Comparison of fungal resistance to gamma and electron beam radiation and the effect of pH, Aw and solute on spore recovery.

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COMPARISON OF FUNGAL RESISTANCE TO GAMMA AND ELECTRON BEAM RADIATION AND THE EFFECT OF pH, Aw AND SOLUTE ON SPORE RECOVERY

BY

DAVID EDWARD CORRIGAN

A Thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

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ABSTRACT

Approximately twenty-five percent of perishable foodstuffs produced around the world are spoiled by fungi and/or by mycotoxins. A proposed method to control fungal spoilage is through the use of high energy electrons or gamma irradiation. Spores from fungi commonly associated with the spoilage of cereals and perishable foods were evaluated for irradiation resistance using either gamma or electron beam irradiation. The D_{10} values of aspergilli ranged from 0.241 kGy (*Aspergillus glaucus*) to 0.318 kGy (*Aspergillus echinulatus*) and 0.184 kGy (*A. echinulatus*) to 0.240 (*A. glaucus*) for gamma or electron beam treatments respectively. For penicillia D_{10} values ranged from 0.249 kGy (*Pencillium granulactum*) to 0.443 kGy (*Penicillium cyclopium*) and from 0.202 kGy (*P. granulactum*) to 0.403 kGy (*Penicillium roqueforti*) for gamma or electron beam treatments respectively. In contrast, *Curvularia geniculata* and *Alternaria alternata* conidia treated with gamma irradiation had D_{10} values of 1.786 and 2.184 kGy but decreased to 1.127 and 1.170 kGys respectively when treated with electron beam irradiation. The effect of media Aw and solute on the recovery of irradiated conidia was also investigated. Untreated conidia of *Aspergillus ochraceus* and *Penicillium cyclopium* exhibited best recovery at 0.87 Aw using sucrose as the controlling solute. Electron beam and gamma treated conidia of *A. ochraceus* were optimally recovered using PDA adjusted with sucrose (0.97 Aw). In contrast *P. cyclopium* conidia treated with gamma radiation were optimally recovered using NaCl (0.97 Aw) while electron beam treated conidia were optimally recovered using sorbitol or glycerol (0.93 Aw). Decreasing the Aw of the recovery media from 0.97 to 0.90 regardless of solute type, resulted in decreased colonial growth rates for both *A. ochraceus* and *P. cyclopium*. The maximum radial growth rate for both fungal organisms was observed on media adjusted to 0.97 Aw. No clear pattern in regards to the growth rates between colonies which developed

from spores following gamma or electron beam treatment was observed. The influence of pH on the recovery and growth of irradiated *A. ochraceus* spores was also investigated. The optimum recovery was observed on PDA (pH 7.3) adjusted to 0.905 Aw using NaCl. The influence of pH, solute and Aw on the growth rate of either untreated or treated *A. ochraceus* condia was not apparent with the exception of PDA containing NaCl; in this case a decrease in growth rate was observed as the pH decreased at 0.96 and 0.90 Aw. The influence of sporulation Aw on the irradiation resistance of *A. ochraceus* and *P. cyclopium* was investigated. The resistance for *A. ochraceus* conidia increased with decreasing Aw; spores produced on sorbitol at 0.95 Aw gave the highest resistance. In contrast, *P. cyclopium* spores grown at 0.99 Aw exhibited the highest irradiation resistance; in this case resistance decreased with decreasing Aw and was especially evident for spores produced on PDA adjusted using glycerol.

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INTRODUCTION

Fungi are considered to be important microorganisms in regard to food spoilage. The Food and Agriculture Organization (IEAE, 1989) indicated that worldwide, twenty-five percent of all post-harvested produce is damaged or destroyed by fungi. As well, many fungi are able to produce secondary products called mycotoxins including aflatoxin, ochratoxin, and trichothecenes. Mycotoxins cause toxicological effects and are considered teratogenic, carcinogenic and immunosuppressive (Robb, 1993; Young and Fulcher, 1984). Many food products such as grain, maize, groundnuts, tree nuts and rice have been found to contain mycotoxins (Robb, 1993; Young and Fulcher, 1984).

In the food industry a number of techniques are used to increase shelf-life by inhibiting or delaying the growth of spoilage and storage organisms. These techniques include heating, refrigeration, the use of chemical additives such as sorbic acid and water activity (Aw) reducing agents such as glycerol, sucrose and sodium chloride. In the case of water activity, the reduction of Aw in a food product below 0.93 inhibits all food poisoning organisms with the exception of *Staphylococcus aureus* (Troller, 1979). Further reduction of a food product below 0.80 Aw inhibits most mold growth and mycotoxin production with the exception of xerophilic molds (Beuchat, 1981; Northolt et al., 1977, 1979; Troller, 1980). Exposing food to radiation treatment also delays spoilage and improves safety by eliminating or reducing pathogenic microorganisms. The effectiveness of the treatment is dependent on several factors including the composition of the food, the number and type of microorganisms and the dose (Diehl, 1990). Food preservation using radiation is principally achieved using a gamma source such as ^{60}Co or electrons generated by high-energy electron beam accelerators. Electron beams and gamma rays differ greatly in their ability to penetrate matter. Generally

gamma rays exhibit higher penetration into food compared to electron beams (Cleland and Pageau, 1985). In foods where surface decontamination and/or disinfestation is desired for example, in the case of cereals containing either molds or insects, the use of electron beam radiation may be preferred. This preference is largely based on economics due in part to the greater efficiency of irradiation (Diehl, 1990; CAST, 1989).

The susceptibility of microorganisms and/or their spores to gamma radiation has been well established (Thayer, 1993; Saleh et al., 1988). In contrast, information pertaining to the resistance of food microorganisms exposed to accelerated electrons is relatively sparse. Therefore the aim of this investigation was to evaluate the resistance of microoganisms to electron beam radiation and compare the results to those obtained by gamma radiation. Spores of commonly occurring fungi with respect to grain contamination, were employed as test microorganisms.

In regards to the decontamination of cereals, studies have focused on the radiation sensitivity of various microorganisms and the influence of environmental factors such as pH and temperature during treatment (Mohyuddin and Skoropad, 1970; Padwal-Desai et al., 1976a, 1976b; Saleh et al., 1988). Little information, however, is available regarding the radiation senstivity of fungi based on their cultural background. In nature fungal spores are formed under diverse conditions and in a mutiplicity of substrates. Treatments aimed to reduce or eradicate fungi and/or their spores may therefore exhibit varying degrees of effectiveness based on their sporogenic conditions. Consequently investigations were also aimed to evaluate the effects of sporulation Aw on spore quality as it relates to radiation sensitivity.

REVIEW OF LITERATURE

Fungi In Food Systems

Microbial presence in foods invariably leads to deterioration and spoilage and infrequently to food-borne illness. Microorganisms of importance include fungi, yeast and bacteria. The Food and Agriculture Organization (FAO) has estimated that approximately twenty-five percent of all food produced worldwide is spoiled after harvesting (IEAE, 1989) due to the action of microorganisms of which fungi are the most important group particularily in products and/or environments with an acid pH and/or reduced free water.

Fungi are a group of microorganisms that can cause deformation, spoilage and/or production of secondary metabolites including mycotoxins or volatile compounds in food items. These organisms can also bring about beneficial effects such as their ability to ripen cheese and synthesize industrially important enzymes including proteases, lipases and organic acids (Campbel-Platt and Cook, 1989). The importance of fungi in food spoilage has been investigated by Young and Fulcher (1984) who stated that food product spoilage by fungi in fruits and fruit products, vegetables, cereals and bakery products is a worldwide problem. Fungi which are most important in relation to foods can be placed into three classes which include: Zygomycotina, Ascomycotina and Deuteromycotina (Pitt and Hocking, 1985a).

Fungal Growth Conditions

Spoilage and/or the production of toxins in food or stored products including cereals involves a wide range of fungi. These fungi differ in their environmental growth requirements including: water activity (Aw), moisture distribution, pH, temperature and

atmospheric gas composition.

Water activity is the measurement of available water in a food product and is an indication of shelf-life stability (Troller, 1980). Filamentous fungi are able to grow over a wide Aw range from 0.60 to 0.99 (Troller, 1979). Fungi that are able to grow below 0.85 Aw are called xerophiles (Pitt, 1975). Lacey (1989) indicated that the minimum Aw for fungal growth was dependent on the temperature, pH and type of solute. A study performed on field and storage fungi by Magan and Lacey (1984) found that as the temperature approached the optimum, the minimum Aw decreased for germination. For example, a decrease in pH from 6.5 to 4.5 resulted in an increase in the minimum Aw required for growth for a wide range of aspergilli and penicillia. This was especially obvious at the lower temperature limits of growth. In addition, it was noted that *Alternaria alternata*, *Penicillium hordei* and *Aspergillus repens* showed an increase in the lag period for germination when the pH decreased from 6.5 to 4. Studies pertaining to the growth rate for various fungi including *Alternaria alternata*, *Cladosporium herbarum*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Fusarium culmorum*, *Verticillium lecanni*, *Penicillium* and *Aspergillus* species indicated that as the optimum temperature was approached the minimum for growth decreased (Magan and Lacey, 1984). The lower Aw limit for growth of fungi is partly influenced by the nature of the solute used to control the water activity. A variety of solutes or a combination of solutes can be used to control the Aw of a food product including fructose, glucose, glycerol, sodium chloride, sorbitol and sucrose. These solutes remove available water from the system by forming hydrogen bonds with the water molecules thus making it unavailable for usage by fungi or other microorganisms (Prior, 1979; Scott, 1957).

Jakobsen and Murrell (1977) reported that glucose, sorbitol and NaCl limited sporulation of *Bacillus cereus* to an Aw of 0.95 but that glycerol allowed sporulation to an Aw of 0.91. Gervais et al. (1988) indicated that solutes which favor fungal spore germination exhibit the greatest (largest) deviation from Raoult's law. Many xerophilic fungi are able to grow at a lower Aw when non-ionic solutes (sugars) are used compared to ionic solutes (salts) (Beuchat, 1981; Beuchat and Pitt, 1990a; Beuchat and Pitt, 1990b;

Pitt and Hocking, 1977). A study performed by Pitt and Hocking (1977) showed that xerophilic fungi including *Aspergillus flavus* and *Aspergillus ochraceus* grew best on glycerol or glucose/fructose based media but were inhibited when NaCl was used as an Aw controlling solute. Also, *Chrysosporium farinicola* was able to grow at the lowest Aw when glucose was used as a solute followed by sorbitol, glycerol and NaCl respectively (Beuchat and Pitt, 1990b). In contrast, certain halophilic fungi such as *Wallemia sebi*, *Basipetospora halophila*, *Polypaecilum pisce* and *Exophiala werneckii* displayed greater growth on media containing NaCl than on media containing non-ionic solutes such as sucrose and glycerol (Andrew and Pitt, 1987; Pitt and Hocking, 1977; Wheeler et al., 1988). An overview of the minimum Aw for growth of various fungi is listed in Table 1.

The moisture content of food products within a closed system will also affect microbial growth. In some instances, localized areas of high moisture may develop and raise the local Aw. Some of these localized areas may contain enough free moisture which will favor the growth of fungi whereas at equilibrium, fungal growth is retarded. As well, the respiration of commodities such as grain during storage may condense and form localized areas of high water activities (Troller, 1979).

Lacey (1989) indicated that the optimal growth pH for the majority of cereal fungi is approximately 5, however, growth normally occurs from pH 3 to 8. At neutral pH, bacterial spoilage usually predominates thereby suppressing fungal growth but as the pH is decreased below 5 fungal spoilage starts to predominate with the exception of a few acidophilic bacteria such as lactobacilli (Lacey, 1989; Pitt and Hocking, 1985a). In many cases the pH range for fungal growth is affected by extrinsic and intrinsic conditions such as temperature, Aw and the presence of additives (Beuchat and Pitt, 1990a; Pitt and Hocking, 1977; Pitt and Hocking 1985a)

The growth temperature varies depending on the fungal species, but most fungi grow at temperatures ranging from 10 to 40°C with the optimum ranging from 25 to 35°C (Lacey, 1989). Some exceptions include *Cladosporium* spp. which are able to grow at temperatures below 0°C while others such as *Aspergillus fumigatus* can grow as

Table 1. Minimum Aw for fungal growth.

Aw	Aw	Reference
Botrytis cinerea	0.93	Snow (1949)
Mucor sp.		Troller (1979)
Cladosporium sp.	0.88	Troller (1979)
Penicillium sp.	0.80	Troller (1979; 1980)
Aspergillus sp.		Troller (1979; 1980)
Erotium sp.	0.70	Troller (1979)
Chrysosporium sp.		Pitt and Christian (1968)
Xeromyces bisporus	0.605	Pitt and Christian (1968)

high as 55.0°C (Lacey, 1989). In many cases decreasing or increasing the pH from the optimum will affect the temperature tolerance and the minimum growth Aw limits (Lacey, 1979; Pitt and Hocking, 1985a). In an investigation to determine the effects of temperature, pH and Aw on the germination of spores for various species of fungi, Magan and Lacey (1984) found that germination occurred at pH 6 with temperatures ranging from 5-30°C. When the pH of the growth media was decreased from 6.5 to 4.5, the minimum Aw generally increased especially at marginal growth temperatures. For example, *Alternaria alternata* at 5°C and pH 6.5 was able to germinate at 0.90 Aw whereas at pH 4.0 the minimum Aw for germination increased to 0.97.

The gas or atmospheric composition of a food product also affects the growth of spoilage fungi. Most spoilage fungi are obligate aerobes; Lacey (1989) stated that the amount of available oxygen and not oxygen tension determines fungal growth. One exception is *Moniliella acetoabutans* which can cause spoilage under anaerobic conditions (Stolk and Dakin, 1966).

Other parameters which may affect fungal growth include the presence of chemical preservatives, microbial interactions, substrate composition and their dispersion by the activity of arthropods (Beuchat, 1981, Lacey, 1989; Troller, 1979, 1980).

Fungal Spoilage

Fungal spoilage consists of damage or disfigurement to fresh and perishable food products. In addition, spoilage can result in the loss of dry matter or product quality (Lacey, 1989). Spoilage fungi can be divided into two groups, (i.e.) those which causes spoilage of fresh or perishable foods and those that can cause the spoilage of processed food products.

The spoilage of fresh and perishable foods include fruits, vegetables, fresh meats and dairy products. The fungal species involved in the damage caused by their growth is noted in Table 2. Fresh fruits have an acidic pH ranging from 1.8 to 5.0 (Splittstoesser, 1978), in addition many varieties have a tough outer skin and inherent defense mechanisms which inhibit both bacterial and fungal growth. During ripening,

Table 2. Spoilage fungi of perishable or fresh food items.

Products	Organisms	References
Bananas	*Colletotrichum musae* and *Fusarium semitectum*	Wallbridge (1981)
Berries	*Botrytis cinerea*	Sommer et al. (1968)
	Rhizopus stolonifer, Rhizoctonia solani, Phytophthora cactorum, Penicillium sp and *Cladosporium sp.*	Ryall and Pentzer (1982)
Citrus Fruits	*Diaporthe citri, Diplodia natalensis, Geotrichium candidum, Penicillium italicum, P. digitatum, Septoria depressa* and *Sphaceloma fawcettii.*	Butler et al. (1965); Splittstoesser (1978); Ryall and Pentzer (1982); Hall and Scott (1977)
Grapes	*B. cinerea*	Ryall and Pentzer (1982); Coley-Smith et al. (1980)
	Penicillium aurantiogriseum	Barkai-Golan (1974)
	P. brevicompactum, P. chrysogenum, P. citrinum, P. expansum and *Aspergillus sp.*	Barkai-Golan (1980)
Pome Fruits	*B. cinerea*	Hall and Scott (1977)
	P. expansum	Pitt and Hocking (1985a)

Table 2. cont.

Products	Organisms	References
Stone Fruit	*Monilia fructicola, M. fructigena* and *M. laxa.* *P. expansum* *Alternaria sp.* and *B. cinerea*	Pitt and Hocking (1985a) Ryall and Pentzer (1982) Splittstoesser (1978)
Beans	*Colletotrichum lindemuthianum, Pythium butleri* and *Rhizoctonic solani*	Pitt and Hocking (1985a)
Cantaloupes and Melons	*Alternaria tenuis* and *Colletotrichum lagenarium*	Pitt and Hocking (1985a)
Carrots	*Stemphylium radicium, Rhizopus sp., B. cinerea,* and *Sclerotinia scerotiorum*	Ryall and Lipton (1979)
Leafy Vegetables	*B. cinerea, Rhizopus stolonier, R. solani*, and *Alternaria sp.*	Pitt and Hocking (1985a)
Melons	*Penicillium sp.* and *Fusarium sp.*	Ryall and Lipton (1979)
Onions	*Aspergillus niger*	Pitt and Hocking (1985a)
Peas	*B. cinerea*	Ryall and Lipton (1979)

Table 2. cont.

