POSTHARVEST ENVIRONMENTAL FACTORS AFFECTING INFECTION OF KIWIFRUIT BY *BOTRYTIS CINEREA.*

A thesis presented in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy in
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at
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Palmerston North
New Zealand.

Silvia Bautista Baños
May 1995

To my husband J. Raúl:

With my deepest gratitude and love, forever.

ABSTRACT

In vitro germination of *B. cinerea* isolates from diseased blueberry, camellia, grapes, kiwifruit and strawberry were compared at Massey University (Palmerston North) and pathogenicity on kiwifruit at both Massey University and at the New Zealand Institute for Crop and Food Research (Levin). An average of 74.4% of spores germinated on agar when the concentration was 5.9×10^4 but only 62.8% when it was 1.5×10^6. Percentage spore germination on agar did not indicate relative pathogenicity on kiwifruit and there was no significant difference in disease incidence of fruit inoculated with different isolates.

The effect of culture medium and of age of culture from which conidial inoculum was prepared were evaluated by growing *B. cinerea* on malt agar, potato dextrose agar and autoclaved kiwifruit leaves for seven, 18 or 28 days. Each fruit was inoculated with one drop of a 0.05% Tween 20 suspension containing 1,000, 5,000, 25,000 or (Levin only) 125,000 spores per drop. Disease incidence was proportional to inoculum concentration. There was no significant effect of colony age. The highest disease incidence at Massey University was with inoculum produced on malt agar whereas at Crop and Food Research it was with inoculum produced on autoclaved kiwifruit leaves. All further inoculation work was carried out using the K3 isolate from Massey University grown on Malt agar for 10-14 days.

The ability of *B. cinerea* conidia to survive temperature/humidity regimes that could be used for curing kiwifruit was tested by exposing conidia on glass slides to combinations of 0, 10, 15, 25 or 30°C with low (<50%), medium-low (64-80%), medium-high (80-90%) or high (>90%) relative humidities for two, four, six or eight days. Both the percentage germination and the speed of germination decreased at the higher temperatures and with longer exposure times.

The effect of temperature during curing on subsequent infection levels was investigated in 1992, of humidity in 1993 and of both temperature and humidity in 1994. After harvest, each fruit was inoculated with 125,000 spores (1992) or 25,000 spores (1993 and 1994). In 1994 dry conidial application using a paintbrush was also included. The greatest curing effect was obtained at 10°C. Disease incidence increased at 0°C and the curing effect diminished at temperatures above 10°C. Fruit cured at 20°C and at 30°C softened rapidly and developed a high incidence of disease. In 1994 a three day curing period was used and 10°C again gave the lowest subsequent disease incidence. After twelve weeks coolstorage (1993) there was less disease in fruit cured at 89-95% relative humidity than at lower humidities. In 1994 comparable results were obtained.

The effect of curing regimes on fruit physiology showed that ethylene production increased and rate of respiration decreased with higher curing temperature but both increased with incubation time. There was no consistent pattern of treatment effect on ethylene production or on rate of respiration during subsequent coolstorage. Fruit firmness decreased with higher curing temperatures and as the curing period was extended. Firmness fluctuated with harvest and in general decreased with storage although a satisfactory firmness was maintained throughout coolstorage from all treatments. There was no consistent relationship between temperature/time of incubation and total soluble solids content during curing and during storage. As the period of storage increased glucose and fructose content of fruit increased. pH remained constant in fruit from all treatments and there was no consistent relationship between acid buffering capacity measured as citric acid equivalent and curing temperature/incubation times during subsequent coolstorage. For all experiments, weight loss increased with increased curing temperature or with decreased relative humidity.

Kiwifruit stem scars consisted of two main tissue systems: Ground and

vascular. Parenchyma, collenchyma and idioblasts containing raphides were the main components of the ground tissue. The vascular system consisted of xylem vessels, phloem and cambium. There was no evidence of anatomical structures blocking the xylem vessels in *Botrytis* infected fruit cured at 0°C or at 10°C. Samples from both showed some evidence of thickening of the parenchyma cell walls in contact with conidial hyphae. Positive reactions to lignin, suberin and reducing compounds were observed in all treatments. Suberin development in xylem and parenchyma scar tissue was found at 10°C but not at 0, 20 or 30°C.

Initial relative humidity ranges of 34-80%, 75-90% and 100% were tested during coolstorage at 0°C in 1992 and 40-59%, 65-80% and 92-97% in 1994. Inoculum levels applied to the stem scar were 5000 and 25000 spores/ml respectively and infection levels were evaluated after 12 weeks coolstorage. There was no definite pattern in ethylene production and rate of respiration during the incubation period. In both, 1992 and 1994 experiments weight loss increased as relative humidity decreased. TSS increased during incubation for all treatments. Firmness decreased with incubation time and after three months coolstorage for all treatments. In the second experiment of 1994 there was a more marked effect of relative humidity on firmness. Fruit firmness decreased with harvest maturity. In the 1992 experiment fruit disease decreased as incubation time increased and in 1994, infection levels decreased as relative humidity increased.

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CHAPTER ONE

GENERAL INTRODUCTION

World market and economic importance

Worldwide, kiwifruit (*Actinidia deliciosa* (A. Chev.) C.F. Liang *et* A.R. Ferguson var. *deliciosa*) production has become one of the fastest-developing horticultural industries of the last 10-15 years. In New Zealand, the kiwifruit industry has developed as one of the most important horticultural crops. In 1992 world production of kiwifruit was 793,000 tonnes with Italy and New Zealand each producing about 270,000 tonnes (Costa *et al.* 1991). At present, New Zealand's share of the international kiwifruit market returns NZ $500 million per year. The main international importers are Japan, The United States and several European countries (Anonymous 1995).

Commercial orchards in New Zealand

In New Zealand, kiwifruit have traditionally been grown in citrus growing areas of the Auckland province, but commercial plantings can now be found from Northland to Nelson and even in some areas of Marlborough and Canterbury. There are some commercial orchards of kiwifruit in the Wanganui and Horowhenua regions and more recently, in Hawkes Bay. However the largest area of kiwifruit plantings are located in the various districts of the Bay of Plenty (eg. Te Puke, Opotiki, Tauranga) where almost 90% of New Zealand commercial kiwifruit plantings are to be found (Fig.1-1) (Ferguson & Bollard 1990).

Cultural, Management and Harvesting Practices for kiwifruit in New Zealand

In earlier years, varieties such as "Bruno", "Abbot/Allison", "Monty", "Hayward", "Gracie", "Brodie", "Wilkins super", were selected and propagated throughout New Zealand, but in recent years the storage qualities of cv "Hayward" made this selection the most important variety. At present it is almost (98%) the sole variety of commerce both in New

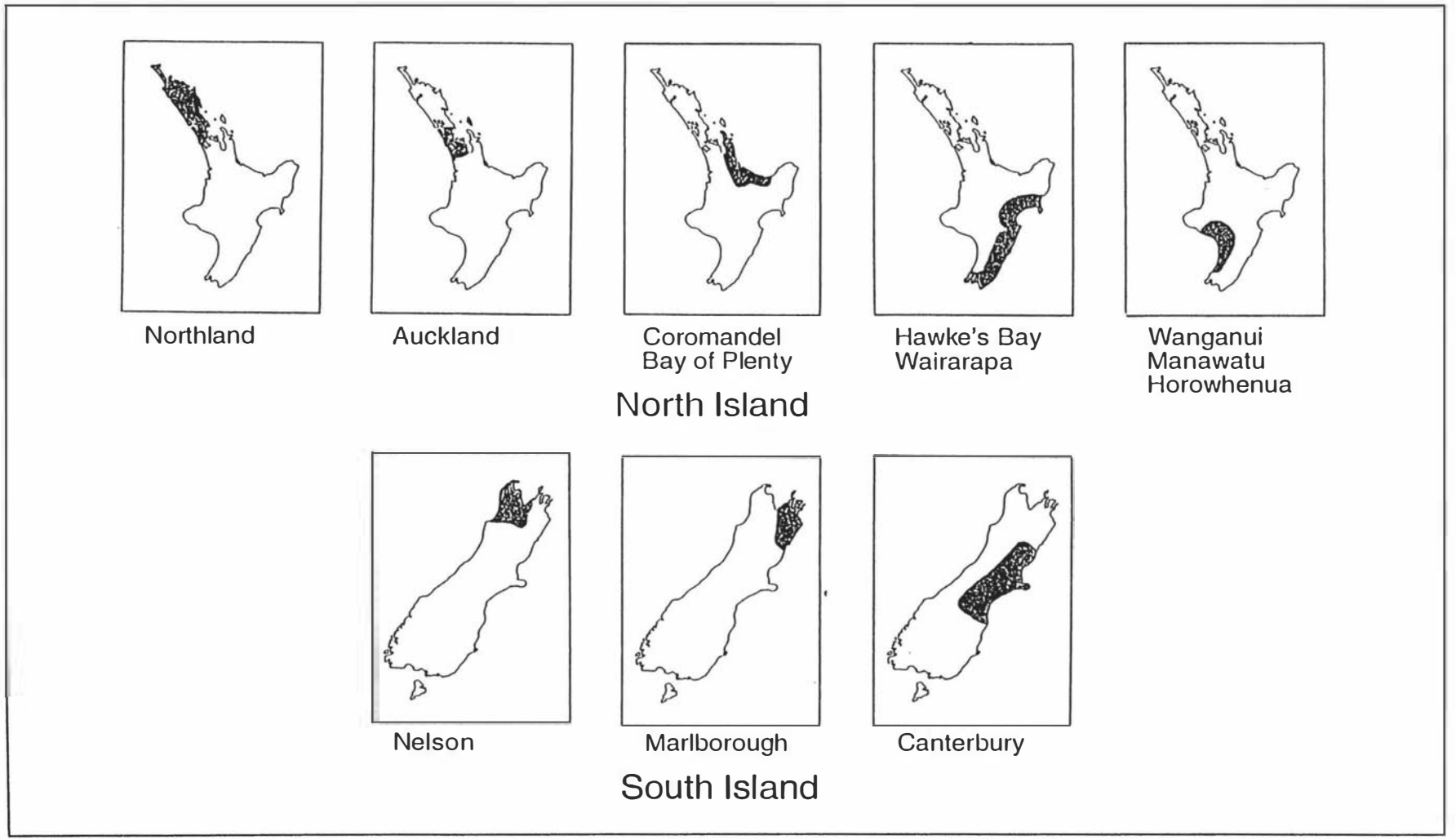


Figure 1-1. Kiwifruit growing areas in New Zealand (compiled from various sources).

Zealand and overseas (Sale 1990). There is concern about an industry based on one cultivar only and Crown Research Institutes such as the Horticultural Research Institute of New Zealand are proceeding with the selection and development of new varieties. For comparison, the apple industry is of a similar size but is based on a number of varieties which include early, mid-season and late varieties and red, yellow or green fruit coloration. Since kiwifruit can be successfully stored for many months there is less of a market requirement for an extension of season, both earlier and later, although this could reduce peak season labour requirements for harvesting. The current research interest in the development of new varieties is for cultivars that are hairless, and for varieties with a different coloured flesh (Pringle *et al.* 1991;).

Among the wide range of cultural and management practices of kiwifruit, training and pruning have become the most important aspects of vine management (Sale 1990). The development of training and pruning methods to control the vigorous vegetative growth of kiwifruit has increased fruit yields throughout the years. After extensive experimentation, the most popular commercial kiwifruit training methods reported are T-bar (Fig.1-2) and Pergolas (Fig.1-3). In 1980 more than 60% of the plantings in the Bay of Plenty were on T-bars (Sale & Lyford 1990). Clark & Gravett (1992) found in a one year survey that those losses caused by *B. cinerea* from orchards utilising pergola support structures were greater than those with T-bars.

Summer and winter prunings are important aspects of vine management. Winter pruning aims to retain an optimum number of evenly distributed one-year old replacement canes, while summer pruning is essential to maintain order in the vine structure, proper spacing of laterals and canes and maximum light penetration into the canopy (Fig.1-4). The most important objectives of this practice are to allow air movement and penetration of light around the vines to minimize the conditions which favour fungal diseases

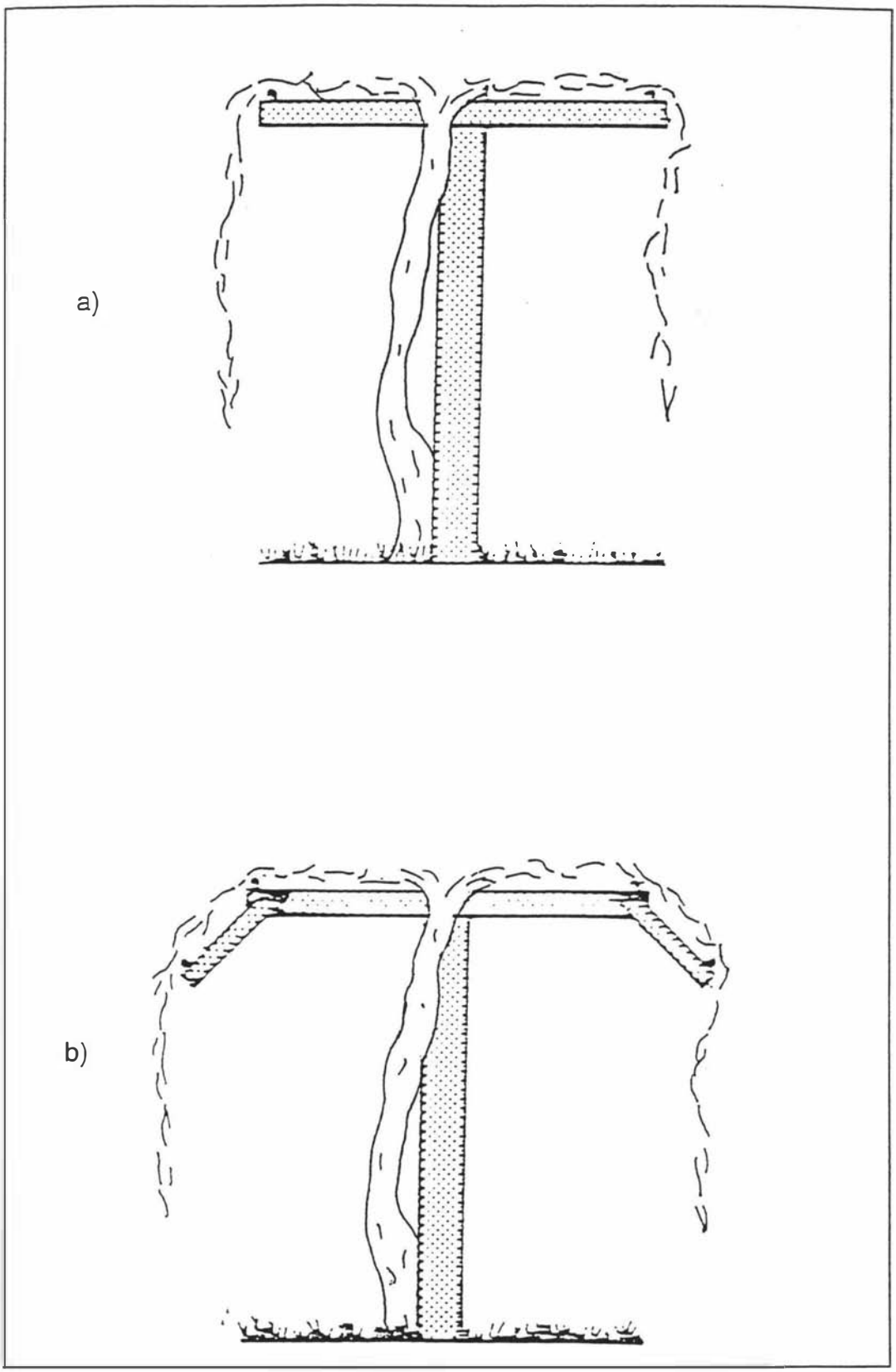


Figure 1-2. T-bar support system for kiwifruit. a) Standard T-bar and b) Winged T-bar (Alexander 1985; Sale and Lyford 1990).

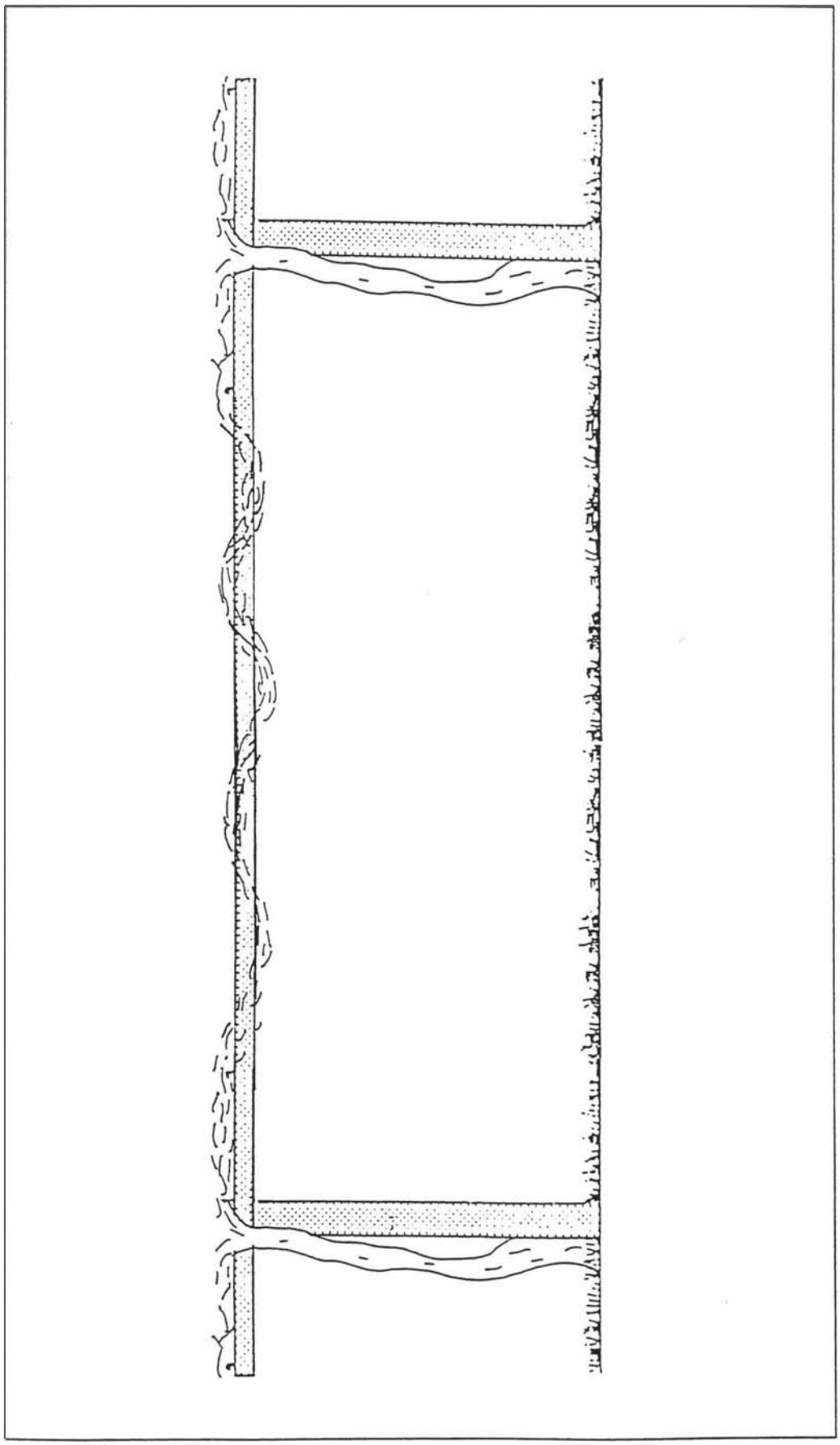


Figure 1-3. Pergola support system for Kiwifruit (Sale and Lyford 1990).

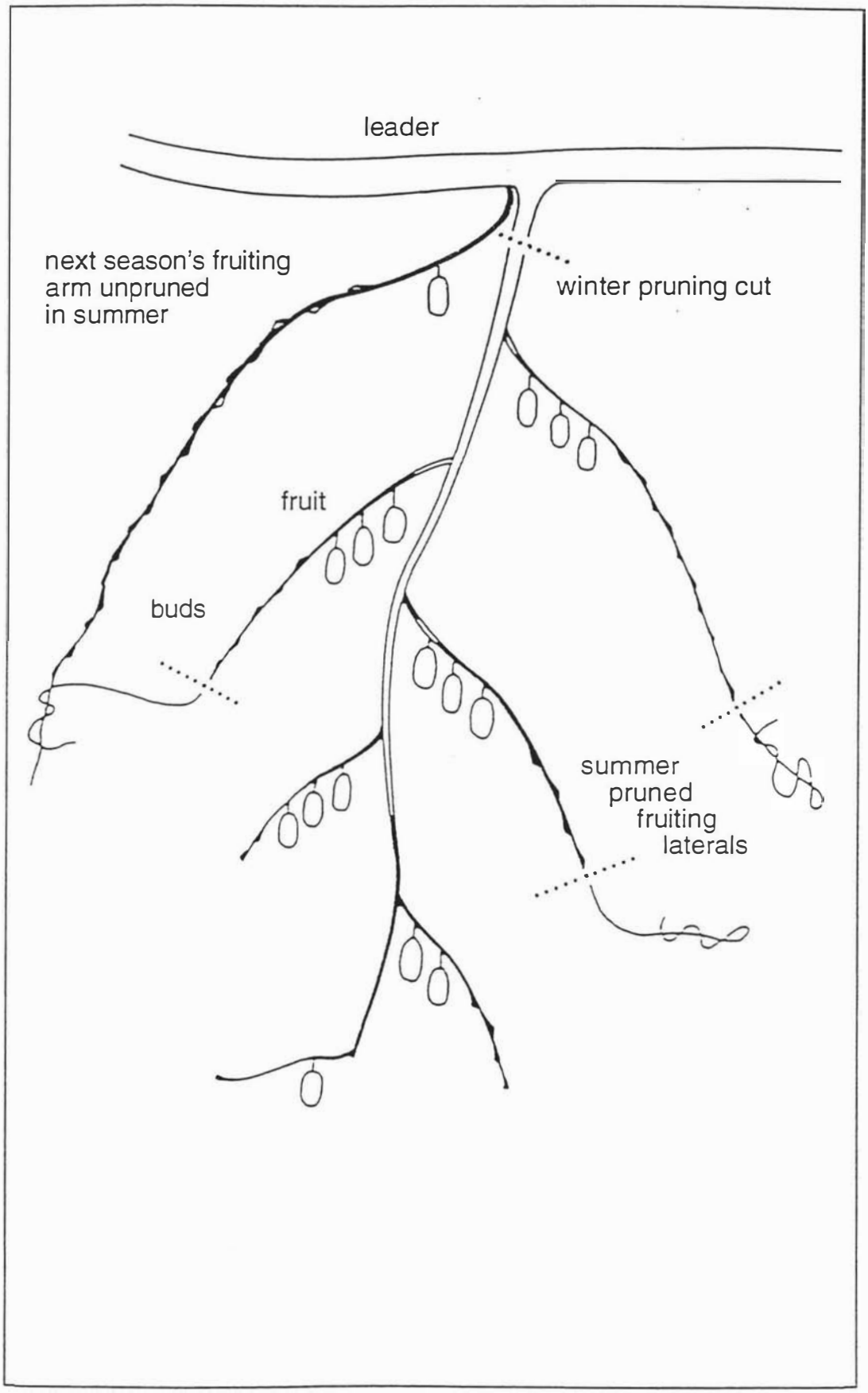


Figure 1-4. Kiwifruit pruning methods (Sale 1990).

such as *Botrytis* and to increase fruit yields (Sale & Lyford 1990).

Commercially kiwifruit are harvested when the soluble solids content (TSS) of the fruit has reached 6.2% (Sale 1990; Hopkirk 1992). Harvesting starts at the end of April/beginning of May and continues until the middle/end of June. Fruit is hand harvested, by snapping off the fruit at the stalk. During harvest, workers wear cotton gloves to avoid bruises or blemishes to fruit and to protect hands from scratches and sap. In general, two pickings are normally made, with the larger fruits harvested at the first picking and the remainder during the subsequent harvest (Sale 1990). During harvest, fruit are placed into an apron bag which, when full is emptied into a bin. Bins are generally made of wood and hold 250 kg of fruit. Although bins do not have any special size or shape a maximum depth of 40 cm has been recommended to minimize crushing of the fruit in the bottom layers by the weight of fruit above them (McDonald 1990).

Transportation of the fruit from orchard to packinghouse is by either trailer or truck. Bins are emptied and returned to the orchard for refilling (Harvey *et al.* 1983; McDonald 1990; Lenting 1991).

Grading and sorting

Once the fruit reaches the packinghouse (Fig.1-5), the main activities before packing are inspection and grading. The first activity, is carried out by trained operators to remove damaged or blemished fruit. The subsequent grading for size is done automatically by machine, each fruit being weighed separately. Some grading machines are of the orbital type in which the fruit is thrown away through the air, the distance projected depending on weight and fruit are caught in various canvas chutes (McDonald 1990; Sale 1990).

