THE POTENTIAL OF HOT WATER TREATMENTS FOR CURTAILING SEED-ASSOCIATED MYCOFLORA

by

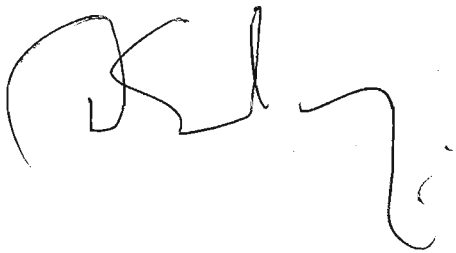## Déon Philip Erdey

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PREFACE

The experimental work described in this thesis was carried out in the Department of Biology, University of Natal, Durban, under the supervision of Professor P Berjak, with Dr DJ Mycock (Department of Botany, University of the Witwatersrand) acting as co-supervisor.

This study represents original work by the author and has not been submitted in any form to another University. Where use was made of the work of others, it has been duly acknowledged in the text.

Déon Philip Erdey

December 1995

Dedicated in loving memory to Shane Gregory Krüger,
my life and inspiration always.

ABSTRACT

The consequences of toxigenic fungi associated with stored seed have stimulated these investigations aimed at developing treatments to minimise this mycoflora, without significantly reducing seed quality or viability. The effects of immersion in water at 55, 57 and 60 ^{0}C for durations of 5 to 60 min were assessed for maize (*Zea mays* L.) seed in terms of fungal status, water uptake, electrolyte leakage, germination and seedling establishment. These assessments were conducted immediately after treatment, after re−dehydration for 2 days in an ambient air stream, and following a 1 month storage period under either cold (4 ^{0}C) or ambient (25 ^{0}C) conditions (33% and 91% RH, respectively). In all cases, the results are compared with those of control seeds and seeds pre−imbibed for 4 h at ambient temperature.

The level of internal contamination, represented almost entirely by *Fusarium moniliforme* Sheldon, declined significantly when assessed immediately after treatment, the efficacy of which increased with increasing temperature and duration of treatment. Seeds immersed in water at 55 ^{0}C for a duration of 15 min exhibited an 85% reduction in infection levels, when compared with those of the control, while those treated at 57 and 60 ^{0}C (same duration) were uninfected. Immersing seeds in hot water, however, resulted in a lag in germination rate and drop in germination totality, the degree of which was enhanced by increasing duration and temperature of treatment, suggesting the status of the manipulation to be an accelerated ageing treatment. The electrolyte leakage studies indicated that the reduced germination performance of these seeds was not due to plasmalemma disorganisation. These deleterious effects, however, were counter−balanced as seeds treated at 55, 57 and 60 ^{0}C for durations up to 60, 30 and 10 min, respectively, produced plants of superior quality than those of the control, which is ascribed to the reduction of systemically transmitted pathogens. The efficacy of the hot water treatment in reducing the levels of seed infection and improving seedling quality was enhanced by subsequent re−dehydration. The reduction in seed−associated mycoflora was maintained following storage for 1 month at both 4 ^{0}C (33% RH) and 25 ^{0}C (91% RH). However, both seed and seedling quality were adversely affected following storage even under cold, dry conditions, which may be a consequence of the pre−treatment history of the seeds,

which had been cold–stored for two years prior to the experiments. Applied as a pre–sowing treatment, therefore, hot water treatment shows promise for producing a crop of superior quality, less prone to fusarial pathogenesis. This treatment may be of particular importance to Third–World subsistence communities.

ACKNOWLEDGEMENTS

LIST OF ABBREVIATIONS

A.N.O.V.A.	analysis of variance
DM:	dry mass
GI:	germination index
mc(s):	moisture content(s)
MDG:	mean daily germination
PV:	peak value
%WMB:	percentage wet mass basis
RH:	relative humidity

TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

LIST OF FIGURES

LIST OF TABLES

Chapter 1.

INTRODUCTION

Maize (*Zea mays* L.) is one of the most important cereal crops grown worldwide, with over 443 million metric tonnes produced annually. In developed countries the bulk of the maize yield is used for livestock feed, whereas this crop forms a major part of the staple diet of the human population in many underdeveloped countries (Benson and Pearce, 1987). From the earliest stages of their formation until the time they germinate, seeds* are susceptible to pests. A seed serves as a concentrated reservoir of nutrients and many insects and microorganisms have mechanisms that allow their utilisation of this food source. Fungal infection, facilitated by insects, usually ultimately damages the embryo thereby reducing seed vigour and viability (Agarwal and Sinclair, 1987; Mycock and Berjak, 1995). This problem is intensified during storage with deterioration by fungal pathogens being particularly prevalent in poor, underdeveloped countries where hot, humid conditions prevail and the availability and maintenance of the correct storage conditions are not economically possible for the average farmer (Bailey, 1982). In addition, many of the fungi that infect seeds both prior to, or during, storage produce toxins that are harmful to both man and stock animals. Fungicidal treatments capable of penetrating the infected seed without affecting seed quality or rendering the seed unfit for consumption, must therefore be developed. Treatments that comply with these criteria, but are easy to apply at little or no cost, would be of tremendous benefit for, in particular, subsistence communities. One such potential treatment involves the immersion of seeds in heated water (hot water treatment), usually to minimise or eliminate seed–borne plant pathogens (Agarwal and Sinclair, 1987).

In this Chapter various aspects of maize seed (including its production, processing, and storage), its associated pathogens, and eradicative treatments are described.

1.1. Maize seed: production, processing and storage

Maize is a tall graminaceous annual, that requires warm temperatures and abundant sunlight

* Although maize caryopses are fruits *sensu stricto*, they are referred to as "seeds" in this dissertation according to common usage (Mycock and Berjak, 1995).

to grow successfully. This plant is monoecious and is generally cross–pollinated. The male flowers are borne on terminal inflorescences (the tassel) while the female or ear flowers, the ovules of which produce tube–like silks that emerge from the husks surrounding the ear tip, are located lower down on the plant. The ears develop to contain 300–1000 mature seeds arranged in rows along a rachis (cob) (Benson and Pearce, 1987).

The mature maize seed consists of an embryo, endosperm, aleurone and pericarp. The embryo is composed of an embryonic axis, differentiated into a plumule at one end and a radicle at the other, surrounded by a single cotyledon called the scutellum. The plumule and radicle are ensheathed by the coleoptile and coleorhiza, respectively. The bulk of the seed (usually comprising 80 – 85%, by weight) consists of the starch–filled endosperm. The aleurone is a protein enriched single layer of cells situated immediately beneath the pericarp. The pericarp, which is derived from the ovary wall and testa, covers the seed and serves to protect the underlying tissues. The entire caryopsis is attached to the cob via the pedicel, which contains vascular conductive tissues referred to as the hilar layer. During seed development, transport of water and nutrients through the pericarp into the seed is facilitated via the micropyle (Watson, 1987a; Zuber and Darrah, 1987).

During seed development, a number of environmental factors including amongst others temperature, rainfall, photoperiod, soil and atmospheric moisture, and soil mineral status can affect seed vigour and viability (Austin, 1972). In addition, physical damage to the developing seeds by birds and insects may adversely affect seed quality (Smith and Berjak, 1995), as well as facilitate invasion by pathogenetic fungi (Agarwal and Sinclair, 1987).

Physiological maturity, when seed vigour is at its greatest, is reached when the seeds have achieved maximum dry matter accumulation (Justice and Bass, 1978; Benson and Pearce, 1987, Bewley and Black, 1994) and is usually confirmed by the formation of the "black layer", which develops between the basal endosperm and the vascular area in the pedicel (Daynard and Duncan, 1969) or the kernel milk line, the boundary between the solid and liquid phases of the maturing endosperm (Afuakwa and Crookston, 1984). In dry climates the seeds will dehydrate naturally while still on the ear, whereas in areas of high rainfall or where

the growing season is short, the ears are usually harvested and dried artificially (Foster, 1982). Roberts (1973) has termed such seeds, tolerant to desiccation without loss of viability, as orthodox.

To prevent excessive loss in the field, modern commercial practice encourages seed harvesting shortly after physiological maturity. However, as the seeds are still wet, rapid drying of the seed is necessary to retain germinability and to prevent deterioration by fungi (McLean, 1980; Foster, 1982). The commercial processing of grain, which embodies numerous operations including harvesting, cleaning and separation, drying, conveying, and transport, has been well described (McLean, 1980; Herum, 1987; Justice and Bass, 1978; Williams; 1991a). In some countries, there may be over 30 steps in grain handling between the producer and the consumer (Williams, 1991a). However, any one stage in the handling of grain may damage the seeds by way of cracks or breakages (Herum, 1987; Justice and Bass, 1978; Williams, 1991a). Damage, especially to the embryo, can adversely affect seed germination, seedling development, plant growth and development, and subsequent grain yield (Hampton, 1992; Jahufer and Borovoi, 1992). In addition, cracks and breakages also render the seeds vulnerable to fungal invasion, which could lead to serious problems during storage (Christensen and Kaufmann, 1969, 1974; Agarwal and Sinclair, 1987; Friday *et al.*, 1989; Jahufer and Borovoi, 1992).

As the production of grains is seasonal, storage of these seeds is essential in order to provide planting material for the next growing season, as well as to meet the continuous demands of consumption (Bailey, 1982). Storage facilities take many forms, ranging from piles of unprotected grain on the ground, underground pits or containers, piles of bagged grain to storage bins including upright elevators and silos. The type of seed store used in any one area is dependent on cultural practices and the availability of technological and financial resources. Ideally, the type of seed store used must ensure the preservation of quality and the prevention of losses (Bailey, 1982; Justice and Bass, 1978; Williams, 1991b). Any seed placed into storage will age naturally, this process being manifested initially as a reduction in vigour and leads, ultimately, to the total loss of viability (Berjak and Villiers, 1970, 1972a,b,c,d; Roberts, 1972; Justice and Bass, 1978). The rate at which seeds age is dependent on the temperature

and relative humidity of the storage environment (Justice and Bass, 1978). These two factors are interdependent and, due to the hygroscopic nature of seeds, a change in either may elevate the moisture content of stored seed (Roberts, 1972). Harrington (1963) proposed that the storage life of seeds is halved by each 5 ^{0}C increase in temperature or by each 1% increase in seed moisture content. When both parameters come into play they are additive, thereby accelerating the rate of seed deterioration. The cellular events associated with seed deterioration include chromosomal abberations and damage to DNA, changes in the synthesis of RNA and proteins, changes in enzymes and food reserves, differences in respiratory activity and ATP production and alterations in membrane integrity (reviewed by Smith and Berjak, 1995). Membrane damage as a consequence of accelerated seed ageing has been demonstrated by electron microscopical (Berjak and Villiers, 1972a,b,d; Berjak et al., 1986) and electrolyte conductivity studies (Pandey, 1989; Bruggink et al., 1991), and is proposed to be as a result of free radical mediated peroxidation of the membrane lipids (Priestley and Leopold, 1983; Berjak et al., 1986, Priestly, 1986; Hendry, 1993; Smith and Berjak, 1995).

Besides controlling the rate of physiological deterioration, temperature and relative humidity, however, are also instrumental in determining the rate at which microorganisms will degrade the seed, thereby exacerbating the effects of seed ageing (Mycock and Berjak, 1995). Under conditions of cold, dry storage, those being described as optimal (Justice and Bass, 1978; Berjak et al., 1986), physiological deterioration is retarded and fungal activity reduced, thereby increasing the life–span of air–dry seeds.

In a good seed store, apart from regulating the effects of temperature and relative humidity, the action of insects, rodents and birds must be prevented. These animals can cause physical damage to the seed, exposing the nutrient–rich endosperm, and act as vectors for fungal propagules. In addition, the metabolic activity of infesting insects may increase both the relative humidity and temperature of the seed store, thereby encouraging fungal growth (Christensen and Kaufmann, 1969; Agarwal and Sinclair, 1987; Williams, 1991b).

1.2. Seed pathogens
Based on the seed moisture content requirements for growth, the fungi that infect seeds have

been divided into two ecological groups – the field and the storage fungi, (Christensen and Kaufmann, 1969, 1974; Roberts, 1972; Christensen and Sauer, 1982). Pelhate (1979, 1981) further suggested that some of the genera previously described as field fungi belong to an intermediate group based on the thermal gradient and moisture content requirements of the species concerned.

1.2.1. Field fungi

The field fungi, consisting of members of the genera *Alternaria*, *Cladosporium*, *Culvalaria*, *Epicoccum*, *Fusarium* and *Verticillium*, are recognised plant pathogens that reportedly invade seeds before harvest or after cutting and swathing, but before the harvest is threshed (Christensen and Kaufmann, 1969, 1974). These fungi require high relative humidities (RH), in excess of 95%, and seed moisture contents above 25% to grow (Christensen and Kaufmann, 1969, 1974; Roberts, 1972; Christensen and Sauer, 1982). Spores and mycelial fragments present in and on the soil, plant débris, seeds, or standing crops, are transported by wind, rain or insects to initiate infection in the developing host plants, particularly in immature seeds (Agarwal and Sinclair, 1987; Mills, 1989). In maize, access to the developing seeds in the husks by *Fusarium* spp. may be via the silks (Headrick and Pataky, 1989, 1991; Headrick *et al.*, 1990) or insect mediated damage (Zummo and Scott, 1990; Sayer, 1991). These fungi grow over, and infect, the surface of the seeds (Kommedahl and Windels, 1981) and penetrate into the seeds via the pedicel (Zummo and Scott, 1990) or cracks in the pericarp (Agarwal and Sinclair, 1987) ultimately gaining access to the embryonic tissues (Bacon *et al.*, 1992). In seasons when harvest is delayed due to excessive rain, fungal activity is enhanced and may cause discolouration of the grain and adversely affect germination and seedling vigour (Watson, 1987b, Clear *et al.*, 1989). Most of the quality loss in maize in the field by fungal pathogens is caused by *Fusarium* species (Watson, 1987b). In southern Africa *Fusarium* species, most notably *F. moniliforme* Sheldon, *F. subglutinans* (Wollen. and Reinking) Nelson, Tousson and Marasas, and *F. graminarium* Schwabe, together with *Diplodia maydis* (Berk.) Sacc. [= *Stenocarpella maydis* (Berk.) Sutton] and *D. macrospora* Earle [= *S. macrospora* (Earle) Sutton], are responsible for the ear–rot complex of maize (Marasas *et al.*, 1979; Rheeder *et al.*, 1990a; Rheeder *et al.*, 1993; Rheeder *et al.*, 1995). Some of the field fungi may continue to grow during storage if the seeds are not dried

sufficiently, but usually when seed moisture content becomes the limiting factor as a consequence of drying, the activity of the field fungi is reduced and they are no longer able to perpetuate (Christensen and Kaufmann, 1974). Once these fungi are no longer able to dominate the microenvironment within the stored seed, the storage fungi can become established (McLean and Berjak, 1987).

1.2.2. Storage fungi

The storage fungi, described as saprophytes and opportunistic invaders of dried seeds or dead organic matter (Hudson, 1986) or alternatively as pathogens (Mycock *et al*, 1990, 1992), are represented by xerotolerant members of the genera *Aspergillus* and *Penicillium*. Members of this group can be metabolically active in stored seed with moisture contents as low as 13% (Christensen and Kaufmann, 1969, 1974). According to Christensen and Kaufmann (1974), fungi of this group do not infect seed to any great extent prior to storage, invading only under conditions generally prevailing in the seed store, although Mycock *et al.* (1990, 1992) have observed systemic transmission of such fungi. Seeds may be damaged during processing into storage (see earlier). High levels of storage fungal propagules have been detected during processing and transport (Christensen and Kaufmann, 1969) and these may be introduced into the storage container as surface contaminants on whole seeds, broken kernels and débris (Christensen and Kaufmann, 1969, 1974; Berjak, 1987) or within seeds that were damaged during processing and not dried sufficiently (Siriacha *et al.*, 1989). Fungal spores may also be transported to, and dispersed by, insects during storage. Insects may further physically damage the seed as well as increase the moisture content, via metabolic water, to levels that will allow for the proliferation of the fungal propagules (Christensen and Kaufmann, 1969; Agarwal and Sinclair, 1987; Williams, 1991b). Christensen *et al.* (1990) have also reported that infected, relatively high–moisture seeds introduced into a store of dry seed, may also initiate infection during storage. Access of germinating fungal propagules to the interior of seeds may be gained via discontinuities in the pericarp such as cracks (Christensen and Kaufmann, 1974; Mycock *et al.*, 1988; Siriacha *et al.*, 1989) or the micropyle (Mycock *et al.*, 1988, Mycock and Berjak, 1992b). The deterioration of stored seed is characterised by a succession of species, starting with *A. restrictus* and *A. glaucus*, followed by *A. versicolor* and *A. ochraceus*, *A. candidus*, *A. flavus* and, finally, *Penicillium* species (Christensen and

Kaufmann, 1974). Each member of this succession characteristically invades and degrades specific seed tissues, depending on both the moisture content of the seeds (Christensen and Kaufmann, 1969, 1974; Christensen and Sauer, 1982) and the extracellular enzyme capabilities of each species (McLean *et al.*, 1985; Mycock and Berjak, 1992a,b). As a consequence, these fungi cause insidious decay first characterised by a loss of vigour, and then of germinability of the stored seed (Christensen and Kaufmann, 1969, 1974; Christensen and Sauer, 1982).

1.2.3. Seed–associated mycoflora

In recent years it has become evident that the categorisation of seed infecting fungi as either field or storage fungi is tenuous.

Many workers have reported the persistence of various field fungi, in particular the fusaria, during protracted cold storage (e.g. Russell *et al.*, 1982; Russell and Berjak, 1983; Agarwal and Sinclair, 1987; Marasas *et al.*, 1979; McLean and Berjak, 1987; Sayer, 1991; Berjak *et al.*, 1992). Thus, in addition to their rôle as plant pathogens these fungi may also be considered as facultative storage fungi (Mycock and Berjak, 1992a). As these fungi may be systemically transmitted from the germinating seed into the developing plant (Daniels, 1983; Agarwal and Sinclair, 1987), the survival of seed–borne field fungi during storage may have important implications in control measures against these plant pathogens.

Contrary to their previously proposed, wholly saprophytic nature (Christensen and Kaufmann, 1969; 1974; Hudson, 1986) there are several reports of storage fungi infecting pre–harvest seed. Notably, *A. flavus*, infection has been reported in developing cotton (Cotty, 1989; Huizar *et al.*, 1990), groundnuts (Mehan *et al.*, 1988, 1991), wheat (Hyder–Ali and Fakir, 1992) and maize (Zummo, 1991; Zummo and Scott, 1992). In maize, *A. flavus* is responsible for ear–rot in the southern states of the USA and, although field infection by this fungus causes little or no loss in yield (Zummo, 1991) high levels of aflatoxin may occur in freshly harvested seeds (Lillehoj *et al.*, 1980). Ears are infected from the late milk to dough growth stages, particularly if the plants are stressed by drought and high temperatures (Jones *et al.*, 1980; Payne *et al.*, 1988; Lacey and Magan, 1991). As in the case of fusarial infections, the

infection of maize ears by *A. flavus* can be via the silks (Marsh and Payne, 1984a,b; Payne *et al.*, 1988) which is dependent on the condition of the silks (Marsh and Payne, 1984b), virulence of the pathogen (Zummo, 1991), the presence of antagonistic mycoflora (Zummo and Scott, 1992) and insect damage (Lacey and Magan, 1991). Chatterjee *et al.* (1990) have proposed that deterioration of maize by *A. flavus* during storage can be reduced if pre–harvest infections are eliminated. In addition, during germination, seeds may also be susceptible to infection by *Aspergillus* spp. (Mycock *et al.*, 1990) which may be transmitted systemically through the growing plant to the next seed generation (Mycock *et al.* 1992).

As a consequence of this dichotomy, Mycock and Berjak (1992b) have suggested that the term seed–associated mycoflora is more appropriate than those of field and storage fungi.

1.2.4. Mycotoxins

In addition to affecting seed quality adversely, many of the seed–associated fungi are capable of producing secondary metabolites called mycotoxins. These toxins, particularly those produced by the fusaria and aspergilli, have been associated with a wide variety of human and animal–stock pathologies, including carcinomas. Exposure to mycotoxins can occur directly via contact and by consumption of contaminated seeds or their products. The nature, type, distribution, toxicology and ecology of the toxins produced by *Fusarium* (Joffe, 1986; Mills, 1989; Marasas, 1991; Shotwell, 1991; Norred, 1993) and *Aspergillus* (Abramson, 1991; Shotwell, 1991; Payne, 1992; Luchese and Harrigan, 1993; Cotty *et al.*, 1994) species has been extensively reviewed. Subsistence farming communities are particularly susceptible to mycotoxin exposure as the bulk of their daily nutritional intake consists of poorly stored, contaminated seed. In southern Africa, fumonisin has been associated with oesophageal cancer (Rheeder *et al.*, 1992) while indirect evidence suggests that aflatoxin may contribute to kwashiorkor (Ramjee *et al.*, 1992). In addition, recent studies have suggested that these toxins may also be phytotoxic (Sinha and Kumari, 1989; Van Asch *et al.* 1992; McLean *et al.*, 1992a,b; Lamprecht *et al.*, 1994) and may contribute to the physiological deterioration of seeds during storage (McLean *et al.*, 1992b).

1.3. Eradicative treatments

The control of seed–borne pathogens is attained through integrated disease management systems, usually involving both methods to prevent seed infection in the field and the eradication of pathogens already within the seeds (Agarwal and Sinclair, 1987). Preventative action in the field includes the selection of seed production areas where the pathogens of major concern are unable to establish or maintain themselves at critical levels during the periods of seed development, use of fertilisers to maintain a balanced soil fertility, water management practices and the use of foliar fungicide sprays, amongst others (Agarwal and Sinclair, 1987). While these practices are viable at the commercial level, the costs involved in disease management in the field would be impractical to the subsistence farmer. On the other hand, eradicative seed treatments are usually easy to apply, have little or no effect on the environment, may involve only a small monetary outlay, if at all, and may give virtual freedom from disease in crops (Maude, 1983; Agarwal and Sinclair, 1987). Such treatments are most useful where the seed is the main or only source of a disease (Maude, 1983). The types of eradicative seed treatments, summarised in Figure 1, include biological, mechanical, chemical or physical processes.

1.3.1. Biological control

Biological control involves the introduction of one or more non–pathogenic antagonists that inhibit or reduce the activity of seed–borne pathogens without adversely affecting grain quality (Agarwal and Sinclair, 1987; Tuite, 1988). While showing tremendous potential as an eradicative treatment, the acceptability of such treatments on a commercial basis, however, is complicated by marketing and quality requirements (Tuite, 1988).

1.3.2. Mechanical methods

Following harvest, seed lots are generally not uniformly sound, containing seeds that are discoloured, distorted, small or enlarged due to infections (Agarwal and Sinclair, 1987) and which may harbour inert inoculum, colonised plant débris, or pathogenic fungal propagules. The removal of all inert material, plant débris and fungal propagules by processing, screening and sieving, and separating out visibly infected seed may help to reduce seed–borne inoculum, but does not eliminate the problem (Agarwal and Sinclair, 1987, Nakagawa and

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│          Eradicative seed treatments          │
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┌──────────────┐   ┌──────────────┐   ┌──────────────┐   ┌──────────────┐
│ Biological │   │ Mechanical │   │   Chemical   │   │   Physical   │
│   control   │   │   │   │   │   │   │
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                                             │   │
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                                      ┌──────────────┐   ┌──────────────┐
                                      │ Protectant │   │   Gamma   │
                                      │ fungicides │   │ irradiation │
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                                      ┌──────────────┐   ┌──────────────┐
                                      │   Systemic   │   │   Microwave │
                                      │ fungicides │   │ irradiation │
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                                      │   Natural   │   │   Hot air   │
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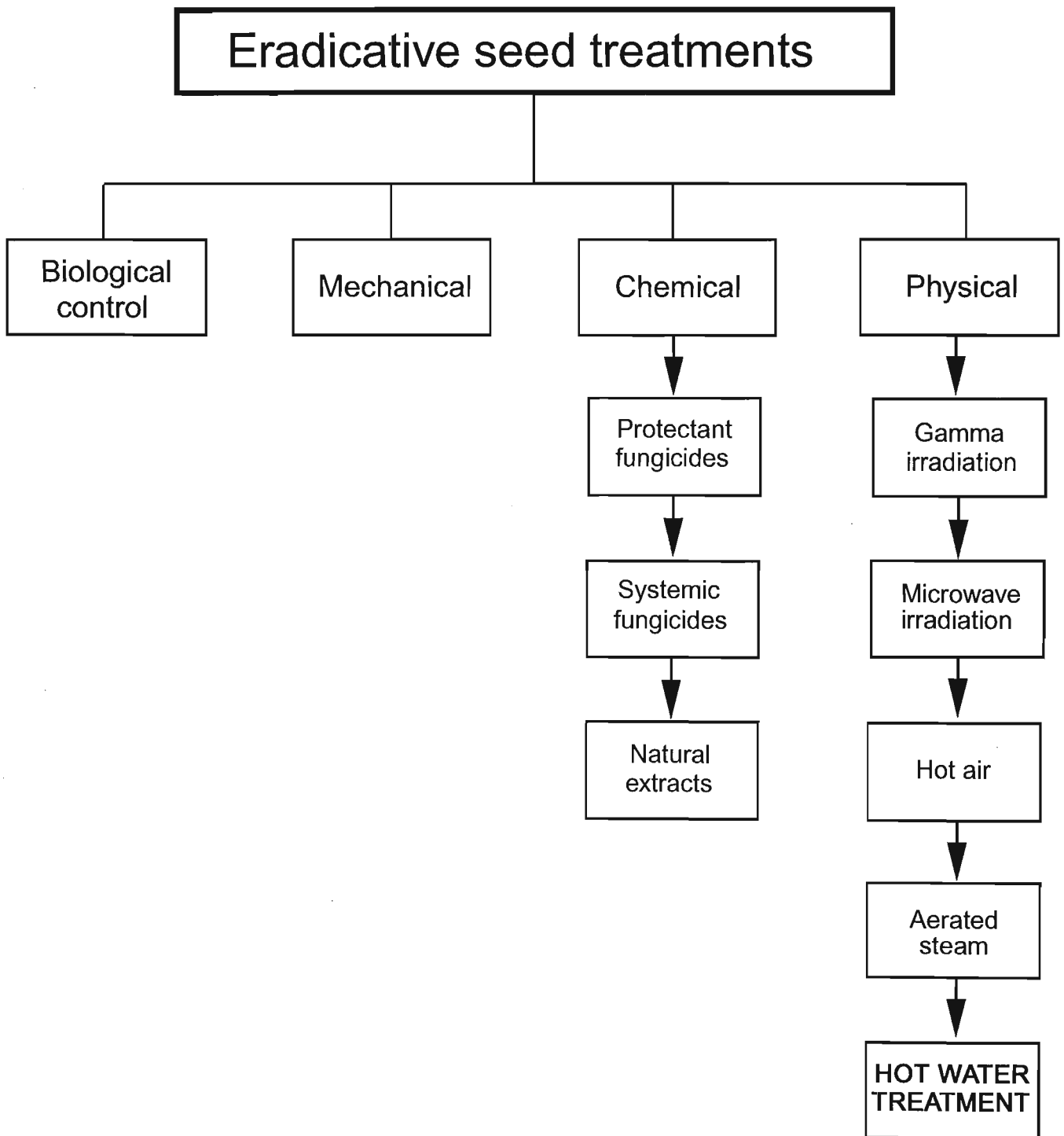
Figure 1. Summary of some treatments used for the eradication of seed-associated fungi.