Products	Organisms	References
Potatoes	*Fusarium sp.*	Pitt and Hocking (1985a)
Sweet Potatoes	*R. stolonifer*	Ryall and Lipton (1979)
	Ceratocystis fimbriata	Pitt and Hocking (1985a)
Tomatoes	*Alternaria tenuis, Cladosporium herbarum* and *B. cinerea*	Pitt and Hocking (1985a)
	Rhizopus sp.	Ryall and Lipton (1979)
Meats	*Cl. cladsporioides, Penicillium hersutum* and *Aureobasidium pullulans.*	Gill et al. (1981)
Butter and Margarine	*Geotrichum canididum, Moniliella sauveolans, Cl. herbarum, and Cladosporium. butryi.*	Muys et al. (1966)

however, pH increases and the skin softens or is more easily damaged. In addition there is often an increase in soluble carbohydrate concentration allowing for growth by fungi (Pitt and Hocking, 1985a). The spoilage of vegetables is different from that of fruit in that the inherent pH of many vegetables is near neutral which allows for equal spoilage by bacteria and fungi (Pitt and Hocking, 1985a). The spoilage of fresh meats by fungi, bacteria and yeasts can occur at temperatures approaching -5°C but fungi mainly predominate as temperatures approach -12°C (Michener and Elliott, 1964). The spoilage of fresh dairy products by fungi is rare since they are crowded out by vigorous bacterial growth due in part to relatively high (0.98-0.99) Aw (Pitt and Hocking, 1985a). Other dairy products and substitutes such as butter or margarine are susceptible to fungal spoilage due to the lower pH and Aw of the products (Muys et. al., 1966).

A list of spoilage fungi of stored and processed foods including grains, intermediate moisture or heat processed foods including certain dairy products is presented in Table 3. Low and intermediate moisture foods such as grains, spices and nuts have Aw levels ranging from 0.1-0.85 which inhibit growth of spoilage organisms (Beuchat, 1981; Mossel, 1975). Spoilage occurs in these products generally due to localized areas of high moisture in which fungi are able to grow (Troller, 1979). Heat processed products such as juices are heated to 70-75°C in order to inhibit/kill bacterial, fungal growth and enzymes. The spoilage of dairy products such as cheese occurs by the growth of psychrotolerant fungi including *Cladosporium* and *Penicillium* which are also able to grow at low Aw and pH (Pitt and Hocking, 1985a).

Fungal Intoxication

Fungal growth not only contributes to food spoilage but also to intoxication by the production of secondary metabolites known as mycotoxins (Padwal-Desai et al., 1976a). Mycotoxins are known to have caused disease outbreaks in both farm animals and humans (CAST, 1989). A number of factors which control the production of mycotoxins incude temperature, Aw, pH, nutrients and carbon to nitrogen levels (Robb, 1993).

Table 3. Spoilage fungi of processed food products.

Products	Organisms	References
Rice	*A. candidus*, *A. flavus*, *A. glaucus*, *A. halophilicus*, *A. ochraceus*, *A. restrictus*, *A. versicolor*, *P. aurantiogriseum*, *P. citreonigrum*, *P. citrinum*, *P. islandicum* and *Wellemia sebi*.	Christensen (1987); Saito et al. (1971)
Spices	*Aspergillus glaucus*, *A. restrictus*, *A. flavus*, *A. fumigatus*, *A. niger* and *Penicillium sp.*	Lebai Juri et al. (1986)
Salted Fish	*Wellemia sebi* *A. clavatus*, *A. flavus* and *A. niger* *Polypaecilum pisce*	Frank and Hess (1941) Townsend et al (1971) Pitt and Hocking (1985b)
Canned Fruits (including baby food)	*Byssochlamys fulva* and *B. nivea* *Neosartorya fishceri*	Put and Kruiswijk (1964); Hocking and Pitt (1984) Kavanah et al. (1963)
Dairy	*Cladosporium sp.* and *Penicillium sp.*	Pitt and Hocking (1985a)

Table 3. cont.

Product	Organisms	References
Dried Cereals (Low Aw)	*Aspergillus sp., Eurotium sp., Wallemia sp., Penicillium aurantiogriseum, P. chrysogenum, P. breviocompactum, P. crustosum* and *P. citrinum*	Pitt and Hocking (1985a)
Dried Fish	*A. flavus, A. wentii* and *Basipetospora halophila* *B. halophila* and *Polypaecilum pisce*	Wheeler et al. (1988) Pitt and Hocking (1985b)
Dried Meats	*Aspergillus sp., Eurotium sp.*, and *Penicillium sp.*	Van der Riet (1976)
Hazelnuts	*R. stolonifer* and *P. aurantiogriseum*	Senser (1979)
Peanuts	*A. flavus* and *A. niger*	Joffe (1969)
Pecans	*A. expansum, A. ficuum, A. niger, A. parasiticus, E. repens,* and *E. rubrum*	Huang and Hanlin (1975); Wells and Payne (1976)

Mycotoxins

Trichothecenes consist of tricyclic ring systems which contain a 12,13-epoxide group. Eppley (1975) cited a number of mycotoxicoses caused by trichothecenes which include alimentary toxic aleukia, stachybotryotoxicosis, red mold poisoning and moldy corn toxicoses. There are over 100 types of trichothecene toxins with deoxynivalenol and T-2 toxin being the most important. Deoxynivalenol, otherwise known as vomitoxin acts on the central nervous system and is immunosuppressive at chronic doses. The toxin has been found in various cereals including wheat and is produced by *Fusarium culmorum* and *Fusarium graminearum* (Gilbert, 1989). T-2 has also been detected in a number of cereal grains and can cause inflammation and hemorrhaging of the gastrointestinal tract, dermal necrosis, edema, low levels of leukocytes and bone marrow degeneration as well as inhibition of protein synthesis (Davis and Diener, 1987). Trichothecenes are produced by a variety of *Fusarium* spps. which include *F. tricinctum*, *F. nivale*, *F. poae*, *F. solani* as well as by *Trichoderma lignorum* (Young and Fulcher, 1984).

Mycotoxins containing a bisdifuranocoumarin ring consist of alfatoxin, aflatoxicol and dihydroaflatoxicol (Heathcote, 1984). The most important of this group are the aflatoxins, which consist of fifteen types with B_1, G_1 and M_1 being the most important; B_1 has been reported to be most toxic (Moss, 1989). Moss (1989) and Robb (1993) stated that aflatoxins can produce a wide variety of toxic effects and that they are carcinogenic and teratogenic; they are also immunosuppressive to man and animals. *Aspergillus flavus* and *Aspergillus parasiticus* represent the primary aflatoxin producers. The main targets are the liver and the kidney. Aflatoxins have been isolated in various agricultural products including peanuts (Chiou et al., 1990, figs (Steiner et al., 1988), rice, sorghum (Stoloff, 1976), sunflower seeds, corn, tree nuts, cereals and animal feeds (Moss, 1989; Robb, 1993).

Heathcote (1984) indicated that there are a number of mycotoxins which are chemically related to aflatoxins. These toxins include: difuroxanthones and quinonoids. The first type include sterigmatocystin and is produced by *A. flavus*, *A. parasiticus*,

Aspergillus nidulans and *Aspergillus rugulosus*. The toxin has been found in grain and rice (Robb, 1993). Sterigmatocystin produces toxicological effects similar to aflatoxins but with less potency. The quinonoids include difuroanthraquinones such as versicolorin A and versicolorin B which are produced by *A. flavus* and *A. parasiticus* (Heathcote and Dutton, 1969; Lee et al. 1975)

Dihydroisocoumarins are mycotoxins which consist of ochratoxin, viomellein and viriditoxin. Ochratoxin is the most important of this group and consists of forms A and B, with ochratoxin A being the most toxic (Madhyastha et al., 1990; Steyn, 1984). Chronic effects of ochratoxin A can result in nephrotoxicity and enteritis; the toxin is also immunosuppressive, carcinogenic, teratogenic, and results in acute toxicity, decreased blood clotting and depressed red blood cell counts (Steyn, 1984 and Robb 1993). Robb (1993) indicated that ochratoxin attacks the kidneys and has been associated with Endemic Balkan Nephropathy. Harwig and Chen (1974) stated that organisms which produce ochratoxins include *Aspergillus* and *Penicillium* species. Harwig and Chen (1974) and Stoloff (1976) indicated that ochratoxin A has been found in cereal grains, white beans, feed grains and corn.

Citrinin which is associated with toxic yellow rice exhibits affects both the kidney and liver. Chronic effects of citrinin toxicity are due to it's nephrotoxic effects which include swelling of the kidney, acute tubular necrosis as well inhibition of cholesterol and triglyceride formation in the liver; it is also a suspected mutagen (Betina, 1984a). Organisms which produce citrinin include *Penicillium* and *Aspergillus* species as well as *Pythium ultimum* (Betina, 1984a; Davis and Diener, 1987; Harwig and Chen, 1974). Citrinin has been isolated in baked goods, cereals, corn and rice (Davis and Diener, 1987; Saito et al., 1975).

Rubratoxins have been associated with moldy corn toxicosis in domestic farm animals (Davis and Diener, 1987; Davis and Richard, 1984). Rubratoxins include rubratoxin A and B with rubratoxin B being the most toxic. Rubratoxins cause liver haemorrhages, inhibition of ATPases and chromosomal damage (Davis and Richard, 1984). These toxins are produced by *Penicillium rubrum* and *Penicillium pupurogenum*

(Davis and Diener, 1987; Natori et al., 1970).

Mycotoxins which contain lactone moieties include patulin and penicillic acid and are produced by *Penicillium* species such as *P. expansum*. Patulin causes damage to the liver, spleen, kidneys and results in edema of the lungs and brain (Engel and Teuber, 1984). Patulin has been detected in fruits such as apples, pears, pineapples, grapes, peaches, apricots and apple juice (Buchanan et al., 1974; Scott et al., 1972; Engel and Teuber, 1984). Penicillic acid is considered carcinogenic (Dickens and Jones, 1961). Penicillic acid has been detected in animal feed, oats, beans, apple, juices, cheeses, corn, rice and crushed coconuts (Davis and Diener, 1987; Engel and Teuber, 1984). The toxin is produced by aspergilli and penicillia species which include: *A. malleus*, *A. ochraceus*, *A. ostinanus*, *P. cyclopium*, *P. martensii*, *P. roqueforti*, *P. viridicatum* and *Paecilomyces ehrlichii*.

Skyrines or hydroxyanthraquinone containing mycotoxins which include luteoskyrin and rugulosin are suspected of causing a condition known as yellow rice (Davis and Diener, 1987; Uneo, 1984). Luteoskyrin can be produced in high concentrations by *P. islandicum* and *Mycelia sterilia*. This toxin causes hepatotoxic-centrolobular necrosis; it is cytotoxic and inhibits mitochondrial enzymes. It has also been reported to induce chromosomal breakages and interferes with DNA repair (Davis and Diener, 1987; Ueno, 1984). Chronic exposures have been linked to tumor formation in livers and it is considered highly carcinogenic and possibly mutagenic. Ueno (1984) indicated that rugulosin produced by *Penicillium sp.*, *Endothia parasitica*, *Endothia fluens* and *Sepedonium ampullosprorum*, have similar hepatotoxic effects as luteoskyrin but exhibits lower cytotoxicity. It is considered highly carcinogenic and causes cirrhosis and tumor formation in the liver.

Tremorgenic mycotoxins include approximately twenty types such as aflatrem, paspaline, paspalicine and paspalinine (Betina, 1984a). These mycotoxins produce tremors and convulsions at low doses but can be lethal at higher doses (Davis and Diener, 1987; Betina, 1984a). A number of food products have been found to contain tremorgenic mycotoxins including oats, commercial feed, millet, rice, corn, peanuts and

potatoes (Betina, 1984a; Cole et al., 1972; Cole et al., 1974). The primary toxin producing fungi include: *Aspergillus fumigatus*, *Claviceps paspali*, and *Penicillium* sp. such as *P. paxilli* and *P. verrucolosum* (Betina, 1984b; Cole et al., 1972; Cole et al., 1974) .

Food Irradiation

Irradiation of food products has been proposed for a variety of applications which include sprout inhibition of tubers, extension of shelf-life, sterilization and the elimination of food pathogens, insects and/or parasites (Diehl, 1990). Additional proposed uses of irradiation include the reduction or elimination of food additives such as sodium nitrite which is used to inhibit the outgrowth of *Clostridium* spores (Wierbicki and Heiligman, 1973) and maleic hydrazide which is used to inhibit sprouting in tubers and onions (Loaharanu and Urbain, 1982). The FOA/IAEA/WHO Joint Committee (WHO, 1981) has recommended a number of ionizing irradiation sources suitable for food applications. Food irradiation includes the use of low, medium and high dose treatments (Diehl, 1990).

Low dose irradiation treatments have been proposed for a number of applications including sprout inhibition, elimination of parasites or insects in grain and delay of maturation of fruit. Low dose treatments range from 0 to 1.0 kGy.

Treatments using a medium dose have two main applications which are: the elimination of pathogens and the extension of product shelf-life. The elimination of pathogenic microorganisms is also known as radicidation (Diehl, 1990). Radicidation normally uses doses between 2.0-8.0 kGys. Treatments used to increase the shelf-life of products by reducing the number of viable spoilage organisms is called radurization. This involves the application of dosages of 0.4-10 kGys (Diehl, 1990).

The use of high dose levels has been proposed for food sterilization or commercial sterility as it is understood in the canning industry. This procedure is known as radappertization (Diehl, 1990) and proposed foods for it's use include meats, poultry and seafoods. Radappertization has been proposed for foods consumed by people with

supressed or impaired immune systems. The dosages proposed by Diehl (1990) are between 10-45 kGys.

Sources of Irradiation

Two main sources of ionizing irradiation recommended by FOA/IAEA/WHO Joint Committee and Codex General Standard for food irradiation include a gamma source consisting of either cobalt-60 or cesium-137 and high speed electrons or x-rays (Diehl, 1990). Sources for ionizing irradiation deemed unsuitable for food are those which have poor penetration such as alpha particles or sources which impart products radioactive such as high energy X-Rays (Diehl, 1990).

Cobalt-60 is a non fission product and ca. 90 percent of the emitted irradiation can be used for food irradiation. The usable energy, for irradiation purposes, consists of two photons produced during decay with energies of 1.17 and 1.33 MeV (Diehl, 1990). Cobalt-60 has a half-life of 5.27 years. Although Cesium-137 has a half-life of 30 years (Jarrett, 1982) only 70 percent of the irradiation produced can be used for food irradiaton; it also has lower penetration than cobalt-60. Cesium-137 produces a single photon with an energy of 0.66 MeV (Jarrett, 1982). Among the drawbacks to the use of this radioactive material is that the isotope source emits rays in all directions, therfore it is less efficient compared to machine sources (Diehl, 1990).

The machine sources used for ionizing irradiation consists of two types. One type produces X-rays by bombarding target sites with high energy electrons while the other source consists of high energy electron beam accelerators (Diehl, 1990). Two types of accelerators are commonly used: direct current (dc) and a linac or radio frequency linear accelerator (Ramler, 1982). Both types of irradiators accelerate electrons to a very high speed (approaching the speed of light) which imparts a high level of kinetic energy to the electrons. The penetration of the electrons into a target depends on the amount of kinetic energy imparted to the electron. In general, for every MeV imparted, the penetration depth increases by five millimeters. Therefore, a five MeV beam can penetrate up to a depth of 2.5 cm (Diehl, 1990). Penetration ability of gamma rays depends on the density

of the material being irradiated. The efficiency of accelerators varies from 30 percent for the Linac type to 50 percent for dc accelerators (Ramler, 1982). X-rays have the same depth of penetration as gamma rays i.e. 30 cm (Diehl, 1990).

Observed differences in electron beam accelerators, which have an efficiency rating (amount of radiation absorbed by the irradiated product) of 50% as compared to 20-30% for gamma sources, are due to two main reasons (Diehl, 1990). Encapsulation and the capsule size of the radioactive source results in self-absorption of some of the radiation. Therefore, 95% of the radiation produced by colbalt-60 is available for irradaition and 70% for cesium-137. Additional absorption by the irradiator shielding, conveyor and storage pool results in an efficiency of 30% for cobalt-60 and 25% for cesium-137. In the case of electron beam radiation, shielding or encapsulation is not ncessary therefore self-absorption is minimal.

Interaction of Irradiation with Matter

Gamma irradiation produces three types of reactions: photoelectric effect, Compton effect and pair (electrons and positrons) production (Diehl, 1990). Only the Compton effect is important in food irradiation. In this process, the gamma photons collide with atoms resulting in the ejection of an electron and the loss of kinetic energy. The photons, after colliding with an atom, are randomly scattered. The electrons which are ejected are called Compton electrons.

