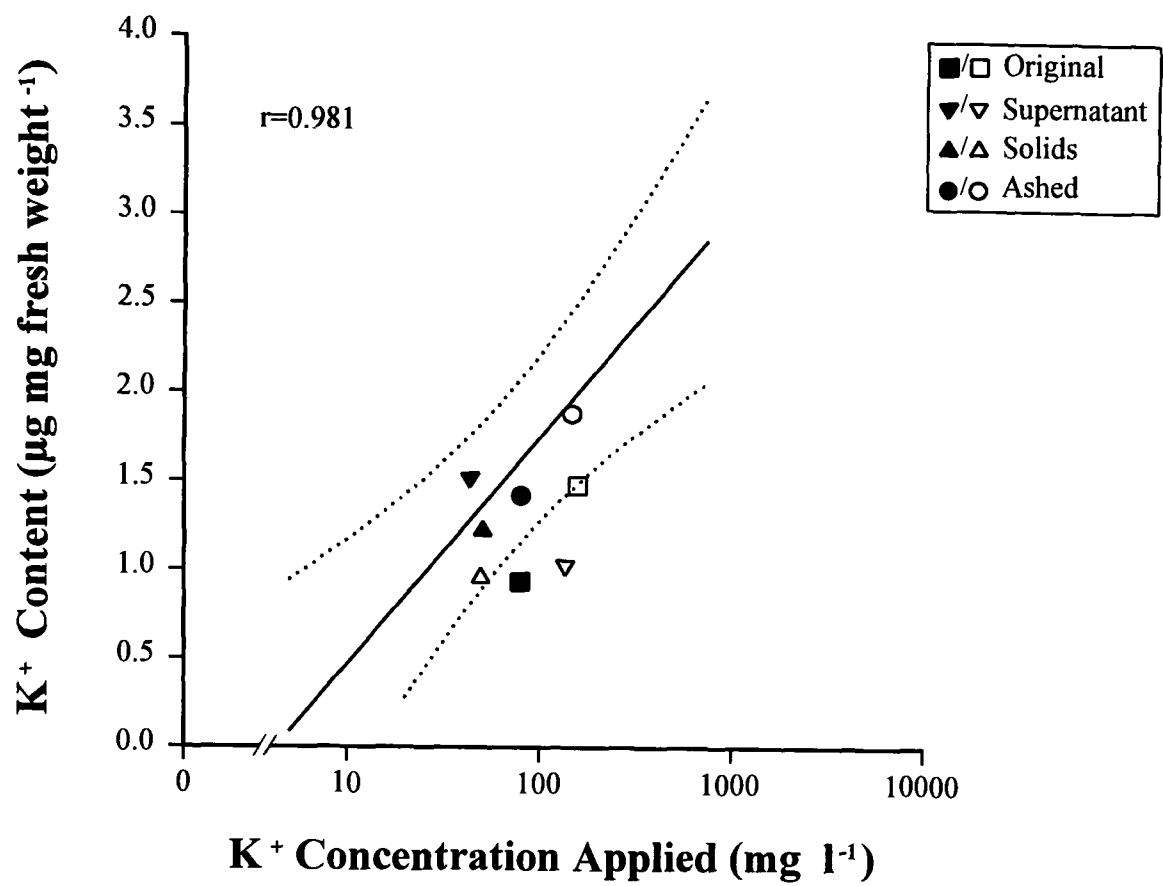


Figure 4.7.6: The regression curve for the effect of KCl on total seedling $[K^+]$ of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days (solid line). Confidence limits ($p=0.05$) indicated by dotted lines. Symbols indicate the responses of original or modified ANS (solid symbols), or LHS (open symbols) in a.) the light or b.) dark. $n=3$.

a.)



b.)

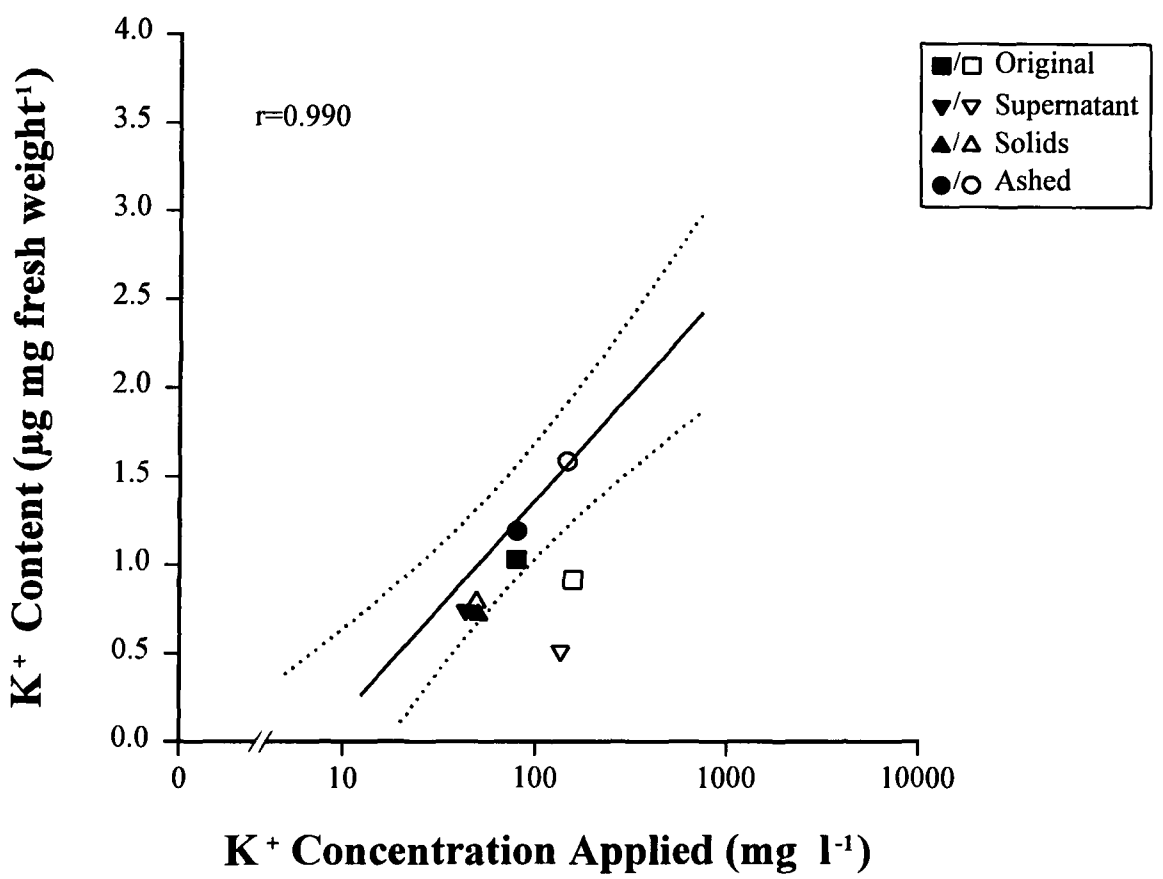
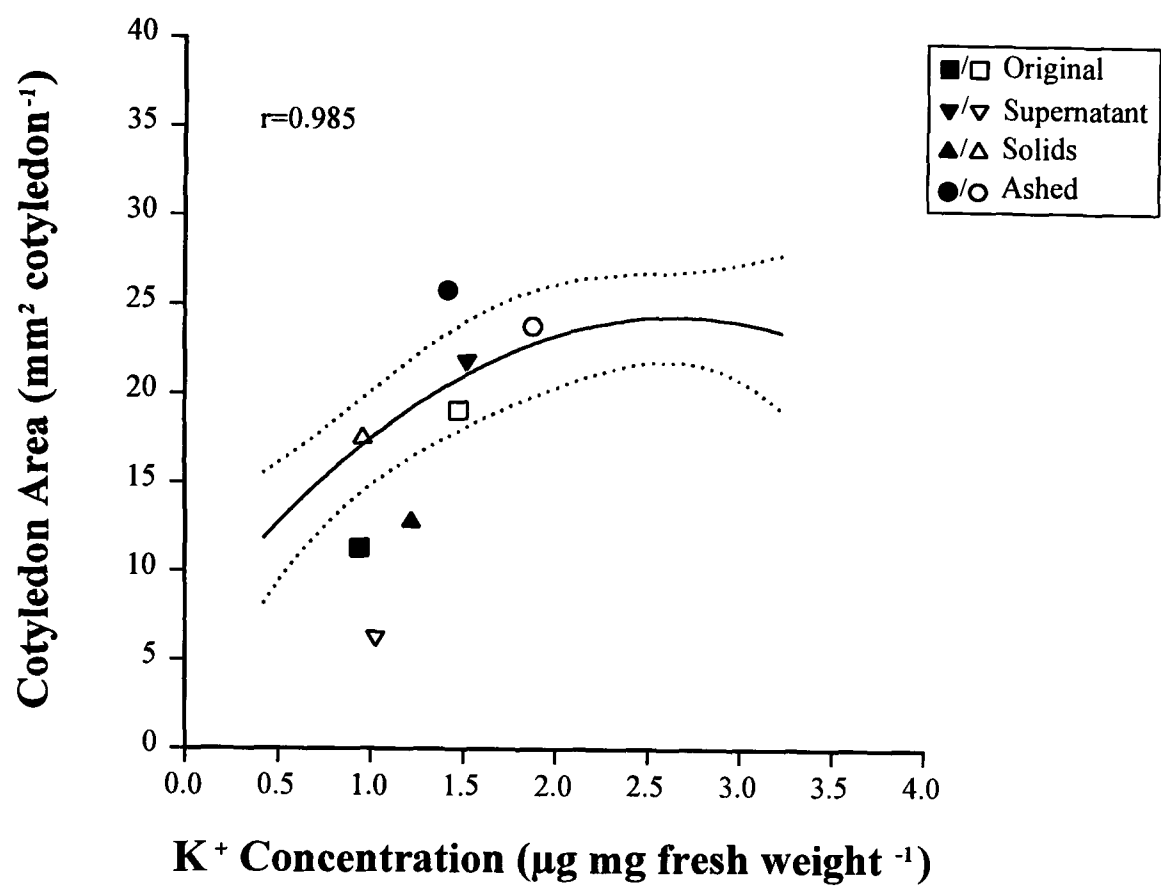


Figure 4.7.7: The regression curve for the effect of KCl on [K⁺] per mg fresh weight of a.) cotyledons in the light or b.) hypocotyls in the dark of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days (solid line). Confidence limits (p=0.05) indicated by dotted lines. Symbols indicate the responses of original or modified ANS (solid symbols), or LHS (open symbols). n=3.

a.)



b.)

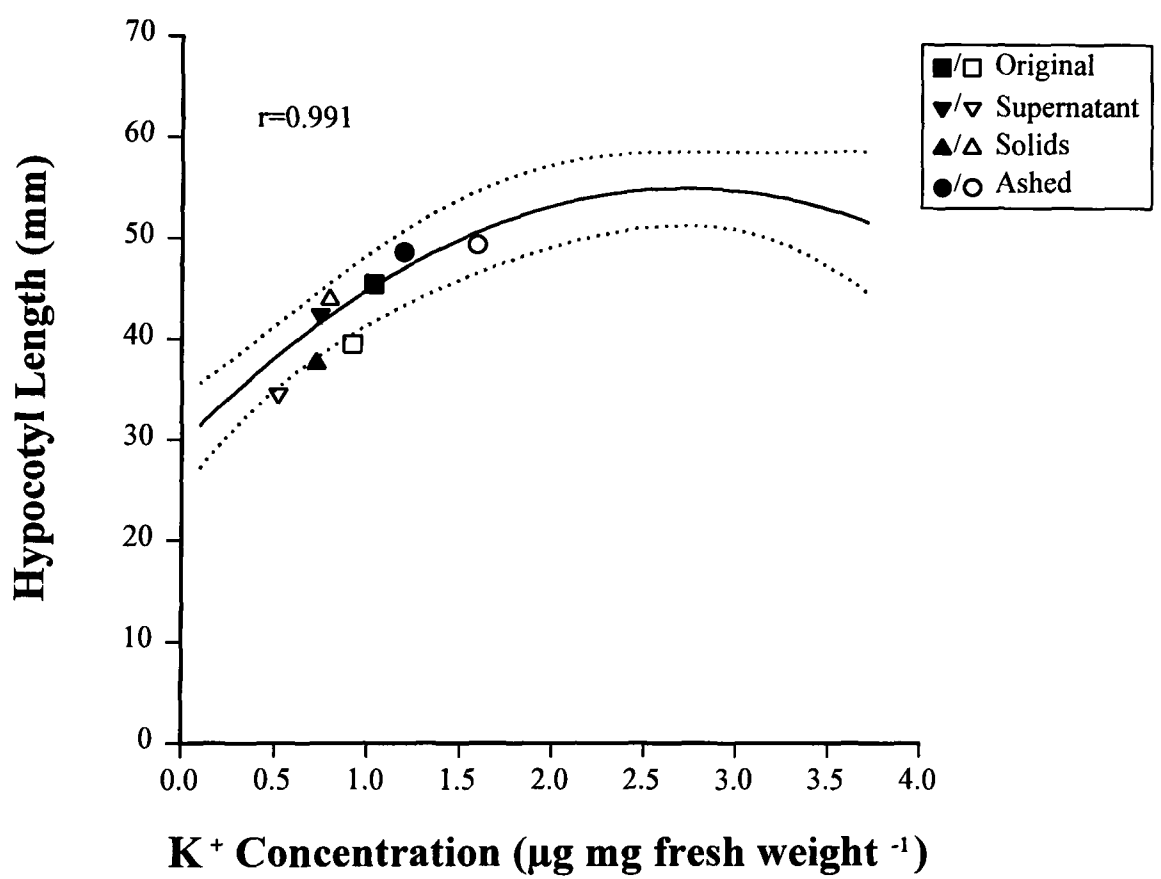


Figure 4.7.8: The regression curve between the $[K^+]$ per mg fresh weight of a.) cotyledons in the light or b.) hypocotyls in the dark, and the growth response of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days (solid line). Confidence limits ($p=0.05$), indicated by dotted lines. Symbols indicate the responses of original and modified ANS (solid symbols, or LHS (open symbols). $n=3$.

response curves of various KCl treatments, together with the calculation of expected values for SWS treatments, it was possible to quantify differences in K^+ uptake.

In the light, original ANS or ANS solid fraction treated seedlings accumulated 55 % or 41 % ($p < 0.001$) less K^+ than seedlings treated with KCl (Figure 4.7.6.a). For ANS supernatant treated seedlings, however, the lower seedling K^+ content was due to a lower K^+ content of this fraction (Table 3.3.2.), hence application. When either ashed ANS or LHS was applied to lettuce seedlings K^+ uptake was restored to levels of KCl treatments. The $[K^+]$ of seedlings grown in the dark, however, varied only due to differences in the inherent K^+ levels of the ANS treatments (Figure 4.7.6.b).

Seedlings subjected to treatments with original LHS, or LHS supernatant in the light, contained 38 % or 83 % ($p < 0.001$) less K^+ than expected. Similar treated seedlings kept in the dark, contained 52 % or 71 % ($p < 0.001$) less K^+ , respectively (Figure 4.7.6.b). In these cases the LHS treatments inhibited K^+ accumulation. Seedlings treated with ashed LHS or the LHS solid fraction accumulated K^+ in line with their inherent K^+ content, under light or dark conditions (Figure 4.7.6). The pattern of total $[K^+]$ in whole seedlings was also reflected in increased $[K^+]$ in either the cotyledon in the light, or the hypocotyl in the dark (Figure 4.7.7).

Cotyledon expansion in light grown seedlings treated with ANS supernatant, original LHS, ashed LHS or the LHS solid fraction was as expected due to $[K^+]$ present in the cotyledon tissue (Figure 4.7.8). Seedlings treated with ashed ANS exhibited a 25 % ($p < 0.01$) higher growth response than expected. In contrast, cotyledons of seedlings treated with the ANS solid fraction, original ANS, or LHS supernatant were 35 %, 36 % or 66 % ($p < 0.001$) below the expected values, respectively (Figure 4.7.8.a).

Hypocotyl expansion in the dark was within the expected range for all treatments, except when original LHS, the ANS or LHS solid fraction was applied.

In these cases the hypocotyl elongation was inhibited 15 %, 16 % or 18 % ($p<0.05$) (Figure 4.7.8.b).

4.8 Experiment 4.16: The effect of original, modified SWS or KCl on cotyledon chlorophyll content of lettuce

The application of KCl solution at $[K^+]$ of 80 mg l⁻¹ or 160 mg l⁻¹, increased the amount of chlorophyll in cotyledons 93 % or 92 % ($p<0.001$), respectively, over the control (Figure 4.8.1.a). In the presence of original ANS, ANS supernatant or ashed ANS the total chlorophyll increased 41 % ($p<0.05$), 75 % ($p<0.01$), or 109 % ($p<0.001$). Seedlings treated with original LHS, the LHS solid fraction, or ashed LHS, cotyledon chlorophyll content was 138 %, 111 % or 124 % ($p<0.001$) higher compared to corresponding control seedlings (Figure 4.8.1.a).

Chlorophyll accumulation expressed on cotyledon fresh weight increased with all treatments (Figure 4.8.1.b). KCl applied at 80 mg l⁻¹ or 160 mg l⁻¹ increased the cotyledon chlorophyll content 48 % or 44 % ($p<0.001$), over the corresponding controls. In the presence of original ANS, ANS supernatant, ashed ANS or the ANS solid fraction, seedlings contained 51 %, 49 %, 39 % ($p<0.001$) or 34 % ($p<0.01$) more chlorophyll per mg fresh weight (Figure 4.8.1.b). Similarly, original LHS, LHS supernatant, the LHS solid fraction, or ashed LHS promoted chlorophyll content 81 %, 63 %, 53 % or 42 % ($p<0.001$) (Figure 4.8.1.b).

Seedling chlorophyll content per cotyledon area was reduced 16 %, 20 % or 19 % ($p<0.05$) respectively, when KCl, ANS supernatant or ashed ANS were applied. The application of original ANS, however, resulted in an increase in chlorophyll content per unit area of 24 % ($p<0.001$). When original LHS, LHS supernatant, or the LHS solid fraction were applied to pre-germinated seeds, an increase in seedling chlorophyll content of 30 % ($p<0.05$), 73 % ($p<0.001$), or 25 % ($p<0.05$) was observed (Figure 4.8.1.c).

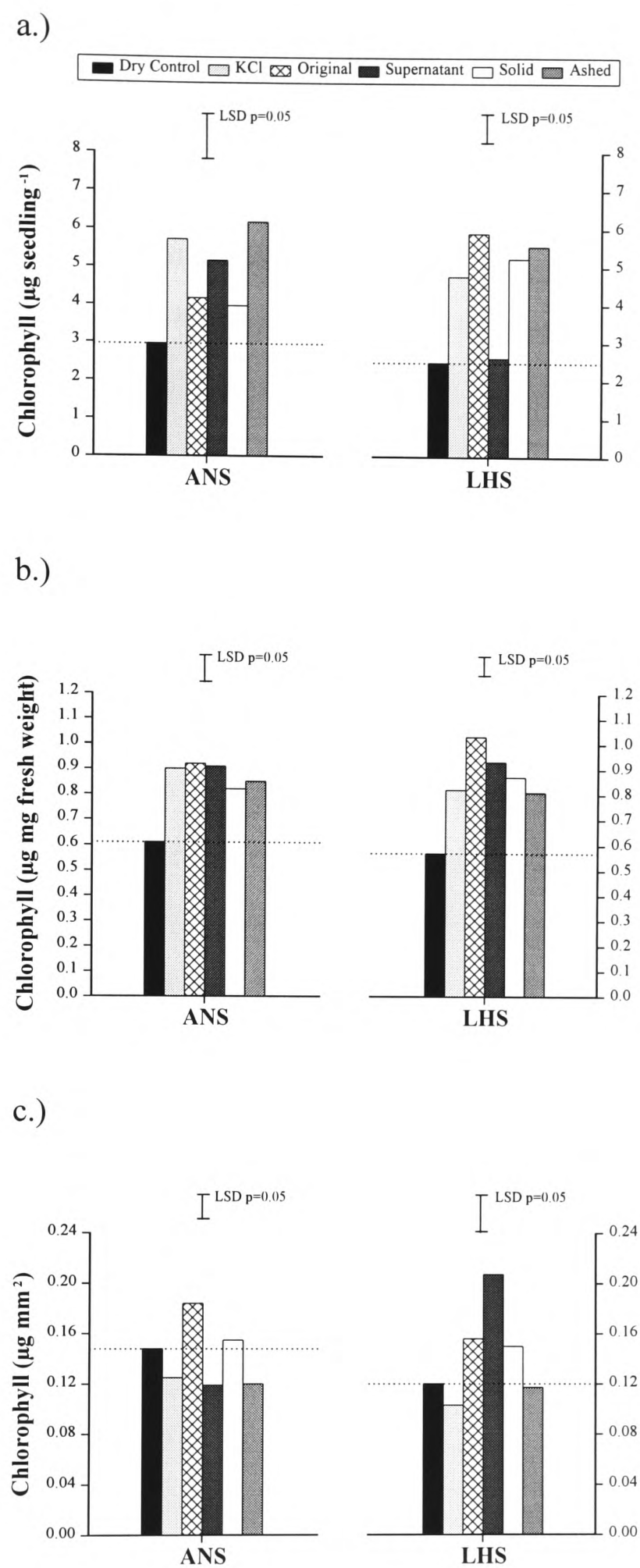


Figure 4.8.1: The effect of KCl, SWS or modified SWS at 10 % on a.) the total chlorophyll content per pair, b.) per mg tissue fresh weight, and c.) per area unit of cotyledons of pre-germinated seeds of lettuce cv. Reskia(B), after 4 days. n=3.

4.9 Discussion

The two seaweed suspensions prepared from brown algae, ANS and LHS, did not, regardless of concentration applied, improve the germination of barley or lettuce seeds, in terms of germination percentage or mean germination time, when dry seeds were germinated on top of SWS soaked filter paper (Figures 4.3.1, and 4.3.2). In contrast, Button and Noyes (1964) observed higher germination rates after the application of a 1 % seaweed extract, **Chase S-M3**, to the germination medium. However, they used seeds of red fescue (*Festuca rubra*) which has a germination behaviour different from the seeds tested in this work. Red fescue seeds germinate slowly, and the final germination percentage is comparably low. Their results also indicated that the applied SWS had detrimental effects on seed germination at higher application rates and lethal to seedlings above extract concentrations of 18 %. This implies that the seaweed extract they used was concentrated. The application of ANS and LHS in this study, however, did not reduce the germination percentage of lettuce or barley seeds, even when applied at half strength (Figure 4.3.1).

However, ANS and LHS did delay the initiation of seed germination, observed as delayed radicle emergence. This delay was mainly due to an inhibition of water uptake by the seed (Figures 4.3.4, and 4.3.5), caused by the inherent high osmotic potential of the SWS (Figure 3.2.1). The inhibitory effect was more SWS dependent than species dependent, as similar inhibitory effects were observed for lettuce and barley for identical SWS treatments (Figure 4.3.2). The difference in the extent of the delay between the two SWS was due to the difference in osmotic potential between the SWS. *Laminaria hyperborea* suspension exhibited a twofold higher osmotic potential, than ANS. Thus the inhibitory effect on water uptake was more pronounced with LHS.

Radicle emergence depended on the seed moisture content, which needed to reach a species specific level before radicle protrusion could proceed. Sodium

chloride and polyethylene glycol, applied to mimic the osmotic effect of the SWS, delayed radicle emergence (Figures 4.3.4, and 4.3.5). Seeds of both species tested were more sensitive to PEG, that caused an increased mean time to radicle emergence (MGT) more than NaCl. With LHS, however, the extent of inhibition of the radicle protrusion was delayed even longer than it would be expected from inherent osmotic potential alone (Figure 4.3.3.b). This indicated the presence of a component or components in LHS that may inhibit physiological events leading to radicle emergence of lettuce and barley seeds.

The physiological response of barley and lettuce seedlings to both SWS was observed in experiments where pre-germinated seeds were chosen, to circumvent the inhibitory effects of SWS in the imbibition phase. Seedlings of both species tested responded to SWS in a similar way with respect to root growth inhibition (Figures 4.4.1, and 4.4.4), and root hair appearance (Figure 4.4.6, and 4.4.7). In attempts to characterise the inhibitory compound(s) in the SWS detailed experiments were carried out, with the aim to mimic SWS properties. The osmotic potential of the SWS was based on dissolved ions (Figure 3.2.2), as well as larger organic molecules. Thus, a range of electrolytes and two different osmotica were tested. Many brown algae also contain an array of different phenolic compounds (Ragan and Glombitza, 1986). The physiological activity in the lettuce bioassay of the different phenolic compounds present in algae and therefore in both SWS, however, is not known. Tannins are abundant in brown seaweeds (Ragan, and Glombitza, 1986), and are constructed of phloroglucinol units (McInnes *et al.* 1984). Thus, it was used in the growth assays to investigate on the scale of root growth inhibition probably attributable to the phenolic compounds in the SWS. In these comparative experiments it was revealed that root morphology of lettuce seedlings was affected by a range of different treatments (Table 4.5.1). Evidence for the involvement of the

polyphenol component of SWS in the effect of growth inhibition comes from analyses on the number and length of root hairs of seedlings. Osmotica alone, at comparable OP's to SWS, had none or little inhibitory effects on root hair number and root hair length (Figures 4.5.2, and 4.5.3), unlike phloroglucinol, that reduced these parameters in a similar manner compared to SWS (Figure 4.5.5). Additionally, osmotica never resulted in the development of necrotic root tissue, unlike phloroglucinol or SWS treatments at higher concentrations.

Although osmotica inhibited root growth, the major component in root growth inhibition was due to polyphenol concentrations in the original SWS, as a close correlation was found between root growth inhibition by SWS and phloroglucinol treatments at comparable phenol concentrations (Table 4.5.1). For LHS treatments in the light, however, the polyphenol content was of lesser importance, than the osmotic potential. This SWS contained around 14 times less polyphenols than ANS. However, the inhibitory effects on lettuce root growth did not reflect this difference (Figure 4.4.4). Therefore, the presence of other inhibitory compounds in LHS was likely. This was illustrated in experiments where modified SWS were applied to lettuce seedlings (Table 4.5.2). Less growth inhibition occurred with the solid fraction of LHS due to the volume re-adjustment performed during fraction preparation. This effectively resulted in a dilution of compounds. However, LHS supernatant contained the lowest amount of polyphenols (Table 3.3.6), but inhibited root growth to the greatest extent (Table 4.5.2). As this effect disappeared when LHS was applied as ashed compounds, the inhibitor must be organic in nature. The auxin-like activity exhibited by this SWS (Section 3.3.2.2) may have accounted for perhaps part of this effect, but the inhibitory activity of LHS supernatant was only found under illumination and not in the dark. This was not an auxin-like response, as it has been shown that illumination facilitates auxin degradation (Galston and Baker, 1951).

The inhibitory effect of LHS supernatant on lettuce seedlings was investigated in more detail. The methodology chosen for experiments in Sections 4.3 and 4.4 involved roots of seedlings being exposed to light as well as the shoots. Light has been shown to inhibit the elongation of roots of a number of species (Torrey, 1952; Eliasson and Bollmark, 1988). However, in this study lettuce root growth of control seedlings was found to be unaffected by exposure to light (Figure 4.4.4). While original LHS caused root growth inhibition and necroses at 50 % concentration, LHS supernatant elicited this effect at 10 %, but only in the light. Original LHS at this concentration did not cause this effect. This was probably due to the preparation of LHS supernatant (Section 2.2.1). As mentioned earlier, the comparable concentration to 10 % original LHS was 8.2 % LHS supernatant. At those concentrations both treatments inhibited root growth to a similar extent (Figure 4.6.1). The effect of LHS supernatant was dose dependent. Concentrations above 5 % were inhibitory to root growth, and lethal to rhizodermal cells at 10 %. This effect was not variety specific, and occurred in seedlings of three different varieties (Table 4.6.1). The results of light exclusion experiments (Table 4.6.2) indicated a correlation between root illumination and root inhibition of LHS supernatant treated seedlings, irrespective of the light exposure of the hypocotyl plus cotyledon sections. The inferior inhibition of root growth without the appearance of necroses in the dark was probably due to other components present in LHS, such as phenols.

The inhibitory compound(s) in LHS supernatant were not of microbial origin and was an intrinsic property of LHS, that was capable of being degraded by microbial activity over time (Figure 4.6.4). The compound(s) in question were found to be heat labile above 60°C (Figure 4.6.3). This is a typical value for most plant proteins. However, the protein fraction of LHS supernatant was found not to be growth inhibiting in the lettuce growth experiments, unlike the residual fraction after protein precipitation (Table 4.6.3). Other organic compounds found in brown

seaweeds and consequently in seaweed products, such as amino acids (Table 1.3.2) or carbohydrates such as mannitol (Table 1.3.1) were not inhibitory in growth test (Blunden *et al.* 1968). Further investigations to reveal the nature of the inhibitory compound(s) would be necessary, which is beyond the aim of this study.

Besides root growth inhibition of lettuce seedlings promotion of cotyledon expansion in the light, and hypocotyl elongation in the dark was observed after the application of both SWS (Figure 4.4.5). The extent of the response was found to be dose related. However, at higher SWS concentrations the correlation was lost. This was especially true for ANS treatments, where lettuce root physiology at high concentrations was impaired (Figure 4.4.4). Obviously this has had an effect on the development of the seedling as a whole, which is illustrated by the reduced cotyledon size and hypocotyl length of seedlings treated with ANS (Figure 4.4.5). The growth promotion of lettuce seedlings after the application of SWS and modified SWS was also observed after the application of ashed SWS. As this fraction elicited growth promotion, results point to a mineral or minerals as the active compound. Similar growth promotion was observed after the application of KCl solutions, but not with any other mineral solution tested. This result strongly indicated that potassium was the compound in SWS responsible for growth promotion.

The promotion of cotyledon expansion by potassium has been previously reported, mainly in connection with work on cucumber cotyledons, used to investigate chlorophyll evolution and hormonal effects on chloroplast development (Haru *et al.* 1982; LePabic *et al.* 1983; Green, 1983; Arnold and Fletcher, 1986). But Knypl and Chylinska (1972) applied K^+ at $\sim 400 \text{ mg l}^{-1}$ to lettuce seedlings and obtained a cotyledon expansion similar to that reported here.

When KCl solutions were applied in this study, K^+ uptake per seedling was proportional to the K^+ concentration applied (Figure 4.7.2.a). The growth response

of cotyledons and hypocotyls was found to be correlated with the $[K^+]$ per mg fresh weight in the growth responding seedling parts up to tissue concentrations of $2.5 \mu\text{g mg}^{-1}$ fresh weight, where $[K^+]$ of 400 mg l^{-1} was applied (Figure 4.7.5). However, 'luxury' K^+ uptake occurred when higher $[K^+]$ were applied (Figure 4.7.5). For the application of modified SWS the $[K^+]$ applied with the SWS treatments varied between 43.6 mg l^{-1} (ANS supernatant) and 158.8 mg l^{-1} (original LHS). At these concentrations no 'luxury' uptake occurred, and thus in theory, the growth response should reflect K^+ uptake (Figure 4.7.5). The application of modified SWS resulted in similar responses to KCl treatments at equivalent $[K^+]$, and varied between SWS treatments. This was due to differences in the $[K^+]$ of the SWS treatments (Table 3.3.5). The $[K^+]$ in the supernatant and solid fractions of both SWS were different from their original suspensions due to the K^+ adsorption capacity of the cell debris of the SWS. This was different for each algal preparation used. Further, solid fractions obtained after centrifugation were re-suspended to the initial volume, thus resulting in a dilution of the K^+ content. The different K^+ levels in the test solutions were reflected in the $[K^+]$ content of seedlings (Figure 4.7.6.a). However, K^+ uptake by light grown seedlings treated with original ANS, or ANS solid fraction was lower than expected (Figure 4.7.6.a), and also the cotyledon growth response (Figures 4.7.8.a). Therefore K^+ uptake by seedlings treated with these preparations was inhibited. A possible explanation for this effect is that K^+ ions were electrostatically fixed to the organic matter, in this case algae cell wall debris. This would result in a reduction in its availability to seedling roots. This mechanism of K^+ immobilisation was indicated by data obtained during $[K^+]$ determination of various fractions (Table 3.3.5). These demonstrated a fourfold higher affinity for K^+ by solid particles of original ANS compared with ANS supernatant. This was indicated earlier in results of plant growth experiments by Blunden *et al.* (1968). In various preparations of different brown algae, alginic acid, as an ion-exchange medium, was thought of

competing with the plants for uptake of cations. It has a theoretical ion-exchange capacity of five times higher than soils (Blunden *et al.* 1968). Alginic acid is present in seaweeds mainly in an insoluble form in cell walls as a structural component (Percival and McDowell, 1990). Apparently, the alginic acid content of *A. nodosum* was found to be twice as high as in *Laminaria* species (Blunden *et al.* 1968), that would explain the twofold higher adsorption capacity of algal cell wall debris in ANS compared to LHS observed in this study (Table 3.3.4). A different mode of action, however, may have been involved in preventing effective K^+ uptake (Figure 4.7.6.a) and growth response in seedlings treated with LHS supernatant (Figure 4.7.8.a). The possible active compound(s) in this preparation that caused root growth inhibition and necroses, therefore indicated that severe damage to root metabolism had occurred. Thus, not only K^+ uptake was inhibited, but so also was cotyledon expansion (Figure 4.7.8.a). Possibly, reduced cytokinin from the roots prevented cotyledon expansion.