Yamaguchi, 1989).

1.3.3. Chemical treatments

Treating seeds with synthetic chemicals is reported to be the most effective method for controlling seed–associated mycoflora (Agarwal and Sinclair, 1987). These chemicals include broad–spectrum protectant fungicides, such as captan and thiram, and systemic fungicides, such as benomyl, carbendazim, imazalil and thiabendazole. Reports on the efficacy of protectant fungicides as treatments against seed–borne pathogens are variable; being reported as ineffectual in some cases (Halfon–Meiri and Solel, 1989), and only partially effective in others (Ellis et al., 1975; Vidhyasekaran, 1983; Haware and Kannaiyan, 1992). The activity of protectant fungicides appears to be limited to seed surfaces and the tissues of the seed coat (Ellis et al., 1975, Agarwal and Sinclair, 1987) which may account for the inability of these chemicals to completely eradicate seed–borne pathogens. While the efficacy of these fungicides may be enhanced by prolonged immersion of seeds in aqueous solutions of these chemicals, this practice is undesirable as the hydrated seeds have to be re–dried (Maude, 1983). This problem may be overcome by dissolving the fungicides in volatile organic solvents (Maude, 1983), but the solvents must not be phytotoxic (Hung et al., 1992). Systemic fungicides, on the other hand, have been reported to be effective in reducing or eliminating deep–seated seed–borne pathogens, even when applied to the seed surface as dusts, slurries or sprays (Ellis et al., 1975; Vidhyasekaran, 1983; Halfon–Meiri and Solel, 1989; Haware and Kannaiyan, 1992; Moreno–Martinez et al., 1994). However, these fungicides can be phytotoxic (Ellis et al., 1975; Halfon–Meiri and Solel, 1989; Kelly, 1993) and their use is limited by their selectivity for specific fungi (Maude, 1983, Agarwal and Sinclair, 1987). Recent studies have shown that combinations of both protectant and systemic fungicides are the most effective means of eliminating seed–borne pathogens (Halfon–Meiri and Solel, 1989; Nakagawa and Yamaguchi, 1989; Haware and Kannaiyan, 1992; Wilson et al., 1993) and improving crop yields due to the control of both seed–borne and soil–borne pathogens (Wilson et al., 1993). Long term application of fungicides, however, may lead to the development of fungal strains resistant to specific chemicals (Agarwal and Sinclair, 1987; Sundas and Raj, 1989; Hamamura et al., 1989; Wada et al., 1990). This problem can be overcome by substituting the chemicals against which resistance has developed by other

fungicides of known activity against the resistant fungal strains (Agarwal and Sinclair, 1987; Leadbeater *et al.*, 1990; Wada *et al.*, 1990). However, while the use of chemical treatments is acceptable for seed destined for planting, these fungicides may be toxic (Kelly, 1993) and of little use, therefore, as treatments for stored seed destined for consumption.

In recent years, the tendency to minimise or even eliminate chemicals from agricultural practices appears to be developing worldwide (Paster *et al.*, 1992). Studies using natural extracts and/or their derivatives (Shetty *et al.*, 1989; Chatterjee, 1990; Dube *et al.*, 1990; Malhotra and Rai, 1990; Paster *et al.*, 1990; Hall and Harman, 1991; Garg and Siddiqui, 1992; Weidenborner *et al.*, 1992) have shown these to be effective alternatives to the application of conventional fungicides. These fungicidal extracts may be of particular importance to subsistence farmers in underdeveloped countries due to the availability of the source material within their environments.

1.3.4. Physical treatments

Some of the oldest methods used for the control of seed–borne pathogens are physical treatments, in particular thermotherapy (Agarwal and Sinclair, 1987). These treatments include radiation, and the use of microwaves, hot air, aerated steam or hot water treatments.

The effects of gamma irradiation in reducing seed–associated mycoflora both *in vitro* and *in vivo* have been previously reported (Russell and Berjak, 1983; Borsa *et al.*, 1992; Menasherov *et al.*, 1992; Paster *et al.*, 1992). However, the doses necessary to control seed–associated fungi effectively are usually much greater than doses permitted for grain for human consumption (Paster *et al*, 1992) and may also result in inhibition of seed germination (Russell and Berjak, 1983). Recent work has shown that the doses required for fungal eradication can be reduced if applied in combination with other physical and chemical treatments (Menasherov *et al.*, 1992; Paster *et al.*, 1992).

The use of microwave technology to eliminate seed–associated fungi is relatively new (James *et al.*, 1988; Conkerton *et al.*, 1991; More *et al.*, 1992a). The advantage of this process is that the microwave energy facilitates both rapid and apparently uniform heating of the seeds

(More *et al.*, 1992a). Such treatments have been shown to reduce *Fusarium* (James *et al.*, 1988), *Aspergillus* and *Penicillium* (More *et al.*, 1992a) infection levels. However, James *et al.* (1988) maintain that a "fine line" exists between effectively reducing seed fungi and seed death, which is exacerbated by microwaves at high seed moisture contents (More *et al.*, 1992a).

The main advantages of dry heat treatments are that they are easy to apply and the seeds are less damaged (Agarwal and Sinclair, 1987). Russell and Berjak (1983) have reported the elimination of *Fusarium moniliforme* from maize seed exposed to air heated to $70\,^{0}$C without adversely affecting germination. However, the efficacy of hot air as an eradicative treatment appears to be dependent on the pathogen type (More *et al.*, 1992b) and the seed moisture content. Such treatments applied to seed at low moisture contents apparently have little eradicant effect on the associated mycoflora (Maude, 1983; Agarwal and Sinclair, 1987). On the other hand, Fourest *et al.* (1990) found dry heat to be an effective method for eliminating bacterial infection in barley seed.

Aerated steam treatments are said to be safer than hot water treatments, and more effective than hot air, with shorter required durations of subsequent drying and low loss of germination (Agarwal and Sinclair, 1987). Aerated steam is more effective if the moisture content of the seed is raised to facilitate heat dispersal, but the efficacy of this treatment is reduced with increasing seed size (Maude, 1983). In addition, this treatment may cause discoloration of certain seeds, thereby reducing their market value (Senter *et al.*, 1984).

Hot water treatments have been widely used for the control of seed–borne pathogens (Agarwal and Sinclair, 1987). Although Maude (1983) has proposed that this treatment is not viable on a commercial scale, as only small quantities of seed can be treated at any one time, Berjak and co–workers (1992) consider the hot water treatment to have definite potential at the subsistence level. This proposal was based on the facts that the costs are negligible, the technique easy to apply, and no extraneous substances are introduced that may render the seed unfit for consumption. In addition, hot water treatments may circumvent the development of resistant fungal strains (Hayden and Maude, 1994). Such treatments have been shown to

reduce the systemic transmission of seed-borne pathogens (Daniels, 1983; Hayden and Maude, 1992, Aveling *et al.*, 1993), may facilitate improved seedling emergence (Daniels, 1983; Berjak *et al.*, 1992), and enhance the storability of seeds under both cold (Berjak *et al.*, 1992) and ambient (Strandberg and White, 1989) conditions.

The efficacy of hot water treatments for reducing or eradicating seed-associated pathogens has been reported for a variety of seeds, including those of carrot (Strandberg and White, 1989), onion (Aveling *et al.*, 1993; Hayden and Maude, 1992, 1994), anemone (Doornik *et al.*, 1992) and maize (Daniels, 1983; Berjak *et al.*, 1992). The precise temperature and duration of treatment at which seed-borne pathogens are eradicated, however, varies among different seed species.

Seeds are usually pre-soaked in water for 4 to 12 hours at ambient temperature in order to facilitate improved conduction of heat during treatment and to initiate the germination of seed-borne fungal propagules, thereby making them more susceptible to eradication (Agarwal and Sinclair, 1987). During subsequent immersion in hot water, the seeds may imbibe further water (Berjak *et al.*, 1992), the extent of which is dependent on both the temperature and duration of imbibition (Oguntunde and Adebawo, 1989). The amount and rate of water uptake by seeds is species dependent (Oguntunde and Adebawo, 1989; Bewley and Black, 1994) and is influenced by seed size, seed coat permeability and the chemical composition of the seed tissues (Bewley and Black, 1994). Smaller seeds with permeable coats, for example, may imbibe water rapidly and are, therefore, predisposed to respond to hot water treatments aimed at successful elimination of seed-borne pathogens. Seed morphology and chemical composition also affect the pattern of water uptake (Bewley and Black, 1994; McDonald *et al.*, 1994). In maize seed, water is taken up preferentially by the embryonic tissues (McDonald *et al.*, 1994), thus it is probable that the treatment would be primarily directed against pathogens located within these tissues.

The duration and temperature of hot water treatment at which seed-associated pathogens are eliminated is also influenced by the location of the pathogens within the seed (Berjak *et al.*, 1992) and interspecific fungal competition (Berjak *et al.*, 1992; Hayden and Maude, 1994).

Treatment at 55 °C for 30 min of maize seed reduced the incidence of peripherally located *Fusarium* spp., but there was a concomitant increase in the incidence of *Aspergillus glaucus* contamination. Berjak and co–workers (1992) ascribed this phenomenon to the elimination of suppression of *A. glaucus*, which is also associated within the seed periphery, by *Fusarium* spp. Longer durations of treatment were required to reduce the incidence of deep–seated *Aspergillus* spp. infections (Berjak *et al.*, 1992).

More recently, however, Hayden and Maude (1994) have suggested that such effects may be largely dependent on the temperature at which the isolation studies took place. Techniques assessing the incidence and range of seed–borne fungi usually involve halving the seeds under sterile conditions and aseptically plating them out onto nutrient medium, usually selected for its specificity towards particular fungal species (Agarwal and Sinclair, 1987). Hayden and Maude (1994) reported the absence of *A. fumigatus* and the predominance of *A. niger* in onion seeds that were hot water treated at 50 °C for durations up to 60 min. In that study, the plates were incubated at 30 °C, well below the temperature optimal for the growth of *A. fumigatus*. However, when seeds were treated at 60 °C for more than 5 min, the incidence of *A. niger* decreased dramatically, while that of *A. fumigatus* showed a sustained increase due to the absence of the faster growing *A. niger*.

A reduction in seed–borne inoculum without affecting seed vigour or viability, however, is not always attained (Agarwal and Sinclair, 1987). The tolerance of seeds to hot water immersion is species dependent. While treatment at 55 °C for 30 min was effective in eliminating almost completely the incidence of *Alternaria dauci* from carrot seed, only a small proportion of the seeds germinated (Strandberg and White, 1989). On the other hand, the effect of treatment under similar conditions on the germination of maize seed was not as severe and, despite a slight decline in germination rate and drop in germination totality, these seeds produced seedlings of a greater dry mass than those that developed from untreated (control) seeds (Berjak *et al.*, 1992). Seeds with high moisture contents may be damaged at lower temperatures than those of a low moisture content (Agarwal and Sinclair, 1987). Thus smaller seeds (e.g. carrot seeds), which have a higher surface to volume ratio, may take up more water during treatment than larger seeds and, as a consequence be more susceptible to

damage. Physical damage to the seed coat, as is the case of legumes, e.g. soybean seed, may also render certain seed intolerant to hot water treatment (Agarwal and Sinclair, 1987). Other factors that may influence the effects of hot water treatment on seed germinability include seed age and vigour (Agarwal and Sinclair, 1987; Berjak *et al.*, 1992; Doornik, 1992).

1.4. Aims and Objectives of the Present Study

Since it has been proposed that hot water treatments may be a useful technique for the eradication of seed–associated mycoflora in subsistence communities (see above), it was decided to investigate the efficacy of this treatment for maize, which constitutes the staple diet for many people in southern Africa. The experimental approach used in this study is summarised in Figure 2, and took the following into consideration:

The effects of hot water treatment on both seed germination and internal fungal status is dependent on both the temperature and duration of treatment (Agarwal and Sinclair, 1987). For maize seed, Berjak *et al.* (1992) proposed that treatment in water heated to 55 ^{0}C for durations of up to 30 min could be effective in reducing the level of seed–associated fungi without adversely affecting seedling establishment, while Daniels (1983) has shown the same to be true for maize seeds treated at 60 ^{0}C for 5 min. One of the aims of this investigation, therefore, was to determine the effects of treatment at both these temperatures, and including a mid–range temperature (57 ^{0}C), on both seed internal fungal status and seed germination and seedling establishment.

As suggested by Berjak *et al.* (1992) seeds were pre–imbibed for 4 h prior to the hot water treatment. However, studies employing this technique have reported only the effects of hot water treatment following re–dehydration of the treated seeds. In addition, those studies have only used unimbibed (control) seeds for comparison (e.g. Daniels, 1983; Berjak *et al.*, 1992). Seed imbibition, followed by re–dehydration, is a well known technique for priming seeds (Coolbear, 1992). In addition, hot water treatments have been used to age seeds artificially (Bhattacharyya *et al.*, 1985; Furbeck *et al.*, 1989) and the efficacy of priming treatments has been reported to be influenced by the physiological status of the seed (Pandey, 1989; Basu, 1994). In this study, therefore, the effects of hot water treatment on seed germination and

Hot water treatment *

*Pre-imbibed for 4 h, then immersed in hot water at 55, 57 and 60 °C for durations of 5, 10, 15, 30, 45 and 60 min. Results are compared with unimbibed (control) seeds and those seeds pre-imbibed for 4 h.

| Moisture content (Embryo & Endosperm) | % Infection (Internal, 10 d) | Conductivity | Germination (Gl & Root & Shoot, 5 d) | Seedling Growth (Dry Mass, 18 d) |

Assessed immediately

Assessed after re-dehydration
(2 days)

Assessed after storage for 1 month at either:
4 °C (33% RH) or 25 °C (91% RH)
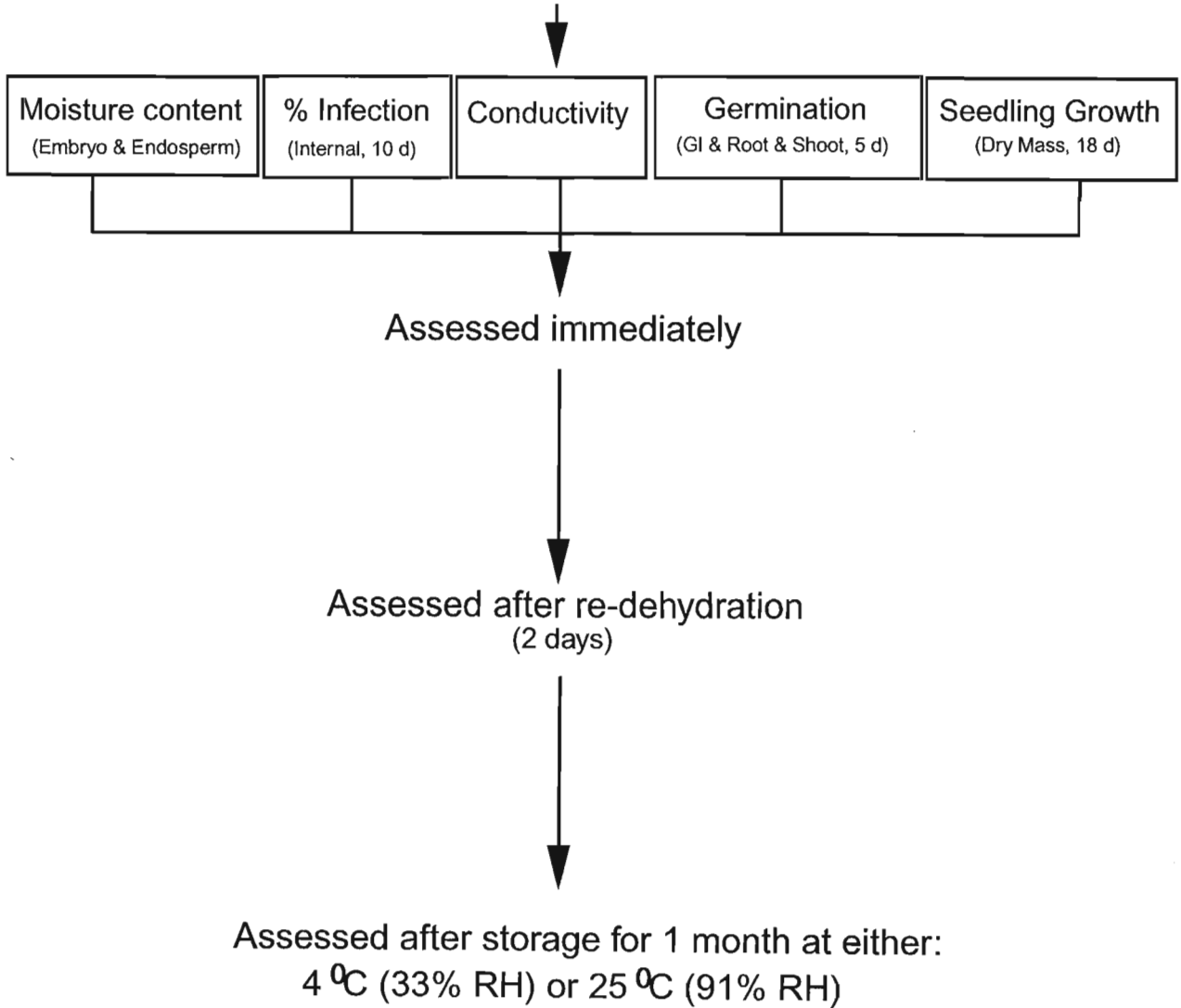
Figure 2. Schematic diagram of the experimental approach used in this study.

fungal status were determined both immediately after treatment and following re–dehydration. These results are compared with those of unimbibed (control) seeds and seeds pre–imbibed for 4 h at ambient temperature. As the response of seeds to either priming or ageing, or both, has been suggested to be influenced at the level of the cell membrane, demonstrated by the amount of electrolytes leached (Pandey, 1989; Nath *et al.*, 1990; Bruggink *et al.*, 1991; Nath *et al.*, 1991; Davidson *et al.*, 1994), electrolytic conductivity tests were also performed.

Berjak *et al.* (1992) reported that a possible 'cold shock' stress, as a consequence of storing hot–water treated maize seed after re–dehydration under cold, dry conditions, may enhance the efficacy of the treatment for reducing the incidence of seed–borne pathogens. In addition, the germination of carrot seed treated at 50 ^{0}C for 20 min was enhanced following storage at ambient temperature and relative humidity (Strandberg and White, 1989), conditions conducive to fungal proliferation (Christensen and Kaufmann, 1969, 1974). Thus the effects of storage for one month at either 4 ^{0}C (33% RH) or 25 ^{0}C (91% RH) of heat treated, pre–imbibed and unimbibed seeds on the level of fungal infection, seed germination and electrolytic leakage were also determined.

Chapter 2.

MATERIALS AND METHODS

2.1. Seeds

Caryopses (seeds) of a yellow maize variety (*Zea mays* var. PAN 6480) were obtained from the Pannar Seed Company, Greytown, KwaZulu–Natal, South Africa and maintained in hermetic storage at 4 ^{0}C for 2 years until used.

2.2. Hot water treatment

As previously described by Berjak *et al.* (1992), seeds were soaked in sterile, distilled water for 4 hours at ambient temperature, after which they were transferred to boiling tubes (12 seeds per tube) containing 20 ml of pre–heated (either at 55, 57 or 60 ^{0}C) sterile distilled water. The tubes were maintained in a water bath at the appropriate temperature and sampled after 5, 10, 15, 30, 45 and 60 min. Thereafter, the seeds were removed from the tubes and seed temperature was gradually reduced to ambient by continuous washing with sterile distilled water at room temperature.

As summarised in Fig. 2, the effect of the treatment on seed quality in terms of internal fungal status, moisture content, electrolytic conductivity, germination and seedling establishment was assessed either immediately, or after re–dehydration in an ambient air stream for 2 days and after subsequent storage for 1 month under either cold or ambient conditions. These parameters were also assessed for control seeds and seeds that were imbibed for 4 h. Results of control seeds represent the mean of 3 replicates, so as to determine variability of the seed lot at any sampling.

2.3. Internal infection

For each sampling, 100 seeds (control, n = 300) were surface sterilised in a 2% solution of sodium hypochlorite and 1% sodium dodecyl sulfate (Mycock *et al.*, 1988) for 20 min, then rinsed three times with sterile distilled water. The seeds were then halved longitudinally through the embryo, aseptically plated onto PDA containing 6% NaCl and incubated at 25 ^{0}C for 10 days (Mycock *et al.*, 1988). This medium is routinely used for the isolation of storage

fungi (Berjak, 1984). Fungi were identified using known cultures previously isolated from local maize, and identified by the Mycology Research Unit, Plant Protection Research Institute, Pretoria, South Africa.

2.4. Moisture content (mc)

The moisture contents (mcs) of both the embryo (scutellum and embryonic axis) and endosperm (pericarp, aleurone layer and endosperm) were determined for 25 individual seeds (control, 75 seeds) per sample. The material was dried in an oven at 80 ^{0}C for 2 days and the percentage mc, expressed on a wet mass basis (%WMB).

2.5. Conductivity

Electrolyte leakage (conductivity) of 25 individual seeds (control, n = 75) per treatment was measured using a multiprobe conductivity meter (CM 100 V1F, Reid and Associates C.C.) at the setting of 1V. Each cell contained 3 ml distilled water. In the case of seeds assessed immediately after immersion in hot water, the seeds were first rinsed rapidly with distilled water, then blotted dry, prior to conductivity measurements. This was done in order to remove any electrolytes from the surface of the seeds. Measurements were taken at intervals of 15 min over a 10 h period, following which the seeds were removed and dried in an oven at 80 ^{0}C for 2 days. The mean total leakage per sample after the 10 h measurement was expressed on a dry mass basis ($\mu A.g^{-1}DM$). In addition, regression analyses were performed to determine the leakage rate, also expressed on a dry mass basis ($\mu A.g^{-1}DM.s^{-1}$).

The amount of leakage that occurred during immersion in the water at the various temperatures and exposure times was also determined. Following an initial 4 h imbibition period, seeds were rapidly washed with distilled water and blotted dry. 10 seeds per treatment were each placed in a test tube containing 5 ml of distilled water pre-heated to the requisite temperature. Following treatment in a water bath at the appropriate temperature and duration, the seeds were removed from the tubes, and the dry mass individually determined. The tubes were allowed to cool to room temperature and the conductivity of the soak water was measured. These treatments are compared with those of similar seeds which had been imbibed for 5 to 60 min at room temperature. In all cases, the results are expressed on a dry mass basis ($\mu A.g^{-1}DM$).

2.6. Germination

Following surface sterilisation (Mycock *et al.*, 1988) for 20 min, 100 seeds (control, n = 300) were set to germinate aseptically at 25 ^{0}C in Petri–dishes containing filter paper dampened with 12 ml sterile distilled water. Germination was assessed over a period of 5 days and the germination index (Czabator, 1962) was calculated as follows:

$$GI = MDG \times PV$$

GI = germination index
MDG = mean daily germination (final percent germination divided by the length of the test period)
PV = peak value (percentage germination on a particular day divided by the number of days taken to achieve that percentage. The highest value achieved over the 5 day experimental period is used as the indication of seed vigour)

Root and shoot lengths were also determined on day 5.

2.7. Seedling establishment

Following the 5 day germination assessment (see 2.6), the seeds were planted in vermiculite and grown under greenhouse conditions. After a total of 18 days from the start of imbibition, the number of seedlings per treatment was scored and the mean dry mass of the shoot and leaves of those seedlings that had germinated was determined. The results of the control material represents the mean of 3 replicates.

2.8. Storage

Following re–dehydration, seeds from each treatment (including the control material) were dusted with Benlate (benzimidazole 500g/kg, Dupont) and separated into 2 sub–samples. The sub–samples were placed in a sterile vial over a saturated solution of either $MgCl_2$ or KNO_3 (equivalent to 33% and 91% RH) (Vertucci and Roos, 1993) and stored for 1 month at 4 ^{0}C and 25 ^{0}C, respectively. After storage, seeds were first rinsed with sterile distilled water prior to assessments of seed fungal status, conductivity and germination.

2.9. Statistical analyses

Any statistical tests carried out on the results were performed using the A.N.O.V.A. multivariate function of Statgraphics at the 0.05 confidence level.

Chapter 3.

RESULTS

3.1. The effects of hot water treatment on the internal fungal status and quality of *Zea mays* seeds, assessed immediately after treatment.

3.1.1. Seed internal fungal status

At the start of this study the seeds exhibited relatively low levels of infection, with only 20% of the unimbibed (control) seeds being infected (Fig. 3). Soaking seeds for 4 h at ambient temperature did not alter the level of infection, which remained the same as that of the control seeds (Fig. 3).

However, subsequent immersion of the seeds in water at 55, 57 and 60 ^{0}C for durations of 5 to 60 min each, decreased the level of internal infection considerably, the efficacy of which increased with increasing temperature and duration of treatment. The level of internal infection of seeds treated at 55 ^{0}C was reduced by 50% after only 5 min, and by 85% after 15 min treatment duration, when compared with the control material (Fig. 3). On the other hand, seeds treated at 57 and 60 ^{0}C, showed no infection after a treatment duration of only 15 min.

Although the seeds used in this investigation had been hermetically stored for 2 years at 4 ^{0}C prior to use, the spectrum of fungi isolated from untreated seeds consisted entirely of the field fungi; *viz. Fusarium* spp., *Diplodia* spp. and *Cladosporium* sp. (Fig. 3). Of these, *Fusarium moniliforme* Sheldon predominated, constituting 89% of the total infection levels in the control seeds (Fig. 3). In fact, *F. moniliforme* remained the dominant fungus isolated from any of the treatments that yielded infection. Consequently, the effect of immersion in hot water on total seed infection levels reflected almost entirely the response of *F. moniliforme* only.