Irradiation Resistance of Asporogenic Microorganisms

Irradiation resistance of asporogenous microorganisms has been investigated in terms of their D_{10} value. The D_{10} value is the irradiation dose (kGy) required to inactive 1 log cycle or 90 percent of an initial microbial population (Diehl, 1990). The D_{10} value is normally calculated from the linear portion of a survivor curve where the log of the surviving numbers is plotted versus the irradiation dose. Most asporogenous bacteria exhibit D_{10} values below 1.00 kGy. For example, the D_{10} values (kGys) for a few potential and known pathogens irradiated in buffer solution are: *Listeria monocytogenes*,

0.18 (Farag et al., 1990); *Salmonella enteriditis*, 0.172; *S. typhimurium* 0.199 (Thayer et al., 1990). Some microorganisms, however, including *Micrococcus* and *Moraxella-Acinetobacter* are highly resistant to irradiation. *Micrococcus radiodurans* and *Moraxella-Acinetobacter* were reported to have D_{10} values of 3.5 and 4.7 kGys respectively when irradiated in nutrient broth (Ma and Maxcy, 1981). Maxcy (1982) indicated these highly resistant bacteria are of no health significance and are primarily involved in the spoilage of fresh meats and fish.

A number of enviromental conditions have been found to affect the resistance of bacteria to irradiation. In one case, the reduction of temperature at which irradiation occurred resulted in an increase in resistance. The use of low temperatures during irradiation results in decreased movement of free radicals and peroxides which are produced during irradiation and are neutralized upon thawing by either recombination or neutralization before they damage the cell (Anellis et al., 1973). In addition, the ability of the bacteria to produce endospores results in higher resistance as endospores generally have higher resistance to irradiation than the vegetative cells with the exceptions of *Micrococcus radiodurans* (Duggan et al., 1963a and 1963b) and *Moraxella* sp. (Bruns and Maxcy, 1978). The complexity of the substrate and/or nutritional adequacy can also effect the resistance of bacteria as reported by Thayer et al. (1990). Thayer et al. (1990) found that samonellae irradiated in phosphate buffer were more susceptible to irradiation than salmonellae irradiated in brain heart infusion broth or deboned chicken; the latter menstruum provided the highest protection against irradiation. The stage of growth of bacteria also effects irradiation sensitivity as stationary and lag phase cultures are more resistant to irradiation than are cells in the logarithmic stage of growth (Diehl, 1990). Other factors which can effect bacterial resistance to irradiation include the age of the cells, concentration, atmosphere composition and DNA content (Duggan et al, 1963a; Hastings et al., 1986).

Irradiation Resistance of Spores

Spore producing bacteria which are important in the food industry include *Bacillus*

and *Clostridium*. Botha and Holzapfel (1988) reported that spores have higher irradiation resistance than their vegetative counterparts. The most important spore producing bacterium from a public health standpoint is *Clostridium botulinum*. The D_{10} values for *C. botulinum* type A and B varies from 2.2-3.3 and 1.3-3.3 kGy respectively (Anellis and Koch, 1962). Kim et al. (1987) reported that the D_{10} values of gamma irradiated spores of *Cl. thermosaccharolyticum* and *Desulfotomaculum nigrificans* both of which represent thermophilic, anaerobic sporeformers, were 2.54 and 2.20 kGy respectively.

In addition to bacterial endospores, fungal conidia are important due to their ability to infect and destroy large quantities of fresh and stored products especially fresh vegetables and fruits as previously discussed. Numerous studies have investigated the potential use of radiation in order to reduce or limit spoilage by fungi (Beraha et al., 1959; O'Neill et al., 1991; Padwal-Desai et al., 1976a and 1976b; Saleh et al., 1988; Sommer et al., 1966; 1967). Saleh et al. (1988) using gamma irradiation noted that the D_{10} values of *Aspergillus niger*, *A. flavus*, *Cladosporium cladosporioides* and *Curvularia geniculata* were 0.42, 0.55-0.6, 0.25-0.3 and 2.42-2.90 kGys respectively. O'Neill et al. (1991) reported the sensitivity of various cereal fungi on grain and in phosphate-buffered saline. Species of *Fusarium* and *Alternaria* were more resistant to irradiation than *Aspergillus* spps and *Penicillium* spps. Generally D_{10} values determined on grain were lower than the corresponding values in buffered saline.

Factors Affecting Irradiation Resistance of Spores

The lethal effects of irradiation are dictated by a number of variables in addition to the dose level. Some of these parameters include; DNA content, temperature and atmosphere during irradiation, age, pH, spore type and Aw. The combination of one or more of these factors can readily increase or decrease the resistance of spores to irradiation.

(1) DNA

DNA is considered to be the main irradiation target for three reasons (Diehl,

1990). First, DNA is used as a storage molecule for genetic information as well as a template for DNA, RNA and protein synthesis. Second, DNA is a relatively large molecule in comparison to other cellular molecules. Finally, there are limited copies of chromosomal DNA in a cell. In addition, Diehl (1990) indicated that the size of the DNA is a factor in resistance as the larger the DNA molecule, the more sensitive the organism is to irradiation. For example bacteria are less sensitive to irradiation compared to yeast. In addition, single stranded DNA is more sensitive to irradiation compared to double stranded DNA (Diehl, 1990).

(2) Temperature

The use of low temperatures during irradiation has been reported to result in an increase in the D_{10} values for microorganisms and spores. Since it results in a decreased movement of free radicals and peroxides which are produced during irradiation (Anellis et al., 1973). Upon thawing, the resultant radiolytic products either recombine or become neutralized into non-toxic compounds before causing lethal cell damage. Lowering the temperature of application also results in a decrease in radiation induced off-flavors caused by lipid oxidation and nutrient losses (Anellis et al., 1973; Diehl, 1990; Ma and Maxcy, 1981; Previte et al., 1970). For example, spores of *Bacillus cereus* exhibited increased sensitivity as the temperature increased from -30°C to 37°C with D_{10} values of 0.32 to 0.23 Mrad (Ma and Maxcy, 1981). Grecz et al. (1971) noted that spores of *Clostridium botulinum* showed an linear decrease with some discontinuity in resistance at temperatures corresponding to a phase change (i.e. solid to liquid). Resistance decreased from 0.55 to 0.159 Mrad as the irradiation temperature was increased from -195°C to 95°C. The use of heat pretreatment has also been shown to affect microbial resistance to radiation (Barkai-Golan et al., 1969; Duggan et al., 1963b; Maxcy and Rowley, 1978; Morgan and Reed, 1954; Padwal-Desai et al., 1976a, 1976b; Sommer et al., 1967; Sommer et al., 1968). For example, the use of preheating at 57.5°C for 2.5 min on *Micrococcus radiodurans* was found to decrease radiation resistance (Duggan et al., 1963b). The use of a pre-irradiation treatment on bacterial

spores sensitized them to subsequent heat treatment (Kan et al., 1957; Kempe, 1955; Kim et al., 1987) but preheating resulted in no increase in spore sensitivity to irradiation (Grecz et al., 1967; Kan et al., 1957). The pre- or post irradiation temperature played a major role in the subsequent sensitivity to irradiation or heating. The use of irradiation-heating or heating-irradiation has also been reported to decrease conidial resistance. Sommer et al. (1967) reported on the efficacy of various combinations protocols: heating conidia prior to irradiation, irradiation followed by heating and irradiation alone. It was found that *Cladosporium herbarum* and *Monilinia fructicola* conidia showed greater sensitivity when heated at 46-49°C for four minutes and then treated with irradiation as compared to the other two treatments. In contrast, *Rhizopus stonlonifer* was most susceptible to irradiation when conidia were first treated to irradiation then heated. In general heating before irradiation has been shown to induce greater sensitivity to cells and spores (Barkai-Golan et al., 1969; Padwal-Desai et al., 1976a; Sommer et al., 1967).

(3) Age of Culture

Another important factor which has been known to influence the irradiation resistance of organisms is the growth phase or age of the culture. Vegetative cells in the actively growing or logarithmic phase are more susceptible to irradiation than those at the stationary or lag phase (Hastings et al., 1986; Stapleton, 1955). Vegetative cells during the logarithmic phase of growth normally consist of a single stranded DNA whereas in the lag or stationary phase the cell is double stranded (Diehl, 1990). With regards to age, Proctor et al. (1958) noted that bacterial spores of *Bacillus thermoacidurans* lose their resistance to irradiation over time, however, age of *Aspergillus flavus* conidia exhibited no effect on irradiation resistance (Padwal-Desai et al., 1976a). In contrast, Munzer (1969) reported that conidia of *Penicillium viridicatum* and *Aspergillus flavus* did exhibit an increase in sensitivity to radiation with an increase in age.

(4) Atmosphere

In general organisms irradiated in the absence of oxygen were observed to have higher resistances to ionizing irradiation (Hastings et al., 1986). Padwal-Desai et al. (1976a) found that irradiating toxigenic and non-toxigenic *Aspergillus flavus* types under anoxic conditions required twice the dose as compared to conidia irradiated under normal atmospheric conditions. Similarly, Hastings et al. (1986) found that *Lactobacillus sake,* isolated from radurized meat, exhibited maximum resistance under a nitrogen atmosphere while carbon dioxide provided the least protection. In contrast reference strains of *Lactobacillus curvatus*, *Lactobacillus sake* and *Lactobacillus alimentarius* showed only a slightly higher resistance under anoxic and nitrogen atmospheres as compared to air. Diehl (1990) indicated that when using electron beam irradiation, near or anaerobic conditions developed unless oxygen was replaced during treatment. The primary products produced during irradiation treatment in oxygen and water containing environments are superoxide anion radicals which result in the production of hydrogen peroxide (Alpher, 1948; Diehl, 1990). Peroxides have an inhibitory effect on remaining microorganisms (Alpher, 1948) while superoxide anion radicals result in cellular damage (Ewing and Jones, 1987).

(5) pH

The effect of menstruum pH on the irradiation resistance of bacterial cells was shown to be minimal (Duggan et al., 1963b; Edwards et al., 1954; Morgan and Reed, 1954; Wolin et al. 1957). Duggan et al. (1963b) reported that no differences in the number of *Micrococcus radiodurans* survivors was observed following irradiation at pH ranging form 5.3 to 9. As well, results from endospore studies have yielded variable survival results depending on the microogranism (Edwards et al., 1954; Upadhyay and Grecz, 1969). Padwal-Desai et al. (1976a) demonstrated that *Aspergillus flavus* and *A. flavus oryzae* conidia were treated, showed a greater sensitivity to irradiation as the substrate pH was increased from pH 2.5 to 10. In general, the effects of pH on irradiation resistance tend to be regulated by the sensitivity of the organism to changes

in the hydrogen ion concentration of the substrate being irradiated.

(6) Type

Most fungi including aspergilli and penicillia produce spores which are generally haploid but certain genera produce macroconidia which are multicelled and thick walled. These fungi include *Fusarium*, *Curvularia* and *Alternaria* spp. (Cove, 1977; Saleh et al., 1988; O'Neill et al., 1991). O'Neill et al. (1991) and Saleh et al. (1990) found that fungal species which contain haploid conidia were more sensitive to irradiation than conidia which were multicellular. They indicated that multiple hits were required to inactivate multicellular macroconidia as compared to a single hit for a haploid conidia due to multiple target sites present in macroconidia.

(7) Aw

The effect of Aw during irradiation was investigated by Moussa and Diehl (1979) who showed that as the menstruum Aw increased from 0.0 to 0.8 *Salmonella senftenberg* irradiation resistance decreased. Harnulv and Snygg (1973) reported that irradiation resistance of spores of *B. subtilis* and *B. stearothermophilus* increased slightly with decreasing Aw. In glycerol solutions, a phase of rapid increase in resistance with decreasing Aw was followed by a slower increase on further reduction of the Aw. The effect of Aw on the recovery of heat stressed *A. parasiticus* was observed by a decrease in survivors on NaCl supplemented media (Adams and Ordal, 1976). The recovery of bacteria following irradiation generally increased as the Aw decreased from 0.99 on sucrose and glycerol containing media (Harnuv and Snygg, 1973; Moussa and Diehl, 1979).

MATERIALS AND METHODS

Fungal Cultures and Maintenance

The fungal organisms used in this investigation included: *Aspergillus echinulatus* ATCC 1021, *Aspergillus niger* ATCC 52172, *Curvularia geniculata* ATCC 11153 and *Penicillium roqueforti* ATCC 10110. These organisms were obtained from the American Type Culture Collection, Rockville, Md. *Aspergillus ochraceus* NRRL 3174 and *Aspergillus versicolor* NRRL 573 were obtained from National Regional Research Laboratories (Agricultrual Research Service, USDA, Peoria, Ill). *Aspergillus fumigatus* (832), *Aspergillus glaucus* (838), *Penicillium aurantiogriseum* (3298), *Penicillium granulactum* (526), *Penicillium verrucosum* (798) and *Penicillium viridicatum* (1117) were obtained from the Plant Pathology Lab, Manitoba Agriculture, Winnipeg, MB. *Alternaria alternata* and *Cladosporium cladosporiodes* were isolated from grain and were identified by Dr. Mills (Agriculture Canada, Winnipeg, MB). *Penicillium cyclopium* was isolated locally from grain and identification was confirmed by the USDA Agriculture Research Service, Peoria, Ill. All cultures were grown on potato dextrose agar (PDA, Difco) slants at room temperature for 14 d and then maintained at 4°C. Cultures were transfered to fresh slants every 30 d.

Spore Preparation and Harvest

Spores from maintenance cultures were inoculated onto PDA slants and incubated at 25°C for 14 d in the dark (O'Neil, 1991; Saleh, 1988). Resultant spore crops were harvested in the following manner: sterile distilled water (5 mL) was added to culture slants and the conidia were gently dislodged using a sterile glass rod. The spore suspensions were collected in sterile screw-cap test tubes (16 x 100 mm) containing 15

mL of sterile distilled water and filtered twice using sterile Pasteur pipettes (4.62 mm) containing loosely packed glass wool. This procedure was used to remove mycelial fragments and spore clumps (Saleh et al., 1988). The suspensions were checked microscopically. The spore suspensions were diluted to 10^{-5}-10^{-6} colony forming units (CFU)/mL using sterile distilled water. Spore concentrations were confirmed using a serial dilution technique. CFU were enumerated in duplicate on PDA (25°C , 5 d).

Evaluation of Recovery Media for Irradiated Spores

Individual spore suspensions (10^5-10^6 CFU/mL) of either *P. roqueforti, A. glaucus* or *Cl. cladosporiodes* were prepared in sterile distilled water as outlined previously. Triplicate suspensions of each organism were irradiated (0.38 kGy) using electron beam treatment and then serially diluted using Butterfield's phosphate buffered dilution water (PDW; Acuff, 1992). The linear accelerator (Impela I-10/I; AECL, Whiteshell Laboratories, Pinawa MB.) used in this study produced 10-MeV electrons, with a nominal total beam power of 1 kW. Dose rate at the sample position was ca. 1 kGy/s. Survivors were enumerated in duplicate using a pour plate method employing the following media: PDA-non acidified (pH 6.3, Becton Dickinson Microbiology Systems, Cockeysville, MD.), PDA-acidified (0.1% tartaric acid; pH 3.5). Czapek-Dox agar (Difco Laboratories, Detroit, MN), standard plate count agar (SPC, Becton Dickinson Microbiology Systems), SPC fortified with chloramphenicol (Sigma Chemical Corporation St. Louis Mo.; 100 mg/L) and Sabouraud dextrose agar (Difco). All plates were incubated at 25°C for 5 d. The entire protocol was repeated using an irradiation dose of 0.61 kGy.

Spore Irradiation and D_{10} Determination

Aqueous spore suspensions (5 mL, 10^5-10^6 CFU/mL) contained in sterile screw-cap test tubes (16 x 100 mm) were randomly coded for either electron or gamma irradiation treatment and dosage level. Spores treated with electron beam irradiation were exposed to the following nominal doses: 0, 0.3, 0.6 and 1.0 kGy at 20-22°C. All

irradiation trials were performed on samples which were laid horizontally on foam insulation.

For gamma irradiation (Gammacell 220; AECL, Whiteshell Laboratories, Pinawa, MB.) test tubes containing the samples were supported in an aluminum disc assembly with holes at the circumference. The assembly was packed in a 2-L beaker of crushed ice; nominal irradiation doses of: 0, 0.3, 0.6 and 1.0 kGy were used. The gamma ray dose rate was 12.2 kGy/hr. Irradiation treatments for both gamma and electron beam were performed using in duplicate.

Absorbed radiation doses were determined by using radiochromic dye films (GaF, Miller and McLaughlin, 1981) enclosed in test tubes and irradiated along with the sample tubes. The absorbance of the irradiated films was measured at 600 nm and the absorbed dose calculated from a calibration curve. Following irradiation, the suspensions were serially diluted using PDW and pour plated in duplicate using PDA. Incubation was carried out at 25°C for 5 d.