With most SWS treatments, promotion of cotyledon growth occurred in the light, whereas promotion of hypocotyl elongation was observed in the dark, in line with the seedling $[K^+]$ (Figure 4.7.8.b). Despite comparably high K^+ contents in the original LHS and LHS supernatant, a promotion of hypocotyl extension did not occur (Figure 4.7.8.b), due to a prevention of K^+ accumulation (Figures 4.7.6.b., and 4.7.7.b). Most likely, this was due to the presence of the growth inhibiting compound(s) found in LHS.

The promotion of hypocotyl elongation by K^+ has been reported for a range of plant species; for lettuce (*Lactuca sativa*) (Stuart and Jones, 1977), sunflower (*Helianthus annuus*) (McIntyre and Boyer, 1984), tomatoes (*L. lycopersicum*) (Kordan, 1987), amaranth (*Amaranthus caudatus*) (Guruprasad and Guruprasad, 1988), and sugar beet (*B. vulgaris* var. *altissima*) (Durrant and Mash, 1989). However, the mode of action of K^+ in this process is unclear. Potassium ions do not

have a significant effect on cell wall extensibility (Stuart and Jones, 1977), and hence might not be the 'driving' force in hypocotyl elongation. Purves (1966), and Stuart and Jones (1977) found that elongation in hypocotyl segments was promoted by a range of other monovalent cations beside K^+ , including Na^+ . However, in this study Na^+ or Ca^{++} did not promote lettuce hypocotyl elongation in the dark when applied to the root (Figure 4.7.1), but a very specific response to K^+ was observed. This is in close agreement with results published by Guruprasad and Guruprasad (1988).

These findings suggest, that the potential for eliciting hypocotyl elongation is given by a range of cations, but is only expressed when the cations were applied directly to hypocotyl tissue. In intact plants, however, the selective permeability of radicles by the cell wall membranes (Müller and Rudin, 1967) will promote K^+ uptake and accumulation in the hypocotyl, whilst other cations, are taken up at a lower rate, preventing their accumulation, and consequently, the growth response. According to Müller and Rudin (1967), Na^+ transportation across bimolecular lipid membranes is 300 times less than K^+ transport.

Alongside cotyledon enlargement, Knypl and Chylinska (1972) reported an increase in the total chlorophyll content per cotyledon of lettuce seedlings treated with KCl solutions. This effect was also found in this work (Figure 4.8.1.a). The chlorophyll content of lettuce cotyledons increased with KCl solutions, as well as with most SWS treatments, and correlated with increases in cotyledon area. The increased chlorophyll concentration on a fresh weight basis of all treatments over controls could be attributed to stimulated cotyledon expansion growth in conjunction with a higher chlorophyll synthesis. Thus, the increase in cotyledon weight was not simply attributable to increased water uptake, due to an osmotic effect of K^+ ions. However, when results were expressed per cotyledon area, KCl, ashed ANS and ANS supernatant treatments reduced chlorophyll accumulation (Figure 4.8.1.c). This

was due to a disproportionate increase in cotyledon area, compared with the equivalent stimulation of chlorophyll synthesis. Only the application of original SWS or the LHS supernatant and LHS solid fraction resulted in an increase in chlorophyll content per cotyledon area unit (Figure 4.8.1.c). In the cases of original SWS and the LHS solid fraction, this was the result of a combination of a cotyledon growth promotion and a stimulation of chlorophyll synthesis at the same time. Thus, the net increase in chlorophyll content was due to an organic SWS factor, rather than entirely due to the mineral effect of K^+ ions. For a further isolation and identification of this compound, the need for further investigations is recognised.

In the case of LHS supernatant, however, despite an unaltered total cotyledon chlorophyll content of seedlings, a high value for chlorophyll concentration per mm^2 cotyledon was calculated. This might indicate a water stress response by the seedlings, most likely caused by a reduced water uptake by the distorted radicle, and resulted in smaller cotyledons, containing an unchanged total chlorophyll content.

Chapter 5

**Results of investigations on the effect of seed
priming in SWS on barley and lettuce
seed germination and seedling growth**

5.1 Introduction

In this chapter pre-sowing priming treatments (osmo-conditioning) using the two seaweed extracts (SWS) were investigated for their ability to improve lettuce (*Lactuca sativa*) and barley (*Hordeum vulgare*) seed performance. Conditions tested included optimum and adverse germination situations, as well as seedling emergence from soil in the greenhouse. The effect of SWS seed soaks on the occurrence of normal and abnormal seedlings was also monitored.

As shown in Section 3.2.1, LHS has an osmotic potential (OP) of -0.5 MPa in the undiluted state. This inhibited the moisture uptake and delayed radicle emergence accordingly (Section 4.3). However, higher OP are usually applied during commercial priming treatments, allowing biochemical events associated with germination to take place, but restricting radicle emergence for between 7-14 days. Thus, it was important to determine water uptake and loss in relation to radicle emergence when seeds were subject to the priming treatments.

Therefore, the inhibition of imbibition with SWS was compared with water priming treatments. In contrast to polyethylene glycol (PEG) the SWS are not inert, but contain substantial amounts of dissolved minerals as well as carbohydrate molecules. Thus, the mineral content of treated and untreated seeds was compared to obtain information on the amount of minerals leaching from the seeds during priming, and the addition of minerals to the seeds due to adhering SWS material during priming.

5.2 The physical effect of SWS priming treatments on seeds

5.2.1 Experiment 5.1: The SWS priming effect on water relations, and the adhesion of SWS cell wall debris to seeds

Barley

The initial water content of the seeds was 12.8 % (dw). When barley seeds were placed in distilled water the seed moisture content reached 47.0 % (dw) after 12 hours and 58.0 % after 24 hours (Figure 5.2.1). The uptake rate decreased from 5.1 % h⁻¹ after 6 hours to 0.8 % h⁻¹ after 24 hours. Seeds primed in SWS and rinsed prior moisture determination had 2.3 % (ANS) and 4.0 % (LHS) ($p < 0.001$) lower values than control seeds after this period. The difference did increase marginally over time to 4.7 % (ANS) or 6.0 % (LHS) ($p < 0.001$) after 48 hours, and 4.8 % (ANS) or 7.2 % (LHS) ($p < 0.001$) after 72 hours of priming. The rate of water loss of seeds primed for 12 hours and then rinsed was identical in all treatments (Figure 5.2.1). After 24 hours of re-drying under uncontrolled conditions, the seed moisture content was 12.0 % and 13.0 %.

If seeds were not rinsed after 12 hours priming treatments with SWS the moisture content was 97.1 % (ANS) ($p < 0.001$), or 75.1 % (LHS) ($p < 0.001$), almost half of this was contained in the adhering algal cell wall debris (Figure 5.2.2). The moisture content of those seeds was 2.2 % ($p < 0.01$), or 1.4 % ($p < 0.05$) higher after 24 hours re-drying, compared to rinsed seeds. Adhering SWS added 1.7 % (ANS) ($p < 0.01$) or 0.8 % (LHS) ($p < 0.05$) dry matter to seeds (Figure 5.2.2) (Plate 5.2.1).

Lettuce

Dry seeds contained 6.5 % moisture. Control seeds primed in water reached a moisture content of 82.9 % after 6 hours, with a moisture uptake rate of 12.9 % h⁻¹. After 12 hours soaking the rate of moisture uptake reduced to 1.8 % h⁻¹, the seed moisture was 93.8 % (Figure 5.2.3). Radicle emergence occurred after 20 hours.

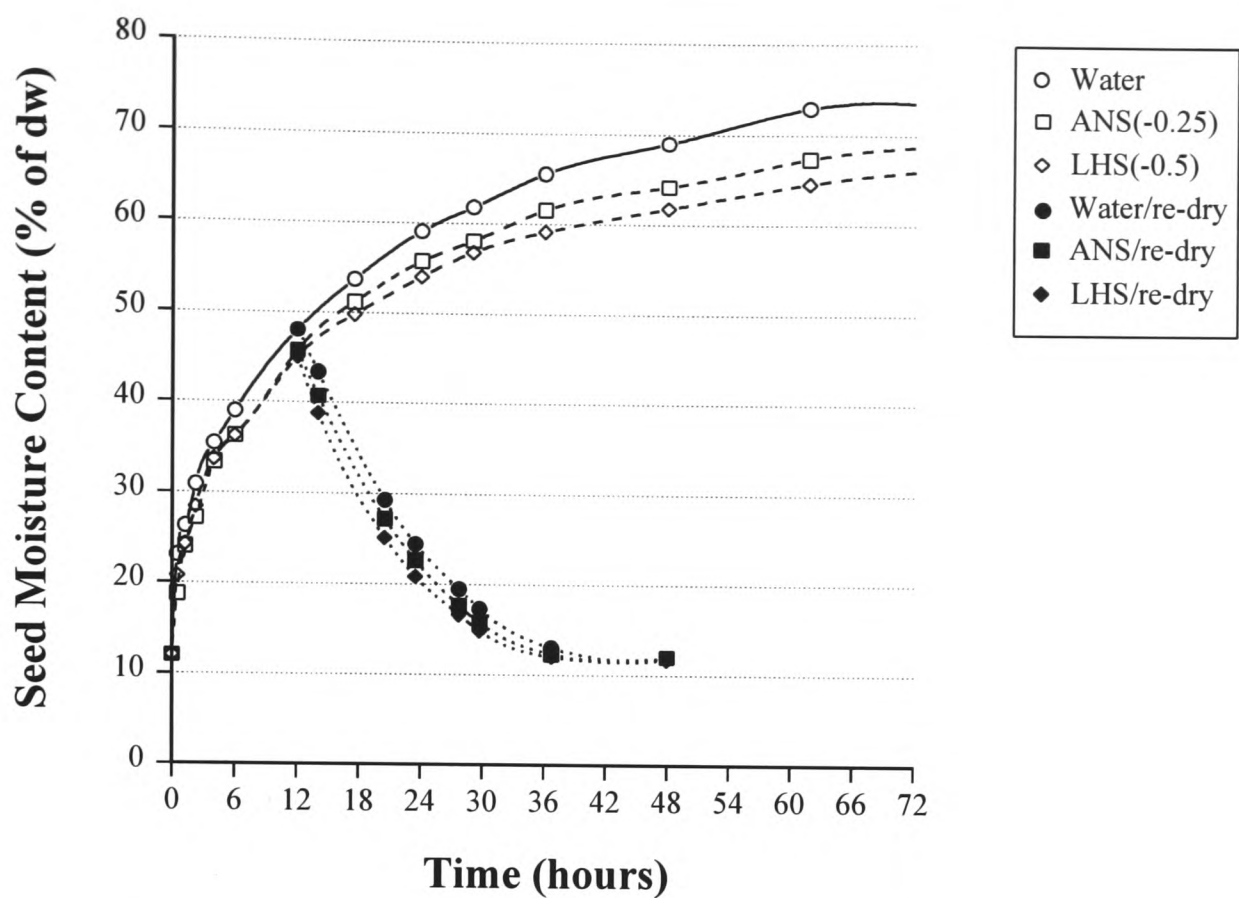


Figure 5.2.1: The effect of soaking in distilled water or undiluted SWS on the water uptake and the moisture loss during consecutive re-drying of barley cv. Triumph(A) seeds. n=3.

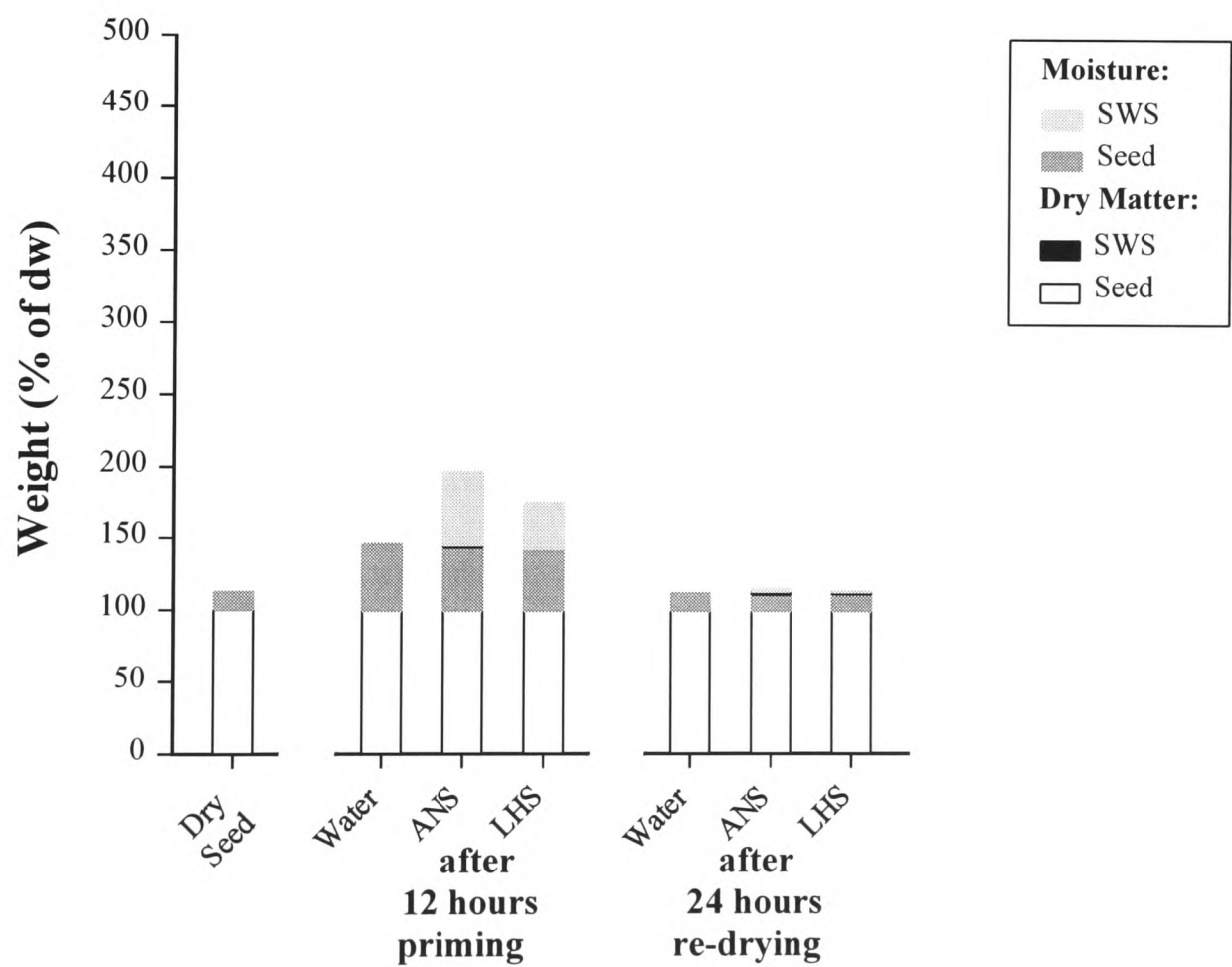


Figure 5.2.2: The dry matter and seed moisture content of barley cv. Triumph(A) seeds after a 12 hour priming period in water, ANS or LHS, and after a consecutive 24 hour re-drying period. n=3.

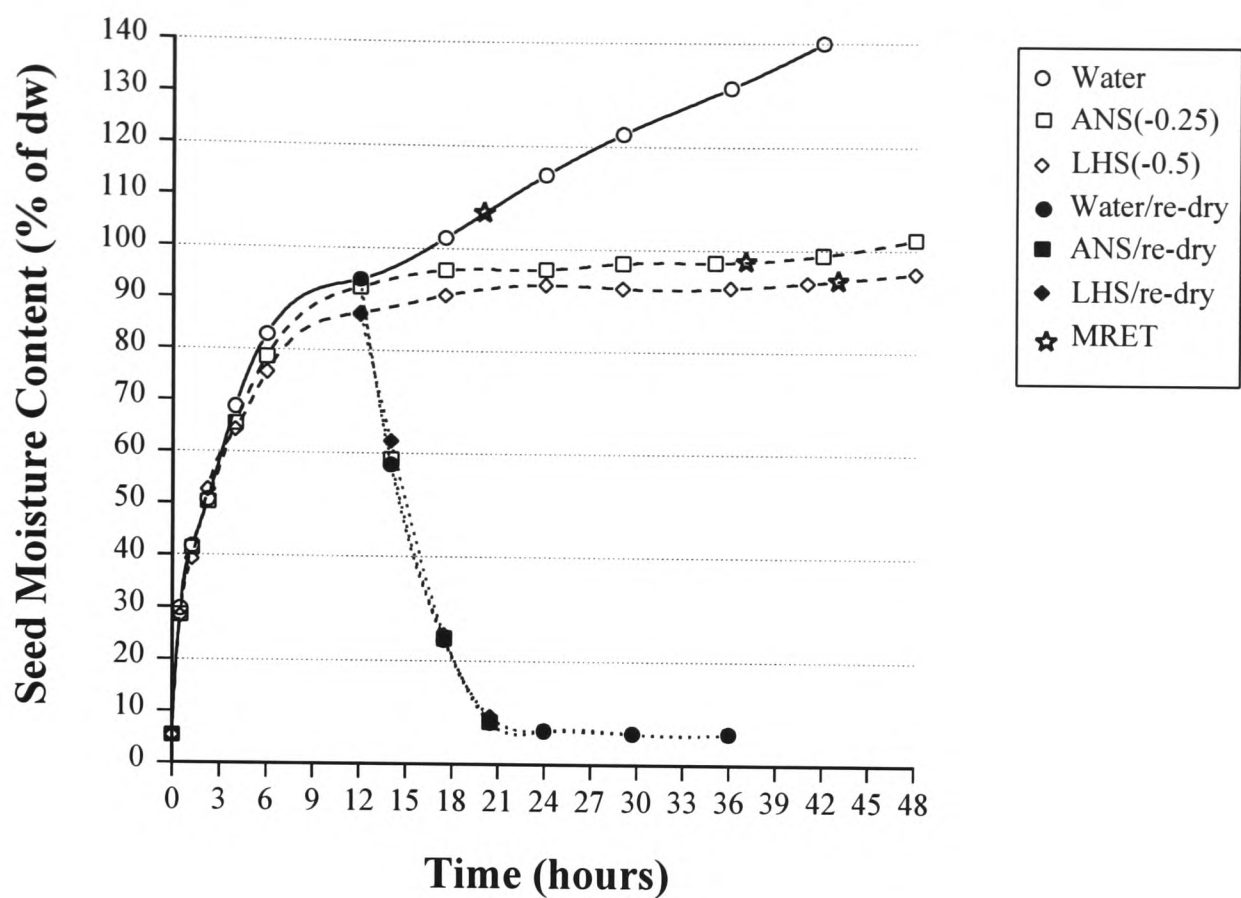


Figure 5.2.3: The effect of soaking in distilled water or undiluted SWS on the water uptake and the moisture loss during consecutive re-drying of lettuce cv. Reskia(B) seeds. n=3.

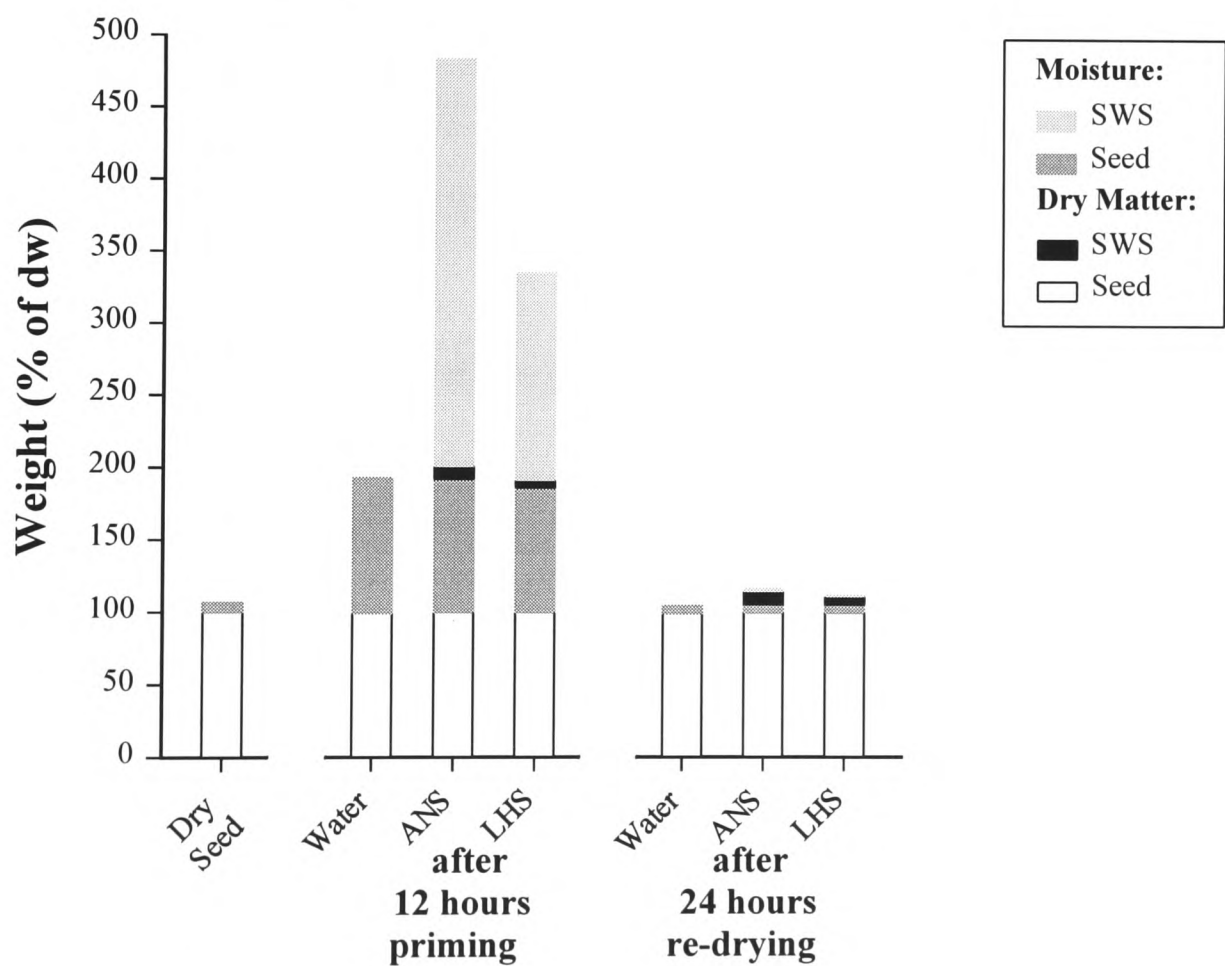


Figure 5.2.4: The dry matter and seed moisture content of lettuce cv. Reskia(B) seeds after a 12 hours priming period in water, ANS or LHS, and after a consecutive 24 hours re-drying period. n=3.

For the first 4 hours, seeds primed in SWS and rinsed after treatment had a similar rate of water uptake compared to those soaked in water. After 6 hours priming, however, treated seeds contained 4.3 % (ANS) and 7.3 % (LHS) ($p < 0.01$) less water, but only 6.9 % (LHS) ($p < 0.01$) after 12 hours, compared to water primed seeds. Beyond 12 hours priming periods the difference between SWS soaked seeds and control seeds increased steadily. Radicle emergence of water soaked seeds was also initiated at this time. In contrast, SWS primed seeds did not germinate until after 36 hours (ANS) or 42 hours (LHS) had elapsed (Figure 5.2.3). The rate of water loss on drying of seeds that were rinsed after priming, was the same between SWS treated and water control seeds (Figure 5.2.3). After 24 hours, seed moisture content of all seeds had dropped to between 6.0 - 6.1 %.

Seeds with adhering SWS had 283 % (ANS) ($p < 0.001$) and 144 % (LHS) ($p < 0.001$) more water after 12 hours priming, compared to water soaked seeds (Figure 5.2.4). The ANS primed seeds after 24 hours re-drying had 1.7 % ($p < 0.05$) more moisture than control seeds. Adhering algal cell wall debris contributed an extra 5.3 % (LHS) or 9.0 % (ANS) ($p < 0.001$) dry matter to the seeds (Plate 5.2.2).

5.2.2 Experiment 5.1: The SWS priming effect on the mineral composition of seeds

Barley

Mineral composition did not change after seed priming in water, except for the potassium concentration that was reduced from 0.36 % - 0.32 % (dw) ($p < 0.01$) (Table 5.2.1). The mineral composition of seeds rinsed after SWS treatment was generally no different to dry seeds, except for the phosphorus content that increased 10 % ($p < 0.05$) after priming in LHS, regardless of rinsing treatment. Adhering ANS cell wall debris increased sodium levels fivefold ($p < 0.01$), manganese 5 % ($p < 0.05$), zinc 15 % ($p < 0.05$), and boron 78 % ($p < 0.001$) (Table 5.2.1).



Plate 5.2.1: The appearance of barley cv. Triumph(A) seeds after priming in ANS (top) or LHS (left) for 12 hours, and 24 hours re-drying. Dry untreated seeds (right).

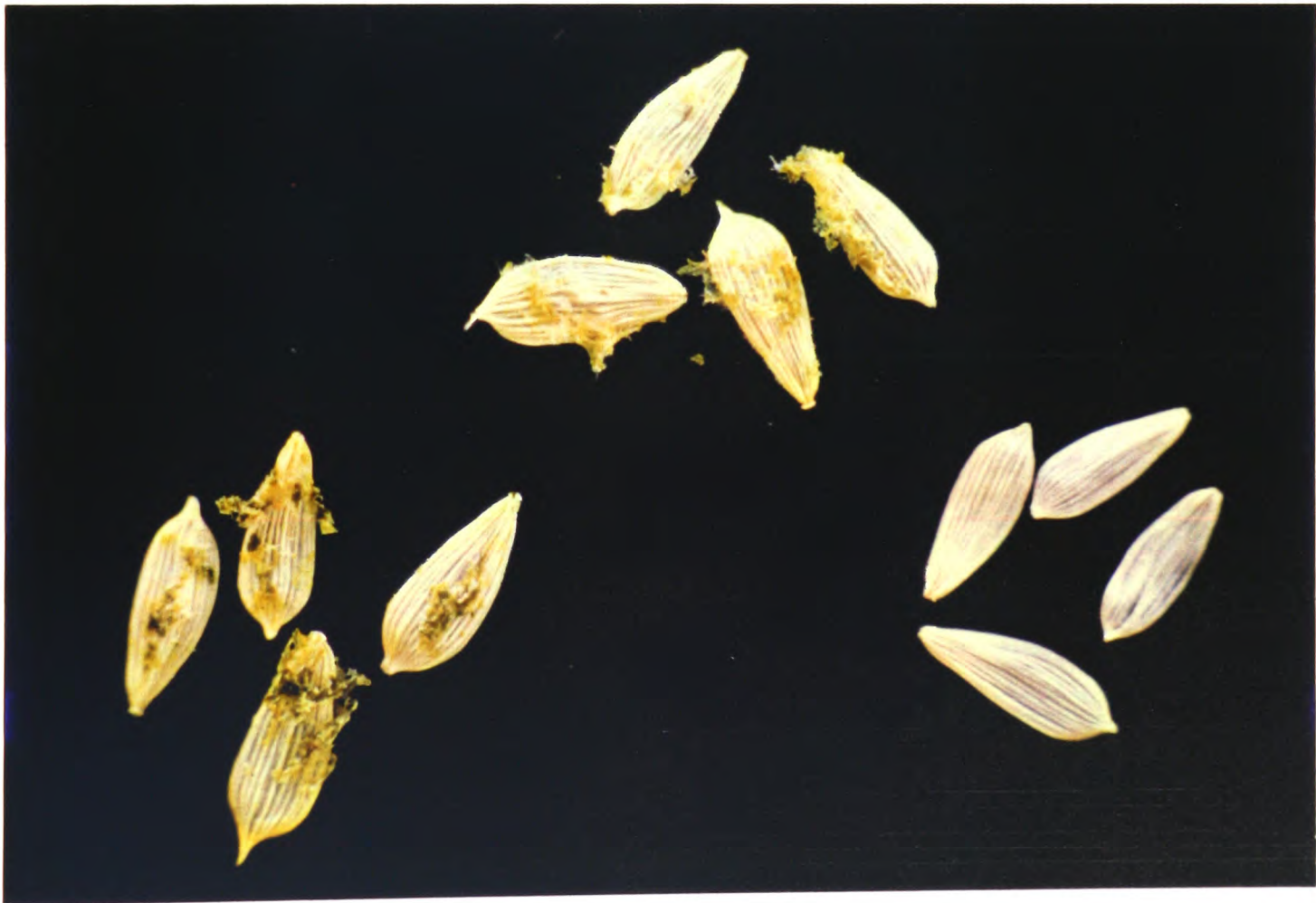


Plate 5.2.2: The appearance of lettuce cv. Reskia(B) seeds after priming in ANS (top) or LHS (left) for 12 hours, and 24 hours re-drying. Dry untreated seeds (right).

Table 5.2.1: The effect of 12 hours SWS seed priming, with or without rinsing, on the mineral composition of seeds of barley cv. Triumph(A). Priming for 12 hours, with 24 hours re-drying period. n=3.

Priming treatment	Control Dry seed	Seed primed in					LSD (DF 5) p= 0.05 / 0.01 / 0.001
Surface		H ₂ O	ANS	LHS	ANS	LHS	
	(% dw)	(% dw)	(% dw)	(% dw)	(% dw)	(% dw)	
Mineral							
P	0.31	0.32 NS ²	0.33 NS	0.34 * ²	0.34 *	0.34 *	0.02 / 0.03 / 0.04
K	0.36	0.32 **	0.35 NS	0.37 NS	0.37 NS	0.38 NS	0.02 / 0.03 / 0.05
Ca	0.03	0.03 NS	0.03 NS	0.03 NS	0.04 NS	0.04 NS	NS
Mg	0.11	0.11 NS	0.12 NS	0.12 NS	0.12 NS	0.12 NS	NS
S	0.12	0.12 NS	0.13 NS	0.13 NS	0.15 NS	0.14 NS	NS
Na	0.01	0.00 NS	0.02 NS	0.02 NS	0.05 **	0.02 NS	0.02 / 0.03 / 0.05
	(ppm dw)	(ppm dw)	(ppm dw)	(ppm dw)	(ppm dw)	(ppm dw)	
Fe	43.9	52.7 NS	45.4 NS	40.4 NS	44.3 NS	41.4 NS	NS
Mn	10.0	10.1 NS	10.0 NS	10.3 NS	10.5 *	10.1 NS	0.4 / 0.7 / 1.1
Cu	5.0	5.1 NS	5.0 NS	5.0 NS	5.1 NS	5.0 NS	NS
Zn	23.2	24.3 NS	23.9 NS	24.0 NS	26.6 *	25.0 NS	2.3 / 3.6 / 6.2
B	0.9	0.8 NS	0.9 NS	0.9 NS	1.6 ***	1.0 NS	0.2 / 0.3 / 0.5

(² - different from the control at * = 5 %, ** = 1 %, and *** = 0.1 % probability level, NS = not significant)

Lettuce

A 12 hour soak in water reduced the potassium content of seeds 6 % (p<0.01), and iron 26 % (p<0.001) (Table 5.2.2). Removal of adhering SWS after priming reduced all minerals except sodium and boron. Sodium concentration increased six fold (ANS) (p<0.001) or fivefold (LHS) (p<0.01), whereas boron concentration was increased 9 % (p<0.05) with both SWS treatments (Table 5.2.2). Adhering ANS compensated for soaking losses of phosphorus, potassium, calcium, and magnesium, while LHS compensated for magnesium losses, and increased potassium 12 % (p<0.001) and calcium levels 19 % (p<0.01) above that of dry control seeds. Sulphur levels were also increased 7 % (p<0.05) for LHS or 25 % (p<0.001) for ANS treatments (Table 5.2.2). Adhering ANS and LHS material increased sodium levels

between 2.5 - 2.0 fold ($p<0.001$). Boron was increased 44 % ($p<0.001$) (ANS), or 23 % ($p<0.001$) (LHS), over SWS primed and rinsed seeds (Table 5.2.2).

Table 5.2.2: The effect of 12 hours SWS seed priming, with or without rinsing, on the mineral composition of seeds of lettuce cv. Reskia(B). Priming for 12 hours, with 24 hours re-drying period. $n=3$.

Priming treatment	Control Dry seed	Seed primed in					LSD (DF 5) $p=0.05/0.01/0.001$
Surface		H ₂ O	ANS	LHS	ANS	LHS	
	(% dw)	(% dw)	rinsed (% dw)	(% dw)	not rinsed (% dw)	(% dw)	
Mineral							
P	0.86	0.86 NS ²	0.80 ***	0.82 **	0.86 NS	0.83 *	0.02 / 0.03 / 0.05
K	0.65	0.61 **	0.58 ***	0.61 **	0.67 NS	0.73 ***	0.02 / 0.03 / 0.05
Ca	0.16	0.16 NS	0.13 **	0.13 **	0.17 NS	0.19 **	0.01 / 0.02 / 0.04
Mg	0.40	0.40 NS	0.37 **	0.37 **	0.41 NS	0.40 NS	0.01 / 0.02 / 0.04
S	0.28	0.27 NS	0.27 NS	0.26 *	0.35 ***	0.30 *	0.01 / 0.02 / 0.04
Na	0.01	0.01 NS	0.06 ***	0.05 **	0.16 ***	0.10 ***	0.02 / 0.03 / 0.04
	(ppm dw)	(ppm dw)	(ppm dw)	(ppm dw)	(ppm dw)	(ppm dw)	
Fe	171.6	127.2 ***	111.0 ***	108.3 ***	123.6 ***	115.4 ***	5.3 / 8.3 / 14.2
Mn	28.2	27.8 NS	23.9 ***	23.8 ***	26.2 *	24.2 **	1.6 / 2.4 / 4.2
Cu	11.5	11.5 NS	11.0 NS	10.9 NS	11.7 NS	11.6 NS	NS
Zn	72.5	71.6 NS	65.5 ***	64.4 ***	69.8 *	66.8 ***	1.8 / 2.8 / 4.8
B	8.9	8.3 NS	9.7 *	9.7 *	14.0 ***	11.9 ***	0.7 / 1.2 / 2.0

(² - different from the control at * = 5 %, ** = 1 %, and *** = 0.1 % probability level, NS = not significant)

5.3 The effect of SWS seed priming parameters on germination

Introduction

Based on results in Section 5.2.1, appropriate priming time ranges were selected to determine an optimum soaking / re-drying regime for maximum effect, but with the prevention of radicle emergence. Further investigations on re-drying of primed seeds were conducted to investigate the effect on seed performance. The following experiments were aimed to provide information on a standard priming regime. This would then be used for further germination experiments under stress conditions.