Of the field fungi isolated, *Cladosporium* sp. appeared the most sensitive to the hot water treatment. While constituting only 1.5 and 5% of the total level of infection for both control and 4 h imbibed seeds, respectively, immersion of the infected seeds in hot water completely
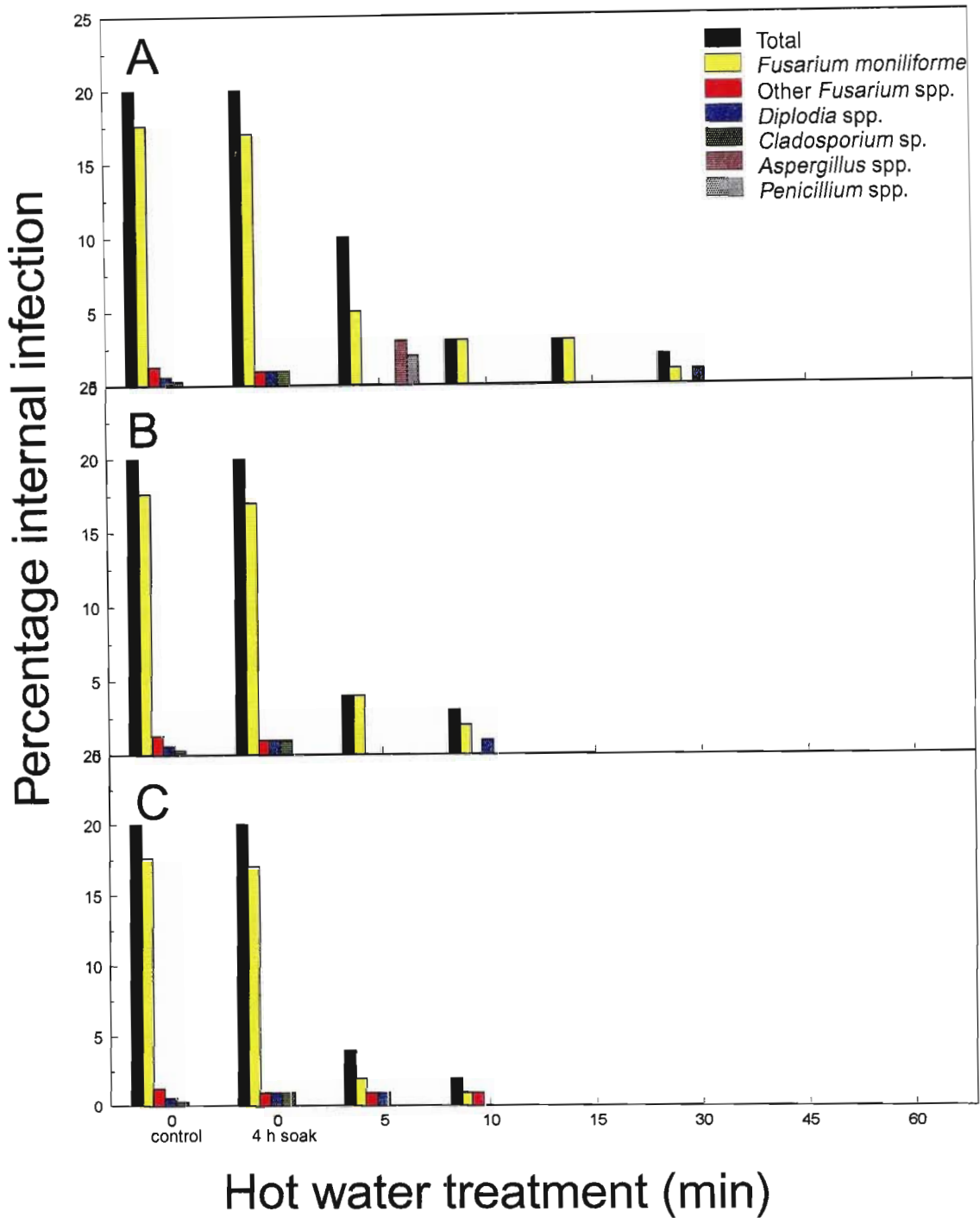
Figure 3. Percentage internal infection of *Zea mays* seeds following immersion in hot water at 55 (A), 57 (B) and 60° C (C) for durations of 5 to 60 min, compared with those of unimbibed (control) seeds and seeds pre-imbibed for 4 h at room temperature. n = 100 (control = 300).

eradicated this fungus (Fig. 3).

In contrast, although never occurring at levels greater than 1%, *Diplodia* spp. were more resistant to the treatment, being able to survive the longest duration of treatment that yielded infection when the seeds were treated at 55 and 57 ^{0}C (Fig. 3).

Members of the storage fungus group, as represented by *Aspergillus* spp. (most notably *A. flavus*, not shown separately) and *Penicillium* spp., were also isolated but never at levels greater than 3% (Fig. 3). In both cases, these fungi were isolated only from seeds immersed in water at a temperature of 55 ^{0}C (Fig. 3).

3.1.2. Moisture Content

Seed moisture content (mc), expressed on a percentage wet mass basis (%WMB), was determined separately for seed embryos and endosperm.

The embryo and endosperm mcs of unimbibed seeds, which had been previously stored for 2 years at 4 ^{0}C, were 9.22 and 11.88%, respectively (Table 1). Imbibing seeds for 4 h at room temperature resulted in a significant increase in the water content of both these tissues, with more water being taken up by the embryo (3–fold) than the endosperm (2–fold), when compared with unimbibed seeds (Table 1). The higher levels of water uptake by the tissues of the embryo was also apparent after further immersion in hot water at 55, 57 and 60 ^{0}C, at all treatment durations tested. Both the embryo and endosperm showed a significant increase in water content at all temperatures after only 5 min, and again after 30 min treatment, when compared with seeds imbibed for 4 h (Table 1). The increase in water uptake by both tissues during immersion in hot water appeared to be dependent on the duration, rather than the temperature, of treatment.

3.1.3. Conductivity

Both the level of electrolytes and the rate at which they were leaked from unimbibed (control) seeds was significantly higher than that of seeds that had been imbibed for 4 h (Table 2).

Table 1. Effect of imbibition for 4 h at room temperature, followed by hot water treatment at 55, 57 and 60 ^{0}C on water uptake by *Zea mays* seeds, compared with those of unimbibed (control) seeds.

	Duration of hot water treatment (min)	Moisture content (%WMB)[a]	
		Embryo	Endosperm
Unimbibed	0	9.22 a	11.88 a
Imbibed 4 h	0	28.98 b	22.60 b
Imbibed 4 h; hot water treated (55 ^{0}C)	5	31.48 c	23.96 c
	10	32.40 c	24.09 c
	15	32.79 c	24.07 c
	30	35.36 d	25.93 de
	45	36.90 de	26.49 def
	60	39.95 ef	27.98 gh
Imbibed 4 h; hot water treated (57 ^{0}C)	5	31.59 c	23.59 c
	10	32.04 c	24.36 c
	15	32.62 c	24.11 c
	30	36.50 de	25.91 de
	45	37.15 de	26.64 ef
	60	40.20 f	27.02 f
Imbibed 4 h; hot water treated (60 ^{0}C)	5	32.54 c	23.59 c
	10	32.24 c	24.47 c
	15	32.29 c	24.16 c
	30	36.02 d	25.59 d
	45	38.18 ef	27.05 fg
	60	41.31 f	28.52 h

[a] Represents the mean of 25 seeds (except control, n = 75). Values with different letters within columns are significantly different (P< 0.05).

Table 2. Effect of imbibition for 4 h at room temperature, followed by hot water treatment at 55, 57 and 60 ^{0}C on electrolyte leakage[a] from *Zea mays* seeds, compared with those of unimbibed (control) seeds.

	Duration of hot water treatment (min)	Total leakage ($\mu A. g^{-1}$ DM)	Leakage rate ($\times 10^{-2} \mu A.g^{-1} DM.s^{-1}$)
Unimbibed	0	7.10 a	100.4 a
Imbibed 4 h	0	5.92 bcd	65.5 b
Imbibed 4 h; hot water treated (55 ^{0}C)	5	6.00 bcd	41.0 bcde
	10	4.50 ghi	31.3 cdef
	15	5.52 bcdefg	30.9 cdef
	30	5.51 bcdefg	31.6 cdef
	45	6.08 bcd	43.5 bcd
	60	5.81 bcdef	56.5 bc
Imbibed 4 h; hot water treated (57 ^{0}C)	5	5.68 bcdefg	11.8 ef
	10	4.45 ghi	9.3 f
	15	4.64 fghi	9.0 f
	30	5.09 defgh	9.3 f
	45	5.40 cdefgh	12.2 ef
	60	6.61 abc	15.1 def
Imbibed 4 h; hot water treated (60 ^{0}C)	5	5.97 bcde	9.0 f
	10	4.80 efghi	5.4 f
	15	4.18 hi	8.2 f
	30	3.78 i	6.8 f
	45	5.52 bcdefg	8.2 f
	60	6.72 ab	9.3 f

[a] Represents the mean of 25 seeds (except control, n = 75) measured over a 10 h period, expressed on a dry mass (DM) basis. Values with different letters within columns are significantly different ($P < 0.05$).

The amount of electrolytes leaked from seeds that had been immersed in water from 5 to 60 min at 55, 57 and 60 °C, respectively, was generally similar to that of seeds imbibed for 4 h only, except for seeds treated for 60 min at 57 and 60 °C, which were characterised by leakage levels similar to that of the control (Table 2). In contrast, while the rate at which electrolytes were leaked from seeds treated at 55 °C for durations of 5, 45 and 60 min were similar to that of seeds imbibed for 4 h only, seeds treated for 10, 15 and 30 min (same temperature) and at 57 and 60 °C (all durations) were characterised by leakage rates significantly lower than that of seeds imbibed for 4 h (Table 2).

When assessing the effects of temperature and duration of immersion on the amount of electrolytes leached into the soak water during immersion, it appeared that the duration of immersion alone was not an important factor in influencing the amount of leakage, as the level of electrolytes leached from seeds imbibed for 5 to 60 min at room temperature were not significantly different (Table 3). On the other hand, the additive effect of increased temperature resulted in increased levels of leakage from seeds imbibed at 55 and 60 °C for durations of 30 to 60 min, when compared with that of seeds imbibed for 5 min only per temperature, as well as with the equivalent duration of immersion for seeds treated at 25 °C (Table 3). Seeds imbibed for 60 min at both 57 and 60 °C leaked significantly higher levels of electrolytes than any of the other treatments.

3.1.4. Germination

The effect of imbibing seeds for 4 h at ambient temperature, followed by immersion in water from 5 to 60 min at 55, 57 and 60 °C, respectively, on germination was expressed in terms of the germination index (GI), which combines both seed vigour (PV) and germination totality (MDG)(Czabator, 1962).

Imbibing seeds for 4 h at ambient temperature enhanced seed vigour and viability (Fig. 4C). The GI of these seeds was 11% greater than that of the unimbibed, control material. The effect of imbibition on both these parameters, however, appeared to be due to an improved rate of emergence (Fig. 4A), more so than an increase in germination totality (Fig. 4B). Seeds immersed in water at 55 and 57 °C for periods of 15 and 5 min, respectively, also exhibited

Table 3. The effect of temperature and duration of immersion of *Zea mays* seeds[a] on the level of electrolytes in the soak water (expressed as $\mu A. \, g^{-1}$ DM).

Duration of treatment (min)	Temperature of treatment (^{0}C)			
	25	55	57	60
5	3.43 def	3.46 def	4.17 bcde	3.35 ef
10	2.86 f	3.43 def	3.33 ef	2.79 f
15	3.11 ef	3.18 ef	3.54 cdef	3.72 cdef
30	3.18 ef	4.59 bcd	3.96 cdef	4.71 bc
45	3.08 ef	4.67 bc	4.72 bc	4.90 b
60	3.92 bcdef	4.99 b	6.62 a	6.51 a

[a] Represents the mean of 10 seeds per sampling, expressed on a dry mass (DM) basis. Values with different letters are significantly different ($P < 0.05$).
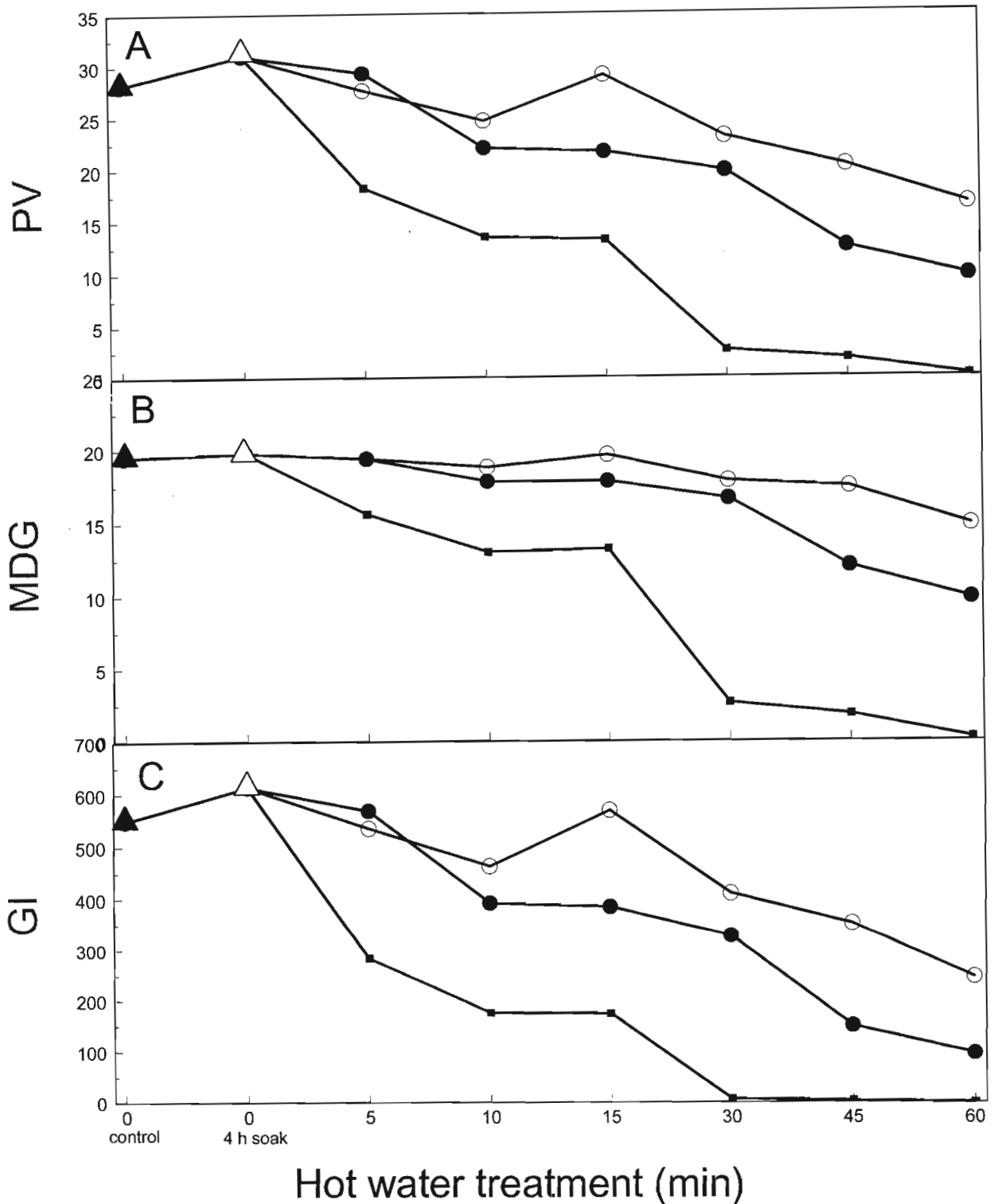
Figure 4. Rate of emergence (A), germination totality (B) and germination index (C) of *Zea mays* seeds following immersion in hot water at 55 (○), 57 (●) and 60 ⁰C (■) for durations of 5 to 60 min, compared with those of unimbibed (control) seeds (▲) and seeds pre-imbibed for 4 h at room temperature (△). n = 100 (control = 300).

enhanced germination performance (Fig. 4C). However, although the GI of these seeds was greater than that of the control seeds, the vigour and germination totality of these seeds did not exceed that of seeds imbibed for 4 h (Fig. 4C). This implies that the improved germination performance of material treated at 55 and 57 ^{0}C for durations of 15 and 5 min, respectively, was a consequence of the 4 h pre–soak, and not the hot water treatment. Immersing seeds for longer periods at both these temperatures, however, proved undesirable as seed germination was negatively affected (Fig. 4C). Seed vigour appeared to be more sensitive to prolonged hot water treatment than seed viability, as these parameters declined by 30 and 14%, respectively, compared with the control, when seeds were treated for 30 min at 57 ^{0}C (Fig. 4A and B). Immersing seeds in water at 60 ^{0}C proved too severe, as these seeds exhibited a 48% reduction in the GI after only 5 min treatment, when compared with the GI of the control material (Fig. 4C).

The improved germination performance of seeds that had been imbibed for 4 h at ambient temperature was also evident by the roots and shoots these seeds produced, when assessed 5 days after initiating of the germination trial (Fig 5A and B). Both these structures were significantly longer (7 and 12%, respectively) than those produced from unimbibed seeds. Root and shoot emergence by seeds that had been immersed in hot water, however, was negatively affected, the extent of which increased with increasing temperature and duration of treatment (Fig. 5A and B).

3.1.5. Seedling establishment

The effect of immersing seeds in hot water from 5 to 60 min at 55, 57 and 60 ^{0}C, respectively, on seedling quantity (expressed as the number of seedlings after a total of 18 days from the start of germination) is shown in Figure 6. The number of seedlings established from seeds that had imbibed for 4 h was less than that of the control seeds (93 and 97, respectively). Immersion in water at 55, 57 and 60 ^{0}C for durations up to 60, 30 and 5 min, respectively, did not further affect seedling quantity (Fig. 6). In fact, seeds immersed in water at 55 and 57 ^{0}C for durations of 15 and 5 min, respectively, were characterised by seedling establishment levels greater than that of the control.
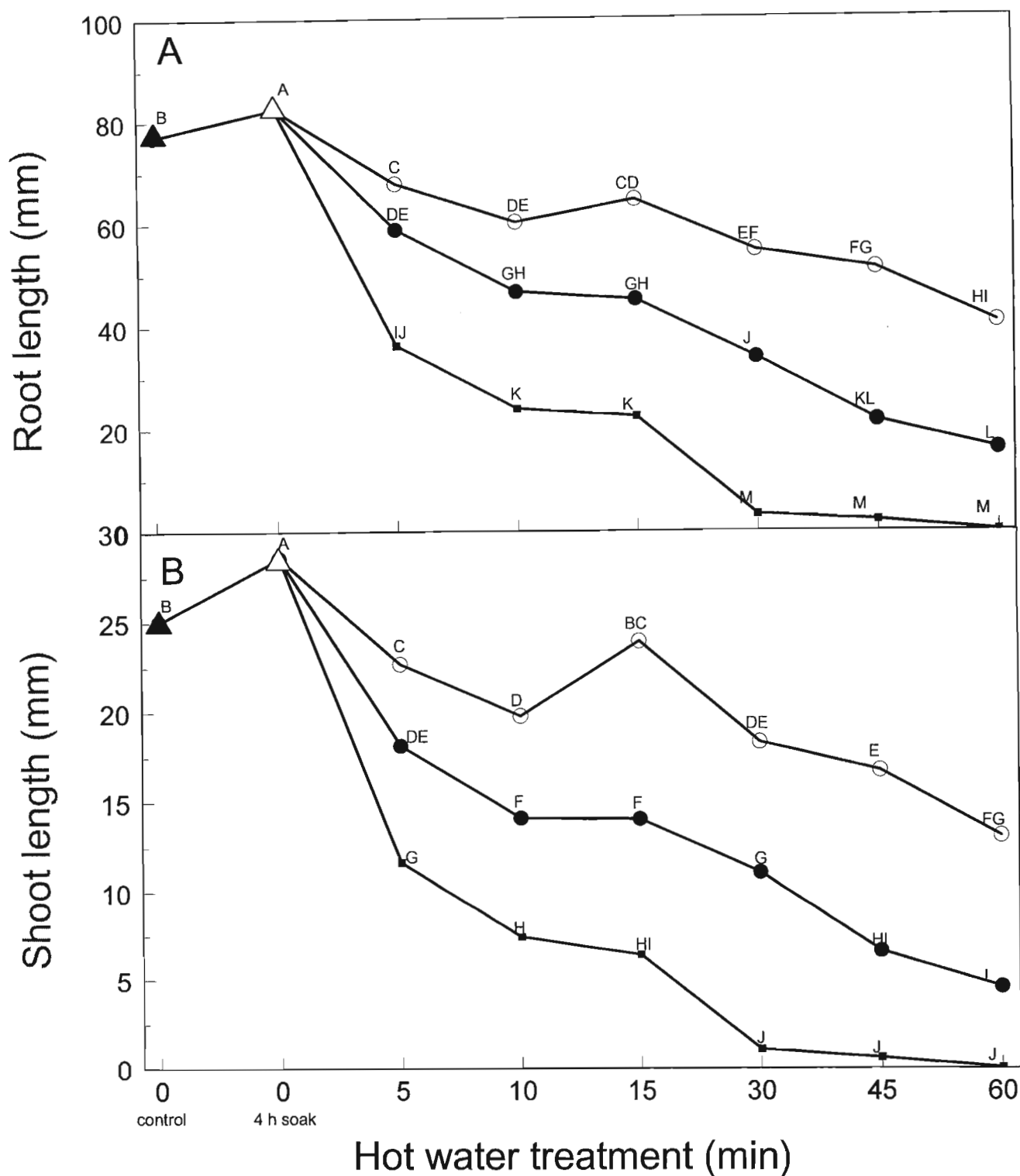
Figure 5. Root (A) and shoot (B) growth from *Zea mays* seeds following immersion in hot water at 55 (○), 57 (●) and 60 ^{0}C (■) for durations of 5 to 60 min, compared with those of unimbibed (control) seeds (▲) and seeds pre-imbibed for 4 h at room temperature (△). n = 100 (control = 300). Data points with different letters are significantly different (P<0.05).
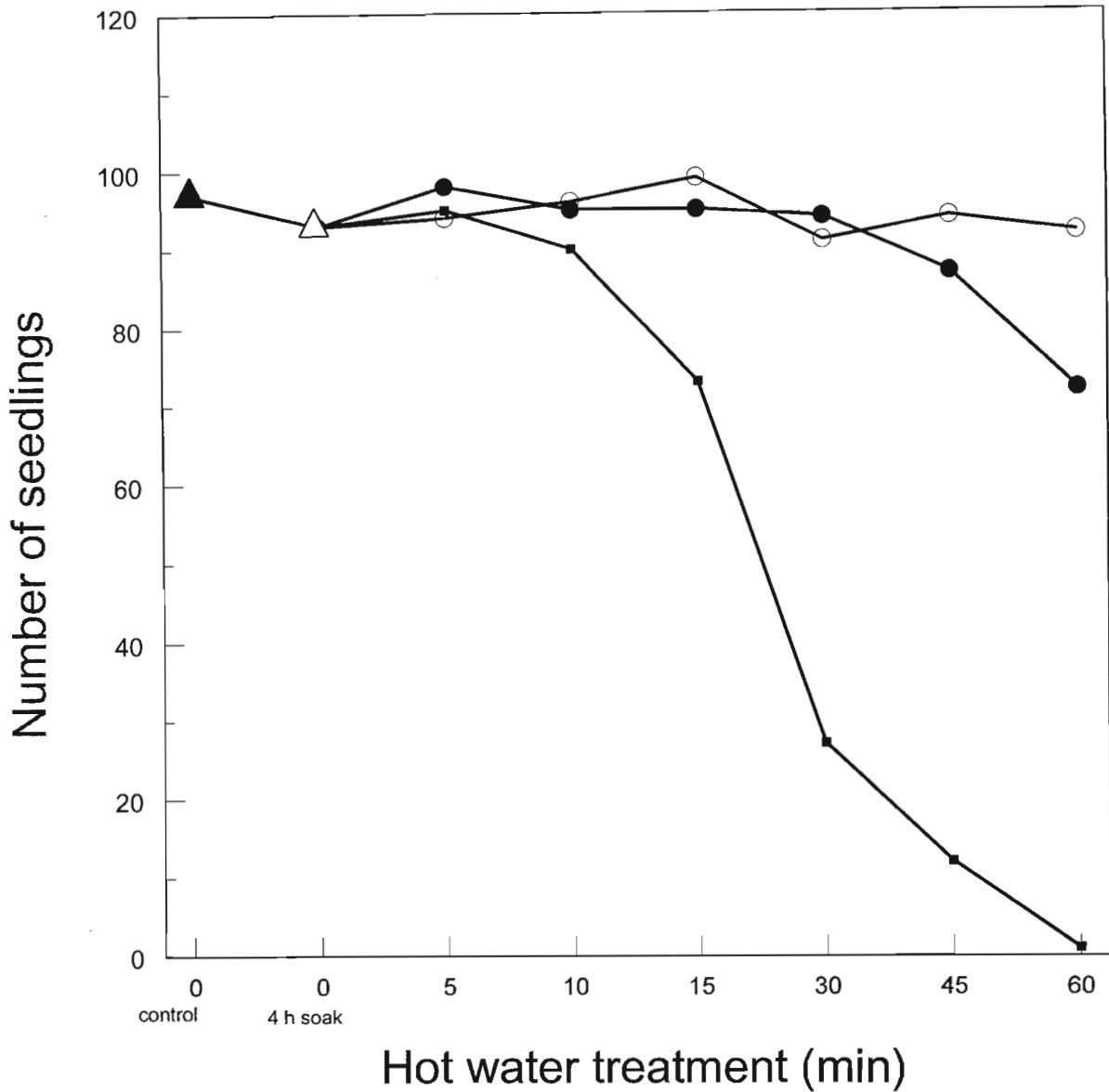
Figure 6. Number of seedlings, assessed 18 days after the start of germination, that developed from *Zea mays* seeds which had been immersed in hot water at 55 (○), 57 (●) and 60°C (■) for durations of 5 to 60 min, compared with those of unimbibed (control) seeds (▲) and seeds pre-imbibed for 4 h at room temperature (△). n = 100 seeds (control = 300 seeds).