Survival curves were constructed by plotting the log of the survivor CFU/mL versus radiation dose. Curves were fitted by linear regression using Figure Plotter (Fig. P Software Corporation, Durham, NC; 1990). Radiation sensitivity was expressed in terms of D_{10} values. A D_{10} value is defined as the dose required to reduce a given population by 90% of it's initial value; The D_{10} value was determined from the recipical of the slope for the straight-line portion of the survival curve (Ley, 1983).

Spore Recovery Following Irradiation on Aw Adjusted PDA and Rate of Colony Development

Spore suspensions of *A. ochraceus* (10^5 CFU/mL; 15 mL distilled water) contained in screw-cap test tubes were irradiated using either electron beam or gamma ray (0.40 kGy). Following treatment, the spore suspensions were serially diluted using PDW and plated on Aw adjusted PDA. All plates were incubated at 21-22°C for up to 11 d prior to enumeration. Spore suspensions which were not irradiated but which were enumerated on the same Aw adjusted medium served as controls. The Aw adjusted

media consisted of PDA to which sucrose was added such that final Aw levels (nominal) of 0.97, 0.93 and 0.90 were obtained. All media were sterilized for 15 min. at 121°C. The Aw of the media were verified using a water activity meter (Decagon, model CX-1; Pullman, Washington). Similar Aw adjusted PDA media were prepared using either glycerol, sorbitol or NaCl. PDA without solute adjustment had an Aw=0.99. The formulation of the Aw adjusted media is given in Appendix I. This entire protocol was repeated with *P. cyclopium*. Recovery results were expressed as the average of two treatments performed in duplicate. In addition, recovery values were calculated for the survivors by dividing the CFU/mL obtained at a specified Aw/solute by the CFU/mL obtained at Aw=0.99. The value assigned to CFU/mL obtained at Aw=0.99 was 1.0.

In order to assess colony development, the diameters of three isolated colonies were measured on each of the Aw adjusted media for each fungal organism (Brancato and Goulding, 1953). The colony diameters were measured at 24 and 48 h of incubation. The growth rate was expressed as the increase (mm/24h) in colony diameters.

Effect of pH and Aw on Recovery of *A. ochraceus* Spores and Colony Growth Following Gamma Irradiation

Spore suspensions of *A. ochraceus* (10^5 CFU/mL; 15 mL distilled water) contained in screw-cap test tubes were gamma irradiated (0.40 kGy). Following treatment the spore suspensions were serially diluted using PDW and plated on Aw and pH adjusted PDA. All plates were incubated at 25°C for up to 11 d prior to enumeration. Spore suspensions which were not irradiated but which were enumerated on the same Aw and pH adjusted medium served as controls. The Aw adjusted media consisted of PDA to which either sucrose, glycerol or NaCl was added. Nominal Aw levels of 0.99, 0.95, 0.90 and 0.85 were aimed for. The media were pH adjusted prior to sterilization using tartaric acid. The actual pH and Aw of the media were determined from samples of sterilized hardened agar. PDA without adjustment had an Aw=0.99 and a pH=5.5. All results represent the average of two trials performed in duplicate.

In order to assess colony development, the diameters of three isolated colonies were measured for each Aw and pH adjusted medium. The growth rate was expressed as the increase in colony diameter from 24 to 48 h of incubation (mm/24 h).

Influence of Sporulation Aw on Spore Irradiation Resistance

A. ochraceus and *P. cyclopium* were sporulated on a series of PDA media adjusted to the following nominal Aw values: 0.99, 0.95, 0.90 and 0.85 using either sucrose sorbitol, glycerol or NaCl (Appendix I) at 21-22°C. Spores produced on media with an Aw of 0.99 to 0.90 were harvested after 24 d; spores produced on media with an Aw of 0.85 were harvested after 8 wks. The harvesting technique consisted of lightly scrapping off the conidia from the surface of the culture medium using a sterile metal spatula. The spores were then mixed with 50.0 g sterile (121°C @ 20 min., on each of three consecutive days) screened soil (mesh size 100). The spore-soil suspensions were further shaken for 30 min. at 22-25°C using a wrist-action shaker (140 rpm). In order to inactivate any mycelia which may have been carried over with the spores, the spore-soil suspensions were heated at 70°C for 10 min. with agitation in a thermostatically controlled water bath. This treatment was repeated three times; following each heat treatment, the soil-spore suspension was maintained at 22-25°C for 1 h. The spore-soil preparations were adjusted to c. 10^5 CFU/g soil by the addition of sterile soil. Viable counts using PDA (5 d, 22-25°C) were used to verify spore numbers.

To determine irradiation resistance, triplicate spore-soil mixtures (1.0g) from each Aw/solute combination were asceptically transferred to sterile screw-cap test tubes and gamma irradiated (0, 0.3, 0.6, 0.9 and 1.2 kGy; Gamma cell 220). Dosimeters, placed in empty test tubes were used to determine the actual dose. All samples were serially diluted using PBW and enumerated on PDA (5 d, @ 20-23°C). Plating was performed in duplicate. The D_{10} value was determined from the slope of the linear regression line of the survival curves and was calculated for each Aw/solute combination.

Statistical Analysis

A general linear models procedure using Duncan's multiple range Test was used to determine if experiment was significant at $P<0.05$. Duncans Multiple Range Test was used to analyze growth rate results, efficacy of gamma irradiation versus electron beam irradiation, and effect of sporulation Aw on fungal D_{10}values.

RESULTS

Evaluation of Recovery Media for Irradiated Spores

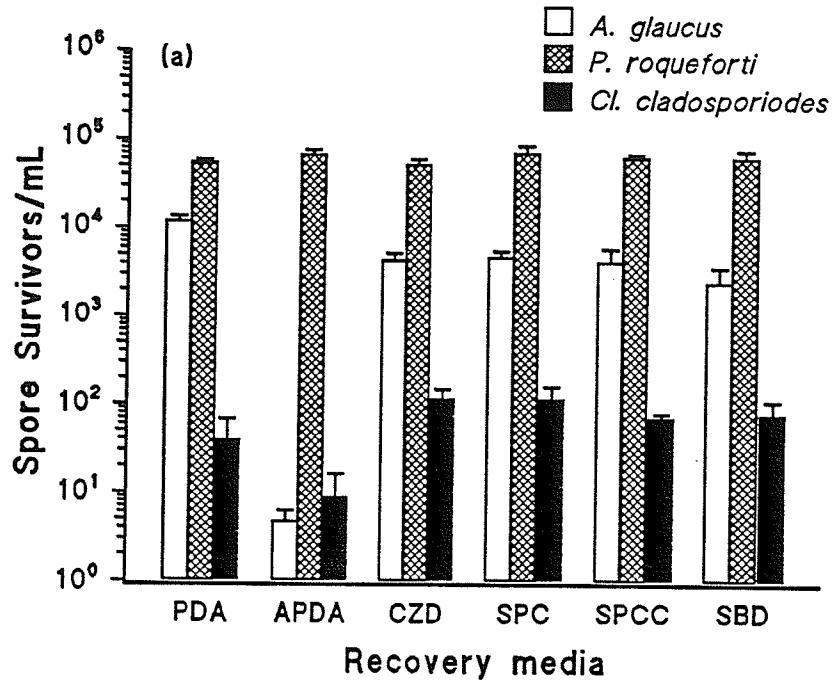
The recovery profile of spores following irradiation treatment at 0.38 kGy on various media is shown in Figure 1a. Among the spore species examined *A. glaucus* exhibited the highest recovery following irradiation which occurred on PDA; the remaining media, with the exception of acidified PDA yielded similar recovery profiles. *P. roqueforti* showed similar recovery patterns on all media. In contrast *Cl. cladosporiodes* exhibited optimum recovery on either SPC or CZD media; similar to *A. glaucus*, poorest recovery of *Cl. cladosporiodes* was observed on acidified PDA.

When the irradiation dose was increased to 0.61 kGy, the recovery patterns for all spore species appeared similar to those patterns obtained with the lower dose (Figure 1b). Overall acidified PDA exhibited the poorest spore recovery with the exception of *P. roqueforti*. Based on these results, PDA was chosen as the recovery medium for future studies.

Determination of D_{10} values for Spores Treated to Either Gamma or Electron Beam Irradiation

The survival curves for *Aspergillus* spores following either gamma or electron beam irradiation are shown in Figure 2. Overall spores appeared more sensitive to electron beam treatment. Similar sensitivity results were obtained with the penicillia spores (Figure 3). Although survivor curves for *A. alternata* and *C. geniculata* indicated that electron beam irradiation also appeared more effective compared to gamma treatment (Figure 4) results with *Cl. cladosporiodes* were less conclusive. Over the dose range (0.0-1.0 kGy) used, it appeared that this organism was equally resistant to both types of

Figure 1. Evaluation of recovery media for irradiated (0.38 kGy, a; 0.61 kGy, b) spores. Potato dextrose agar, PDA; acidified PDA, APDA; Czapek-Dox agar, CZD; Standard plate count agar, SPC; SPC fortified with chlorampenicol, SPCC; Sabouraud dextrose agar, SBD. Bars represent the mean $\pm$s.d., n=6.


(a)
A. glaucus
P. roqueforti
Cl. cladosporiodes
Spore Survivors/mL
10⁶
10⁵
10⁴
10³
10²
10¹
10⁰
PDA
APDA
CZD
SPC
SPCC
SBD
Recovery media

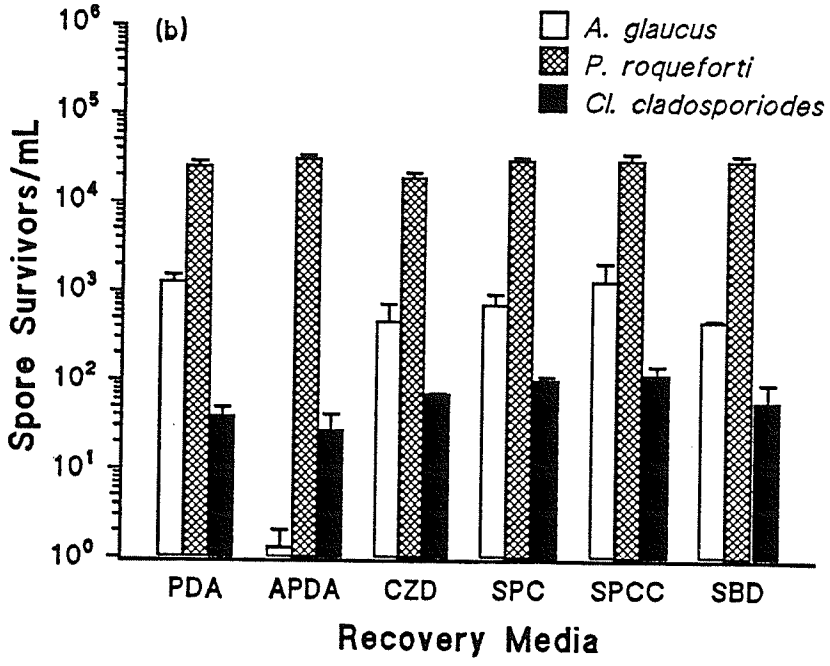
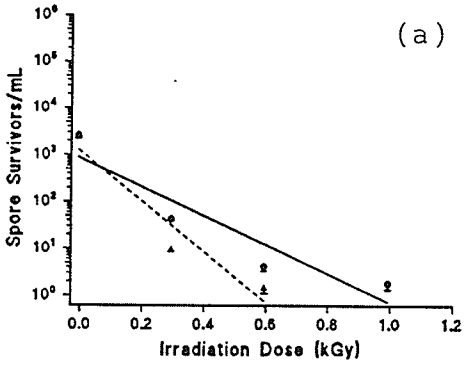
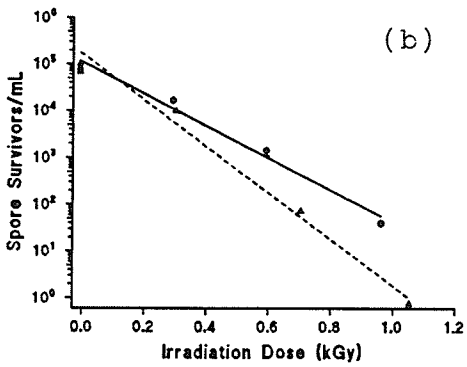
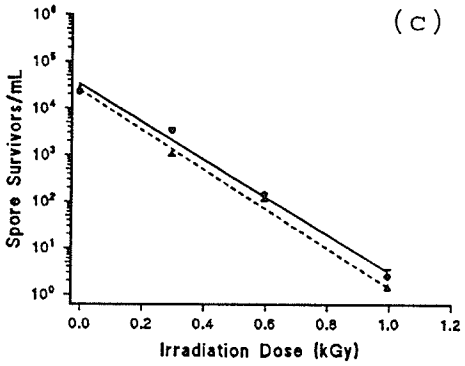
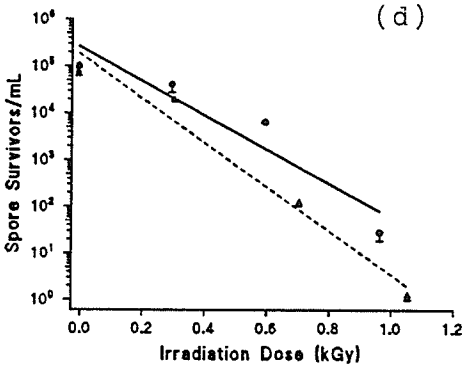

(b)
A. glaucus
P. roqueforti
Cl. cladosporiodes
Spore Survivors/mL
10⁶
10⁵
10⁴
10³
10²
10¹
10⁰
PDA
APDA
CZD
SPC
SPCC
SBD
Recovery Media

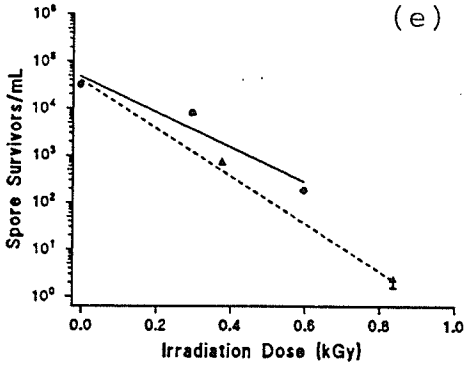
Figure 2. Suvival curves for aspergilli spores following either gamma (——) or electron beam (---) treatment. *A. echinulatus*, a; *A. fumigatus*, b; *A. glaucus*, c; *A. niger*, d; *A. ochraceus*, e; *A. versicolor*, f. Bars represent means ±s.d., n=4.


(a)
Spore Survivors/mL
Irradiation Dose (kGy)


(b)
Spore Survivors/mL
Irradiation Dose (kGy)


(c)
Spore Survivors/mL
Irradiation Dose (kGy)


(d)
Spore Survivors/mL
Irradiation Dose (kGy)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Spore Survivors/mL
Irradiation Dose (kGy)

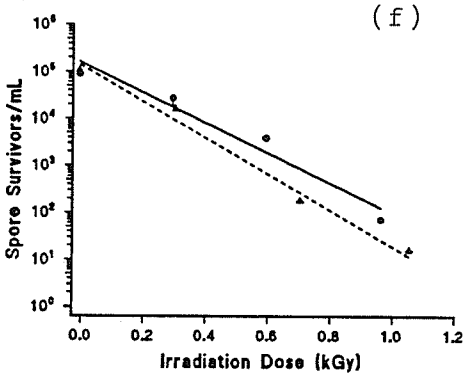
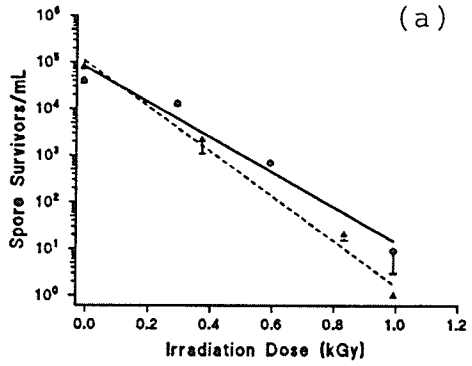
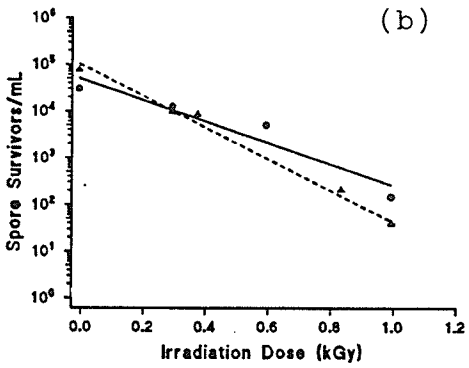
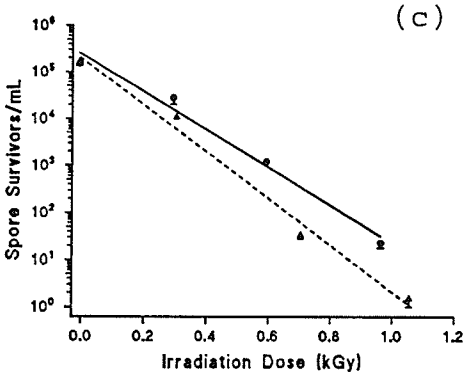
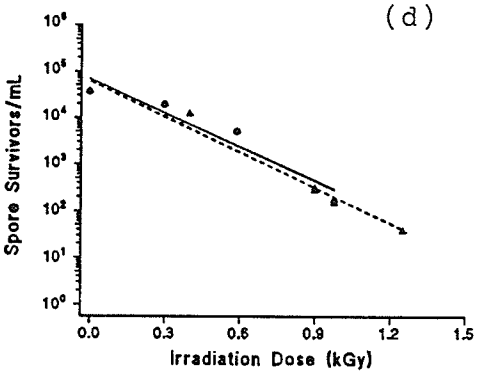

(f)
Spore Survivors/mL
Irradiation Dose (kGy)

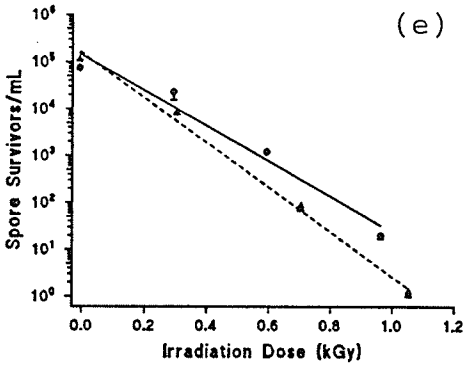
Figure 3. Survival curves for penicilli spores following either gamma (——) or electron beam (---) treatment. *P. aurantiogriseum*, a; *P. cyclopium*, b; *P. granulatum*, c; *P. roqueforti*, d; *P. verrucosum*, e; *P. viridicatum*, f. Bars represent means $\pm$s.d., n=4.