Packing

Fruit for export is packaged as specified by The New Zealand Kiwifruit Marketing Board. The more common packages are: a single-layer tray of

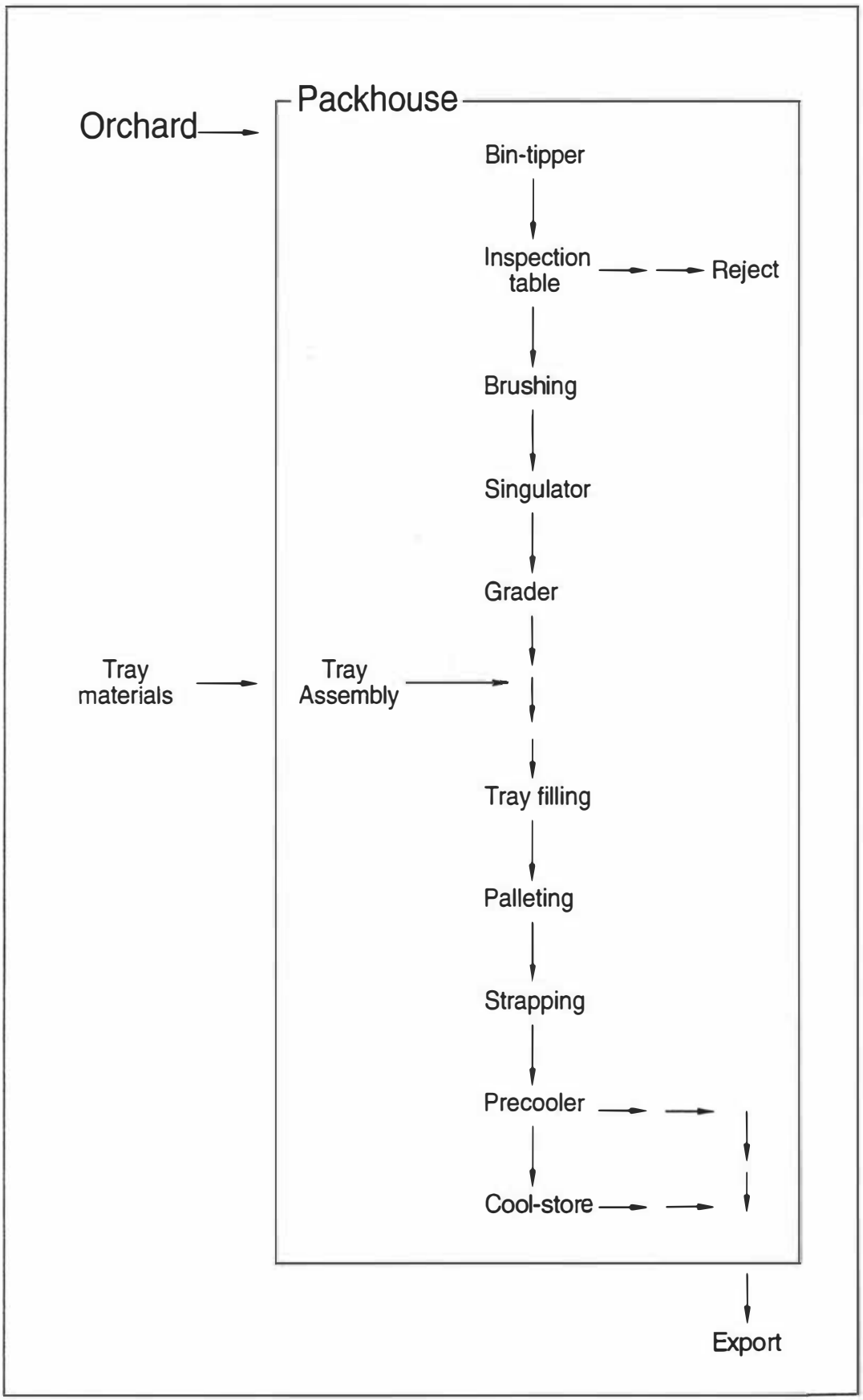


Figure 1-5. Kiwifruit handling system. (Modified from McDonald 1990).

approximately 3.5 Kg of fruit; tripack, containing 10.2-11.0 Kg fruit and bulkbins equivalent to 15 or 31 single layer trays. Although the material of fruit containers has been continuously modified in the last few years to meet the environmental requirements from European countries, in general fruit is placed into the preformed plastic pocket tray-packs, wrapped in polyethylene liners and packed in cardboard trays (Sale & Lyford 1990).

Storage and Marketing

Once the fruit are packed into the appropriate container, they are often immediately precooled (Fig.1.5). The most common precooling technique is forced air where cool air is forced between the fruit to remove heat and bring it more rapidly (8h from ambient to 2°C) to near coolstore temperature. After precooling, fruit are stored in coolrooms at 0.5-1°C and a high relative humidity (at least 95%) to be kept for long term storage of 4-6 months (McDonald 1990). With the exception of fruit going to Australia, kiwifruit can only be exported by The New Zealand Kiwifruit Marketing Board (Earp 1990).

Diseases of kiwifruit

Major diseases of kiwifruit and a brief explanation of symptoms are given in Table 1-1.

BOTRYTIS CINEREA

Taxonomy, morphology and general life cycle

B. cinerea is the asexual stage of *Botryotinia fuckeliana* (de Bary) Whetzel. *Botrytis* is classified in the true fungi Eumycota, subdivision Deuteromycotina, Class Hyphomycetes and Order Hyphales (Agrios 1988). *B. cinerea* produces abundant grey mycelium and long branched conidiophores with rounded apical cells bearing clusters of colourless or grey, one-celled, ovoid conidia which contain 4-18 nuclei. (Jarvis 1980; Agrios 1980). The fungus overwinters in the soil as mycelium growing on

Table 1-1. DISEASES OF KIWIFRUIT

Diseases	*Symptoms*
Storage	
Botrytis storage rot (*Botrytis cinerea*)	Extreme softness localized at the stem end. The fungus first visible on the fruit surface as a white fluffs spreading alongside the fruit. Mycelium turns grey with sclerotia formation (Brook 1990b).
Ripe rot or Dothiorela rot (*Botryosphaeria dothidea*)	Fruit rotting after coolstorage. A portion of the fruit collapses (Sommer *et al.* 1992).
Field	
Phytophthora root rot (*Phytophthora cactorum, Phytophthora cinnamomi*)	Drought and starvation symptoms on the leaves, plants become weak and susceptible to attack by other pathogens. Necrotic brown lesions on roots (Agrios 1988).
Honey fungus (root rot) (*Armillaria* spp)	Plant growth reduction, smaller yellowish leaves, dieback of twigs and branches and gradual or sudden death of the tree. Development of white mycelial mats on roots (Agrios 1988).

Table 1-1. (Cont.)

Sclerotinia fruit rot (*Sclerotinia sclerotiorum*)	Appearance of a white fluffy mycelial growth. Firstly developing of a white sclerotia and later becoming black (Agrios 1988). Infected fruit often fall but sometimes the infection will heal leaving an unsightly scar on the fruit (Sale 1990).
Leaf spot (*Alternaria alternata*)	Numerous leaf spots and blight dark brown to black colour. Progression of disease cause leaves to dry and fall off eventually (Agrios 1988).
(*Glomerella* spp., *Colletotrichum* spp.)	The fungus can be present in plants at all stages of growth. In leaves the fungus attacks the veins of the underside of the leaves causing lesions dark, brick-red to purplish (Agrios 1988).
Bacterial blossom rot (*Pseudomonas* spp.)	Development of necrotic spots on leaves and buds. When disease is advanced necrosis leads to blights and death of the plant partially or total (Agrios 1988).

decaying plant debris and as sclerotia. Sclerotia are generally considered to be the most important structures involved in the survival of this fungus (Coley-Smith 1980). Sclerotia almost invariably germinate to produce mycelial threads that can infect plant tissue directly but in a few instances sclerotia have been found germinating to produce apothecia and ascospores (Agrios 1980). The conditions for producing the sexual stage form sclerotia has been studied and an appropriate laboratory procedure devised (Faretra & Antonnaci 1987).

Pathogenicity of B. cinerea on different commodities

B. cinerea is probably the most common and most widely distributed disease of vegetables, ornamentals, fruits and field crops throughout the world. Grey mould caused by *B. cinerea* is a serious disease of lettuce (Wheeler 1969), grapes (Mustonen 1992) and several species of berries like raspberries (Knight 1980), and strawberries (Jarvis 1962). Several species of *Botrytis* cause plant diseases, for example *B. alli*, *B. byssoidea* and *B. squamosa* cause neck rots of onions and *B. tulipae*, tulip fire. Leaf spot diseases of economic importance are chocolate spot of broad beans caused by *B. fabae* and *B. cinerea* (Wheeler 1969). In apples, blossom-end rot caused by *B. cinerea* can lead to considerable economic losses (Lakshminarayana *et al.* 1987). In the last decade, stem-end rot caused by *B. cinerea* has been reported as one of the major causes of rotting of stored kiwifruit (Hawthorne *et al.* 1982). *B. cinerea* is a common fungus of greenhouse-grown crops. Grey mould of ornamentals is reported as a serious disease of begonias, roses, cyclamen and several cut flower crops (Agrios 1980; Fletcher 1984; Hammer *et al.* 1990).

The infection process by Botrytis

Germination of *Botrytis* conidia on the host surface is the first step in the infection process from conidia. Certain conditions such as availability of nutrients on the plant or fruit surface, production of toxic substances by the host, nutritional status of the plant etc. are required for the success or

failure of *Botrytis* germination and penetration into the host (Kamoen 1992). Once the fungus has germinated, penetration is considered as the second important step of the infection process. Penetration of *Botrytis* is carried out by the formation of a narrow infection hypha which may form from an appressorium or from the tip of a germtube. Kamoen (1992) reported this type of hyphal swelling during penetration in onions, tomatoes and gerbera ray flowers. Invasion of *Botrytis* can also occur through natural openings such as stomata and on carpels. *B. fabae* and *B. cinerea* on broad beans and strawberries respectively are examples of this type of penetration (Verhoeff 1980). Wounds produced on vegetable crops during cultural practices such as pruning and grafting and during the produce harvesting are also important points of entry for *Botrytis*. After penetration into the host, lesions expanded as a result of both intracellular and/or extracellular hyphal growth, spreading throughout the whole plant organ at a rate that varies according to the compatibility of the host/pathogen combination (Kamoen 1992).

Effect of environment during the infection process by Botrytis

Relative humidity and temperature are considered the most important environmental factors that affect the initial infection and spread of *Botrytis.* Infection by this fungus can take place in the field, greenhouse or storage rooms. In the latter although the development of disease occurs during storage of the produce, the infections most probably occur in the field and may involve a latent stage. Most reports of *B. cinerea* epidemiology mention that a high relative humidity (92%) is necessary for *B. cinerea* infection (Jarvis 1980). Optimum temperatures for *B. cinerea* infection in the field or at greenhouses vary according to each specific crop and the time of exposure. Jarvis (1980) gives as optimum temperatures for germination of *B. cinerea* on strawberries as 20°C to 30°C for 3-20h or 3°C to 20°C for 3h with high relative humidity in grapes (Nelson 1951). Thomas *et al.* (1988), found more conidial formation on the surface of grapes at 21°C with 94% relative humidity and 0.6 m/sec wind speed than on grapes held at the

same temperature and relative humidity and zero wind speed. Conversely, in macadamia flowers *B. cinerea* was depressed at temperatures higher than 20°C and relative humidity of 95-100% (Hunter *et al.* 1972). In other crops such as tomatoes, tulips and onions, it was necessary for periods of high rainfall or long persistent water films and high humidity for aggressive lesions caused by this pathogen to develop (Price 1970; Verhoeff 1970; Jarvis 1980). Similar environmental conditions plus air movement were necessary for *B. cinerea* infection on greenhouse grown cucumbers (Yunis *et al.* 1990).

B. cinerea can germinate at 0°C and high relative humidities and cause infection. During storage of several vegetable crops growth of *B. cinerea* and infection were enhanced by the high relative humidity and condensed water (Jarvis 1980). In strawberries *B. cinerea* develops during transit and storage at 0°C. Stem-end rot of kiwifruit during storage can also occur at 0°C (Sharrock & Hallet 1991).

Kiwifruit and B. cinerea

Prior to 1978 *B. cinerea* was not a commercial problem on kiwifruit but as the kiwifruit industry developed in New Zealand, it became a disease problem during storage (Pennycook 1985; 1990). At present, *Botrytis* stem-end rot is one of the major problems facing the kiwifruit industry (Manning & Pak 1993). For example, in 1991 postharvest losses of kiwifruit from *Botrytis* rots were evaluated at NZ $6 million with labour and repacking costs at a further NZ $4.5 million (Personal communication: Peter Bull). *Botrytis* is a major contributor to the wastage through fruit loss, lost revenue and loss of confidence in the marketplace (Anonymous 1994a).

It has been widely reported that *B. cinerea* gains entrance into various fruits, including kiwifruit through the stem-end wound produced during the snapping of the fruit from the pedicel at harvest (Sommer *et al.* 1983; Poole & McLeod 1991). Sharrock & Hallet (1992); Hallet & Sharrock (1993)

reported that the primary route for postharvest infection from *B. cinerea* spores is through the stem-end vasculature which has been exposed by picking the fruit from the pedicel. Increased contamination of the fruit by this fungus, is likely during the subsequent handling operations such as grading and packing (Pennycook 1990; Brook 1991).

Numerous studies have shown that primary *Botrytis* rot in kiwifruit generally appears after 4-5 weeks but before 12-13 weeks coolstorage at 0°C. Secondary *Botrytis* rots (commonly termed "nesting") develop when the *Botrytis* hyphae spread across the trays from rotten fruit to adjacent healthy fruit (Lallu 1989b; Beever 1991; Sommer *et al.* 1992).

CONTROL MEASURES

Alternatives for control of B. cinerea

Chemical control:

Control of *B. cinerea* in the field and during storage has been achieved using various fungicides. The dicarboximides (iprodione, procymidone, vinclozolin) have been extensively used in spray programmes in the orchard but only for experimentation during coolstorage of the fruit. For example a significant reduction of *B. cinerea* (1.1%) after 12 weeks storage was achieved when 0.05% of vinclozolin as "Ronilan" was applied at harvest compared with control fruit (6.6%) (Pommer & Lorenz 1982). However there would be little future in postharvest use of such fungicides because of the ability of *B. cinerea* to develop resistance to them and the lack of a complete protection against *B. cinerea* during coolstorage (Brook 1990a). Postharvest fumigation methods under investigation for kiwifruit include treatments with ozone (O_3) and sulphur dioxide (SO_2). In preliminary experiments application to the stem-scar of 250 ppm of O_3 reduced stem-end rot to 0.25% compared with 1.5% in the control fruit (Brook 1990a). Cheah *et al.* (1992a) reported complete inhibition of germination *in vitro* of *B. cinerea* spores at SO_2 concentrations of 400-3200 ppm applied for 10,

20 or 30 min and reduction of stem-end rot with time of treatment increased within levels between 800 to 3200 ppm.

Although the above mentioned methods have shown promise for control of *B. cinerea* during coolstorage of the fruit, the increasing worldwide trend to consume commodities free of chemical residues means such postharvest treatments do not now have general international approval (Hammer & Marois 1989; Johnston 1994). Moreover, in the near future there will be new legislations concerning the use of pesticides in the kiwifruit industry in New Zealand (Holland *et al.* 1994). Therefore at this stage, there is a trend to concentrate experimental work on the control of *B. cinerea* by means of non-chemical agents.

Non-chemical control methods:

Heat treatment by dipping the fruit in hot water or by dry air treatments at 44-50°C reduced stem-end rots during storage (Brook 1990a), Similar *Botrytis* control (92%) but at temperatures between 50-54°C, for 2, 4 or 8 min, 42 and 46°C for 8 min (64%), 46°C for 15 min and 48°C for 8 min (100%) have been reported by Cheah *et al.* (1992b; 1993a; 1993b) but at the same time, they reported that certain disorders of the fruit such as physiological breakdown and rapid loss of firmness were encouraged at the highest temperatures.

Reports associated with biological control experiments in which microorganism such as *Cladosporium cladosporioides*, *Bacillus subtilis*, *Erwinia herbicola, Trichoderma* spp. were applied to kiwifruit to stem scars before inoculation with *B. cinerea* spores, achieved a reduction of stem-end rots caused by this fungus (Harvey *et al.* 1991). A decrease of *B. cinerea* infection after 10-12 days incubation at 20-22°C was shown in fruit artificially inoculated at the stem scar by various isolates of *B. subtilus* and spores of *B. cinerea* previously grown on agar or in liquid culture. *B. subtilus* isolates grown on agar gave between 10 to 55% infection, while

those grown on the liquid media showed *B. cinerea* reduction between 0 to 15% (Brook 1990a). Additional investigations carried out by Cheah *et al.* (1992c) found an antagonist effect between various isolates of *Trichoderma* and *B. cinerea*. In that study five isolates of *Trichoderma* significantly reduced *B. cinerea* sporulation (0-13.9%) on kiwifruit stem scars after ten days incubation at 15°C.

Manipulation of controlled atmosphere storage of kiwifruit to reduce *B. cinerea* infections is another approach still under experimentation (Manning & Lallu 1993; Lallu & Manning 1994). However, in general results have shown an increase (2.0-2.9%) in *both B. cinerea* stem-end and other rots such as those caused by *Phomosis*, *Fusarium* and *Crytosporiopsis* in fruit stored under modified atmospheres compared with air stored fruit.

Another natural method for control of stem-end rots of kiwifruit during coolstorage has been to hold the fruit at ambient temperature for several days before storage (Evans 1992). This preconditioning practice carried out soon after harvest is commonly named "curing" (Brook 1990a; Hallet *et al.* 1991; Beever 1992; Pennycook & Manning 1992).

Definition of curing

Curing is a pre-storage practice widely used to reduce spoilage by microorganism during the normal storage period of a number of commodities such as fruits (Hopkins & Loucks 1948; Ben-Yehosua *et al.* 1987a; Pennycook & Manning 1992) and vegetables (Passam *et al.* 1976; Baudoin & Eckert 1985).

In root and tuber crops curing refers to the process of wound healing with the development and suberization of new epidermal tissue called wound periderm whereas in bulb crops, curing is the process of drying the external layers of those vegetables to reduce neck rot diseases (Kasmire & Cantwell 1992). Beever (1991) has defined the term curing "as the encouragement

of the natural processes of repair that take place in a fruit or vegetables, following physical damage".

Commercial curing of fruits and vegetables is carried out by holding the produce in conditions of relative humidity and temperature which are different (usually higher temperatures) from those in which the commodity is held for long term storage (Kitinoja & Kader 1994).

Impact of curing to control postharvest diseases.
In tubers, bulbs and roots:
Most tubers and roots have the ability to heal cuts, wound and bruises if provided with a suitable environment (Ryall & Lipton 1980). Meijers (1987) found that in root and tuber crops the curing process or wound healing is developed in a two stage process which result in the development of new epidermal tissue called wound periderm.

A number of diseases can spread through potato tubers in storage, e.g. gangrene (*Phoma* spp.), dry rot (*Fusarium caerulum*), black scurf (*Rhizoctonia solani*), late blight (*Phytophthora infestans*), blackleg (*Erwinia carotovora* pv. *atroseptica*) and soft rot (*Erwinia carotovora* pv. *carotovora*). Curing is an important procedure in limiting the losses caused by these postharvest disease problems.

The development of gangrene in potato was studied by Malcomson & Gray (1968). They found suppression of gangrene disease when tubers were held for three, seven or ten days at 18-23°C and concluded that suppression of gangrene disease increase with length of the curing period. Similarly Adams & Griffith (1978) found that a curing period of ten days, together with the time of harvest and previous wounding of the tubers were the main factors which affected the incidence of gangrene in potato. Stewart *et al.* (1983) cured potatoes at 15°C and 100% relative humidity for one day to control tuber blight and also found that susceptibility to this

disease decreased with late harvest. Morris *et al.* (1989) found stimulation of suberin development and periderm formation when wounded potato tubers were held at 25°C and 98% relative humidity for seven days. Snowdon (1991) reported differences in curing time according to the holding temperature. Three to six days were necessary to cure tubers at 20°C, one to two weeks at 10°C and three to six weeks at 5°C, while there was no curing effect at lower temperatures.

Improvement in caladium tubers by means of curing has been reported by Marousky & Raulston (1973) and Marousky & Harbaugh (1976). They reported that the suberization process was increased in tubers incubated at 26.6°C and at relative humidities of 75% compared with tubers held at the same temperature and 90% relative humidity.

Bulbs of various cultivars of the Dutch iris (*Iris hollandica* Hoog) plants were improved by means of ethephon treatments together with curing (Cascante & Doss 1988). The time to flowering was shortened and there was a reduction of the numbers and length of leaves when bulbs were dipped for 1h in ethephon at 0.25 g/l and then heat cured at 32°C for three days.

Curing of zantedeschia tubers (*Zantedeschia elliottiana*) is also an important commercial practice to protect them against microorganisms and desiccation. Funnell *et al.* (1987) reported development of suberized cells and periderm formation in tubers cured at 30°C and 80% relative humidity for periods longer than three days. Further research carried out by Funnell & McKay (1988) reported that although suberization could take place at 30°C and at both 80 or 40% relative humidity, less weight loss was reported in those tubers incubated at the highest relative humidity.

The beneficial effects of curing has also been reported in tropical roots, tubers and corms such as cassavas (*Manihot esculenta* C.) boniatos sweetpotatoes and yams (*Dioscorea* spp.), for control of postharvest

diseases such as black rot (*Ceratocystis fimbriata* Ell. & Halst.) and various storage rots (*Rhizopus* spp. and *Diplodia* spp.) (Snowdon 1991). In sweetpotatoes decay was reduced by 19%, according to the harvest date and cultivar when held for 1 week at 30°C, 100% relative humidity (Delate *et al.* 1985), whilst Snowdon (1991) reported the best curing temperature for this commodity as 35°C for two to ten days. Walter *et al.* (1989), reported that wound healing in this commodity was closely related to the soil temperature in which the crop was grown. They found that the best curing results i.e. less rots and less weight loss, occurred on tubers of sweetpotato harvested at soil temperatures of 15-17°C and cured for one to seven days at 30°C and 90-95% relative humidity.

Storage quality of commodities such as cormels (*Xanthomona caracu* Kouch and Bouche) can also be improved by curing. According to Mbonomo & Brecht (1991) cormels cured at 30 or 35°C and 95-100% relative humidity for seven days had less weight loss and decay caused by soil-born pathogens such as *Erwinia carotovora* and *Erwinia chrysanthemi* during storage than uncured cormels.

Snowdon (1991) reported an increase in the quality of carrots (*Daucus carota* L.) following a brief curing period at ambient temperature and high relative humidity to promote wound healing.

Postharvest diseases of shallots, onions and garlics such as black mould (*Aspergillus niger* v. Tieghem) and bacterial rots (*Erwinia* spp., *Lactobacillus* spp. and *Pseudomonas* spp.) can be successfully controlled by curing (Snowdon 1991). According to Kasmire & Cantwell (1992) and Kitinoja & Kader (1994), for these two commodities, five to ten days were necessary to allow the external layers of the skin and neck tissue to dry when held at ambient temperature, whilst one day or less was sufficient using forced air at 35 to 45°C with relative humidities at 60 to 75%. Snowdon (1991) recommended 27-30°C as the most optimum temperature range for curing.

In vegetables:

Curing is recommended for vegetables such as squash and pumpkin. Snowdon (1991), reported curing temperatures of 20°C or higher for one week, to reduce rots caused by *Alternaria alternata* (Fr.) Keissler and *Alternaria cucumerina* (Ell. & Ev.) Elliot, and to develop wound healing.

In leafy vegetables:

Bae (1989) found that leaf rotting caused by *Botrytis* spp. decreased in a number of cultivars of tobacco when leaves of this commodity were cured at temperatures of 25, 30 and 35°C and relatives humidities of 82, 92 and 100%. This researcher also found that development of *Botrytis* disease increased during the rainy season compared with the dry season. Other studies on this commodity have shown that content of leaf fatty acids such as palmitic, oleic, linoleic and linolenic increased with curing (Srivastova & Chaudhary 1990).

In tropical and subtropical fruits:

Control of green mould (*Penicillium digitatum*) in "Valencia" oranges (Brown & Barmore 1983), sealed lemons, and pummelo fruit (*Citrus grandis* L. *Osbeck*) (Ben Yehoshua *et al.* 1987a 1987b; 1988b) was reduced about 12%, 2- 4% and 5% respectively when fruit were cured at 30°C for 24h in combination with high relative humidities. In lemons, the incidence of active lesions of sour rot (*Geotrichum candidium* Link ex Pers) were reduced about 8% following 5 days of curing at 25°C and 100% relative humidity (Baudoin & Eckert 1985; Predebon & Edwards 1992). Curing of sealed citrus (pummelo fruit) at 32-36°C enhanced lignification and antifungal metabolite production (Ben-Yehoshua *et al.* 1988a).

In grapefruits (Schiffmann-Nadel *et al.* 1971) the incidence of storage rots during their shelf life and cold storage period was reduced about 5% when they had been cured at 12°C for several days or weeks.

In temperate fruits:

The process of wound healing in apples has also been studied (Skene 1981; Lakshminarayana *et al.* 1987). Although in these studies the term *curing* was not used, In wounded apples, the development of periderm tissue, synthesis of phenolic substances, tannins, lignins and callose varied with temperature, time of incubation and harvest date. They reported that 38 days at 5°C were necessary to develop resistance compared with only 14 days when incubated at 20°C. They also reported less decay caused by *B. cinerea* and *P. expansum* inoculated onto healed wounds than onto fresh wounds.

Curing as an alternative for control of B. cinerea during kiwifruit storage

Several workers have found that a delay between harvesting the fruit and inoculation results in a decrease in infection. There is a considerable body of evidence that once the pedicel has been removed, the stem scar develops a high level of resistance (Sharrock & Hallet 1991; 1992). Penycook & Manning (1992) and Beever (1992), reported that the incidence of stem-end rot in this commodity was reduced (from 46% to 6%) progressively with increasing duration of a holding period at ambient temperature. Microscope studies by Hallet *et al.* (1991) showed that there was little germination of *Botrytis* spores on stem scars of cured kiwifruit compared with those on uncured ones. Collapse of *Botrytis* spores on the stem scar were frequently observed in the cured fruits.

Long-term storage conditions to reduce B. cinerea on kiwifruit.