In contrast, the quality of seedlings (i.e. shoot dry mass determined as the mean for those seeds germinated after 18 days from the start of imbibition) of those seeds subjected to the hot water treatment was not as greatly affected (Fig. 7). Seeds immersed in water at 55, 57 and 60 °C for durations up to 60, 30 and 10 min, respectively, produced plants with a mean dry mass similar to that of the control material (Fig. 7). As the same was evident for those seeds imbibed for 4 h only, the apparent improvement in seedling quality, as a consequence of treating seeds in hot water at the above-mentioned temperatures and durations, may partially be a consequence of the 4 h soak. In all cases, however, as the total number of seedlings was, in general, less than that of the control (Fig. 6), it suggests that seedling quality (Fig. 7) was improved in all the above-mentioned treatments.

3.2. The effects of re-dehydration on the internal fungal status and quality of *Zea mays* seeds previously imbibed for 4 h, then hot water treated.

3.2.1. Seed internal fungal status

Re-dehydrating seeds that had been previously imbibed for 4 h at room temperature did not affect the level of internal infection, this parameter being the same as that of unimbibed (control) seeds (Fig. 8). However, re-dehydration following immersion in hot water appeared to enhance the reduction in infection levels imposed by the treatment, as seeds immersed at 57 and 60 °C showed no infection after a treatment duration of only 10 min (Fig. 8, compare with Fig. 3).

In all the treatments where infection persisted, it was again represented almost entirely by the field fungi. Of these *F. moniliforme* remained the dominant and, together with *Diplodia* spp., the most persistent of the field fungi isolated. Neither *Cladosporium* sp., nor any of the other *Fusarium* spp. isolated from seeds previously imbibed for 4 h, survived the hot water immersion/re-dehydration treatment (Fig. 8). The storage fungi, represented in this case by *Aspergillus* spp., were isolated only from seeds previously treated at 55 °C for 5 min.
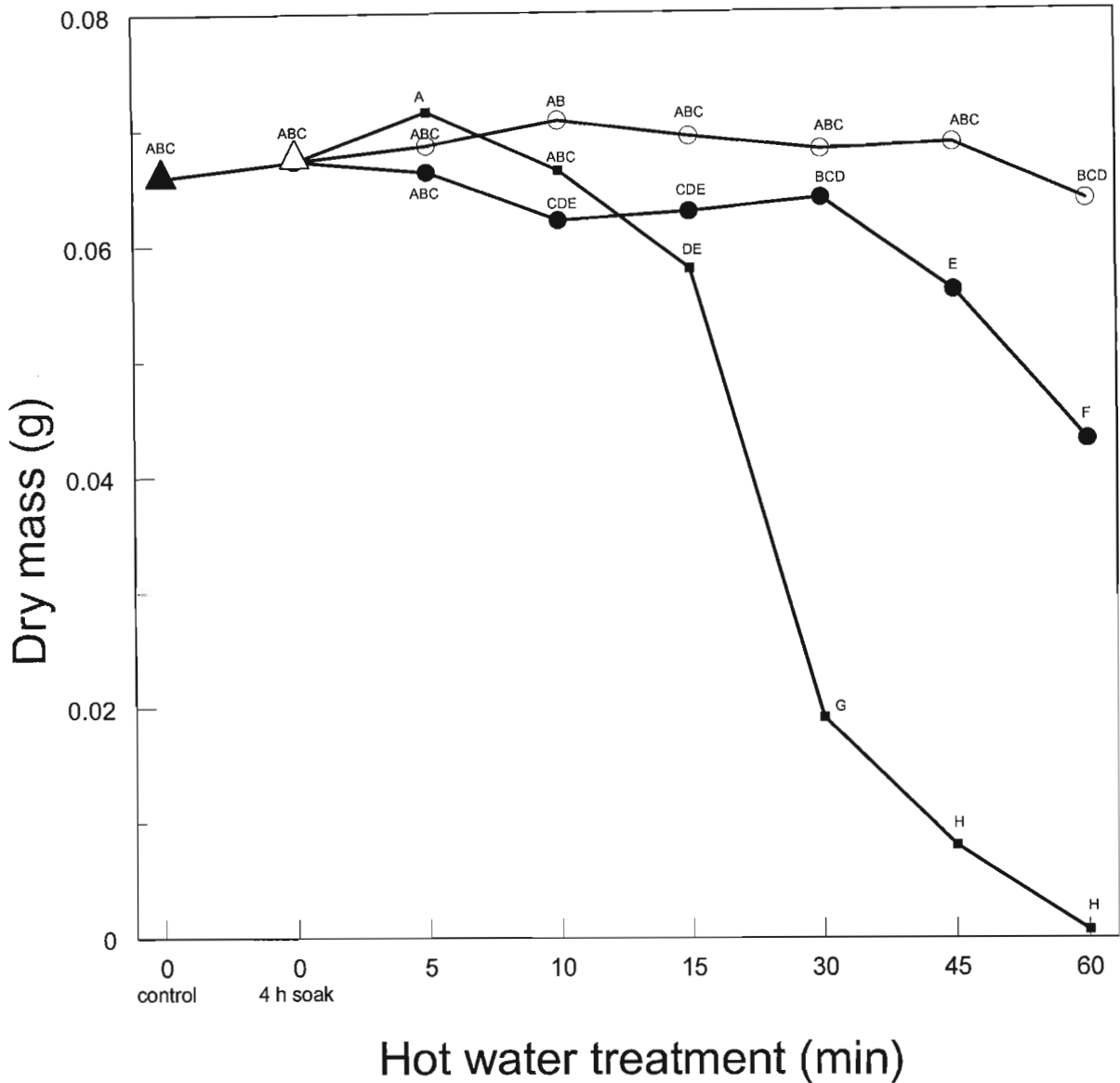
Figure 7 . Seedling dry mass, assessed 18 days after the start of germination, from *Zea mays* seeds that had been immersed in hot water at 55 (○), 57 (●) and 60 ^{0}C (■) for durations of 5 to 60 min, compared with those of unimbibed (control) seeds (▲) and seeds pre-imbibed for 4 h at room temperature (△). n = 100 seeds (control = 300 seeds). Data points with different letters are significantly different (P<0.05).

Figure 8. Effect of re-dehydration on percentage internal infection of *Zea mays* seeds previously immersed in hot water at 55 (A), 57 (B) and 60 ^{0}C (C) for durations of 5 to 60 min. These results are compared with those of unimbibed (control) seeds and seeds pre-imbibed for 4 h at room temperature, then re-dehydrated, n = 100, (control = 300).

3.2.2. Moisture content

Water loss, as a consequence of re-dehydration for 2 days, of seeds previously immersed in hot water, was greater by the tissues of the embryo than that of the endosperm (Table 4). In most cases, the embryonic tissues were re-dehydrated to levels similar to, and even lower than, those of the unimbibed (control) material. In contrast, the mcs of the endospermal tissues were, for the most part, greater than that of the control seeds (Table 4). Seeds previously imbibed for 4 h only, however, appeared the most resistant to water loss, achieving embryo and endosperm mcs of only 10.08 and 13.4%, respectively; water contents significantly higher those that of any of the other treatments (Table 4).

3.2.3. Conductivity

Both the level and rate at which electrolytes were leaked from seeds that had been imbibed for 4 h, then re-dehydrated were significantly lower than those of unimbibed (control) seeds (Table 5). Seeds that had been treated in hot water for 55, 57 and 60 ^{0}C (all durations) attained total leakage levels similar to those of seeds imbibed for 4 h, and similar to (or even greater) than those of the control seeds when treated for durations of 45 min or longer at 55 ^{0}C, or 30 min and longer at both 57 and 60 ^{0}C (Table 5).

While the rate at which seeds treated at 55 ^{0}C for durations of 5, 10 and 45 min leaked electrolytes was similar to that of seeds imbibed for 4 h, those seeds treated for 60 min (same temperature) leaked electrolytes at a higher rate, similar to that of the control seeds (Table 5). In contrast, seeds treated at 55 ^{0}C for durations of 15 and 30 min, and those treated at 57 and 60 ^{0}C (all durations) leaked electrolytes at rates significantly lower than that of seeds imbibed for 4 h.

3.2.4. Germination

Following re-dehydration for 2 days the GI of seeds previously imbibed for 4 h was 8% greater than that of the control material (Fig. 9 C). This appeared to be as a consequence of an improved rate of emergence (Fig. 9A), rather than an increase in germination totality (Fig. 9B), and was apparent also for seeds that had been previously immersed in water at 55 ^{0}C for 5 min (Fig. 9A, B and C). While germination totality of seeds treated for 15 min at both 55

Table 4. Effect of re-dehydration on the water content of *Zea mays* seeds previously imbibed for 4 h and hot water treated, compared with those of unimbibed (control) seeds.

	Duration of hot water treatment (min)	Moisture content (%WMB)[a]	
		Embryo	Endosperm
Unimbibed	0	9.22 ghi	11.88 c
Imbibed 4 h; re-dehydrated	0	10.08 l	13.40 l
Imbibed 4 h; hot water treated (55 ^{0}C); re-dehydrated	5	9.75 jk	12.24 ef
	10	9.09 fgh	12.04 d
	15	9.43 hij	12.59 h
	30	9.52 hij	12.76 ij
	45	9.57 ij	12.96 k
	60	9.27 ghi	12.32 fg
Imbibed 4 h; hot water treated (57 ^{0}C); re-dehydrated	5	8.35 bc	12.33 fg
	10	8.89 efg	12.40 g
	15	8.90 efg	11.99 cd
	30	8.79 cde	12.00 cd
	45	8.46 bcd	12.07 de
	60	9.19 fghi	12.91 jk
Imbibed 4 h; hot water treated (60 ^{0}C); re-dehydrated	5	7.77 a	11.17 a
	10	7.78 a	11.27 a
	15	9.28 ghi	12.36 fg
	30	9.75 jk	12.06 hi
	45	9.40 hij	12.61 hi
	60	8.20 ab	11.72 b

[a] Represents the mean of 25 seeds (except control, n = 75). Values with different letters within columns are significantly different (P < 0.05).

Table 5. Effect of re–dehydration on electrolyte leakage[a] from pre–imbibed and hot water treated *Zea mays* seeds, compared with those of unimbibed (control) seeds.

	Duration of hot water treatment (min)	Total leakage ($\mu A.\,g^{-1}$ DM)	Leakage rate ($\times 10^{-2}\,\mu A.g^{-1}\,DM.s^{-1}$)
Unimbibed	0	7.10 cd	100.4 a
Imbibed 4 h; re–dehydrated	0	5.58 efgh	64.8 b
Imbibed 4 h; hot water treated (55 ^{0}C); re–dehydrated	5	5.07 fghi	58.3 b
	10	5.68 efgh	47.1 bc
	15	4.78 ghi	36.7 cd
	30	4.15 hi	36.3 cde
	45	6.07 defg	51.1 bc
	60	8.72 ab	89.2 a
Imbibed 4 h; hot water treated (57 ^{0}C); re–dehydrated	5	4.44 hi	9.3 f
	10	5.16 efghi	7.9 f
	15	4.40 hi	17.2 ef
	30	7.64 bcd	15.1 f
	45	7.51 bc	17.6 def
	60	9.37 a	9.3 f
Imbibed 4 h; hot water treated (60 ^{0}C); re–dehydrated	5	4.50 hi	9.7 f
	10	3.84 i	7.2 f
	15	4.51 hi	11.1 f
	30	6.57 cdef	15.4 f
	45	6.62 cde	8.2 f
	60	5.19 efghi	8.6 f

[a] Represents the mean of 25 seeds (except control, n = 75) measured over a 10 h period, expressed on a dry mass (DM) basis. Values with different letters within columns are significantly different (P < 0.05).
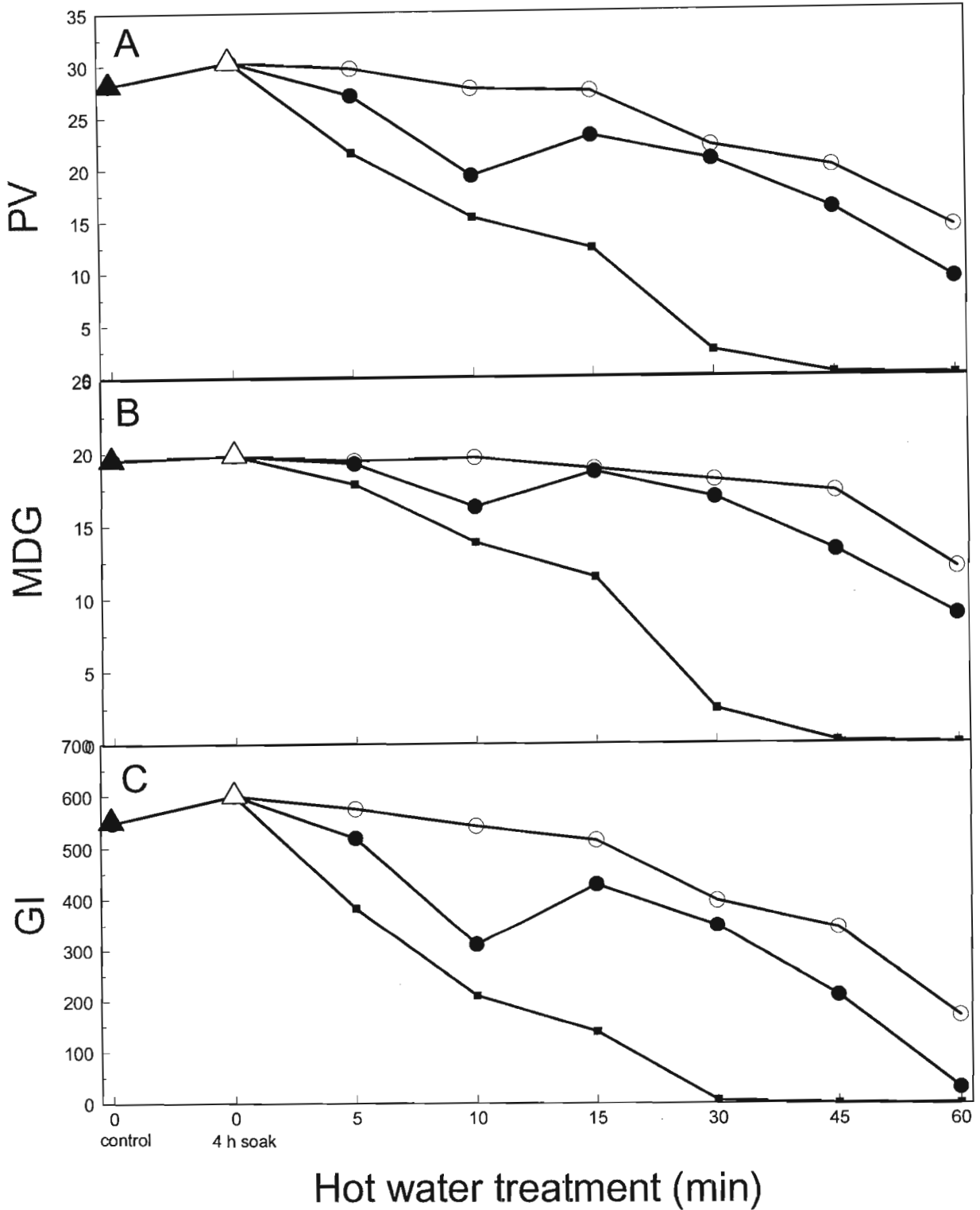
Figure 9. Effect of re-dehydration on emergence rate (A), germination totality (B) and germination index (C) of *Zea mays* seeds previously immersed in hot water at 55 (○), 57 (●) and 60 °C (■) for durations of 5 to 60 min. These results are compared with those of unimbibed (control) seeds (▲) and seeds pre-imbibed for 4 h, then re-dehydrated (△). n = 100 (control = 300).

and 57 $^{\circ}$C was similar to that of the control seeds (Fig. 9B), the vigour of these seeds was, however, reduced (Fig. 9A) when assessed after re–dehydration. Both seed vigour and viability were negatively affected by treatment at 60 $^{\circ}$C (all durations), with no germination occurring after 60 min (Fig. 9C).

The apparent lack of damage as a consequence of the hydration/re–dehydration treatment was also reflected by the roots and shoots these seeds produced (Fig. 10). Seeds previously imbibed for 4 h at ambient temperature, in fact, produced shoots that were significantly longer than those of the control seeds not subjected to the hydration/re–dehydration treatment (Fig. 10B). On the other hand, root and, to a greater extent, shoot growth of seeds previously immersed in hot water was negatively affected, the extent of which increased with increasing temperature and duration of treatment.

3.2.5. Seedling establishment

The number of seedlings produced from seeds imbibed for 4 h, then re–dehydrated for 2 days, was 6% lower than that of untreated (control) seeds (Fig. 11). On the other hand, the quantity of seedlings from seeds previously immersed in hot water at 55, 57 and 60 $^{\circ}$C for durations not exceeding 30, 10 and 5 min, respectively, was similar to the control material, when assessed after re–dehydration (Fig. 11).

The decreased performance in seedling establishment by seeds previously imbibed for 4 h was also reflected by seedling quality (expressed as dry matter accumulated in the shoots), being 15% lower than that of the control seeds (Fig. 12). In contrast, seeds treated at 55, 57 and 60 $^{\circ}$C for durations not exceeding 60, 10 and 15 min, respectively, were not similarly affected (Fig. 12). In fact, treatment at both 55 and 60 $^{\circ}$C for durations of up to 10 min, produced plants of significantly higher quality than those of the control seeds, when assessed after re–dehydration.
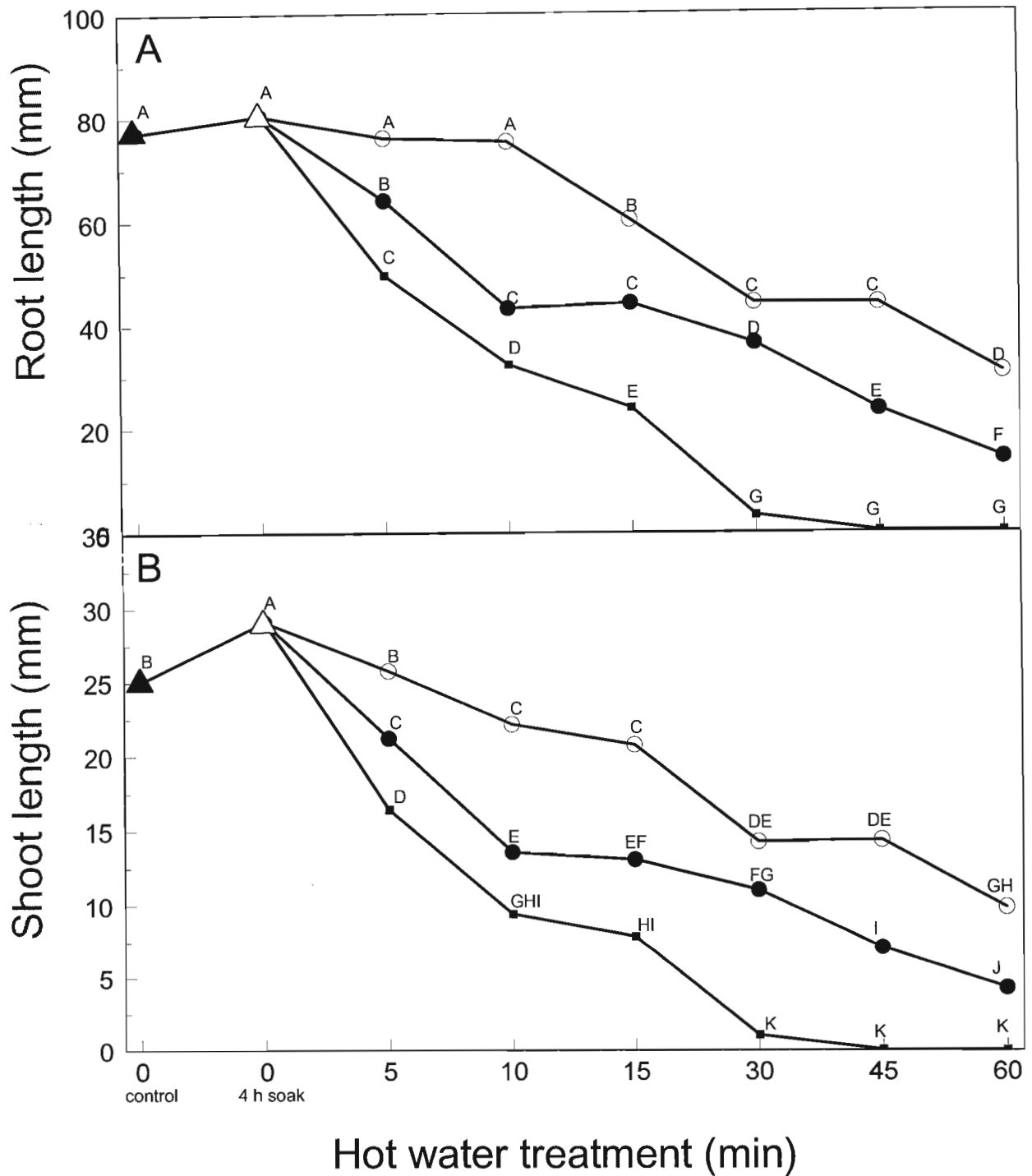
Figure 10. Effect of re-dehydration on the root (A) and shoot (B) growth from *Zea mays* seeds previously immersed in hot water at 55 (○), 57 (●) and 60 °C (■) for durations of 5 to 60 min. These results are compared with those of unimbibed (control) seeds (▲) and seeds pre-imbibed for 4 h at room temperature, then re-dehydrated (△). n = 100 (control = 300). Data points with different letters are significantly different (P<0.05).
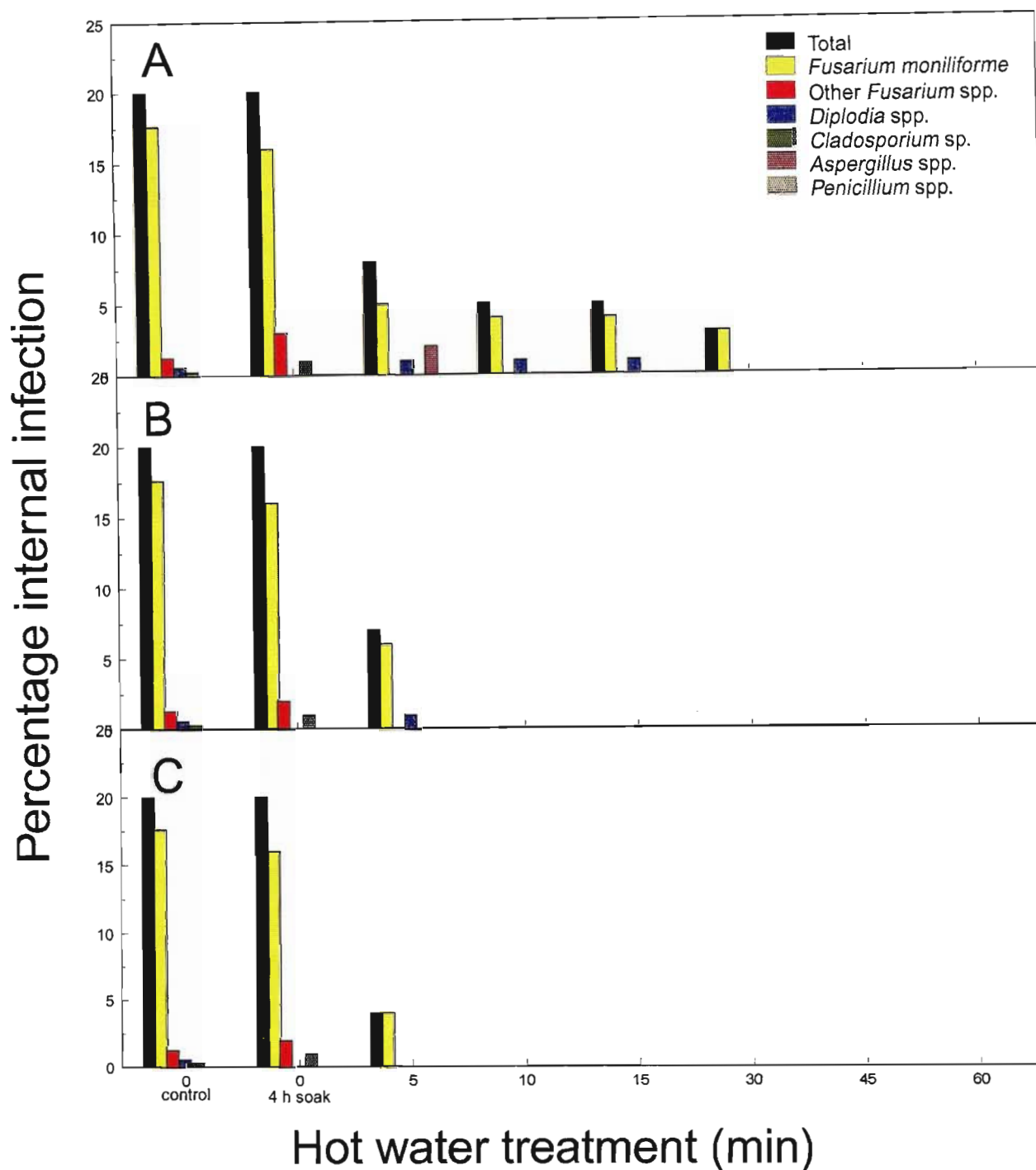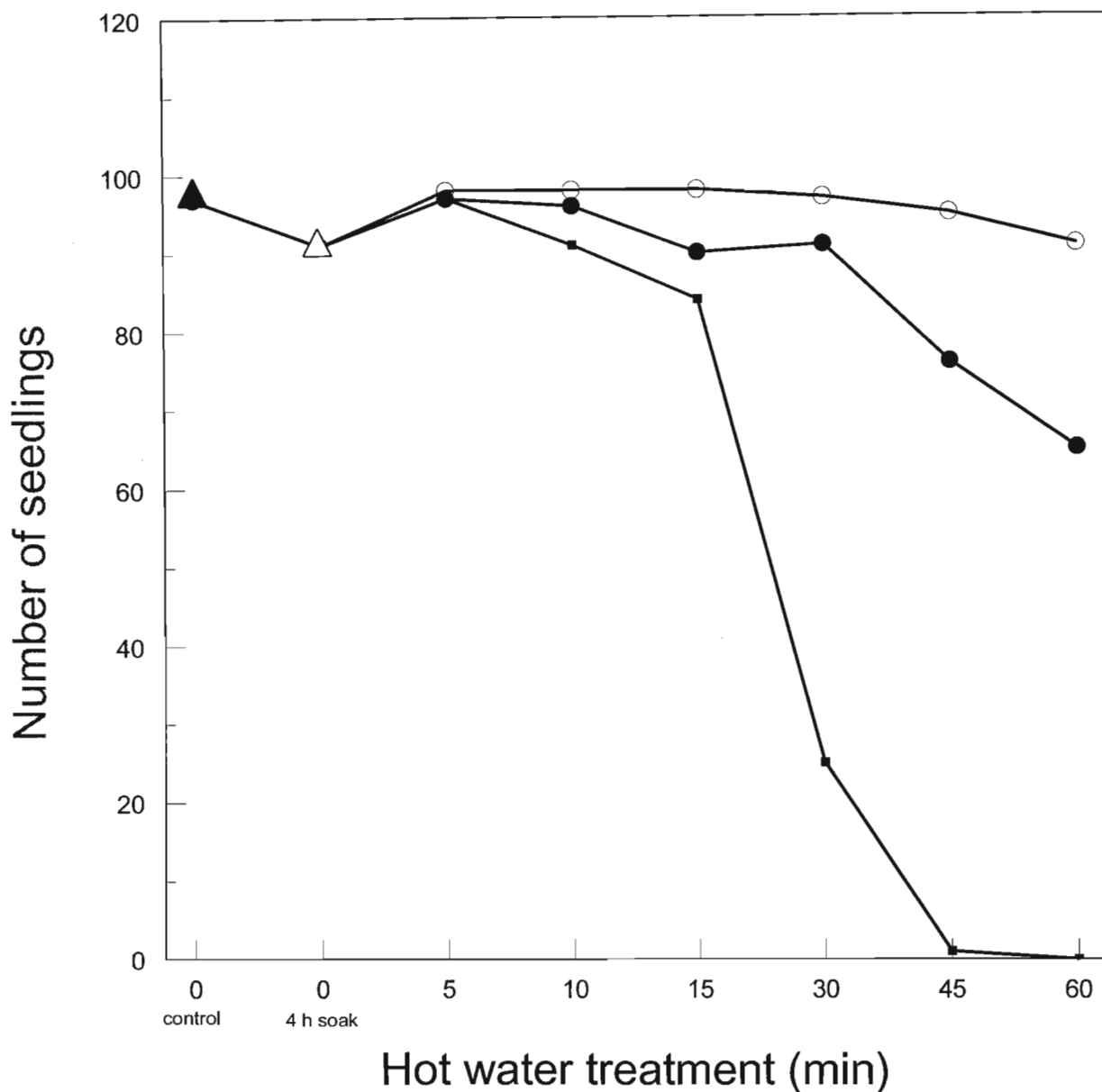
Figure 11. Effect of re-dehydration on the number of seedlings, assessed 18 days after the start of germination, that developed from _Zea mays_ seeds previously immersed in hot water at 55 (○), 57 (●) and 60°C (■) for durations of 5 to 60 min. These results are compared with those of unimbibed (control) seeds (▲) and seeds pre-imbibed for 4 h at room temperature, then re-dehydrated (△). n = 100 seeds (control = 300 seeds).
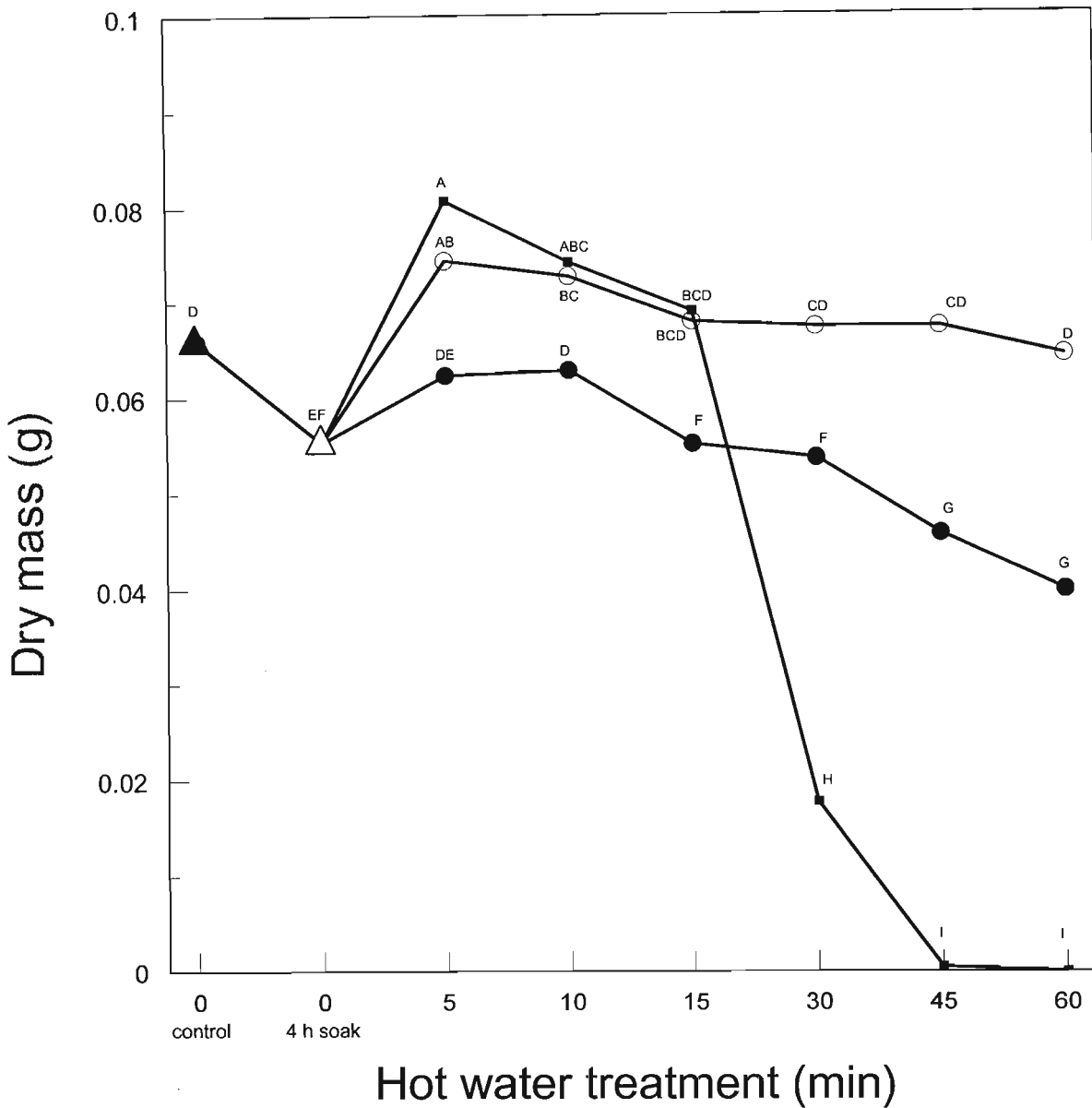
Figure 12 . Effect of re-dehydration on seedling dry mass, assessed 18 days after the start of germination, from *Zea mays* seeds previously immersed in hot water at 55 (o), 57 (●) and 60 °C (■) for durations of 5 to 60 min. These results are compared with those of unimbibed (control) seeds (▲) and seeds pre-imbibed for 4 h at room temperature, then re-dehydrated (△). n = 100 seeds (control = 300 seeds). Data points with different letters are significantly different (P<0.05).