(a)
Spore Survivors/mL
Irradiation Dose (kGy)


(b)
Spore Survivors/mL
Irradiation Dose (kGy)


(c)
Spore Survivors/mL
Irradiation Dose (kGy)


(d)
Spore Survivors/mL
Irradiation Dose (kGy)


(e)
Spore Survivors/mL
Irradiation Dose (kGy)

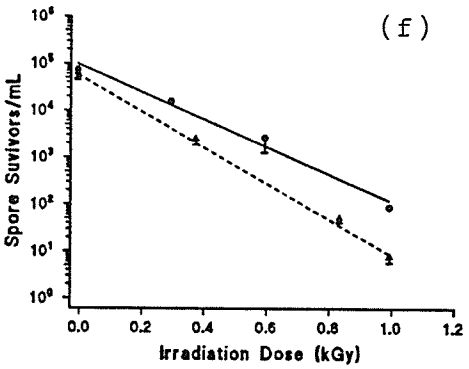
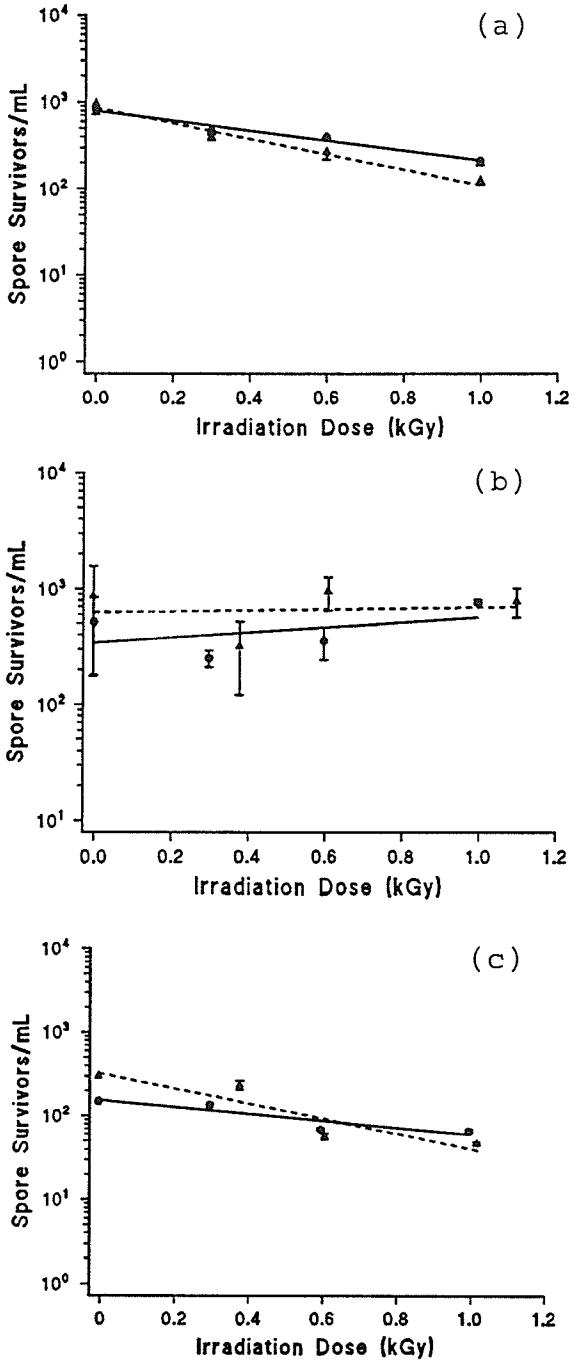

(f)
Spore Suvivors/mL
Irradiation Dose (kGy)

Figure 4. Survival curves for *C. geniculata*, a; *Cl. cladosporiodes*, b; *Alt.alternata*, c; following either gamma (——) or electron beam (---) irradiation. Bars represent means ±s.d., n=4 with the exception of *A. alternata* where n=2.


(a)
Spore Survivors/mL
10^4
10^3
10^2
10^1
10^0
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Irradiation Dose (kGy)
(b)
Spore Survivors/mL
10^4
10^3
10^2
10^1
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Irradiation Dose (kGy)
(c)
Spore Survivors/mL
10^4
10^3
10^2
10^1
10^0
0
0.2
0.4
0.6
0.8
1.0
1.2
Irradiation Dose (kGy)

treatment. The D_{10} values for all organisms were determined from the slopes of the survival curves and are summarized in Table 4. The D_{10} values for the aspergilli ranged from 0.241 kGy (*A. glaucus*) to 0.318 kGy (*A. echinulatus*) for gamma irradiation and from 0.184 kGy (*A. echinulatus*) to 0.240 kGy (*A. glaucus*) for electron beam treatment. For the penicillia spps the D_{10}values ranged from 0.249 kGy for *P. granulatum* to 0.443 kGy for *P. cyclopium* with gamma irradiation. For electron beam treatment the D_{10} values ranged from 0.202 kGy (*P. granulactum*) to 0.403 kGy (*P. roqueforti*). Overall, it appeared that the penicillia were more resistant to irradiation when compared to the aspergilla regardless of the treatment source. When *C. geniculata* and *Alt. alternata* were gamma irradiated, the D_{10} values obtained were 1.786 and 2.184 kGy respectively. Both values decreased when spores were subjected to electron beam treatment (1.127 and 1.170 kGy respectively).

Spore Recovery Following Irradiation on Aw Adjusted PDA and Rate of Colony Development

The recovery profiles for irradiated *A. ochraceus* spores, on Aw adjusted PDA, are given in Table 5. Generally the recovery rate was higher with spores which had been gamma irradiated. Among the control spores, recovery rates appeared highest in PDA adjusted to 0.97 Aw. This was particularly noted in PDA containing sucrose (R=1.54) and to a lesser extent in PDA containing sodium chloride (R=1.29) and sorbitol (R=1.13). Spores surviving either gamma or electron beam irradiation exhibited optimum recovery in PDA containing sodium chloride (0.97 Aw). Overall decreasing the Aw of the recovery media, regardless of the solute species, resulted in a concomitant decrease in survivor numbers except for NaCl 0.97 Aw. Neither control nor treated spores formed colonies on sodium chloride (Aw=0.90) within the appointed incubation period (11 d at 21-22°C).

The recovery profiles for *P. cyclopium* spores following irradiation on Aw adjusted PDA are given in Table 6. Similar to *A. ochraceus* spores, *P. cyclopium* were more sensitive to electron beam treatment. In addition, the control spores exhibited the

Table 4. Summary of D_{10}Values for irradiated spores.

Microorganism	Irradiation Treatment (kGy)			
	Gamma	r^2 value	Electron Beam	r^2 value
A. echinulatus	0.318	0.874	0.184	0.922
A. fumigatus	0.294	0.983	0.203	0.990
A. glaucus	0.241	0.991	0.240	0.995
A. niger	0.274	0.898	0.215	0.971
A. ochraceus	0.268	0.932	0.201	0.996
A. versicolor	0.313	0.954	0.265	0.988
P. aurantiogriseum	0.259	0.966	0.212	0.996
P. cyclopium	0.443	0.918	0.306	0.990
P. granulactum	0.249	0.984	0.202	0.990
P. roqueforti	0.414	0.922	0.403	0.975
P. verrucosum	0.266	0.966	0.212	0.995
P. viridicatum	0.343	0.986	0.266	0.996
C. geniculata	1.786	0.971	1.170	0.979
Cl.cladosporiodes	[1]ND		ND	
Alt. alternata	2.184	0.844	1.127	0.816

[1] D_{10} values were not determined.

Table 5. Recovery of *A. ochraceus* spores in Aw adjusted PDA following irradiation treatment (0.4 kGy).

Solute	Aw	Control		Gamma		Electron beam	
		CFUx10^4/mL	R	CFUx10^4/mL	R	CFUx10^4/mL	R
Sorbitol	0.99	[1]120.0±20.0	[2]1.00	20.0±3.0	1.00	3.5±0.2	1.00
	0.97	135.0±25.0	1.13	13.5±5.5	0.68	2.9±0.1	0.83
	0.93	85.0±25.0	0.71	14.0±4.0	0.70	2.1±0.7	0.60
	0.90	40.5±0.5	0.34	6.0±0.8	0.30	1.6±0.5	0.46
Sodium	0.99	120.0±20.0	1.00	20.0±3.0	1.00	3.5±0.2	1.00
chloride	0.97	155.0±5.0	1.29	28.5±0.5	1.43	4.6±0.6	1.31
	0.93	60.0±10.0	0.50	15.0±3.0	0.75	0.4±0.1	0.11
	0.90	ng[3]		ng		ng	
Glycerol	0.99	120.0±20.0	1.00	20.0±3.0	1.00	3.5±0.2	1.00
	0.97	125.0±5.0	1.04	15.0±1.0	0.75	2.9±2.5	0.83
	0.93	55.0±25.0	0.46	16.0±1.0	0.80	3.9±0.3	1.11
	0.90	83.5±7.5	0.70	15.0±1.0	0.75	2.9±0.7	0.83

Table 5. cont.

Solute	Aw	Control		Gamma		Electron beam	
		CFUx10^4/mL	R	CFUx10^4/mL	R	CFUx10^4/mL	R
Sucrose	0.99	120.0±20.0	1.00	20.0±3.0	1.00	3.5±0.2	1.00
	0.97	185.0±75.0	1.54	12.1±1.0	0.61	2.2±0.2	0.63
	0.93	41.5±5.5	0.35	6.7±0.7	0.34	2.1±0.3	0.60
	0.90	80.0±10.0	0.67	7.5±0.5	0.38	3.5±0.5	1.00

[1][2]Values are expressed as means ±s.d.; n=4.

$$R=\frac{\textit{CFU/mL at either 0.97, 0.93 or 0.90 Aw}}{\textit{CFU/mL at 0.99 Aw}}$$

[3]No growth within the appointed incubation time (11 d at 21-22°C)

Table 6. Recovery of *P. cyclopium* spores in Aw adjusted PDA following irradiation treatment (0.4 kGy).

Solute	Aw	Control		Gamma		Electron Beam	
		CFUx10^4/mL	R	CFUx10^4/mL	R	CFUx10^4/mL	R
Sorbitol	0.99	[1]265.0±75.0	[2]1.00	60.5±4.5	1.00	29.5±5.5	1.00
	0.97	290.0±30.0	1.09	59.0±7.0	0.98	24.5±2.5	0.83
	0.93	245.0±15.0	0.92	78.5±2.5	1.38	25.0±3.0	0.85
	0.90	190.0±40.0	0.72	65.0±5.0	1.07	20.5±5.5	0.69
Sodium	0.99	265±75.0	1.00	60.5±4.5	1.00	29.5±5.5	1.00
chloride	0.97	260±80.0	0.98	60.5±4.5	1.00	35.0±3.0	1.19
	0.93	200±20.0	0.75	67.0±3.0	1.11	27.5±2.5	0.93
	0.90	ng[3]		ng		ng	
Glycerol	0.99	265.0±75.0	1.00	60.5±4.5	1.00	29.5±5.5	1.00
	0.97	320.0±30.0	1.21	64.0±1.0	1.06	21.0±1.0	0.71
	0.93	265.0±35.0	1.00	81.5±9.5	1.35	28.5±1.5	0.97
	0.90	240.0±20.0	0.91	55.0±7.0	0.91	26.5±6.5	0.90

Table 6. cont.

Solute	Aw	Control		Gamma		Electron Beam	
		CFUx10^4/mL	R	CFUx10^4/mL	R	CFUx10^4/mL	R
Sucrose	0.99	265±75.0	1.00	60.5±4.5	1.00	29.5±5.5	1.00
	0.97	780±80.0	2.94	61.0±5.0	1.01	30.0±7.0	1.02
	0.93	335±105.0	1.26	64.5±19.5	1.07	30.0±1.0	1.02
	0.90	110±20.0	0.42	30.0±6.0	0.50	7.0±1.0	0.24

[1][2]Values are expressed as means ±s.d.; n=4.

$$R=\frac{CFU/mL \text{ at either } 0.97,\ 0.93 \text{ or } 0.90\ Aw}{CFU/mL \text{ at } 0.99\ Aw}$$

[3]No growth within the appointed incubation time (11 d at 21-22°C).

highest recovery rate (R=2.94) on PDA containing sucrose (0.97 Aw). Survivor levels following gamma irradiation were highest on 0.93 Aw PDA especially when either sorbitol or glycerol were used as solutes. Recovery values for *P. cyclopium* spores following electron beam treatment were highest (R=1.19) in PDA containing sodium chloride (0.97 Aw).

The rate of growth for *A. ochraceus* colonies on Aw adjusted PDA media is given in Table 7 . Decreasing the Aw of the recovery media from 0.97 to 0.90 regardless of the solute type, decreased rate of colonial growth. In all cases, the growth rate of the colonies appeared maximum on media adjusted to 0.97 Aw. The minimum growth rate was observed on PDA adjusted with sodium chloride (0.9 Aw). A comparison of the growth rates between colonies which developed from spores following either gamma or electron beam irradiation indicated no clear pattern. It was observed, however, that the colony growth rates were consistently the highest at 0.97 Aw following gamma irradiation. The growth rate of colonies originating from the non irradiated spores also exhibited no clear pattern when compared to their irradiated counterparts.

The rate of growth for *P. cyclopium* colonies on Aw adjusted PDA media is given in Table 8. Overall it was observed that decreasing the Aw from 0.97 to 0.90, regardless of solute type, decreased the rate of colony development. The growth rate was invariably highest at 0.97 Aw particularly when sorbitol was used to control the water activity. The lowest growth rates were observed on PDA containing sodium chloride (0.93 Aw). A comparison of the growth rates between colonies which developed following either gamma or electron beam irradiation indicated no clear pattern. The growth rate of control colonies similary exhibited no clear pattern when compared to the growth rates of colonies which developed from either gamma or electron beam treated spores.

Influence of solute Aw and pH on the Recovery and Growth Rate of Spores Following Irradiation

The percent recovery of *A. ochraceus* spores following irradiation was highest (c.

Table 7. The effect of Aw on the growth rate (mm/24hrs) of *A. ochraceus* colonies on PDA medium following irradiation treatment (0.4 kGy).