5.3.1 Experiment 5.2: The effect of SWS priming duration on germination

Barley

Dry untreated seeds of cv. Triumph(A) had 96 % germination after three days. The SWS priming treatments had no effect on the percentage seeds that germinated after three or seven days (Figure 5.3.1).

The MGT of control seeds was 50 hours (Figure 5.3.2.a). Priming in ANS for 6 hours reduced the MGT 8 % ($p<0.05$). Priming for 12 hours (36 %), 24 hours (44 %) or 48 hours (44 %) all significantly reduced ($p<0.001$) MGT. Priming in LHS for 24 hours (12 %) or 48 hours (24 %) also reduced ($p<0.001$) MGT. The number of abnormal seedlings was not affected by any treatment. Soaking seeds in ANS (10 %) or LHS (9 %) ($p<0.01$) for 48 hours increased the number of dead seeds (Figure 5.3.2.c).

Lettuce

Priming lettuce cv. Reskia(B) seeds in ANS for 24 hours reduced germination by 10 % ($p<0.01$) after day three, but was not different from the control after seven days (Figure 5.3.3). After 48 hours in SWS, germination already had occurred, all seeds were dead after 24 hours re-drying, and thus these treatments were excluded.

Seeds primed for 12 hours (20 %), or 24 hours (44 %) ($p<0.001$) in ANS germinated faster. LHS treatments reduced MGT 22 % (6 hours), 28 % (12 hours) or 33 % (24 hours) ($p<0.001$) (Figure 5.3.4.a). The occurrence of abnormal seedlings and dead seeds in the germination test was not influenced by the priming treatments (Figures 5.3.4.b, and 5.3.4.c).

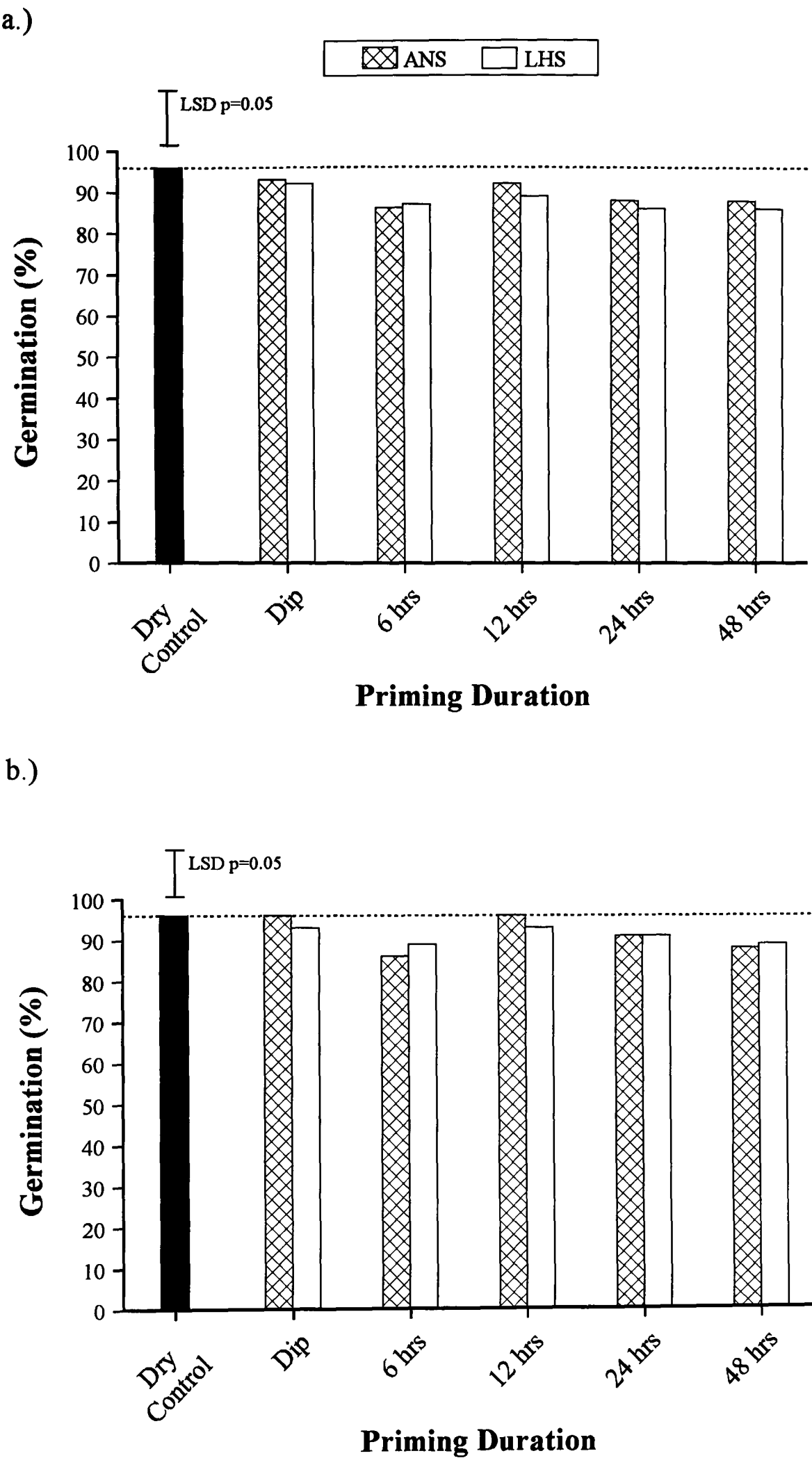


Figure 5.3.1: The effect of priming duration in undiluted SWS on the percentage germination of barley cv. Triumph(A), a.) after 3 days, and b.) after 7 days. Seeds were re-dried for 24 hours. n=3.

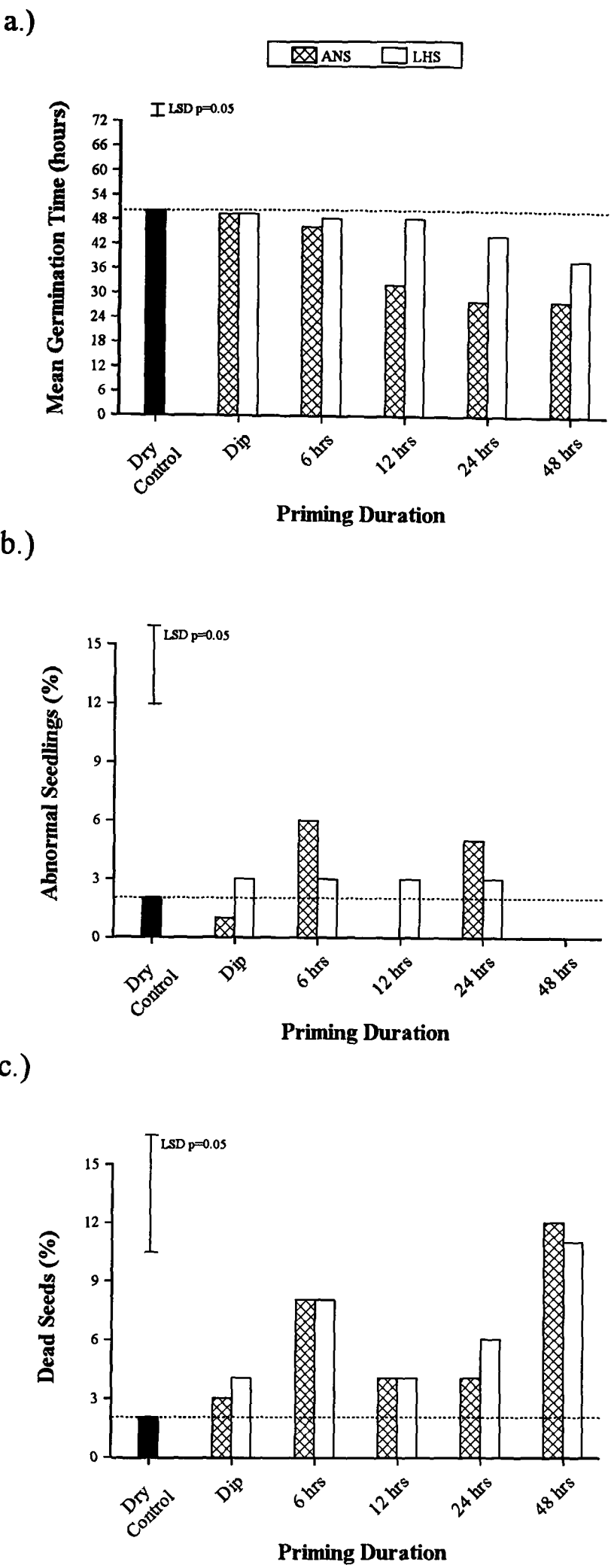


Figure 5.3.2: The effect of priming with undiluted SWS on a.) mean germination time, b.) percentage abnormal seedlings, and c.) dead seeds of barley cv. Triumph(A). Seeds were re-dried for 24 hours. n=3.

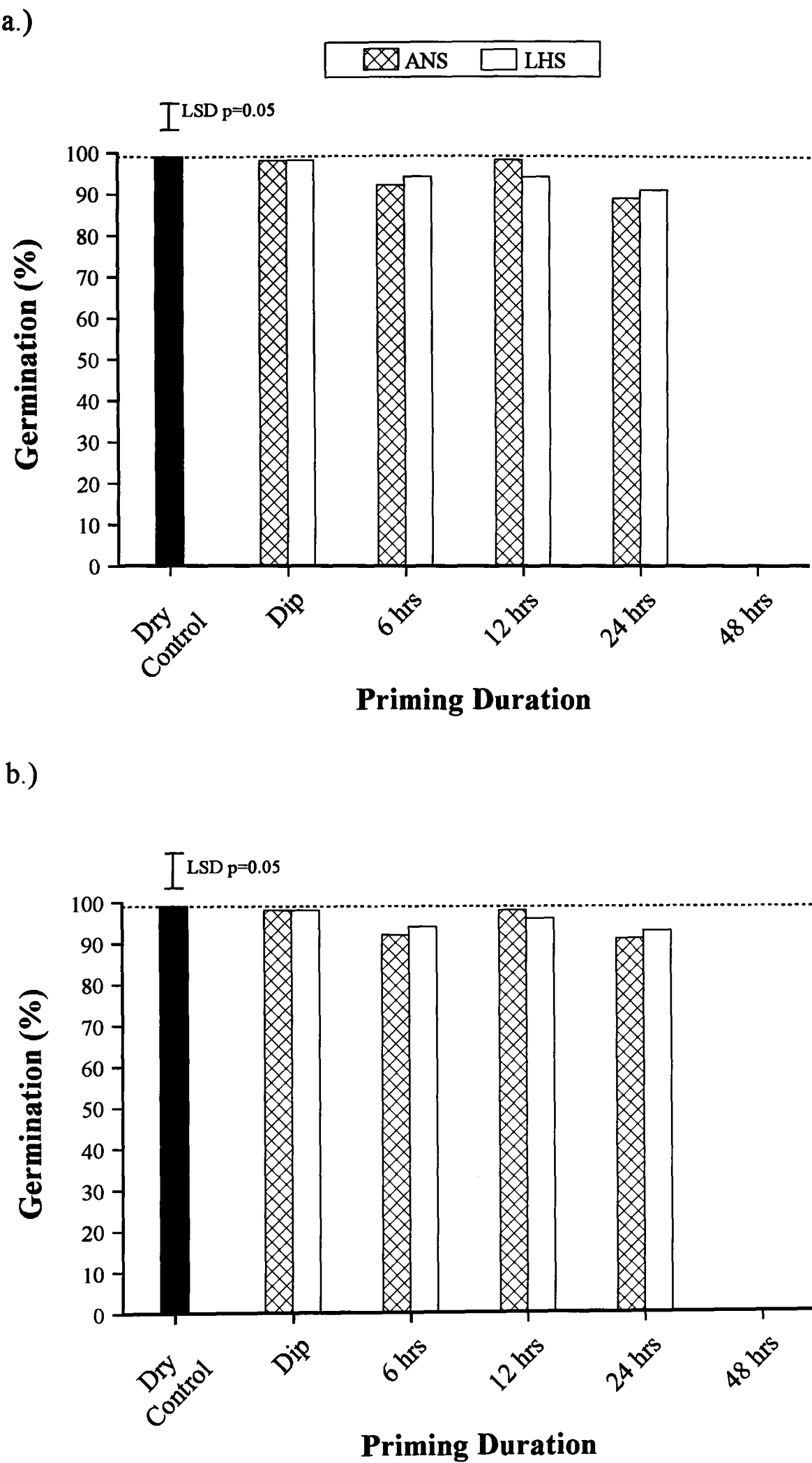


Figure 5.3.3: The effect of priming duration with undiluted SWS on the percentage germination of lettuce cv. Reskia(B), a.) after 3 days, and b.) after 7 days. Seeds were re-dried for 24 hours. n=3.

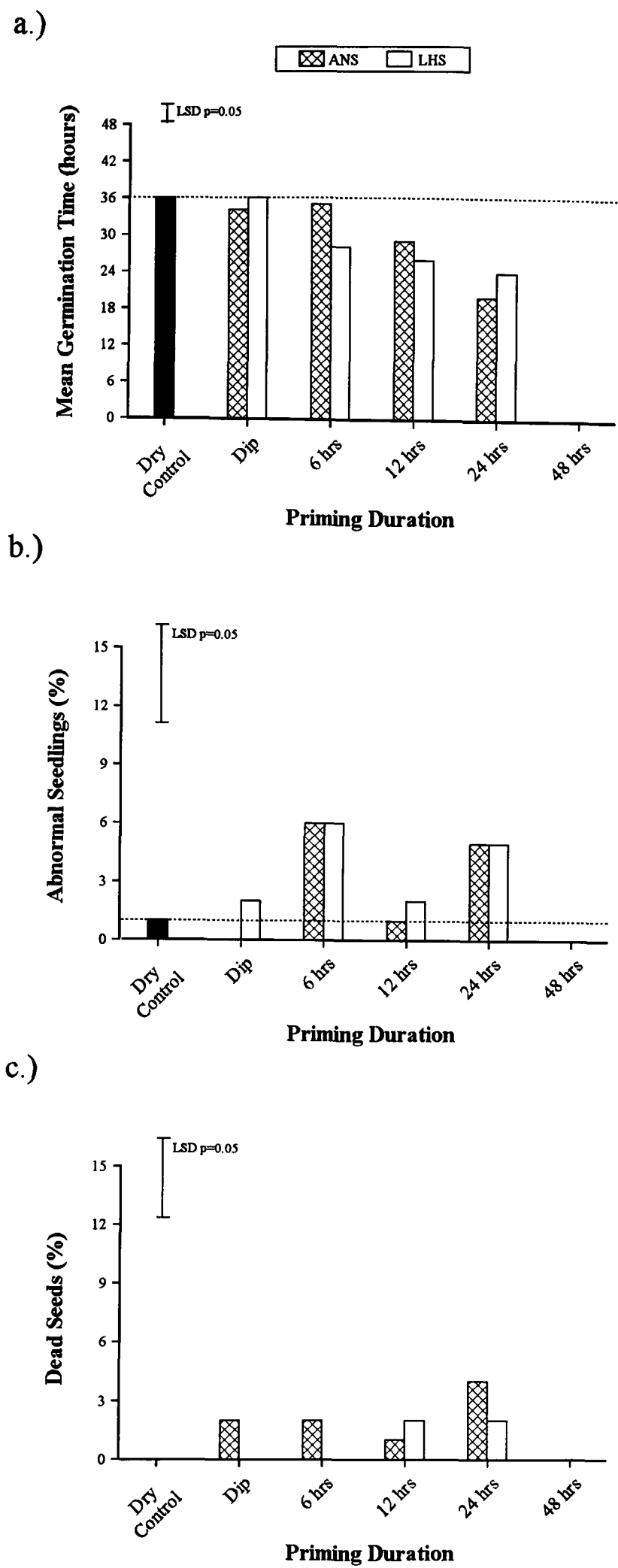


Figure 5.3.4: The effect of priming with undiluted SWS on a). mean germination time, b.) percentage abnormal seedlings, and c.) dead seeds of lettuce cv. Reskia(B). Seeds were re-dried for 24 hours. n=3.

5.3.2 Experiment 5.3: The effect of re-drying of SWS primed seeds on germination

Barley

Untreated barley cv. Triumph(A) seeds had a germination percentage of 93 - 94 % (Figure 5.3.5.a). Seed priming in water did not affect the percentage germination. Priming in undiluted SWS reduced the germination to 48 % (ANS) ($p < 0.001$) or 61 % (LHS) ($p < 0.01$) when they were not re-dried after priming. A re-drying period of one day was sufficient to restore the germination percentage to control levels. A similar result was obtained after a re-drying period of one week (Figure 5.3.5.a).

A seed priming treatment in water reduced the MGT 24 % ($p < 0.01$) when they were not re-dried, while SWS treatments were not effective (Figure 5.3.5.b). However, re-drying the seed reduced the MGT for ANS treated seeds 18 % (1 day) or 19 % (1 week) ($p < 0.001$) compared to the corresponding non re-dried treatment (Figure 5.3.5.b). Re-drying of seeds primed in LHS had no effect on the MGT.

Lettuce

Treatments had no effect on the percentage germination of seeds of lettuce cv. Reskia(B) (Figure 5.3.6.a). A seed treatment with water, ANS or LHS reduced MGT 39 %, 25 % or 29 % ($p < 0.001$), when seeds were not re-dried. With re-drying over 1 week, the MGT of soaked seeds increased 35 % (water) ($p < 0.001$), 23 % (ANS) ($p < 0.01$) or 70 % (LHS) ($p < 0.001$) over corresponding not re-dried priming treatments (Figure 5.3.6.b).

5.3.3 Experiment 5.4: Effect of removing adhering SWS on germination

Barley

SWS treatments with or without rinsing of adhering SWS material had no effect on percentage germination on seeds of barley cv. Triumph(A). The MGT was 44 hours

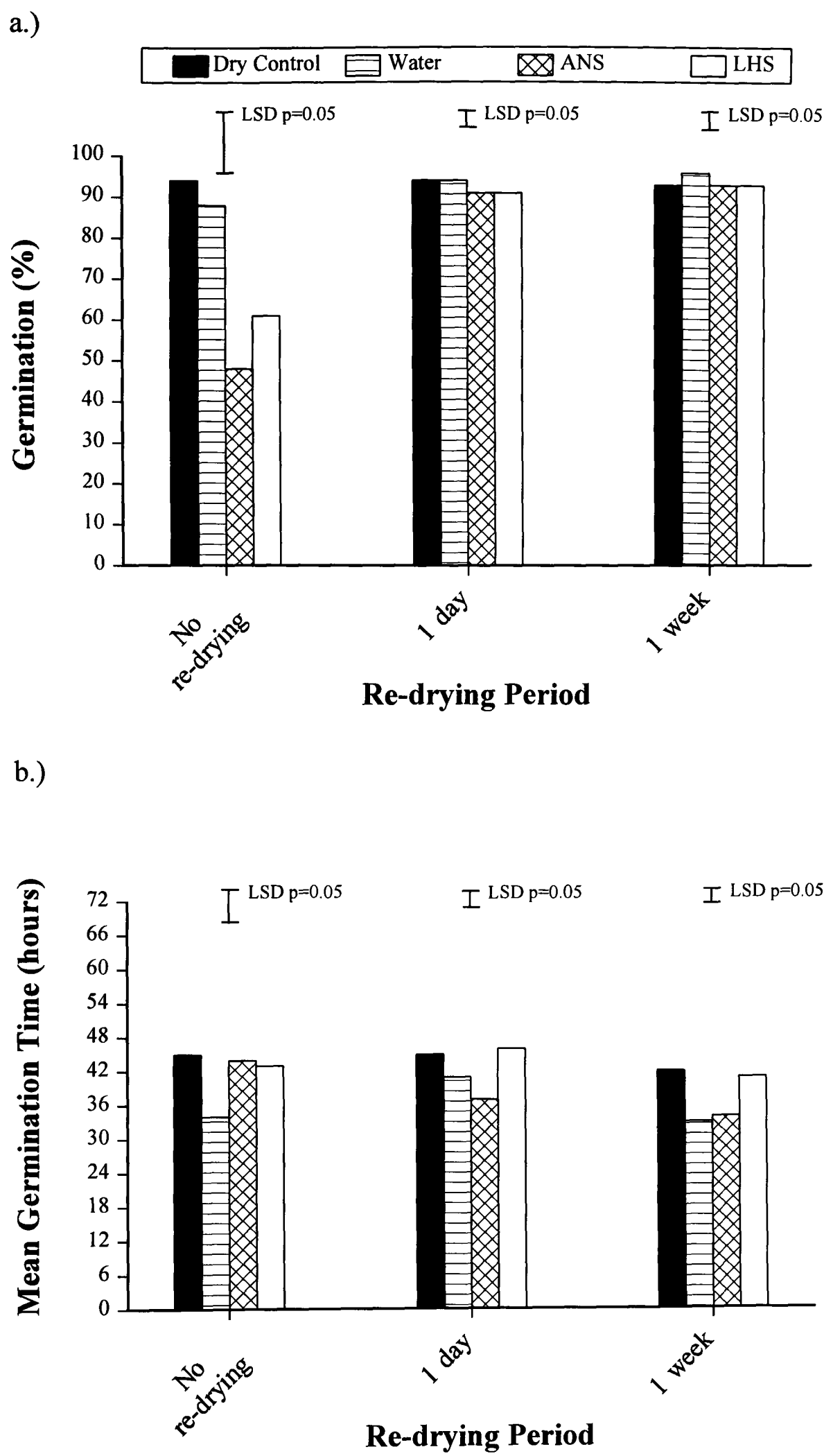


Figure 5.3.5: The effect of re-drying on a.) percentage germination after 3 days and b.) mean germination time over 3 days, of barley cv. Triumph(A) seeds, primed for 12 hours. n=3.

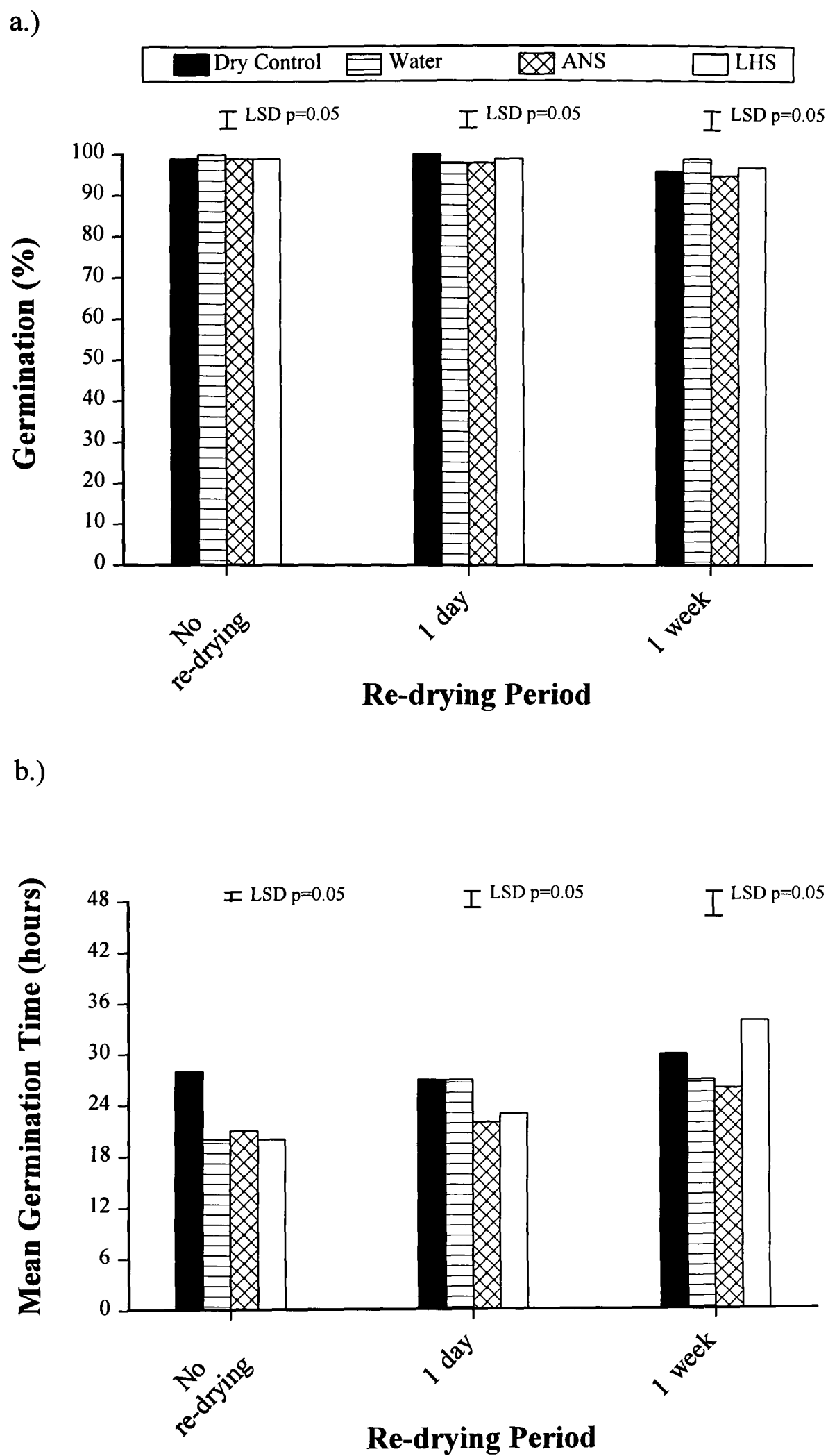


Figure 5.3.6: The effect of re-drying on a.) percentage germination after 3 days and b.) mean germination time over 3 days, of lettuce cv. Reskia(B) seeds, primed for 12 hours. n=3.

for the control, and was reduced by all treatments ($p < 0.01$) (Figure 5.3.7.b).

Removal of adhering SWS increased the MGT of ANS treated seeds ($p < 0.05$) (Figure 5.3.7.b).

Lettuce

No treatment effect on the percentage germination of lettuce cv. Reskia(B) was observed (Table 5.3.8.a). The MGT was reduced with ANS (19 %) ($p < 0.001$) and LHS (15 %) ($p < 0.01$), only when the seeds were not rinsed (Figure 5.3.8.b).

5.4 The effect of controlled deterioration on seed performance

Introduction

To test seedling performance various vigour tests have been developed. In this study, seeds that had been aged by controlled deterioration (CD) were tested for their vigour potential measured by changes in the MGT, and the percentage germination after three days. The root, shoot and hypocotyl growth were used as additional vigour indices.

5.4.1 Experiment 5.5: The effect of controlled deterioration on seed germination

Barley

Control seeds of cv. Triumph(B) had a germination of 96 % (Figure 5.4.1.a). Controlled deterioration for one or two days had no effect on the percentage germination. Seeds deteriorated for three days had a germination of 57 % ($p < 0.001$).

The MGT of control treatments was 38 hours (Figure 5.4.1.a). The MGT was not affected by CD for one day, but increased after two days CD 45 %, and after three days CD 63 % ($p < 0.001$).

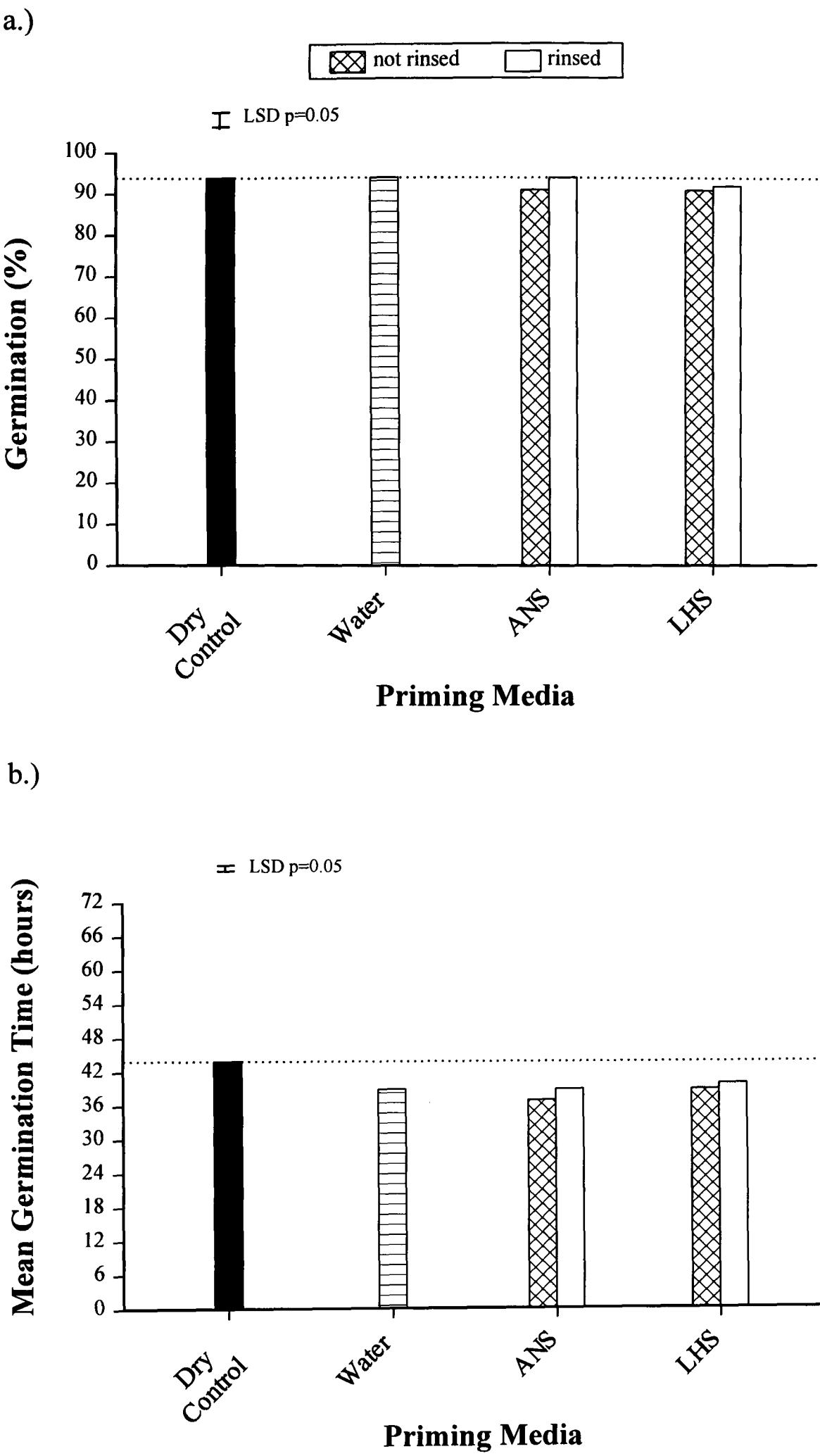


Figure 5.3.7: The effect of removal of adhering SWS after priming on a.) the percentage germination after 3 days, and b.) mean germination time over 3 days, of seeds of barley cv. Triumph(A). Priming for 12 hours and re-drying for 24 hours. n=3.