3.3. The effect of storage for 1 month at 4 ^{0}C (33% RH) on the internal fungal status and quality of unimbibed (control) *Zea mays* seeds, and seeds previously imbibed for 4 h, and hot water treated.

3.3.1. Seed internal fungal status

The level of total internal infection of seeds previously imbibed for 4 h, then dried and stored for 1 month under cold, dry conditions was 19% (Fig. 13). In contrast, unimbibed (control) seeds exhibited a 37% lower infection level, compared with the 4 h imbibed treatment (Fig. 13). Low infection levels were maintained in those seeds which had been immersed previously in hot water.

The spectrum of fungi isolated from seeds subjected to any of the treatments, including the control material, was greatly reduced following 1 month storage at 4 ^{0}C (33% RH), and was represented almost entirely by *F. moniliforme* (Figure 13). *Aspergillus* spp. were isolated at low levels (1%) from seeds that had been treated at 55 ^{0}C for a duration of 10 min only.

3.3.2. Moisture content

Following storage for 1 month under cold (4 ^{0}C), dry (33% RH) conditions seeds that had been previously imbibed for 4 h had embryo water contents similar to those of control (unimbibed) seeds stored under the same conditions (Table 6). The same was true for seeds that had been immersed in water at 55 ^{0}C. On the other hand, seeds treated at 57 ^{0}C and, to a greater extent, at 60 ^{0}C exhibited an increased incidence of embryos with water contents significantly lower than that of the control seeds (Table 6).

3.3.3. Conductivity

While the total amount of electrolytes leaked by seeds imbibed for 4 h was similar to that of unimbibed seeds, when assessed after 1 month storage at 4 ^{0}C, seeds that had been immersed in hot water at 55 ^{0}C and, to a lesser extent, at 57 and 60 ^{0}C, were characterised by leakage levels higher than that of the control seeds (Table 7). In contrast, the rate at which electrolytes were leaked by unimbibed seeds was higher than that by seeds from any of the other treatments (Table 7). Conversely, seeds that had been imbibed for 4 h achieved the lowest
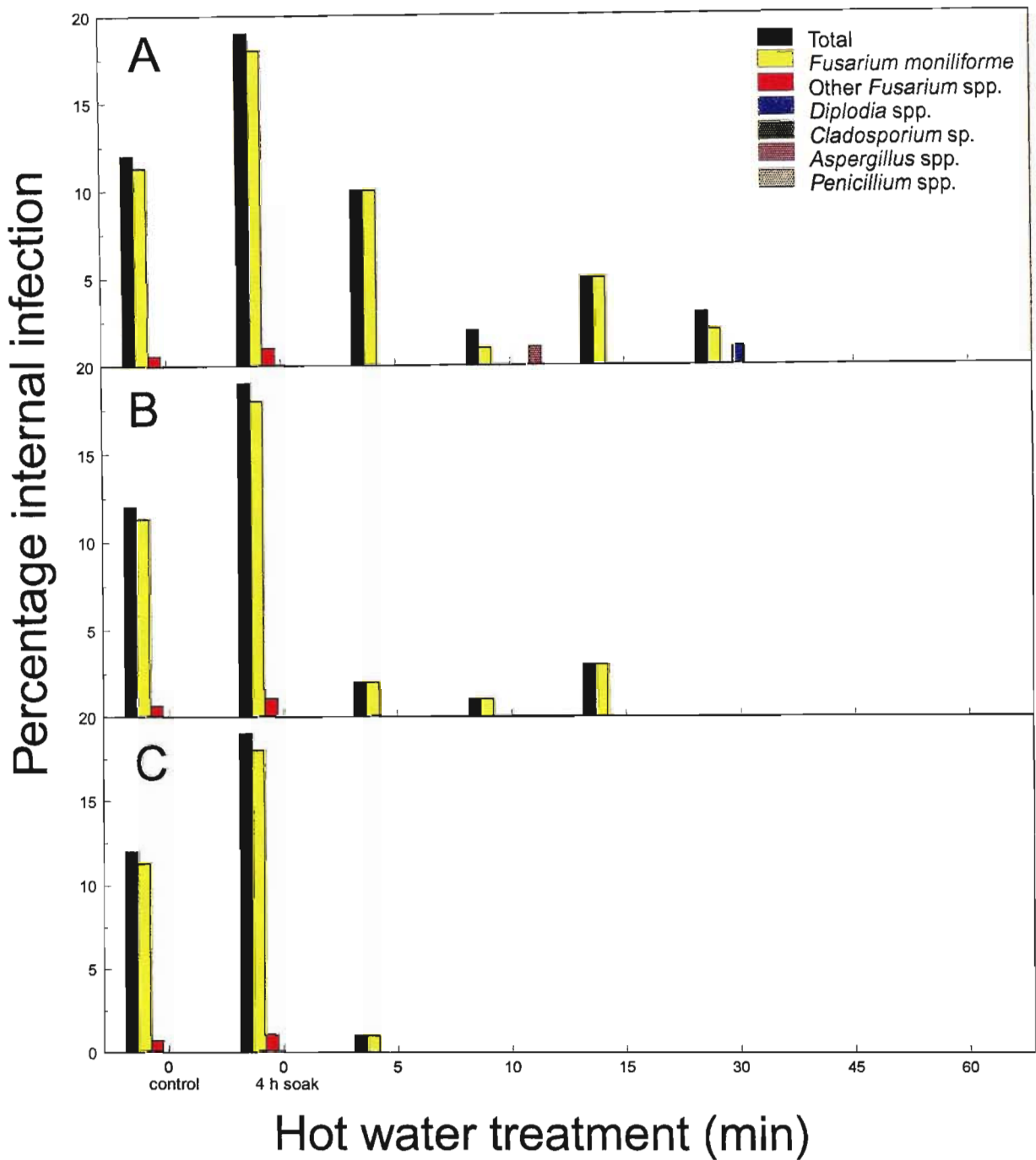
Figure 13. Effect of storage for 1 month at 4 ^{0}C (33% RH) on the percentage internal infection of *Zea mays* seeds previously immersed in hot water at 55 (A), 57 (B) and 60 ^{0}C (C) for durations of 5 to 60 min, then dehydrated. These results are compared with those of unimbibed (control) seeds and seeds pre-imbibed for 4 h/dehydrated and stored under the same conditions. n = 100 (control = 300).

Table 6. Effect of storage for 1 month at 4 ^{0}C (33% RH) on the water contents of re-dehydrated *Zea mays* seeds that were previously pre-imbibed and hot water treated, compared with those of unimbibed (control) seeds stored under the same conditions.

	Duration of hot water treatment (min)	Moisture content (%WMB)[a]	
		Embryo	Endosperm
Unimbibed; stored at 4 ^{0}C (33% RH)	0	9.15 ghi	11.80 def
Imbibed 4 h; re-dehydrated; stored at 4 ^{0}C (33% RH)	0	9.17 fghi	12.39 klm
Imbibed 4 h; hot water treated (55 ^{0}C); re-dehydrated; stored at 4 ^{0}C (33% RH)	5	8.87 efgh	11.72 cdef
	10	8.44 bcde	11.85 efg
	15	9.35 hi	12.55 lm
	30	9.46 i	12.33 jkl
	45	8.79 defg	12.60 m
	60	8.81 defg	12.13 hij
Imbibed 4 h; hot water treated (57 ^{0}C); re-dehydrated; stored at 4 ^{0}C (33% RH)	5	8.87 efgh	12.20 ijk
	10	9.02 fghi	12.10 hi
	15	7.98 ab	11.53 c
	30	8.00 ab	11.68 cde
	45	8.36 bcd	11.52 c
	60	8.88 efgh	12.50 lm
Imbibed 4 h; hot water treated (60 ^{0}C); re-dehydrated; stored at 4 ^{0}C (33% RH)	5	7.76 a	11.00 a
	10	8.00 ab	11.28 b
	15	8.28 bc	11.94 fgh
	30	8.37 bcd	11.62 cd
	45	9.17 fghi	12.06 ghi
	60	7.67 a	11.65 cde

[a] Represents the mean of 25 seeds (except control, n = 75). Values with different letters within colums are significantly different (P < 0.05).

Table 7. Effect of storage for 1 month at 4 ^{0}C (33% RH) on the leakage of electrolytes[a] from re–dehydrated *Zea mays* seeds that were previously pre–imbibed and hot water treated, compared with those of unimbibed (control) seeds stored under the same conditions.

	Duration of hot water treatment (min)	Total leakage ($\mu A. g^{-1}$ DM)	Leakage rate ($x10^{-2}$ $\mu A.g^{-1}$ $DM.s^{-1}$)
Unimbibed; stored at 4 ^{0}C (33% RH)	0	5.43 efgh	4.1 a
Imbibed 4 h; re–dehydrated; stored at 4 ^{0}C (33% RH)	0	4.41 h	1.6 g
Imbibed 4 h; hot water treated (55 ^{0}C); re–dehydrated; stored at 4 ^{0}C (33% RH)	5	7.70 bc	3.0 bcde
	10	7.17 bcd	3.4 bc
	15	5.21 fgh	2.3 efg
	30	6.86 bcdef	2.7 cdef
	45	7.14 bcd	2.1 fg
	60	7.35 bcd	3.2 bcd
Imbibed 4 h; hot water treated (57 ^{0}C); re–dehydrated; stored at 4 ^{0}C (33% RH)	5	4.19 h	2.2 efg
	10	6.36 cdefg	1.8 g
	15	6.34 cdefg	2.8 bcdef
	30	8.16 b	2.3 efg
	45	8.34 ab	2.1 fg
	60	7.44 bc	1.9 g
Imbibed 4 h; hot water treated (60 ^{0}C); re–dehydrated; stored at 4 ^{0}C (33% RH)	5	5.40 fgh	2.4 defg
	10	5.69 defgh	2.4 defg
	15	5.17 gh	2.4 defg
	30	6.81 bcdefg	2.8 bcdef
	45	9.95 a	3.6 ab
	60	5.74 defgh	2.1 fg

[a] Represents the mean of 25 seeds (except control, n = 75) measured over a 10 h period, expressed on a dry mass (DM) basis. Values with different letters within columns are significantly different (P < 0.05).

rate of leakage, when compared with any of the other treatments.

3.3.4. Germination

Seed vigour and viability, as represented by the germination index (GI), was negatively affected by cold storage for all the treatments, when compared with control seeds stored under the same conditions (Fig. 14C). Seeds previously imbibed for 4 h were characterised by a GI value 30% lower than that of the control material. The effect of cold storage on both these parameters was corroborated by the increase in time required for the onset of germination (decline in rate) and by the decline in the total number of seeds germinating (Fig. 14A and B). While seeds that had been treated at 55 ^{0}C for 15 min had a germination performance similar to that of the 4 h imbibed material, the negative effect of prolonged duration and increased temperature of treatment on the GI of these seeds was also apparent when assessed after 1 month cold storage. All the seeds that had been treated for 60 min at 60 ^{0}C were dead.

In contrast, the quality of roots and shoots produced from seeds previously imbibed for 4 h, as well as those immersed in hot water, appeared to be less affected than the germination process when assessed after cold storage (Fig. 15). Root and shoot lengths of seeds imbibed for 4 h were similar to, and in the former case significantly greater than, that of control seeds stored under the same conditions. Similarly, root length was unaffected for seeds treated at 55 ^{0}C for 15 min and shoot length unaffected when treated at 55 and 57 ^{0}C for the same duration.

3.3.5. Seedling establishment

Following cold storage, the number of seedlings produced from seeds previously imbibed for 4 h was similar to that of the control, being 97 and 98, respectively (Fig. 16). In contrast, seeds previously treated at 55 and 57 ^{0}C for durations up to 10 and 5 min, respectively, were characterised by seedling emergence levels lower than that of the control. This decline in seedling quantity, however, was not apparent in those seeds that had been treated for longer durations (up to 15 and 10 min at 55 and 57 ^{0}C, respectively) or at higher temperatures (60 ^{0}C for 5 min)[Fig. 16]. Treatment for durations in excess of 15 min dramatically decreased the number of seedlings these seeds produced. As there was virtually no variability in the
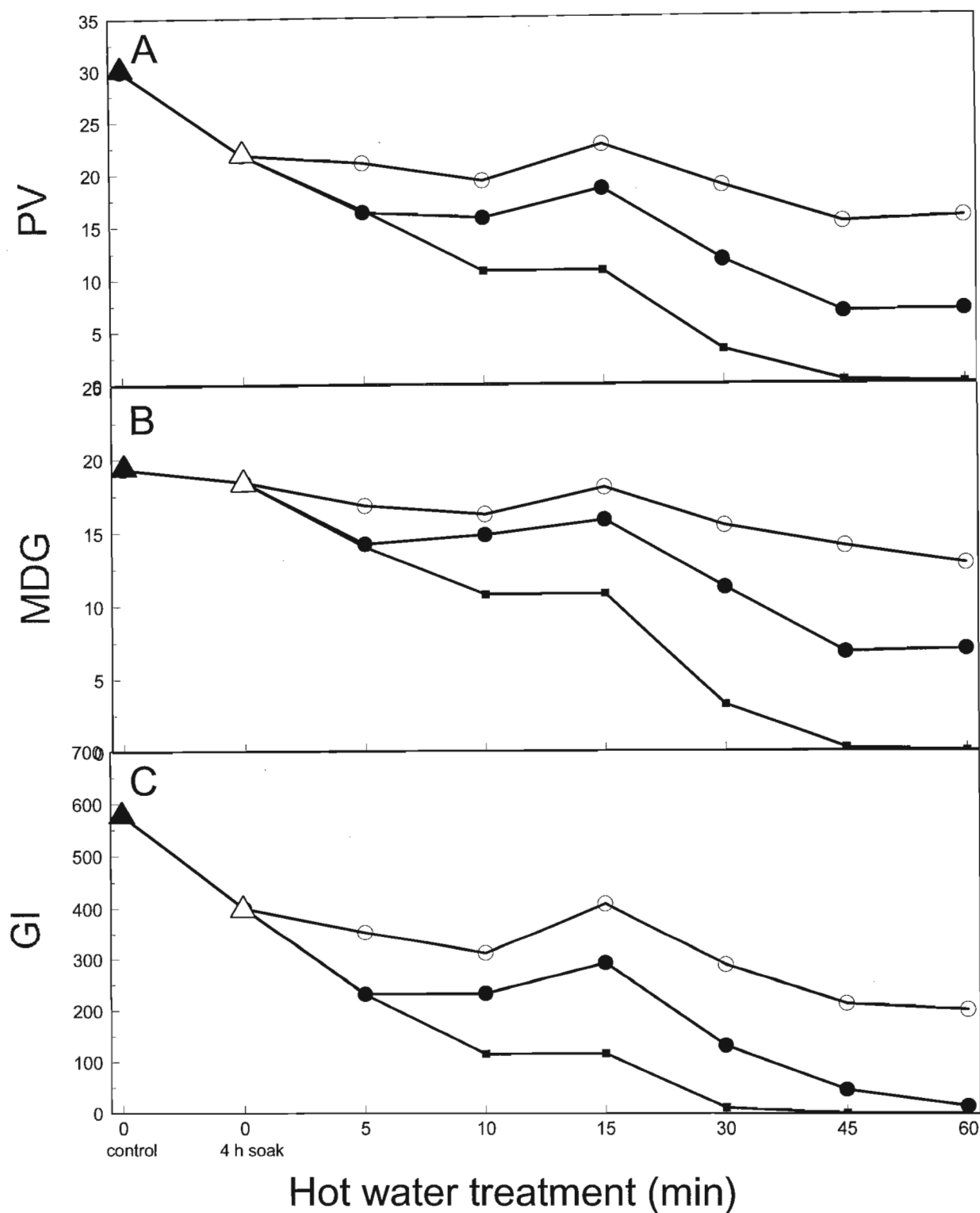
Figure 14. Effect of storage for 1 month at 4 ⁰C (33% RH) on emergence rate (A), germination totality (B) and germination index (C) of *Zea mays* seeds previously immersed in hot water at 55 (O), 57 (●) and 60 ⁰C (■) for durations of 5 to 60 min, then dehydrated. These results are compared with those of unimbibed (control) seeds (▲) and seeds pre-imbibed for 4 h/dehydrated (△),and stored under the same conditions. n = 100 (control = 300).
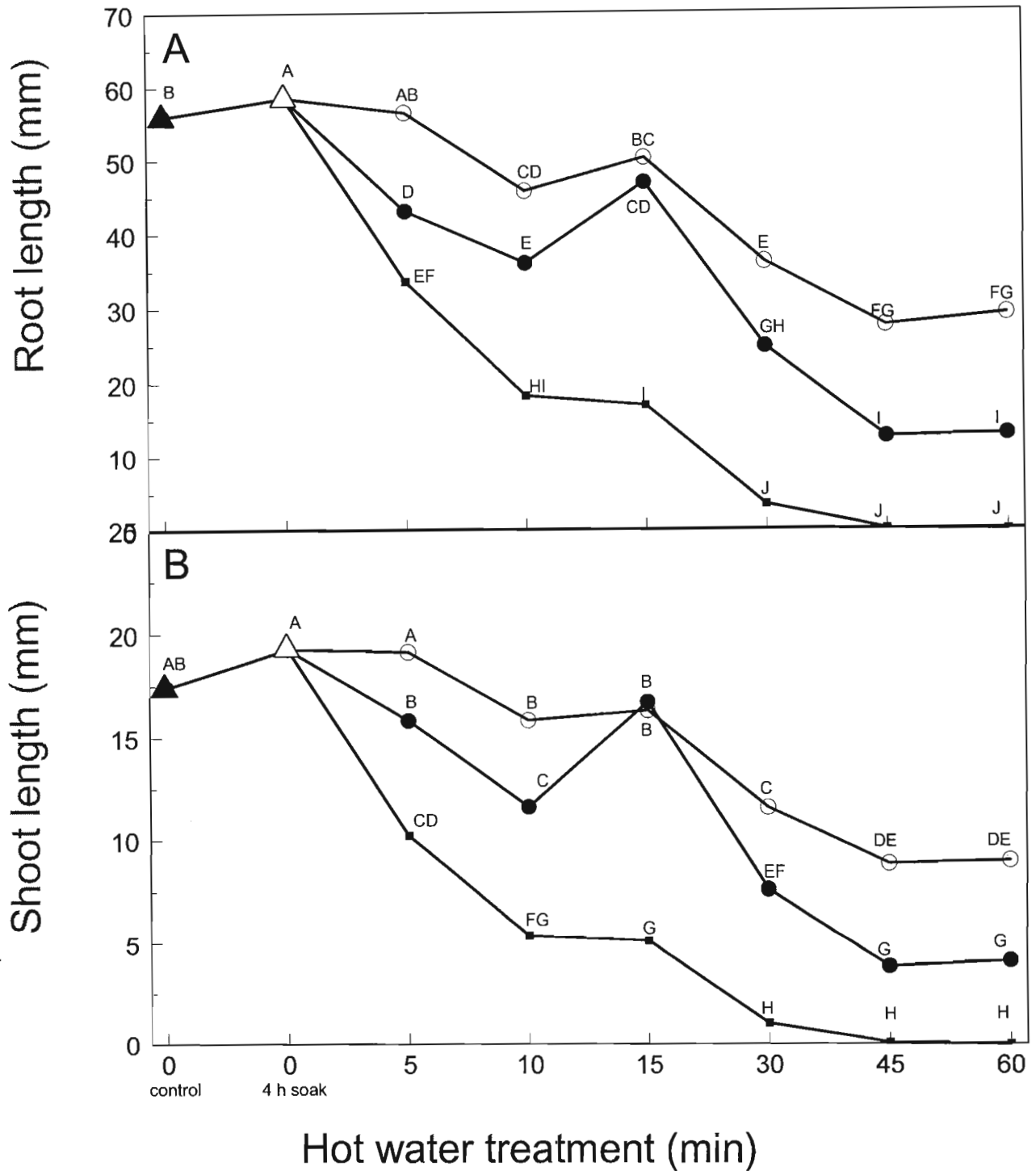
Figure 15. Effect of storage for 1 month at 4°C (33% RH) on root (A) and shoot (B) growth from *Zea mays* seeds previously immersed in hot water at 55 (○), 57 (●) and 60°C (■) for durations of 5 to 60 min, then dehydrated. These results are compared with those of unimbibed (control) seeds (▲) and seeds pre-imbibed for 4 h/dehydrated (△), and stored under the same conditions. n = 100 (control = 300). Data points with different letters are significantly different (P<0.05).
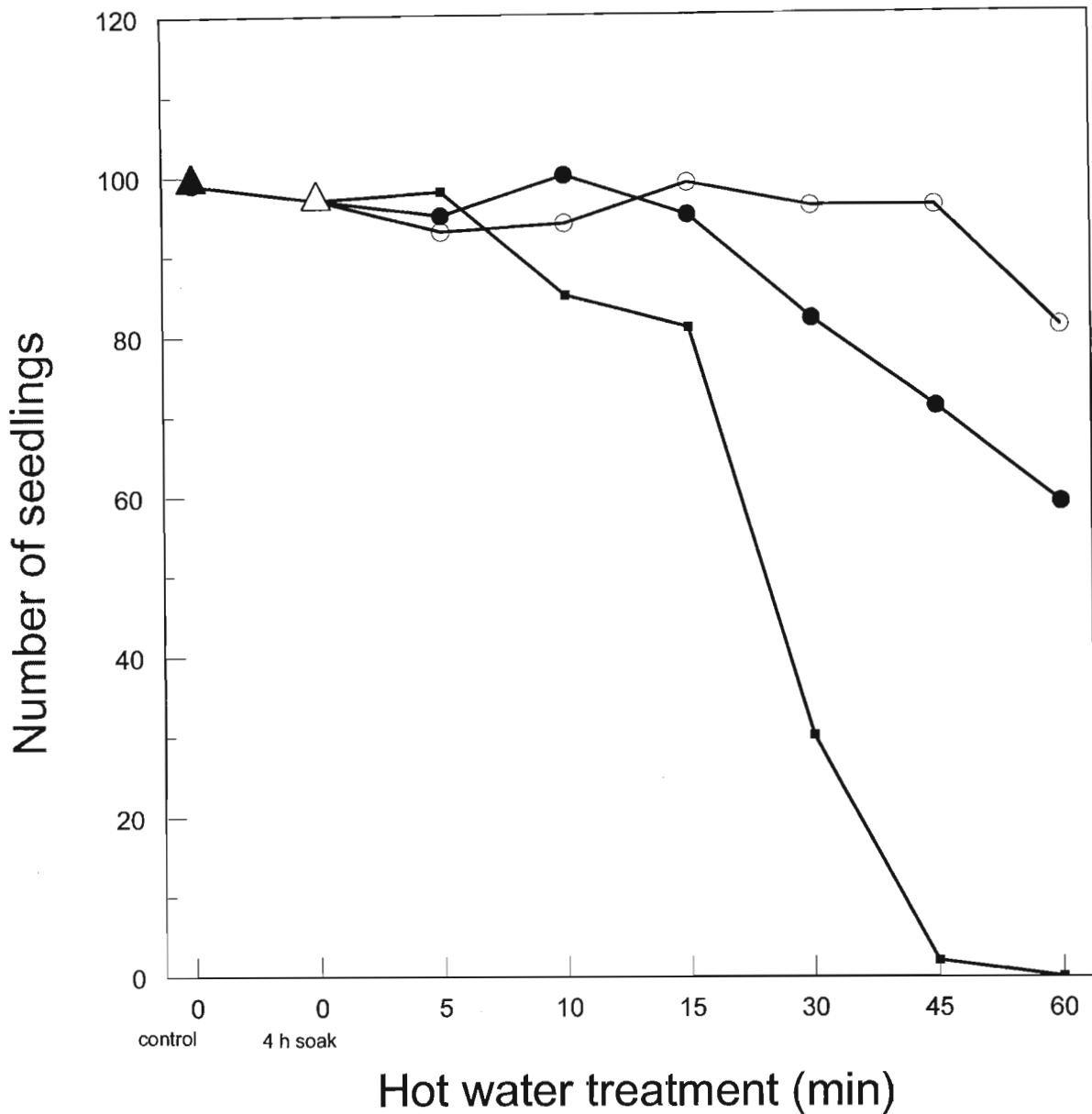
Figure 16. Effect of storage for 1 month at 4^0C (33% RH) on the number of seedlings, assessed 18 days after the start of germination, that developed from Zea mays seeds previously immersed in hot water at 55 (○), 57 (●) and 60^0C (■) for durations of 5 to 60 min, then dehydrated. These results are compared with those of unimbibed (control) seeds (▲) and seeds pre-imbibed for 4 h/dehydrated (△), and stored under the same conditions. n = 100 seeds (control = 300 seeds).