Solute	Aw	[1]Irradiation Treatment		
		Control	Gamma	Electron
None	0.99	6.38 ± 0.95^{A}	6.05 ± 0.50^{A}	5.18 ± 1.23^{A}
Sucrose	0.97	9.08 ± 0.98^{A}	9.50 ± 1.00^{A}	[2]9.33 ± 0.29^{A}
	0.93	[2]7.93 ± 0.81^{A}	8.20 ± 0.36^{A}	8.50 ± 1.22^{A}
	0.90	4.38 ± 0.48^{A}	3.15 ± 0.66^{B}	2.43 ± 0.83^{B}
Glycerol	0.97	10.13 ± 0.63^{B}	11.05 ± 0.42^{A}	10.85 ± 0.47^{AB}
	0.93	6.65 ± 2.11^{A}	6.50 ± 1.70^{A}	8.93 ± 0.73^{A}
	0.90	2.50 ± 0.82^{A}	2.18 ± 1.25^{A}	[3]1.30 ± 0.99^{A}
Sorbitol	0.97	9.18 ± 1.48^{A}	11.25 ± 1.26^{A}	10.13 ± 1.03^{A}
	0.93	8.20 ± 1.80^{A}	8.00 ± 0.82^{A}	9.33 ± 0.91^{A}
	0.90	4.80 ± 0.36^{B}	6.23 ± 0.76^{A}	6.33 ± 0.70^{A}
NaCl	0.97	6.53 ± 0.78^{A}	5.75 ± 1.50^{A}	[2]4.97 ± 1.12^{A}
	0.93	1.95 ± 0.10^{A}	3.27 ± 1.37^{A}	[2]3.00 ± 0.50^{A}
	0.90	[4]NG	NG	NG

[1] Values represent the means ±s.d., n=4.
[2] Values represent the means ±s.d., n=3.
[3] Values represent the means ±s.d., n=2.
[4] No growth.
[A, B] Means followed by a different superscript within a row are significantly different ($P<0.05$).

Table 8. The effect of Aw on the growth rate (mm/24hrs) of P. cyclopium colonies on PDA medium following irradiation treatment (0.40 kGy).

Solute	Aw	Irradiation Treatment[1]		
		Control	Gamma	Electron
None	0.99	2.25 ± 0.65^{A}	2.50 ± 0.91^{A}	2.25 ± 1.28^{A}
Sucrose	0.97	4.25 ± 1.19^{A}	5.25 ± 0.64^{A}	5.10 ± 1.90^{A}
	0.93	4.83 ± 0.47^{A}	4.35 ± 0.51^{AB}	3.88 ± 0.25^{B}
	0.90	1.90 ± 0.74^{A}	2.38 ± 0.48^{A}	2.50 ± 0.58^{A}
Glycerol	0.97	5.20 ± 0.47^{A}	5.45 ± 1.05^{A}	5.60 ± 0.74^{A}
	0.93	3.05 ± 0.74^{A}	3.75 ± 0.87^{A}	2.50 ± 0.87^{A}
	0.90	1.15 ± 0.82^{A}	1.78 ± 0.22^{A}	1.48 ± 0.55^{A}
Sorbitol	0.97	6.00 ± 0.41^{A}	5.50 ± 0.41^{A}	6.38 ± 1.11^{A}
	0.93	3.35 ± 1.54^{A}	2.88 ± 0.91^{A}	3.05 ± 0.82^{A}
	0.90	2.13 ± 0.95^{AB}	1.80 ± 0.24^{B}	2.93 ± 0.51^{A}
NaCl	0.97	3.43 ± 0.51^{A}	3.05 ± 0.10^{A}	3.40 ± 0.80^{A}
	0.93	0.88 ± 0.25^{A}	1.25 ± 0.56^{A}	1.00 ± 0.00^{A}
	0.90	[2]NG	NG	NG

[1] Values represent the means $\pm$s.d., n=3.

[2] No growth.

[A, B] Means followed by a different superscript within a row are significantly different ($P < 0.05$).

24%) on PDA (pH 7.3) adjusted to 0.905 Aw using NaCl. Recovery rates on the remaining media ranged from 8 to 15% (Table 9).

Within each Aw adjusted medium increasing the pH from c. 5.7 - 6.5 to 7.5 - 7.8 appeared to decrease the recovery of control spores. This was especially evident when either glycerol (0.907 and 0.855 Aw) or NaCl (0.905 Aw) were used as humectants. In the case of glycerol (0.855 Aw) or NaCl (0.905 Aw) increasing the pH of the medium to 7.3 - 7.4 resulted in a c. 0.5 log reduction in log survivors. Increasing the pH of the medium for treated spores, however, appeared to have little effect on their recovery (equal to or less than a 0.25 log increase/decrease). The influence of pH, Aw and solute on the growth of *A. ochraceus* on PDA is given in Table 10. Generally no distinct trends or patterns were observed with either the control or treated colonies with the exception of those developing on PDA containing NaCl. In this case the growth rate of both the control and treated colonies decreased with decreasing pH at 0.96 and 0.90 Aw.

Influence of Sporulation Aw on Spore Irradiation Resistance

The survival curves for *A. ochraceus* and *P. cyclopium* spores following gamma treatment are shown in Figures 5 and 6 respectively. The D_{10} values calculated from the slopes of the survival curves are presented in Tables 11 and 12.

In the case of *A. ochraceus* it was observed that conidia produced on 0.99 Aw (PDA without solute added) appeared most sensitive to irradiation. Among spore crops produced on PDA - sorbtiol those conidiated at 0.95 Aw exhibited the highest ($P < 0.05$) irradiation resistance. In the case of PDA - glycerol, spores produced at 0.90 Aw appeared to exhibit the highest resistance. However, the D_{10} values were not significantly ($P < 0.05$) different from spores produced at 0.85 Aw. Spore crops could not be produced on PDA - NaCl 0.85 Aw. Among spore crops produced at 0.95 Aw those sporulated on PDA - sorbitol exhibited the highest resistance. At 0.90 Aw the highest resistance was exhibited by spores produced on either PDA - glycerol or PDA-sodium chloride. Resistance of spore crops produced at 0.895 Aw was not significantly ($P < 0.05$) different between PDA - sorbitol and PDA - glycerol.

Table 9. The influence of solute, Aw and pH on recovery of *A. ochraceus* spores following irradiation (0.4 kGy) on PDA.

Solute	Aw	pH	Spore Survivors/mL		[2]% Survival
			Control	Irradiation	
Control	0.999	6.50	$84.3x10^4$	$88.0x10^3$	10.4
		6.98	$84.3x10^4$	$93.0x10^3$	11.9
		7.58	$77.7x10^4$	$10.6x10^4$	12.5
Sucrose	0.961	6.03	$88.7x10^4$	$57.0x10^3$	11.2
		6.73	$87.0x10^4$	$81.7x10^3$	9.0
		7.43	$86.3x10^4$	$82.7x10^3$	8.9
	0.912	6.08	$53.0x10^4$	$99.3x10^3$	10.6
		6.63	$75.0x10^4$	$78.3x10^3$	10.8
		7.51	$80.7x10^4$	$78.0x10^3$	10.9
	0.827	6.07	[1]NG	NG	
		6.70	NG	NG	
		7.83	NG	NG	
Glycerol	0.955	5.93	$90.7x10^4$	$81.7x10^3$	9.0
		6.55	$74.0x10^4$	$96.7x10^3$	13.1
		7.30	$89.3x10^4$	$86.0x10^3$	9.6
	0.907	5.70	$97.7x10^4$	$81.3x10^3$	8.4
		6.52	$93.7x10^4$	$10.7x10^4$	11.4
		7.44	$61.0x10^4$	$90.7x10^3$	15.0

Table 9. cont.

Solute	Aw	pH	Spore Survivors/mL		[2]% Survival
			Control	Irradiation	
Glycerol	0.855	5.71	$82.3x10^4$	$85.3x10^3$	10.4
		6.74	$73.3x10^4$	$10.1x10^4$	13.8
		7.46	$33.3x10^4$	$50.0x10^3$	15.0
NaCl	0.960	6.29	$11.1x10^5$	$10.0x10^4$	10.8
		6.88	$12.0x10^5$	$10.5x10^4$	8.8
		7.40	$10.0x10^5$	$10.2x10^4$	10.1
	0.905	6.00	$84.7x10^4$	$93.0x10^3$	11.2
		6.24	$79.0x10^4$	$75.7x10^3$	9.6
		7.37	$33.3x10^4$	$80.3x10^3$	23.9

[1]No growth within the appointed time (21-22°C; 14 d).
[2]CFU irradiation/CFU Control x 100.

Table 10. The influence of solute, Aw and pH on the growth rate (mm/24hr) of *A. ochraceus* spores following irradiation (0.4 kGy) on PDA.

			[1]Irradiation Treatment	
Solute	Aw	pH	Control	Gamma
None	0.999	6.50	5.83 ± 0.29^{B}	7.00 ± 0.00^{A}
		6.98	6.33 ± 0.58^{A}	6.00 ± 1.00^{A}
		7.58	7.00 ± 0.00^{A}	4.67 ± 0.58^{B}
Sucrose	0.955	6.03	6.67 ± 4.16^{B}	13.83 ± 1.44^{A}
		6.73	12.33 ± 2.08^{A}	13.33 ± 2.08^{A}
		7.43	8.33 ± 1.53^{A}	7.33 ± 2.89^{A}
	0.912	6.08	4.33 ± 1.53^{A}	1.33 ± 0.58^{B}
		6.63	2.33 ± 1.53^{A}	2.00 ± 0.00^{A}
		7.51	3.00 ± 1.00^{A}	3.00 ± 1.00^{A}
	0.827	6.07	[2]NG	NG
		6.98	NG	NG
		7.58	NG	NG
Glycerol	0.955	5.93	7.33 ± 1.15^{A}	8.67 ± 1.15^{A}
		6.55	5.50 ± 0.87^{A}	6.33 ± 0.58^{A}
		7.30	4.50 ± 0.50^{B}	6.33 ± 0.58^{A}
	0.907	5.70	1.00 ± 0.00^{A}	3.33 ± 2.31^{A}
		6.52	1.00 ± 1.00^{A}	3.00 ± 1.00^{A}
		7.44	3.00 ± 1.00^{A}	3.67 ± 2.08^{A}

Table 10. cont.

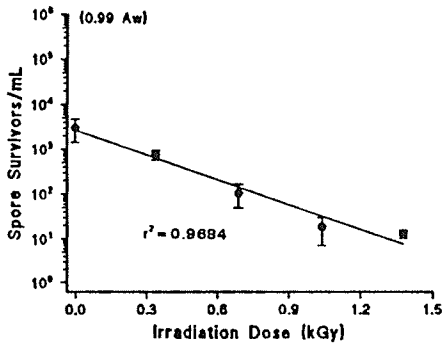
Solute	Aw	pH	[1]Irradiation Treatment None	Gamma
Glycerol	0.855	5.71	0.75 ± 0.25^{A}	0.42 ± 0.29^{A}
		6.74	1.17 ± 0.58^{A}	1.67 ± 0.29^{A}
		7.46	0.25 ± 0.43^{A}	0.25 ± 0.00^{A}
NaCl	0.960	6.29	8.33 ± 0.58^{A}	7.33 ± 2.08^{A}
		6.88	7.67 ± 0.58^{A}	4.33 ± 1.15^{B}
		7.40	5.00 ± 0.00^{A}	3.33 ± 1.15^{A}
	0.905	6.00	1.67 ± 0.58^{B}	3.33 ± 0.58^{A}
		6.24	1.33 ± 0.58^{A}	3.00 ± 1.00^{A}
		7.37	0.75 ± 0.25^{A}	1.25 ± 0.25^{A}

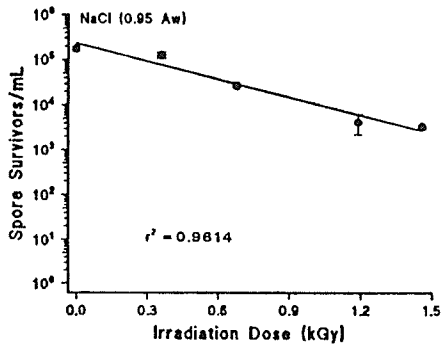
[1] Values represent means$\pm$s.d., n=3

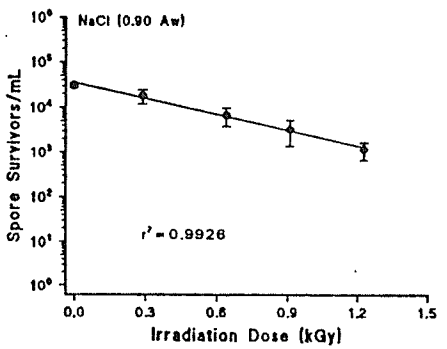
[2] No growth within the appointed time (21-22°C; 14 d).

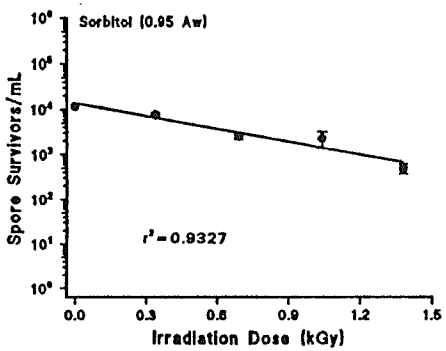
[A, B] Means followed by a different superscript within a row are significantly different ($P<0.05$).

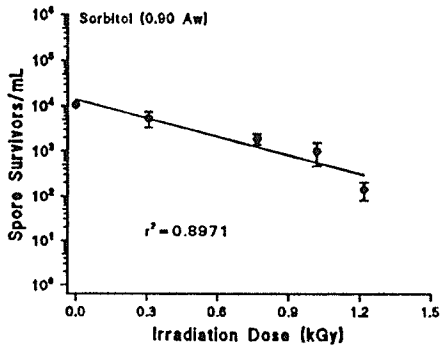
Figure 5. Survival curves for *A. ochraceus* spores following gamma irradiation. Spores produced on Aw adjusted PDA using various solutes. Bars represent the mean $\pm$s.d.; n=6.

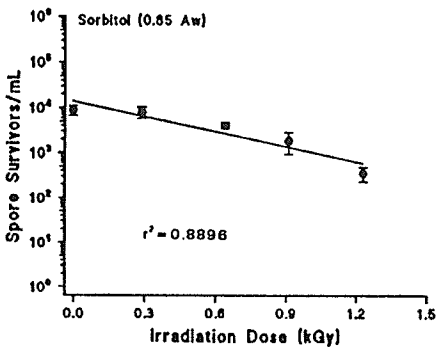

(0.99 Aw)
Spore Survivors/mL
$r^2 = 0.9684$
Irradiation Dose (kGy)

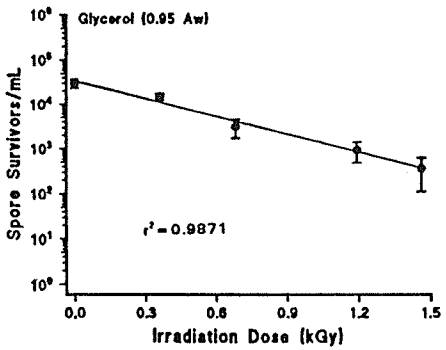

NaCl (0.95 Aw)
Spore Survivors/mL
$r^2 = 0.9614$
Irradiation Dose (kGy)

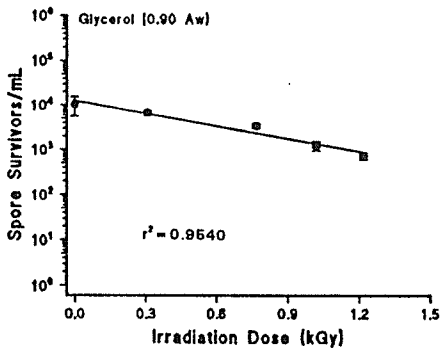

NaCl (0.90 Aw)
Spore Survivors/mL
$r^2 = 0.9926$
Irradiation Dose (kGy)


Sorbitol (0.95 Aw)
Spore Survivors/mL
$r^2 = 0.9327$
Irradiation Dose (kGy)


Sorbitol (0.90 Aw)
Spore Survivors/mL
$r^2 = 0.8971$
Irradiation Dose (kGy)


Sorbitol (0.85 Aw)
Spore Survivors/mL
$r^2 = 0.8896$
Irradiation Dose (kGy)


Glycerol (0.95 Aw)
Spore Survivors/mL
$r^2 = 0.9871$
Irradiation Dose (kGy)


Glycerol (0.90 Aw)
Spore Survivors/mL
$r^2 = 0.9540$
Irradiation Dose (kGy)

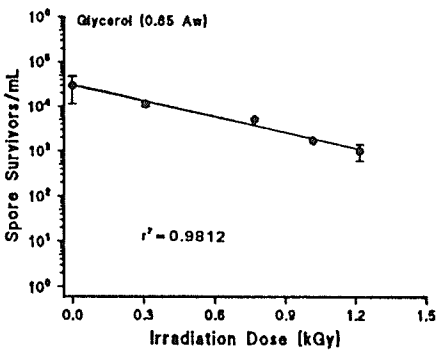
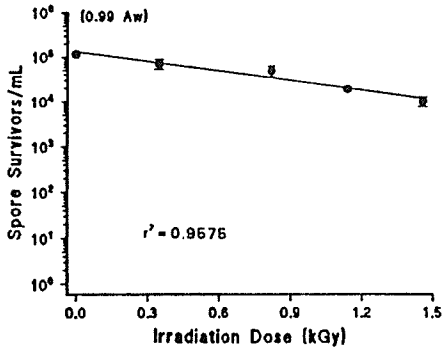
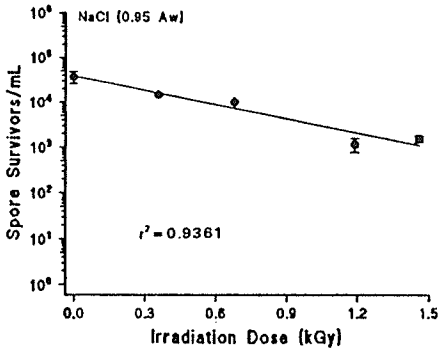
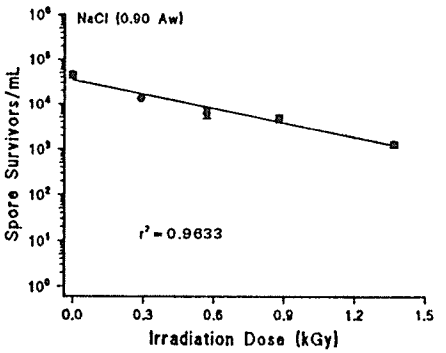
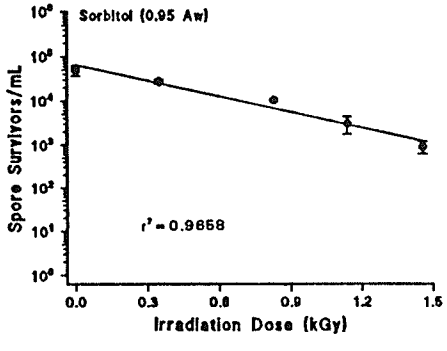

Glycerol (0.85 Aw)
Spore Survivors/mL
$r^2 = 0.9812$
Irradiation Dose (kGy)

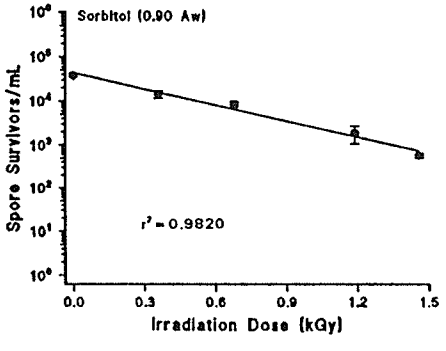
Figure 6. Survival curves for *P. cyclopium* spores following gamma irradiation. Spores produced on Aw adjusted PDA using various solutes. Bars represent the mean $\pm$s.d.; n=6.