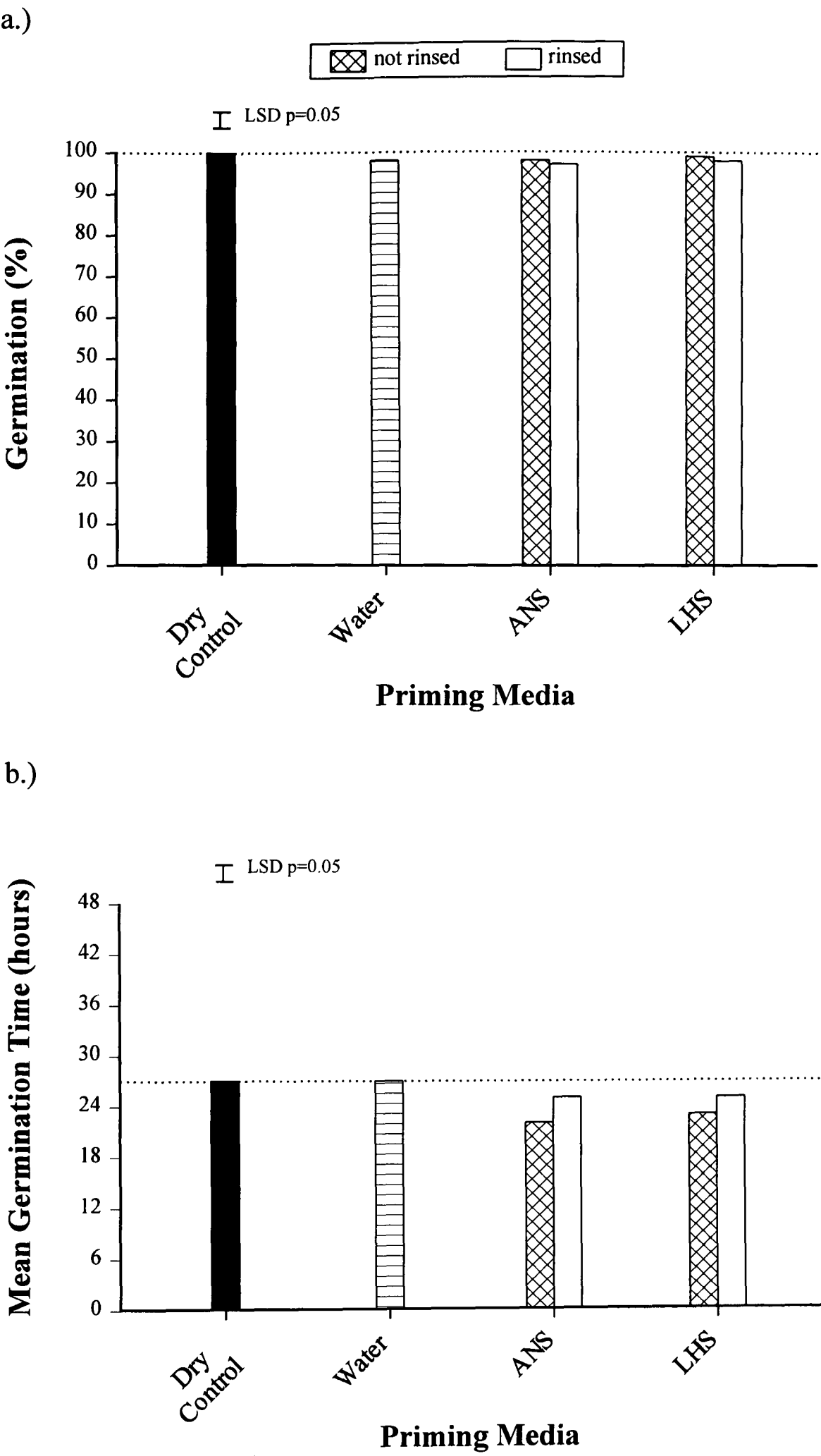


Figure 5.3.8: The effect of removal of adhering SWS after priming on a.) the percentage germination after 3 days, and b.) mean germination time over 3 days, of seeds of lettuce cv. Reskia(B). Priming for 12 hours and re-drying for 24 hours. n=3.

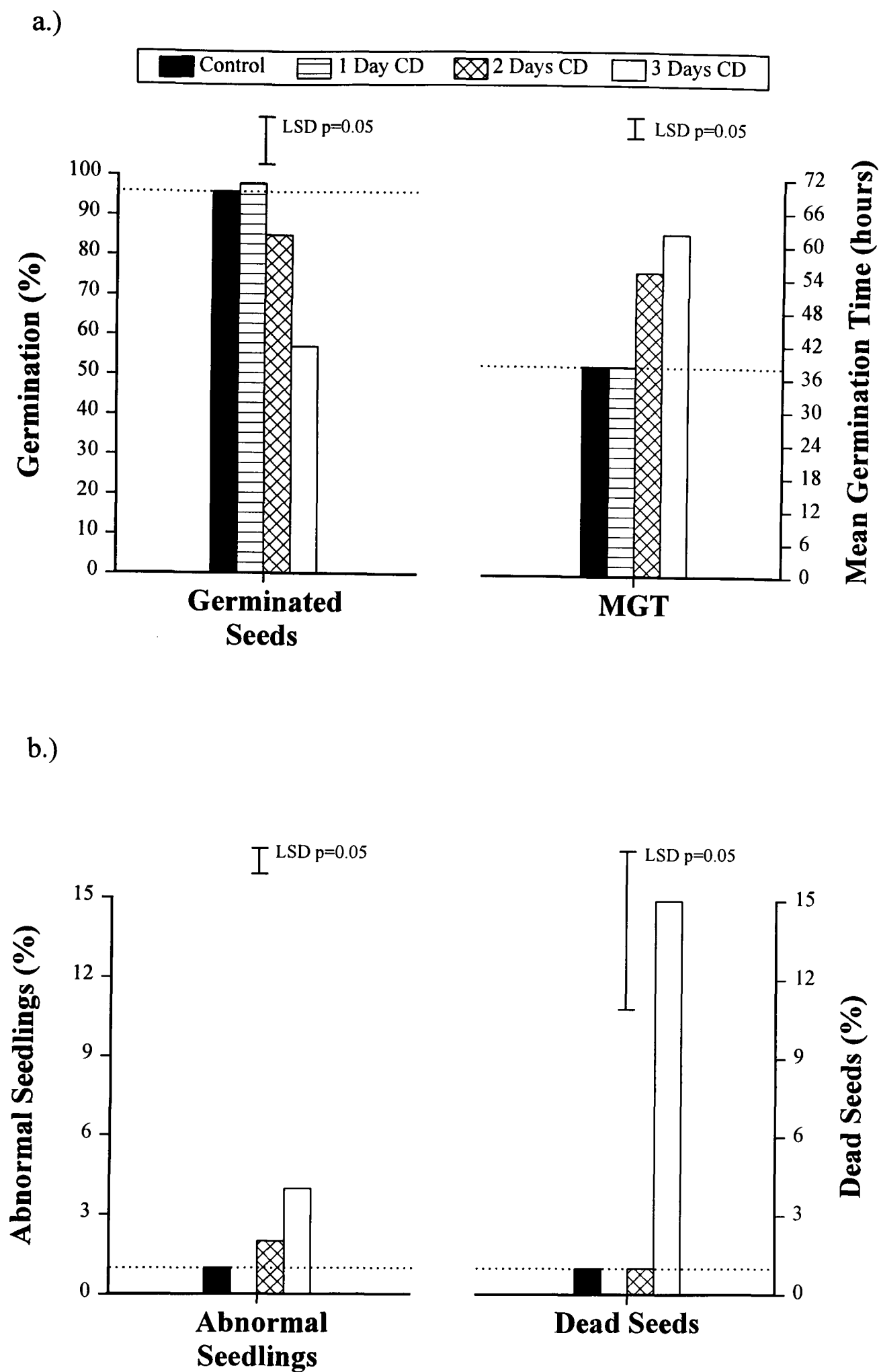


Figure 5.4.1: The effect of controlled deterioration (CD) for 1 to 3 days on a.) the germination percentage and mean germination time, and b.) abnormal seedlings and dead seeds of barley cv. Triumph(B). n=3.

The occurrence of abnormal seedlings (3 %) ($p<0.01$) and dead seeds (14 %) ($p<0.001$) increased only after three days CD (Figure 5.4.1.b). Shorter periods of CD had no effect on these parameters.

Lettuce

Control seeds of cv. Reskia(C) had a germination percentage of 93 % after three days (Figure 5.4.2.a). One or two days CD had no effect on germination. After three days CD germination fell 18 % ($p<0.05$).

The MGT of controls was 31 hours (Figure 5.4.2.a). This increased after CD for one day (23 %), two days (48 %) or three days (55 %) ($p<0.001$).

The number of abnormal seedlings and dead seeds was not affected by CD for one or two days. After three days CD, 5 % abnormal seedlings ($p<0.001$), and 13 % dead seeds ($p<0.01$) were counted (Figure 5.4.2.b).

5.4.2 Experiment 5.5: The effect of controlled deterioration on seedling growth

Barley

Shoot growth was not affected after one day CD (Figure 5.4.3.a). However, the shoot length was reduced 12 % (2 days CD) ($p<0.01$) or 31 % (3 days CD) ($p<0.001$).

Lettuce

The root length of seedlings was reduced 30 % (1 day CD), 66 % (2 days CD) or 64 % (3 days CD) ($p<0.001$) (Figure 5.4.3.b). The hypocotyl length was progressively reduced 24 % (1 day CD), 51 % (2 days CD) or 53 % (3 days CD) ($p<0.001$) (Figure 5.4.3.b).

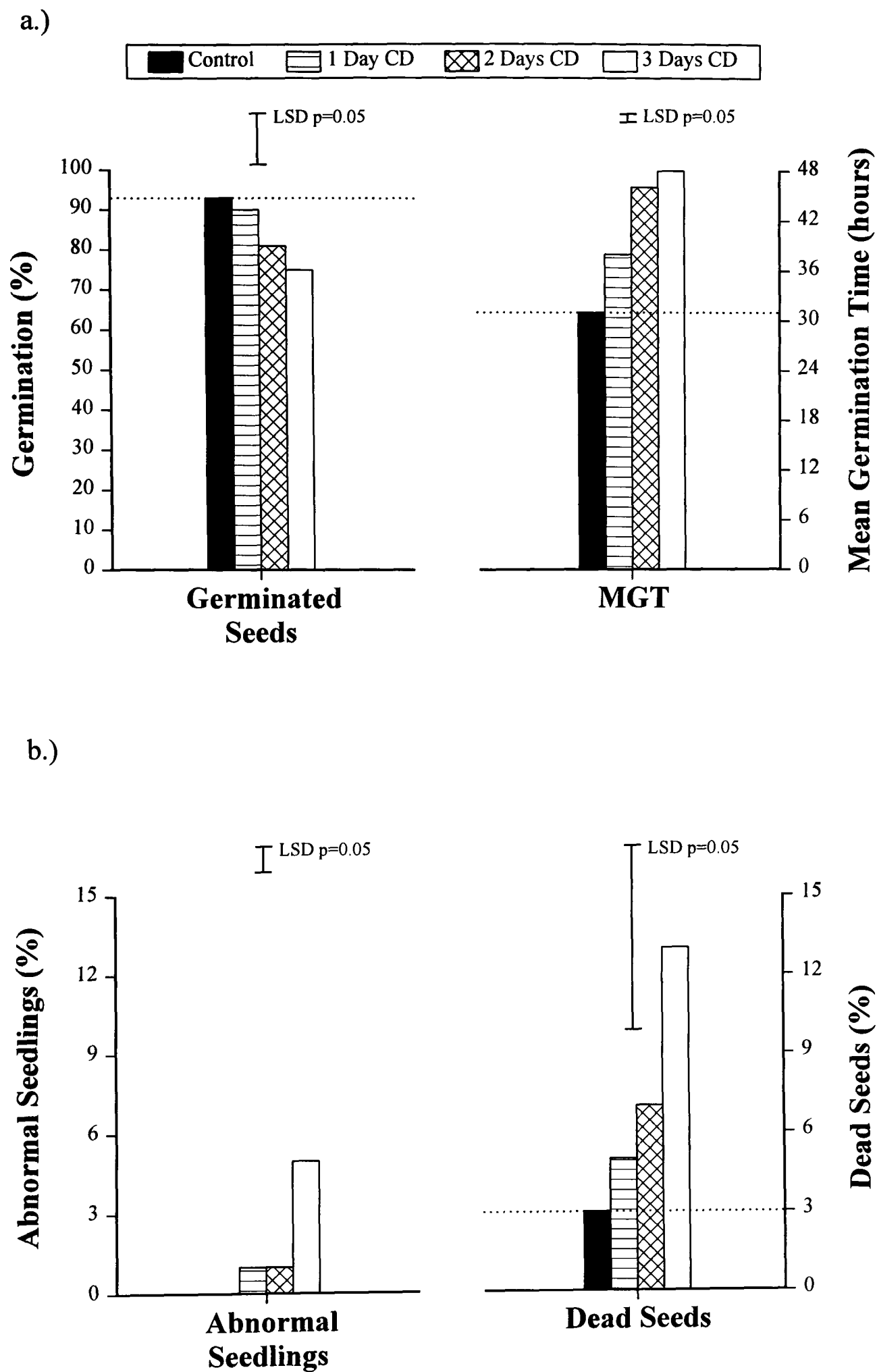


Figure 5.4.2: The effect of controlled deterioration (CD) for 1 to 3 days on a.) the germination percentage and mean germination time, and b.) abnormal seedlings and dead seeds of lettuce cv. Reskia(C). n=3.

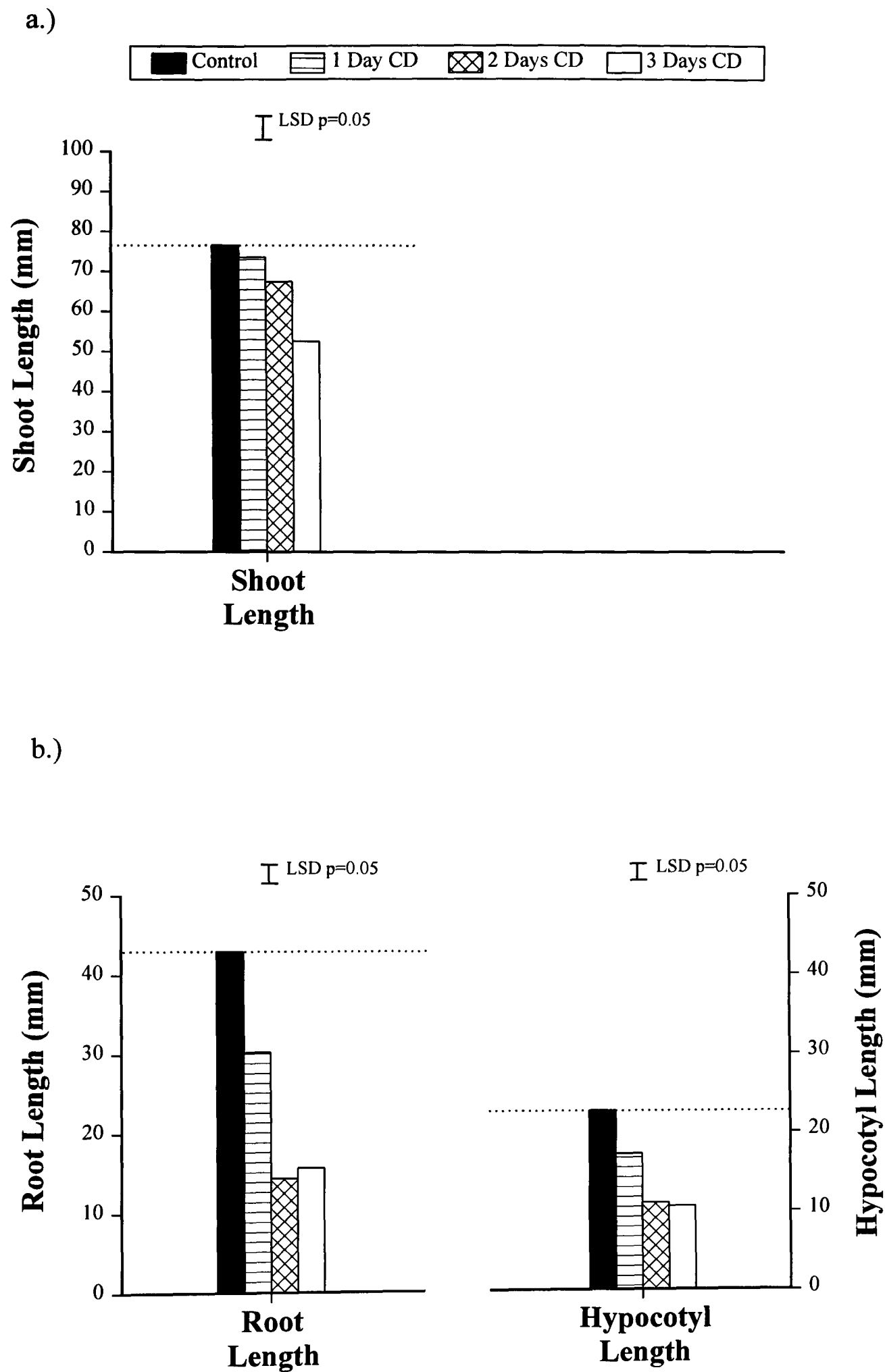


Figure 5.4.3: The effect of controlled deterioration (CD) for 1 to 3 days on a.) the shoot length of barley cv. Triumph(A) (plumule growth test), and b.) the root and hypocotyl length of lettuce cv. Reskia(B) (slant test). n=3.

5.5 The significance of initial seed vigour on the effect of SWS seed priming

Introduction

For both species a controlled deterioration of two days under the species specific temperature and seed moisture conditions was found to be appropriate to produce seeds of low vigour without a reduction in viability or an increase in abnormal seedlings (Section 5.4). Low vigour sub-lots so produced were used in consecutive priming experiments with a 12 hours priming period followed by 24 hours re-drying.

5.5.1 Experiment 5.6: The significance of seed vigour prior to SWS priming on germination

Barley

Dry control (high vigour) seeds of cv. Triumph(B) had a germination of 92 % after three days (Figure 5.5.1.a). Germination was 6 % (water control) ($p<0.05$), or 7 % (ANS) ($p<0.01$) higher when seeds were primed. Treatments in LHS had no effect. Seeds primed in PEG had 23 % ($p<0.001$) lower germination. Low vigour (deteriorated) control seeds had 25 % lower germination than high vigour seeds (Figure 5.5.1.a). Seeds treated with PEG had a 24 % ($p<0.001$) lower germination. All other treatments had no effect on this parameter.

The MGT of high vigour seeds was only reduced by water (29 %) or ANS (23 %) ($p<0.001$) (Figure 5.5.1.b). All priming treatments reduced the MGT of low vigour seeds, with water (26 %) or ANS (22 %) ($p<0.001$) being the best (Figure 5.5.1.b).

Low vigour seed lots had a higher number of abnormal seedlings and dead seeds compared to high vigour seeds (Figure 5.5.2.a). Regardless of the vigour status, priming treatment had no effect on the number of abnormal seedlings. However, the number of dead seeds increased in high vigour seeds from 2 % of the dry control to 5 % ($p<0.05$) after PEG treatment (Figure 5.5.2.b).

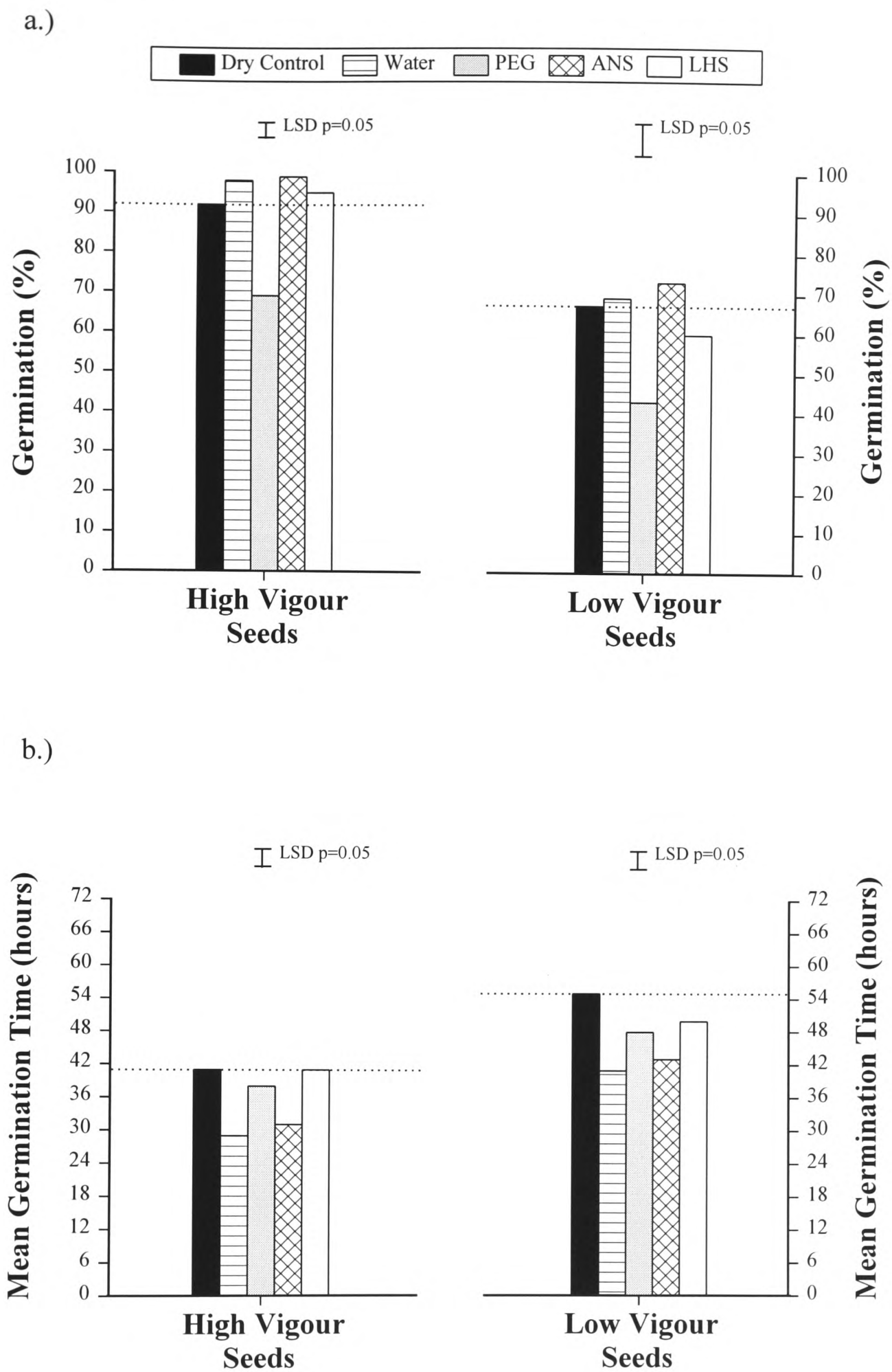


Figure 5.5.1: The effect of priming in water, osmotica or SWS on a.) the percentage germination after 3 days and b.) mean germination time over 3 days, of high and low vigour seeds of barley cv. Triumph(B). Priming for 12 hours, and re-drying for 24 hours. n=3.

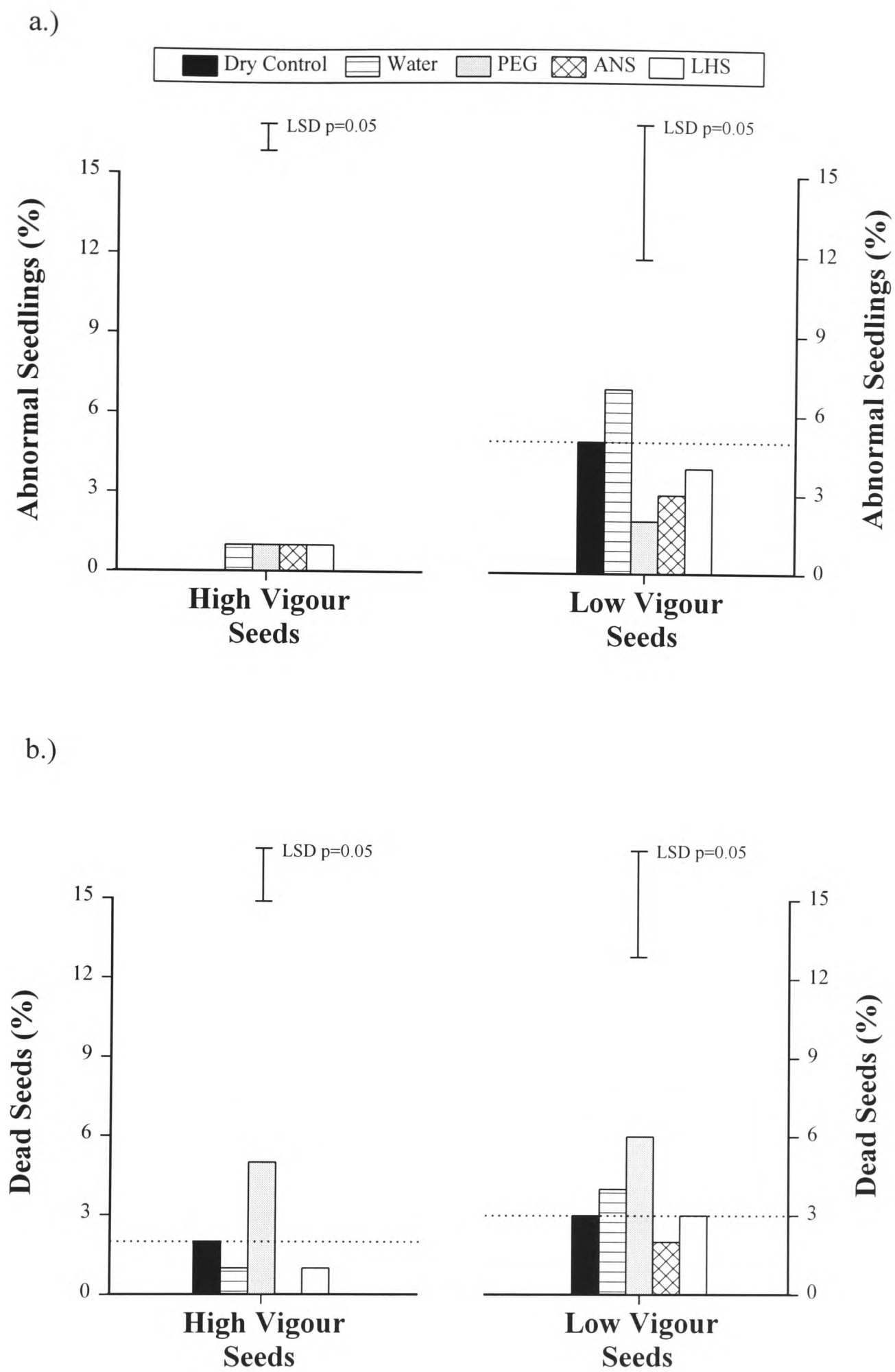


Figure 5.5.2: The effect of priming in water, osmotica or SWS on a.) the percentage abnormal seedlings, and b.) dead seeds, after 7 days of high and low vigour seeds of barley cv. Triumph(B). Priming for 12 hours, and re-drying for 24 hours. n=3.

Lettuce

The germination of high vigour water treated seeds was 7 % ($p<0.05$) lower than of the corresponding control, all other priming treatments had no effect. Low vigour seeds had a germination of 85 % when untreated seeds were used, no treatment had any effect on this parameter (Figure 5.5.3.a).

Water priming of high vigour seeds increased the MGT 8 % ($p<0.01$), while either priming in PEG or ANS reduced the MGT 17 % ($p<0.001$), and priming in LHS 8 % ($p<0.01$) (Figure 5.5.3.b). All priming treatments reduced the MGT of low vigour seeds; water, and SWS treatments reduced the MGT 11 % ($p<0.01$), and PEG priming 16 % ($p<0.001$) (Figure 5.5.3.b).

Priming of high or low vigour seeds had no effect on the occurrence of dead seeds or abnormal seedlings (Figure 5.5.4).

5.5.2 Experiment 5.6: The significance of seed vigour prior to SWS priming on seedling growth***Barley***

Seed priming of high vigour seeds in PEG reduced the length of plumules 10 % ($p<0.05$). In contrast, shoots of ANS primed seeds were 9 % ($p<0.05$) longer. Seed treatment in water or LHS had no effect (Figure 5.5.5).

Priming of low vigour seeds in PEG or LHS had no effect on shoot length, but water priming resulted in 7 % ($p<0.05$) longer shoots, compared to the low vigour control (Figure 5.5.5). After seed priming in ANS, shoots were a further 9 % ($p<0.01$) longer than water primed seeds, and similar to the shoot length of dry controls of high vigour seed (Figure 5.5.5).

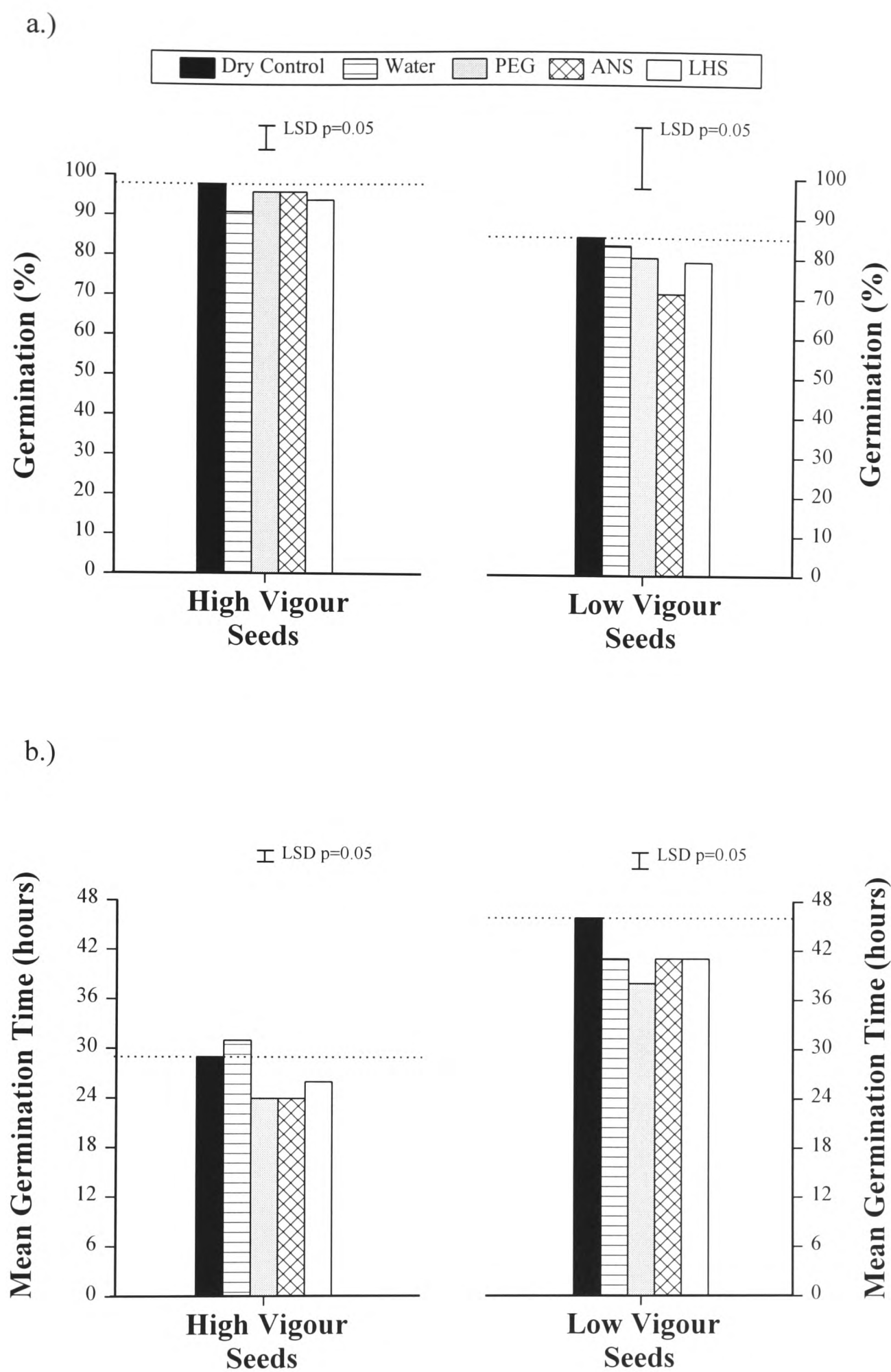


Figure 5.5.3: The effect of priming in water, osmotica or SWS on a.) the percentage germination after 3 days, and b.) mean germination time over 3 days, of high and low vigour seeds of lettuce cv. Reskia(C). Priming for 12 hours, and re-drying for 24 hours. n=3.

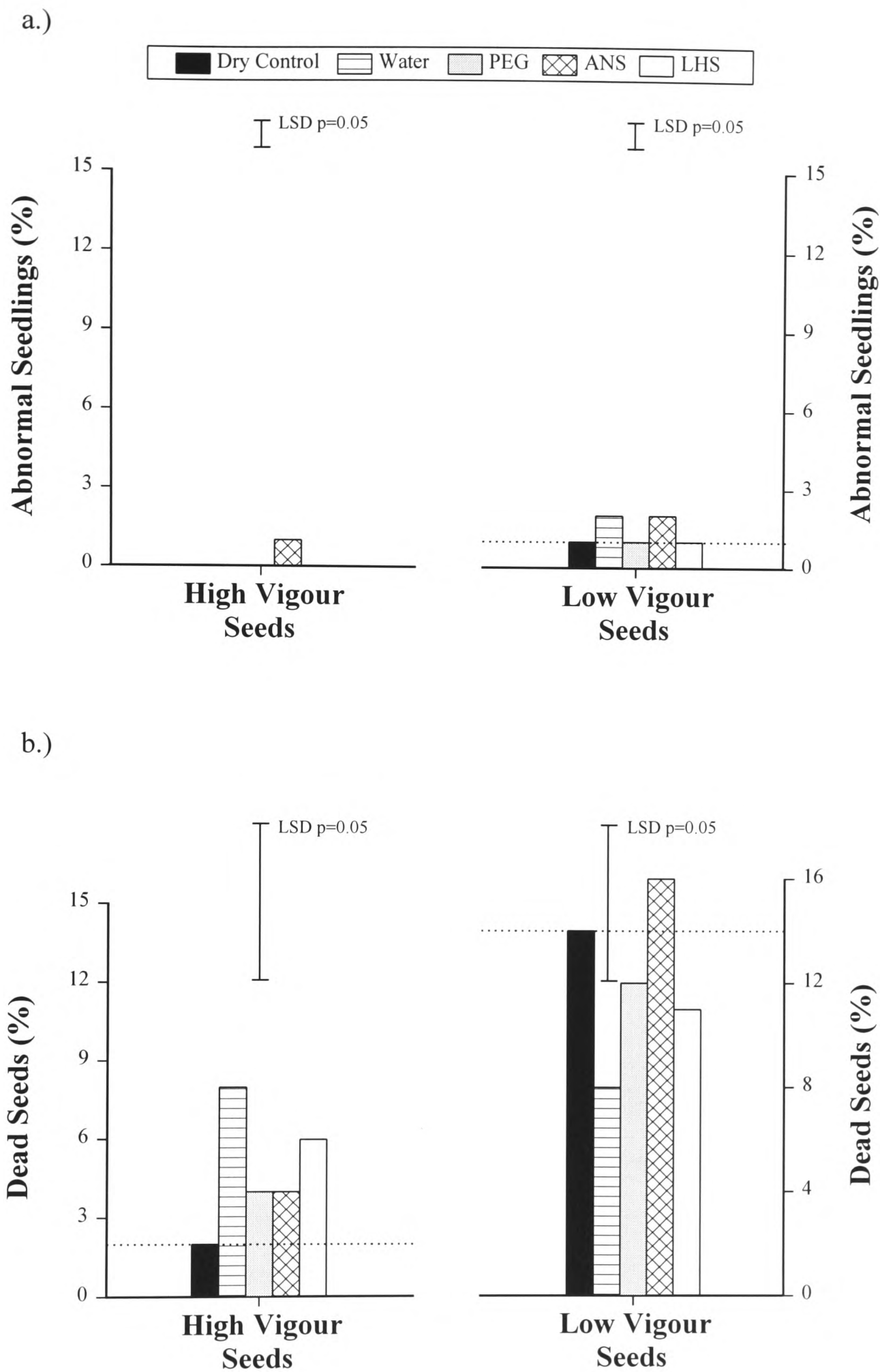


Figure 5.5.4: The effect of priming in water, osmotica or SWS on a.) the percentage abnormal seedlings, and b.) dead seeds, after 7 days of high and low vigour seeds of lettuce cv. Reskia(C). Priming for 12 hours, and re-drying for 24 hours. n=3.