control material, which was replicated 3 times (results not shown), this suggests that the differences between samples was real.

This initial decline in seedling establishment by seeds that had been treated at lower temperatures for short durations, and its absence by seeds treated for longer durations or at higher temperatures, was also reflected in the quality of the seedlings that these seeds produced (Fig. 17). While the seedlings of seeds treated at 55 ^{0}C for 5 and 10 min attained dry mass levels significantly lower than that of the control seeds, seedling quality was unaffected in those seeds treated for durations of 15, 30 and 45 min, at the same temperature (Fig. 17). Similarly, seedling dry mass did not differ significantly from that of the control material for seeds treated at 60 ^{0}C for 5 min. Seeds previously imbibed for 4 h attained seedling dry mass levels similar to that of the control (Fig. 17).

3.4. The effect of storage for 1 month at 25 ^{0}C (91% RH) on the internal fungal status and quality of unimbibed (control) *Zea mays* seeds, and seeds previously imbibed for 4 h, and hot water treated.

3.4.1. Seed internal fungal status
Following storage for 1 month at 25 ^{0}C (91% RH), control (unimbibed) seeds were characterised by infection levels significantly lower than those of seeds previously imbibed for 4 h, then re–dehydrated and stored under the same conditions (Fig. 18). In addition, although storage was under warm, moist conditions, low infection levels were maintained in those seeds previously immersed in hot water. There was no infection in seeds that had been treated for durations of 45, 15 and 10 min at temperatures of 55, 57 and 60 ^{0}C, respectively (Fig. 18).

F. moniliforme again was the dominant fungus isolated from most of the treatments, except for those seeds that had been treated at 55 ^{0}C for durations longer than 5 min, where it was isolated in a 50:50 ratio with *Diplodia* spp.. The appearance of *Aspergillus* spp., although representing only 2.7% of the total infection levels, occurred only in the unimbibed (control) seeds (Fig. 18).
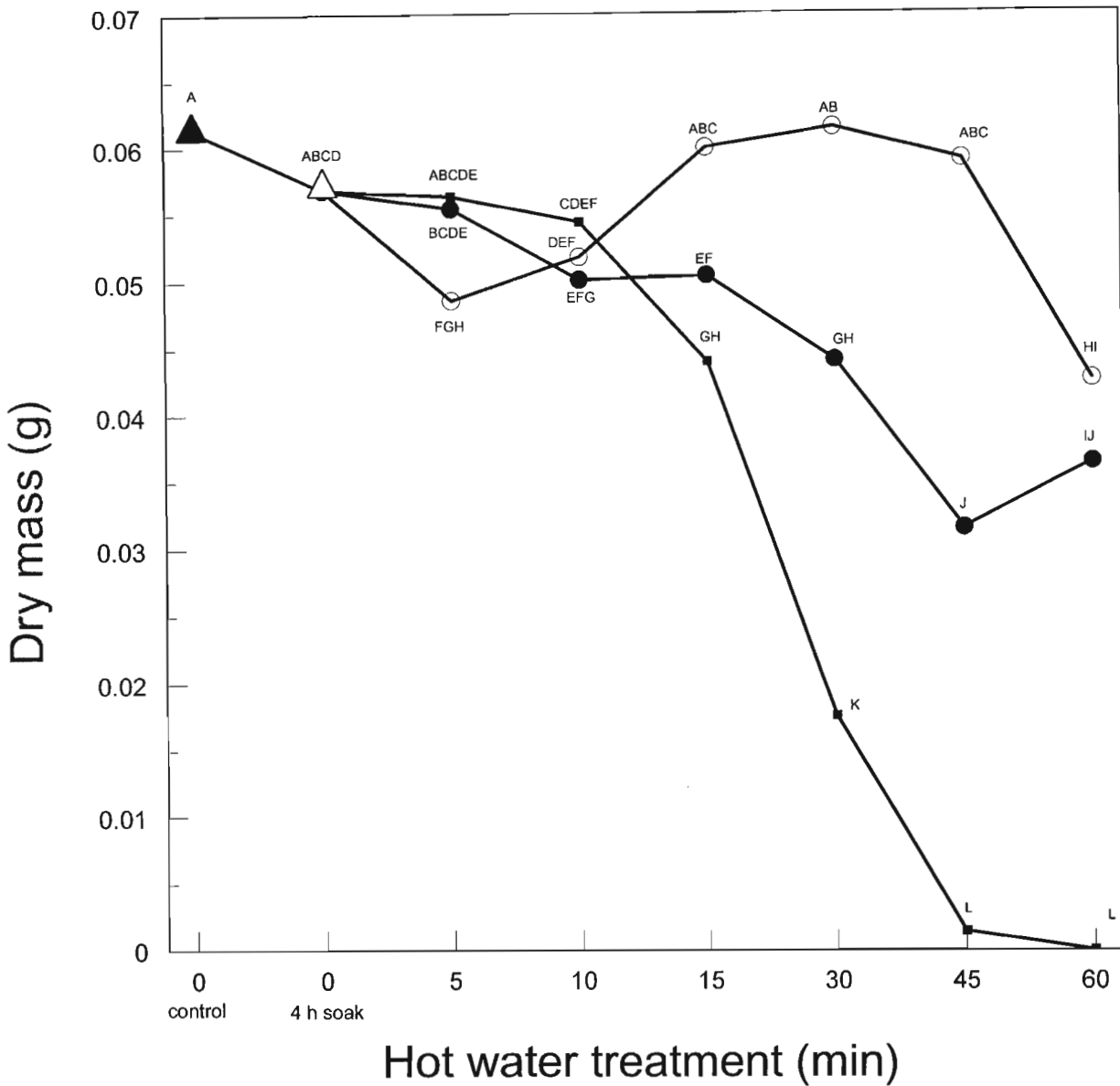
Figure 17 . Effect of storage for 1 month at 4 ^{0}C (33% RH) on seedling dry mass, assessed 18 days after the start of germination, from *Zea mays* seeds previously immersed in hot water at 55 (○), 57 (●) and 60 ^{0}C (■) for durations of 5 to 60 min, then dehydrated. These results are compared with those of unimbibed (control) seeds (▲) and seeds pre-imbibed for 4 h/dehydrated (△), and stored under the same conditions. n = 100 seeds (control = 300 seeds). Data points with different letters are significantly different (P<0.05).
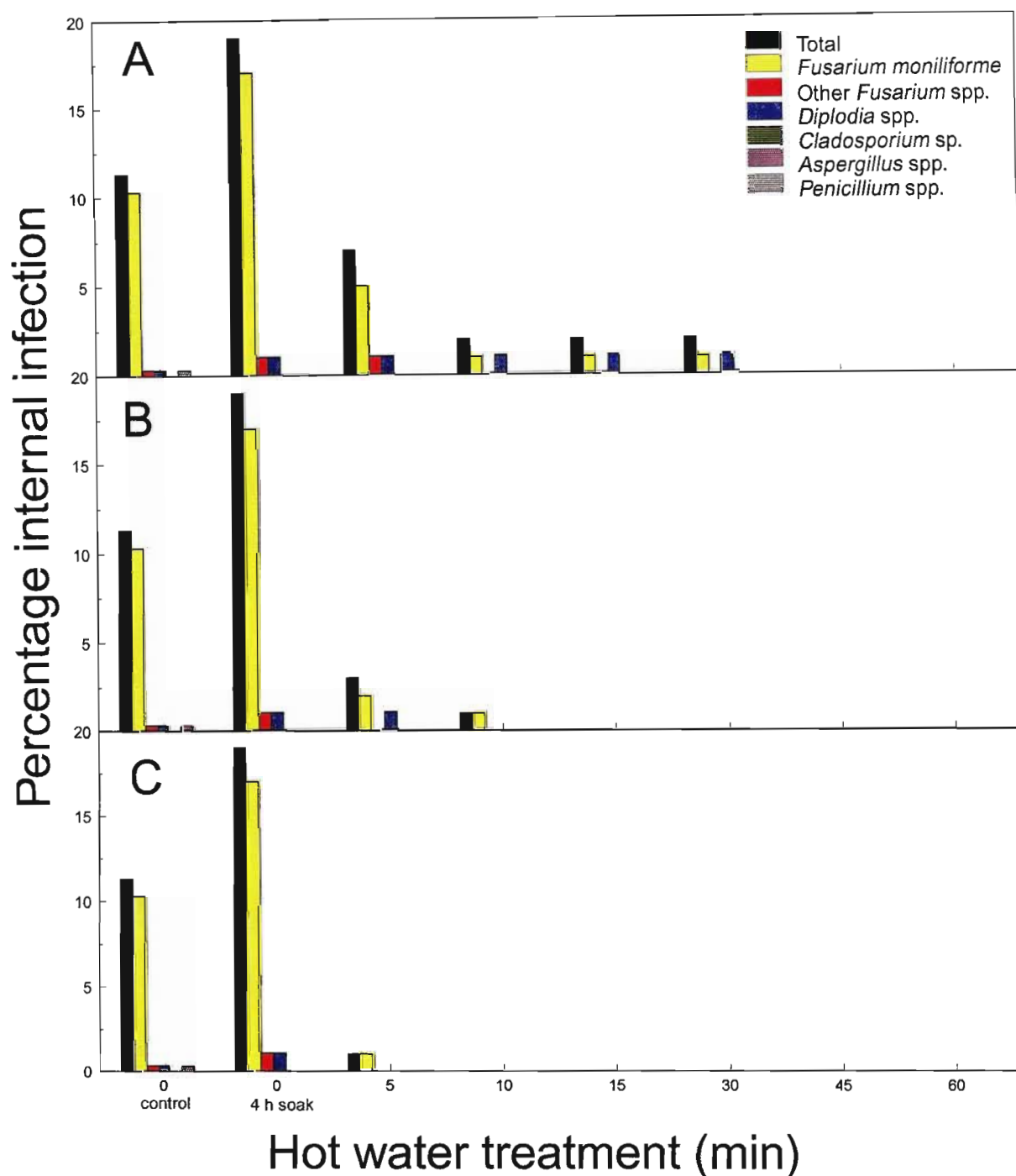
Figure 18. Effect of storage for 1 month at 25⁰ C (91% RH) on the percentage internal infection of *Zea mays* seeds previously immersed in hot water at 55 (A), 57 (B) and 60 ⁰C (C) for durations of 5 to 60 min, then dehydrated. These results are compared with those of unimbibed (control) seeds and seeds pre-imbibed for 4 h/dehydrated, and stored under the same conditions. n = 100 (control = 300).

3.4.2. Moisture content

Unimbibed (control) seeds stored for 1 month under ambient (25 ^{0}C), moist (91% RH) conditions attained embryo and endosperm mcs of 10.98 and 12.70%, respectively (Table 8). Embryos from seeds that had been previously imbibed for 4 h and then stored under the same conditions achieved a mc similar to those of the control seeds (Table 8). On the other hand, the endospermal tissues of these seeds, as well as for those seeds that had been previously hot water treated, were mostly characterised by water contents significantly higher than that of the control material (Table 8).

3.4.3. Conductivity

The total amount of electrolytes leaked by unimbibed (control) seeds after 1 month storage at 25 ^{0}C was significantly greater than that of seeds that had been previously imbibed for 4 h, re–dehydrated and stored under the same conditions (Table 9). Seeds that had been treated with hot water attained leakage levels similar to that of seeds that had been imbibed for 4 h only. In contrast, the rate at which electrolytes were leaked did not differ significantly among any of the treatments assessed, regardless of an almost 2–fold difference between the control seeds and seed that had been treated at 60 ^{0}C for a duration of 45 min (Table 9). This seems to imply a tremendous variability in the rate of leakage within any one treatment.

3.4.4. Germination

Seeds previously imbibed for 4 h, as well as those subsequently immersed in hot water (all temperatures and durations) were characterised by GI levels lower than that of the control seeds, when assessed after storage for 1 month under ambient conditions (Fig. 19C). These findings were corroborated by a decline in germination rate (Fig. 19A). Germination totality, however, appeared to be less affected with seeds previously imbibed for 4 h, as well as those treated at 55 ^{0}C for 5 min attaining levels higher than that of the control (Fig. 19B). Seeds previously treated at 60 ^{0}C for durations of 45 min and longer did not germinate following removal from ambient storage (Fig. 19).

Root and shoot growth appeared to be less affected as seeds previously imbibed for 4 h attained root and shoot lengths significantly longer than that of the control (Fig. 20).

Table 8. Effect of storage for 1 month at 25 ^{0}C (91% RH) on the water contents of re-dehydrated *Zea mays* seeds that were previously pre-imbibed and hot water treated, compared with those of unimbibed (control) seeds stored under the same conditions.

	Duration of hot water treatment (min)	Moisture content (%WMB)[a]	
		Embryo	Endosperm
Unimbibed; stored at 25 ^{0}C (91% RH)	0	10.98 de	12.70 b
Imbibed 4 h; re-dehydrated; stored at 25 ^{0}C (91% RH)	0	11.28 efg	13.72 ijk
Imbibed 4 h; hot water treated (55 ^{0}C); re-dehydrated; stored at 25 ^{0}C (91% RH)	5	10.94 cde	13.26 f
	10	11.64 fgh	13.39 fg
	15	10.41 bc	13.21 ef
	30	10.91 bcde	13.48 gh
	45	10.59 bcd	13.61 hij
	60	11.41 efg	13.61 hij
Imbibed 4 h; hot water treated (57 ^{0}C); re-dehydrated; stored at 25 ^{0}C (91% RH)	5	11.82 ghi	13.53 gh
	10	11.74 ghi	13.65 hij
	15	11.17 ef	13.61 hi
	30	11.36 efg	13.51 gh
	45	11.68 fghi	13.80 jk
	60	12.19 i	13.85 k
Imbibed 4 h; hot water treated (60 ^{0}C); re-dehydrated; stored at 25 ^{0}C (91% RH)	5	9.46 a	12.35 a
	10	10.40 b	12.63 b
	15	12.04 hi	13.59 hi
	30	11.41 efg	13.03 de
	45	11.01 de	12.91 cd
	60	9.60 a	12.80 bc

[a] Represents the mean of 25 seeds (except control, n = 75). Values with different letters within columns are significantly different (P < 0.05).

Table 9. Effect of storage for 1 month at 25 ^{0}C (91% RH) on the leakage of electrolytes[a] from re–dehydrated *Zea mays* seeds that were previously pre–imbibed and hot water treated, compared with those of unimbibed (control) seeds stored under the same conditions.

	Duration of hot water treatment (min)	Total leakage ($\mu A. g^{-1} DM$)	Leakage rate ($x10^{-2} \mu A.g^{-1} DM.s^{-1}$)
Unimbibed; stored at 25 ^{0}C (91% RH)	0	6.54 a	3.8 bcdef
Imbibed 4 h; re–dehydrated; stored at 25 ^{0}C (91% RH)	0	5.30 cde	2.3 ef
Imbibed 4 h; hot water treated (55 ^{0}C); re–dehydrated; stored at 25 ^{0}C (91% RH)	5	4.44 efg	4.7 abcde
	10	6.06 abc	5.4 abcd
	15	3.95 fg	3.9 abcdef
	30	5.65 bcd	5.7 abcd
	45	4.22 efg	3.4 def
	60	5.00 cdefg	5.8 abcd
Imbibed 4 h; hot water treated (57 ^{0}C); re–dehydrated; stored at 25 ^{0}C (91% RH)	5	4.64 defg	2.5 ef
	10	5.57 cd	3.0 def
	15	4.01 fg	5.5 abcd
	30	6.16 abc	3.3 def
	45	4.80 defg	3.2 def
	60	5.03 cdef	3.6 cdef
Imbibed 4 h; hot water treated (60 ^{0}C); re–dehydrated; stored at 25 ^{0}C (91% RH)	5	4.63 defg	5.7 abcd
	10	4.90 defg	1.7 f
	15	3.90 g	3.1 def
	30	5.18 cde	2.3 ef
	45	5.13 cde	6.3 ac
	60	5.24 cde	2.1 ef

[a] Represents the mean of 25 seeds (except control, n = 75) measured over a 10 h period, expressed on a dry mass (DM) basis. Values with different letters within columns are significantly different (P < 0.05).
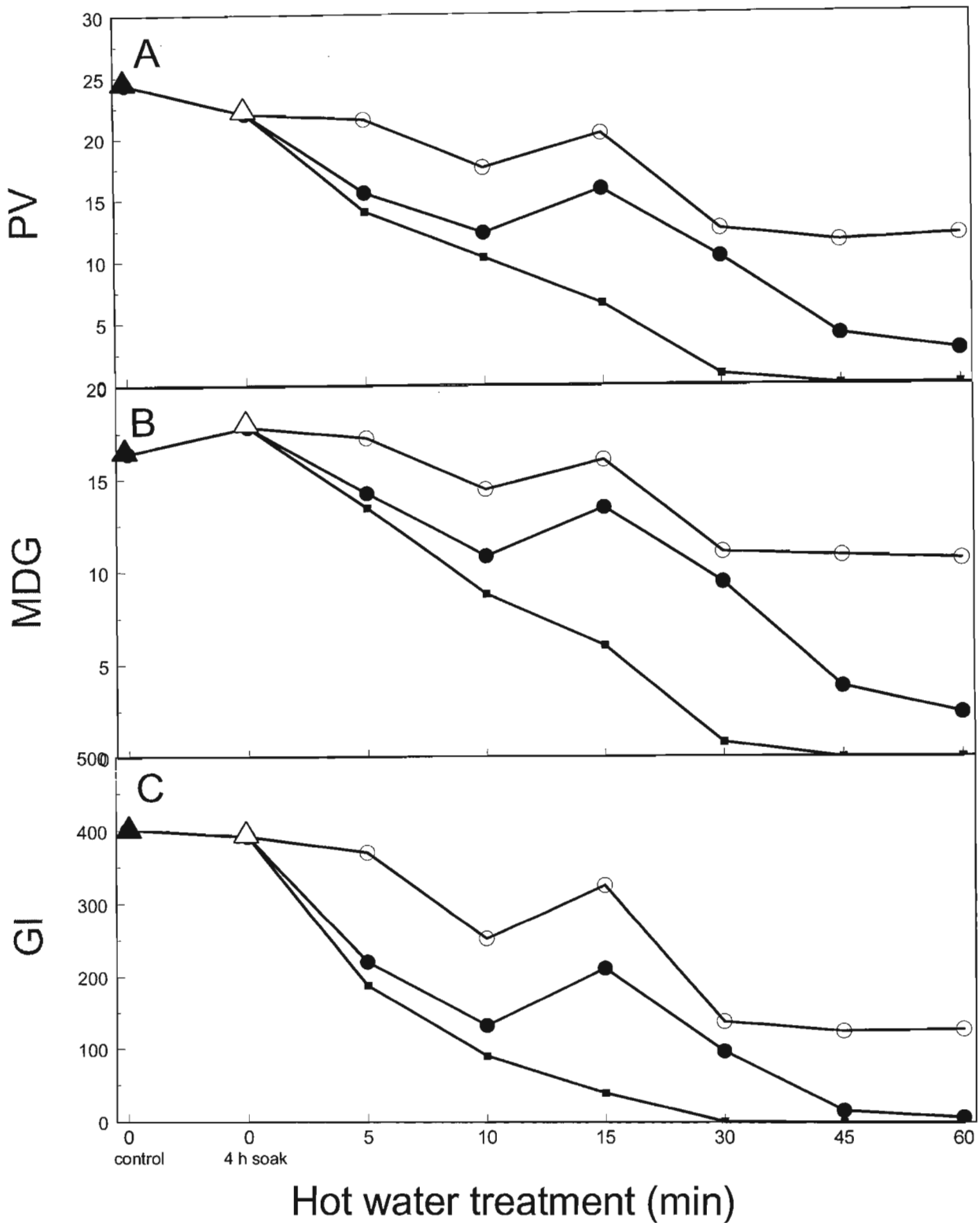
Figure 19. Effect of storage for 1 month at 25 ^{0}C (91% RH) on emergence rate (A), germination totality (B) and germination index (C) of *Zea mays* seeds previously immersed in hot water at 55 (○), 57 (●) and 60 ^{0}C (■) for durations of 5 to 60 min, then dehydrated. These results are compared with those of unimbibed (control) seeds (▲) and seeds pre-imbibed for 4 h/dehydrated (△), and stored under the same conditions. n = 100 (control = 300).
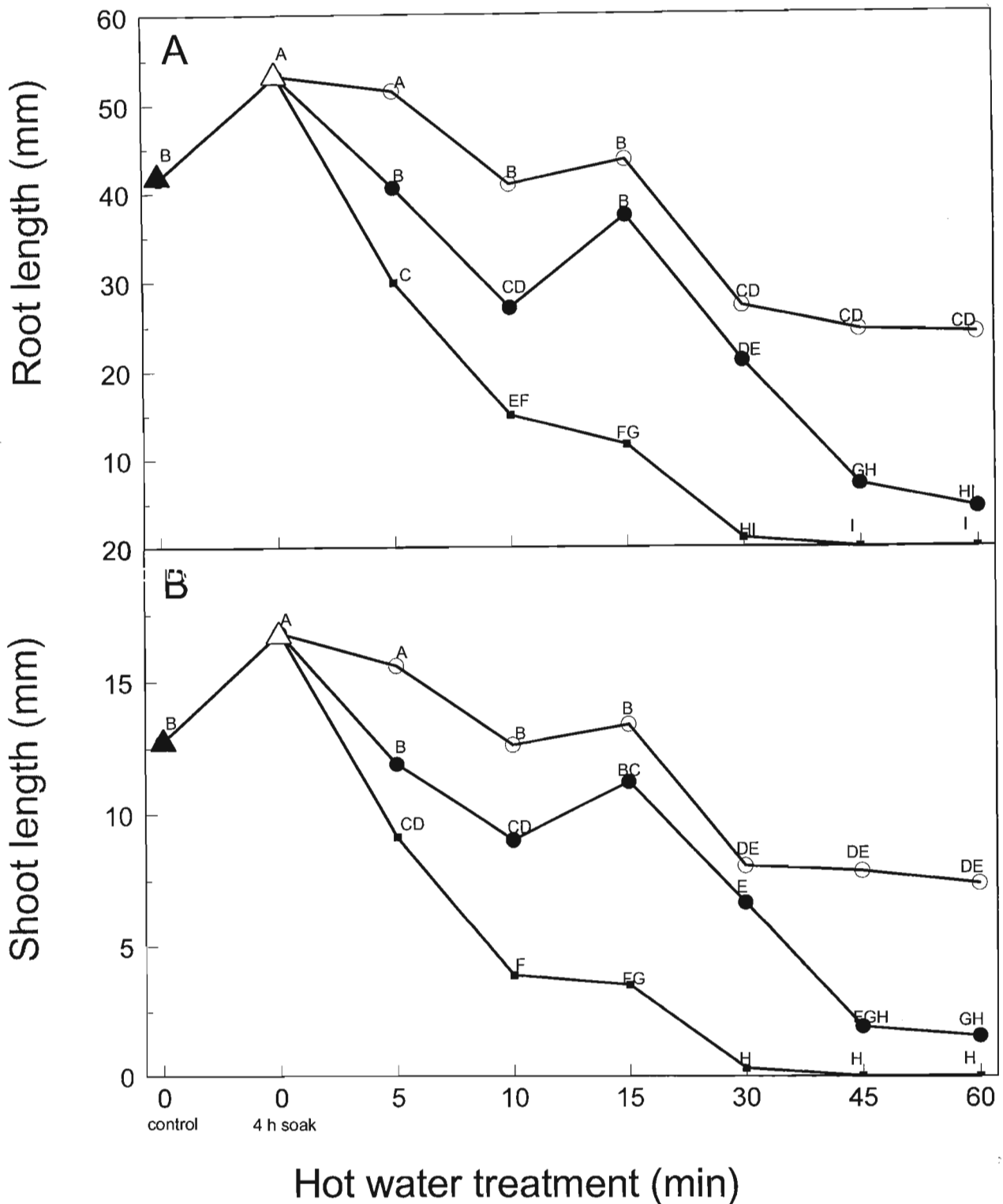
Figure 20. Effect of storage for 1 month at 25 ^{0}C (91% RH) on root (A) and shoot (B) growth from *Zea mays* seeds previously immersed in hot water at 55 (○), 57 (●) and 60 ^{0}C (■) for durations of 5 to 60 min, then dehydrated. These results are compared with those of unimbibed (control) seeds (▲) and seeds pre-imbibed for 4 h/dehydrated (△), and stored under the same conditions. n = 100 (control = 300). Data points with different letters are significantly different (P<0.05).