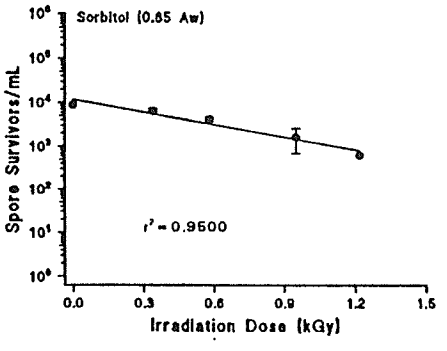

(0.99 Aw)
Spore Survivors/mL
$r^2 = 0.9575$
Irradiation Dose (kGy)

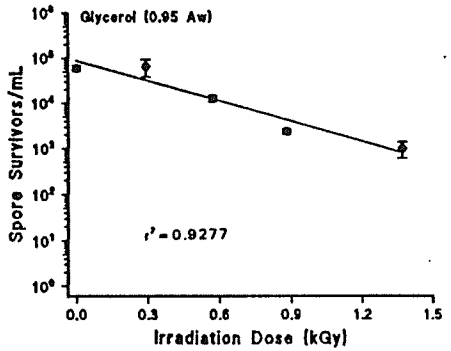

NaCl (0.95 Aw)
Spore Survivors/mL
$r^2 = 0.9361$
Irradiation Dose (kGy)

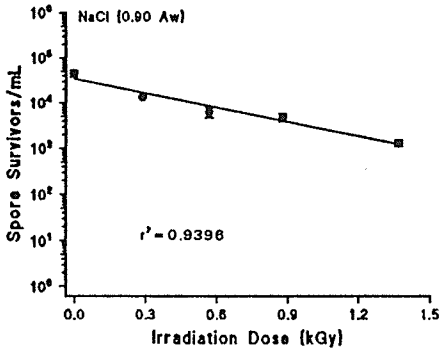

NaCl (0.90 Aw)
Spore Survivors/mL
$r^2 = 0.9633$
Irradiation Dose (kGy)


Sorbitol (0.95 Aw)
Spore Survivors/mL
$r^2 = 0.9658$
Irradiation Dose (kGy)


Sorbitol (0.90 Aw)
Spore Survivors/mL
$r^2 = 0.9820$
Irradiation Dose (kGy)


Sorbitol (0.85 Aw)
Spore Survivors/mL
$r^2 = 0.9500$
Irradiation Dose (kGy)


Glycerol (0.95 Aw)
Spore Survivors/mL
$r^2 = 0.9277$
Irradiation Dose (kGy)


NaCl (0.90 Aw)
Spore Survivors/mL
$r^2 = 0.9396$
Irradiation Dose (kGy)

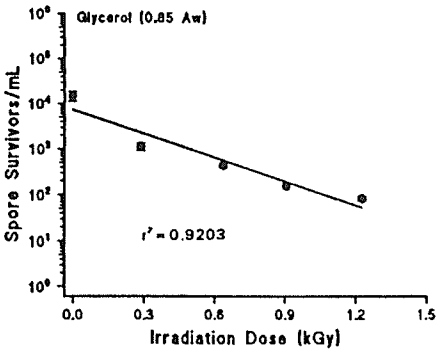

Glycerol (0.85 Aw)
Spore Survivors/mL
$r^2 = 0.9203$
Irradiation Dose (kGy)

Table 11. Effect of sporulation Aw on the radiation resistance (D_{10} values) of *A. ochraceus*.

Solute	[1]D_{10} values for spores produced on Aw			
	0.99	0.95	0.90	0.85
Sorbitol	$0.55 \pm 0.06^{D}_{a}$	$1.05 \pm 0.08^{A}_{a}$	$0.73 \pm 0.09^{C}_{b}$	$0.89 \pm 0.08^{B}_{a}$
Glycerol	$0.55 \pm 0.06^{C}_{a}$	$0.72 \pm 0.11^{BC}_{b}$	$1.09 \pm 0.16^{A}_{a}$	$0.91 \pm 0.18^{AB}_{a}$
NaCl	$0.55 \pm 0.06^{B}_{a}$	$0.75 \pm 0.04^{A}_{b}$	$0.84 \pm 0.07^{A}_{b}$	[2]ND

[A, B, C, D] Means followed by a different superscript within a row are significantly different ($P < 0.05$).

[a,b] Means followed by a different subscript within a column are significantly different ($p < 0.05$).

[1] Values represent the means $\pm$s.d., n=6 (3 trials performed in duplicate).

[2] No sporulation was observed after incubation for 30 d.

Table 12. Effect of sporulation Aw on the radiation resistance (D_{10} values) of *P. cyclopium* spores.

	[1]D_{10} values for spores produced on Aw			
Solute	0.99	0.95	0.90	0.85
Sorbitol	$1.39 \pm 0.14^{A}_{a}$	$0.84 \pm 0.02^{C}_{b}$	$0.83 \pm 0.06^{C}_{b}$	$1.04 \pm 0.07^{B}_{a}$
Glycerol	$1.39 \pm 0.14^{A}_{a}$	$0.67 \pm 0.07^{B}_{c}$	$0.35 \pm 0.05^{C}_{c}$	$0.58 \pm 0.03^{B}_{b}$
NaCl	$1.39 \pm 0.14^{A}_{a}$	$0.95 \pm 0.06^{B}_{a}$	$0.96 \pm 0.07^{B}_{a}$	[2]ND

A, B, C Means followed by a different superscript within a row are significantly different ($P < 0.05$).
a,b Means followed by a different subscript within a column are significantly different ($p < 0.05$).
1 Values represent the means $\pm$s.d., n=6 (3 trials performed in duplicate).
2 No sporulation was observed after incubation for 30 d.

Spores of *P. cyclopium* produced on 0.99 Aw (PDA without solute added) exhibited the highest irradiation resistance ($P < 0.05$). Decreasing the Aw of either PDA - NaCl or PDA - sorbitol from 0.95 to 0.90 did not significantly reduce the irradiation resistance of the spores. With glycerol, however, the reduction in sporulation Aw was accompained by a significant ($P < 0.05$) decrease in resistance. Of the spore crops produced at 0.95 Aw those conidiated on either sorbitol or NaCl significantly had the highest resistance. A similar trend was observed among spore crops conidiated at 0.90 Aw. At an Aw of 0.85 spores produced on PDA - sorbitol displayed a significantly ($P < 0.05$) higher resistance. Spores produced at 0.95 and 0.90 Aw using NaCl were not significantly different. Spores of *P. cyclopium* could not be conidiated on PDA - NaCl 0.85.

DISCUSSION

Media study

Injured microorganisms surviving process treatments including heat or irradiation quite often require exacting recovery conditions in order to undergo repair (Foegeding and Busta, 1983). In an effort to assess the post-irradiation recovery of spores it was deemed necessary to evaluate various commonly used mycological media. Based on the results, all media investigated with the exception of acidified PDA yielded similar recovery profiles. In the present study, non acidified PDA was arbitrarily chosen as the recovery medium In the case of acidified PDA it is likely that the pH and/or acidulant interfered with spore repair. Increasing the dose or changing the radiation source did not appear to alter the recovery profile on the media examined. O'Neill et al. (1991) investigated the effect of common grain fungi to gamma irradiation and reported no significant differences in survivors based on recovery media. Munzner (1969) also reported that when *Aspergillus flavus* spores were subjected to gamma irradiation, only slight differences in survivors were observed among Sabourand, potato dextrose and malt extract agars. Czapek agar, however, exhibited the poorest growth. Buechat (1979) found that acidified PDA resulted in lower recovery of fungi as compared to nonacidified PDA containing only chloramphenicol. In another study (Munzner, 1969), the use of various recovery media was assessed using *P. viridicatum* and *A. flavus*. In the case of *P. viridicatum*, the use of PDA resulted in the highest recovery as compared to Czapek which exhibited a substantially lower recovery. *Aspergillus flavus* also showed a similar recovery profile of irradiated conidia on PDA and Czapek agar.

Irradiation Resistance

Differences in fungal resistance to irradiation may be due to a number of factors including the dose and the nature of the species being treated (Saleh et al., 1988). However, the most important or critical factor which affects irradiation survival of fungi is the level of chromosomal DNA (Diehl, 1990). Aspergilli, penicillia and *Cladosporium* produce conidia which normally contain a single chromosome (haploid), however, conidia containing multiple chromosome sets (polyploid) may be present (Diehl, 1990; Mohyuddin and Skoropad, 1970; Saleh et al., 1988). The presence of multiple chromosomes within a conidium would result in a higher resistance to irradiation (Saleh et al., 1988). In addition, fungal species which produce multicellular, macroconidia such as *Curvularia* or *Alternaria* are more likely to survive irradiation treatment as compared to unicellular conidia due to the increased number of critical target sites (Mohyuddin and Skoropad, 1970; Saleh et al., 1988).

Several other factors which can effect irradiation resistance of conidia include wall thickness and pigment content e.g. melanin (Saleh et al. 1988). Fungi which are capable of producing thick walls and melanin pigment include *Alternaria* and *Curvularia*. In addition, it was found that spores undergoing nuclear division during germination are more sensitive to irradiation (Padwal-Desai et al., 1976b).

Information regarding the effect of the irradiation type on the resistance of microorganisms is scant. D_{10} values obtained from the dose-response curves in this study were higher in all cases with gamma irradiation compared to electron beam treatment. Chelack et al. (1991) working with *Aspergillus alutaceus* var. *alutaceus* compared the irradiation efficiency between gamma and electron beam treatment. The authors also reported higher D_{10} values when gamma irradiation was used. Conidiospores of *Alternaria* and *Curvularia* did, however, exhibit significantly higher resistances to gamma treatment as compared to aspergilli or penicillia species. The resistance (D_{10} values) of penicillia and aspergilli to gamma irradiation were comparable to those reported by O'Neill et al. (1991) and Saleh et al. (1988). In some instances, however, direct comparison of D_{10} values could not be made because of differences in experimental

conditions. Spores of *Alternaria* and *Curvularia* exhibited higher resistances compared to the penicillia and aspergilli regardless of radiation source. As pointed out by Saleh et al. (1988), the presence of multicelled thick-walled macrocondia may impart radiation protection to these fungi. *Cladosporium* which also produces multicellular thick-walled spores (Mallock, 1981) appeared to be radiation resistant within the applied dose range.

Influence of Aw and pH on Recovery and Growth of Irradiated Conidia

The recovery of irradiated fungal spores on media having reduced water activity (Aw) has not been previously investigated. In this study, the effects of solute in the recovery medium was investigated using irradiated conidia of *A. ochraceus* and *P. cyclopium*. It was found that a reduction in Aw resulted in an increase in the recovery of *A. ochraceus* spores at 0.97 Aw but decreased with decreasing Aw. In contrast, *P. cyclopium* demonstrated an increase in recovery as the Aw decreased to 0.93 but there after it also decreased. The optimal Aw for recovery as noted in this study may be close to the optimal Aw for growth in which case the stress caused by the relatively low Aw would be reduced or eliminated. Gamma and electron beam treated *A. ochraceus* spores were best recovered on media containing sodium chloride (0.97 Aw) while untreated conidia were optimally recovered using sucrose. *P. cyclopium* conidia at 0.93 were best recovered using sorbitol or glycerol for gamma treated spores but electron beam treated spores were best recovered at 0.97 Aw using sodium chloride while untreated conidia were optimally recovered using sucrose (0.97 Aw). The influence of solute on the recovery of conidia for all solutes was generally a decrease in recovery below 0.97 for *A. ochraceus* and 0.93 for *P. cyclopium* but the use of NaCl resulted in a large reduction in recovery. The stress produced by the presence of NaCl at high concentrations within the recovery media appeared to inhibit injured conidia to germinate. The influence of NaCl may be due to the fact that some xerophilic molds tend to be more sensitive to salts as compared to sugars or polyalcohols (polyols) which are used to lower the Aw (Beuchat, 1981). In a study by Beuchat and Pitt (1990b), the recovery of heat treated *Chrysosporium farinicola* aleuriospores was found to be highest in reduced Aw media

which used sorbitol followed by glycerol with least recovery on NaCl adjusted media. In another study by Beuchat and Pitt (1990a), the recovery of heat stressed *Wallemia sebi* was optimally recovered using sorbitol followed by glucose in which little variation in recovery was noted as Aw decreased. But when NaCl was used as the humectant, the recovery decreased as Aw was reduced below 0.92 Aw. In a study by Adams and Ordal (1976), it was found that thermally injured conidia of *A. parasiticus* were recovered equally at 0.96 Aw on NaCl, glycerol and sucrose adjusted media but at 0.90 Aw recovery was lowest in media containing NaCl. But the enhanced recovery of irradiation injured *A. ochraceus* conidia and electron beam treated *P. cyclopium* conidia in this study demonstrates that at lower concentrations NaCl may somehow enhance recovery.

The effect of pH on the recovery of heat treated spores is extremely important especially in media where the Aw has been reduced. In this investigation, the affect of pH on spore recovery of *A. ochraceus* varied depending on the solute used for the reduction of Aw. In most cases, the percent survival tended to increase with increasing pH for a given Aw but some variability was noted at 0.95 - 0.96 Aw and 0.90 (NaCl). The tendency for recovery to increase from pH 5.7-6.0 to pH 7.3-7.5 may reflect that pH 7.3-7.5 is near the optimum for growth of *A. ochraceus* thus one would expect higher recovery at this pH value. In a study by Nelson (1972), it was found that untreated (non heated) yeast showed little difference in their ability to grow in various pH adjusted media while heat stressed yeast showed a reduction in recovery numbers as the pH varied past the optimum for growth. The effect of low pH on the recovery of heat stressed conidia was also demonstrated by Beuchat (1979), Beuchat and Pitt (1990a) and Flannigan (1974). It is suspected that at low pH the hydrogen ion concentration is sufficient to inhibit or delay the repair of sublethally injured conidia but has little or no deliterious effect on uninjured conidia (Beuchat and Pitt, 1990a).