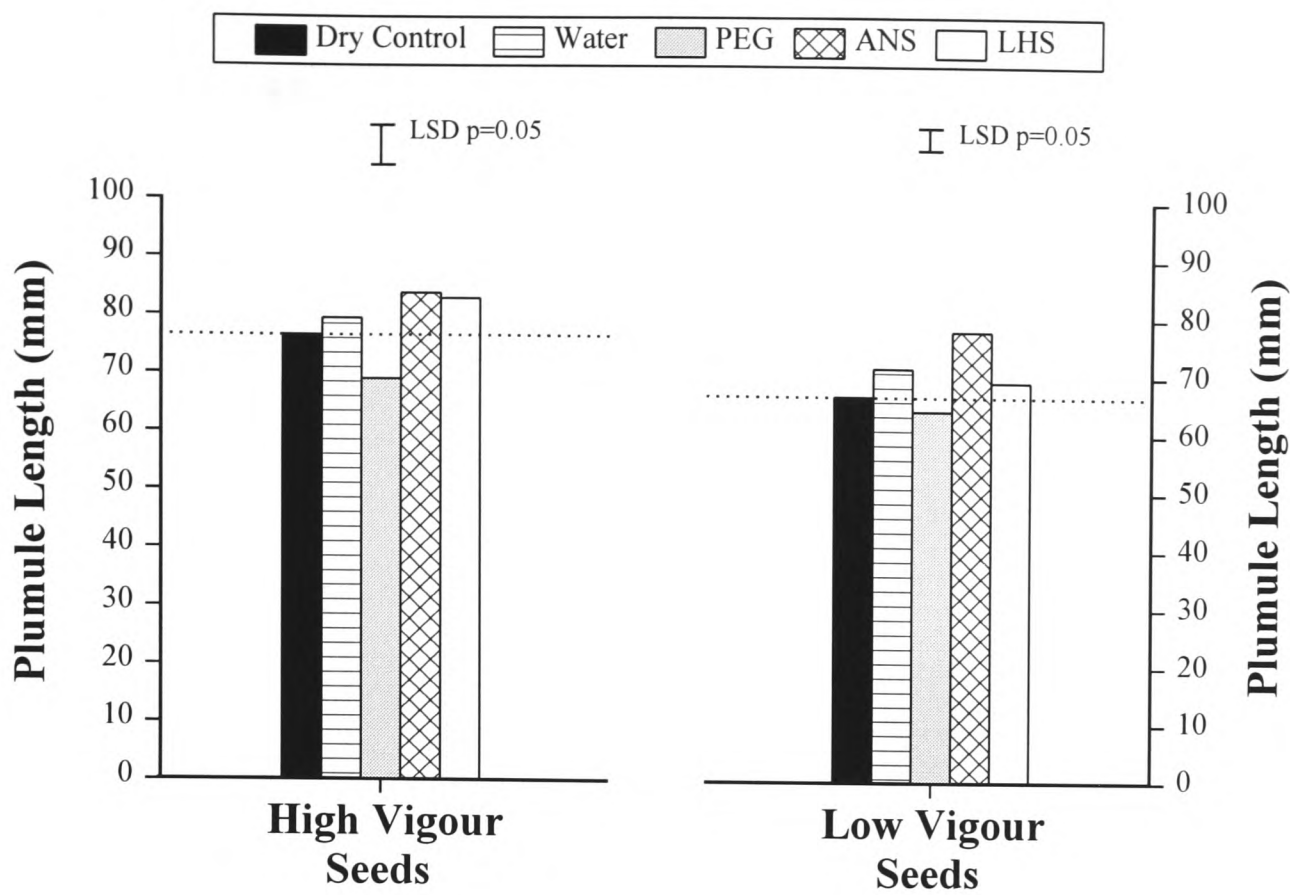


Figure 5.5.5: The effect of priming in water, osmotica, or SWS on the shoot length of high and low vigour seeds of barley cv. Triumph(B), after 7 days in the plumule growth test. Priming for 12 hours, and 24 hours re-drying. n=3.

Lettuce

The hypocotyl length of seedlings grown from high vigour seeds was not affected by a water treatment, but PEG priming reduced the length by 27 % ($p<0.001$). In contrast, after seed priming in ANS or LHS hypocotyl length increased 9 % ($p<0.01$), or 10 % ($p<0.001$), respectively (Figure 5.5.6.a). The dry control seedlings of low vigour seeds had hypocotyls 50 % shorter than high vigour controls. Seed priming in PEG increased the hypocotyl length 38 % ($p<0.001$), while SWS or water treatments had no effect (Figure 5.5.6.a).

No treatment increased the root length (Figure 5.5.6.b). However, high vigour seeds primed in LHS (4 %, $p<0.05$), water (6 %, $p<0.01$), ANS (9 %, $p<0.001$) or PEG (11 %, $p<0.001$) all developed shorter roots (Figure 5.5.6.b).

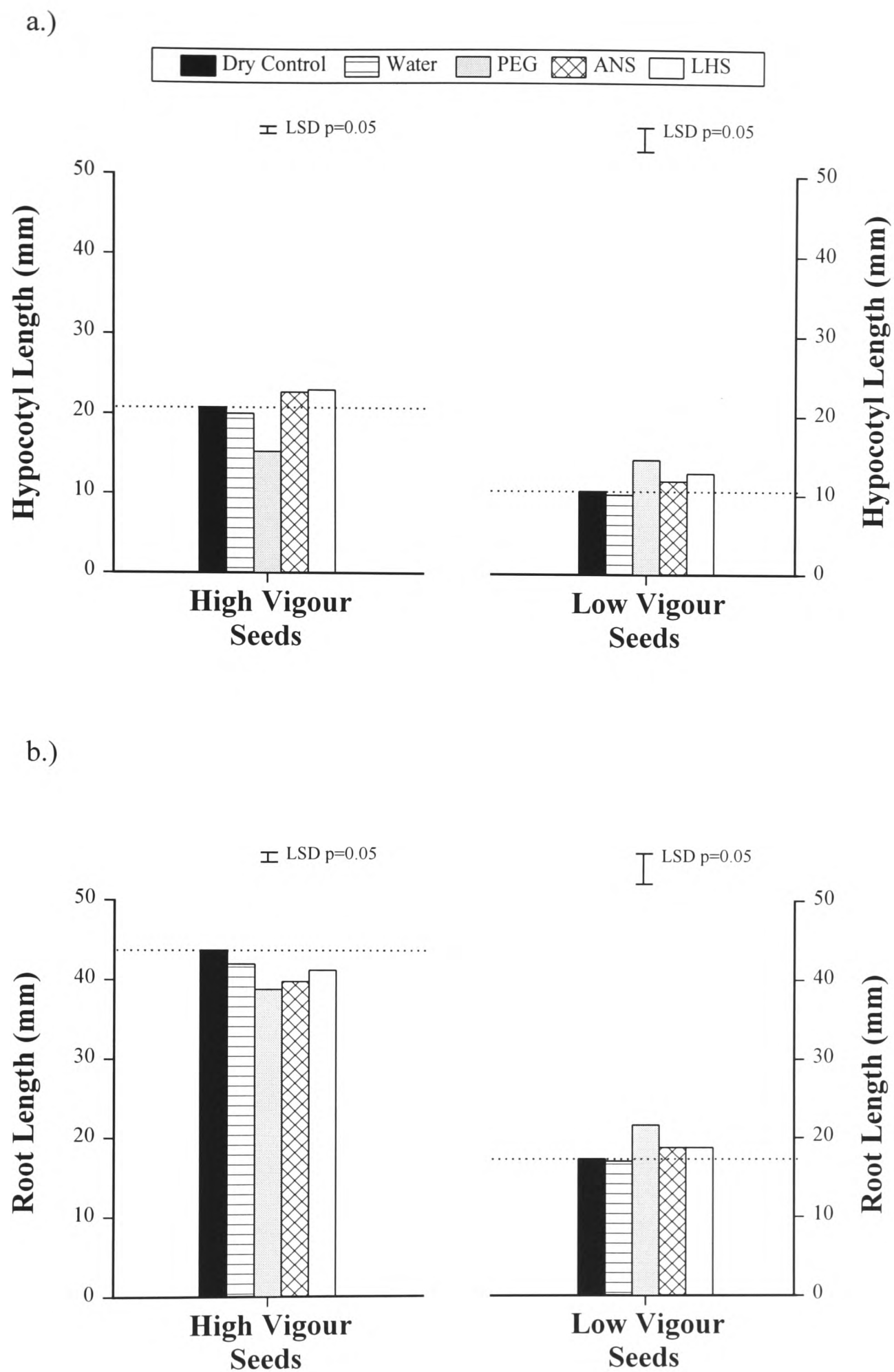


Figure 5.5.6: The effect of priming in water, osmotica, or SWS on a.) the hypocotyl and b.) root length of high and low vigour seeds of lettuce cv. Reskia(C), in a slant test after four days. Priming for 12 hours, and re-dried for 24 hours. n=3.

Low vigour control seeds produced roots with only 40 % of the root length of dry control seedlings grown from high vigour seeds (Figure 5.5.6.b). None of the SWS treatments or the water control reduced this parameter, but PEG priming increased the root length 25 % ($p<0.001$) (Figure 5.5.6.b).

5.5.3 Experiment 5.7: The significance of seed vigour prior to SWS priming on seedling emergence, establishment and growth

Barley

In a greenhouse experiment over 21 days 97 % of dry control seeds of both high or low vigour seed lots emerged (Figure 5.5.7.a). The mean emergence time (MET) was 192 hours (high vigour) or 209 hours (low vigour). Only ANS reduced MET of low vigour seeds 5 % ($p<0.05$) (Figure 5.5.7.b).

Priming high vigour seeds in PEG caused a 10 % ($p<0.05$) reduction in shoot length. When low vigour seeds were primed in ANS shoot length increased 8 % ($p<0.05$). Other priming treatments had no effect on the shoot length (Figure 5.5.9.a).

Lettuce

Seedling emergence of the dry controls was 89 % for high vigour or 82 % for low vigour seeds (Figure 5.5.8.a). None of the treatments, regardless of vigour level, had an effect on the final percentage of emerged seedlings. The MET of the dry controls of high vigour seeds was 137 hours or low vigour seeds was 173 hours (Figure 5.5.8.b). Low vigour seeds primed in LHS and high vigour seeds primed in PEG had a MET increased by 14 % or 20 % ($p<0.05$), while other treatments had no effect (Figure 5.5.8.b).

No priming treatment had an effect on shoot fresh weight, irrespective of vigour level (Figure 5.5.9.b).

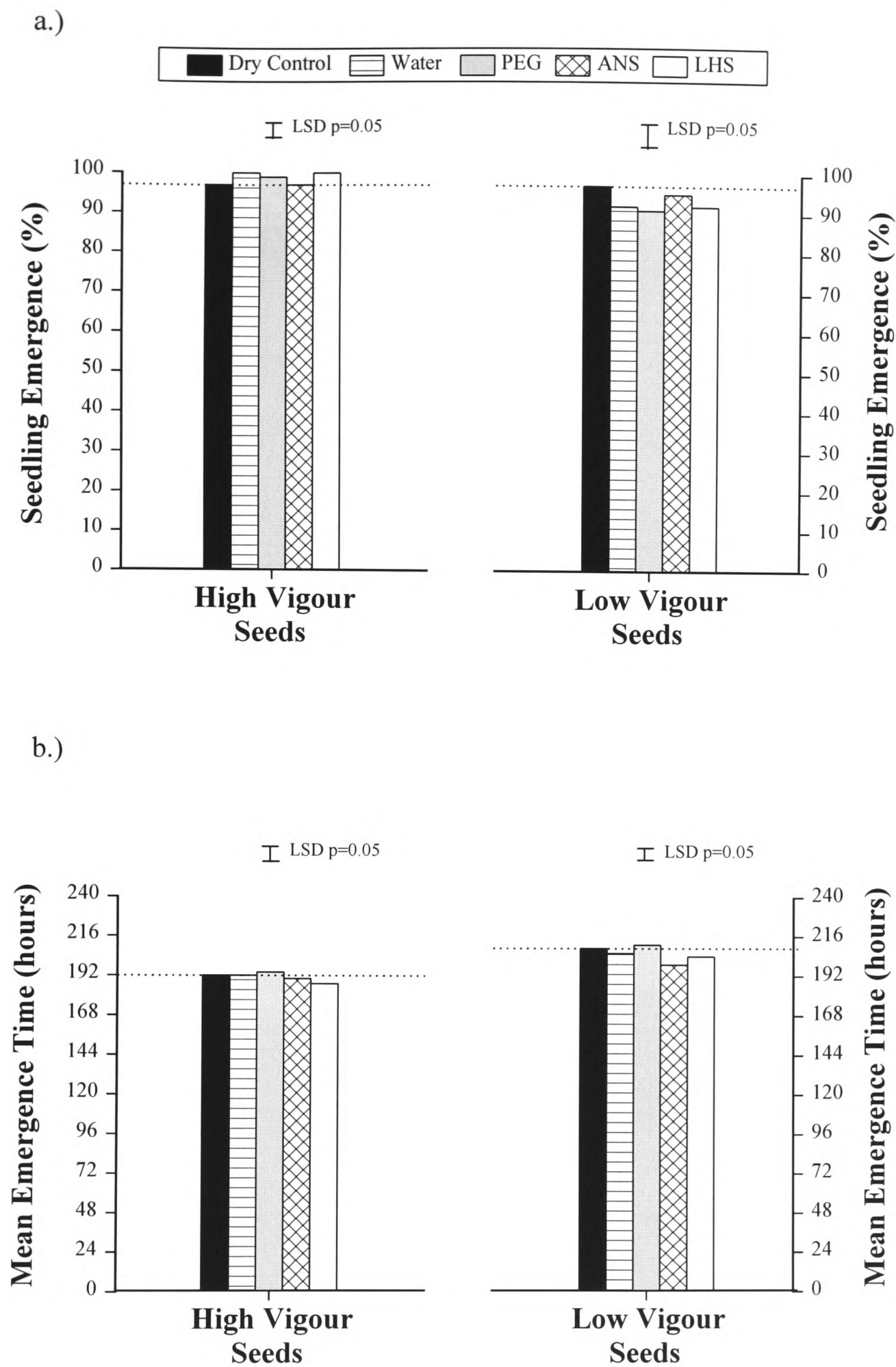


Figure 5.5.7: The effect of priming in water, osmotica (PEG), or SWS on the a.) seedling emergence, and b.) mean emergence time, of high and low vigour seeds of barley cv. Triumph(B), after 21 days in a greenhouse experiment. Priming for 12 hours, and re-drying for 24 hours. n=4.

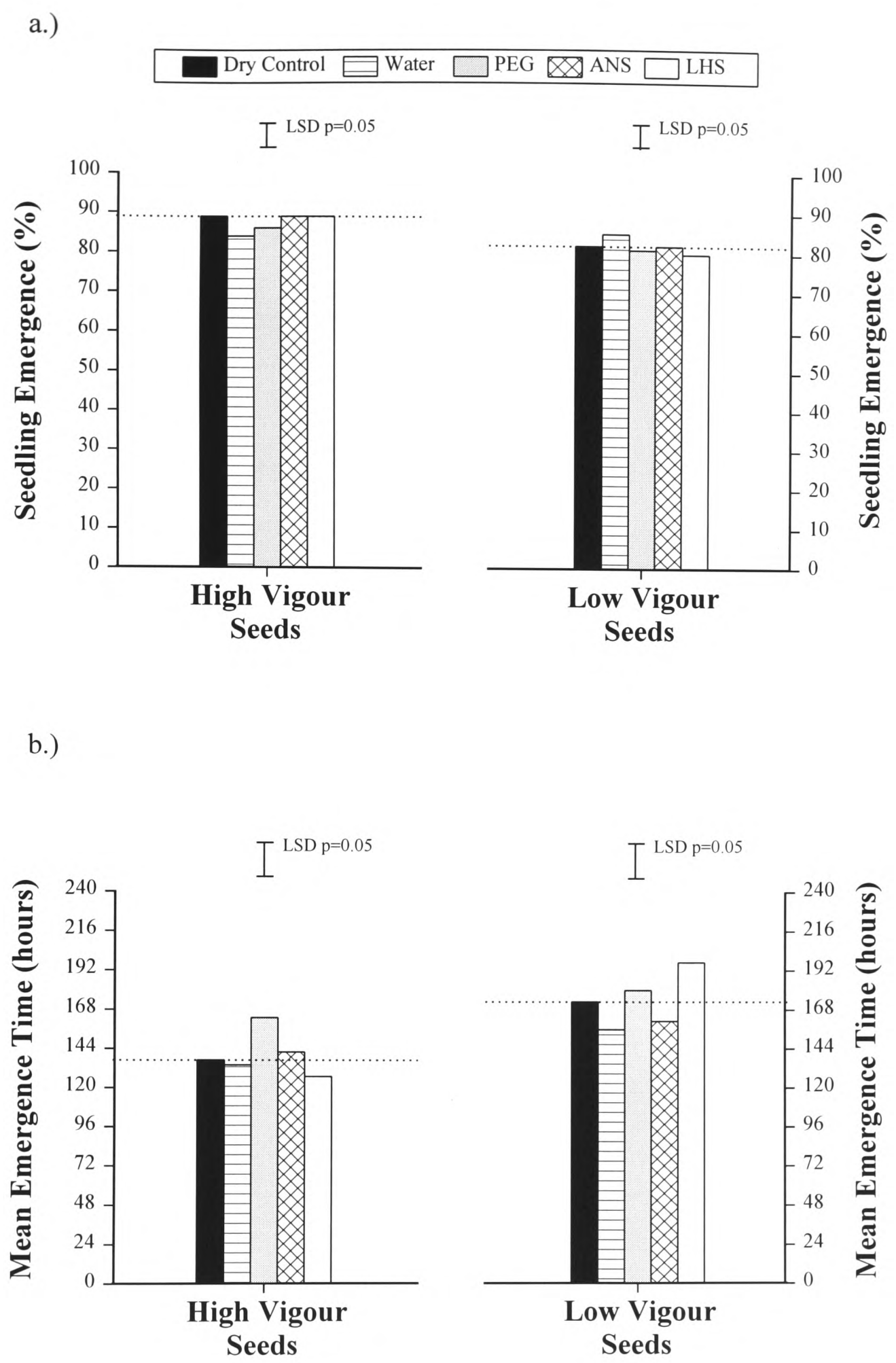


Figure 5.5.8: The effect of priming in water, osmotica (PEG), or SWS on a.) the seedling emergence, and b.) mean emergence time, of high and low vigour seeds of lettuce cv. Reskia(C), after 21 days in a greenhouse experiment. Priming for 12 hours, and re-dried for 24 hours. n=4.

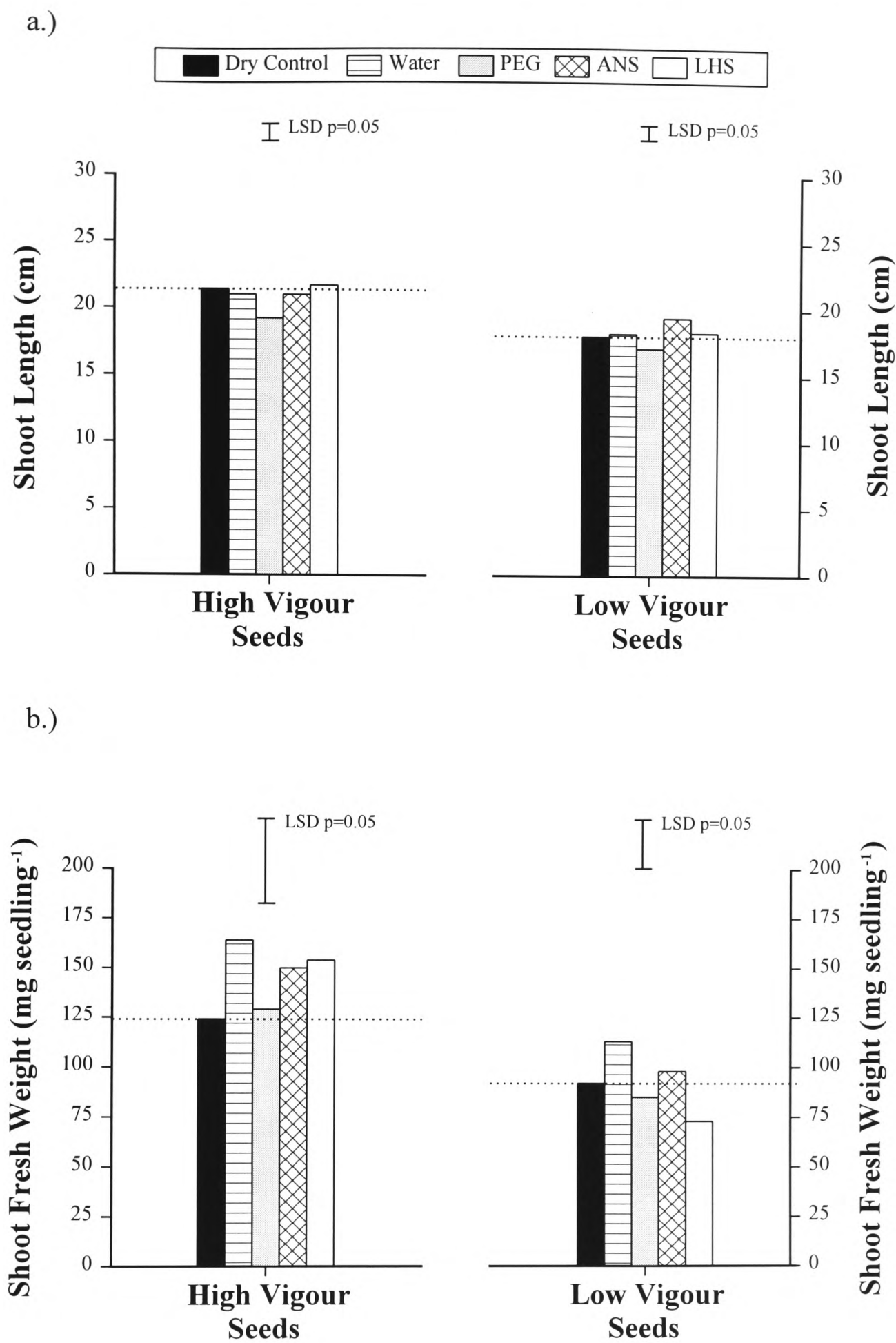


Figure 5.5.9: The effect of priming in water, osmotica (PEG), or SWS of high and low vigour seeds on a.) the shoot length of barley cv. Triumph(B) seedlings, and b.) the shoot fresh weight of lettuce cv. Reskia(C) seedlings, after 21 days in a greenhouse experiment. Priming for 12 hours, and re-dried for 24 hours. n=4.

5.6 The effect of SWS seed priming on lettuce thermo-dormancy

5.6.1. Experiment 5.8: The determination of thermo-inhibition of lettuce cv.

Reskia

At temperatures ranging from 10°C - 20°C, no effect on the percentage germination was observed, irrespective of light supplement (Figure 5.6.1). At 26°C germination was 7 % in the dark, and 3 % in the light, while at 30°C no seed germinated. These values were not improved after seven days.

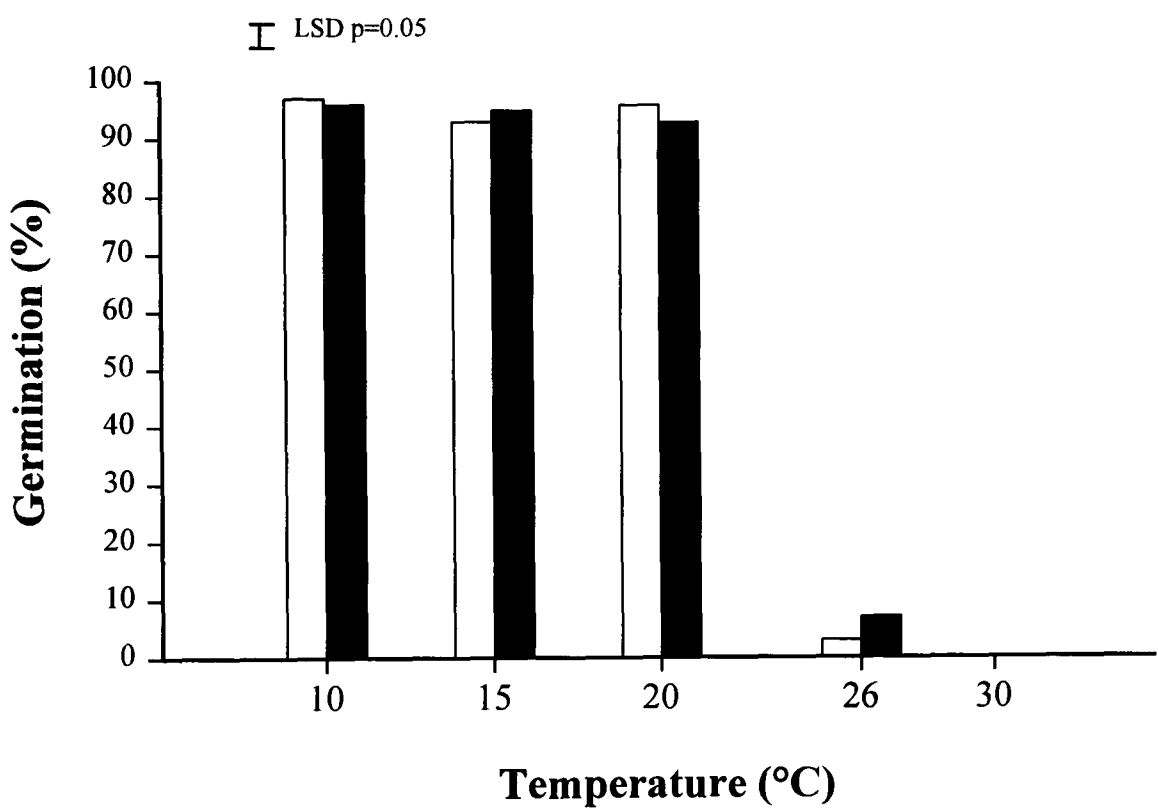


Figure 5.6.1: The effect of temperature on the germination of lettuce cv. Reskia(B) seeds after 3 days, in the light (□) or dark (■). n=3.

5.6.2 Experiment 5.9: The thermo-sensitivity of lettuce cultivars

While cv. Great Lakes showed no inhibition of germination (99 % after three days), cv. Columbus only had a germination of 57 % ($p<0.001$). After seven days, however, this cultivar had a germination of 94 % (Figure 5.6.2.a). This indicated lesser thermo-sensitivity. Both seed lots of cv. Reskia exhibited a higher degree of thermo-inhibition, with only 8 % or 10 % germination after seven days.

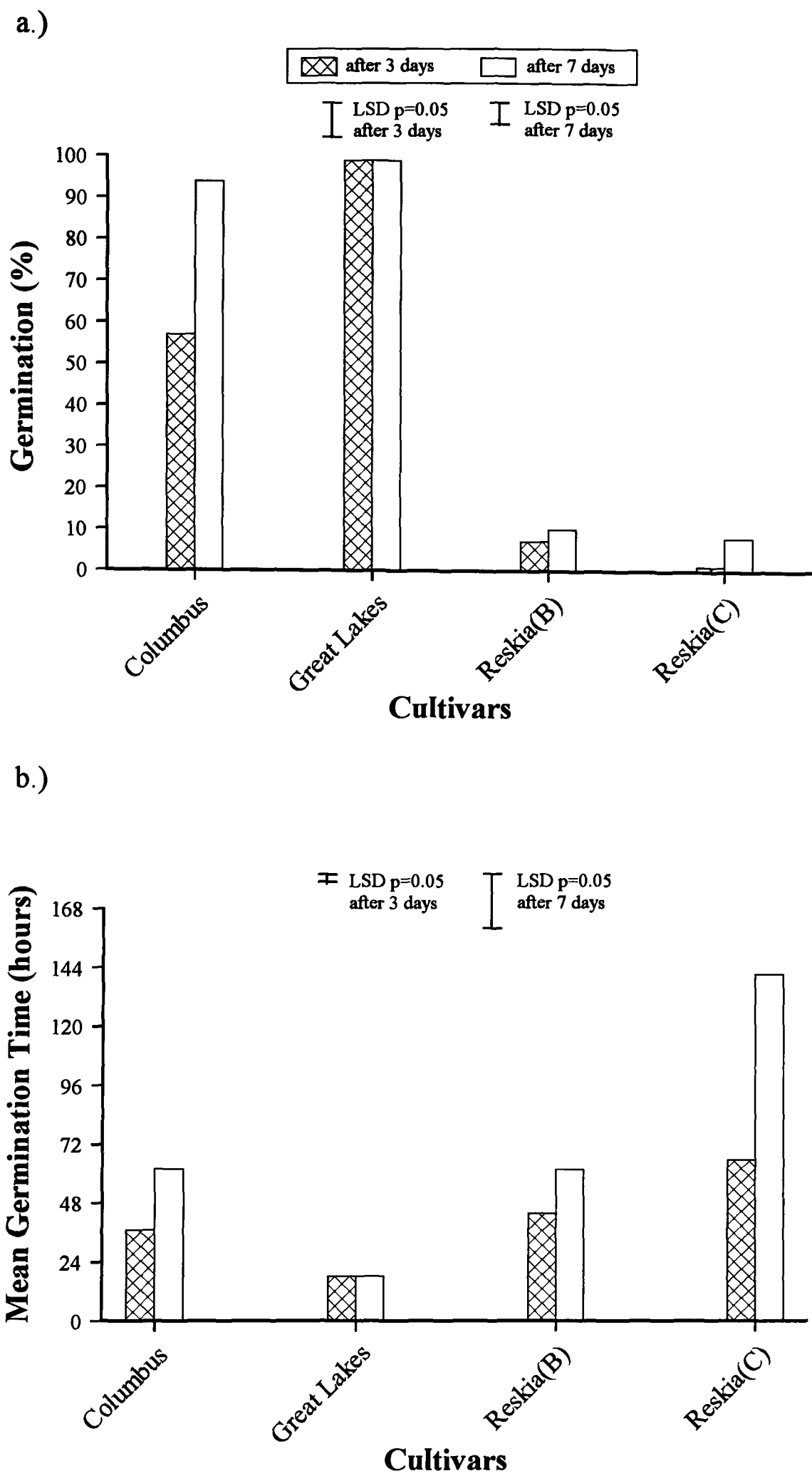


Figure 5.6.2: The percentage germination of diverse lettuce cultivars at 26°C a.) after 3 and 7 days, and b.) the mean germination time over 3 and 7 days, in the dark. n=3.

The MGT over three days for cv. Great Lakes was 18 hours, and was twice as high ($p<0.001$) for the thermo-sensitive cv. Columbus. The MGT for the two seed lots of the thermo-inhibited cv. Reskia was 44 hours (lot B) and 66 hours (lot C) ($p<0.001$) (Figure 5.6.2.b). The MGT over seven days increased 68 % for cv. Columbus and 41 % (seed lot B) or 115 % (C) for cv. Reskia (Figure 5.6.2.b).

5.6.3 Experiment 5.10: The effect of SWS priming on thermo-inhibition of lettuce

After 12 hours soaking and 24 hours re-drying, seeds of cv. Reskia(B) were incubated at 26°C. Dry untreated control seeds had 10 % germination after three days, water primed seeds had 33 % germination ($p<0.05$). In contrast, priming seeds in ANS resulted in 63 % ($p<0.001$) germination (Figure 5.6.3.a). The MGT of control treatments was 39 hours. Priming in SWS reduced the MGT around 38 % ($p<0.05$) (Figure 5.6.3.a).

After seven days, the remaining seeds were checked for fresh and dead seeds; all treatments contained on average 2 % dead seeds (Figure 5.6.3.b).