Similarly, for seeds that had been hot water treated at 55 and 57 ^{0}C for durations not exceeding 15 min the lengths of both these structures did not differ significantly from that of control seeds.

3.4.5. Seedling establishment

Unimbibed (control) seeds were characterised by 100% seedling establishment following storage for 1 month under ambient conditions (Fig. 21). Although seeds previously imbibed for 4 h, as well as those hot water treated for a duration of 5 min at both 55 and 57 ^{0}C, attained seedling emergence levels similar to that of the control (98 and 99%, respectively), seedling quantity was reduced for seeds treated for longer durations and increased temperature (Fig. 21).

The similarity between the control and 4 h treatments was also reflected in the quality of plants these seeds produced (Fig. 22). Interestingly, seeds treated at 55 and 60 ^{0}C for 15 and 5 min, respectively, also attained dry mass values similar to that of the control (Fig. 22), despite a decline in seedling number (Fig. 21), implying that the quality of plants produced from these seeds was superior to that of the control.
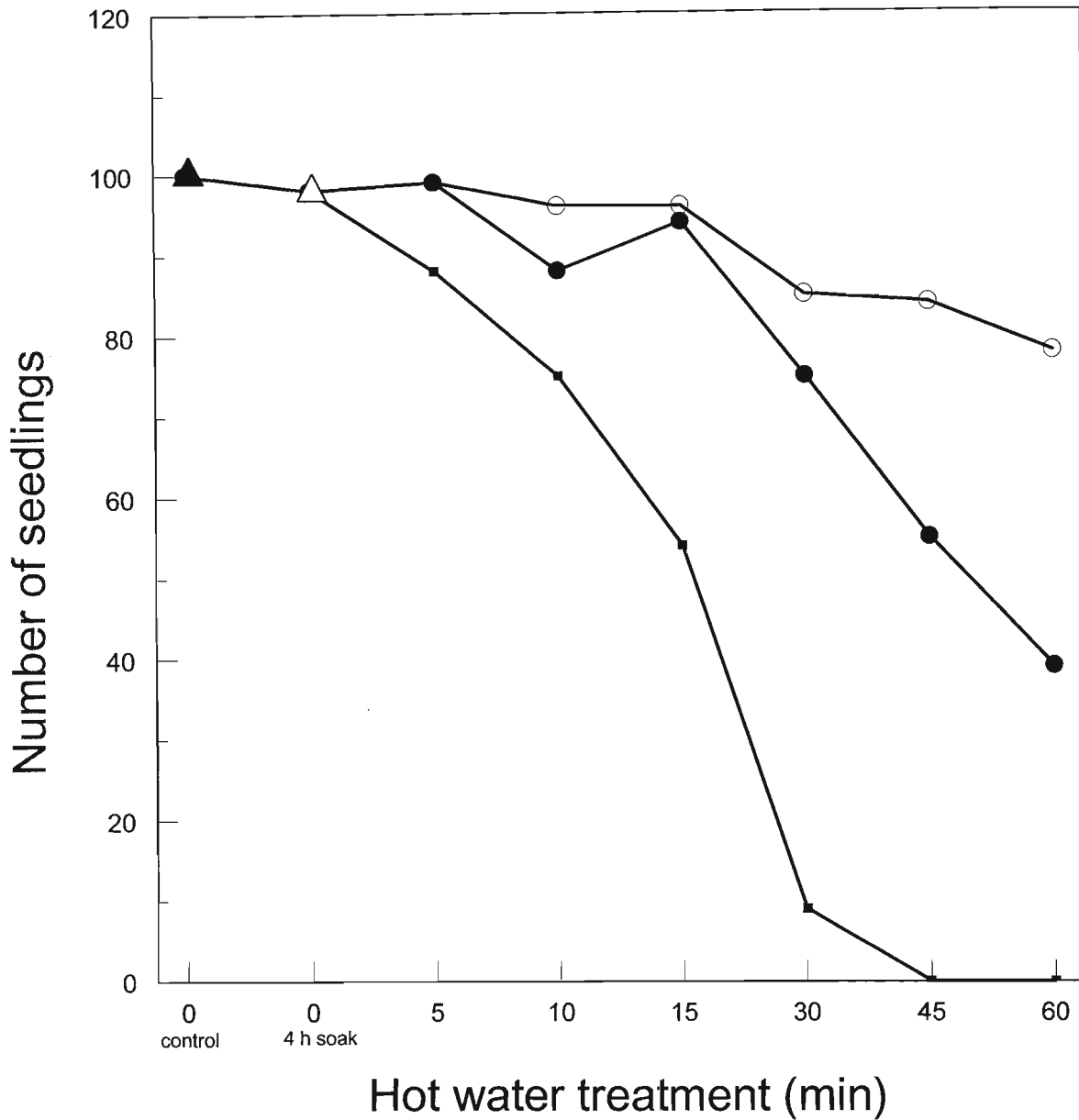
Figure 21. Effect of storage for 1 month at 25 °C (91% RH) on the number of seedlings, assessed 18 days after the start of germination, that developed from *Zea mays* seeds previously immersed in hot water at 55 (O), 57 (●) and 60 °C (■) for durations of 5 to 60 min, then dehydrated. These results are compared with those of unimbibed (control) seeds (▲) and seeds pre-imbibed for 4 h/dehydrated (△), and stored under the same conditions. n = 100 seeds (control = 300 seeds)
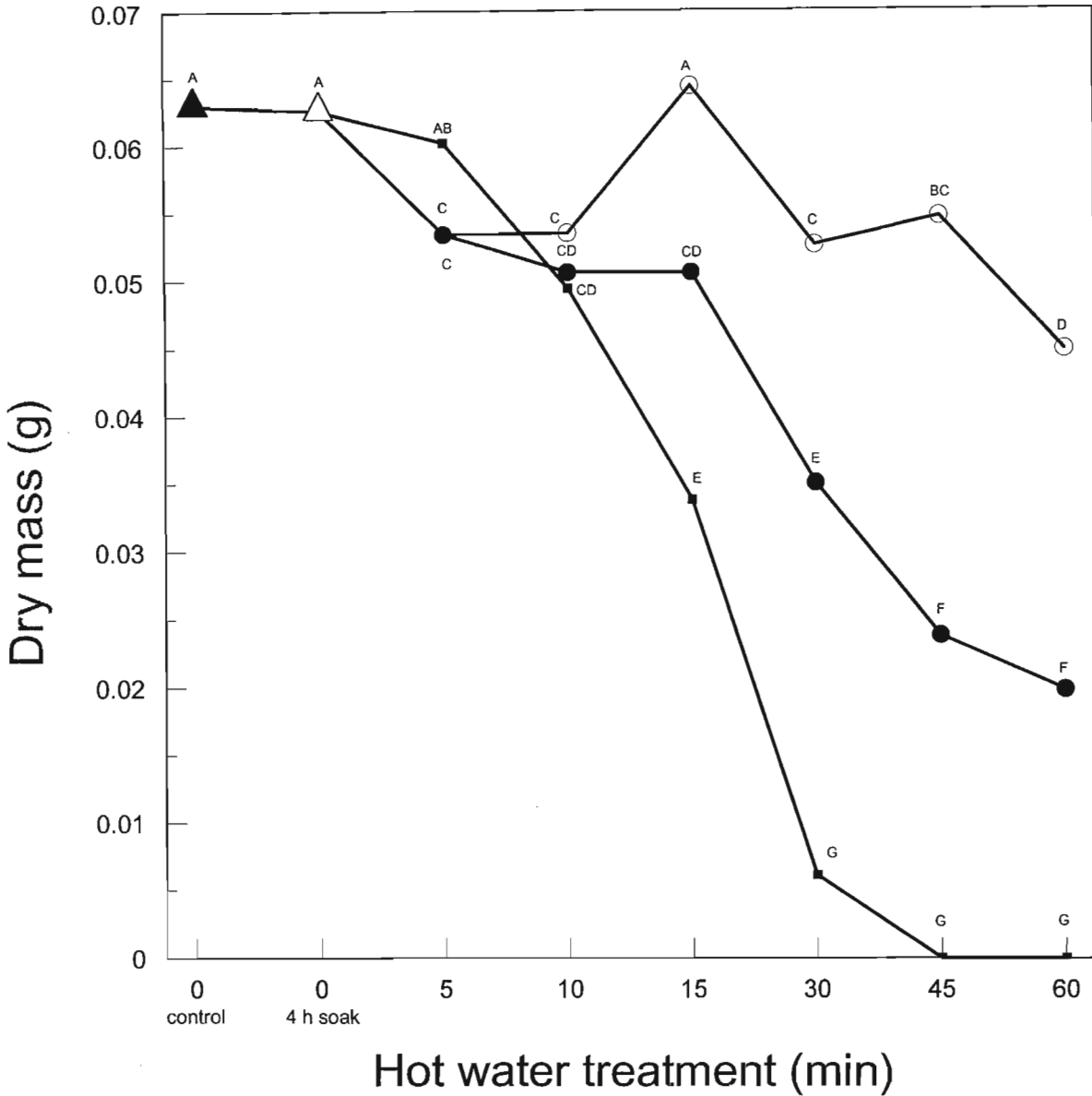
Figure 22 . Effect of storage for 1 month at 25 ^{0}C (91% RH) on seedling dry mass, assessed 18 days after the start of germination, from *Zea mays* seeds previously immersed in hot water at 55 (○), 57 (●) and 60 ^{0}C (■) for durations of 5 to 60 min, then dehydrated. These results are compared with those of unimbibed (control) seeds (▲) and seeds pre-imbibed for 4 h/dehydrated (△), and stored under the same conditions. n = 100 seeds (control = 300 seeds). Data points with different letters are significantly different (P<0.05).

Chapter 4.

4.1. DISCUSSION

The seeds used in this study exhibited relatively low levels of infection at the start, as only 20% of the unimbibed (control) seeds were infected (Fig. 3). The spectrum of fungi isolated from these seeds consisted of *Fusarium* spp., *Cladosporium* sp. and *Diplodia* spp. These fungi are members of the field fungus group (Christensen and Kaufmann, 1969; 1974; Christensen and Sauer, 1982), so-called as they are primarily associated with freshly harvested seed and require high moisture contents (facilitated by a relative humidity (RH) in excess of 90%) to grow. Of those fungi isolated, *Fusarium moniliforme* (Sheldon) predominated, constituting 89% of the total infection levels (Fig. 3). *F. moniliforme* is one of the most prevalent fungal spp. associated with maize seed and its products worldwide (Marasas *et al.*, 1984; Bullerman and Tsai, 1994). Recent surveys of South African commercial maize by Rheeder and co-workers have shown this to be the case locally, particularly for that maize grown in hot, dry areas of the country (Rheeder *et al.*, 1990a; Rheeder *et al.*, 1993; Rheeder *et al.*, 1995), conditions with which this fungus is usually associated (Marasas *et al.*, 1979). In addition, the seeds used in this study were of a yellow maize variety, which has been shown to harbour higher levels of this fungus than, for example, white maize (Rheeder *et al.*, 1990a; Rheeder *et al.*, 1993; Bullerman and Tsai, 1994; Rheeder *et al.*, 1995).

These seeds, however, had been hermetically stored at 4 ^{0}C for 2 years prior to use. According to Christensen and Kaufmann (1969; 1974), once the RH during storage declines below 90%, the field fungi are said to die out and the storage fungi, consisting of members of the genera *Aspergillus* and *Penicillium*, appear (Christensen and Kaufmann, 1969; 1974). However, the persistence of the field fungi, in particular the fusaria, during protracted cold storage has been previously reported (Agarwal and Sinclair, 1987; Marasas *et al.*, 1979; Russell *et al.*, 1982; Russell and Berjak, 1983; McLean and Berjak, 1987; Sayer, 1991; Berjak *et al.*, 1992). The longevity of seed-borne fungi is dependent on numerous factors, including the virulence of the fungus itself, the presence of antagonistic mycoflora and the nature of the storage environment, with cool, dry conditions being favourable for survival of the inoculum (Agarwal and Sinclair, 1987). The moisture contents of the control seed tissues (Table 1), as

well as the temperature at which these seeds had been stored, were below that required for fungal proliferation (Christensen and Kaufmann, 1969; 1974). In addition, the presence of *F. moniliforme* has been reported to be an important deterrent to the proliferation of other seed infecting fungi, including other *Fusarium* spp. (Van Wyk *et al.*, 1988; Rheeder *et al.*, 1990b), *Diplodia* spp. (Rheeder *et al.*, 1990b) and *Aspergillus flavus* (Zummo and Scott, 1992). Under these conditions, therefore, the levels of seed infection by the field fungi, particularly, as in this case, by *F. moniliforme*, are maintained during protracted storage. As suggested by Mycock and Berjak (1992a), the field fungi could also, therefore, be considered as facultative storage fungi.

In fact, *F. moniliforme* predominated in any sampling that yielded infection (Figs 3, 8, 13 and 18) and, consequently, the effect of any of the treatments used in this investigation on seed infection levels appeared to reflect almost entirely the response of this fungus.

Storage of the control seeds for 1 month at both 4 ^{0}C (33% RH) and 25 ^{0}C (91% RH) resulted in infection levels being reduced by 40% (Fig. 13) and 45% (Fig. 18), respectively, when compared with those seeds at the start of the investigation (Fig. 3). This was unexpected, particularly for those seeds that had been stored at 25 ^{0}C (Fig. 18), conditions that would normally encourage fungal proliferation (Christensen and Kaufmann, 1969; 1974). In order to assess the response of internally located seed mycoflora only, the seeds were dusted with Benlate prior to storage under either regime. Benlate is one of many commercially available systemic fungicides which, even when applied as dusts or slurries, are able to reduce fungal infections within seeds (Maude, 1983). The active ingredient, benzimidazole, has been shown to reduce *Fusarium* (Vidhyasekaran, 1983; Nakagawa and Yamaguchi, 1989; Champawat, 1990; Hawara and Kannaiyan, 1992; Wilson *et al.*, 1993), *Penicillium* (Wilson *et al.*, 1993) and *Aspergillus* (Gupta *et al.*, 1993) infection within seeds.

While the spectrum of fungi isolated in both cases (4 ^{0}C [33% RH] and 25 ^{0}C [91% RH]) again consisted almost entirely of *F. moniliforme* (Figs 13 and 18), *Aspergillus* spp. were isolated, although at low levels, from seeds that had been stored at 25 ^{0}C (91% RH)(Fig. 18). Seeds are hygroscopic, hence their moisture content comes to equilibrium with that of their

surrounding atmosphere (Roberts, 1972; Justice and Bass, 1978). While storage at 4 °C (33% RH) did not alter the mc of the seed tissues (compare Tables 1 and 6), those seeds stored at 25 °C (91% RH) were characterised by embryo and endosperm mcs as much as 16 and 6% (respectively) higher than those of seeds at the start of the investigation (compare Tables 8 and 1). Under such conditions of storage, the storage fungi are said to proliferate (Christensen and Kaufmann, 1969; 1974). When compared with those seeds that had been previously imbibed and stored under the same conditions (Fig. 18), it is apparent that the appearance of *Aspergillus* spp. in these seeds was associated only with reduced levels of *F. moniliforme* infection. Thus, as suggested by McLean and Berjak (1987), the succession of the seed-associated mycoflora during storage may not be dependent on the storage environment alone, as proposed previously by Christensen and Kaufmann (1969; 1974), but on interspecific competition as well.

In order to facilitate improved heat conduction during the hot water treatment, the seeds were pre-imbided for 4 h (Agarwal and Sinclair, 1987). Also, fungal propagules within the seed become metabolically active upon imbibition and, as a consequence, will be sensitive to heat damage (Agarwal and Sinclair, 1987). However, as some fungal spp. are tolerant to high temperatures, including some members of the genus *Aspergillus* (Hudson, 1986), it is imperative that the seeds be rapidly re-dehydrated to low moisture contents (McLean, 1980; Foster, 1982) so as to avoid deterioration by fungal propagules not eradicated during treatment (Agarwal and Sinclair, 1987). This is of particular importance for those seeds destined for storage (Christensen and Kaufmann, 1969, 1974). The level of internal infection was unaffected by either the 4 h hydration (Fig. 3) or 4 h hydration-dehydration (Fig. 8) treatments. However, the spectrum of fungi was reduced following dehydration, primarily due to the absence of *Cladosporium* sp. (Fig. 8). This corresponds with the proposal of Basu (1994) that germinating fungal propagules in hydrated seed may be killed upon re-dehydration.

Subsequent storage for 1 month at either 4 °C (33% RH) or 25 °C (91% RH) did not further affect the total levels of fungal infection within these seeds (Figs 13 and 18, respectively). As in the case of the control seeds, only members of the fusaria were isolated from the pre-

imbibed seeds, when assessed after cold, dry storage (Fig. 13), and this may be due to the Benlate dusting. Following 1 month of storage under ambient conditions, however, *Diplodia* spp. were isolated from the pre-imbibed seeds as well, possibly due to the higher mcs attained by these seeds (Table 8). In both cases, the high levels of *F. moniliforme* appeared to preclude the growth of *Aspergillus* spp.

Hot water treatments have been used widely for the control of seed–borne pathogens (Agarwal and Sinclair, 1987). The exact temperature and duration of treatment at which seed–associated fungi are eliminated, however, varies according to seed type and the species of pathogen with which it is infected (Daniels, 1983; Agarwal and Sinclair, 1987; Strandberg and White, 1989; Berjak *et al.*, 1992; Doornik, 1992; Hayden and Maude, 1992, Aveling *et al.*, 1993; Hayden and Maude, 1994). For this reason, a range of temperatures (55, 57 and 60 ^{0}C) and durations (5 to 60 min) of treatment were selected.

As previously reported (Daniels, 1983; Agarwal and Sinclair, 1987; Strandberg and White, 1989; Berjak *et al.*, 1992; Doornik, 1992; Hayden and Maude, 1992, Aveling *et al.*, 1993; Hayden and Maude, 1994), the efficacy of the hot water immersion in reducing seed infection levels increased with increasing temperature and duration of treatment (Fig. 3).Compared with unimbibed (control) material, seeds immersed in water for 5 min at 55 ^{0}C exhibited a 50% reduction in contamination, which decreased by a further 35% after an immersion duration of 15 min (same temperature). On the other hand, seeds treated at 57 and 60 ^{0}C exhibited no infection after a treatment duration of only 15 min (Fig. 3). As suggested by Berjak *et al.* (1992), the varying ability of both temperature and duration of the hot water treatment in reducing seed infection may be related to the location of the fungal propagules within the seed. The marked reduction in infection levels after only 5 min treatment (all temperatures) may be explained in terms of the peripheral location of *Fusarium* spp., particularly *F. moniliforme*, within the pericarp (Russell and Berjak, 1983; Gopinath and Shetty, 1985; Agarwal and Sinclair, 1987) and pedicel (Zummo and Scott, 1990; Bacon *et al.*, 1992) of maize seeds. Some members of the genus *Aspergillus* have also been found to be peripherally located (Mycock *et al.*, 1988; Smart *et al.*, 1990; Zummo and Scott, 1990, 1991; Mycock and Berjak, 1992), which appears to be the case in these seeds, as members of this genus did not

survive a treatment duration of 10 min at 55 °C. The elimination of *Cladosporium* sp. by only 5 min treatment at 55 °C would suggest this fungus to also be located peripherally and may explain its intolerance to the hydration–dehydration treatment evidenced in those seeds pre-imbibed for 4 h (Fig. 8)[see above]. Both the embryo and endosperm of seed immersed in hot water (all temperatures) exhibited a slight, though significant, increase in water content after only 5 min immersion, when compared with those seeds imbibed for 4 h only (Table 1). This is in agreement with Oguntunde and Adebawo (1989) who demonstrated that the temperature of imbibition also affected the level of water uptake. Immersion for 30 min at all temperatures resulted in an even further increase in water content of both the embryonic and endospermal tissues (Table 1) which was correlated with 2 % infection at 55 °C and no infection either at 57 or 60 °C. As *Fusarium* spp. and *Aspergillus* spp. are also reportedly located within, or at least adjacent to, the seed embryonic tissue (Gopinath and Shetty, 1985; Agarwal and Sinclair, 1987; Mycock *et al.*, 1988; Smart *et al.* 1990; Zummo and Scott, 1990, 1991; Bacon *et al.*, 1992; Mycock and Berjak, 1992b) it is probable that longer durations of treatment are required for the elimination of embryo associated infections. The persistence of *Diplodia* spp. (Fig. 3) suggests these fungi to be located within or near the tissues of the embryo as well.

Re–dehydration appeared to enhance the efficacy of the hot water treatment for controlling seed–associated mycoflora. Seeds treated at both 57 and 60 °C exhibited no apparent infection after a treatment duration of only 10 min (Fig. 8). It is possible that those germinating fungal propagules that survived the hot water treatment were adversely affected, thus becoming more susceptible to eradication upon subsequent re–dehydration. In addition the activity of fungi within seeds is reported to be dependent on the water content of the seed tissues, with dry seeds yielding lower levels of infection (Christensen and Kaufmann, 1969, 1974). These seeds were, in most cases, dried to mc levels lower than those of the control (Table 4).

Berjak *et al.* (1992) have reported that cold storage following heat treatment retained seed infection at low levels (c.f. Fig. 13) and ascribed this effect to a possible 'cold shock' stress induced by the low temperature at which the treated seeds were stored. This, however, was also the case following ambient storage (Fig. 18), during which time seed mcs increased (Table 8); conditions reported to encourage fungal proliferation (Christensen and Kaufmann,

1974). As suggested by Daniels (1983), therefore, it would appear that the effect of the hot water treatment on reducing infection levels is by way of elimination, rather than fungistasis. However, due to the uncontrolled possible effects of Benlate on seed internal fungal status during storage (Figs 13 and 18), any conclusions concerning the effects of the hot water treatment only must, therefore, be treated with caution.

Placing seeds directly into water, as was done in this study, may induce soaking injury which is manifested as a reduction in germination due to both metabolic and physical disruptions upon imbibition (Ellis *et al.*, 1990; Martin *et al.*, 1991; Pretorius and Small, 1991, 1992; Bewley and Black, 1994). However, this was not the case in the present study as seeds imbibed for 4 h exhibited higher rates of emergence (Fig. 4A) and longer root and shoot lengths (Fig. 5A and B) than those of the unimbibed (control) seeds. These effects were coupled with an increase in the water contents of both the embryo and endosperm to levels greater (by 3 and 2-fold, respectively) than those of the unimbibed (control) seeds (Table 1). The enhanced rate of water uptake by the embryos of maize seed has been previously reported (McDonald *et al.*, 1994) and described as a consequence of both physical (pericarp) and chemical (seed part composition) factors. As metabolic processes are initiated upon the re-hydration of seed tissues (Bewley and Black, 1994) and the early stages of germination appear to be dependent on energy reserves from the embryo (McDonald *et al.*, 1994), the preferential hydration of the embryonic tissues could have caused these seeds to be in a more advanced state of germination than those of the control (McDonald *et al.*, 1994).