The postharvest storage life of most commodities for short or long-term storage is dependant on the environment. The storage system in use should be able to reduce or minimize postharvest losses due to the normal physiological responses of the fruit to environment and to infection by microorganisms.

Among the principal objectives of much long-term storage research are the reduction of commodity metabolism to delay senescence, minimization of moisture loss to reduce shrinkage and loss of turgor and reduction of growth and spread of microorganisms. Davis (1980), considered that produce should be kept at lower temperatures for long-term storage than those used for short-term storage. Once fruit or vegetable produce is separated from the plant or dug from the soil, the postharvest life is short at ambient temperature. Low temperatures serve to greatly extend the storage life of the commodity by suppressing disease and extending host resistance by means of delaying the commodity senescence (Sommer 1985; 1989).

In contrast to the uniformity of an optimum requirement for low temperatures for long-term storage, there are some contradictions about the optimum relative humidity for low temperature storage of vegetable crops. Mann (1954) and Thompson (1992) considered that the relative humidity should be maintained at 90-95% for most perishable commodities because higher levels of relative humidity in the storage room would encourage high levels of decay.

Similar optimum storage relative humidities of 90-95% have been reported for potatoes, sugar beets, carrots and cabbages (Ryall & Lipton 1980). However, studies on carrots showed that lower or equal levels of infection were found when they were stored at relative humidity ranges of 98-100% than at 92-96% (Van den Berg & Lentz 1966; 1973a; 1973b; 1974). Further research (Van den Berg & Lentz 1977a; 1978) with parsnips, rutabagas, carrots, celery and other vegetable crops also showed that relative humidities between 98-100% reduced decay levels during storage. Studies on kiwifruit (McDonald 1990; Mitchell 1990; Lallu *et al.* 1992) have shown that 0°C and relative humidities above 95% are the most suitable environmental conditions to store this commodity in order to avoid a high incidence of disorders and high moisture loss. Differences in this

recommended storage temperature are slight: Sale (1990) recommended temperature ranges between -0.5 to 0.5°C as the optimum for kiwifruit storage. It is unlikely that a more accurate control of temperature under commercial conditions could be achieved.

GENERAL OBJECTIVES

In this study, three related areas of the kiwifruit - *B. cinerea* pathosystem were investigated:

- First, some factors which could affect the pathogenicity of *B. cinerea* were investigated. These included an evaluation of isolate-to-isolate variability in pathogenicity to kiwifruit using isolates obtained from various infected crops from different areas of New Zealand, and several factors affecting inoculum potential (culture age, inoculum concentration and growing media).

- Second, *in vitro* survival of conidia of *B. cinerea* incubated over various periods of time at several temperatures and relative humidities that could be used during curing were studied.

-Third, environmental prestorage curing conditions (temperature and relative humidity) were assessed for effects on general fruit characteristics during storage,for effects on stem scar structure and for suppression of storage rots of kiwifruit.

CHAPTER TWO

GENERAL MATERIALS AND METHODS

The materials and methods described here are those used in most of the present study. Where procedures differed, or are specific to each chapter then they are explained in the materials and methods section of that chapter.

Fruit harvesting

Kiwifruit used in these experiments were harvested from vines trained on a winged T bar system at Massey University Fruit Crops Orchards, Palmerston North (Lat S 40 21, Long E 175 37), New Zealand. Fruit were harvested between 7:30 to 10:30 am and the relative humidity and temperature in the orchard were recorded at the start of harvesting. Fruit were snapped from the pedicel and placed into a picking bag which was emptied into a cardboard carton at the end of the row. The harvested fruit were taken to the laboratory and the sepals removed with a soft nail brush to provide a more uniform environment at the picking scar. One 17 µl droplet of the appropriate spore suspension was placed on each picking scar wound. After the droplet had dried (about 30-45 minutes) fruit were placed in plastic plix trays in commercial cardboard kiwifruit trays with a polyethylene liner. Unless otherwise stated they were immediately stored at 0°C in the Massey University Plant Growth Unit coolstores.

Preparation of inoculum

A single spore isolate of *B. cinerea* (isolate K3) which was originally obtained from diseased kiwifruit at Massey University was maintained on malt agar and subcultured at 3-4 week intervals. Inoculum was prepared from 10-14 day old cultures.

Spore suspensions were prepared by flooding cultures with sterile 0.02% Tween 20 (Polyoxyethylene sorbitan monolaurate by Serva,

Feinbiochemica), rubbing with a sterile bentglass rod and filtering through glass wool to remove hyphal fragments. Spore concentrations were measured using a haemocytometer and adjusted to the required spore concentrations. These varied between experiments but included one or more of 1000, 5000, 25000 or 125000 per 17 µl droplet of suspension corresponding to 5.9×10^4, 2.9×10^5, 1.5×10^6 or 7.4×10^6 spores per ml respectively. Plastic dropper bottles fitted with a micropipette tip were much quicker to use than micropipettes and they delivered a 17 µl droplet. Since one droplet was placed on each stem scar the number of spores per droplet was a convenient way of expressing inoculum loadings (this technique was developed by Dr. Greg Tate of Crop Health Services, Hawkes Bay).

Quality measurements

For each experiment initial firmness and total soluble solids of 10 to 15 fruits were measured at harvest on fruit taken one metre along the vine stem and one metre from the row. Firmness (kgf) was measured with a penetrometer (R. Bryce Mod. FT327) at the mid point of each side of each fruit after removing a 2-3 cm diameter disc of peel. Juice was squeezed from a slice cut from each end of the fruit and total soluble solids (TSS) concentration (%) was measured using a hand-held Atago N-20 refractometer (Mod. N. McCormick Fruit Tech., brix range from 0-30%). Both fruit firmness and total soluble solids were calculated as the mean of the two measurements.

Defined relative humidity

The principle of incubating fruit in air of a defined relative humidity is simple. Air from an aquarium pump is bubbled through water or saturated salt solution, into an entry chamber where the input humidity can be measured, through the chamber containing the fruit and into an exit chamber where the relative humidity of the air leaving the fruit chamber can be checked. In practice, the aquarium pumps used in this work (Mod. Elite 802 or Etema II) produced flow rates of 2.5 or 3.5 l/min respectively as measured by

collecting air exiting the fruit chamber and collecting it over a specified time in a measuring cylinder inverted in a water bath. The input and output chambers were 500 ml "Agee" preserving jars with input and output tubing fixed to the metal lids and with a 2 cm hole that could be plugged with a rubber bung or with a humidity probe with a sealing strip to the same diameter. Initially, the water and salt solutions were placed in one litre "Agee" jars with 5-10 mm internal diameter plastic tubing as inlet and outlet tubes. The inlet tubes ended at the bottom of the jars and the outlet tubes were flush with the lids. Three problems were encountered with this design:

1. Salt crystallised in tubes which soon became blocked.
2. The vigorous bubbling of the salt solutions forced liquid up into the tubes.
3. With a full flow rate from the pumps, the air bubbles were large with a small volume:surface ratio and were in contact with the salt solutions for such a short time that it was impossible to maintain a constant humidity in the desired range. At lower flow rates there was insufficient air passing through to cope with the water loss from the fruit.

After considerable experimentation, these problems were overcome by:

1. Using tubing with a larger (15-20 mm) internal diameter.
2. Placing the water or saturated salt solutions in vertical polythene tubes 1 m long and 35 mm diameter. With the tubes two thirds to three quarters full there was sufficient headspace to prevent liquid bubbling up into the outlet tubing and this combined with the larger diameter outlet tubing eliminated the blocking by salt crystallisation. The great depth of liquid through which the bubbles had to pass gave a longer contact time for the humidity in the bubble to stabilise.
3. Connecting two tubes in series provided a stable, defined humidity output of 2.5 l/min for experiments *in vitro* and 3.5

l/min per series for the remaining experiments.

Analytical grade Calcium Chloride ($CaCl_2$) was used for the lowest relative humidity range, Sodium Chloride (NaCl) for the lower intermediate relative humidity range, Potassium Chloride (KCl) for the higher intermediate range and water for the saturated atmosphere.

Relative humidity and temperature were monitored using a Squirrel meter/logger device mod. Grant 1200 with a humidity probe (Gaffney 1978; Talbot & Baird 1991) or by a manual mercury thermometer respectively.

Measurement of ethylene and carbon dioxide production

Fruit were held overnight at 20°C to stabilise their temperature. Ethylene and carbon dioxide production were measured by placing individual fruit in separate air-tight glass jars, of approximately 700 ml capacity. Jars were sealed, incubated at 20°C for 1h and 1 ml gas samples were then taken from each jar using an air tight syringe to withdraw the sample from the headspace above the fruit. Ethylene and carbon dioxide measurements were made from the same sample. A Varian 3400 or Pye Unicam series 104 gas chromatographer with flame ionization detector (FID) and N_2 as the carrier gas was used for ethylene and a GC-8A Shimadzu gas liquid chromatographer for CO_2.

Respiration rate and ethylene production were calculated according to the following formulae (Shusiri 1992):

Carbon dioxide:

$$FCO_2 = \frac{[CO_2]final - [CO_2]initial}{100} \times (Vjar - Vfruit) \times \frac{1000}{wfruit} \times \frac{60}{T}$$

Ethylene:

$$FC_2H_4 = \frac{[C_2H_4]final - [C_2H_4]initial}{1000} \times (Vjar - Vfruit) \times \frac{1000}{wfruit} \times \frac{60}{T}$$

Where:

FCO_2 = rate of carbon dioxide production ($cm^3kg^{-1}h^{-1}$).

FC_2H_4 = rate of ethylene production ($\mu lKg^{-1}h^{-1}$).

$[CO_2]initial$ = initial carbon dioxide concentration (%).

$[C_2H_4]initial$ = initial ethylene concentration (μl^{-1}).

$[CO_2]final$ = final carbon dioxide concentration (%).

$[C_2H_4]final$ = final ethylene concentration (μl^{-1}).

$Vjar$ = jar volume (cm^3).

$wfruit$ = fruit weight (kg).

T = time.

Titratable acidity was calculated as % (w/v) of citric acid in juice according to the following formulae (Shusiri 1992):

$$\%citric\ acid = \frac{mlNaOH * N(0.1) * 64}{1ml\ juice * 10}$$

where:

64 = molecular weight of citric acid divided by 3

Fruit weight loss

Total and daily weight loss was measured according to the following formulae:

$$\%weight\ loss = \frac{wt\ initial - wt\ final}{wt}\ initial \times 100$$

where:

wt initial = Initial weight.

wt final = Final weight.

Assessment of infection

B. cinerea storage rot was assessed at six and 12 weeks of coolstorage. Infected fruit were removed at six weeks to prevent secondary spread of infections to neighbouring fruit. The *Botrytis* stem-end rot is quite distinctive but samples were tested for *Botrytis* to confirm diagnosis.

Statistical Analysis

The SAS System programmes (SAS/Stat User's Guide, 1988) were used to analyze data from each experiment for Analysis of Variance (ANOVA), means, standard errors and standard deviations. If required, data were appropriately transformed to satisfy the basic assumptions for analysis of variance. Means separation was performed by Duncan's multiple range test ($P < 0.05$). When data were unbalanced only means and standard errors were evaluated.

CHAPTER THREE

INOCULUM VARIABLES AFFECTING PATHOGENICITY OF *BOTRYTIS CINEREA* INFECTION OF KIWIFRUIT

INTRODUCTION

To induce *Botrytis* rot in kiwifruit during storage by artificial inoculation with spores or mycelium still remains a problem. The incidence of rot can vary considerably from one experiment to another even when the same strain of *Botrytis* is used as inoculum at the same spore concentration. For experimental work on infection it is important to identify and quantify sources of variability. Nutrient status, age of spores, isolate to isolate variation and inoculum concentration can all affect infectivity of *B. cinerea* (Lorbeer 1980).

Nutrient availability is known to affect colony morphology and growth of *B. cinerea*. For example, studies *in vitro* with a range of *B. cinerea* isolates showed significant differences in sporulation and sclerotial formation according to the peptone concentration of the agar medium on which the fungus was grown (Stewart 1986).

Conidia of this fungus are dependent on exogenous nutrients for *in vitro* sporulation and germination (Leach & Moore 1966; Lorbeer 1980). The pathogenicity of *B. cinerea* conidia is known to be stimulated by high levels of nutrients (Kosuge & Dutra 1962; Kosuge & Hewitt 1964; Köhl & Fokkema 1994). Nutrients required for infection can be supplied in the form of glucose, fructose, potato dextrose agar (PDA), malt extract agar (MA) or extracts of leaves or fruits (Clark & Lorbeer 1977a). Significant infection was recorded in cabbage (*Brassica oleracea* L), onion leaves (*Allium cepa* L), tomato (*Lycopersicum esculentum* M) and gerbera flowers (*Gerbera*

jamesonnii) when glucose, pollen extracts or sucrose were added to the *B. cinerea* spore suspension (Chu-Chou & Preece 1968; Chou 1972a; Yoder & Whalen 1975; Clark & Lorbeer 1977b; Gorfu 1986; Salinas *et al.* 1989). A stimulation of germination of *B. cinerea* conidia suspended in aqueous suspensions of pollen grain extracts of various small fruits, fruit trees and ornamentals was reported by Borecka & Millikan (1973). Van den Heuvel (1981) reported that development of lesions on leaves of French beans caused by *B. cinerea* was dependant on the presence of various factors such as pH, type and molarity of the buffer, presence of glucose and inoculum concentration. Further histological studies on bean, inoculated with *B. cinerea* spore suspension amended with glucose and other nutrients have shown variations in the penetration structures formed on the surface of the leaves according to the nutrients in which the fungus was suspended (Van den Heuvel & Waterreus 1983). Recent studies on kiwifruit have also demonstrated a significant increase in disease incidence when the spore suspension was amended with a yeast extract (Long & Wurms 1993).

The effect of spore age and inoculum level on germination and on infection has been studied by Last (1960). He compared *B. fabae* conidia of various ages for ability to germinate on leaves of a range of varieties of beans and found that percentage germination decreased with spore age. He also found that when the spore concentration reached a critical level, increased percentage germination was reduced due to self-inhibition of germination. This self-inhibition was not apparent on kiwifruit where high levels of inoculum were required to achieve good infectivity by *B. cinerea* (Long & Wurms 1993). Mansfield & Hutson (1980) found that infections by high inoculum levels (200 conidia/droplet) of suspensions of *B. fabae* and *B. tulipae* on broad beans and tulip leaves grew at rapid linear rates compared with the low rate found when plants were inoculated with a lower spore concentration (20 conidia/droplet).

B. cinerea isolates may differ in their pathogenicity according to the origin

of isolates. In red raspberry canes (*Rubus idaeus* L.) significant differences were found in the lesion lengths inoculated with 31 different *B. cinerea* strains (Williamson & Jennings 1986). However, Bryk (1985a), inoculated apples with 80 *B. cinerea* strains from different hosts and found that all isolates caused apple fruit rot.

OBJECTIVE

To investigate the effect of *Botrytis* isolate, culture age, culture media and inoculum level on pathogenicity and infection of kiwifruit.

MATERIALS AND METHODS

Experiment No. 1

Title: Effect of *B. cinerea* isolates, culture age and inoculum concentration on *in vitro* conidial germination.

The experiment was carried out in the Plant Health laboratory of Massey University, Palmerston North New Zealand. *B. cinerea* isolates were grown on malt agar (MA) for 7 or 28 days. Spore concentrations from each isolate/age combination were adjusted to 1000 or 25000 spores/ 17 µl droplet of suspension (equivalent to 5.9×10^4 and 1.5×10^6 spores/ml). Inoculated malt agar disks (20 mm diameter) in Petri plates were incubated at 20°C for 12 hours to examine germination *in vitro*.

One isolate of *B. cinerea* was obtained from each of infected strawberry (Palmerston North), blueberry (Palmerston North), grapes (Hawkes Bay) and camellias (Palmerston North). The remainder of the isolates were obtained from infected kiwifruit at the New Zealand Institute for Crop and Food Research, Levin (CFRI), at Massey University Fruit Crops Unit (Palmerston North) (D^s), Hort Research, Mt. Albert (Auckland) and Hort Research (Lincoln).

Statistical Analysis

The experiment was designed as a 8x2x2 factorial with three replications. Data required a square root transformation before Analysis of Variance.

Experiment No.2

Title: Pathogenicity of *B. cinerea* isolates from a variety of sources on kiwifruit.

The experiment was carried out at the Massey University Fruit Crops Orchard (MUFCO), Palmerston North and duplicated at the New Zealand Institute for Crop and Food Research (CFRI), Kimberley Road, Levin by Dr. L. Cheah. Fruit was harvested on June 2nd 1992 at both sites. At harvest, temperature and relative humidity for MUFCO experiment were 13°C and 80% respectively while at the CFRI ambient environment was 13-15°C and 65-70% relative humidity. MUFCO fruit had a total soluble solids content (TSS) of 11.6% and a firmness of 7.8 kgf, while those at CFRI had a TSS of 11.0% and a firmness of 8.5 kgf.

Cultures of *B. cinerea* isolated from infected kiwifrut were obtained from research centres in four different areas of New Zealand; at CFRI (Levin), Hort Research (Lincoln), Hort Research, Mt. Albert (Auckland) and Massey University Fruit Crops Unit (Palmerston North) (D^S). A fifth isolate was obtained from diseased strawberry fruit and a sixth from diseased grapes (Hort Research, Hawkes Bay). A laboratory-induced dicarboximide resistant strain (D^R) of the Massey isolate was also used. All isolates were grown on MA for 10-14 days. Spores suspensions were adjusted to the required concentration to give 1000, 5000, 25000 and 125000 spores per 17µl droplet of water (corresponding to 5.9×10^4, 2.9×10^5, 1.5×10^6 and 7.4×10^6 spores/ml). All cultures were prepared and grown at Massey University and those required at CFRI were couriered overnight.

After inoculation, fruit were packed in commercial single layer 36 count trays and coolstored at 0°C (90-98% R.H.). *B. cinerea* storage rot from both

MUFCO and CFRI was assessed at 6 and 12 weeks of coolstorage.

Statistical Analysis

The MUFCO experiment was analyzed as a two factor experiment with four replications and the CFRI experiment was analyzed as a completely randomized (one factor) experiment since results for each tray within a treatment were not separated out. Square root transformation was carried for both MUFCO and CFRI data before analysis of variance.

Experiment No.3

Title: Effect of growth media, culture age and inoculum level on pathogenicity of *B. cinerea* to kiwifruit.

The experiment was carried out with fruit harvested at Massey University Fruit Crops Orchard (MUFCO), Palmerston North and at the New Zealand Institute for Crop and Food Research (CFRI), Kimberley Road, Levin by Dr. L. Cheah. Fruit was harvested on May 20th 1992 at both sites. At harvest temperature and relative humidity from MUFCO were 11°C and 80% relative humidity while at the CFRI ambient environment was 13-15°C and 65-70% relative humidity. MUFCO fruit had an average total soluble solids content (TSS) of 9.69% and a firmness of 8.5 kgf, while those at CFRI had a TSS of 7.6% and a firmness of 9.2 kgf.

B. cinerea (Massey isolate K3) was grown on PDA, MA and autoclaved kiwifruit leaves (LVS) for 7, 18 or 28 days. Spore concentrations from each media/age combination were adjusted to 1000, 5000 or 25000 spores per 17µl droplet of suspension (corresponding to 5.9 x 10^4, 2.9 x 10^5 and 1.5 x 10^6 spores/ml) respectively. An additional concentration of 125000 per droplet (7.4 x 10^6 spores /ml) was also tested at CFRI. Aliquots of each spore suspension were incubated at 20°C on MA, PDA, LVS and water agar to evaluate *in vitro* percentage germination.

After inoculation, fruit were packed in commercial single layer 36 count trays and coolstored at 0°C (90-98% R.H.). *B. cinerea* storage rot was assessed at 6 and 12 weeks of coolstorage at both research centres.

Statistical Analysis

The experiment was designed as a 3x3x3 factorial with three replications, at MUFCO and as a 4x3x3 with one replication at CFRI. Data from the MUFCO required a log transformation while data from CFRI required a square root transformation before Analysis of Variance.

RESULTS

Experiment No.1

Significantly higher ($P < 0.001$) conidial germination rates were found from cultures of *B. cinerea* isolated from diseased kiwifruit (Levin, Lincoln, Auckland and Palmerston North) than from cultures originating from other crops. The lowest germination rates were found in cultures isolated from diseased camellia and grapes (Table 3-1).

Spores prepared from young (seven day old) cultures of *B. cinerea* showed a higher germination rate ($P < 0.001$) compared with those from older (28 days) cultures (Table 3-1).

Spore concentrations significantly ($P < 0.001$) influenced germination. The lower spore concentration (1k) gave the highest percentage germination (Table 3-1).

The lowest spore load gave highest percentage conidial germination for most isolates, a trend particularly marked with the strawberry and blueberry isolates (Fig.3-1). The significant interaction between isolates and inoculum level in Table 3-1 is because two isolates (Lincoln and Massey) showed the reverse of this trend with most spores germinating at the higher

Table 3-1. SUMMARY OF *IN VITRO* GERMINATION OF *B. cinerea* CONIDIA AFTER 12h INCUBATION AT 20°C ON MA.

Source	Level	Mean[x] germination (%)
Main effects		*P < 0.001*
Isolates	Auckland	76.15 (1.9)[az]
	Blueberry	46.23 (3.7)[c]
	Camellia	34.61 (2.7)[d]
	Grape	47.71 (4.0)[d]
	Levin	92.27 (1.7)[a]
	Lincoln	86.20 (1.7)[b]
	Massey D[S]	90.74 (1.3)[a]
	Strawberry	74.81 (2.5)[b]
		P < 0.001
Culture Age (Ca) (days)	7	78.23 (1.6)[a]
	28	58.95 (1.5)[b]
		P < 0.001
Spore concentration (K) (nos. 17µl/droplet)	1000	74.40 (1.5)[a]
	25000	62.78 (1.6)[b]
Interactions		
Isolates x Ca		*P < 0.001*
Isolates x K		*P < 0.001*
Ca x K		*P < 0.001*

[x] = *P* values after square root transformation.
[z] = Mean separation within columns by Duncan's multiple range test, *P < 0.05*.
Values enclosed in parenthesis indicate overall standard error of the mean.

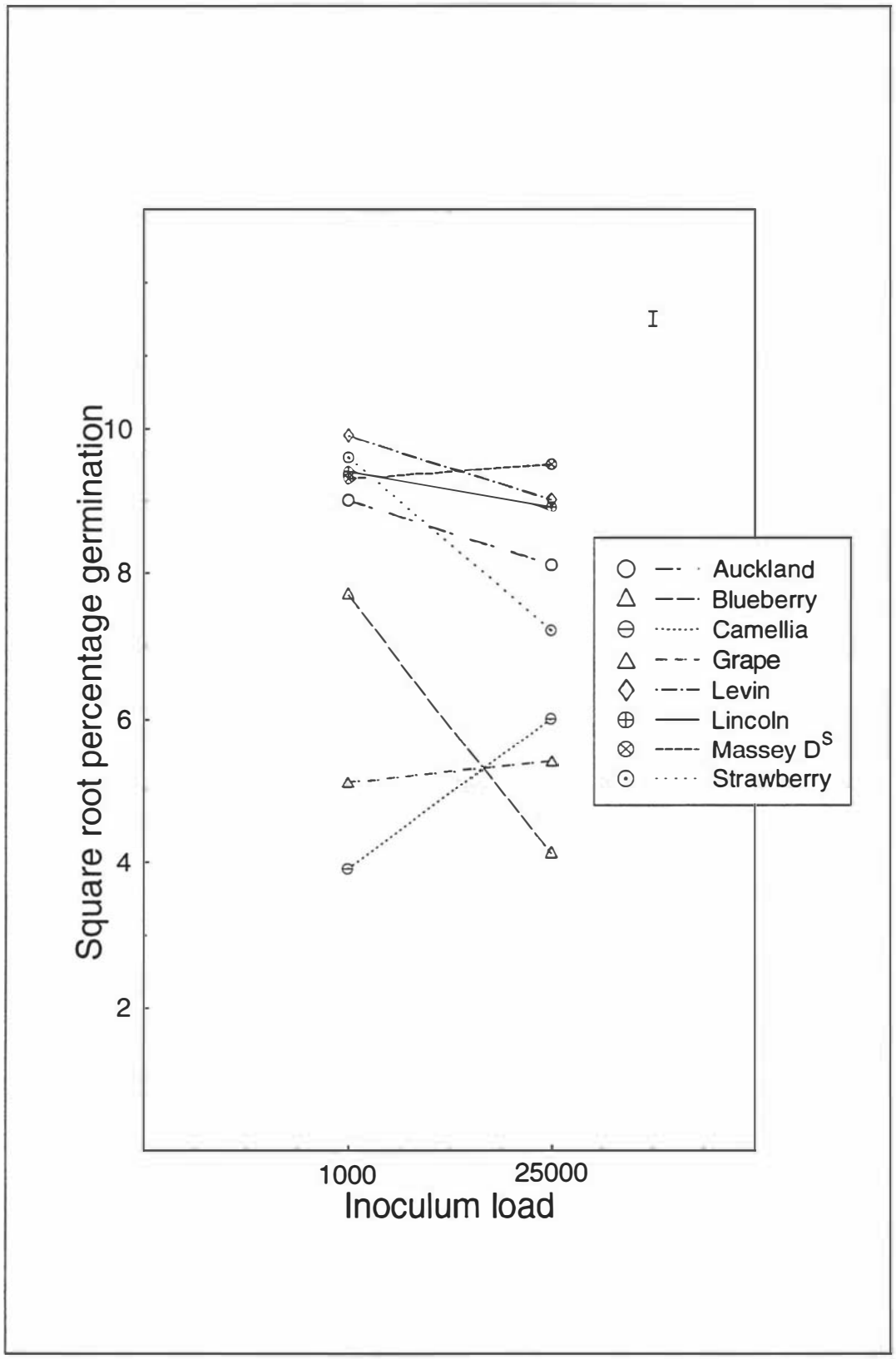


Figure 3-1. Interaction between isolates and spore concentration during *in vitro* conidial germination on MA. Vertical bar indicates overall standard error of the mean (SEM).

concentration as showing in Fig. 3-1. The lower overall germination of isolates from blueberry, grapes and camellia can be clearly seen. With the exception of the isolate obtained from infected kiwifruit at Levin, there was higher germination *in vitro* when conidia were obtained from seven day old cultures than when they were obtained from 28 day old cultures (Fig.3-2).