5.6.4 Experiment 5.11: The effect of priming in modified ANS on thermo-inhibition of lettuce

If seeds were not rinsed after priming, then water-primed seeds had a germination of 31 %, and PEG primed seeds 55 % ($p<0.001$). Seeds primed in ashed ANS had a germination of 22 % ($p<0.01$) (Figure 5.6.4.a). Germination was 55 % ($p<0.001$) with seeds soaked in the ANS solid fraction, 61 % ($p<0.001$) in original ANS, and 63 % ($p<0.001$) in ANS supernatant. No difference in percentage germination was observed between unrinsed or rinsed seeds, soaked in water or ashed ANS. Removal of the adhering material after soaking reduced the germination promotion of seeds soaked in PEG (41 %, $p<0.001$), in the ANS solid fraction

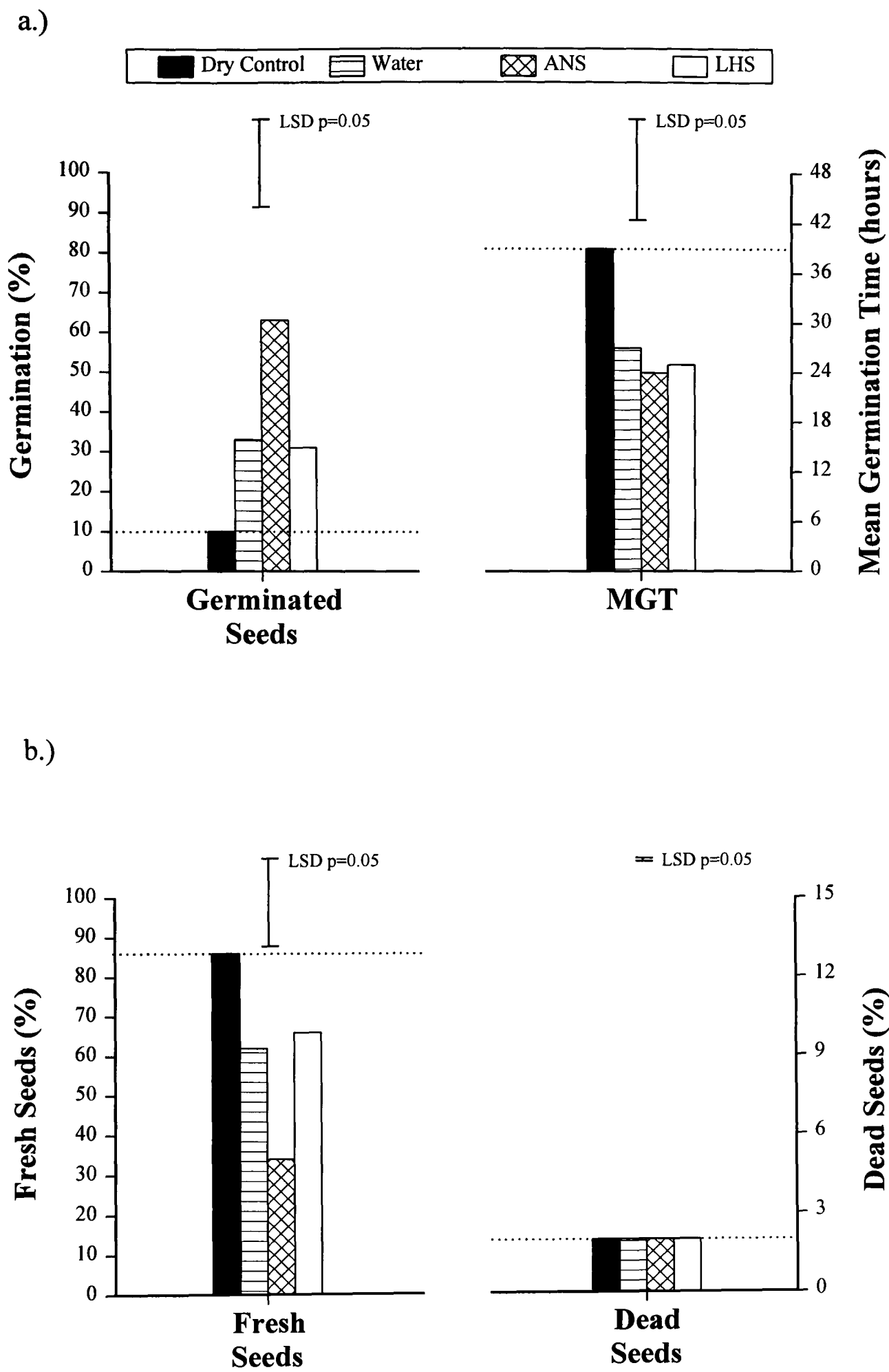


Figure 5.6.3: The effect of seed priming in water or SWS on a.) the percentage germination and mean germination time after 3 days, and b.) percentage of fresh and dead seeds after 7 days, of lettuce cv. Reskia(B) seeds at 26°C in the dark. Priming for 12 hours, and re-drying for 24 hours. n=3.

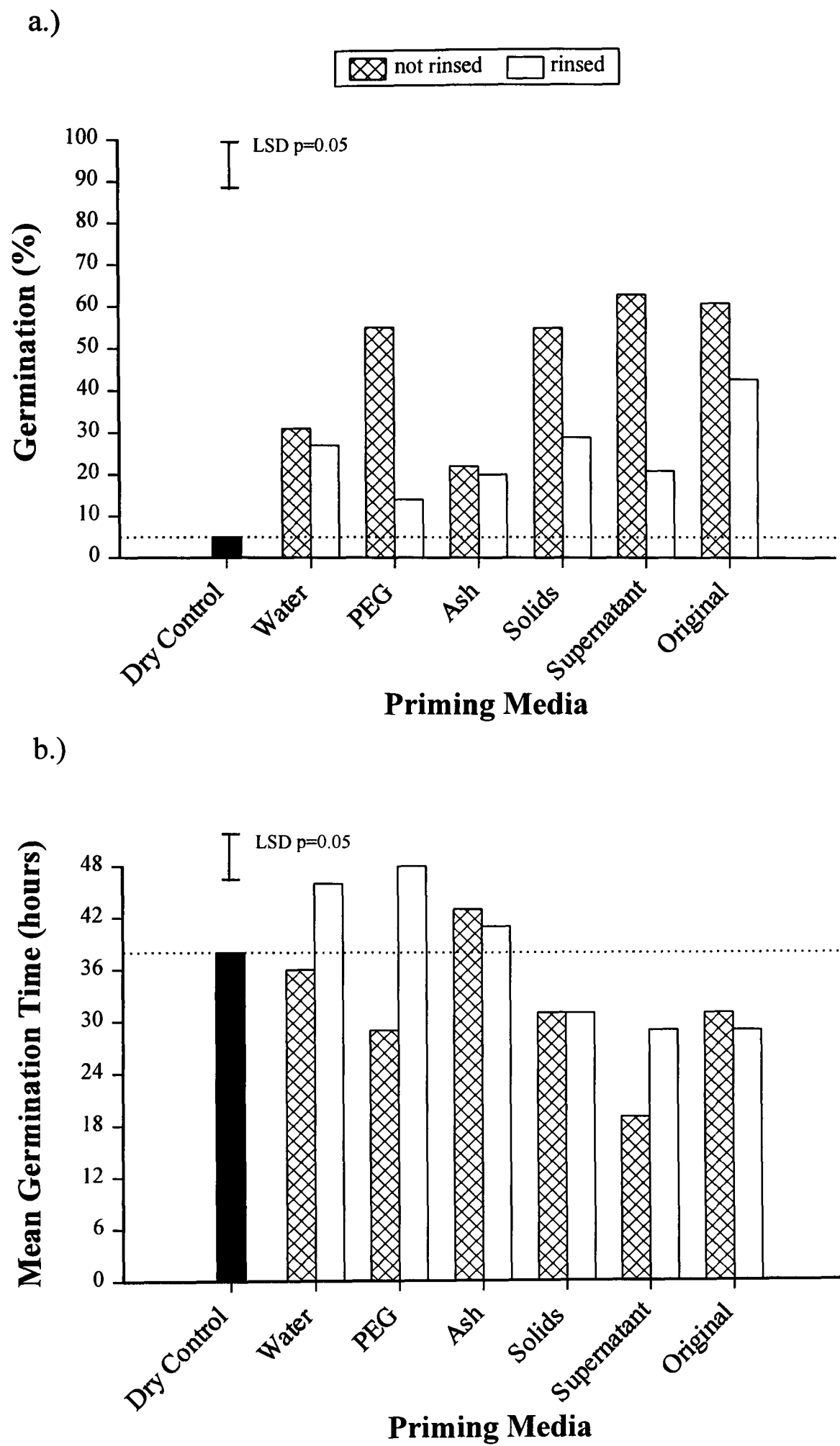


Figure 5.6.4: The effect of seed priming in water, osmotica (PEG), ANS or modified ANS on a.) the percentage germination after 3 days, and b.) the mean germination time over 3 days, of lettuce cv. Reskia(B) seeds at 26°C in the dark. Priming for 12 hours, and re-drying for 24 hours. n=3.

(26 %, $p<0.001$), in ANS supernatant (42 %, $p<0.001$) and in original ANS (18 %, $p<0.01$) (Figure 5.6.4.a).

The percentage germination was reflected in the MGT figures. All ANS treatments, except for ashed ANS, reduced MGT, with the greatest effect recorded for ANS supernatant (50 % faster, $p<0.001$) treated seeds not rinsed after priming (Figure 5.6.4.b). Seeds primed in PEG and sown without rinsing germinated 25 % ($p<0.01$) faster. Rinsing after soaking increased the MGT for seeds soaked in water 27 % ($p<0.001$), in ANS supernatant 50 % ($p<0.001$), and in PEG 67 % ($p<0.001$) compared to the respective un-rinsed treatments (Figure 5.6.4.b). Priming in ashed ANS had no effect on the MGT.

5.6.5 Experiment 5.12: The effect of storage after ANS priming on thermo-inhibition of lettuce

While only 5 % of dry control seeds germinated after three days at 26°C in the dark, 95 % ($p<0.001$) of seeds, primed in undiluted ANS and tested without re-drying, germinated as long as adhering SWS material was not rinsed off (Figure 5.6.5.a). Germination dropped progressively as storage time increased and reached 26 % ($p<0.01$) after 20 weeks. If seeds were rinsed after priming and sown without re-drying, 73 % ($p<0.001$) germinated. Germination was on average 18 % lower compared to un-rinsed seeds. After 20 weeks of storage the priming effect disappeared (Figure 5.6.5.a).

Seeds treated with ANS and sown without re-drying germinated in half the time ($p<0.001$) of dry control seeds, irrespective of rinsing. The MGT of seeds re-dried for one day was 25 % lower than dry control seeds. Storage from one week to 20 weeks did not alter the MGT to that observed after one day (Figure 5.6.5.b).

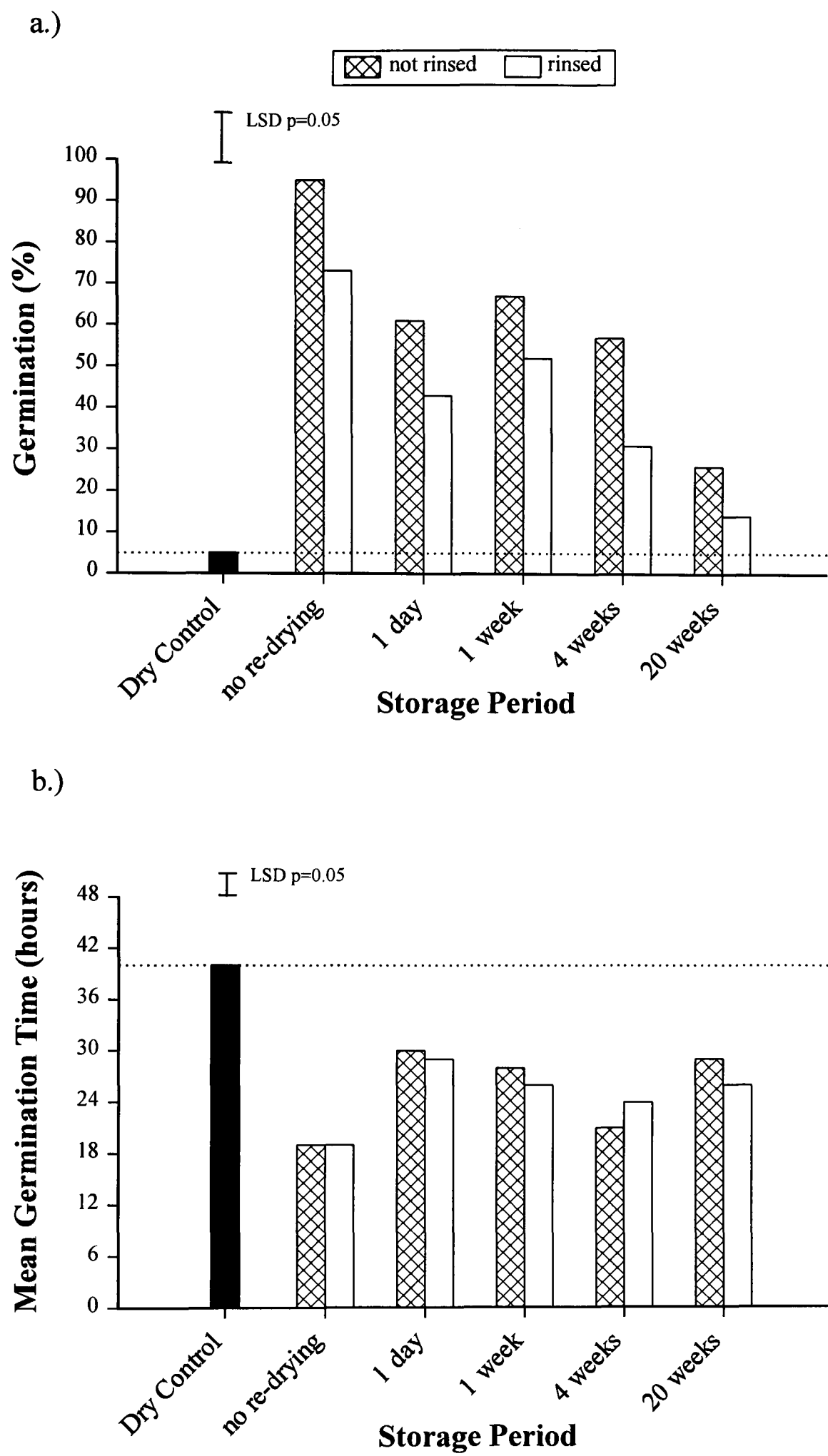


Figure 5.6.5: The effect of storage after ANS seed priming on a.) the percentage germination after 3 days, and b.) mean germination time over 3 days, of lettuce cv. Reskia(B) seeds at 26°C in the dark. Priming for 12 hours, with storage periods as indicated. n=3.

5.7 The effect of SWS seed priming on water sensitivity of barley

5.7.1 Experiment 5.13: The determination of water sensitivity of barley cultivars

Cultivars tested generally exhibited a high proportion of water sensitive seeds as only between 10 % - 21 % of seeds germinated if incubated in 8 ml water. An exception was cv. Triumph(B), which had a germination of 57 % ($p < 0.001$) (Figure 5.7.1.a) after three days. The two seed lots of cv. Blenheim had the highest water sensitivity, with germination only between 10 % - 14 %. These figures did not change markedly after seven days, except for the dormant cultivar, Pastoral (Figure 5.7.1.a).

The MGT varied from 41 hours to 53 hours, over the first three days (Figure 5.7.1.b). Germination performance was reflected in the MGT, and seed lots with a higher germination percentage had a lower MGT. Due to the high number of germinated seeds after three days, the MGT of cv. Pastoral increased from 46 to 65 hours (Figure 5.7.1.b).

The total viability of seeds after seven days was between 93 % - 98 % (Figure 5.7.1.c). An exception was cv. Blenheim(A) with only 84 % ($p < 0.01$) viable seeds.

5.7.2 Experiment 5.14: The effect of SWS priming on water sensitivity of barley

Seeds of barley cv. Triumph seed lot (A), which showed a high, but not extreme degree of water sensitivity, were chosen for the following set of experiments that investigated the effect of SWS priming on the release of seeds from water sensitivity.

The percentage germination of dry control seeds incubated at a water level of 8 ml was 23 % after three days (Table 5.7.1). Where primed seeds were not re-dried after soaking either in water or both SWS the seed lots exhibited a higher water

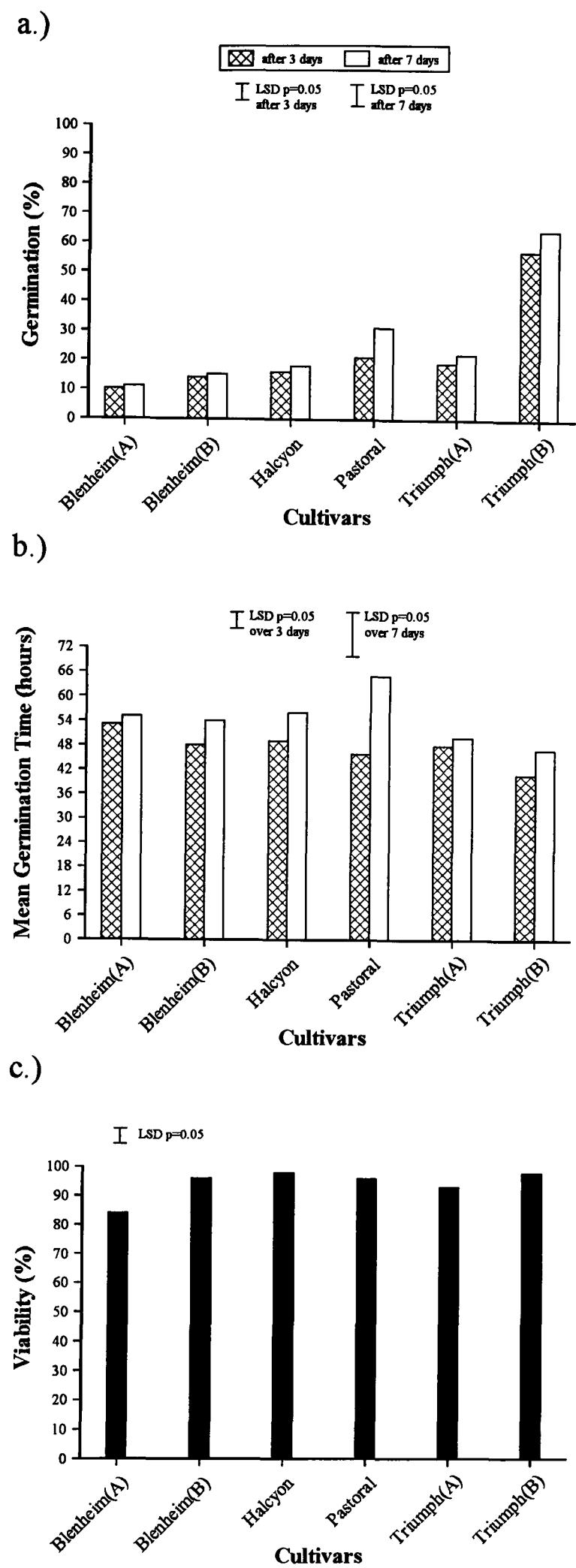


Figure 5.7.1: The percentage germination a.) after 3 and 7 days, b.) the mean germination time over 3 and 7 days, and c.) total viability after 7 days of barley cultivars in 8 ml water (water sensitivity test), at 20°C. n=3.

sensitivity, and only between 5 % - 17 % ($p<0.05$ - $p<0.001$) seeds germinated, irrespective of priming medium, SWS concentration, or priming duration. Re-drying seeds to 50 % of the water gained during priming resulted in improved germination rates between 11 % - 38 % ($p<0.01$ - $p<0.001$). Re-drying the seeds to the original moisture content further increased the germination to figures above 40 % ($p<0.001$) (Table 5.7.1). On average, a 12 hour soaking treatment and re-drying to the original seed moisture gave the highest germination, irrespective of priming treatment. Where seeds were primed in SWS for 12 hours with complete re-drying, undiluted ANS treatments caused 19 % ($p<0.001$) higher germination compared to dry untreated seeds germinated in 8 ml of water, whereas LHS had no effect on germination at this treatment combination (Table 5.7.1).

Table 5.7.1: The effect of various combinations of priming and re-drying periods in water or SWS at different concentrations, on the germination of barley cv. Triumph(A) seeds at 8 ml water levels. n=2.

Priming duration (hours)			0	6	12	24	6	12	24	6	12	24	
Moisture loss (% dw)				0 %			50 %			100 %			
Priming treatment	Conc. (%)	ml H ₂ O dish ⁻¹											
Dry Control			8	23									
Water Control			8		15* ²	13*	5***	26 NS	29 NS	19 NS	29 NS	23 NS	22 NS
LHS	1	8		13**	9***	14*	23 NS	23 NS	31*	28 NS	31*	25 NS	
LHS	10	8		9***	12**	8***	30 NS	32*	27 NS	23 NS	32*	30 NS	
LHS	100	8		6***	8***	5***	22 NS	25 NS	11**	17 NS	23 NS	16 NS	
ANS	1	8		12**	12**	10**	22 NS	28 NS	28 NS	29 NS	34**	36**	
ANS	10	8		13**	17 NS	16 NS	30 NS	37***	29 NS	30 NS	35**	28 NS	
ANS	100	8		13**	11**	15*	31*	38***	30 NS	29 NS	42***	30 NS	
LSD (DF 64)													
p=0.05													
p=0.01													
p=0.001													

(² - different from the dry control at * = 5 %, ** = 1 %, and *** = 0.1 % probability level, NS = not significant; bold - significant increased germination over the dry control)

5.7.3 Experiment 5.15: The significance of ANS application method on water sensitivity of barley

A seed dip in ANS had no effect on the percentage germination after three days. Water primed seeds had a germination of 42 % ($p<0.001$). Priming in ANS resulted in 16 % higher germination ($p<0.001$). Removal of the adhering ANS material after priming resulted in a drop in germination of 12 % ($p<0.01$) (Figure 5.7.2.a). Germination was on average 9 % higher after seven days, but not preferential for a particular treatment (Figure 5.7.2.a).

Water priming reduced the MGT 13 % ($p<0.05$), while either ANS dip or ANS priming treatments reduced the MGT 11 % ($p<0.05$) (Figure 5.7.2.b).

The total viability of dry control seeds was 97 % after seven days, and was not affected by any treatment (Table 5.7.2.b).

5.7.4 Experiment 5.16: The effect of priming in modified ANS on water sensitivity of barley

After three days 17 % of dry control seeds of barley cv Triumph(A) had germinated. Priming with PEG had no effect on the release of seeds from water sensitivity. Whereas for water-primed seeds 58 % ($p<0.001$) germinated (Figure 5.7.3.a). Seeds primed in ANS supernatant germinated 10 % ($p<0.05$), in original ANS 13 % ($p<0.01$) and in the ANS solid fraction 20 % ($p<0.001$) higher than water treated seeds. Seeds primed in ashed ANS had 59 % germination, similar to water primed seeds (Figure 5.7.3.a).

The MGT increased 18 % ($p<0.01$), when seeds were primed in PEG. Water, or any of the ANS treatments had no effect (Figure 5.7.3.b).

The total viability of seeds in dry controls after seven days was 98 %, this decreased to 92 % ($p<0.001$) after PEG priming (Figure 5.7.3.b).

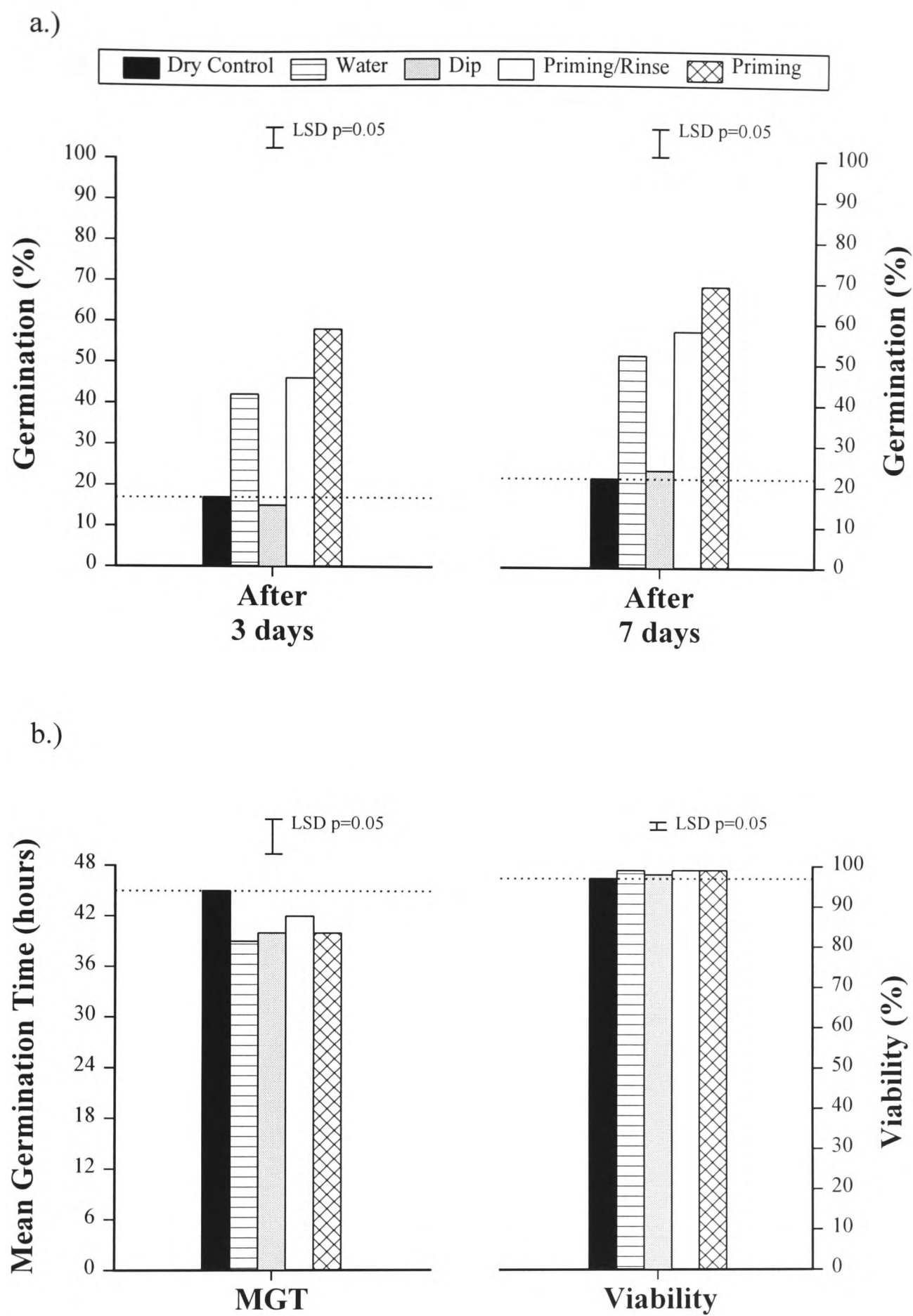


Figure 5.7.2: The effect of application method of ANS on a.) the percentage germination after 3 and 7 days, and b.) the mean germination time over 3 days and the total viability after 7 days, of barley cv. Triumph(A) seeds, in 8 ml water (water sensitivity test), at 20°C. Priming for 12 hours, and re-drying for 24 hours. n=3.

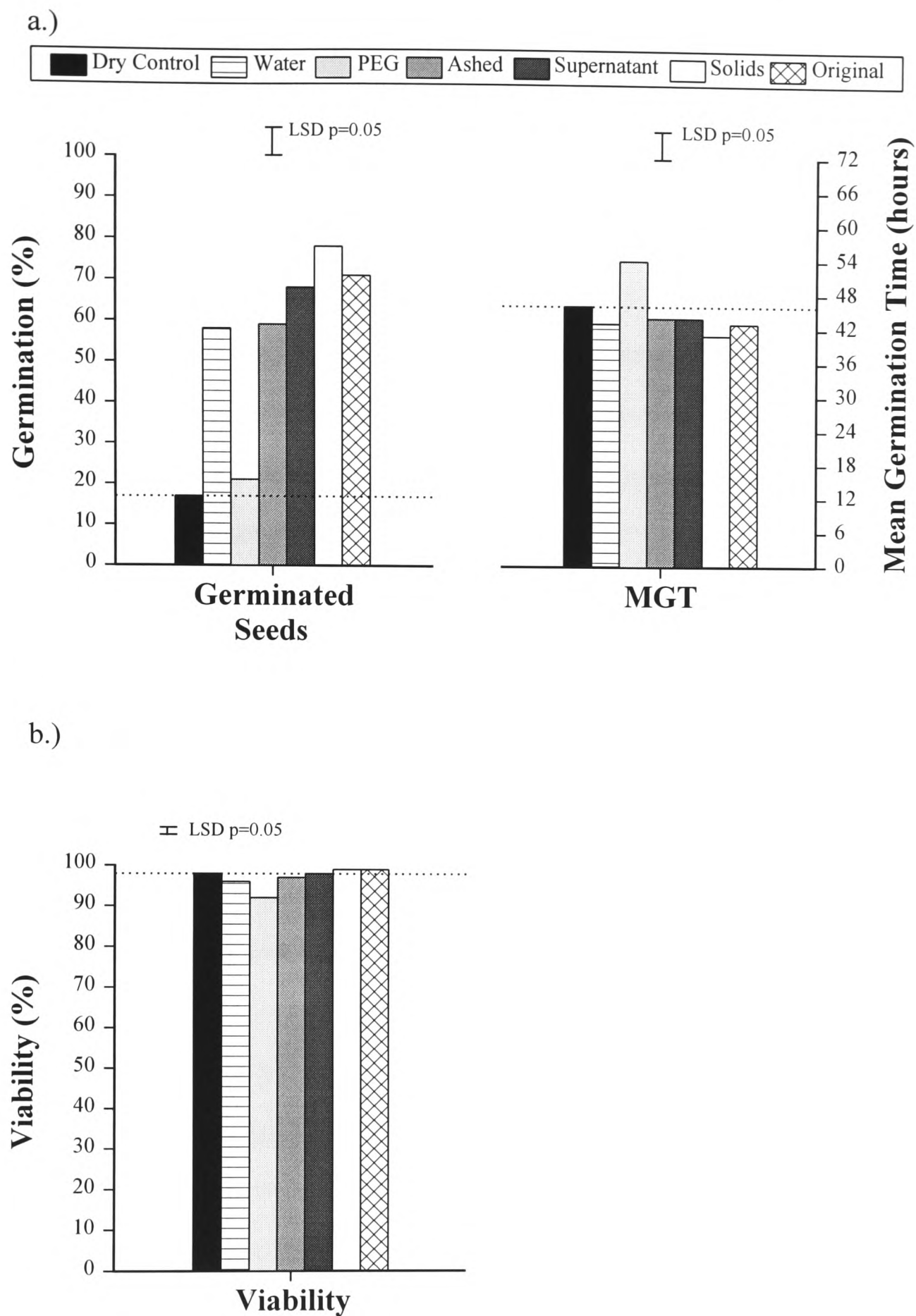


Figure 5.7.3: The effect of priming in water, osmotica (PEG) or modified ANS on a.) the percentage germination after 3 days and the mean germination time over 3 days, and b.) the total viability after 7 days, of barley cv. Triumph(A) seeds, in 8 ml water (water sensitivity test), at 20°C. Priming for 12 hours, and re-drying for 24 hours. n=3.

5.7.5 Experiment 5.17: The effect of seed to ANS ratio on water sensitivity of barley

Dry control seeds of barley cv. Triumph(A) germinated 21 % at 8 ml water level. Water priming of seeds resulted in 54 % germination ($p<0.001$), whereas ANS (seed to ANS ratio 1 : 4) priming resulted in a further 17 % ($p<0.001$) germination (Figure 5.7.4.a). A ratio of 1 : 1 resulted in the same germination as water priming.

The MGT of dry control seeds was 47 hours, and was reduced by 9 % ($p<0.05$) with water and ANS treatments (ratio 1 : 4) (Figure 5.7.4.a). No treatment had an effect on the total viability of the seeds (Figure 5.7.4.a).

5.7.6 Experiment 5.18: The significance of seed storage prior to ANS priming on water sensitivity of barley

The water sensitivity of seed lot (A) of barley cv. Triumph was monitored from week 78 - week 95 after harvest. Over time the water sensitivity of seeds stored at 5°C did not change, with germination in 8 ml water between 17 % - 23 % (Figure 5.7.5.a). However, the sensitivity of the seed lot to priming treatments changed over time and the germination of seed primed in water increased from 23 % at week 78 to 54 % ($p<0.001$) at week 95. The sensitivity to ANS treatments also increased over storage time. This treatment was superior to water treatments by between 12 % ($p<0.01$) and 19 % ($p<0.001$) over the storage period (Figure 5.7.5.a).

The MGT of the control treatments was 46 - 50 hours, over the test period. This parameter reduced over time from 55 hours to 43 hours ($p<0.001$) when water was used, or from 53 hours to 43 hours ($p<0.001$) when ANS was used for priming (Figure 5.7.5.b). After 78 weeks of storage, primed seeds germinated 15 % (water, $p<0.01$), and 10 % (ANS, $p<0.05$) slower than dry control seeds. After 86 weeks, however, germination was 17 % (water, $p<0.001$) and 11 % (ANS, $p<0.01$) faster (Figure 5.7.5.b).