The maximal rate of colonial growth was noted at 0.97 Aw, with the colonial growth rate decreasing from 0.97 to 0.90 regarless of solute type for both *A. ochraceus* and *P. cylopium*. In addition, no treatment effects were noted. The lack of difference between treatments and the control maybe due to the nature of the repair mechanisms taking place

before germination or outgrowth. The effect of Aw on the growth rate of injured conidia has been previously demonstrated. The growth rate of *Wallemia sebi* conidia following heat treatment was greatest when sorbitol was used to control the Aw in the recovery medium particularly from 0.8 to 0.97 Aw. Over this Aw range, NaCl had a deleterious effect (Beuchat and Pitt, 1990a). In another investigation, it was reported that heat treated and nontreated conidia of *A. parasiticus* had similar radial growth rates (Adams and Ordal, 1976). It was concluded that the damage caused by heating was repaired before germination; outgrowth took place after an extended lag period which was much shorter for untreated spores. The influence of pH on the growth rate of *A. ochraceus* showed no distinct patterns for control or treated conidia with the exception of media containing NaCl. The lack of distinction may be due to the narrow pH range used in this study. Further investigations using a wider variation in pH should be investigated in order to determine the effects of pH on the recovery of irradiated spores at stressed water activity levels.

Effect of Sporulation Aw on the Irradiation Resistance of Conidia

Based on the results of this study it would appear that the sporulation Aw and the nature of the solute used to control the Aw had an effect on conidial resistance to radiation treatment. In addition, a genus effect was observed, viz, *A. ochraceus* exhibited higher D_{10} values at Aw 0.95-0.85 while *P. cyclopium* exhibited the highest D_{10} value when produced at Aw 0.99. Environmental conditions prevailing during sporogenesis such as temperature have been reported to affect spore quality including heat resistance (El-Bisi and Ordal, 1956; Yokoya and York, 1965). The composition of the sporulation medium has also been reported to impact on the spore heat resistance, germination capacity and pathogenicity of spores (Jackson and Schisler, 1992).

In addition, the water activity of the suspension medium used resulted in an increase in the overall D_{10} values as compared to conidia suspended in distilled water. When a comparison was made between the D_{10} values determined in this study a 2 to 3 fold increase in resistance was noted for conidia irradiated in a dry state. Irradiation

efficiency would require higher treatment dosages for dry or Aw reduced conditions in order to achieve similar radiolytic effects as observed in a liquid suspension. Diehl (1990) indicated that the primary radiolytic product which could lethally damage a cell was hydrogen peroxide which is formed in the presence of water. The radiation treatment in a dry state would require direct damage to the chromosome to produce lethal effects or the production of hydrogen peroxide within the spore if any free water was present. Harnulv and Snygg (1973) found that irradiation resistances of *Bacillus subtilis* and *Bacillus stearothermophilus* spores irradiated in glycerol at Aw's ranging from 0.0 to 1.0 found that the spores resistance to irradiation increased with decreasing Aw. Similarly, the thermal resistance of fungal conidia decreased with increasing humidity (Lubieniecki-Von Scehlhorn, 1973).

SUMMARY

I. All media investigated in this study with the exception of acidified PDA yielded similar recovery patterns of conidia following radiation treatment.

II. Conidiospores of *Alternaria* and *Curvularia* showed significantly higher resistance to gamma treatment as compared to electron beam. All species of aspergilli and penicillia showed higher resistance (D_{10}) to radiation when treated with a gamma source. In addition, *Alternaria* and *Curvularia* exhibited much higher resistances then aspergilli or penicillia.

III. Recovery of irradiated (either gamma or electron beam) *A. ochraceus* spores was optimum at 0.97 Aw especially on media adjusted using NaCl. *P. cyclopium* conidia treated with electron beam irradiation showed optimum recovery at 0.97 Aw adjusted using NaCl while gamma treated spores were best recovered at 0.93 Aw adjusted with sorbitol or glycerol.

IV. The colonial growth rates of *A. ochraceus* and *P. cyclopium* decreased as Aw decreased from 0.97 to 0.90 Aw. No clear patterns were observed between gamma and electron beam treated conidia.

VI. The influence of pH on the recovery of *A. ochraceus* spores was especially evident on PDA (pH 7.3) adjusted to 0.905 Aw using NaCl. In general, an increase in pH resulted in a concomitent decrease in the number of survivors for each Aw and solute combination tested. No trends were observed in regards to

colonial growth rate with the exception of NaCl in which case the growth rate of treated and untreated conidia decreased with decreasing pH at 0.96 and 0.90 Aw.

VII. The nature of the solute used to control the Aw during sporulation affected spore resistance to irradiation treatment. In addition, a genus effect was observed in which *A. ochraceus* demonstrated a higher resistance to sporulation as the Aw decreased; whereas *P. cyclopium* resistance decreased with decreasing sporulation Aw.

VIII. Further work should focus on the hydration of fungal conidia following sporulation in low Aw environments and the effect of substrate composition during sporulation such as pH and antimicrobials on irradiation resistance.

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APPENDICES

Appendix I. Mycological growth media formulations.

Solute Type	Water Activity	Solute (g)	[1]Distilled water (mL)
Sucrose	1.00	0	1000
	0.97	314.9	851.1
	0.95	422.2	740.7
	0.93	414.2	647.2
	0.90	659.8	573.8
	0.85	857.1	476.2
Sorbitol	1.00	0	1000
	0.97	247.1	882.6
	0.95	315.8	789.5
	0.93	400.8	715.7
	0.90	516.8	654.2
	0.85	787.1	645.2
Glycerol	1.00	0	1000
	0.97	117.4	917.4
	0.95	186.4	847.5
	0.93	236.2	787.4
	0.90	328.4	746.3
	0.85	437.5	656.9

Appendix I. cont.

Solute Type	Water Activity	Solute (g)	[1]Distilled Water (mL)
NaCl	1.00	0	1000.0
	0.97	43.3	985.2
	0.95	68.0	970.9
	0.93	97.6	956.9
	0.90	132.1	943.4
	0.85	186.9	934.6

[1] 15 g PDA (15 g, Difco) was added to distilled water prior to the addition of solute. Final volume was ca. 1000 mL.

Appendix II. Evaluation of recovery media for spores following electron beam irradiation.[1]

Media	Dose (kGy)	Organisms (CFU/mL)		
		A. glaucus	*Cl. cladosporoides*	*P. roqueforti*
PDA	0.38	$1.08 \pm 0.17 \times 10^{4}$	$3.72 \pm 2.70 \times 10^{1}$	$4.93 \pm 0.41 \times 10^{4}$
APDA	0.38	$4.50 \pm 1.50 \times 10^{0}$	$8.50 \pm 6.93 \times 10^{0}$	$6.03 \pm 0.74 \times 10^{4}$
CZD	0.38	$3.99 \pm 0.76 \times 10^{3}$	$1.09 \pm 0.30 \times 10^{2}$	$4.63 \pm 0.70 \times 10^{4}$
SPC	0.38	$4.30 \pm 0.70 \times 10^{3}$	$1.09 \pm 0.40 \times 10^{2}$	$6.27 \pm 1.33 \times 10^{4}$
SPCC	0.38	$3.81 \pm 1.54 \times 10^{3}$	$6.84 \pm 0.63 \times 10^{1}$	$5.60 \pm 0.49 \times 10^{4}$
SBD	0.38	$2.26 \pm 1.08 \times 10^{3}$	$7.38 \pm 2.88 \times 10^{1}$	$5.54 \pm 1.17 \times 10^{4}$
PDA	0.61	$1.17 \pm 0.25 \times 10^{3}$	$3.88 \pm 0.98 \times 10^{1}$	$2.32 \pm 0.30 \times 10^{4}$
APDA	0.61	$1.25 \pm 0.75 \times 10^{0}$	$2.68 \pm 1.33 \times 10^{1}$	$2.78 \pm 0.28 \times 10^{4}$
CZD	0.61	$4.30 \pm 2.40 \times 10^{2}$	$6.88 \pm 0.13 \times 10^{1}$	$1.71 \pm 0.25 \times 10^{4}$
SPC	0.61	$6.60 \pm 2.13 \times 10^{2}$	$9.90 \pm 0.90 \times 10^{1}$	$2.67 \pm 0.23 \times 10^{4}$
SPCC	0.61	$1.20 \pm 0.70 \times 10^{3}$	$1.15 \pm 0.28 \times 10^{2}$	$2.72 \pm 0.49 \times 10^{4}$
SBD	0.61	$4.37 \pm 0.15 \times 10^{2}$	$5.65 \pm 3.29 \times 10^{1}$	$2.71 \pm 0.38 \times 10^{4}$

[1]Results are the average of three trials performed in duplicate (n=6) ± standard deviation.

Appendix III. *Aspergillus* spore survivors following either gamma or electron beam irradiation.

	Electron Beam	
Organism	Dose (kGy)	Survivors ±s.d./mL[1]
A. echinulatus	0.00	$2.3\pm0.3\times10^{3}$
	0.30	$8.6\pm1.1\times10^{0}$
	0.60	$1.4\pm0.6\times10^{0}$
	1.00	0.0
A. fumigatus	0.00	$9.9\pm1.6\times10^{4}$
	0.31	$9.5\pm0.6\times10^{3}$
	0.71	$7.1\pm0.6\times10^{1}$
	1.06	$0.8\pm0.5\times10^{0}$
A. glaucus	0.00	$3.5\pm1.0\times10^{4}$
	0.38	$1.0\pm0.1\times10^{3}$
	0.84	$3.3\pm0.6\times10^{1}$
	1.00	$2.3\pm1.5\times10^{0}$
A. niger	0.00	$7.6\pm2.1\times10^{4}$
	0.31	$1.9\pm0.1\times10^{4}$
	0.71	$1.2\pm0.2\times10^{2}$
	1.06	$1.3\pm1.0\times10^{0}$

Appendix III. cont.

Organism	Dose (kGy)	Survivors ±s.d./mL[1]
	Electron Beam	
A. ochraceus	0.00	$3.2\pm0.4\times10^{4}$
	0.38	$7.1\pm2.0\times10^{2}$
	0.84	$2.3\pm1.3\times10^{0}$
	1.00	0.0
A. versicolor	0.00	$1.1\pm0.1\times10^{5}$
	0.31	$1.5\pm0.1\times10^{4}$
	0.71	$1.8\pm0.1\times10^{2}$
	1.06	$1.6\pm1.2\times10^{1}$
	Gamma	
A. echinulatus	0.00	$2.5\pm0.3\times10^{3}$
	0.30	$4.1\pm0.5\times10^{1}$
	0.60	$4.0\pm1.7\times10^{0}$
	1.00	$2.9\pm1.7\times10^{0}$
A. fumigatus	0.00	$7.5\pm2.0\times10^{4}$
	0.30	$1.6\pm0.1\times10^{4}$
	0.60	$1.4\pm0.4\times10^{3}$
	0.97	$4.0\pm0.5\times10^{1}$

Appendix III. cont.

Organism	Gamma	
	Dose (kGy)	Survivors ±s.d./mL[1]
A. glaucus	0.00	$2.7\pm0.1\times10^{4}$
	0.30	$9.1\pm1.4\times10^{3}$
	0.60	$8.9\pm0.9\times10^{2}$
	1.00	$2.4\pm0.5\times10^{1}$
A. niger	0.00	$1.0\pm0.1\times10^{5}$
	0.30	$4.0\pm1.5\times10^{4}$
	0.60	$6.5\pm1.0\times10^{3}$
	0.97	$2.8\pm1.3\times10^{1}$
A. ochraceus	0.00	$3.1\pm0.1\times10^{4}$
	0.30	$7.9\pm1.1\times10^{3}$
	0.60	$1.8\pm0.3\times10^{2}$
	1.00	0.00
A. versicolor	0.00	$8.9\pm0.6\times10^{4}$
	0.30	$2.7\pm0.9\times10^{4}$
	0.60	$3.8\pm0.6\times10^{3}$
	0.97	$7.0\pm0.3\times10^{1}$

[1] Values represent the means±s.d.; n=4.

Appendix IV. *Penicillium* spore survivors following either gamma or electron beam irradiation.

	Electron Beam	
Organism	Dose (kGy)	Survivors ±s.d./mL[1]
P. aurantiogriseum	0.00	$7.8\pm1.3\times10^{4}$
	0.38	$2.1\pm1.2\times10^{3}$
	0.84	$2.0\pm2.2\times10^{1}$
	1.00	$1.0\pm0.0\times10^{0}$
P. cyclopium	0.00	$7.4\pm0.6\times10^{4}$
	0.38	$8.3\pm1.2\times10^{3}$
	0.84	$2.0\pm0.2\times10^{2}$
	1.00	$3.9\pm0.5\times10^{1}$
P. granulactum	0.00	$1.6\pm0.3\times10^{4}$
	0.31	$1.1\pm0.1\times10^{3}$
	0.71	$3.4\pm0.8\times10^{1}$
	1.06	$1.5\pm1.9\times10^{0}$
P. roqueforti	0.00	$3.0\pm0.5\times10^{4}$
	0.40	$9.8\pm0.9\times10^{3}$
	0.90	$2.6\pm0.8\times10^{2}$
	1.25	$3.2\pm0.3\times10^{1}$

Appendix IV. cont.

Organism	Electron Beam	
	Dose (kGy)	Survivors ±s.d./mL[1]
P. verrucosum	0.00	$1.1\pm0.1\times10^{5}$
	0.31	$8.2\pm1.1\times10^{3}$
	0.71	$8.4\pm2.1\times10^{1}$
	1.06	$1.3\pm1.0\times10^{0}$
P. viridicatum	0.00	$4.8\pm0.8\times10^{4}$
	0.38	$2.4\pm0.7\times10^{3}$
	0.84	$5.0\pm3.2\times10^{1}$
	1.00	$7.5\pm3.1\times10^{0}$
	Gamma	
P. aurantiogrieseum	0.00	$4.0\pm0.7\times10^{4}$
	0.30	$1.3\pm0.2\times10^{4}$
	0.60	$6.9\pm1.4\times10^{2}$
	1.00	$9.0\pm7.0\times10^{0}$
P. cyclopium	0.00	$3.0\pm0.4\times10^{4}$
	0.30	$1.2\pm0.5\times10^{4}$
	0.60	$5.0\pm0.2\times10^{3}$
	1.00	$1.5\pm0.1\times10^{2}$

Appendix IV. cont.

	Gamma	
Organism	Dose (kGy)	Survivors ±s.d./mL[1]
P. granulactum	0.00	$1.5\pm0.0\times10^{5}$
	0.30	$2.7\pm1.0\times10^{4}$
	0.60	$1.2\pm0.2\times10^{3}$
	0.97	$2.3\pm0.7\times10^{1}$
P. roqueforti	0.00	$3.0\pm0.4\times10^{4}$
	0.30	$1.6\pm0.2\times10^{3}$
	0.59	$4.2\pm0.5\times10^{3}$
	0.98	$1.4\pm0.4\times10^{2}$
P. verrucosum	0.00	$7.4\pm1.8\times10^{4}$
	0.30	$3.4\pm0.9\times10^{4}$
	0.60	$1.2\pm0.1\times10^{3}$
	0.97	$2.0\pm0.7\times10^{1}$
P. viridicatum	0.00	$7.1\pm2.0\times10^{4}$
	0.30	$1.5\pm0.1\times10^{4}$
	0.60	$2.5\pm1.5\times10^{3}$
	1.00	$8.7\pm0.7\times10^{1}$

[1] Values represent the means ±s.d.; n=4.

Appendix V. Spore survivors following either gamma or electron beam irradiation.

Organism	Electron Beam	
	Dose (kGy)	Survivors ±s.d./mL[1]
Alt. alternata[2]	0.00	$3.0\pm0.0\times10^{2}$
	0.38	$2.3\pm0.3\times10^{2}$
	0.61	$5.6\pm0.5\times10^{1}$
	1.02	$4.7\pm0.1\times10^{1}$
Cl. cladosporoides	0.00	$3.7\pm0.4\times10^{3}$
	0.30	$2.2\pm0.1\times10^{2}$
	0.60	2.2±0.1x102
	1.00	1.9±0.2x102
C. geniculata	0.00	9.4±0.2x102
	0.30	3.9±0.8x102
	0.60	2.7±1.1x102
	1.00	1.2±0.3x102
	Gamma	
Alt. alternata[2]	0.00	$1.5\pm0.0\times10^{2}$
	0.30	$1.4\pm0.1\times10^{2}$
	0.60	$6.8\pm0.3\times10^{1}$
	1.00	$6.5\pm0.4\times10^{1}$

Appendix V. cont.

Organism	Gamma	
	Dose (kGy)	Survivors ±s.d./mL[1]
Cl. cladosporoides	0.00	$1.6\pm0.2\times10^3$
	0.30	$3.0\pm0.6\times10^3$
	0.60	$3.2\pm0.1\times10^3$
	1.00	$1.5\pm0.2\times10^3$
C. geniculata	0.00	$8.1\pm2.1\times10^2$
	0.30	$4.6\pm0.8\times10^2$
	0.60	$3.9\pm0.4\times10^2$
	1.00	$2.1\pm0.5\times10^2$

[1] Values represent the means ±s.d.; n=4.
[2] Values represent the means ±s.d.; n=2.