The spores obtained from seven day old cultures had a high percentage germination at both spore concentrations. Spores from 28 day cultures had a lower germination and this decreased at the high spore concentration (Fig.3-3).

Experiment No.2

At MUFCO most fruit (74.7%) ultimately diseased (Table 3-2) developed symptoms after six weeks coolstorage, but at CFRI most infected fruit (58.6%) developed disease symptoms within six weeks coolstorage (Table 3-3). At MUFCO there was considerable variability in the proportion of infected fruit showing symptoms after six weeks with a range from 36.2% (strawberry isolate) to 100% (Levin isolate). The final disease incidence was considerable higher, especially at low inoculum levels, at CFRI than at MUFCO.

After six weeks coolstorage there were significant differences between isolates at MUFCO *($P < 0.05$)* and at CFRI (*$P < 0.01$*) (Tables 3-2 & 3-3).

At MUFCO *B. cinerea* isolates from infected kiwifruit at Lincoln and from infected grapes had significantly higher infection levels than that from strawberry. At CFRI, isolates from infected kiwifruit at Lincoln and Auckland caused significantly more disease than those from grapes, Levin and the Massey D^R isolate. By 12 weeks of coolstorage, there were no statistical differences between isolates at either MUFCO or at CFRI. The relative ranking of isolates had also changed.

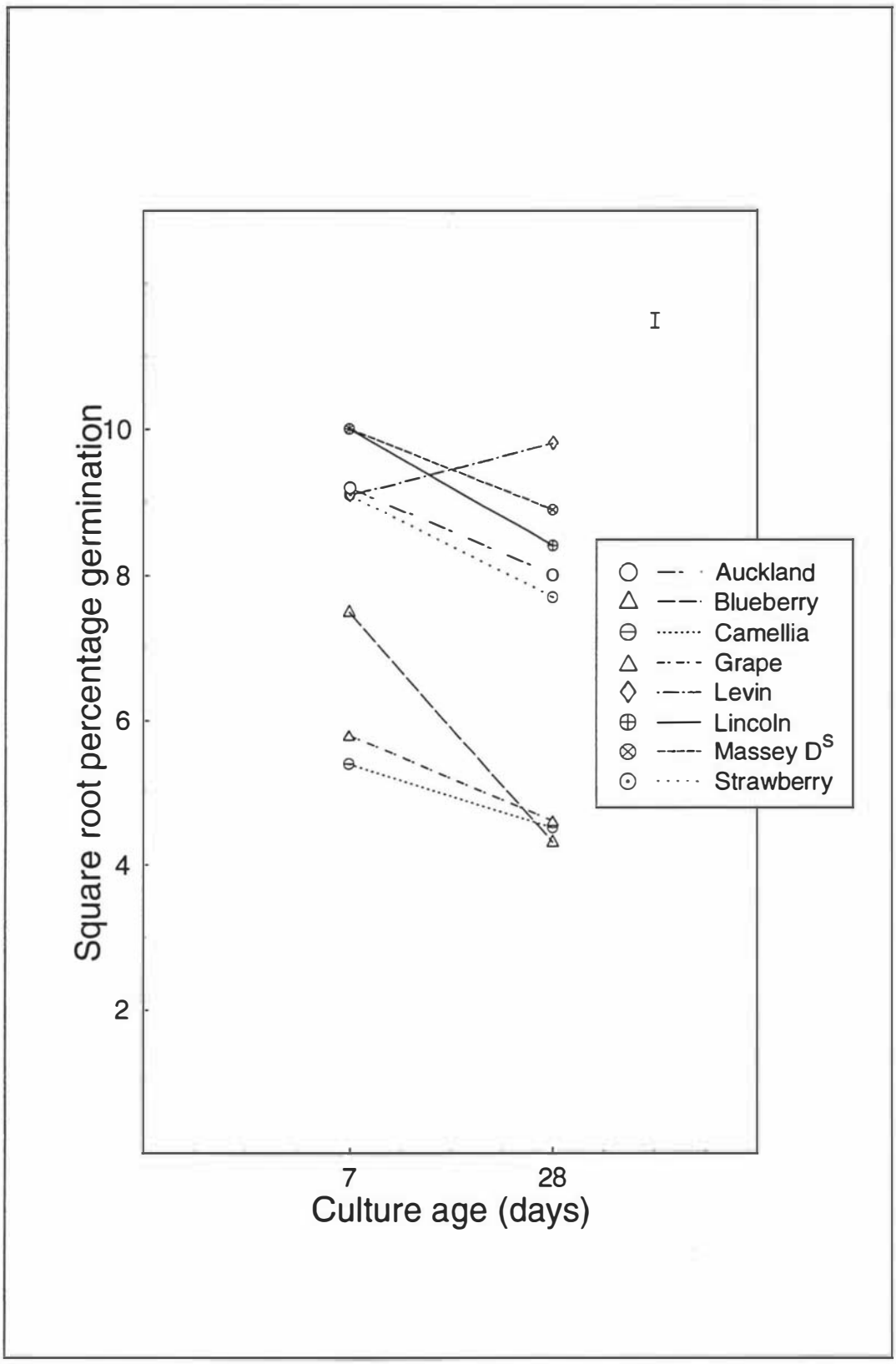


Figure 3-2. Interaction between isolates and culture age during *in vitro* conidial germination on MA. Vertical bar indicates overall standard error of the mean (SEM).

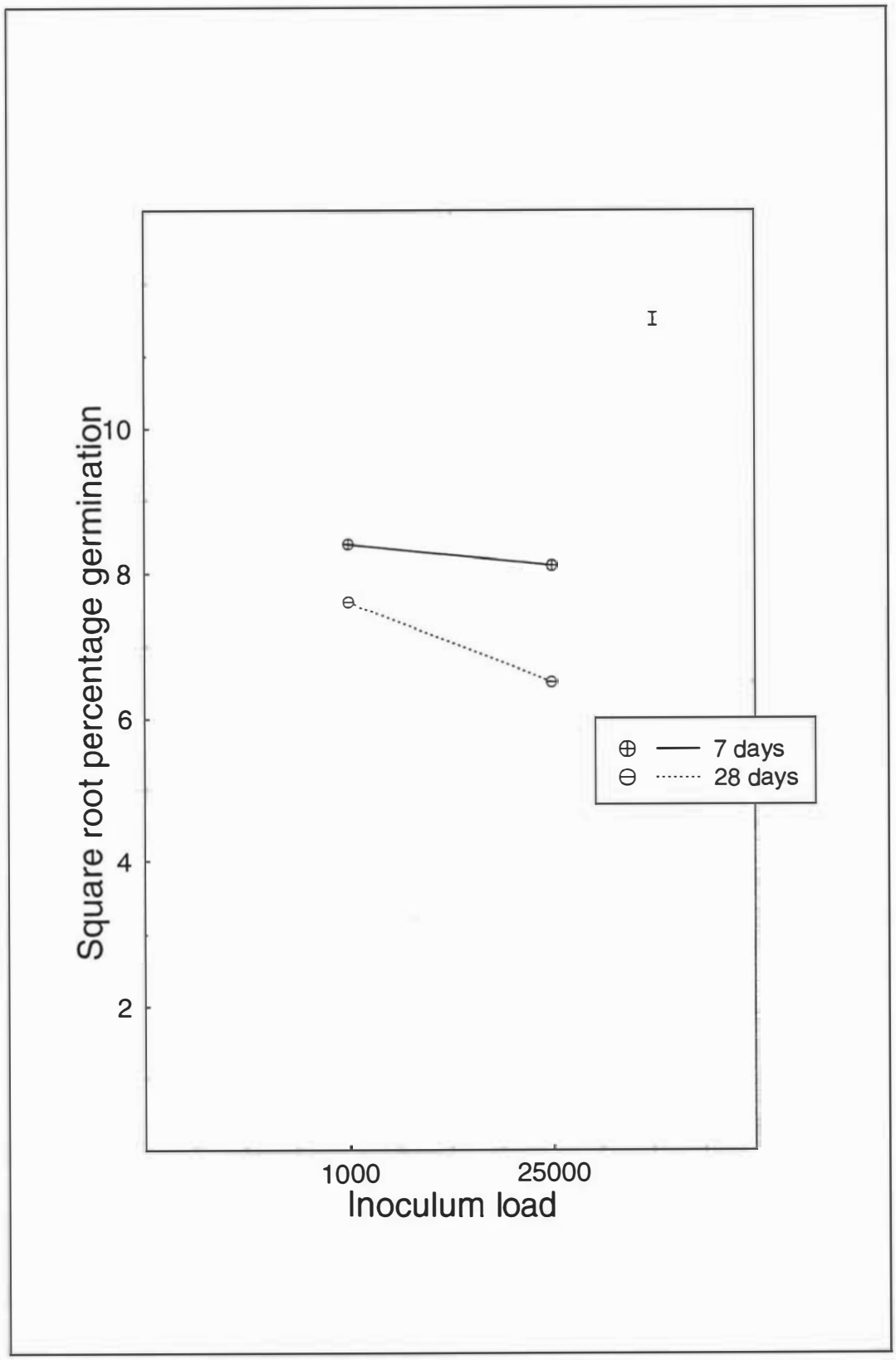


Figure 3-3. Interaction between culture age and spore concentration during *in vitro* conidial germination on MA. Vertical bar indicates overall standard error of the mean (SEM).

Table 3-2. SUMMARY OF THE EFFECT OF *B. cinerea* ISOLATES AND SPORE CONCENTRATIONS ON PERCENTAGE INFECTION OF KIWIFRUIT AFTER STORAGE AT 0°C AT MASSEY UNIVERSITY FRUIT CROPS ORCHARD.

Source	Level	Mean [x] infection 6 weeks (%)	Mean [x] infection 12 weeks (%)
Main effects		*P < 0.05*	*NS*
Isolates	Auckland	5.18 (2.0)^{abz}	7.77 (2.6)^{a}
	Grape	7.61 (2.9)^{a}	11.08 (4.0)^{a}
	Levin	4.51 (1.6)^{ab}	4.51 (1.8)^{a}
	Lincoln	7.78 (2.6)^{a}	8.57 (3.0)^{a}
	Massey D^{R}	3.96 (1.1)^{ab}	5.17 (1.2)^{a}
	Massey D^{S}	3.27 (1.2)^{ab}	4.49 (1.6)^{a}
	Strawberry	1.18 (0.4)^{b}	3.26 (1.0)^{a}
		P < 0.001	*P < 0.001*
Spore concn. (K)	1000	0.09 (0.1)^{a}	0.09 (0.1)^{a}
(nos. 17µl/droplet)	5000	0.87 (0.3)^{b}	1.17 (0.4)^{b}
	25000	6.70 (1.8)^{c}	9.57 (2.1)^{c}
	125000	11.47 (1.5)^{c}	13.55 (2.0)^{c}
Interactions			
Isolates x K		*NS*	*P < 0.05*

[x] = *P* values after square root transformation.
NS = not significant.
[z] = Mean separation within columns by Duncan's multiple range test, *P < 0.05.*
Values enclosed in parenthesis indicate overall standard error of the mean.

Table 3-3. SUMMARY OF THE EFFECT OF DIFFERENT *B. cinerea* ISOLATES AND SPORE CONCENTRATIONS ON PERCENTAGE INFECTION OF KIWIFRUIT AT CROPS AND FOOD RESEARCH LEVIN AFTER STORAGE AT 0°C.

Source	Level	Mean [x] infection 6 weeks (%)	Mean [y] infection 12 weeks (%)
Main effects		*P < 0.01*	*NS*
Isolates	Auckland	20.12 (0.8)[az]	27.30 (3.0)[a]
	Grape	2.75 (1.9)[b]	13.17 (4.5)[ab]
	Levin	8.27 (6.5)[b]	21.87 (11.9)[ab]
	Lincoln	21.48 (7.2)[a]	26.72 (7.4)[ab]
	Massey D^R	4.13 (1.6)[b]	13.55 (7.8)[b]
	Massey D^S	10.02 (6.2)[ab]	14.45 (5.3)[ab]
	Strawberry	14.20 (7.1)[ab]	21.20 (8.3)[ab]
		P < 0.05	*P < 0.001*
Spore concn. (K)	1000	5.31 (3.0)[b]	10.31 (4.5)[b]
(nos. 17µl/droplet)	5000	5.91 (2.8)[b]	11.30 (8.3)[b]
	25000	15.42 (4.8)[a]	23.34 (11.9)[a]
	125000	19.62 (4.5)[a]	34.05 (5.3)[a]

[x y] = *P* values after log and square root transformation respectively.
NS = not significant.
[z] = Mean separation within columns by Duncan's multiple range test, *P < 0.05.*
Values enclosed in parenthesis indicate overall standard error of the mean.

Inoculum levels influenced disease incidence with significant differences (*P < 0.001*) at both, six and 12 weeks storage at MUFCO and at *P < 0.05* (six weeks) and *P < 0.001* (12 weeks) at CFRI (Tables 3-2 & 3-3).

A significant interaction between isolates and concentration at *P < 0.05* was found after 12 weeks of kiwifruit storage at MUFCO only (Table 3-2). The highest spore load for each isolate gave the highest infection levels in kiwifruit, but the curve for inoculum vs disease increased sharply at low inoculum levels and levelled off at high inoculum levels for some isolates (e.g. Levin and Massey D^R) while the reverse happened with others (e.g. grape and Lincoln) hence the significant interaction (Fig.3-4).

Experiment No.3

The percentage germination of conidia tested *in vitro* on MA, PDA, LVS and water agar was 100% on all four media tested.

There was rapid disease development at both sites with 60% of the fruit ultimately diseased at MUFCO showing symptoms within six weeks coolstrage while at CFRI the corresponding figure was 77%. As in the previous experiments disease, incidence at CFRI was higher (four and a half more times) than at MUFCO (Tables 3-4 & 3-5).

At MUFCO, there were no significant differences from preparing inoculum cultures on different media when fruit had been coolstored for six weeks but after 12 weeks inoculum produced on malt agar had caused significantly more infection (*P < 0.01*) than that from the other two media. At CFRI inoculum prepared from colonies on autoclaved kiwifruit leaves caused significantly more infections (*P < 0.001*, *P < 0.01*) than those prepared from the other media after both six and 12 weeks of coolstorage.

There was no significant effect of the age of cultures from which inoculum had been prepared at either MUFCO or at CFRI.

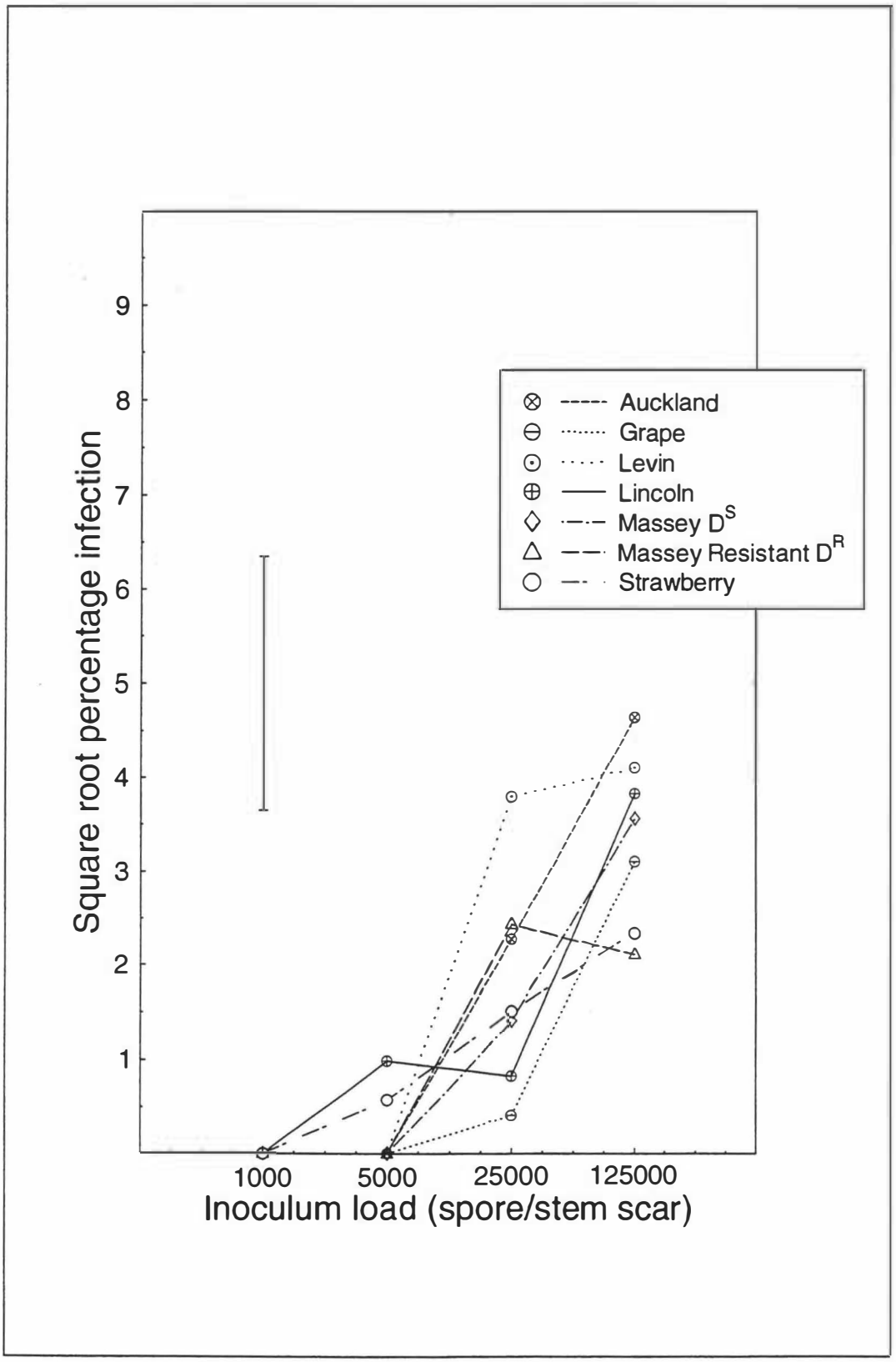


Figure 3-4. Interaction between isolates and spore concentration on storage rot incidence at MUFCO. Vertical bar indicates overall standard error of the mean (SEM).

Table 3-4. SUMMARY OF THE EFFECTS OF MEDIA, CULTURE AGES AND INOCULUM LEVEL ON INCIDENCE OF STEM END ROT CAUSED BY *B. cinerea* AT MASSEY UNIVERSITY FRUIT CROPS ORCHARD AFTER STORAGE AT 0°C.

Source	Level	Mean [x] infection 6 weeks (%)	Mean [x] infection 12 weeks (%)
Main effects		*NS*	*P < 0.01*
Media	PDA	5.32 (1.9)[az]	7.17 (2.3)[b]
	MA	6.35 (2.0)[a]	11.17 (2.3)[a]
	Kwlv	3.58 (1.1)[a]	6.95 (1.4)[b]
		NS	*NS*
Culture Age (Ca)	7	4.60 (1.3)[a]	8.1 (1.5)[a]
(days)	18	5.95 (2.0)[a]	9.4 (2.5)[a]
	28	4.71 (1.7)[a]	7.6 (2.1)[a]
		P < 0.001	*P < 0.001*
Spore concn. (K)	1000	0.10 (0.1)[a]	2.33 (0.4)[a]
(nos. 17µl/droplet)	5000	2.04 (0.9)[b]	6.95 (1.3)[b]
	25000	13.13 (2.1)[c]	16.0 (3.0)[c]
Interactions			
Me x Ca		*P < 0.001*	*P < 0.001*
Me x K		*NS*	*NS*
Ca x K		*NS*	*NS*

[x] = *P* values after log transformation.
NS = not significant.
[z] = Mean separation within columns by Duncan's multiple range test, *P < 0.05.*
Values enclosed in parenthesis indicate overall standard error of the mean.

Table 3-5. SUMMARY OF THE EFFECTS OF MEDIA, CULTURE AGES AND INOCULUM LEVEL ON INCIDENCE OF STEM END ROT CAUSED BY *B. cinerea* AT CROPS AND FOOD RESEARCH LEVIN AFTER STORAGE AT 0°C.

Source	Level	Mean [x] infection 6 weeks (%)	Mean [x] infection 12 weeks (%)
Main effects		*P < 0.001*	*P < 0.01*
Media	PDA	23.31 (6.4)[bz]	31.25 (6.3)[b]
	MA	23.75 (8.5)[b]	34.28 (7.8)[b]
	Kwlv	42.32 (6.4)[a]	50.50 (5.7)[a]
		NS	*NS*
Culture Age (Ca)	7	29.04 (4.3)[a]	37.63 (4.0)[a]
(days)	18	28.56 (7.7)[a]	37.64 (7.3)[a]
	28	31.78 (7.0)[a]	40.76 (6.7)[a]
		P < 0.001	*P < 0.001*
Spore concn. (K)	1000	7.84 (2.8)[c]	19.44 (3.6)[c]
(nos. 17µl/droplet)	5000	18.17 (4.8)[b]	27.33 (4.4)[bc]
	25000	28.67 (6.9)[b]	37.66 (6.9)[b]
	125000	64.89 (4.2)[a]	70.27 (3.7)[a]
Interactions			
Me x Ca		*NS*	*NS*
Me x K		*NS*	*NS*
Ca x K		*NS*	*NS*

[x] = *P* values after square root transformation.
NS = not significant.
[z] = Mean separation within columns by Duncan's multiple range test, *P < 0.05.*
Values enclosed in parenthesis indicate overall standard error of the mean.

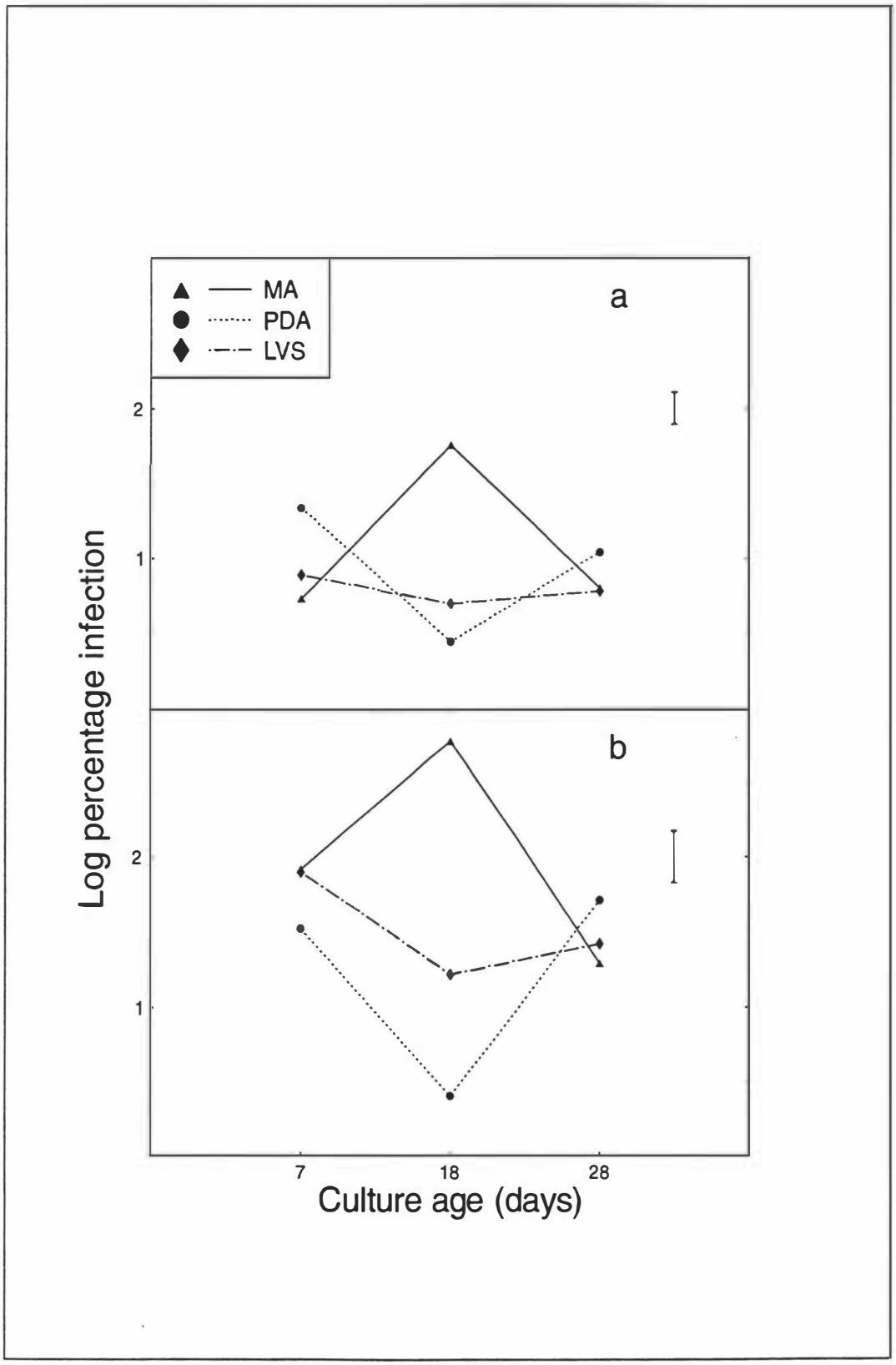


Figure 3-5. Interaction between media and *B. cinerea* culture age on storage rot incidence at MUFCO. a) 6 weeks, b) 12 weeks of storage. Vertical bars indicates overall standard error of the mean (SEM).