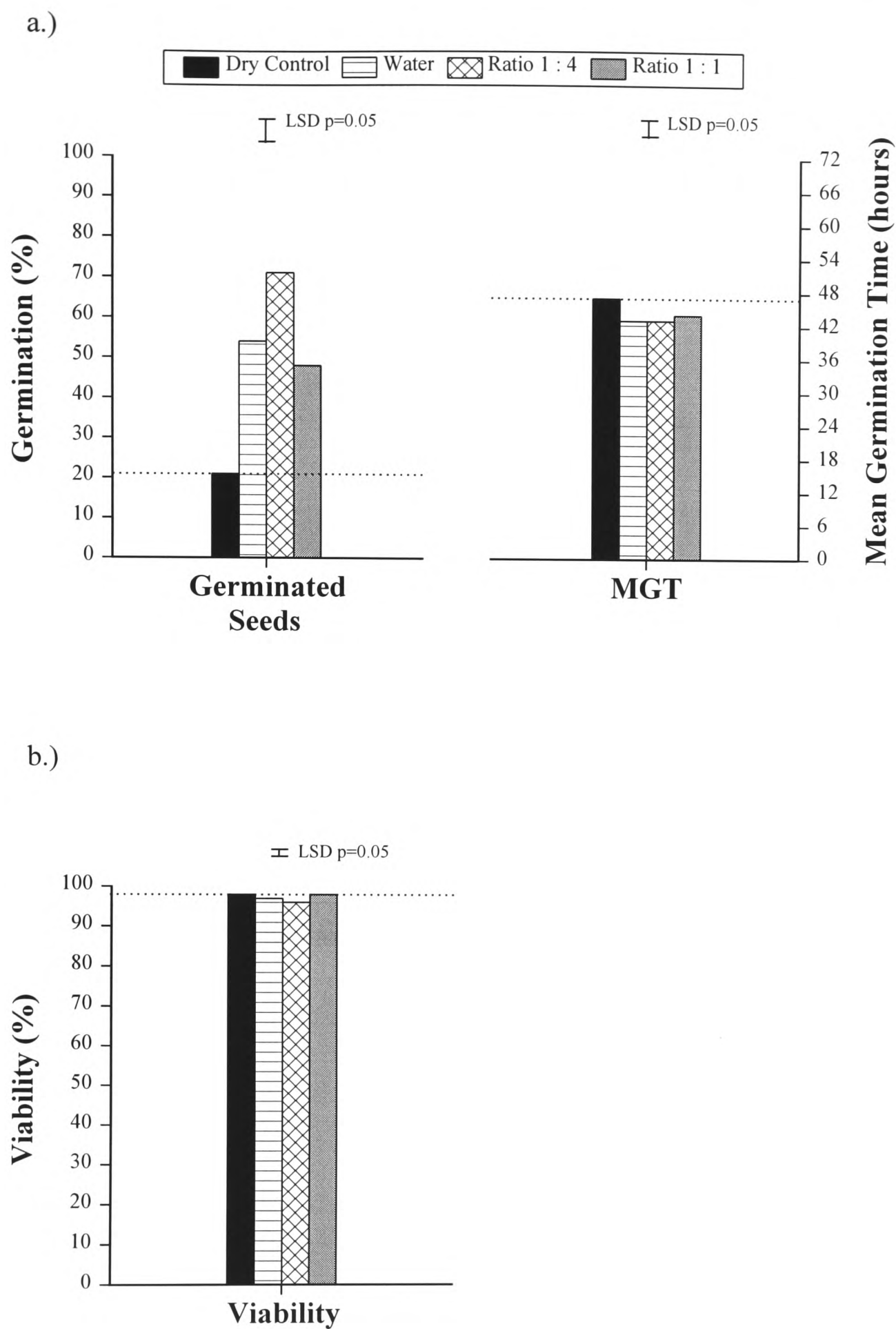


Figure 5.7.4: The effect of seed : ANS ratio on a.) the percentage germination after 3 days and the mean germination time over 3 days, and b.) the total viability after 7 days, of barley cv. Triumph(A) seeds, in 8 ml water (water sensitivity test), at 20°C. Priming for 12 hours, and re-drying for 24 hours. n=3.

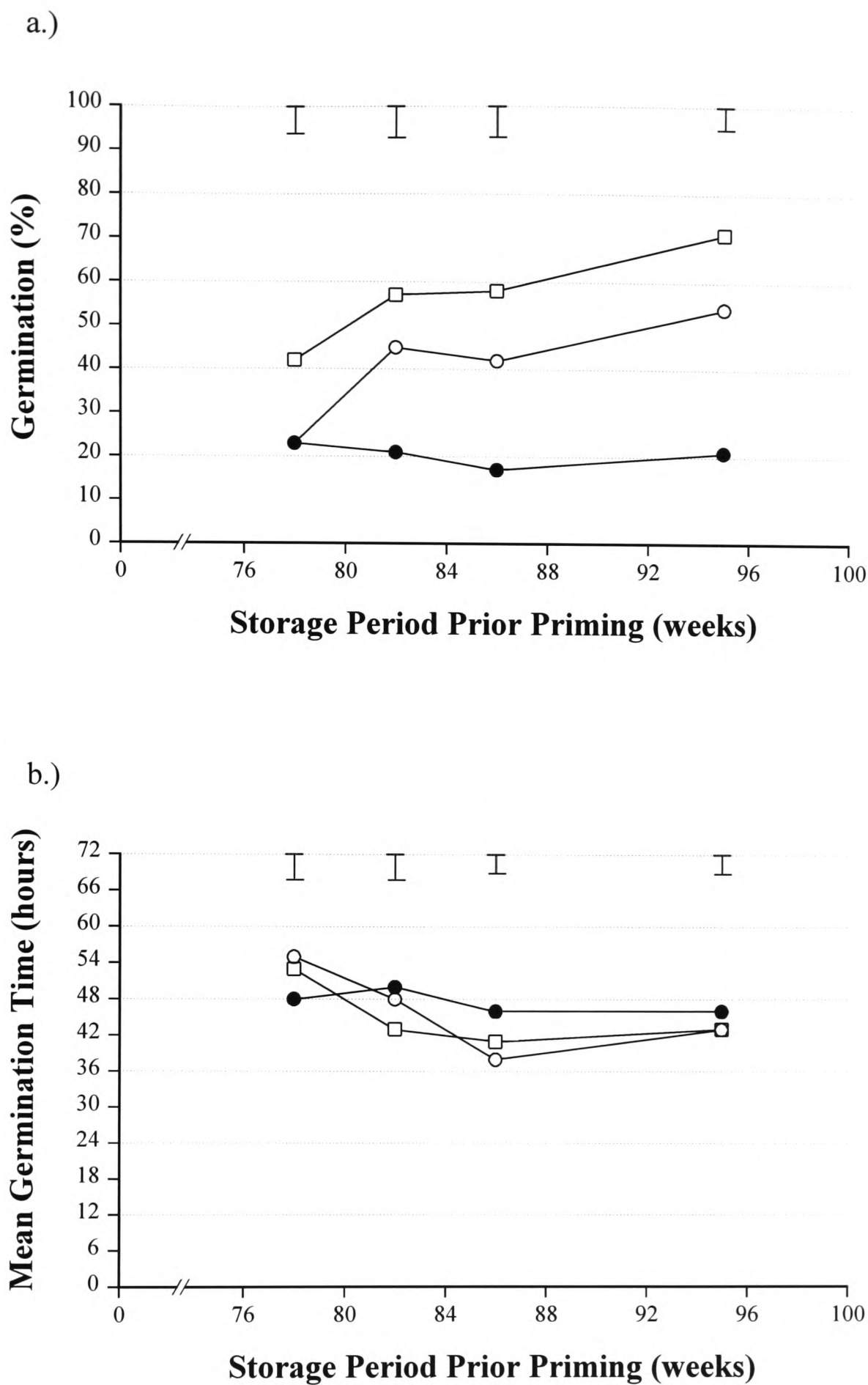


Figure 5.7.5: The effect of storage at 5°C prior to water (○) or ANS (□) priming on a.) the percentage germination after 3 days, and b.) the mean germination time over 3 days, of barley cv. Triumph(A) seeds, in 8 ml water (water sensitivity test), at 20°C. Priming for 12 hours, and re-drying for 24 hours. Dry Control (●). LSD (p=0.05) represented as bars. n=3.

5.7.7 Experiment 5.19: The effect of seed storage conditions after ANS priming on water sensitivity of barley

The water sensitivity of untreated dry control seeds of barley cv. Triumph(A) stored at 5°C did not change over the storage period and was between 17 % - 24 % (Figure 5.7.6.a). Untreated seeds stored at 20°C showed a progressive release from water sensitivity. This release first occurred after 8 weeks of storage, at which point germination increased to 42 % ($p<0.001$) (Figure 5.7.6.a). Germination after 20 weeks of storage at 20°C had further increased to 67 % ($p<0.001$). For water primed seeds 33 % ($p<0.001$) greater germination occurred with storage at 20°C for one day, compared to dry control seeds. This difference increased to 65 % ($p<0.001$) at the end of the storage period. Seeds primed in ANS germinated 17 % ($p<0.001$) higher after one day storage, and 8 % ($p<0.05$) higher after one week storage, compared to water primed seeds. After 20 weeks of storage ANS primed seeds had a 7 % better germination ($p<0.05$) than water treated seeds (Figure 5.7.6.a).

The MGT of dry control seeds ranged between 38 - 50 hours at 5°C, or 43 - 48 hours at 20°C storage. In general, water or ANS primed seeds germinated faster than dry untreated seeds. The MGT decreased over the storage period, from 43 hours to 38 hours ($p<0.01$) for water and from 43 hours to 34 hours ($p<0.001$) for ANS primed seeds (Figure 5.7.6.b). No difference between the priming treatments was observed.

5.7.8 Experiment 5.20: The effect of seed surface sterilisation and suppression of microbial proliferation on water sensitivity of barley

Water soaking had no effect on the water sensitivity of barley cv. Triumph(A) seed, while a 5 minute soak in 10 % calcium hypochlorite increased germination in 8 ml water to 46 % ($p<0.001$) after three days (Figure 5.7.7). Adding antibiotics to the germination medium increased germination of dry control seeds by 44 %

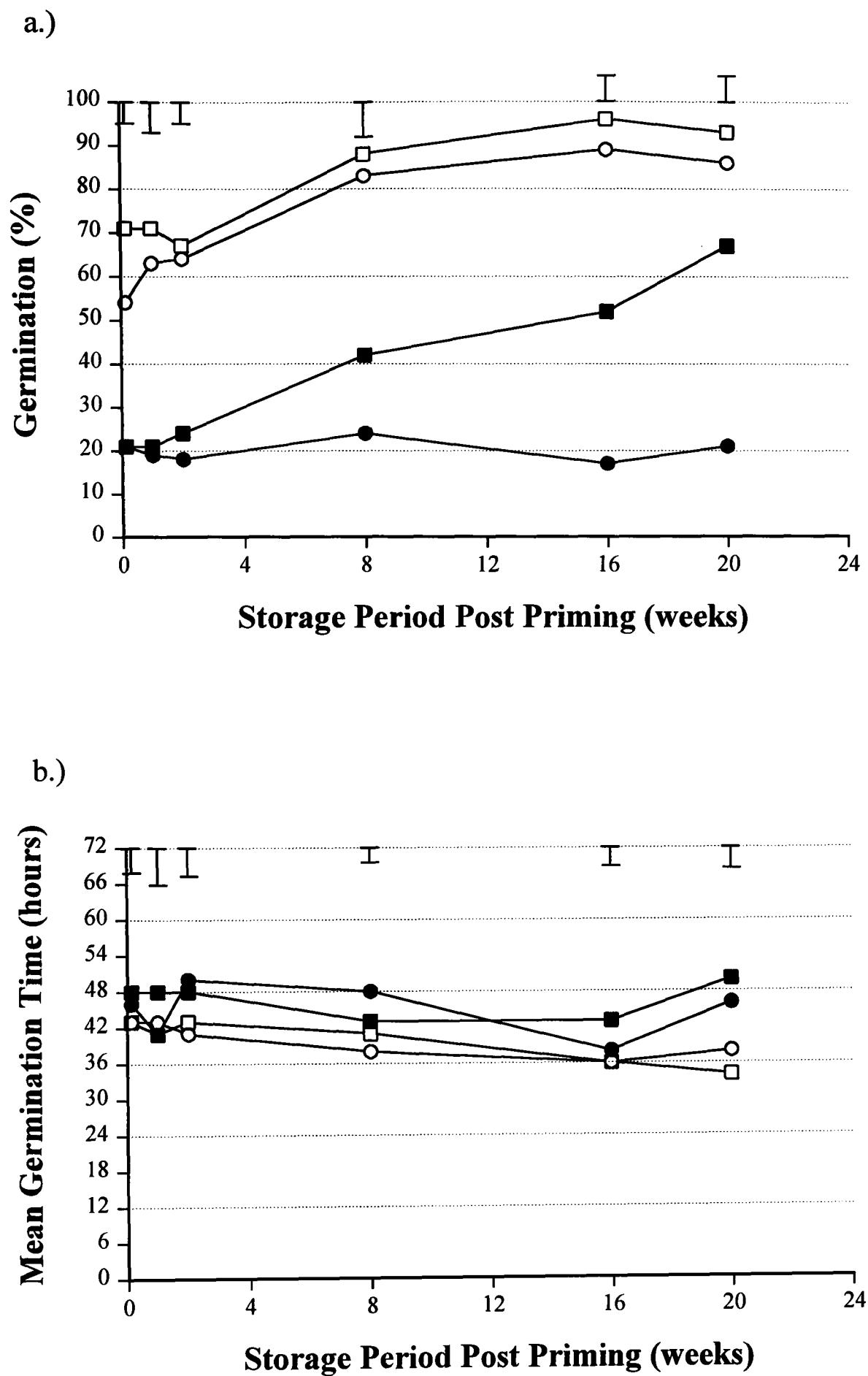


Figure 5.7.6: The effect of storage of dry seeds at 5°C (●), or 20°C (■), or after priming in water (○) or ANS (□) on a.) the percentage germination after 3 days, and b.) the mean germination time over 3 days, of barley cv. Triumph(A) seeds, in 8 ml water (water sensitivity test), at 20°C. Priming for 12 hours, and storage as indicated. LSD (p=0.05) represented as bars. n=3.

($p<0.001$). After a water treatment this parameter increased by 23 % when antibiotics were added. Antibiotics had no enhancing effect on the germination of seeds surface sterilised with calcium-hypochlorite (Figure 5.7.7).

Germination results after seven days were similar to figures obtained after three days, where untreated or water soaked seeds were sown in the absence of antibiotics. Germination after seven days was markedly higher when antibiotics were present in the germination medium, and the difference between treatments with or without antibiotics increased to 66 % ($p<0.001$), 39 % ($p<0.001$), for dry control seeds, or water treated seed, respectively (Figure 5.7.7).

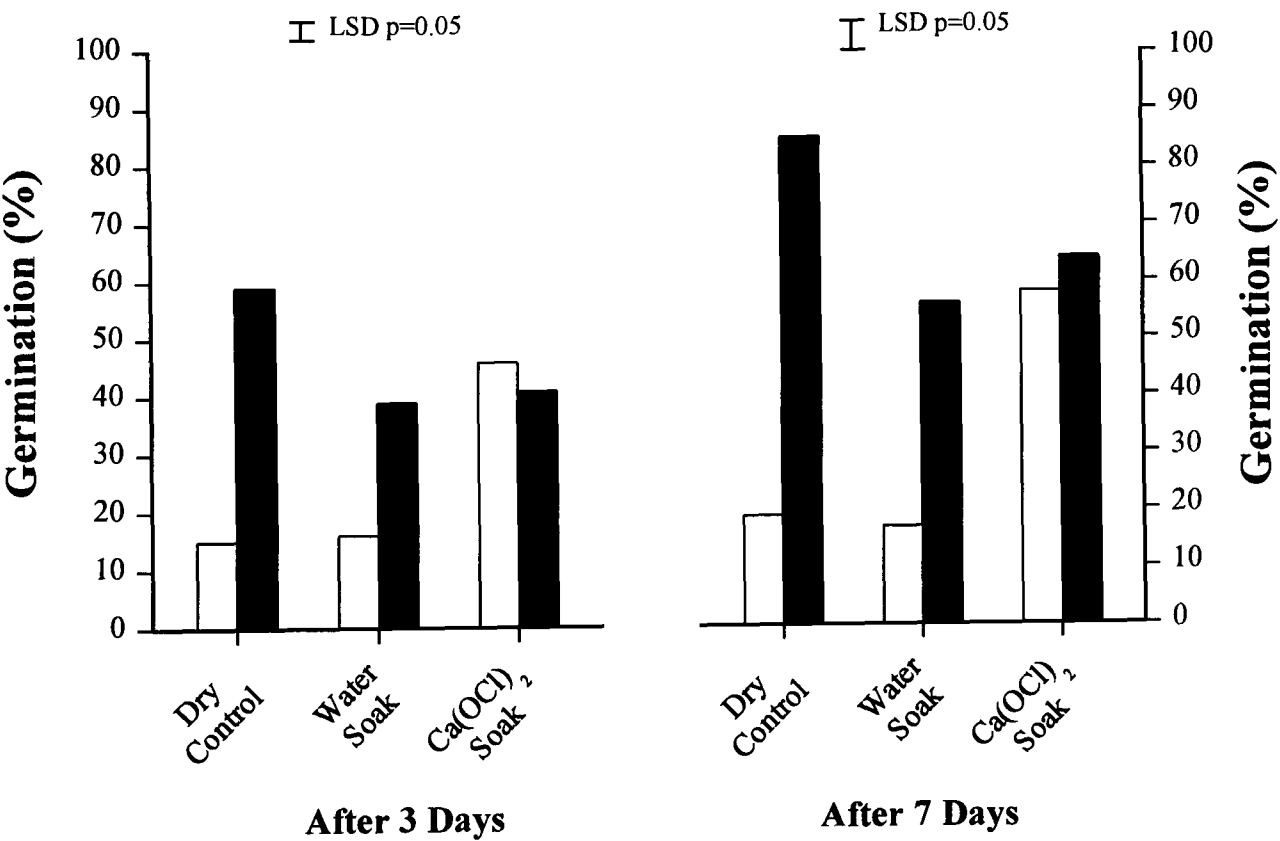


Figure 5.7.7: The effect of seed surface sterilisation (Ca(OCl)_2) on the germination of barley cv. Triumph(A) seeds in the absence (□) or presence (■) of antibiotics in 8 ml water (water sensitivity test), after 3 and 7 days. Seed soak for 5 minutes, and re-drying for 24 hours. $n=3$.

5.8 The anti-microbial activity of SWS to microbes of external source

5.8.1 Experiment 5.21: The effect of SWS on seed microbes of barley

The initial microbial load of barley cv. Triumph(A) seeds was 48.3×10^6 colony forming units (cfu) g^{-1} dry seed. Water (26 %, $p < 0.05$) or PEG (27 %, $p < 0.05$) priming reduced the population of microbes. After priming with ANS the number of microbes dropped by 86 % ($p < 0.001$), while LHS had no effect. Seed surface sterilisation with 10 % calcium hypochlorite removed 99.8 % of the microbes (Figures 5.8.1).

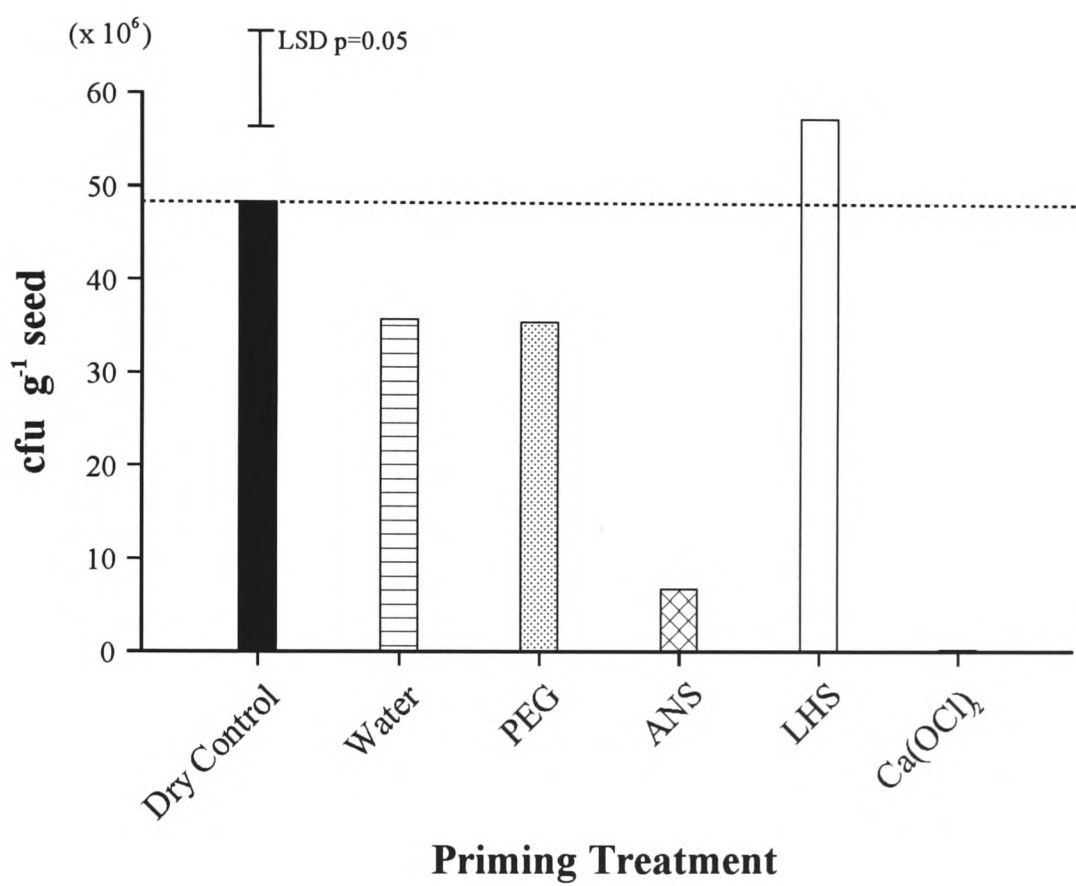


Figure 5.8.1: The effect of priming in water, PEG or SWS, or the seed surface sterilisation ($\text{Ca}(\text{OCl}_2)$) on the population of microbes on barley cv. Triumph(A) seeds. Priming for 12 hours, and re-drying for 24 hours. $n=3$.

5.8.2 Experiment 5.21: The fate of microbial contaminants in priming media

The microbial load was determined in the seed priming media after a 12 hours priming period. In water 2.5×10^6 cfu ml^{-1} were present (Figure 5.8.2). In LHS 30 % ($p < 0.001$) more microbes were found. In contrast, in ANS the number of microbes detected was 99.9 % ($p < 0.001$) lower than in the water priming solution (Figure

5.8.2). The population of microbes on the seeds was found to be closely correlated with the number of microbes in the priming medium ($r=0.947$) (Figure 5.8.3).

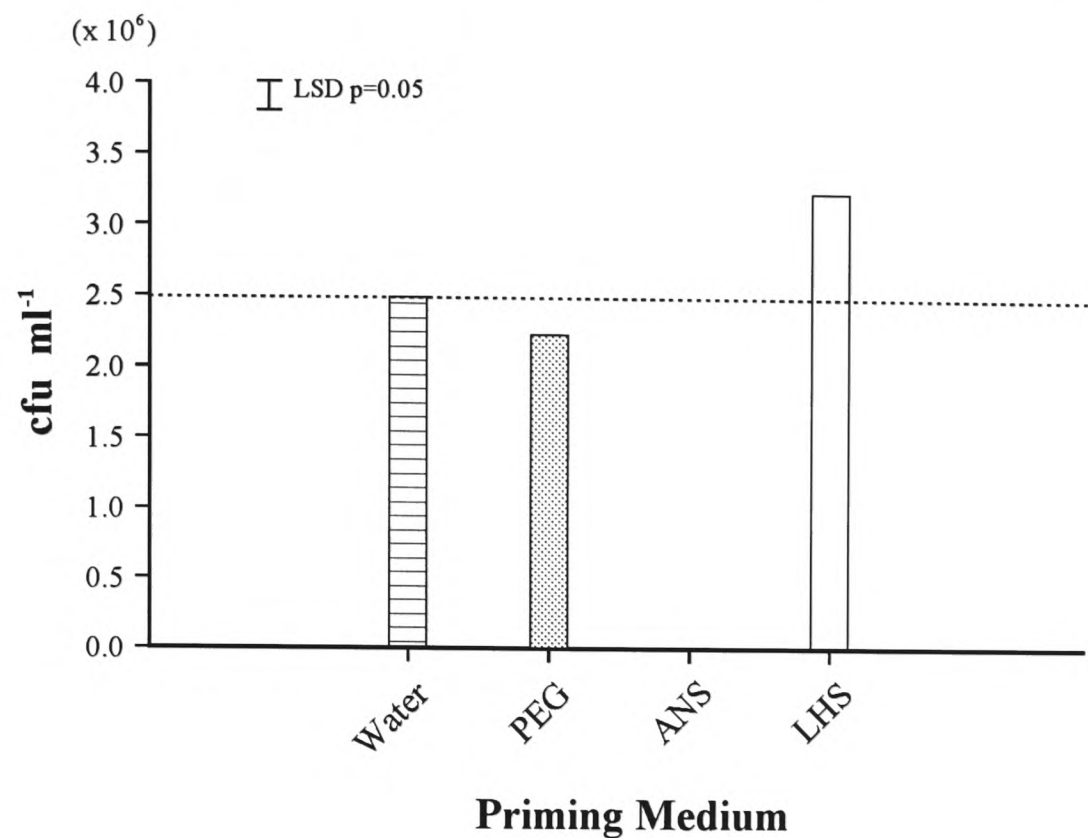


Figure 5.8.2: The microbial load (total colony forming units) of priming media after 12 hours priming at 20°C in the presence of barley cv. Triumph(A) seeds. Priming for 12 hours, and re-drying for 24 hours. $n=3$.

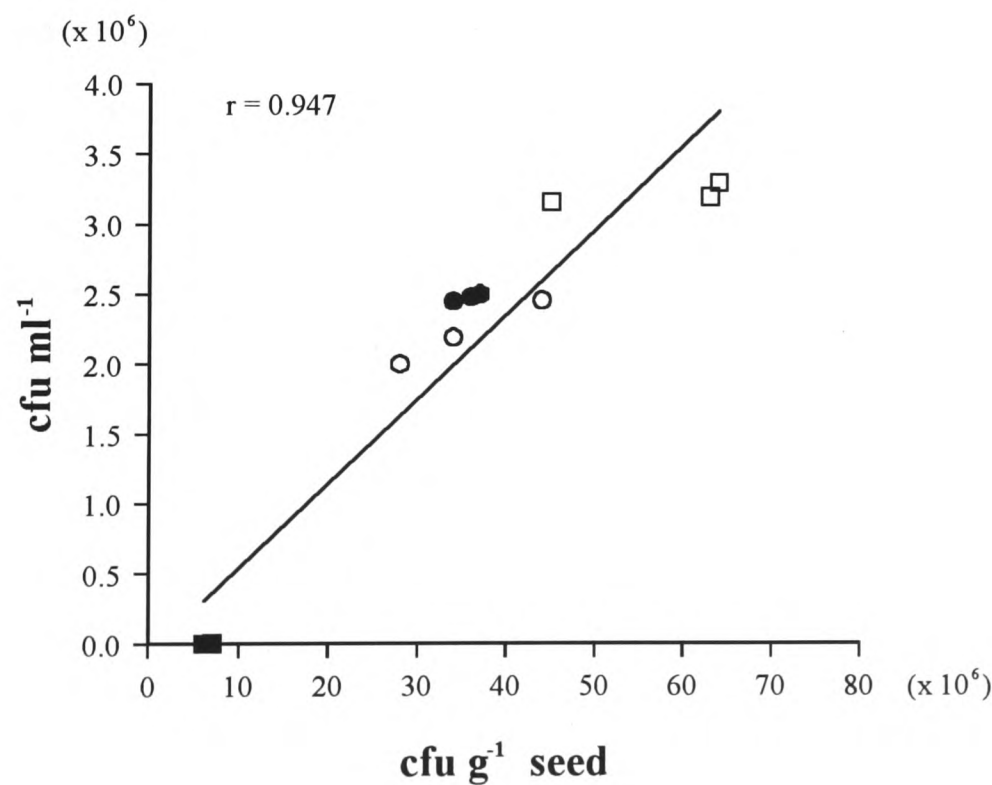


Figure 5.8.3: The correlation between the microbial load of primed seeds and used priming media. Water control (●), PEG (○), ANS (■) or LHS (□) priming for 12 hours, and re-drying for 24 hours.

5.9 Discussion

As for the imbibition experiments on SWS soaked filter paper (Section 4.3.3), water uptake of seeds primed in SWS was inhibited, due to the osmotic potential of the two seaweed extracts. LHS was more inhibitory to moisture uptake than ANS. This was expected. However, the osmotic potentials (OP) of the SWS were not especially low, with view to priming requirements (Khan *et al.* 1980/81), thus lettuce seeds germinated within 48 hours of being treated with either SWS (Figures 5.3.3, and 5.3.4).

The priming media were not aerated as the viscosity of the SWS made the maintenance of a constant stirring motion or even aeration by the use of air pumps virtually impossible. Thus, a minimal suspension depth was chosen for the priming treatments, to allow maximum gas exchange. However, under the priming set up chosen, barley seeds failed to germinate when submerged in priming media. This was probably due to a low oxygen availability, and not due to the prevention of moisture uptake by the SWS (Figure 5.2.1). However, the potentially anoxic conditions during priming were not detrimental when applied for 12 hours, as Gibbons (1983) demonstrated that anaerobic imbibition for up to 24 hours had little inhibitory effects on shoot and root growth during subsequent germination. It was also shown that aeration of lettuce seeds during priming had no effect on consecutive germination performance (Guedes and Cantliffe, 1980).

During priming in water, seeds leaked minerals (Tables 5.2.1, and 5.2.2). Barley seeds, leaked few minerals during a 12 hours priming period (Table 5.2.1). The proportion of SWS material adhering to barley seeds in relation to the size of the seeds was small (Figure 5.2.2; Plate 5.2.1). Thus, the impact of this material on the total mineral composition of SWS primed seeds was low. Conversely, lettuce seeds were much smaller, with intrinsically less storage tissue. Thus, any loss of nutrients during the priming treatment could be potentially detrimental. In this study, water

primed lettuce seeds showed leakage of potassium and iron. Priming with SWS caused even higher losses of a range of minerals, but exceptions included sulphur, sodium and copper (Table 5.2.2). The leakage of potassium could have a detrimental effect on germination, as it is known to be a non-specific promoter of a number of enzyme systems (Evans and Sorger, 1966), and it has been shown in this study (Section 4.5), that potassium is important in the initial seedling growth phase of lettuce seedlings. However, SWS priming with consecutive rinsing, that resulted in lowest potassium levels in lettuce seeds, did not affect the germination percentage or MGT (Figure 5.3.8). Weges and Karssen (1990), using potassium leakage as a measurement of membrane integrity, found a drop in the potassium content of re-dried water primed seeds. No further potassium leakage, however, was found during a second priming treatment. They concluded from the experiments that the initial potassium leakage originated from the pericarp. A membrane barrier prevented a further leakage of potassium from the embryo. This barrier was located in the envelope of the seeds, and Weges and Karssen (1990) presumed that it was suberized semi-permeable integumentary membrane, that was in close association with the endosperm (Borthwick and Robbins, 1928). They further concluded from their experiments, that the integrity of the membrane was not affected by the water priming treatment. The greater potassium leakage of primed lettuce seeds in this study (Table 5.2.2) thus could indicate an effect of the SWS on membrane integrity. Interestingly, while the potassium concentration decreased the sodium concentration in lettuce seeds, primed in SWS and rinsed, increased 5 to 6-fold over control seeds (Table 5.2.2). This was due to a concentration gradient between the initially low sodium concentration in seeds and the high sodium concentration in the SWS. A reverse situation was present for potassium, where the concentration in seeds was around 4 to 8 times higher than in the SWS. This difference could have attributed to increased potassium leakage. The results indicate a mineral diffusion in both

directions, further pointing towards a possible effect on the permeability of the seed envelope by priming in SWS. However, as the potassium or sodium content of isolated embryos was not analysed this conclusion might be premature, and further work is needed to verify the SWS effect on membrane integrity. However, adhering SWS added minerals, compensating for leakage losses occurring during priming, and in some instances the mineral concentration of non-rinsed seeds increased, especially with LHS treatments. This was simply a reflection of the mineral concentration in LHS (Table 3.3.1) and the amount of cell walls debris adhered to the seed coat (Figure 5.2.4).

In general, 12 hour priming was most effective for both species. Prolonged priming allowed radicle of lettuce seeds to emerge, and these were damaged upon re-drying. Longer exposure of barley seeds was not effective in further reducing the MGT, without the risk of increasing the number of dead seeds (Figure 5.3.2).

In this study, some well-known features of seed priming were observed. The treatments did not increase the final percentage germination under optimum germination conditions (Figures 5.3.1.a, and 5.3.3.a), and a reduced MGT was observed (Figures 5.3.2.a, and 5.3.4.a). The MGT of primed lettuce seeds was shortest where the seeds were not re-dried, irrespective priming treatment (Figure 5.3.6.b). This was simply due to a reduction of the imbibition phase, reducing the time to radicle emergence. Another common observation of primed seeds is the increase in MGT with increasing re-drying periods (Bodsworth and Bewley, 1981; Gray *et al.* 1991; Pill *et al.* 1991). This can be explained by a simple reversal of the reduction in the imbibition and lag-phase of primed seeds (Bodsworth and Bewley, 1981; Brocklehurst and Dearman, 1983). However, primed barley and lettuce seeds, re-dried and stored for one week, still exhibited a reduced MGT compared to untreated control seeds. This was entirely due to a reduced lag-phase and could

indicate that the seeds had been invigorated. The MGT was found to be a good indicator of vigour, as it was closely related with physiological and biochemical parameters occurring during germination (Dell' Aquila, 1987; Ram and Wiesner, 1988; Dell'Aquila and Tritto, 1990). These were found to be affected by artificial ageing treatments, or priming treatments. Thus a reduction in MGT reflects an invigoration of seeds. In general, seed priming advances the physiological processes that precede radicle emergence (Khan *et al.* 1978), hence allowing germination to proceed in less time (Coolbear *et al.* 1980; Clarke and James, 1991; Smith and Cobb, 1992). Controlled deterioration experiments in this study (Section 5.4) supported these observations, as aged seeds showed an increased MGT, and primed seeds a reduced MGT (Figures 5.5.1.b, and 5.5.3.b). The most effective priming media was different for the two species. Neither seaweed suspension was superior to control or water treatments in terms of germination percentage. However, priming of low vigour seeds in water or ANS improved barley seed performance to similar levels of untreated high vigour control seeds, as indicated by the MGT. The mode of action of SWS priming seemed to lie in an other effect than that of an osmotic priming effect, as PEG treatments were detrimental to barley germination performance (Figure 5.5.1.a), and shoot growth (Figure 5.5.5).