As seeds take up water, solutes such as sugars, organic acids, ions, amino acids, and proteins are leaked into the surrounding medium (Simon, 1974; Bewley and Black, 1994). The time course of leakage from imbibing seeds has been shown to be comparable to that of water uptake (Simon and Mathavan, 1986; Bruggink *et al.*, 1991). The rate of water uptake by dry seeds during imbibition is very rapid at first (Oguntunde and Adebawo, 1989; Bewley and Black, 1994; McDonald *et al.*, 1994) due to the high matric potential of the dry seed tissues (Bewley and Black, 1994). Similarly, there is a corresponding rapid loss of electrolytes during this first phase of imbibition (Simon, 1974; Senaratna and McKersie, 1983; Simon and Mathavan, 1986; Bruggink *et al.*, 1991) as electrolytes are leached from extracellular sites

such as the seed surface, intercellular spaces, and ruptured cells (Senaratna and McKersie, 1983; Lott *et al.*, 1991). For maize seed the pericarp has been shown to be the main source of electrolytes during the first hour of imbibition (Bruggink *et al.*, 1991; Beecroft and Lott, 1993). As the cells become progressively hydrated the rate at which water is taken up slows down until a steady state is reached (Oguntunde and Adebawo, 1989; Bewley and Black, 1994; McDonald *et al.*, 1994). During this phase the leakage of electrolytes slows down (Simon, 1974; Senaratna and McKersie, 1983; Simon and Mathavan, 1986; Bruggink *et al.*, 1991) as the plasma membrane regains its function as a diffusion barrier (Simon, 1974; Senaratna and McKersie, 1983; Bewley and Black, 1994), until a steady rate of leakage is achieved. The extent of leakage during this phase, therefore, reflects the ability of cell membranes to re-organise and repair themselves (Roberts, 1981; Nath *et al.*, 1990; 1991, Basu, 1994; Davidson *et al.*, 1994).

The enhanced germination performance (Figs 4 and 5) of seeds imbibed for 4 h was associated with a reduction in both the rate and level of electrolytes leached from these seeds (Table 2), when compared with those of the control. It is probable that membrane control of permeability in those seeds imbibed for 4 h was re-established, at least partially, during the pre-soak treatment, thereby resulting in lower levels of leakage during conductivity measurement. Seeds pre-imbibed for 4 h therefore, had a longer time for membrane repair mechanisms to operate which, as suggested by Davidson *et al.* (1994), could result in seed invigoration. Membrane repair mechanisms are suggested to be one of the underlying factors responsible for seed priming (Pandey, 1989). The reduced leakage of electrolytes by those seeds imbibed for 4 h may also reflect the loss of readily leachable electrolytes (Davidson *et al.*, 1994), for example those located in the pericarp (Bruggink *et al.*, 1991), during the pre-imbibition treatment.

Seed invigoration, as a consequence of the 4 h soak, was retained in these seeds after they had been re-dehydrated. The vigour and viability of these seeds (Fig 9C) and the quality of roots and shoots they produced (Fig. 10A and B) was similar to those soaked for 4 h only (Figs 4C, 5A and B, respectively). This suggests that the re-dehydration regime used was not too severe. The maintenance of seed invigoration effects upon re-dehydration has been

previously reported (Indris and Aslam, 1975; Nath *et al.*, 1990, 1991; Basu, 1994; Davidson *et al.*, 1994; Jansen and Ison, 1994) and is limited by the rate of drying (Justice and Bass, 1978; McLean, 1980; Foster, 1982) and the duration of imbibition (Senaratna and McKersie, 1983; Leprince *et al.*, 1990, Coolbear, 1992; Hong and Ellis, 1992; Leprince *et al.*, 1992; Davidson, *et al.*, 1994; Bewley and Black, 1995). If the duration of imbibition exceeds the time at which germination processes are initiated, the seeds become intolerant to subsequent dehydration (Senaratna and McKersie, 1983; Leprince *et al.*, 1990, Coolbear, 1992; Hong and Ellis, 1992; Leprince *et al.*, 1992; Davidson, *et al.*, 1994; Bewley and Black, 1995). As in the case of seeds assessed immediately after the 4 h soak, both the level and rate of leakage from seeds assessed after subsequent re–dehydration remained low (compare Tables 2 and 5, respectively). The change in the nature of the cell membranes, as a result of the 4 h soak, was, therefore, retained in these seeds upon re–dehydration, providing support for the theory of repair mechanisms (Roberts, 1981; Nath *et al*, 1990, 1991; Basu, 1994; Davidson *et al.*, 1994). As a result, maize seed has been shown to be tolerant to successive hydration–dehydration cycles (Davidson *et al.*, 1994).

Despite these invigoration effects, however, seedling emergence was negatively affected in both the 4 h hydration (Fig. 6) and hydration–dehydration (Fig. 11) treatments. This decline in seedling emergence could possibly be attributed to post emergence damping off (Agarwal and Sinclair, 1987). As mentioned previously, imbibing seeds, either for germination or pre–soaking treatments, may stimulate pathogen growth (Agarwal and Sinclair, 1987) and, in fact, actively growing mycelia could be seen in some cases in this study. Neither the level of fungal infection nor the incidence of *F. moniliforme* was affected by the 4 h hydration (Fig. 3) and 4 h hydration–dehydration treatments (Fig. 8), being similar to those of the control material in both cases. The reported effects of seed infection by *F. moniliforme* on germination and seedling emergence, however, are conflicting. While some workers have shown these to be adversely affected by *F. moniliforme* (Kulik and Schoen, 1982; Forbes *et al.*, 1989; Headrick and Pataky, 1989; Headrick *et al.*, 1990), others have found seed germination to be unaffected when caryopses are infected with this fungus (Kulik and Schoen, 1982; Naik *et al.*, 1982; Rheeder *et al.*, 1990b, 1995). These differences could, in part, be accounted for by differences in environmental factors and the virulence of the *F. moniliforme*

strain with which the seeds are infected (Kulik and Schoen, 1982). On the other hand, other *Fusarium* spp. (Nakagawa and Yamaguchi, 1989; Khanam, 1990; Rheeder *et al.*, 1990b; Rosas Romero, 1991; Wong *et al.*, 1992) and *Diplodia* spp. (Rheeder *et al.*, 1990b), which were also isolated from these seeds, have been reported to be aggressive pathogens, adversely affecting germination and seedling establishment. Therefore, as seeds imbibed for 4 h, and those subsequently re-dehydrated were characterised by moisture contents higher than those of the control (Tables 1 and 4, respectively), the reduction in post-emergence seedling survival may have been due to damping off by pathogenic fungi found within these seeds. As a consequence, seedling quality was also adversely affected in those seeds assessed after re-dehydration (Fig. 12).

Seed vigour and viability, expressed as the germination index, was negatively affected by immersion in hot water, the extent of which increased with increasing duration and temperature of treatment (Fig. 4C). Although seeds treated at 55 and 57 ^{0}C for durations of 15 and 5 min, respectively, attained GI levels similar to that of the control, these seeds were characterised by GI levels lower than those of seeds imbibed for 4 h only (Fig. 4C). The negative effect of hot water immersion was greater for the rate of emergence than germination totality (compare Fig. 4A and B), and was reflected in the quality of roots and shoots these seeds produced (Fig. 5A and B). A reduction in seed vigour prior to any loss of viability is one of the characteristic manifestations of seed ageing (Berjak and Villiers, 1970, 1972a,b,c,d; Roberts, 1972; Justice and Bass, 1978). Work by Bhattacharyya *et al.* (1985) and Furbeck *et al.* (1989) have shown the effects of hot water immersion to be comparable to that of accelerated ageing treatments. One of the consequences of accelerated ageing is that membranes undergo deteriorative changes that are manifested upon imbibition (Berjak and Villiers, 1972a,b,c,d; Berjak *et al.*, 1986). Electrolyte conductivity measurements have confirmed this, with more electrolytes being leached with increasing seed age (Pandey, 1989; Bruggink *et al.*, 1991).

In the present study, however, this was not the case. The levels of electrolyte leakage from seeds that had been imbibed in hot water were similar to those that had been imbibed for 4 h only (Table 2). In fact, seeds immersed in hot water leached electrolytes at lower rates than

those imbibed for 4 h only, the degree of which decreased with increasing temperature of treatment. This seems to suggest that the ability of the membranes to regain selective permeability, as a consequence of the 4 h imbibition, was enhanced by the temperature of hot water immersion, thus negating possible membrane effects. When determining the amount of leakage during treatment, however, it was apparent that leakage increased with increasing temperature of treatment, particularly for those seeds treated for longer durations (Table 3). The loss of essential solutes necessary for germination (Lott *et al.*, 1991; Davidson *et al.*, 1994) may also have contributed towards the reduced germination performance of the hot water treated seeds. Membrane alterations, however, are not the only events associated with seed deterioration. Other events, as reviewed by Smith and Berjak (1995), include chromosomal abberations and damage to DNA, changes in the synthesis of RNA and proteins, changes in enzymes and food reserves, and differences in respiratory activity and ATP production. Dreyer and Van de Venter (1992) have reported reduced mitochondrial activity in the shoots of maize kernels immersed for 2 h at 46 ^{0}C.

These proposed accelerated ageing effects appear to be short term, however, for those seeds that had been immersed in water at 55, 57 and 60 ^{0}C for durations up to 60, 30 and 10 min, respectively, as these seeds achieved seedling dry mass levels similar to those of the control (Fig. 7). Similar findings were reported by Berjak *et al.* (1992) for maize seeds treated at 55 ^{0}C for durations up to 45 min. In fact, seeds subjected to the above–mentioned temperatures and durations of treatment achieved seedling emergence levels similar to those of the seeds imbibed for 4 h (Fig. 6) thereby suggesting that seedling quality (Fig. 7) was enhanced as a consequence of the hot water immersion at these treatments. Daniels (1983) found that reduced seed infection by *F. moniliforme*, as a consequence of hot water immersion, was correlated with reduced levels of subsequent seedling infection. Thus considering the potential pathogenic ability of this fungus, as well as that of the persistent *Diplodia* spp (Rheeder *et al.*, 1990b), the reduction of these pathogens as a consequence of the hot water immersion could have improved seedling quality. This was particularly evident in those seeds treated for 15 and 5 min at 55 and 57 ^{0}C, respectively, probably due to reduced embryo associated infections. Contaminants located within these tissues may be systemically transmitted to, and as a result, could affect the growth of, developing seedlings (Agarwal and Sinclair, 1987). In

this regard, recent reports suggest that members of the storage fungi, most notably *Aspergillus* spp., can also be systemically transmitted to (Mycock *et al.*, 1990, 1992) and affect the growth of (Hyder–Ali and Fakir, 1992; Gupta *et al.*, 1993) developing seedlings. Berjak *et al.* (1992) have reported the efficacy of hot water treatments for reducing *Aspergillus* infection in seeds within which these species predominated. In addition, such treatments have been shown to reduce the systemic transmission by members of this genus (Hayden and Maude, 1992). It is possible, therefore, that seedling quality could also be improved in seeds infected predominantly by *Aspergillus* spp. due to the elimination of these fungi by hot water treatments.

Despite a slight decline in emergence rate (Fig. 9A), re–dehydration of those seeds previously immersed in hot water yielded improved vigour and viability (Fig. 9C), root and shoot growth (Fig. 10A and B), seedling emergence (Fig. 11) and seedling quality (Fig. 12) for most treatments when compared with those of similar seeds assessed immediately after treatment (Figs 4C, 5A and B, 6 and 7). The invigoration of aged seeds by hydration–dehydration treatments has been reported (Pandey, 1989; Nath *et al.*, 1990, 1991; Penaloza and Eira, 1993; Basu, 1994) and, as in the case of seeds imbided for 4 h then re–dehydrated, could to be due to the retention of metabolic repair mechanisms, activated during hydration, upon re-hydration. Basu (1994) has proposed that a reduction in free radical–induced lipid peroxidation is instrumental, at least in part, for the beneficial effects of seed invigoration treatments. However, this must be accompanied by repair mechanisms of previously accumulated damage. It is generally agreed that free radicals, produced during seed ageing, may react with the lipids of membranes ultimately leading to membrane deterioration (Priestley and Leopold, 1983; Priestly, 1986; Wilson and McDonald, 1986; Bajavarajappa *et al.*, 1991; Vertucci, 1992; Hendry, 1993; Smith and Berjak, 1995). The proposed mechanisms by which such deterioration is reduced by hydration–dehydration treatments includes the activation of free radical scavenging enzymes and the leaching out of these toxins upon imbibition (Basu, 1994). These seeds were characterised by leakage levels similar to, and leakage rates lower than, those seeds imbided for 4 h, followed by re–dehydration (Table 5). In addition the enhanced quantity (Fig. 11) and quality (Fig. 12) of the seedlings these seeds produced may also be due to the reduced infection levels (Fig. 8).

The lifespan of seed is limited, thus during storage seeds will age naturally, the rate of which is dependent on the species, the seed moisture content and the temperature of storage environment (Justice and Bass, 1978; Berjak et al., 1986). Seed ageing is usually manifested by a reduction in seedling vigour, a narrowing of the environmental range over which the seeds germinate, and ultimately, as total loss of viability (Berjak and Villiers, 1970; 1972a,b,c,d; Roberts, 1972; Justice and Bass, 1978). Under conditions of cold, dry storage, those being described as optimal (Justice and Bass, 1978), the vigour and viability of maize seed has been shown to be unaffected by storage of up to 8 years (Berjak et al., 1986). It is not surprising, therefore, that the seeds used in this study, being of a high quality when received, maintained germination levels of 98% (results not shown) even after 2 years cold, dry storage. Also, the vigour and viability of these seeds, as represented by the germination index (GI), was not affected after further storage for 1 month at 4 $^{\circ}$C (33% RH) (compare Figs 4C and 14C). Those seeds stored under cold, dry conditions, however, were characterised by reduced root and shoot growth (Fig. 15A and B) when compared with those of similar seeds assessed at the start of the investigation (Fig. 5A and B). In addition, although the number of seedlings produced after cold storage was similar to those of seeds at the start of the experiment (compare Figs 16 and 6), the quality of these seedlings was reduced, attaining mean dry mass levels lower than those of the unstored control seeds (compare Figs 17 and 7). This may be as a result of the Benlate dusting, which has been reported to affect seed germination adversely (Champawat, 1990; Aveling et al., 1993). In fact, the use of Benlate in agricultural practices has diminished as this fungicide has been associated with a number growth abnormalities, included stunted growth (Kelly, 1993). These effects were exacerbated by storage of the control seeds at 25 $^{\circ}$C (91% RH). When compared with those stored under cold, dry conditions, seeds stored under ambient conditions attained a GI value 27% lower than those seeds assessed at the start (compare Figs 4C and 19C). This decline was associated with an increase in the time taken for emergence (Fig. 19A) as well as a reduction in germination totality (Fig. 19B), and was reflected in the roots and shoots these seeds produced (Fig. 20A and B). This may be ascribed to accelerated seed ageing due to the higher temperature and RH of the environment in which these seeds were stored (Justice and Bass, 1978). As in the case if the control seeds assessed after cold storage, seeds stored under ambient conditions exhibited reduced seedling quality (Fig. 22), despite improved levels of

seedling emergence (Fig. 21) when compared with those seeds at the start of the experiment (Figs. 7 and 6, respectively). The increase in seedling number following both cold and ambient storage (Figs 16 and 21, respectively) corresponds with the decline in infection levels (Figs 13 and 18, respectively) which, as mentioned previously, appeared to be possibly a result of the Benlate treatment.

Despite the initial invigoration of the seeds used in this study by the 4 h hydration–dehydration treatment, subsequent storage of these seeds at 4 ^{0}C (33% RH) resulted in reduced vigour and viability (Fig. 14C) and hence root and shoot growth (Fig. 15A and B) when compared with those assessed immediately after the priming treatment (Figs 9C, 10A and B, respectively). According to Basu (1994), unless seeds subjected to hydration–dehydration treatments are thoroughly dried back, storage may accelerate deterioration. In the present study, seeds imbibed for 4 h were not dried back to the mc of the control seeds (Table 4). However, after 1 month of storage at 4 ^{0}C (33% RH) the embryo had reached a mc comparable to the control seeds, but the endosperm retained a higher mc (Table 6). Despite the final mc of the embryo, the time taken to reach the mc level of the control seeds may have allowed for physiological deterioration of the embryo and would account for the reduced vigour. These effects were exacerbated in similar seeds stored at 25 ^{0}C (91% RH) for 1 month. These seeds attained GI values (Fig. 19C) and root and shoot lengths (Fig. 20A and B) lower than those of similar seeds stored under cold conditions (Figs 14C, 15A and B, respectively), which would be associated with the higher seed mcs (compare Tables 6 and 8).

The number of seedlings produced after storage under both regimes was greater than those assessed immediately after the hydration–dehydration treatment (compare Figs 11, 16 and 21). This increase in seedling number was not associated with decreased levels of infection, but, as a systemic fungicide, Benlate may have prevented the systemic transmission of pathogens from infected seed to the growing plant, thereby preventing seedling death. Despite the increase in seedling number, those seeds that had been cold stored produced seedlings with a mean dry mass similar to those assessed immediately after the hydration–dehydration treatment (compare figures 17 and 12), suggesting that seedling quality was ultimately reduced. On the other hand, those seeds stored at 25 ^{0}C (91% RH) produced more plants (Fig.

21) of superior quality (Fig. 22).

Storage under both cold and ambient conditions resulted in reduced vigour and viability of those seeds that had been wet heat treated, more so under conditions of ambient storage (Fig. 19C) than cold storage (Fig. 14C) when compared with seeds assessed immediately after re-dehydration (Fig. 9C). The lag in germination rate (Figs 14A and 19A) and decline in germination totality (Figs 14B and 19B) exhibited by heat treated seeds, as previously reported by Berjak *et al.* (1992) and evident in those seeds assessed immediately after re-dehydration (Fig 9A and B), was exacerbated as a consequence of storage under both of the regimes tested. Root and shoot growth was also reduced by storage under both cold (Fig. 15A and B) and ambient (Fig. 20A and B) conditions, when compared with heat treated seeds assessed after re-dehydration (Fig. 10A and B). Seeds heat treated at 55 ^{0}C for 15 min attained GI values similar to those of 4 h pre-imbibed seeds when both were assessed after cold storage (Fig. 14C). Unlike those from the latter, the hot water treated seeds attained mcs similar to those of the control seeds when assessed following both dehydration (Table 4) and subsequent cold storage (Table 6). The reduced germination performance of the heat treated seeds following cold storage, therefore, does not appear to be correlated with the accelerated deterioration proposed for those seeds pre-imbibed for 4 h and stored under the same conditions. It is possible that this decline, as previously suggested to the case for the control seeds, could, in part, be attributed to adverse effects of the Benlate treatment, and was also reflected in the quality of the seedlings these seeds produced (Fig. 17). Despite the slight enhancement in seedling number produced by heat treated seeds after cold storage (Fig. 16), when compared with that of similar seeds assessed after re-dehydration (Fig. 11), the mean dry mass attained by these seedlings was lower than those assessed after re-dehydration (compare Figs. 12 and 17). However, when compared with control seeds stored under the same conditions, seeds heat treated at 55 and 60 ^{0}C for durations of between 15 and 45 min, and 5 min, respectively, attained similar dry mass values (Fig. 17). Berjak *et al.* (1992) have reported that cold storage enhanced the quality of seedlings produced from heat treated seeds. However, those authors have also shown this not to be the case if the seeds are debilitated which, in this study, could have been as a result of the Benlate dusting. The reduced germination performance of heat treated seeds stored at 25 ^{0}C (91% RH), conditions at which

seed deterioration is enhanced (Justice and Bass, 1978), was also evident in the quality of the seedlings these seeds produced (Fig. 22) when compared with those of similar seeds assessed prior to storage (Fig. 12) and those stored under cold, dry conditions (Fig. 17). Similar findings were reported by Strandberg and White (1989), who showed also that the adverse effect of ambient storage on seeding quality was exacerbated with increasing RH of the seed store. However, seeds treated at 55 and 60 ^{0}C for 15 and 5 min, respectively, attained seedling dry mass values similar to those of control seeds stored under the same conditions (Fig. 22), despite a decline in seedling number (Fig. 21). This implies that the quality of plants produced by these seeds were superior to those of the control. Hydration–dehydration treatments have been reported to enhance the storability of medium vigour seeds under ambient conditions (Pandey, 1989; Penaloza and Eira, 1993; Basu, 1994; Jansen and Ison, 1994). As hot water treatments have been shown to be comparable to accelerated ageing treatments (Bhattacharyya et al., 1985; Furbeck et al., 1989) and, as mentioned previously, appears to be the case in the present study (Fig. 4), the enhanced seedling quality exhibited by those seeds treated at 55 and 60 ^{0}C for 15 and 5 min, respectively, may be as a consequence of the hydration–dehydration treatment.

The reduced germination performance by seeds from any of the treatments following storage under both cold and ambient conditions did not appear to be as a result of membrane damage. In most cases, except for heat treated seeds assessed after cold storage (Table 7), the seeds were characterised by electrolyte leakage levels lower than those of similar seeds assessed after re–dehydration (Table 5). In all cases, including those of the control, the rates at which these seeds leached electrolytes were greatly reduced as a consequence of storage under either regime (compare Tables 5, 7 and 9). Bruggink et al. (1991) reported that aged maize seed leached more electrolytes than those of unaged seeds. Both control seeds and seeds pre-soaked for 4 h exhibited reduced germination and leaked slightly more than those of similar seeds stored under cold conditions, when assessed after storage at 25 ^{0}C (91% RH), conditions conducive to enhanced seed ageing (Justice and Bass, 1978)(Tables 7 and 9). Thus comparisons between either of the storage regimes may lead to the conclusion that some evidence for membrane damage as a consequence of storage at ambient conditions was apparent. However, heat treated seeds stored under cold conditions were characterised by

electrolyte leakage levels greater than those of similar seeds stored under ambient conditions (compare Tables 7 and 9), despite the poorer germination performance of those seeds stored under ambient conditions (compare Figs 14C and 19C). According to Bruggink *et al.* (1994), increased electrolyte leakage as a consequence of ageing treatments became apparent only after a conductivity measurement duration greater than 10 h. As conductivity trials used in this study did not exceed this duration, it may account, in part, for the lack of correlation between leakage and seed ageing effects when assessed after storage.

4.2. CONCLUDING COMMENTS

It is apparent from this study that immersion of maize seeds in hot water, for all temperatures and durations tested, proved to be an effective method for reducing or eliminating seed–associated mycoflora. These effects were maintained during storage at both 4 ^{0}C (33% RH) and 25 ^{0}C (91% RH) which, coupled with the possible synergistic effects of coating the seeds with Benlate prior to storage, suggests the efficacy of the hot water treatment to be by way of elimination, rather than fungistasis. However, although the hot water treatment proved effective in eliminating seed–associated mycoflora, it is unlikely that these treatments would detoxify or reduce the levels of mycotoxins already present within the seeds, as these metabolites, in general, are reported to be unaffected by temperatures lower than 100 ^{0}C (Scott, 1991; Samarajeewa, 1991; West and Bullerman, 1991). On the other hand, by preventing the mycotoxin levels from increasing as a consequence of the reduced incidence of toxigenic fungi, the hot water treatment may have its benefits if the seed is destined for consumption after storage. Obviously, the sooner after harvest such treatments could be applied, the less the accumulation of mycotoxins.

Despite an initial lag in germination rate and drop in germination totality, seeds treated at 55, 57 and 60 ^{0}C for durations of up to 60, 30 and 10 min, respectively, produced plants of a quality superior to those of untreated (control) seeds, when assessed after re–dehydration. Should the main source of infection be within seeds these treatments would, therefore, have distinct potential as pre–sowing manipulations not only for improving crop yields, but also for producing a crop only minimally infected, as a consequence of the elimination of systemically transmitted pathogens within those seeds destined for planting. In addition, this

practice may, therefore, lessen the hazard to the consumer of the mycotoxins produced by these fungi, particularly if the seed is destined for storage.

As the treatment described here has the advantages of both simplicity and negligible cost, it could, therefore, be of tremendous benefit to subsistence farming, particularly in those areas with a high incidence of mycotoxin related pathologies.

4.3. FUTURE PROSPECTS

As the beneficial effects of immersing seeds in hot water as a pre–sowing treatment is apparent, it would be of interest to determine the impact of the reduction of systemically transmitted pathogens by this treatment on the fungal status of the next seed generation. Such studies should include comparisons between plants grown under sterile, greenhouse conditions and those grown in the field in order to discern the invasion of pathogens in the field.

Also, as the seeds used in this study were stored for 2 years prior to use, it is possible that some inherent fungal activity may have contributed to the reduced vigour and viability evidenced in these seeds when assessed immediately after treatment. It would be of interest, therefore, to assess the effects of this treatment on freshly harvested seed. As seeds are reported to harbour a spectrum of both field and storage fungi at harvest (McLean and Berjak, 1987), and these fungi are active in wet seeds (Christensen and Kaufmann, 1969, 1974), it is possible that heat–treating freshly harvested seed, prior to drying, may enhance the efficacy of the treatment. In other words, the fungi, being in a metabolically active state may be more sensitive to heat, and the seeds could, therefore, possibly be eradicated at lower temperatures and for shorter durations of treatment, thereby reducing the negative effect of heat treatment on seed vigour and viability. While soaking and re–drying freshly harvested seed has been reported to reduce the germination performance of seeds following storage (Basu, 1994), such treatments may be of great significance for seed destined for consumption due to the reduced incidence and, therefore, activity of toxigenic fungi both prior to and during storage. Chatterjee *et al.* (1990) have reported that reducing the incidence of *A. flavus* infections in freshly harvested seed by seed treatments, minimised the proliferation of this fungal species during storage.

The efficacy of the hot water treatment may be enhanced by the addition of fungal growth promoters, such as sugar, during the 4 h pre-imbibition period. Stimulating fungal growth in such a manner may result in these pathogens becoming more susceptible to eradication by subsequent immersion of these seeds in hot water (McDonald, *pers. comm.*). In addition, although lacking the simplicity or potential acceptability of hot water treatment required for success in subsistence farming practices, the addition of chemicals, for example calcium hydroxide, that are known to be efficacious in degrading fungal toxins (Smith and Moss, 1985; Pemberton and Simpson, 1991) during the hot water immersion, may help to detoxify infected seeds. This would be of particular importance for that seed destined for consumption.

It is also possible that heat treating seeds in an osmotic priming medium may reduce the effects of accelerated seed ageing. Klein and Hebbe (1994) have reported that tomato seeds primed in 0.1 or 3% $CaCl_2$ at 50, 60 and 70 ^0C for durations up to 60 min did not affect seed vigour and viability, as occurred in the present study. In addition, those authors reported that the treated seeds produced plants that were taller than those of untreated (control) seeds. Thus, an osmotic priming medium such as $CaCl_2$ may enable treatment at higher temperatures and for longer durations than those proposed in the present study, enabling the complete eradication of seed associated mycoflora without adversely affecting seed quality.

Due to the difficulty in differentiating between the effects of the hot water treatment and later, of Benlate during storage, an investigation of the efficacy of the treatment during storage, without Benlate, is suggested in order to determine its benefits as a pre-storage treatment. In addition, it would be of interest to ascertain the effects of such treatments during long term storage under both cold and ambient conditions.

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