Inoculum level influenced disease incidence with significant differences at $P < 0.001$ after six and 12 weeks of storage at both MUFCO and at CFRI. The highest spore concentration gave the highest mean percent of diseased fruit at both locations (Tables 3-4 & 3-5) while the lowest spore load gave the lowest disease incidence.

There was a significant interaction between media and culture age ($P < 0.001$) after 6 and 12 weeks of coolstorage at MUFCO (Table 3-4). Spores from 18 day-old cultures grown on malt agar caused significantly more infection than those from other age and media combinations (Fig.3-5). At CFRI there were no significant interactions.

DISCUSSION

In this present study, the growing medium and inoculum levels influenced *B. cinerea* pathogenicity on harvested kiwifruit. Likewise, *in vitro* germination varied between isolates obtained from different infected crops.

In *Experiments 2 & 3*, higher levels of infection were recorded in fruit harvested and inoculated at the CFRI than in fruit from MUFCO. The inoculum used at both sites was prepared at Massey University and inoculated at the packing shed from the same batch of Petri plates incubated under the same conditions than the CFRI plates and sent by overnight courier. Fruit were harvested on the same day and at approximately the same maturity as measured by TSS and firmness. The major difference in procedure was that the fruit were snapped off the pedicel at harvest at MUFCO but at CFRI the pedicels were cut off the vine and the pedicel snapped from the fruit immediately before inoculation. This fresh wound would be more susceptible than those several hours old (at MUFCO) and could be the reason for the different infection levels at the two locations. Differences in a microclimate at the two sites could also have an

influence on susceptibility of the fruit. Regional differences in levels of infection during kiwifruit storage have been reported by Brook (1990a). Consistent differences in amounts of *B. cinerea* between regions and districts within regions were also reported by Hopkirk *et al.* (1990a) who related this variability in infection to climate variability. For example they found fruit from the Northland region had the highest losses (5.9%) while Nelson area had the lowest (1.3%). In that study infection levels in 1989 in fruit from the Southern North Island (Manawatu, Wanganui etc..) were approximately 0.8%, while overall infections in this present study (1992 experiments) after 12 weeks coolstorage were 6.4% and 20% at MUFCO and CFRI respectively. Knight (1980) showed that the virulence of *B. cinerea* on red raspberries can be associated with the susceptibility of each red raspberry cultivar to *B. cinerea.* In this present study cultivar differences in *B. cinerea* infection on kiwifruit can not be accounted for this type of host-pathogen association because the *Hayward* cultivar is the most extensive cultivar grown in New Zealand orchards (Ferguson & Bollard 1990). On balance, the difference in incubation procedures would appear to be the most likely explanation for different infection levels at both sites of study.

The isolates of *B. cinerea* evaluated in this study were all pathogenic to kiwifruit and caused almost 20% of inoculated fruit to become diseased. After 12 weeks coolstorage there were no significant differences between infection levels by different *B. cinerea* isolates at either site and the isolates did not rank in the same order (based on infection levels) at either site. The statistical difference between isolates after six weeks incubation were probably not real effects since the relative virulence of isolates was not the same at each site and significant differences had disappeared by 12 weeks coolstorage.

Other studies, related with *B. cinerea* pathogenicity in different cultivars (Williams & Jennings 1986), found that the length of disease lesions on red raspberry canes inoculated with 31 different isolates of *B. cinerea* obtained

from different localities, significantly varied between 665 to 42 mm. However in that study, the origin of the isolate was not associated with individual pathogenicity. Similar results were obtained evaluating several isolates of *Didymella applanata* on the same cultivar, all 32 isolates were pathogenic regardless the area where they were obtained (Pepin *et al.* 1985).

For experiments 2 & 3 disease incidence varied between the two locations. In those *in vivo* studies, it was evident that as the spore concentrations increased a greater number of infected fruit were recorded at both assessment periods. Similar results have been found by other researchers; Long & Wurms (1993) have reported that in order to achieve a high percentage of infection in kiwifruit it is necessary to apply high doses of spore inoculum. In their experiment, 125000 spores per droplet in the picking scar wound of the fruit was needed to achieve a high percentage of disease fruit. Hallet & Sharrock (1993) reported that infection levels of kiwifruit inoculated after 4h after harvest with a dry spore mass (about 50,000 spores/fruit) were higher (98%) compared with a liquid spore suspension (4000 fruit) where fruit infection was approximately 20%. Segall & Newhall (1960) found that blight disease in onions caused by *B. alli* gradually increased with spore load. They reported that inoculum load of 59,1250 spores/ml gave 222 spots per plants compared with 4 spots per plant observed when inoculum load was of 6678 spores/ml. Coinciding with those results, Last & Hamley (1956) and Price (1970) working with *B. fabae* on broad beans and *B. tulipae* on tulip leaves respectively, reported a direct correlation between spore load (10^2 to 10^6/ml) and droplet size (0.001 to 0.003 ml) with lesions numbers (0 to 500 and 1 to 5 respectively), however in further work, contradictory results were reported by Last (1960). This researcher found that at concentrations of $1x10^5$ conidia/ml or more of *B. fabae* infectivity decreased with culture age up to 40 days old. In this present study, the high spore concentrations required to infect kiwifruit suggests some type of antifungal activity present on the kiwifruit stem scar. The highest percentage of infected fruit came from fruit inoculated at CFRI

with spores from *B. cinerea* grown on autoclaved kiwifruit leaves. Exudates from portions of tomatoes, leaves of different tomato cultivars (Chou 1972b), grape berries (Kosuge & Hewitt 1964) and heads of safflower (*Carthamus tinctorius* L.) (Barash *et al.* 1963; 1964) have been shown to enhance *B. cinerea* germination and infection. Analysis of these exudates showed that carbohydrates such as glucose and fructose were the main compounds, although in tomato exudates inhibitory phenolic substances were also detected. Similar results were reported by Blakeman (1975) when addition of exogenous nutrients equivalent to leaf exudates of beetroot (*Beta vulgaris* L) were added to conidial suspension of *B. cinerea*. In that study the pathogenicity of *B. cinerea* was related not only to the isolates but also to the addition to the growth media of certain amino acids and/or simple sugars such as fructose and sucrose. Possibly in this present study the autoclaving temperatures allowed denaturalization of inhibitors present in the leaves permitting the *B. cinerea* conidia to gain sufficient nutrients and reserves for the infection process.

At MUFCO, the combination malt agar/18 old day culture gave the highest disease incidence. Maas & Powelson (1972) observed that growth of *B. convoluta* was best on a maltose and glucose medium. However Clark & Lorbeer (1977) reported different nutrient dependency between *B. squamosa* and *B. cinerea.* They found that germination of *B. squamosa* conidia on onion leaves was equivalent in water or nutrients (glucose solution, standard nutrient solution, onion leaves diffusate or filtrate of cattail pollen), while germination of *B. cinerea* was lower in water than in nutrient solutions, however the number of lesions produced were higher with nutrients in both species. In that study conidia germination of both species declined with age (21 days) regardless water or nutrient solutions. Salinas *et al.* (1989) found that addition of gerbera pollen diffusates stimulated germination of stored (eight weeks) dry conidia of *B. cinerea* in both *in vitro* (20°C) and on gerbera flowers nevertheless incubation temperatures (4 or 25°C). Choi *et. al.* (1990) observed better spore production on cucumbers

when *B. cinerea* conidia were grown on PDA or Czapeck-Dox media compared with corn meal agar, nutrient agar or lime bean agar. Although Bryk (1985b) reported more *B. cinerea* mycelial growth and sclerotia formation only when grown on PDA. Last & Hamley (1956) reported that *B. fabae* 10 days old cultures produced more lesions (about 70) on broad beans than conidia from 25 days old cultures (about 5). Choi *et al.* (1990) reported disease severity on cucumbers was not affected by *B. cinerea* spore age (20 days old).

The difference in percentage germination of isolates on MA were not reflected by consistent differences in virulence on kiwifruit at the two sites. In the absence of any one isolate of *B. cinerea* showing a marked characteristic of high virulence compared with other isolates, the Massey isolate K3 was selected for future work since it has been used in other studies on kiwifruit at Massey University and would provide continuity between this and other work here. The choice of media on which to produce inoculum does not appear to be critical with MA giving best results at MUFCO and autoclaved kiwifruit leaves at CFRI. MA has been used in other studies at Massey is easier and more uniform over time (seasonal variation) than kiwifruit leaves and was therefore selected as the medium of choice for future work. The age of colonies from which inoculum was prepared did not have a significant effect on virulence of *B. cinerea* to kiwifruit but in view of the lower percentage germination on MA of spores from 28 compared with seven day old colonies and of the literature quoted above it was decided to use 7-14 day old colonies for future work. The high concentration of spores had a lower percentage germination on MA confirming reports of self-inhibition at high concentrations *in vitro.* However, this effect was not found in kiwifruit where increases in spore concentration resulted in increased disease incidence. With no clear resolution to this aspect of inoculation it appeared prudent to continue to use a range of spore concentrations (inoculum loadings).

CHAPTER FOUR

EFFECT OF RELATIVE HUMIDITY AND TIME OF EXPOSURE AT DIFFERENT TEMPERATURES ON SURVIVAL OF CONIDIA OF *BOTRYTIS CINEREA*.

INTRODUCTION

Several factors may influence spore production, survival, germination and infectivity of plant pathogenic fungi. They include inherent fungal factors such as isolate variability, age and nutrient status of the spores and environmental conditions such as temperature, moisture and external nutrient supply. McLaughlin & True (1952) showed that conidia (on glass beads) and mycelium (in wood chips) of *Chalara quercina* survived longer at temperatures of 10 and 20°C than at higher temperatures (25 and 30°C). A relative humidity of 20% was more favourable for survival than higher humidities. More extensive studies of the oak wilt pathogen by Merek & Fergus (1954) using both endoconidia and ascospores confirmed that a cool, dry atmosphere was the most favourable condition for survival of this fungus. Conidia and sclerotia of *B. squamosa* survived longer in soil at low temperatures than in soil held at high temperatures (Ellerbrock & Lorbeer 1977).

Despite this consistent pattern in the above examples of an improved survival of fungi at low temperatures, the relationship between temperature, relative humidity and spore survival is not necessarily so straight forward. Thus Coley-Smith (1980) found that urediniospores of *Melampsora lini* remained viable longer in mid-range humidities (40 and 60%) than in dry (20%) or humid (80%) conditions. A relative humidity of 75% dramatically

reduced survival of conidia of *Aspergillus flavus* and of *A. terreus* compared with humidities of 32% or 85% (Teitell 1958). This lethal effect occurred over a very narrow humidity range since the effect of humidities of 73% and 77% were little different from that of other humidities. In common with the general trend of temperature and survival discussed above, Teitell (1958) also found that spores of the two *Aspergillus* spp. survived for a shorter time at higher temperatures. Mycelium and conidia of *B. cinerea* and mycelium of *Sclerotinia sclerotium* survived longer at 0°C than at 20°C in the absence of nutrients (Van den Berg & Lentz 1968). In contrast to the findings of Coley-Smith (1980) and Teitell (1958), survival was longer at relative humidities of 99-100% than at 85-90% but was dependant on type and strain of organism.

A first step in studying the effect of temperature and relative humidity on infection of kiwifruit by *B. cinerea* is to ascertain the effect of these parameters on the pathogen itself. Given the variability between strains found by Van den Berg & Lentz (1968) and the inconsistencies noted above, it was important to test the characteristics of the isolate of *B. cinerea* used in the rest of this work.

OBJECTIVE

The objective of this study was to evaluate the effect of relative humidity and temperature on survival of conidia of *B. cinerea* over time periods which could be used in a pre-coolstorage curing period for kiwifruit.

MATERIALS AND METHODS

Survival of conidia of *B. cinerea* isolate K3 were assessed in controlled environment chambers at temperatures of 0°C ± 1, 10°C ± 2, 15°C ± 2, 20°C ± 1, 25°C ± 2 and 30°C ± 2.

The four relative humidity ranges of 40-50%, 65-75%, 80-90% and 95-100% were obtained by the methods explained in the *General Materials and Methods.*

Glass microscope cover slips were sterilized by immersion in alcohol and passing through a bunsen flame. They were placed in plastic Petri plates, five per plate. A 14 day old culture of *B. cinerea* was inverted over each Petri plate and tapped to release spores so they fell onto the cover slips. The Petri plates with cover slips were placed in humidity chambers (30 x 30 x 30 cm) (Fig.4-1) maintained at the appropriate temperature/humidity combination with an output air flow of 2.5 l/min. Relative humidities were monitored every day and the relative humidity values given in Fig.4-2 are the mean of at least three measurements per day. Temperature was monitored twice daily using a maximum and minimum thermometer placed on top of the relative humidity chamber. One plate per treatment combination was removed at each assessment time of 2, 4, and 6 days from the 20°C temperature and of 2, 4, 6, and 8 days for the other temperatures. The cover slips removed from the chambers were each inverted onto 20 mm diameter disks of malt agar and incubated at 20°C for 8 or 24 hours to test spore viability. They were then stained with lactophenol acid fuchsin and the percentage germination counted at 100x magnification.

Statistical Analysis

Experiments were carried out as a nested design with fixed effects. Arcsin-transformation was required before analysis of variance. To compare differences in germination between 8 and 24h a two sample t-test was also carried out.

RESULTS

The relative humidities obtained during this experiment remained constant

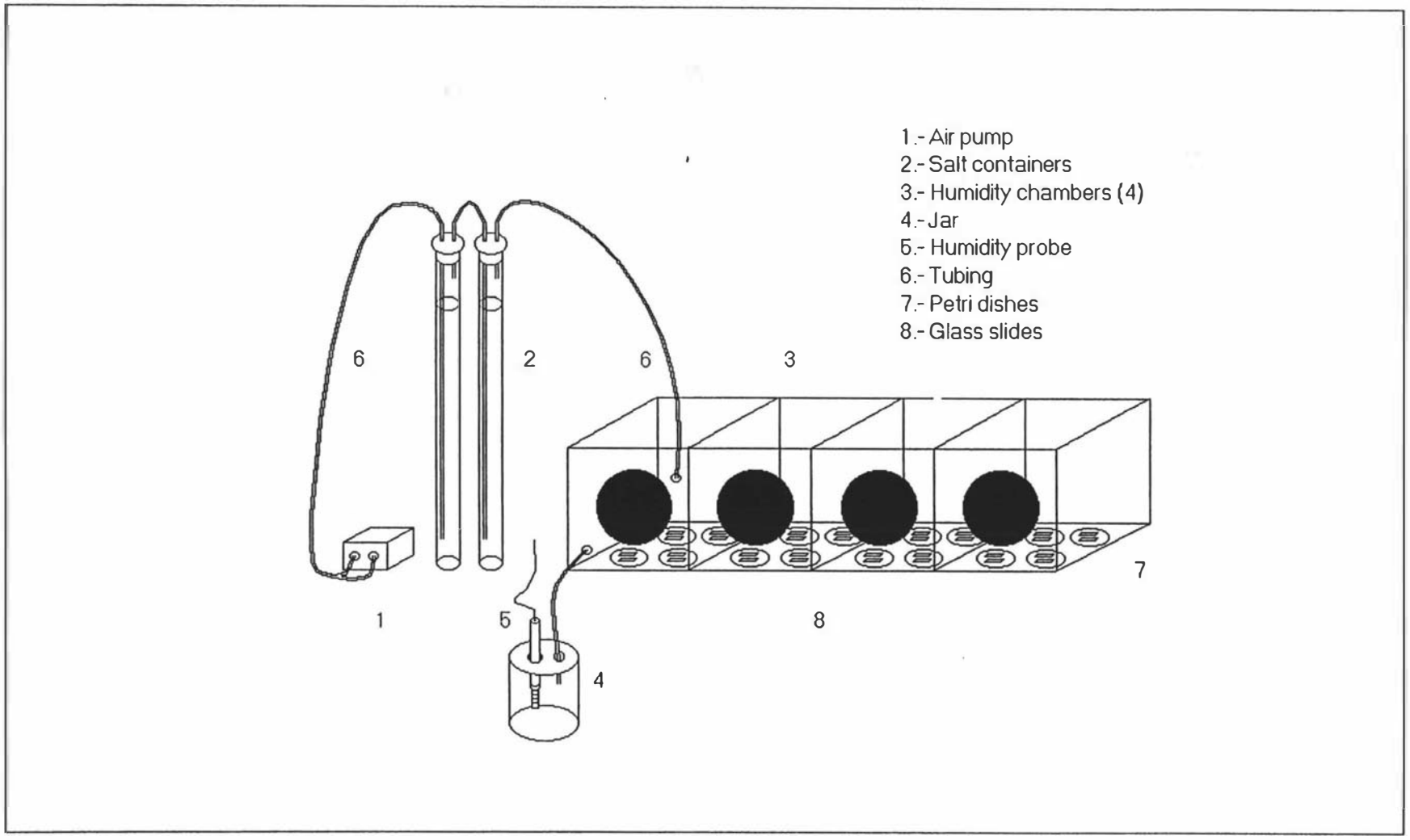


Figure 4-1. Relative humidity system.

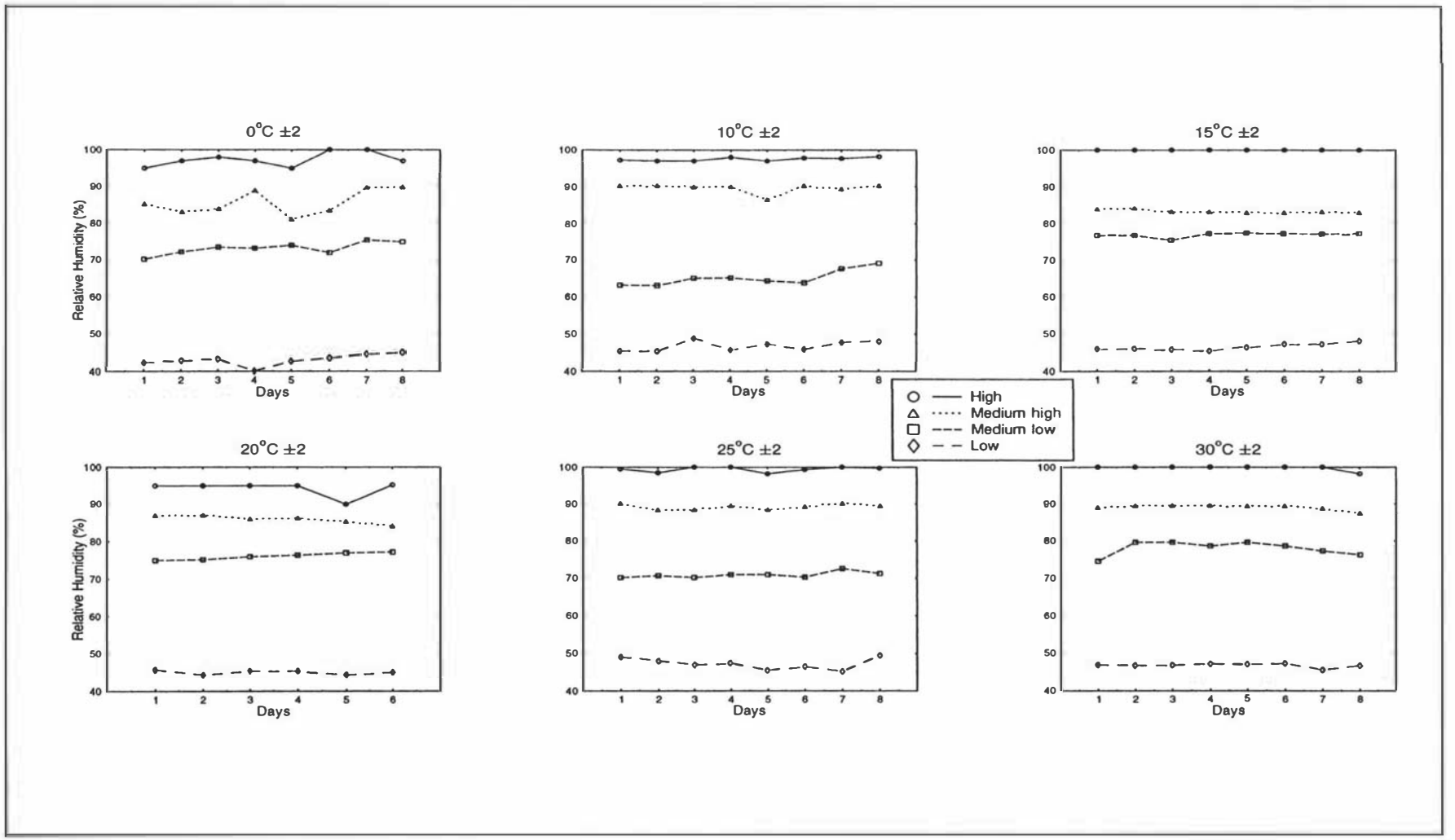


Figure 4-2. Actual relative humidities attained at six temperatures.

and distinct in each chamber throughout the eight days. The humidity ranges were distinct at each temperature although there was some variation in absolute values from one temperature to another (Fig.4-2). The highest RH range remained over 95% (except day 5 at 20°C) and the lowest was always below 50% RH. The second RH range was always between 80 and 92% RH but the third range showed considerable variation between temperatures. It was lowest at 10°C (60-69%) and highest at 30°C (74-79%) with RH at other temperatures falling within the range 70-79%.

Highly significant overall differences ($P < 0.001$) were observed among temperatures, relative humidities, days of treatment and incubation on MA (Table 4-1). Percentage germination decreased with temperature after eight hours incubation. Spores survived better at lower temperatures than at high ones with an approximately 42.7% decrease in survival rates at 30°C compared with that at 0°C after 8h incubation on MA and 14.0% after 24h. There was no similar trend of reduced spore survival with high or low relative humidities. More spores survived exposure to the lowest relative humidity range than any other but fewest spores survived in the medium high range (83-92%) at both germination incubation periods. There was a consistent reduction of spore survival with increasing length of treatment as assessed after both eight and 24h incubation on MA. There was approximately 35% (8h) and 20% (24h) decrease in survival rates after eight days compared with that after two days of treatment. Overall percentage spore germination was higher on MA after 24h (85.6%) than after eight hours (67.2%).

The data in Table 4-2 shows spore survival at each relative humidity/duration combination grouped by temperature. The 20°C treatments were carried out first and on the basis of the results obtained it was decided to add an eight day duration and to assess spore survival for 24h as well as for eight hours for the remainder of the experiment.

Table 4-1. SUMMARY OF *B. cinerea* CONIDIAL SURVIVAL AT DIFFERENT TEMPERATURES AND HUMIDITIES AS ASSESSED BY SUBSEQUENT GERMINATION ON MA.

Environment	Lsmeans ± SE Percentage germination	
Temperature (°C) (*P < 0.001*)	8h on MA	24h on MA
0	86.9 ± 1.6	89.6 ± 1.4
10	82.2 ± 1.6	92.6 ± 1.4
15	61.8 ± 1.6	90.5 ± 1.4
20	73.6 ± 1.9	-
25	48.5 ± 1.7	78.4 ± 1.5
30	49.8 ± 1.6	77.0 ± 1.4
Relative humidity (%) (*P < 0.001*)		
High (90 - 100)	66.2 ± 1.3	88.8 ± 1.2
Medium high (83 - 92)	61.0 ± 1.4	77.3 ± 1.3
Medium low (70 - 79)	66.9 ± 1.3	86.1 ± 1.3
Low (40 - 47)	74.6 ± 1.3	90.3 ± 1.3
Time (days) (*P < 0.001*)		
2	79.4 ± 1.3	93.5 ± 1.2
4	73.2 ± 1.3	88.0 ± 1.3
6	64.5 ± 1.3	86.2 ± 1.2
8	51.6 ± 1.5	74.8 ± 1.3
Incubation on MA (h) (*P < 0.001*)		
	67.2 ± 0.7	85.6 ± 0.6

P values after Arcsin transformation.
- = Missing data.

Table 4-2. SURVIVAL OF *B. cinerea* CONIDIA AS INDICATED BY GERMINATION ON MA AFTER 8h OR 24h FOLLOWING INCUBATION AT VARIOUS COMBINATION OF RELATIVE HUMIDITIES AND TEMPERATURES FOR DIFFERENT PERIODS OF TIME ($P < 0.001$)[z].

Temp. (°C)	Relative Humidity (%)	Duration of treatment (days)	Lsmeans Percentage germination			8h as % of 24h
			Incubation (h)		*t-test* 8 vs 24h	
			8	24		
0	High (100)	2	49.0	100.0	***	49.0
		4	96.4	99.0	*NS*	97.3
		6	98.6	100.0	*NS*	98.6
		8	97.2	98.2	*NS*	98.9
	Medium high (89-92)	2	88.0	94.2	*NS*	93.4
		4	82.8	84.5	*NS*	98.0
		6	55.6	57.0	*NS*	97.5
		8	39.3	77.1	*NS*	50.9
	Medium low (73-75)	2	97.3	98.2	*NS*	99.0
		4	95.3	100.0	*NS*	95.3
		6	97.9	98.7	*NS*	99.1
		8	95.8	98.5	*NS*	97.2
	Low (40-45)	2	69.5	89.6	*NS*	77.5
		4	88.1	96.6	*NS*	91.2
		6	91.6	99.5	*NS*	92.0
		8	93.7	94.9	*NS*	98.7
10	High (97-98)	2	90.1	97.9	*NS*	92.0
		4	93.5	93.7	*NS*	99.7
		6	76.3	96.0	*NS*	79.4
		8	88.5	100.0	*	88.5
	Medium high (86-90)	2	92.0	96.2	*NS*	95.6
		4	77.9	91.1	*NS*	85.5
		6	79.5	87.1	*NS*	91.2
		8	48.1	86.2	*	55.8
	Medium low (60-69)	2	82.0	100.0	***	82.0
		4	87.4	92.9	*NS*	94.0
		6	92.9	94.6	*NS*	98.2
		8	48.7	87.6	*NS*	55.2
	Low (45-48)	2	98.1	100.0	*NS*	98.1
		4	90.5	95.8	*NS*	94.4
		6	65.2	97.2	*	67.0
		8	80.1	87.1	*NS*	91.9

[z] = *P* values, after Arcsin transformation.
NS, *, **, *** = Not significant or significant $P < 0.05$, *0.01* or *0.001* respectively.