Although ANS priming was not superior to water treatments in terms of germination performance of barley seeds (Figure 5.5.1.a), growth of ANS primed barley seeds was greater ($p < 0.05$) than water treated seeds at the low vigour level (Figure 5.5.5). This may be due to priming in combination with the adhering ANS material, that hence maintained a higher moisture content of seeds after the 24 hour re-drying period (Figure 5.2.2). This may have allowed physiological processes to progress further, compared to water treated seeds.

For lettuce, increased root length was observed after priming (Wurr and Fellows, 1984; Tarquis and Bradford, 1992), and was correlated with vigour (Wurr

and Fellows, 1985). However, in this study, priming of high vigour seeds reduced root length (Figure 5.5.6.b). Apparently, events leading to radicle emergence on one side, and radicle growth on the other side, were affected independently by priming treatments. Although radicle emergence proceeded faster after seed priming (reduced MGT) (Figure 5.5.3), radicle growth seemed to be slower than in control seeds. This effect was indicated in previous work by Wurr and Fellows (1984). Tarquis and Bradford (1992) also observed increased lettuce radicle growth with shorter priming periods in water (0.5 to hours), while this effect was partially reversed with treatments of 10 hours. As priming reduced root growth of high vigour seeds in this study, the root length of low vigour seeds may have been reduced further. This was not the case, and the worst treatment for high vigour seeds, PEG, promoted root growth of low vigour seeds. The effect of the SWS on the hypocotyl length of lettuce seedlings of high and low vigour could be explained partially by a nutrient supplement of adhering SWS material, that supplied essential nutrients, especially potassium (Table 5.2.2), that was effective in promoting the elongation of lettuce hypocotyls in the dark (Section 4.7.2).

Under the less controlled conditions of a greenhouse experiment, the effects of priming treatments were less pronounced, and not significant for lettuce. Priming of barley seeds in ANS, however, achieved marginally ($p < 0.05$) faster establishment of low vigour seeds (Figure 5.5.7.b), shown by the production of longer shoots ($p < 0.05$) (Figure 5.5.9.a), indicating the persistence of a beneficial priming effect.

The number of abnormal seedlings and dead seeds was not increased after 12 hours priming in undiluted SWS. This indicated the suitability of these media for pre-sowing treatments, unlike other seaweed products which were shown to adversely affect the viability of seeds and seedlings, even at comparatively low dilutions (Button and Noyes, 1964).

Priming seeds in SWS was beneficial when germination occurred under stress conditions. While untreated seeds of lettuce cv. Reskia(B) were incubated at 26°C, germination was inhibited. They, however, germinated after priming. Borthwick and Robbins (1928) observed that the inhibitory effect of high temperatures operated only during the first hours of imbibition. Thus, a 12 hour priming treatment, prepared the seeds sufficiently for germination, so as to partially escape thermo-inhibition. This effect has been indicated in previous research work (Guedes and Cantliffe, 1980; Cantliffe *et al.* 1981; Wurr and Fellows, 1984).

Priming in ANS was more effective than water or LHS treatments (Figure 5.6.3.a). As the efficacy of the various ANS fractions (except ashed ANS) and the PEG treatment was reduced after a post-priming rinsing treatment, the involvement of an intrinsic ANS factor in the adhering material seems obvious (Figure 5.6.4). However, application of individual ANS fractions did not isolate an effective compound, as all fractions, except ashed ANS, were similarly effective (Figure 5.6.4.a). The promotive effect of adhering ANS could have been due to higher moisture levels around the seed during the re-drying phase, resulting in seeds with around 8 % moisture content after 24 hours drying (Figure 5.2.4). The importance of the seed moisture on the alleviation of thermo-inhibition was documented by Weges and Karssen (1990). They demonstrated that a seed moisture contents of 10 % after re-drying after priming caused full germination at 28°C. There was a gradual reduction in germination with lower seed moisture (Weges and Karssen, 1990).

However, in this study, the presence of a further ANS specific effect, different from a seed moisture effect, was demonstrated by the higher germination percentage of rinsed seeds in a storage experiment. Results of this experiment (Section 5.6.5) substantiated an effect of ANS priming on the seed, effective even after 4 weeks storage (Figure 5.6.5.a), and thus, not in connection with elevated seed moisture. The release of lettuce seeds from thermo-inhibition is complex. The effect

of ANS may be linked to the increased permeability of the integumentary membrane of seeds. This may have resulted in a higher leakage of inhibitors from the seeds as shown by Speer and Tupper (1975). They indicated that a germination inhibiting substance, possibly abscisic acid, leaked from embryos of lettuce seeds imbibed in water. Damaging or removing the pericarp and endosperm can overcome thermo-inhibition, hence indicating the importance of the integumentary membrane (Ikuma and Thimann, 1963; Drew and Brocklehurst, 1984b). Khan *et al.* (1978) also demonstrated the disappearance of abscisic acid (ABA) in primed seeds. However, they also reported that changes in ABA appeared to be related to reversible hydration dehydration processes. This agrees with findings by Weges and Karssen (1990), who found a reversibility of thermo-inhibition upon complete re-drying of primed seeds within 6 hours. Braun and Khan (1975) indicated a correlation between ABA content and thermo-inhibition, but also evidence for a different mode of action, as the levels of ABA were not always correlated with germination. The action of growth regulating substances in ANS promoting high temperature germination is unlikely. Cytokinins, gibberellic acid, and ethylene are capable of promoting lettuce seed germination at higher temperatures (Kahn 1960; Bewley *et al.* 1968; Reynolds and Thompson, 1973; Dunlap and Morgan, 1977; Huang and Khan, 1992). However, gibberellic acid was not found in ANS, and cytokinin levels were too low to have any physiological effect (Section 3.3.2). Although the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC) was found to be present in a seaweed concentrate prepared from *Ecklonia maxima* (Nelson and van Staden; 1985), its presence in *Ascophyllum nodosum* has not been shown.

Guedes and Cantliffe (1980), Cantliffe *et al.* (1981), and Wurr and Fellows (1984), treated lettuce seeds in tripotassium orthophosphate (K_3PO_4) in a similar priming re-drying regime as in the present study, and obtained almost twice as high germination at 30° to 35° C, compared to the water control. This might suggest the

involvement of a mineral compound, beside the osmotic effect, however, there was no evidence for this in the present study as ashed ANS had no extra effect (Figure 5.6.4.a). To elucidate the potential property of ANS to release lettuce seeds from thermo-inhibition, further work should be conducted including germinating experiments in the presence of ANS at elevated temperatures.

Barley seeds were tested under increased water levels, as this can inhibit the germination of barley seeds (Essery and Pollock, 1957). It is nowadays a standard test for barley seeds intended for malting (Anon., 1986). The results of Experiment 4.1 and Experiment 5.13 indicated that water sensitivity of barley cv. Triumph was not due to residual dormancy, as seeds germinated readily at optimum water levels in 5 ml water (Figure 4.2.1.a). The separation of water sensitivity from dormancy was first proposed by Essery and Pollock (1957), who conducted a controlled study on the influence of heat and desiccation treatments on the water sensitivity in germination tests.

In this study, a marked reduction in water sensitivity was achieved by priming in ANS (Section 5.7), however, re-drying was essential to obtain maximum effect (Table 5.7.1). It was found that prolonged storage prior to water or ANS priming added to the reduction of water sensitivity (Figures 5.7.5.a). The reduction in water sensitivity could have been due to an advanced physiological metabolism of primed seeds. This may also have been responsible for the improved performance of primed seeds germinating under low oxygen. However, priming in PEG was ineffective (Figure 5.7.3), indicating a different mode of action for the release of seeds from water sensitivity, beside an osmotic effect. Water or ANS treatments resulted in similar effects when the adhering ANS material was rinsed off (Figure 5.7.2.a). The greater reduction of water sensitivity observed for seeds with adhering algal material could be attributed to the hygroscopic property of the SWS; the algal

material might have taken up excess water, and reduced the water film covering the seeds. This would have allowed an increased gas exchanges of seeds, and thus oxygen availability to the embryo. The water film was shown to be a substantial oxygen barrier, and its removal resulted in an almost four fold increase in the oxygen uptake by barley seeds (Crabb and Kirsop, 1969). In contrast, however, the adhering algal material did not increase germination in 8 ml water of dry seeds (Figure 5.7.2.a). Thus, it seems that the additional effect of adhering ANS material is only apparent in seeds that have advanced metabolic activity, i.e. primed seeds. The physiological mechanisms involved in the expression of water sensitivity are not fully understood. The main factor, however, seems to be the oxygen availability / sensitivity of the embryo (Crabb and Kirsop, 1969). Water sensitive seeds, which did not germinate in 8 ml water, germinated readily under high oxygen tensions (Thunaeus, 1938). The significance of the oxygen sensitivity of the embryo in water sensitive seeds is indicated by findings of Crabb and Kirsop (1969). They could not find differences in the oxygen uptake by intact water sensitive and non-water sensitive seeds. In addition, isolated embryos from water sensitive seeds failed to grow at low oxygen tensions (Crabb and Kirsop, 1969). Removal of the husk, abolished water sensitivity (Jansson *et al.* 1959), and was therefore associated with a large increase in the rate of oxygen uptake. Côme *et al.* (1988) showed that the oxygen uptake by isolated glumellae could be as high as 50 % of the total oxygen uptake.

With respect to the significance of oxygen in the expression of water sensitivity of barley, some researchers point to the importance of microbes present on and under the husk (Petters *et al.* 1988), competing with the embryo for oxygen during germination (Blum and Gilbert, 1957; Gaber and Roberts, 1969; Kelly and Briggs, 1992; Doran and Briggs, 1993b). In this study the effect of a reduction of the microbial contamination of the seed on the expression of water sensitivity was tested.

Surface sterilisation with calcium hypochlorite was beneficial, but was not as effective as antibiotics (Figure 5.7.7). Calcium hypochlorite did not eradicate the microbial population completely (Figure 5.8.1), especially not those presumably concealed under the husk close to the embryo. Furthermore, seeds were not germinated under sterile conditions, hence allowing secondary contamination of seeds to occur and to proliferate. This is because hypochlorite has no persistent antimicrobial effect, unlike antibiotics. Thus, seeds exposed to the water sensitivity test in the presence of antibiotics, were less contaminated with microbes over the duration of the germination experiment and germinated to a higher percentage than surface sterilised seeds. A direct physiological effect of the antibiotics applied on seed germination could be ruled out. Gaber and Roberts (1969) obtained no effect on water sensitivity when the antibiotic substances were applied individually, suppressing only either bacteria, or fungal contaminations, and leaving sufficient microbes on the seed to reduce oxygen availability to the embryo. These results strengthened the theory, that the oxygen competition between micro-organisms and embryo increase water sensitivity, although Jansson *et al.* (1959) suggested that the oxidising capacity or the ability to bind sulphhydryl groups rather than the sterilising properties of hypochlorite are responsible for the efficacy in promoting germination. This was proven otherwise for the seed lot used in this study by employing antibiotics to reduce the microbial contamination. The relative insignificance of the quantitative presence of microbes on primed seeds, in relation to the expression of water sensitivity, is indicated in this work by findings that water priming reduced water sensitivity almost to the same extent as ANS treatments, although the number of microbes on ANS treated seeds was 80 % lower (Figure 5.8.1).

In this study prolonged storage prior to priming added to the reduction of water sensitivity (Figures 5.7.5.a). This indicated changes in the seeds occurred

during storage, resulting in an increased response to priming treatments. The release from water sensitivity of untreated seeds, stored at higher temperatures (Figure 5.7.6.a) has been reported in previous publications (Essery and Pollock, 1957). It was not attributed to a desiccation process, but entirely to a temperature effect (Essery and Pollock, 1957), that diminished the requirement of the embryo for oxygen (Crabb and Kirsop, 1969).

In this study it was found that when storing primed seeds at 20°C, the water sensitivity declined progressively with increased storage periods parallel to untreated seeds (Figure 5.7.6.a). This was a temperature related response (Essery and Pollock, 1957). Kudo and Yoshida (1958) associated higher storage temperatures with a decline of seed microbes, which would result in increased oxygen levels when seeds were subjected to water sensitivity tests, allowing more seeds to germinate. This may be true for dry untreated seeds. In the present work, microbes were counted at the end of the storage period (week 95). Despite a comparable microbial contamination with untreated seeds (Figure 5.8.1), water priming was effective in releasing seeds from water sensitivity (Figure 5.7.6.a). The release from water sensitivity earlier in the storage experiment by ANS priming was not due to a priming effect, as priming in water was not effective at that time (week 78) (Figure 5.7.6.a). It therefore appears that an additional inhibitory compound in barley seeds was mainly responsible for the expression of water sensitivity, declining over storage time. However, it is clear, that the number of microbes present on barley seeds were a modifying factor in the expression of water sensitivity in germination experiments. This was shown by evidence that ANS priming was superior to the water treatments (Figures 5.7.5.a, and 5.7.6.a).

In this study, ANS reduced not only barley seed microbes (Figure 5.8.1), but also inhibited proliferation and reduced the number of external microbial contaminants almost completely (Figure 5.8.2). The source of this antibiotic effect

could be, firstly, the production of antibiotic substances by bacteria. The production of antibiotic substances was observed by Fries (1988), who analysed the symbiosis between *A. nodosum* and an endophytic fungus, and epiphytic bacteria, and found that antibiotics produced by the bacteria kept fungal growth in check. Further evidence comes from work by Lemos *et al.* (1991), who found that antibiotic producing strains of epiphytic marine bacteria not only inhibited the growth, but actually reduced the quantity of other marine bacteria within hours of co-culture. However, it is difficult to conclude that the antibiotic activity of ANS was due to this phenomenon, because the symbiotic bacteria of *A. nodosum* exist on the surface of intact algae (Fries, 1988), and not in direct contact with algal cytoplasm, and as the physical presence of those bacteria in ANS was not tested. The second potential source for an antibiotic activity could be the relatively high osmotic potential, in which terrestrial microbes may not survive. However, the osmotic potential did not seem to be a factor, as LHS was not effective in reducing the number of microbes (Figure 5.8.2), although it possessed a higher OP than ANS. A third source could be SWS intrinsic antibiotic compounds (Table 1.4.1), such as the high concentration of polyphenolic substances in ANS. The polyphenol content of the SWS correlated well with their observed antibiotic activity, and the *Ascophyllum nodosum* suspension contained 14 times more than LHS. This strongly suggests that polyphenol content was responsible for the antibiotic effect.

Chapter 6

General discussion and conclusions

In this study two seaweed suspensions, prepared from the brown seaweeds *Ascophyllum nodosum* (ANS) or *Laminaria hyperborea* (LHS), were tested as priming media for their ability to improve seed germination, and seedling establishment. Their effect on seedling physiology was investigated in short duration bioassays as well, to evaluate their potential value as plant fertiliser. Two distinct different plants, barley, representing monocotyledons, and lettuce, representing dicotyledons, were used as test species.

In initial experiments the physical and chemical properties of the two seaweed products were investigated, to enable characterisation of the suspensions. The manufacturing process, was found to be a comparatively mild procedure, and resulted in a mineral composition (on the dry matter basis) of both SWS similar to fresh algal material (Tables 1.3.3, 3.3.1, and 3.3.7). Due to the source material used, marine macro algae, the products exhibited a relatively high negative osmotic potential of -0.25 MPa (ANS) and -0.5 MPa (LHS). However, these figures are low compared to the OP of commercial concentrates, such as **Maxicrop Triple**, that can reach osmotic potentials of up to -6.5 MPa in undiluted concentrated product, (Section 3.4). However, this concentrated extract is usually applied diluted to 1 : 200 to 1 : 500, at which the osmotic potential is similar to water. The method of production affects the pH. The physical disintegration procedure chosen for the preparation of the suspensions tested in this study resulted in pH's of 5.6 (ANS) and 7.4 (LHS). Alkaline hydrolysis, on the contrary, yields products with higher pH levels, between 9.0 - 10.5 for **Seasol Liquid** (Abetz, 1980), or 8.9 for undiluted **Maxicrop Triple** (Section 3.4). Even diluted to 1 % this extract had a pH of 8.2. Alkaline hydrolysis results in concentrated extracts high in salts, as the conductivity values for **Maxicrop Triple** indicated. Its conductivity was 8-12 times higher than those of the extracts used in this study. Due to the process of alkaline hydrolysis, the resulting extracts are further enriched with minerals, such as nitrogen or potassium

(Table 1.5.2). This points to a general problem often encountered when trying to compare results obtained after the application of seaweed extracts. Differences in the composition of the final product (Section 1.5.2) are partly due to variations in the manufacturing process of different seaweed products (Table 1.5.2), but also due to different algal source material used (Table 1.5.1), and the addition of various compounds to the final product (Section 1.5.1). Furthermore, different plant species may respond differently to the same product, and the same species differently under different experimental conditions or at different growth stages. Thus, results obtained after the use of the two SWS cannot be generalised, and effects expected from the application of seaweed extracts other than those used in this study would most likely be different from those observed here. Similarly, the application of the two SWS to species, other than those tested here might yield different results as well.

In previous publications the ability of seaweed preparations to alter plant growth was attributed to plant growth regulators present in the seaweed products (Section 1.5.2.2). In this study it was demonstrated that unpurified SWS tested exhibited low levels of abscisic acid, medium levels of auxin-like activity, and low levels of cytokinin-like activity only in ANS (Table 3.3.5). It would be wrong to assume, however, that inhibition of root growth in the bioassay was entirely due to the presence of auxin-like substances, as the unpurified SWS were applied. These contained high amounts of phenols, which could have accounted for most of the inhibitory activity in the SWS (Table 4.5.1). The actual levels of auxins in the SWS might therefore be much lower than estimated in the bioassay, as indicated by Buggeln and Craigie (1971) for fresh algal material (Table 1.3.7).

The sensitivity limit of the gibberellin-bioassay was $34.6 \mu\text{g l}^{-1}$ (Figure 3.3.5), while gibberellic acid concentrations found in fresh algae material range from $0.76 - 100 \mu\text{g kg}^{-1}$ (Table 1.3.7). As the SWS were diluted during the manufacture, rather

than concentrated, only low levels of GA-like activity were expected. However, at this level they are most unlikely to be of physiological significance to plants.

Findings by Williams *et al.* (1981) further indicated an instability of GA activity in commercial seaweed extracts over time. They found rapidly declining GA levels within 2 - 4 months of storage.

As commercial seaweed extracts have been found to contain considerable amounts of growth hormone-like substances (Section 1.5.2.2), the effects on plant growth were attributed to the presence of these substances, while the mineral component of seaweed products applied at recommended concentrations was thought not to be high enough to induce the plant responses observed (Blunden, 1977; Temple and Bomke, 1989). However, in this study, using a short duration bioassay with lettuce seedlings, it was demonstrated that the mineral compounds of the SWS tested were responsible for promotion of lettuce seedling growth, as shown by results using ashed SWS (Table 4.7.1). Of the minerals present, only potassium was found to be of importance in the lettuce bioassay. A similar observation was reported by Blunden *et al.* (1968). They found that ashed fractions of fresh *A. nodosum* material, applied regularly as soil flushes, were more growth promotive on mustard seedlings, in terms of fresh and dry weight increase, than aqueous extracts, although the distinctive role of individual monovalent cations, especially K^+ , was not investigated. The significance of the mineral component was also pointed out by Beckett and van Staden (1989). They found greater effects of ashed over fresh **Kelpak** application to K^+ stressed wheat, and no effect of cytokinins. In all cases seaweed products and the ashed fractions were applied in larger quantities. Of the other constituents present in brown algae, and consequently present in seaweed extracts (Section 1.3), Blunden *et al.* (1968) tested amino acids and mannitol on plant growth and found no promotive effect.

In contrast to growth promotion, at higher concentrations both SWS caused root growth inhibition in both plant species tested in this study, although barley seedlings were less sensitive (Figure 4.4.1, and 4.4.4). Growth reduction of bean (*P. vulgaris*) seedlings was observed by Temple and Bomke (1988), when a kelp, *Macrocystis integrifolia*, was applied to soil at high levels of 120 tonnes per hectare. The observed stunted plant growth was attributed to a high salt or chlorine concentration in the soil from the kelp and was eliminated by leaching the soil to remove the soluble salts. In the present study, the salt concentration of SWS was comparably low, and affected root growth only marginally, as shown by results for ashed SWS (Table 4.5.2), while cotyledon and hypocotyl growth was promoted. The major compound(s) responsible for root growth inhibition of SWS was found to be of organic nature, as the inhibitory effect of original SWS was more severe. Whereas it was found that in the case of ANS, phenolic compounds were responsible for the inhibition, an unknown compound was accountable in LHS.

There have been studies published previously, reporting increased chlorophyll accumulation after the application of SWS. Whapham *et al.* (1992) treated tomato plants either with a foliar application or with a root drench using **Maxicrop Triple**, and detected 20-50 % higher chlorophyll levels based on the fresh weight, compared to untreated plants. They attributed this increase in chlorophyll content to the presence of cytokinins in the extract. In a later work, Whapman *et al.* (1993) attributed an increase in chlorophyll content to betaines which were shown to be present in the product used (Blunden *et al.* 1984; 1986), as the cytokinin content of **Maxicrop Triple** was likely to be low (Blunden, 1991). In the present study high cytokinin-like activity was not determined in the SWS tested (Section 3.3.2), and although betaines were found in *A. nodosum* and *L. hyperborea* (Blunden *et al.* 1982; 1985), their presence in the SWS was not investigated. However, treatments with both SWS, modified SWS and KCl treatments increased the chlorophyll

concentration per milligram fresh weight (Figure 4.8.1), and thus indicated an involvement of potassium. The potassium concentration of **Maxicrop Triple** was 25 times higher than in ANS, and might have been partly responsible for the observed increase in chlorophyll by Whapham *et al.* (1992). In contrast, Temple and Bomke (1988), applied *M. integrifolia* and observed the dark green colour of bean plant tissue, in combination with stunted growth, and attributed it to high salt or chlorine concentration (Hajrasuliha, 1980; Taneja *et al.* 1992).

In investigations on seed priming treatments in this study (Chapter 5), a 12 hour seed soak with subsequent re-drying to the original seed moisture content (~24 hours) was the treatment to obtain maximum osmo-conditioning effect before detrimental effects occurred, such as radicle emergence of lettuce seeds, or an increase in dead barley seeds (Figure 5.3.2.c). Under optimum conditions, priming in SWS or water did not improve the final germination percentage of both lettuce or barley seeds, but reduced the mean germination time by 27 - 38 % (Figures 5.3.2.a, and 5.3.4.a). Similar improvements in the speed of germination were observed by Bodsworth and Bewley (1981) who primed barley seeds on PEG (-0.15 MPa) soaked filter paper and found that a 48 hour treatment at 10°C reduced the time to maximum germination by 60 %. In this work, however, PEG treatments were detrimental to germination and MGT. This was probably due to the low oxygen solubility in this priming medium (Mexal *et al.* 1975), as the seeds were submerged for priming. It was shown that reductions in the speed of germination reflected an invigoration of seeds (Dell'Aquila, 1987; Ram and Wiesner, 1988; Dell'Aquila and Tritto, 1990). In this study, the invigoration effect, indicated as a reduced MGT in petri-dish germination experiments, was manifested only in barley seeds, primed in ANS in the plumule growth test by increased shoot length (Figure 5.5.5), as well as under greenhouse conditions by reduced mean emergence times (Figure 5.5.7.b) and longer

shoots (Figure 5.5.7.c). The promotion under greenhouse conditions was less pronounced compared to laboratory tests. However, the mean germination time and plumule length, can be used as predictors for field emergence and yield (Kim *et al.* 1994). The correlation between MGT and plant establishment of winter cereals was further shown by Haastrup-Pedersen *et al.* (1993), although on aged rather than primed seeds. They found progressively increased MGT of controlled-deteriorated seed (low vigour) while the germination was still above commercial standards. However, in field trials they observed a decrease in the number of heads per unit area, and reduced yields of barley or wheat crops under normal cultivation conditions between sub-lots that differed in MGT by 47 % or 90 %. Conversely, in another field experiment 37 % higher yield was observed for barley seeds primed for 6 hours in sodium orthophosphate (NaHPO_4) (Chatterjee and Singh, 1983).

Occasionally, seeds sown under commercial conditions may be exposed to less favourable conditions, detrimental to germination and seedling establishment. Lettuce seed, incubated above 25°C show inhibited germination and poor emergence results. This results in yield losses when sown directly in the field in regions with high soil temperatures (Cantliffe *et al.* 1981; Seale *et al.* 1994). This could be overcome by priming treatments and Cantliffe *et al.* (1981) obtained 35 % to 71 % germination at 30°C and 70 % to 80 % field emergence after a 9 hour seed priming with a 24 hour re-drying treatment in various osmotica (-0.51 MPa), while untreated seeds failed to germinate at 30°C and emerged to 50 % only. With similar priming conditions used in this study, priming in ANS, was found to be superior to water treatments and similar to PEG treatments, and resulted in 61 % germination at 26°C (Figure 5.6.4.a). However the mode of action of ANS in the release of lettuce cv. Reskia seeds from thermo-sensitivity was complex and involved an intrinsic ANS constituent, as well as a direct effect on the primed seeds (Figure 5.6.5.a), not found in PEG primed seeds, when the adhering priming media were rinsed off (Figure

5.6.4.a). The effect of ANS priming decreased over storage time. This could be linked to an accelerated ageing of primed seeds, as lettuce seeds were more susceptible to deterioration in storage after priming (Tarquis and Bradford, 1992).

Barley seed fail to germinate under germination conditions that cause oxygen deficiency, such as elevated water levels (Essery and Pollock, 1957). The oxygen sensitivity of the embryo was shown to be a key factor in the expression of water sensitivity (Crabb and Kirsop, 1969). Priming treatments in this study alleviated germination inhibition in a water sensitivity test (Figure 5.7.2) mimicking an environment of low oxygen tension. It was found that ANS treatments were superior to water and PEG treatments (Figure 5.7.3). This priming effect was partly due to a reduction in the water film covering the seeds by the uptake of excess water by the adhering algal material. It was shown that the removal of the water film from soaked seeds resulted in increased oxygen uptake by barley seeds (Crabb and Kirsop, 1969). The ANS priming effect was further enhanced by the anti-microbial effect of ANS, reducing the microbial population of seeds by 86 % (Figure 5.8.1). This effect is of importance, as it is not only in the water sensitivity tests in a laboratory, that oxygen scavenging organisms reduce the germination of barley seeds. Harper and Lynch (1981), investigated the germination of barley seeds in soil in correlation with fungal colonisation and found suppressed germination by competition of fungi on the surface of seeds with the embryo for available oxygen. Thus, sowing seeds with a reduced microbial load in an unfavourable, low oxygen environment can be beneficial to germination and establishment. The adhering ANS material to seed may further prevent microbial proliferation in the soil in the close proximity of the seed, increasing the oxygen availability to the embryo.

As SWS priming treatments did not increase the occurrence of abnormal seedlings or dead seeds in barley (Figure 5.5.2) or lettuce (Figure 5.5.4) the suitability for priming purposes is indicated. However effective a priming treatment

might be in laboratory experiments, it should be robust enough to allow rapid seedling establishment under commercial growing conditions. Priming treatments in ANS marginally improved barley seed performance in greenhouse conditions. It is, however, doubtful if this invigorating effect was sufficient to result in increased yields under normal growing conditions. This will require further investigations. As indicated by the improved performance of barley seeds under water stress, seeds primed in ANS might be well prepared for sowing under difficult agricultural conditions, that farmers often encounter especially under Scottish climatic conditions.

On the contrary, seed treatments are an extra cost. An attempt to lower the volume of SWS needed, a reduced SWS to seed ratio was tested, but was not successful (Figure 5.7.4.a). The application of lower concentrations of ANS was similarly effective in the water sensitivity test (Table 5.7.1), and could be an alternative to reduce the amount of ANS needed. However, from an economic point of view, a wide adaptation of this priming treatments is not to be expected. Nevertheless, the application of this seed treatment might be feasible to valuable breeding material or small lots of gene bank collections. Lettuce seed, on the other hand, is a more valuable commodity, and the profit margin greater. For this crop the amount of extract needed was less, compared to barley. Additional costs for seed treatments may be therefore more justifiable economically. Temperatures in greenhouses are often unfavourable for proper seed germination, especially during the summer months. The application of ANS priming under these conditions to alleviate thermo-inhibition might be a potential use, which could be investigated further.

The addition of nutrients by SWS priming method, is a novel difference to other priming techniques, such as priming in PEG, which is an inert chemical, where its effectiveness relies solely on an osmotic effect. This difference could be of

importance in situations where nutrient or trace elements are in short supply, such as under commercial production of plantlets. The option of using the SWS as a carrier for the application of added trace elements could also be investigated in future projects.

The applicability of other commercial extracts as priming media is feasible but the effects may not be similar, due to differences in the physical, mineral or biochemical properties of those extracts, as outlined earlier. Concentrated commercial seaweed extracts may exhibit higher negative osmotic potentials, and thus could allow longer priming periods, compared to the SWS tested in this study. However, high salt concentration of those concentrates (Section 3.4) may have detrimental effects, as indicated in previous publications (Manohar, 1966; Brocklehurst and Dearman 1984). This aspect would need separate investigation.

In this study priming of seeds of only two plant species have been tested, and was effective when stress conditions prevailed. Whether an SWS priming would generally be of benefit to seeds of other species, however, would need further investigations. Further, other approaches to the problem of SWS seed priming, such as priming at low temperature, the addition of osmotica to SWS, or SWS to osmotic solutions, that would allow the priming period to be extended, have not been pursued, but might improve the beneficial effects. This would also require further research.

Brown seaweeds are known to produce a range of antibiotic substances (Table 1.4.1) that are able to control diverse organisms (Section 1.4). In this study, the extract based on *A. nodosum* exhibited not only a marked antibiotic effect on barley seed microbes (Figure 5.8.1), but also inhibited proliferation and reduced the number of external microbial contaminants (Figure 5.8.2). The source of this antibiotic effect was most likely a high concentration of polyphenolic substances in

ANS, as the polyphenol content of the SWS correlated with their observed antibiotic activity. Brown algae contain a range of polyphenols, various phlorotannins with varying antibiotic properties (Pesando, 1990), and these compounds in *A. nodosum* most likely serve as defensive agents against herbivores (Section 1.3.5) (Geiselman and McConnell, 1981; Tugwell and Branch, 1989). As the polyphenols were not extracted from ANS and tested for their specific antibiotic activity in this study, confirmation of their involvement in the antibiotic effect of ANS requires further work.

Regardless the mode of action, this is the first reported case of the application of a liquid seaweed product to reduce the population of seed microbes. The actual number of microbes on seeds after an ANS treatment, as well as the external contaminants were reduced. This indicates the potential of ANS as a biological seed treatment with antibiotic properties. Whether this property can be used to effectively control specific seed-borne pathogens would require further detailed investigation. The potential, however, is indicated.

Observations in this study indicated the complexity of the production and application of organic substances such as seaweed extracts or suspensions as plant fertiliser or seed priming media. Various factors were found to affect the activity of those products. These may have to be carefully controlled in the initial manufacturing process to obtain a product of consistent quality and to obtain reproducible treatment effects. If not carried out aseptically, microbes may occur in variable amounts, which may cause degradation processes, thus reducing the shelf life of the product. There was a big difference in the microbial content of the two seaweed suspensions studies here, but in this case the effect was beneficial as the microbial activity removed the inhibitory effect of one product. This is of importance with a view to the production and storage requirement of such organic

products. Immediate freeze-storage or the addition of preservatives would reduce microbial activity, but may also leave the inhibitory compound(s). A limited fermentation step might result in an improvement of the product.

The specific activity of a product depends on the algal source material used. In this study, *Ascophyllum nodosum* yielded a product with a strong antibiotic property, while *Laminaria hyperborea* had a fertiliser effect, mainly due to the high potassium content. The mineral composition of seaweed suspensions reflected the content in the source algae to a great extent. Thus products with a specific mineral profile may be produced by using selected batches of seaweed according to initial mineral analyses of the fresh algal material. Variability between batches of a product may reflect changes in the biochemical or mineral composition of seaweeds due to seasonal changes, or the location of harvest.

Another complication is that different production methods are known to be applied in the manufacturing of seaweed extracts, variously affecting the composition and physical properties of the final product. Further, the addition of preservatives, trace elements or growth regulating substances may enhance the effectiveness of algal products, but the reluctance of manufacturers to disclose these manipulations may lead to a misinterpretation of experimental results. This variability of the products, and with it, the different plant responses observed, make a comparison of different products difficult and the application of standard test methods for the effectiveness of seaweed products not possible. It may be more feasible to apply the knowledge on the algae composition and the effect of specific manufacturing processes available, to produce products for specific purposes, such as fertiliser, or as an antibiotic agent, and to test their activity in individually relevant experiments.

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