Table 4-2 (Cont.)

Temp. (°C)	Relative Humidity (%)	Duration of treatment (days)	Lsmeans Percentage germination			8h as % of 24h
			Incubation (h)		*t-test* 8 vs 24h	
			8	24		
15	High (100)	2	56.3	90.0	*NS*	62.6
		4	68.9	100.0	***	68.9
		6	67.8	67.8	*NS*	75.2
		8	58.1	90.3	*NS*	64.3
	Medium high (83-84)	2	54.6	95.1	*	57.4
		4	75.4	83.3	*NS*	90.5
		6	59.0	84.1	*NS*	70.1
		8	38.6	70.5	*NS*	54.8
	Medium low (75-77)	2	56.2	95.8	*	58.7
		4	76.3	90.1	*NS*	84.6
		6	66.6	100.0	***	66.6
		8	55.2	100.0	***	55.2
	Low (45-48)	2	46.7	92.1	*	50.7
		4	91.5	97.9	*NS*	93.5
		6	46.6	95.9	**	48.6
		8	71.4	73.1	*NS*	97.6
20	High (90-95)	2	89.8	-		
		4	57.8	-		
		6	73.8	-		
	Medium high (84-86)	2	97.2	-		
		4	73.1	-		
		6	56.0	-		
	Medium low (75-77)	2	91.6	-		
		4	96.2	-		
		6	92.0	-		
	Low (44-45)	2	74.2	-		
		4	81.6	-		
		6	62.7	-		

NS, *, **, *** = Not significant or significant $P < 0.05$, 0.01 or 0.001 respectively.
- = Missing data.

Table 4-2 (Cont.)

Temp. (°C)	Relative Humidity (%)	Duration of treatment (days)	Lsmeans Percentage germination			8h as % of 24h
			Incubation (h)		*t-test* 8 vs 24h	
			8	24		
25	High (98-100)	2	92.3	100.0	*NS*	92.3
		4	66.5	83.5	*NS*	79.6
		6	15.1	81.4	*	18.5
		8	7.4	81.1	**	9.1
	Medium high (88-90)	2	58.0	94.1	*	61.6
		4	54.0	61.8	*NS*	87.3
		6	4.1	67.5	**	6.0
		8	1.6	74.5	***	2.1
	Medium low (70-72)	2	79.5	85.2	*NS*	93.3
		4	67.3	85.2	*NS*	78.9
		6	58.0	58.1	*NS*	99.8
		8	5.4	26.7	*	20.3
	Low (45-49)	2	84.9	89.9	*NS*	94.4
		4	54.8	100.0	***	54.8
		6	80.6	93.0	*NS*	86.7
		8	12.1	43.0	*NS*	28.1
30	High (98-100)	2	86.5	91.7	*NS*	94.3
		4	21.0	79.4	*NS*	26.4
		6	36.6	87.8	**	41.6
		8	23.6	23.6	*NS*	100.0
	Medium high (87-89)	2	81.3	93.4	*NS*	87.0
		4	42.4	58.3	*NS*	72.7
		6	50.3	93.1	*	54.0
		8	15.6	73.0	*NS*	21.3
	Medium low (74-79)	2	81.1	84.7	*NS*	85.7
		4	20.2	95.3	***	21.2
		6	2.1	73.5	***	2.8
		8	0.6	40.3	*NS*	1.5
	Low (45-47)	2	93.8	97.9	*NS*	95.8
		4	66.2	95.1	*NS*	69.6
		6	87.2	89.1	*NS*	97.9
		8	63.5	79.9	*NS*	79.5

*NS, *, **, **** = Not significant or significant $P < 0.05$, *0.01* or *0.001* respectively.

After treatment at 0°C or at 10°C, spore survival was high and most spores germinated quickly within eight hours on MA irrespective of the test relative humidity. Treatment at 15°C did not have a marked effect on survival but spores were slower to germinate as shown by the difference in percent germination after eight and 24h. The available data for 20°C is similar to that for 15°C but after treatment at 20 or 30°C there was a decrease in overall germination, especially after 8 days of treatment, and spores were slower to germinate. Of particular note is the very slow germination of spores exposed to the high and medium high humidities at 25°C and to the medium high and medium low humidities at 30°C.

DISCUSSION

Conidia of *B. cinerea* are usually considered to be short-lived although there is evidence that they can survive for some time (Coley-Smith 1980). In substrates such as soil, they are subject not only to the influence of temperature and humidity but also to fungistasis (Ellerbrock & Lorbeer 1977) and to attack by soil microorganisms such as amoeba (Coley-Smith 1980). The effect of temperature and humidity in this type of environment could be either a direct effect on the *B. cinerea* itself or an indirect effect on the general soil microbial population and its interaction with *Botrytis*. The infection court on a kiwifruit is the stem scar where the fruit has been snapped from the pedicel. This site is 'virgin ground' when first exposed and spores deposited on it afterwards, for example in the picking bag, would not encounter a well established microbial community. The effect of the physical environment at this time would therefore probably be primarily to the host, to the pathogen and to their interactions.

In this present study *B. cinerea* conidia survived exposure to a very wide range of temperatures (0 to 30°C) and relative humidities (40-100%) for the eight days of the experiment. Spores of *B. cinerea* do not germinate on glass slides but could well do so on kiwifruit stem scars during the curing

period when the surface of the scar could provide a suitable, moist substrate.

High temperatures can be used to kill fungal spores but there may be a small margin between the heat treatment required to kill the pathogen and that which damages the host. Choi *et al.* (1990) found that cucumber grey mould disease did not develop at temperatures greater than 25°C but Coley-Smith (1980) has shown that conidia of *B. cinerea* can survive for a short time at temperatures of 33-50°C. Temperatures of this magnitude are unlikely to be used for curing kiwifruit because of possible adverse effects on the fruit (Chea *et al.* 1993b)

It is generally recognised that there is a marked effect of temperature on survival of fungal spores. For example, Teitell (1958) found that survival of *Aspergillus flavus* decreased with increasing temperature in the range 29-48°C. Lidell & Burgess (1985) showed that microconidia of *Fusarium moniliforme* Sheldon can survive for extremely long times in soil at 5°C and 75% relative humidity (at least 900 days). At higher temperatures (25°C and 35°C), survival was reduced to less than 70 days - a period still well in excess of those that would be used to cure kiwifruit for resistance to *B. cinerea*. Ellerbrock & Lorbeer (1977) studied the survival of conidia of *B. squamosa* in soil. They found that conidia could be detected in field plots up to two months after infestation. If infested soil was held in the laboratory at 30°C then nearly half had lost viability within one day and almost all were non-viable by 10 days. At 21°C, one third were still viable after 10 days and at 3°C two thirds remained viable. Alternating moist and dry conditions dramatically reduced survival - after six days no conidia had survived alternating wet and dry conditions whereas over half had survived continuously dry or continuously wet conditions. McLaughlin & True (1952) evaluated (using agar plates) the longevity of *Chalara quercina* previously incubated at various temperatures and relative humidities on glass beads or in oak twigs. They found that fewer conidia survived at high temperatures

(25°C and 30°C) than at 10°C or at 20°C where conidia survived for 111 and 173 days respectively. They also reported that conidia on glass slides survived longer than those in plant tissue. Merek & Fergus (1954) evaluated the survival of endoconidia (on glass beads) and ascospores (on glass cover slips) of *Endoconidiophora fagacearum* Brets exposed to temperature and humidity ranges of 3-37°C and 95-100% respectively. The lower the relative humidity and temperature, the greater the survival of these fungal structures - 173 days for endoconidia at 3-9°C and 232 days for ascospores. Wetting the ascospores halved their longevity. In view of these results the possibility that conidia of *B. cinerea* would not survive on kiwifruit stem scars as long as on cover glasses must be borne in mind when interpreting the results obtained in the current study. Nevertheless there is a clear indication from these present results that with an increase in temperature from 0 to 30°C and duration of treatment from two to eight days of exposure, *B. cinerea* spore survival decreased.

The relationship between relative humidity and fungal spore survival is not as simple as that between temperature and survival (Teitell 1958). While endoconidia of *Endoconidiophora fagacearum* survived best under dry conditions, at 12-24°C the ascospores remained viable longer at 95% relative humidity than at 75% (Merek & Fergus 1954). Coley-Smith (1980) showed that urediniospores of *Melampsora lini* remained viable for longer at 40-60% relative humidity than at 20 or 80%. Teitell (1958) found a small band of relative humidity around 75% that reduced viability of *Aspergillus flavus* and of *A. terreus* to zero after one month. In the case of *A. flavus*, at 29°C, viability dropped from 90% to 8% within six days, a time period comparable with that used in this work on *B. cinerea*, and to 0 after 13 days. Viability of spores of *B. cinerea* held for six days at 30°C and 74-79% relative humidity dropped from 100% to 73.5% when viability was assessed after 24h incubation on MA. However, the speed of germination was also affected and only 2.1% of the spores had germinated when examined after 8h incubation on MA. Teitell assessed germination after one incubation time

only (9-14h) on potato dextrose agar and would not have been able to assess speed of germination. His assessment time of 9-14h was intermediate between the two (8h and 24h) used in the current work and the survival rate of *A. flavus* (8%) was intermediate between those found for *B. cinerea* (2.1 and 73.5%). After eight days (the maximum tested) the figures for *B. cinerea* were 0.6 and 40.3. It would appear likely that survival of conidia of *B. cinerea* and of *A. flavus* is affected to a similar extent by relative humidity.

The effect of temperature and humidity on fungal spore germination is quite well documented and a few key issues will be summarised here.

Dennis & Cohen (1976) working with various strains of *B. cinerea* observed different rates of germination and mycelial growth on potato dextrose agar over a temperature ranges of 0-25°C. Percentage germination increased up to 95% as temperature increased. Studies carried out by Yoder & Whalen (1975) found a slow rate of growth at temperatures below 15°C or above 25°C in two strains of *B. cinerea*.

Temperatures greater than those tested in the present study have shown a similar tendency for reduced spore germination (*in vitro*) with increased temperature. Kramer & Eversmeyer (1992) recorded different rates of germination of urediniospores of *P. recondita* and *P. graminis* on water agar which varied to the previous incubation temperature. They found no germination of either *P. recondita* or *P. graminis* at 2°C for the first 4h of incubation but after 17h germination was 98% and 80%. In both species germination was about 98% after 2h incubation at 6°C to 28°C but at 31°C 47% had germinated after 4h and there was no further germination after 17h. There was no germination at 35°C or above.

Greater spore germination with increased temperature has been reported by Estrada *et al.* (1993) for two isolates *C. gloesporoides*. They found a

higher germination from spores incubated on cellulose membranes for about 12h at 25 or 30°C than from those incubated at 20°C. Similarly Van Roermund *et al.* (1984) concluded that germination of *Zoophthora radicans* on different media (Sabourad's maltose yeast extract agar and distilled water agar) was greatly influenced by temperature together with other external factors such as media, light and pH. They found that spores of this fungus when incubated at 0 to 36°C could germinate but no germination took place at a very high (40°C) temperature. They also observed that germ-tubes did not develop at temperatures below 24°C.

Inhibition of *B. cinerea* germination on MA following a low temperature period did not occur in this present study as has been reported in many other fungi. Matsumoto & Sommer (1967) reported a direct effect between spore germination of *R. stolonifer* on potato-dextrose broth (PDB) with low temperatures (-1, 0, 2.5 and 5°C) and time of incubation on spore survival. They reported that spore germination during incubation at 25°C was less than 5% when they were previously held at 0°C for six days compared with a 10% germination at 2.5°C. In that study they also reported that spores of *R. stolonifer* gradually decreased from 100 to 5% with increased incubation period at 0°C from 0 to 48h.

Diversity in results on *in vitro* studies of exposure periods to different temperatures among other fungal species have also been reported by Wells & Forbes (1967); Zentmyer *et al.* (1976); Beckman & Payne (1983) and Phillips & Weste (1985). These authors working on spore germination of *G. cingulata,* germ tube elongation and spore germination of *C. zea-maydis* and colony diameters of various strains of *P. cinnamomi* respectively, found in these fungi a broad optimum temperatures of growth according to time of exposure.

Couey & Uota (1961) have studied the effect of relative humidity on spore germination of *B. cinerea* on cold gelatin. They found that conidial

germination was greatly influenced by the combinations of relative humidity, $S0_2$ concentration and time of exposure. In their study, relative humidities of 93 to 96% and SO_2 concentrations of 100ppm for 0 to 12 min reduced germination by about 24%. Percentage germination gradually increased with a decrease in relative humidity and/or increase in SO_2 concentration i.e. 87% spores germinated when incubated in a relative humidity of 19-20% and SO_2 concentration of 6800 ppm for eight hours compared with 39% in a relative humidity of 93-96% and SO_2 concentration of 100 ppm.

Estrada *et al.* (1993) working with two isolates of *C. gloesporoides* (I_2 and I_4) showed that germination of both isolates took place at relative humidities of 97.5 and 100% after 20h incubation while only 11% (*C. gloesporoides* I_2) and 21% (*C. gloesporoides* I_4) of conidia were able to produce germ tubes after incubation at 95% relative humidity for 30h. They also reported that at relative humidities of 39% for one week no germination of *C. gloesporoides* occurred with either isolates. Germination was significantly delayed when spores were incubated at relative humidities at 62 and 86%. for one to two weeks.

In the present study germination of *B. cinerea* conidia increased when spores were held to 24h on MA following the treatment period. Good & Zathureczky (1967) observed that spores of *B. cinerea* germinated 100% on moist dialysing membranes while *Cercospora musae* and *Monilinia fructicola* germinated about 83-93% after periods of drying for seven, 12 or 19 hours. A second period of drying showed that spore survived was 87-90% for *B. cinerea*, 67-88% for *C. musae* and 61-76% for *M. fructicola*. They reported that spore rehydration occurred in some minutes when placed on diluted juice agar or in a saturated atmosphere for one hour.

The effects of combinations of temperature/relative humidity/time of treatment on germination of *B. cinerea*, *Sclerotium sclerotia* and *Aspergillus* spp. has been reported by others. Van den Berg & Lentz (1968) studying

environmental effects on various strains of *B. cinerea* and *S. sclerotium* germination found that survival of *Botrytis* strains at 0°C and 20°C decreased as relative humidity decreased. They also reported survival of *B. cinerea* conidia for more than one year when they were stored at 0°C, 90-100% relative humidity. Rewal & Grewal (1989), reported that three different strains of *B. cinerea* conidia (B_1, B_4 or B_5) showed differences in germination on filter papers according to incubation temperature, relative humidity and type of strain. For example, they found the highest conidial germination at 20°C compared with no germination at 0°C or 35°C regardless of type of strain. The minimum for germination of B_1 was 93% of B_2 was 81% and of B_5 was 75%.

An environment of 95% relative humidity at 25°C was required for the conidial growth and appressorium formation of *C. zea-maydis.* In that study, continuous relative humidities of 60%, 70%, 80% and 90% for 6, 8, 10 and 15 days respectively, did not reduce the conidial viability (Thorson & Martinson 1993).

The isolate K3 of *B. cinerea* used in this experiment and in subsequent work can survive under a wide range of environmental conditions and percentage spore germination is unlikely to be severely reduced by the temperature and humidity conditions likely to be used for curing kiwifruit. However, the infectivity could be reduced and a slower spore germination would give more time for effective defense mechanisms to develop and thus affect the probability of successful infections taking place.

CHAPTER FIVE

CURING OF KIWIFRUIT TO CONTROL *BOTRYTIS CINEREA* DURING STORAGE.

INTRODUCTION

The curing process in fruits and vegetables involves several complex events including physiological, anatomical and histochemical mechanisms outlined in the *General Introduction*.

Physiological considerations of curing

It has long been recognized that temperature and relative humidity can affect commodity quality. Weight loss, ethylene production, rate of respiration, firmness and soluble solids are among some of the factors that can be greatly influenced by these two factors not only during the time of exposure but also during the subsequent storage life of the commodity. (Bourne 1982; Mitchell 1986; Amand & Randle 1989; Miller *et al*. 1990; Yano & Hasegawa 1992; 1993a; 1993b; Sentana *et al.* 1993).

Picha (1986b) reported a higher rate of weight loss during a ten day curing period than in the subsequent 20 weeks of coolstorage of 6 cultivars of sweet potato (*Ipomea batatas*). Transpiration was the major cause of overall weight loss during curing but respiration was more important in the latter part of coolstorage. In lemons previously held at 2, 5, 8 or 14°C for several weeks, the rate of respiration increased as temperature increased and there was a low ethylene production at all temperatures (Cohen and Schiffmann-Nadel 1978).

Studies on caladium tubers (Marousky & Harbaugh 1976) showed that weight loss during storage at 24°C for eight weeks of tubers cured at low

relative humidities (30-45% and 60-75%) was higher than with those previously cured at 90-100% relative humidity. Mbonomo & Brecht (1991) reported that in cormels, curing temperatures (25, 30, 35 or 40°C) and 95-100% relative humidity for seven days, affected weight loss both during curing and subsequent storage. They reported a direct relation between curing temperature and weight loss during this conditioning period while after four weeks storage at 25°C and 75% relative humidity those cormels cured at 25 or 40°C showed higher weight loss than those cured at 30 or 35°C. They concluded that cured cormels showed 10.8% less weight loss than those uncured. Morris *et al.* (1989) measured weight loss of potatoes during curing at a range of temperatures from 10 to 30°C and of relative humidities from 50 to 98%. Weight loss was inversely related to relative humidity. They also concluded that the best curing temperature/relative humidity combination was 25°C and 98% although weight loss increased more than 50% at temperatures above 10°C. Studies on sweetpotatoes concluded that weight loss during storage at 25°C and 70% relative humidity was affected by curing i.e less weight loss was observed in those cured (30°C and 95-100% relative humidity) and wrapped than in the not cured (Delate *et al.* 1985). Other external factors such as soil temperature at harvest, degree of dissection, bruising and peeling affected weight loss as well (Walter *et al.* 1988; 1989).

Lurie & Klein (1991) observed that ethylene production in tomatoes incubated at 36, 38 or 40°C was lower after three days incubation, compared with the first day incubation. Atta-Aly (1992) reported that maximum ethylene production in greenhouse tomatoes was registered at 20°C, declining with increasing temperatures up to 30°C. Maxie *et al.* (1974) reported similar tendency in pears incubated at 20, 30 or 40°C. In that study, ethylene production was inhibited when fruit were incubated at 30 or 40°C.

The combination of some physiological factors such as ethylene

concentration and carbon dioxide production on fruit quality has also been studied. Metzidakis & Sfakiotakis (1988) reported that firmness of avocado was influenced not only by storage temperature but also by carbon dioxide concentration and internal ethylene. Similarly An & Paull (1990) reported differences in papaya fruit firmness according to incubation temperature and exogenous application of ethylene. They reported that fruit incubated at temperatures of up to 30°C showed abnormal softening, while applications of ethylene (≈100 $\mu l \cdot l^{-1}$) improved fruit quality.

Curing may improve the physiological quality of stored fruit. Fruit of four apple cultivars pretreated for two or four days at 40°C were firmer after 2-4 weeks storage at 21°C than untreated fruit (Liu 1978). Klein & Lurie (1990; 1992) also found apple fruit were firmer after heat treatment whether stored at 0°C or at 20°C.

There is limited information about the physiological implications of a curing period on subsequent storage of kiwifruit. In some preliminary work Beever (1991), reported a high percentage weight loss and a rapid initial loss of firmness when coolstorage of kiwifruit was delayed after harvest.

Because of the importance of quality for the sweetpotato and potato processing industry, the carbohydrate content of these commodities has been widely investigated not only at harvest but also during curing and subsequent storage. In studies on raw sweetpotato carried out by Lambou (1958) sucrose was the main component of the solid solubles not only at harvest but also during the curing (30°C, 80-85% RH for 10 days) and storage periods (15°C for six months). Morris & Mann (1955) obtained similar results for this commodity when they evaluated three different methods of curing (warm-house, field pile and storage house) at a range of temperatures (4.4, 10 and 26.6°C) and relative humidities during curing (30-80%) and subsequent storage (70-90%). However, Walter (1992) reported that curing of sweetpotatoes at 29.5°C, and 90-95% relative humidity for

seven days did not increase the sugar content compared with those non-cured and stored at 14.5°C, 85% relative humidity for 8, 15, 29 and 57 days. This researcher found that differences in sugar content were associated with cultivar rather than with curing. This researcher also reported that sucrose was the major sugar in all cultivars, with glucose and fructose present in lower amounts. However, Picha (1986a) found that the content of sucrose was only high at harvest while fructose and glucose were the main sugars detected in sweetpotatoes when previously cured for 10 days at 30°C, 90% relative humidity and then stored at 15.5°C, 90% relative humidity for four to 46 weeks. Picha (1986a) also found that differences in the amount of sugars associated with different variety but that, in general, sugars increased during both curing and storage periods. Previous studies carried out by McCombs & Pope (1958) did not find changes in the amount of sweetpotato glucose with curing treatment (four or 10 days), but they reported higher glucose content during storage at 12°C in those cultivars cured for 10 days at 30°C than those stored at temperatures of 15.5 and 18°C. Differences in sugar content of different cultivars have also been reported by Sistrunk *et al.* (1953); Scott & Mathews (1957) and Hammet (1961). They concluded that there were general distinct varietal differences in sugar content and that levels changed at harvest, during curing and during storage.

In further research, the effect of external factors such as ethylene exposure on the quality of sweetpotatoes has also been evaluated. In those studies, it has been reported that ethylene exposures in cured or non-cured tubers of two cultivars of sweetpotatoes influenced sugar content (Chegeh & Picha 1993). They found that both sucrose and total sugars increased with increasing ethylene (0, 1, 10, 100 and 1000 ppm) for 15 days at 21°C, but there was no effect on fructose, glucose or maltose concentration.

Samotus *et al.* (1974) studied the effect of storage temperatures, varieties strains and seasonal variations on reducing sugar accumulation in potatoes.

They found that storage at 6°C (after curing at 15-20°C) did not enhance the levels of reducing sugars as occurred if potatoes were stored at the lower temperatures of 1 and 2°C. They also found that glucose and sucrose were the main reducing sugars compared with the non-reducing sugar sucrose. In that study accumulation of sugars depended on the variety, period of low temperature storage and year of production. Dogras *et al.* (1989) also reported higher reducing sugars in potatoes (cured at 15-16°C for one week) stored at a low (6°C) than at higher (10°C) temperature. Similarly they found differences in sugar concentrations both between, cultivars and between growing regions. Further work to investigate storage temperatures and sugar accumulation was carried out by Cottrel *et al.* (1993) who found that concentrations of glucose and fructose in potatoes stored at 4°C for 3 to 139 days were significantly higher than in those stored at 10°C. They also found differences in sugar levels between cultivars.

Nagao *et al.* (1991) studied the effects of curing squash (*Cucurbita maxima* Duch.) at 25°C for 12 days and subsequent storage temperatures of 7.5, 10, 12.5 and 15°C for 12 to 100 days. They reported that reducing sugar content increased more during storage temperatures, although apparently there was no differences in sugar content among storage temperatures. In that study they also reported that the best curing temperature to increase sugars was 30°C compared to those temperatures at 20 or 25°C.

In other commodities such as red beet, parsnip and carrots although no curing practice was carried out, the effects of long-term storage on the chemical composition has also been studied. Phan *et al.* (1973) reported that in raw carrots reducing sugars fluctuated throughout the storage period, (1°C and 98% relative humidity) for a period of time. They found that after eight weeks storage reducing sugars decreased and increased again between 14 and 18 weeks storage. Rutherford (1977) reported differences in glucose and fructose contents of both red beet and parsnips stored at

4°C for six to seven months. In that study no differences were found in sugar content when the whole plant root of either commodity was analyzed, but in the parsnip root dissected plant roots were analyzed and a higher content of sugars, (about 84%) was found in the stele than in the cortex. Shattuck *et al.* (1991) working with field grown parsnip roots stored at 0°C and 93% relative humidity, reported that in parsnips stored at 0°C for two and four weeks the concentration of both fructose and glucose decreased during the coolstorage period.

Control of postharvest diseases by curing

One of the main objectives of the curing practice is to control postharvest diseases during commodity storage, hence the importance of studies associated with effects of temperature and/or relative humidity on infection levels. For example, the influence of various temperature ranges on infections caused by pathogens such as those caused by *B. cinerea*, *A. brassicae*, *R. stolonipher*, *Mucor piriformis* has been widely reported. Pierson (1965) observed reduction of *Rhizopus* rots of peaches of about 10% or 60% when held at 0°C for 7 or 14 days respectively while Spotts & Chen (1987) observed significant reduction of storage rots such as *P. malorum*, *P. expansum*, *M. piriformes* and *B. cinerea* on pears held at 21-38°C for 1-7 days. In tomatoes, the pathogenicity of various isolates of *Fusarium equiseti*, *F. chlamydosporum*, *A. solani*, *G. candidum*, *Acremonium recifei* and others was affected by temperature and relative humidity (Oladiran & Iwu 1993). They reported that the optimum temperature for maximum fruit rot by all isolates tested was between 30 to 35°C compared with the lower rot development at 5 or 10°C. Likewise, the optimum relative humidity to for rots to develop was in the range of 70% to 90% rather than 50% to 60%. In other commodities such as cape gooseberry (*Physalis peruviana* L.), it was reported that increasing the storage duration from 15 to 33 days and increasing temperatures from 0°C to 7°C, increased storage rots caused by *Alternaria*, *Botrytis*, *Cladosporium* and *Penicillium* spp. following storage at 18°C for seven days (Lizana &

Espina 1993).

There is general agreement that curing and film wrapping gives a significant reduction of citrus storage rots such as those caused by *P. digitatum* Sacc., *P. italicum*, *A. citri* and *G. candidum* during fruit storage (Miller *et al.* 1987; Chun *et al.* 1988; Waks *et al.* 1988; Ben-Yehoshua *et al.* 1987a 1987b; 1988a; 1988b; 1989;). Curing condition varied from one citrus species to another but in general, the best curing conditions were achieved with temperatures in the range of 30-34°C and relative humidities of 95% for about three days with subsequent storage at 12°C.

Nagao *et al.* (1991), reported that during storage at 10°C squash fruit developed fewer rots when previously cured at 30°C for 16 days compared with lower temperatures of 20 or 25°C.

OBJECTIVE

To define the environmental conditions that are required to minimize *Botrytis* rot in kiwifruit during a curing and to determine the principal physiological responses of fruit to this preconditioning practice and to the subsequent effects on fruit quality during storage.

MATERIALS AND METHODS

Experiment No.1

Title: Curing temperatures, physiological changes and incidence of *B. cinerea* stem-end rot during subsequent coolstorage in 1992.

Fruit harvesting

Fruit were harvested from the Massey University Fruit Crops Unit on 11th

May 1992. Temperature and relative humidity in the orchard at harvest were 15°C and 85% RH respectively. The initial total soluble solids and firmness of fruit were 10.8% and 6.6 kgf.

Inoculum

Inoculum of *B. cinerea* (K3) was applied to the kiwifruit stem scars as 17 µl droplets each containing 125000 spores (equivalent to 7.4 x 10^6 spores/ml).

Preparation of treatments

The experiment was carried out in incubator rooms at temperatures of 10°C ± 1, 20°C ± 2 or 30°C ± 1. Inoculated fruit were placed in commercial kiwifruit trays stem scars uppermost. Five trays were exposed to each temperature for each of the three incubation periods. A further five inoculated and uninoculated trays of fruit were closed and placed in the coolroom at 0°C as per normal commercial practice to evaluate natural and artificially induced levels of infection. Physiological changes in inoculated fruit was also evaluated and only percentage disease from uninoculated trays.

Assessments

Healthy fruit were selected from each temperature and curing period to evaluate the following physiological parameters: Weight loss (%), ethylene production (C_2H_4) ($\mu l \cdot kg^{-1} \cdot h^{-1}$) and carbon dioxide production (CO_2) ($cm^3 \cdot kg^{-1} \cdot h^{-1}$), total soluble solids (%TSS) and firmness (kgf) were the parameters assessed.

Biochemical assays

Five fruit previously cured at each temperature were taken for analysis of pH, titratable acidity (TTA-calculated as citric acid equivalent), sucrose, fructose and glucose content.

pH and Titratable acidity (TTA)

pH and TTA were measured using a Mettler DL21 auto-titrator. One ml of fruit juice mixed with 40 ml of distilled water was titrated against 0.1M sodium hydroxide (NaOH) to an endpoint of pH 8.2 (Shusiri 1992).

Analysis of sugars

Aliquots of the same batches of juice used for pH and titratable acidity measurements were used to prepare ethanol extracts for separation and estimation of sugars by high pressure liquid chromatography (HPLC) (Pesis *et al.* 1991). The aliquots were mixed with 95% ethanol (1:5 v/v), heated to boiling in a microwave oven, filtered and stored at -20°C for 6 months. Glucose, fructose and sucrose were analyzed by HPLC equipped with a modified C18 column (ODS-224 220 x 4.6mm) and a Waters 490E UV/VIS Detector. Samples of 20 µl were injected and eluted isocratically with 0.013*N* sulphuric acid at a continuous increasing flow of 0.4-0.7 ml/min for 20 min, at 22°C and were detected at 210 nm. Results were expressed in mg/100ml juice.

Assessment period

At harvest 15 fruits were weighed and marked to follow weight loss of individual fruit during the curing period. Ethylene production, rate of respiration, TSS and firmness were also assessed in a further ten fruits. Similar assessments were carried out on ten fruits, on control inoculated fruit and on fruit cured for 2, 4 or 6 days at 10°C, for 2 or 4 days at 20°C and in fruit held for 2 days at 30°C. Similar evaluations in ten fruits for each combination temperature/curing period were carried out after 1, 3 and 6 months of coolstorage. Analyses of sucrose, glucose, fructose, pH and acidity in five fruits were also carried out for each combination of temperature/curing time during the first and third month of storage. Percentage of infection was evaluated after 12 weeks of storage at 0°C.

Statistical Analysis

Curing period data were analyzed separately for each temperature as a complete randomized design to test differences between curing periods for the physiological parameters (except ethylene) and infection levels. There were too many zeros in the ethylene production data to use analysis of variance so one and two sample t-tests were used for this parameter. Data from the storage period were analyzed as a nested design. Before analysis of variance a square root transformation was carried out for firmness data from the curing period and a log transformation for ethylene production, rate of respiration, sugars, pH and acidity from storage data.

Experiment No.2

Title: Curing temperature and incidence of *B. cinerea* stem-end rot of kiwifruit during subsequent coolstorage in 1994.

Fruit harvesting

Fruit were harvested from a commercial orchard near Levin (Lat S 40 37 Long E 175 17), on 16th, 23rd and 27th May 1994. Orchard temperature and relative humidity were 12°C and 78.5% for the first harvest, 11°C and 80% for the second and 14°C and 90.5% for the third. The initial total soluble solids and firmness for each harvest were 6.8% and 9.5kgf, 7.0% and 7.4kgf and 8.3% and 8.7kgf respectively.

Inoculum

The inoculum applied to the kiwifruit stem scar was 25000 spores in a 17 µl droplet (equivalent to 1.5 x 10^6 spores/ml).

Preparation of treatments

The experiment was carried out in control temperature rooms at 0°C, 5°C, 10°C, 15°C or 20°C. Inoculated fruit were placed in the commercial kiwifruit trays stem scars uppermost. To maintain a high relative humidity during the three day curing period trays were wrapped (but not sealed) in black

polyethylene bags. After three days of curing, commercial polyethylene liners were placed in the trays which were closed and stored at 0°C as per normal commercial practice. There were four replicate trays per treatment.

Assessments

Three trays per treatment were used to evaluate levels of infection after 12 weeks of storage and fruit from the fourth replicate were used to evaluate percentage weight loss every day during curing, to assess firmness at the end of curing and after 6 or 12 weeks coolstorage.

Statistical Analysis

Analysis of variance was performed on data using a completely randomized design. Before analysis of variance square root transformation was carried out for percentage infection at both storage periods and for all harvest dates. The experiment was repeated three times.

Experiment No. 3

Title: Relative humidity during curing and *B. cinerea* stem-end rot incidence in kiwifruit during subsequent coolstorage in 1993.

Fruit harvesting

Fruit were harvested from the Massey University Fruit Crop Unit on 23rd May and 4th June 1993. Orchard temperature was 15°C or 17°C respectively and relative humidity as 82-85% at both harvest periods. Initial total soluble solids and firmness at the first harvest were 8.8% and 9.1 kgf and from the second were 10.6% and 9.2 kgf respectively.

Inoculum

The inoculum was as described for *Experiment No.2.*

Treatment preparation

The experiments were carried out in a controlled temperature room at 10°C

and three relative humidities ranges were assessed: 40-59%, 60-80% and 92-97% corresponding to vapour pressure deficits (VPD) of 0.70-0.49, 0.48-0.24 and 0.09-0.03 Kpa respectively. Polythene pipes 2.20 m long x 15 cm wide were used as relative humidity chambers (Fig.5-1). A series of six holes (2.5 mm diameter) were drilled equidistant down the length of each pipe to allow access for relative humidity measurements. Each hole was blocked with a rubber bung that could be removed and replaced with a relative humidity probe inserted through another bung of the same external diameter. Commercial plastic plix trays were cut into single cup strips and stapled end to end to form chains 36 cups long. Fruits were placed stem scar uppermost in alternate cups, inoculated, and when dry, the entire string of cups was carefully pulled into one of the pipes using two, long wires as cords. The ends of the pipes were capped and connected to the tube from the inlet chamber at one end and to the outlet chamber at the other. Relative humidity of inlet, outlet and tube access points were checked every day over the three day curing period.

Assessments

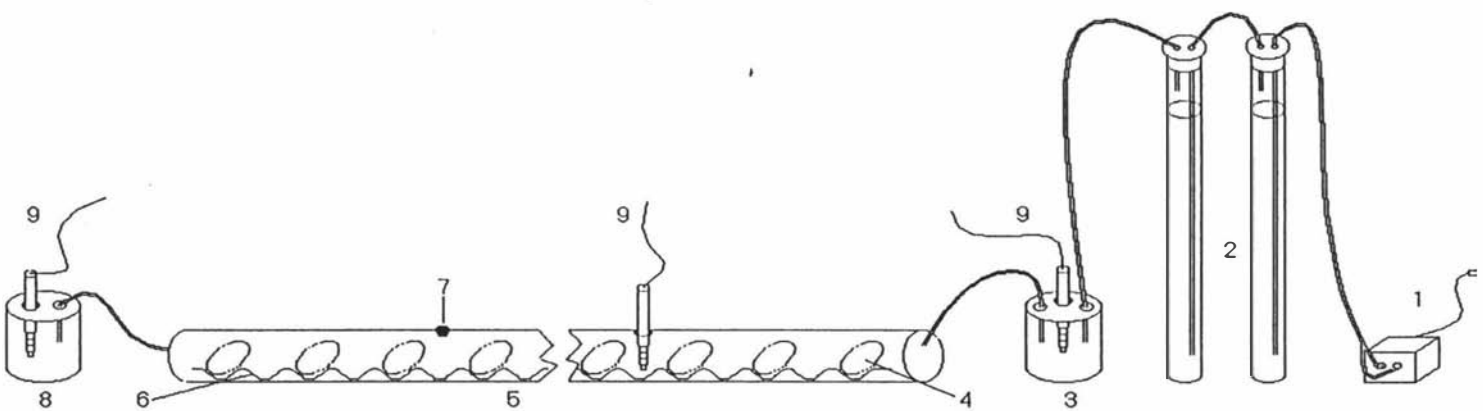
There were eight replicate pipes per treatment. Fruit from seven were packed and coolstored as per normal commercial practice and fruit from the eight was used for measurement of weight loss, TSS and firmness immediately after curing.

Statistical Analysis

The experiment was analyzed as a completely randomized design. Square root transformation was carried out before analysis of variance of percentage infection at both storage periods and the two harvest dates.

Experiment No. 4

Title: Relative humidity, type of inoculum and harvest maturity on incidence of *B. cinerea* stem-end rot in kiwifruit during subsequent coolstorage in 1994.



1.- Air pump
2.- Salt containers
3.- Jar (input)
4.- Kiwifruit
5.- Relative humidity chamber
6.- Plix
7.- Ruber bung
8.- Jar (output)
9.- Relative humidity probe

Figure 5-1. Relative humidity system.

Fruit harvesting

Fruit were harvested on four occasions from a commercial orchard at Levin. Harvest dates were the same as those of *Experiment No.2* but with an additional harvest on 1st June. The orchard temperature and relative humidity for this harvest were 10°C and 56% and the initial TSS and firmness were 9.2% and 8.1 kgf respectively.

Inoculum

Half the fruit were inoculated with a spore suspension prepared from 10-14 day old cultures as in *Experiments 2 and 3*. The remaining half were inoculated with dry conidia by touching a fine artist paint brush on a sporulating 12 day old culture of *B. cinerea* growing on MA and then touching the stem scar of a kiwifruit. An estimate of the inoculum deposited by this procedure was obtained by touching a glass slide in a similar manner to the stem scar and then counting the number of conidia. The mean of three attempts was equivalent to 6700 spores per stem scar.

Treatments and experimental procedure

The experiments were carried out at 10°C using three humidity ranges. Sodium chloride was replaced by potassium chloride to produce a middle humidity range that was higher than that in *Experiment 3*. The ranges achieved were 50-80%, 80-93% and 95-98% resulting in a range of vapour pressure deficits (VPD) of 0.59-0.24, 0.24-0.08 and 0.05-0.02 KPa respectively.

Subsequent packing, coolstorage and measurement of fruit parameters were as described for *Experiment 3*.

Statistical Analysis

The experiment was designed as a 4x3x2 factorial with seven replications per each relative humidity. Data from percentage weight loss and infection levels at both storage periods required square root transformation before

analysis of variance.

RESULTS

Experiment No.1

Physiological changes of fruit during curing and coolstorage in 1992

Temperature and time of curing greatly influenced most of the physiological parameters evaluated in this study.

Weight loss was greatest at the highest temperature (0.79%) and least at 0°C (Table 5-1). The differences in percentage weight loss of fruit held at 0, 10 or 20°C were highly significant (*P < 0.001*). Weight loss increased with time of curing at each temperature. Subsequently measurement of weight loss during storage was not possible because most of the fruit marked for this purpose showed symptoms of *Botrytis.*

Table 5-1. 1992: PERCENTAGE WEIGHT LOSS (MEAN ± SE) OF KIWIFRUIT CURED AT FOUR TEMPERATURES FOR UP TO SIX DAYS.

	Weight loss (%) (days)		
Treatment	2	4	6
Control Inoculated			
0°C *P < 0.001*	0.12 ± 0.01	0.29 ± 0.07	0.58 ± 0.06
10°C *P < 0.001*	0.38 ± 0.01	0.66 ± 0.03	1.10 ± 0.04
20°C *P < 0.001*	0.53 ± 0.01	1.05 ± 0.02	
30°C*	0.79 ± 0.02		

* Only mean and standard error were evaluated for this treatment.

After two days curing there was little difference in firmness between fruit held at 10, 20 or 30°C but those at 0°C were softer (Table 5-2). Firmness decreased with time of curing and the decrease over six days was greater (*P < 0.001*) in the inoculated control fruit than it was in fruit cured at 10°C (19%: *0.05*).

Table 5-2. 1992: FIRMNESS (MEAN ± SE) OF KIWIFRUIT CURED AT FOUR TEMPERATURES FOR UP TO SIX DAYS.

	Firmness (kgf) (days)[z]		
Treatment	2	4	6
Control Inoculated			
0°C *P < 0.001*	7.15 ± 0.54	6.74 ± 0.32	4.50 ± 0.24
10°C *P < 0.05*	8.05 ± 0.35	8.38 ± 0.39	6.53 ± 0.32
20°C *NS*	8.9 ± 0.61	7.71 ± 0.75	
30°C*	8.0 ± 0.30		

NS = No significant.

* Only mean and standard error were evaluated for this treatment.

[z] *P* values after square root transformation.

Within one month of coolstorage, firmness of fruit in all treatments including the inoculated controls, had diminished by about two thirds (Table 5-3). Firmness of fruit cured at 30°C for two days was less than the other temperatures after one, three and six months but there were no consistent differences between the remainder of the treatments. There was no further overall reduction in firmness between one month (mean = 1.92) and six months (mean = 2.02) of coolstorage.

Table 5-3. 1992: FIRMNESS (LSMEAN ± SE) OF KIWIFRUIT CURED FOR UP TO SIX DAYS THEN STORED AT 0°C FOR UP TO SIX MONTHS.

Temperature & Curing period (*P < 0.001*)	Storage Time (months)		
	1	3	6
	Firmness (kgf)		
10°C + 2 days	1.62 ± 0.05	2.12 ± 0.05	2.23 ± 0.05
4 days	2.42 ± 0.05	2.35 ± 0.05	2.13 ± 0.05
6 days	2.23 ± 0.05	2.47 ± 0.05	2.11 ± 0.05
20°C + 2 days	1.90 ± 0.05	2.12 ± 0.05	1.89 ± 0.05
4 days	2.38 ± 0.05	2.60 ± 0.05	2.03 ± 0.05
30°C + 2 days	0.90 ± 0.05	1.83 ± 0.05	1.77 ± 0.05
Control Inoculated 0°C*	2.02 ± 0.59	2.00 ± 0.03	1.98 ± 0.06

* Only means and standard error were evaluated for this treatment.

Table 5-4. 1992: ETHYLENE PRODUCTION (MEAN ± SE) OF KIWIFRUIT CURED AT FOUR TEMPERATURES FOR UP TO SIX DAYS.

	Ethylene production ($\mu l \cdot kg^{-1} \cdot h^{-1}$)[z] (days)		
Treatment	2	4	6
Control Inoculated 0°C *P < 0.001*	0.00	0.00	0.468 ± 0.045
10°C *P < 0.01*	0.00	0.29 ± 0.08	0.462 ± 0.020
20°C *NS*	0.00	1.095 ± 0.71	
30°C *NS*	0.295 ± 0.28		

NS = not significant.
[z] t-test analyses.
C_2H_4 at harvest = zero.

There was no ethylene production at harvest and after two days of curing only fruit held at 30°C produced ethylene (Table 5-4). After four days curing fruit held at 10°C and at 20°C produced ethylene but at 0°C it was six days before ethylene was detected. Ethylene production was significantly greater after six than after four days at 10°C ($P < 0.001$). After two and four days at 20°C and at 30°C respectively, the ethylene production was not significant. Although there was no significant difference between fruit held for two or for four days at 20°C, fruit from these treatments showed the highest levels of ethylene production. Highly significant differences ($P < 0.001$) in ethylene production were found during the subsequent coolstorage (Table 5-5).

Table 5-5. 1992: ETHYLENE PRODUCTION (LSMEAN ± SE) OF KIWIFRUIT CURED FOR UP TO SIX DAYS THEN STORED AT 0°C FOR UP TO SIX MONTHS.

Temperature & Curing period ($P < 0.001$)[z]	Storage Time (months)		
	1	3	6
	C_2H_4 ($\mu l \cdot kg^{-1} \cdot h^{-1}$)		
10°C + 2 days	1.122 ± 0.25	0.492 ± 0.25	0.157 ± 0.25
4 days	0.00	0.456 ± 0.25	0.257 ± 0.25
6 days	0.573 ± 0.25	0.260 ± 0.25	0.061 ± 0.25
20°C + 2 days	0.899 ± 0.25	0.032 ± 0.25	0.295 ± 0.25
4 days	0.384 ± 0.25	0.376 ± 0.25	0.261 ± 0.25
30°C + 2 days	0.359 ± 0.25	0.129 ± 0.25	0.959 ± 0.25
Control Inoculated 0°C*	0.00	0.00	3.383 ± 2.15

* Only means and standard error were evaluated for this treatment.
[z] *P* values after log transformation.

Ethylene production was not detected in fruit from the inoculated control until the six month assessment when it was greater than in any other treatment. Fruit from all treatments except those cured at 10°C for four days

produced ethylene within one month of coolstorage and production was detected in that treatment after three months. There was no consistent relationship between ethylene production, curing temperature and incubation time.

Respiration as measured by CO_2 production was greatest in fruit held at 0°C and decreased with an increase in curing temperature (Table 5-6).

Table 5-6. 1992: RATE OF RESPIRATION (MEAN ± SE) OF KIWIFRUIT CURED AT FOUR TEMPERATURES FOR UP TO SIX DAYS.

	CO_2 production ($cm^3 \cdot kg^{-1} \cdot h^{-1}$) (days)		
Treatment	2	4	6
Control Inoculated			
0°C	18.97 ± 0.78	27.84 ± 1.13	23.17 ± 1.25
P < 0.001			
10°C	15.03 ± 0.38	23.93 ± 1.46	26.86 ± 0.50
P < 0.001			
20°C	13.53 ± 0.42	14.54 ± 1.11	
NS			
30°C*	13.61 ± 0.23		

NS = not significant.

* Only mean and standard error were evaluated in this treatment.

CO_2 at harvest = 13.65.

Over a six day curing period the respiration rate continued to increase and fruit held at 10°C were respiring more after six days than fruit held at 0°C. Overall differences in respiration rate were highly significant (*P < 0.001*) (Table 5-7). After one month of coolstorage respiration rate of fruit from the

inoculated control were less than those from all treatments and 59% less than the treatment average. At three months coolstorage, fruit from the control and curing treatments were comparable and by six months fruit respiration from the control was 35.6% greater than those of the treatment averages.

Table 5-7. 1992: RATE OF RESPIRATION (LSMEAN ± SE) OF KIWIFRUIT CURED FOR UP TO SIX DAYS THEN STORED AT 0°C FOR UP TO SIX MONTHS.

Temperature & Curing period ($P < 0.001$)[z]	Storage Time (months)		
	1	3	6
	CO_2 production		
10°C + 2 days	22.14 ± 0.79	16.05 ± 0.79	14.78 ± 0.79
4 days	18.88 ± 0.79	17.17 ± 0.79	10.11 ± 0.79
6 days	16.92 ± 0.79	14.68 ± 0.79	16.46 ± 0.79
20°C + 2 days	18.94 ± 0.79	18.16 ± 0.79	14.08 ± 0.79
4 days	17.36 ± 0.79	16.17 ± 0.79	15.49 ± 0.79
30°C + 2 days	12.64 ± 0.79	17.02 ± 0.79	15.57 ± 0.79
Control Inoculated 0°C*	10.59 ± 0.31	16.26 ± 0.46	19.55 ± 1.35

* Only means and standard error were evaluated for this treatment.
[z] *P* values after log transformation.

Chemical composition of fruit

There was little effect of curing on total soluble solids at any temperature (Table 5-8). Overall differences in total soluble solids were significant at $P < 0.05$ (Table 5-9). TSS of fruit after one month coolstorage was higher than that immediately after curing but there was no consistent effect of curing temperature or of time of incubation on TSS during storage. As the period of storage increased, glucose and fructose content of fruit increased in 10 of the 12 treatments.

Table 5-8. 1992: TOTAL SOLUBLE SOLIDS (MEAN ± SE) OF KIWIFRUIT CURED AT FOUR TEMPERATURES FOR UP TO SIX DAYS.

	Total soluble solids (%) (days)		
Treatment	2	4	6
Control Inoculated 0°C *NS*	11.1 ± 1.38	11.8 ± 0.99	11.5 ± 1.13
10°C *NS*	10.6 ± 1.45	10.6 ± 1.64	11.4 ± 1.40
20°C *NS*	10.6 ± 1.67	11.4 ± 1.25	
30°C*	10.3 ± 1.66		

NS = not significant.
* Only mean and standard error were evaluated in this treatment.

Table 5-9. 1992: TOTAL SOLUBLE SOLIDS (LSMEAN ± SE) CONTENT OF KIWIFRUIT CURED FOR UP TO SIX DAYS THEN STORED AT 0°C FOR UP TO SIX MONTHS.

Temperature & Curing period (*P < 0.05*)	Storage Time (months)		
	1	3	6
	TSS (%)		
10°C + 2 days	12.66 ± 0.21	12.79 ± 0.21	12.78 ± 0.20
4 days	12.79 ± 0.20	12.37 ± 0.20	12.16 ± 0.20
6 days	12.57 ± 0.20	12.63 ± 0.20	11.93 ± 0.20
20°C + 2 days	13.29 ± 0.20	12.62 ± 0.20	12.43 ± 0.20
4 days	13.11 ± 0.21	13.01 ± 0.20	12.55 ± 0.21
30°C + 2 days	12.77 ± 0.20	12.48 ± 0.20	12.39 ± 0.20
Control Inoculated 0°C*	14.02 ± 0.59	14.05 ± 0.01	14.92 ± 0.32

* Only means and standard error were evaluated for this treatment.

Although the overall difference was not significant, sucrose content of fruit cured at 10°C increased significantly with storage time but this increase was not found in fruit cured at other temperatures. Fruit content of sucrose, glucose and fructose was generally lower in cured than in uncured fruit (Table 5-10). Fruit cured for two days at 30°C had lower sugar levels than those cured for two days at 20°C which in tum had lower levels than those cured for two days at 10°C. A similar comparison can be made between fruit cured at 20°C and at 10°C for four days. There was an inverse relationship between duration of curing and fruit sugar content. Only the data for the sucrose treatment was significant overall at $P < 0.001$.

pH values of fruit were lower after three months storage than after one month (Table 5-11). Although significant differences ($P < 0.001$) were observed, in general pH remained relatively constant in a range of 3.3 to 4.0 in fruit from all the treatments at both storage assessments. Fruit acidity evaluated as a citric acid equivalence increased as the coolstorage period increased. Overall significant differences were $P < 0.001$. There was no consistent relationship between content of citric acid and curing temperature/incubation time.

Infection levels during coolstorage

Fruit held at 20°C for six days and at 30°C for up to two days showed a rapid onset of *Botrytis* rot and a high loss of firmness during the incubation period. These fruit had no potential storage life. Percentage of infection after three months coolstorage was lowest in fruit cured at 10°C (Table 5-12). This was the only treatment with a lower incidence of disease than the inoculated controls. The disease incidence at 20°C was more then double after a four day compared with a two day curing period.

Table 5-10. 1992: SUGAR CONTENT OF CURED KIWIFRUIT DURING SUBSEQUENT STORAGE AT 0°C.

Temperature and Curing time	Lsmeans ± SE					
	Sucrose ($P < 0.001$)[z]		Glucose (NS)[z]		Fructose (NS)[z]	
	mg/100ml of juice					
	1 month	3 months	1 month	3 months	1 month	3 months
10°C + 2 days	*	73.33 ± 7.4	*	226.54 ± 20.5	*	224.30 ± 20.9
4 days	34.89 ± 7.4	46.68 ± 8.3	144.48 ± 20.5	154.59 ± 22.9	139.23 ± 20.9	156.32 ± 23.4
6 days	21.92 ± 7.4	53.73 ± 7.7	86.66 ± 20.5	173.21 ± 21.2	88.36 ± 20.9	167.32 ± 21.7
20°C + 2 days	46.18 ± 8.3	42.31 ± 8.7	153.50 ± 22.9	173.46 ± 23.9	151.81 ± 23.4	176.59 ± 24.4
4 days	34.27 ± 7.7	30.63 ± 7.4	138.64 ± 21.2	117.41 ± 20.5	135.85 ± 21.7	119.25 ± 20.9
30°C + 2 days	42.02 ± 7.7	32.39 ± 8.3	138.76 ± 21.2	145.40 ± 22.9	136.82 ± 21.7	144.47 ± 23.4
Control Inoculated[y] 0°C	66.69 ± 14.8	63.20 ± 18.20	166.88 ± 32.45	215.94 ± 58.38	172.52 ± 34.1	227.94 ± 58.80

* Missing data.
[z] P values after log transformation.
NS = not significant.
[y] Only means and standard error were evaluated for this treatment.

Table 5-11. 1992: ACIDITY OF CURED KIWIFRUIT DURING SUBSEQUENT STORAGE AT 0°C.

Temperature and Curing period	Lsmeans ± SE			
	pH ($P < 0.001$)[z]		Citric Acid equivalent (%w/v) ($P < 0.001$)[z]	
	1 month	3 months	1 month	3 months
10°C + 2 days	4.0 ± 0.07	3.7 ± 0.07	0.0949 ± 0.01	0.1598 ± 0.01
4 days	3.7 ± 0.07	3.7 ± 0.07	0.0913 ± 0.01	0.1948 ± 0.01
6 days	4.0 ± 0.07	3.7 ± 0.07	0.0584 ± 0.01	0.1646 ± 0.01
20°C + 2 days	3.6 ± 0.07	3.8 ± 0.07	0.1586 ± 0.01	0.1252 ± 0.01
4 days	*	3.7 ± 0.07	*	0.1779 ± 0.01
30°C + 2 days	3.4 ± 0.07	3.7 ± 0.07	0.1699 ± 0.01	0.1530 ± 0.01
Control Inoculated[y] 0°C	3.3 ± 0.21	3.8 ± 0.07	0.1563 ± 0.01	0.2072 ± 0.02

* Missing data.
[y] Only means and standard error were evaluated for this treatment.
[z] *P* values after log transformation.

Table 5-12. 1992: PERCENTAGE INFECTION (MEAN ± SE) OF CURED KIWIFRUIT AFTER 12 WEEKS COOLSTORAGE.

	Curing period (days)		
Treatment	2	4	6
10°C *NS*	6.48 ± 1.00	5.78 ± 1.62	5.78 ± 0.83
20°C *P < 0.01*	12.03 ± 3.21	28.56 ± 1.00	
30°C*	15.50 ± 2.95		

Control inoculated 0°C = 7.96 ± 0.56

Control uninoculated 0°C = 0

NS = not significant.

* Only means and standard error were evaluated in this treatment.

Experiment No. 2

Fruit quality after the curing and coolstorage in 1994

Measurement of physiological changes in fruit during curing and coolstorage periods showed that cumulative and daily weight loss during curing and subsequent coolstorage periods were significantly different ($P < 0.05$) for all temperatures at each harvest date (Tables 5-13 & 5-14).

In general, weight loss increased with an increased curing temperature. The lowest cumulative and daily weight loss was observed in fruit from the inoculated control, while the highest weight loss was in fruit cured at 20°C. Cumulative weight loss during the three days of curing was highest after the first day of curing than the subsequent two days of incubation for all fruit from three harvests (Table 5-13).

Table 5-13. 1994: DAILY PERCENTAGE WEIGHT LOSS OF INOCULATED KIWIFRUIT FROM THREE HARVESTS CURED AT FIVE TEMPERATURES.

	Temp.(°C)	Mean weight loss (%)		
		Curing days		
		First[x]	Second[x]	Third[x]
Harvest 1				
16 May	0	0.05^{c} (0.01)	0.07^{c} (0.01)	0.11^{c} (0.008)
	5	0.12^{bc} (0.02)	0.12^{c} (0.02)	0.07^{d} (0.005)
	10	0.17^{b} (0.03)	0.10^{c} (0.02)	0.19^{b} (0.02)
	15	0.43^{a} (0.04)	0.23^{b} (0.04)	0.41^{c} (0.01)
	20	0.42^{a} (0.06)	0.41^{a} (0.05)	0.42^{c} (0.008)
	C. inoc.	0.04^{c} (0.01)	0.04^{c} (0.01)	0.01^{e} (0.003)
Harvest 2				
23 May	0	0.39^{ab} (0.04)	0.12^{c} (0.02)	0.04^{d} (0.006)
	5	0.38^{ab} (0.02)	0.03^{d} (0.02)	0.02^{de} (0.006)
	10	0.38^{ab} (0.05)	0.20^{b} (0.04)	0.12^{c} (0.01)
	15	0.30^{b} (0.03)	0.25^{ab} (0.02)	0.20^{b} (0.008)
	20	0.49^{c} (0.01)	0.32^{a} (0.02)	0.25^{a} (0.01)
	C. inoc.	0.39^{ab} (0.07)	0.04^{d} (0.01)	0.01^{e} (0.007)
Harvest 3				
27 May	0	0.25^{d} (0.01)	0.04^{c} (0.005)	0.01^{c} (0.004)
	5	0.29^{cd} (0.01)	0.03^{c} (0.007)	0.04^{c} (0.01)
	10	0.39^{ab} (0.2)	0.10^{bc} (0.01)	0.03^{c} (0.01)
	15	0.34^{bc} (0.2)	0.15^{b} (0.02)	0.12^{b} (0.01)
	20	0.44^{a} (0.2)	0.38^{a} (0.06)	0.11^{b} (0.04)
	C. inoc.	0.23^{d} (0.02)	0.01^{c} (0.003)	0.28^{a} (0.005)

[x] Mean separation within columns by Duncan's multiple range test at $P < 0.05$.
Values enclosed in parenthesis indicate mean standard error (SEM).

Table 5-14. 1994: CUMULATIVE PERCENTAGE WEIGHT LOSS OF INOCULATED KIWIFRUIT FROM THREE HARVESTS AFTER CURING AND COOLSTORAGE AT 0°C.

	Temperature (°C)	Mean weight loss (%)		
		Curing	Storage 0°C	
		3 days[x]	6 weeks[x]	12 weeks[y]
Harvest 1				
16 May	0	0.24[e] (0.01)	0.92[b] (0.09)	*
	5	0.32[d] (0.01)	0.72[b] (0.07)	1.40 (0.1)
	10	0.46[c] (0.02)	0.77[b] (0.05)	1.86 (0.1)
	15	1.07[b] (0.02)	1.50[a] (0.07)	2.11 (0.1)
	20	1.25[a] (0.03)	1.55[a] (0.06)	1.93 (0.1)
	C. inoc.	0.09[f] (0.01)	0.77[b] (0.08)	*
Harvest 2				
23 May	0	0.56[c] (0.03)	0.79[c] (0.08)	0.94 (0.1)
	5	0.45[c] (0.02)	0.96[bc] (0.06)	1.28 (0.1)
	10	0.71[b] (0.05)	1.24[a] (0.09)	1.35 (0.1)
	15	0.76[b] (0.04)	1.10[ab] (0.06)	1.23 (0.1)
	20	1.07[a] (0.04)	1.29[a] (0.07)	2.49 (0.1)
	C. inoc.	0.42[c] (0.07)	0.52[d] (0.07)	0.58 (0.1)
Harvest 3				
27 May	0	0.34[c] (0.02)	0.93[c] (0.09)	1.54 (0.2)
	5	0.36[c] (0.01)	0.73[c] (0.05)	1.05 (0.1)
	10	0.62[b] (0.03)	1.57[ab] (0.1)	1.78 (0.1)
	15	0.61[b] (0.03)	1.84[b] (0.09)	2.50 (0.1)
	20	1.11[a] (0.1)	1.33[a] (0.1)	2.34 (0.1)
	C. inoc.	0.26[c] (0.02)	0.88[c] (0.07)	1.37 (0.2)

[x] Mean separation within columns by Duncan's multiple range test at $P < 0.05$.

[y] As number of fruit were unbalanced due to losses from disease, only lsmeans and standard errors are shown at this storage period.

* Fruit from these treatments completely rotten.

Values enclosed in parenthesis indicate mean standard error (SEM).

Daily weight loss decreased the curing period for harvests two and three but there was no consistent pattern at harvest one. Total weight loss increased during both curing and subsequent coolstorage. Fruit coolstored for 12 weeks showed the highest weight loss (Table 5-14).

Firmness was significantly different ($P < 0.05$) after both curing and coolstorage, at the second and third harvests with the exception of harvest two after six weeks coolstorage, but at the first harvest significant differences were found only in fruit coolstored for six weeks (Table 5-15). Fruit firmness decreased with curing and storage at all temperatures and at all three harvests. There was little change in firmness over the curing period itself but a drop of 39% (8.6-5.3 kgf) occurred in the first six weeks of coolstorage followed by a further 15.4% in the second six weeks. After 12 weeks coolstorage the average fruit firmness was 3.9 kgf irrespective of harvest date.

Infection levels during fruit coolstorage

Overall infection levels were high in fruit from the first harvest and considerably lower in fruit from the second and third harvests (Figs. 5-2 & 5-3). Most diseased fruit showed symptoms by the six week assessment. There was a significant difference ($P < 0.05$) between temperature treatments after both six and 12 weeks coolstorage for each harvest date. Fruit cured at 0°C and 5°C always had a higher incidence of disease than the inoculated control fruit and in 10 of 12 instances this difference was significant. Fruit cured at 10°C had a lower disease than the inoculated and this was significant in four of the six comparisons. The disease incidence in fruit cured at 15°C was not significantly different from that in the inoculated controls except for the 12 weeks assessment of the third harvest. Fruit cured at 20°C had significantly less disease than the controls at harvest one after six weeks and harvest two after 12 weeks.

Table 5-15. 1994: FIRMNESS OF INOCULATED KIWIFRUIT FROM THREE HARVESTS AFTER CURING AND COOLSTORAGE AT 0°C.

	Temperature (°C)	Mean firmness (Kg/f)		
		Curing	Storage 0°C	
		3 days[x]	6 weeks[x]	12 weeks
Harvest 1				
16 May	0	8.8[a] (0.2)	4.6[cd] (0.2)	*
	5	9.2[a] (0.09)	5.2[bc] (0.2)	3.5 (0.1)[y]
	10	9.2[a] (0.1)	6.0[b] (0.03)	4.0 (0.1)
	15	8.6[a] (0.2)	4.2[d] (0.1)	4.0 (0.1)
	20	9.1[a] (0.2)	7.1[a] (0.4)	3.7 (0.1)
	C. inoc.	9.0[a] (0.2)	6.0[b] (0.2)	*
Harvest 2				
23 May	0	9.2[a] (0.1)	4.5[a] (0.3)	3.5[c] (0.1)
	5	8.5[bc] (0.2)	5.0[a] (0.4)	4.0[ab] (0.1)
	10	8.5[bc] (0.2)	4.5[a] (0.5)	4.1[ab] (0.1)
	15	8.1[c] (0.1)	4.7[a] (0.3)	3.9[abc] (0.1)
	20	7.2[d] (0.2)	5.0[a] (0.4)	4.3[a] (0.2)
	C. inoc.	9.0[ab] (0.1)	4.8[a] (0.2)	3.7[bc] (0.09)
Harvest 3				
27 May	0	9.0[a] (0.1)	5.9[b] (0.3)	3.8[bc] (0.1)
	5	8.3[ab] (0.2)	5.4[cb] (0.2)	3.9[abc] (0.1)
	10	8.6[a] (0.2)	6.0[b] (0.2)	4.3[ab] (0.1)
	15	7.6[b] (0.3)	4.7[c] (0.2)	3.4[c] (0.1)
	20	7.6[b] (0.2)	7.1[a] (0.2)	4.4[a] (0.2)
	C. inoc.	8.5[b] (0.2)	5.4[cb] (0.3)	3.5[c] (0.2)

[x] Mean separation within columns by Duncan's multiple range test at $P < 0.05$.

[y] As number of fruit were unbalanced at this harvest and storage period, only lsmeans and standard errors were calculated.

* Fruit from these treatments completely rotten.

Values enclosed in parenthesis indicate mean standard error (SEM).

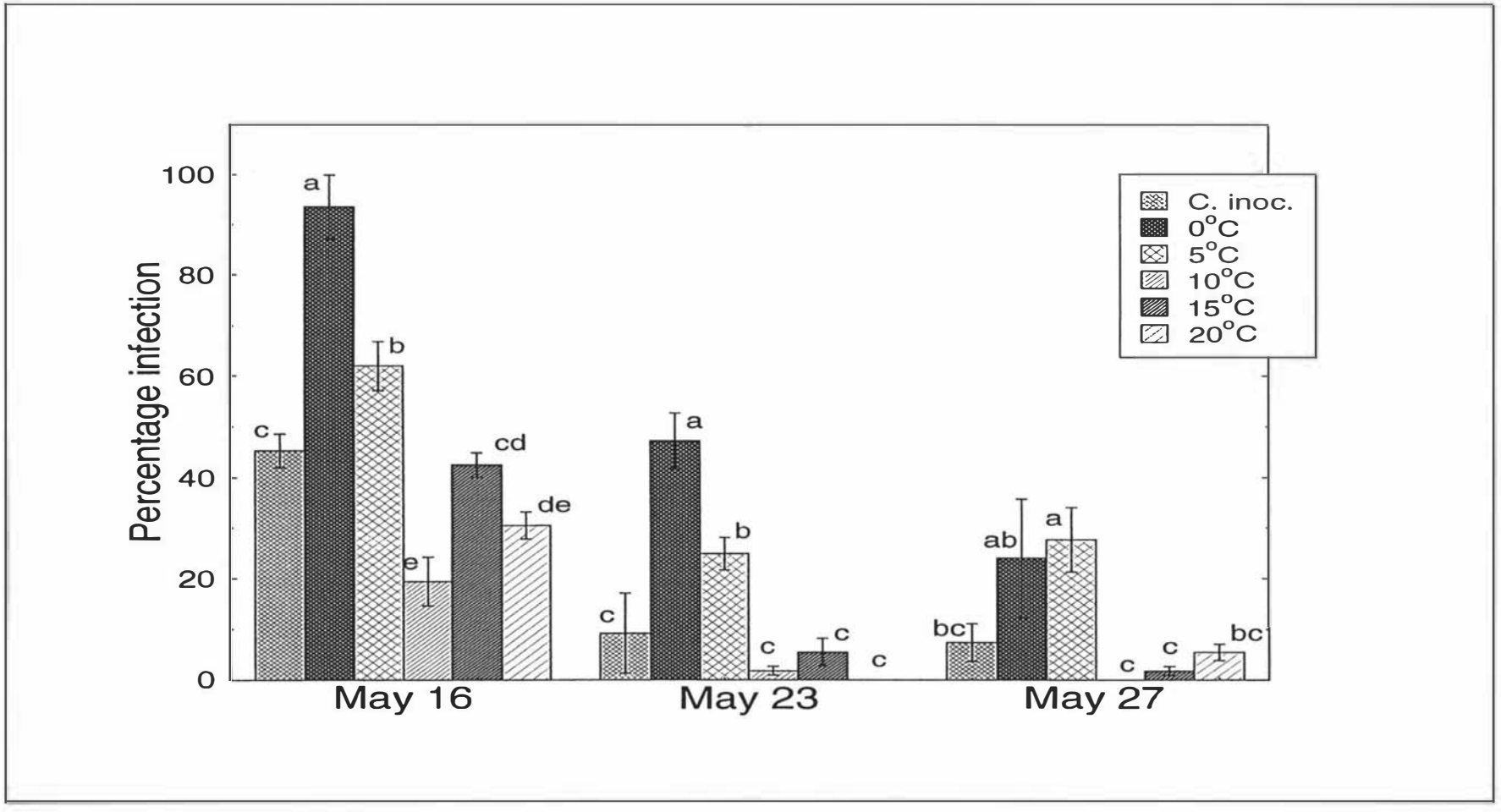


Figure 5-2. 1994: Percentage infection of kiwifruit from three harvests after curing at a range of temperatures and coolstorage for six weeks. Letters a,b c d & e refer to Duncan's test ($P < 0.05$). Vertical bars indicate SEM.

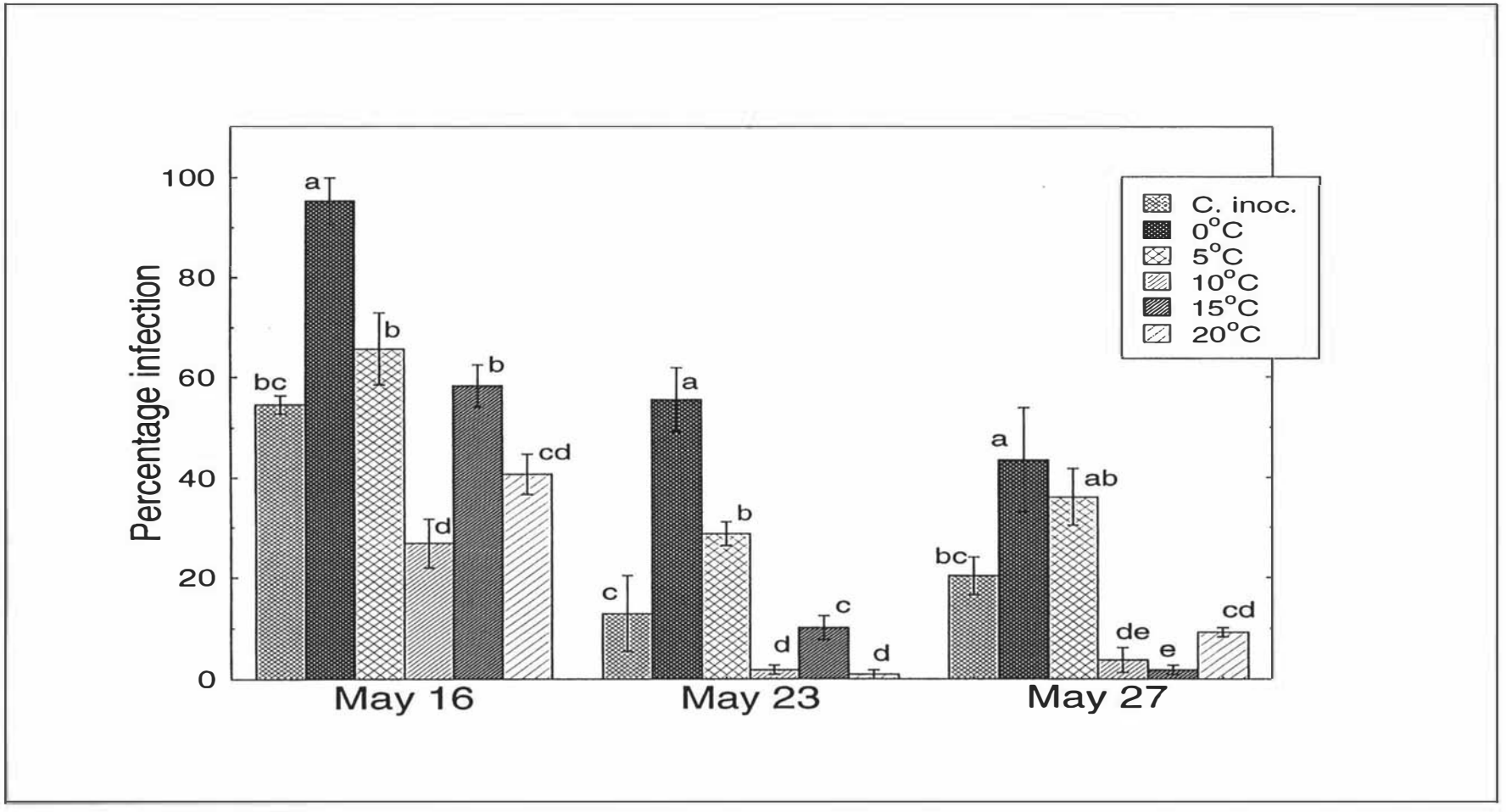


Figure 5-3. 1994: Percentage infection of kiwifruit from three harvests after curing at a range of temperatures and coolstorage for 12 weeks. Letters a, b, c, d & e refer to Duncan's test ($P < 0.05$). Vertical bars indicate SEM.

Experiment No.3

Relative humidity behaviour during the curing period in 1993

At both harvest dates the input/output relative humidity ranges remained constant throughout the three days of curing (Figs. 5-4 & 5-5). With small differences between the relative humidity ranges from the first and second harvest, the lowest relative humidity varied from 40% to 60% (input) and from 41% to 59% (output). The medium range varied from 61% to 80% and 62 to 81% (input/output respectively), while the highest varied from 94-98% and 92-98%.

Fruit quality after the curing period:

After three days curing at 10°C weight loss of fruit was inversely proportional to the relative humidity for all treatments (Fig.5-6). Weight loss of fruit in the high relative humidity treatment was significantly less ($P < 0.05$) than that in the other two treatments at both harvest dates. The highest percentage weight loss was in fruit cured at 40-59% RH although this difference was significantly greater than that in the mid-range (60-80% RH) at the second harvest only.

Firmness of fruit from the first harvest was not affected by the relative humidity during curing (Fig.5-7). At the second harvest, firmness of cured fruit at the highest relative humidity range was significantly higher ($P < 0.05$) than that in the other two treatments.

Total solid solubles were not affected by relative humidity conditions during curing of fruit at either harvest (Fig.5-8).

Infection levels during coolstorage:

Most infections of fruit by *B. cinerea* could be detected after six weeks coolstorage in the high relative humidity treatment but only two thirds of that in the low relative humidity treatment was detected at this time (Figs.5-9 &

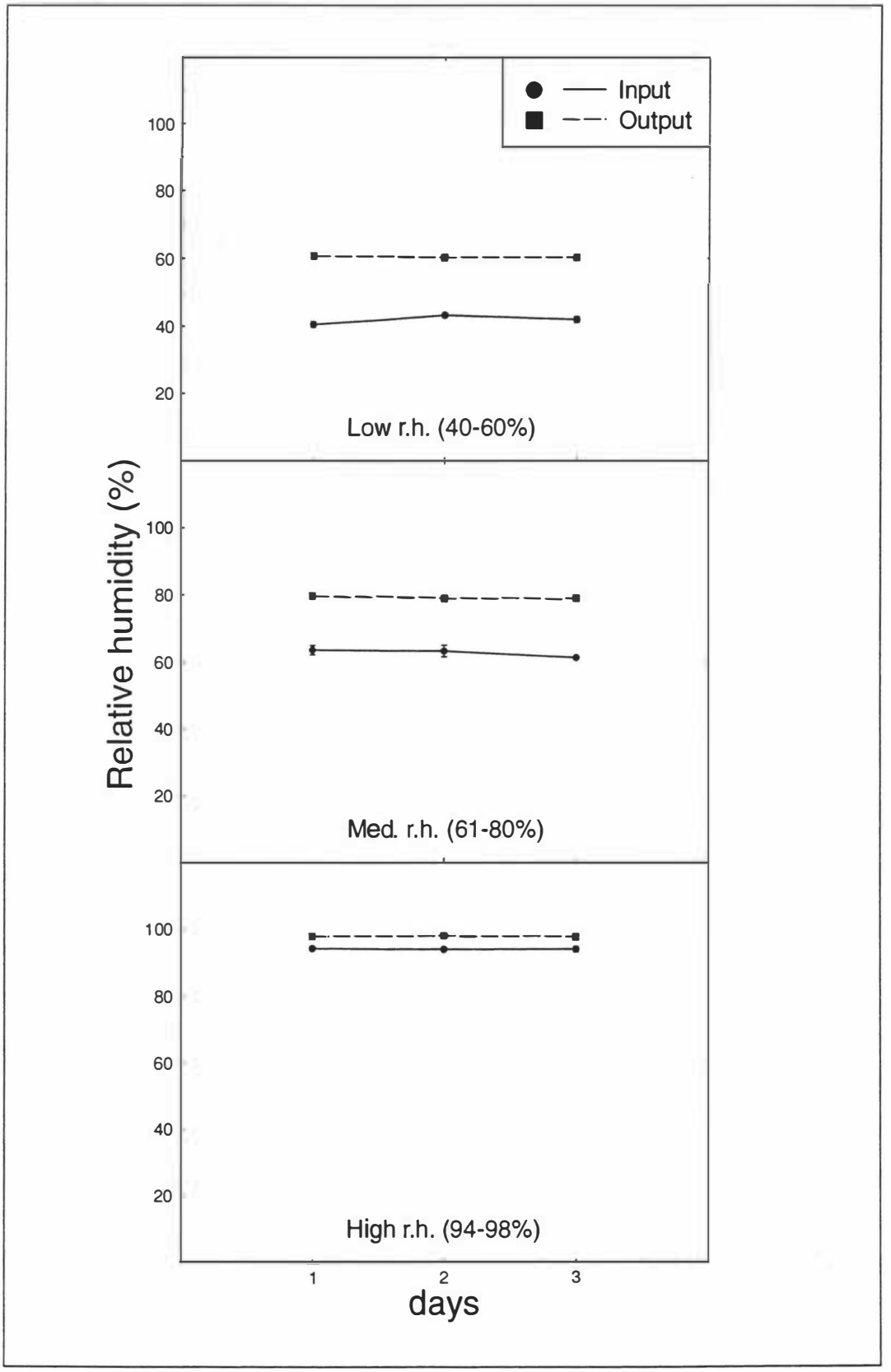


Figure 5-4. 1993: First harvest relative humidity during a three day incubation time at 10°C (Mean ± SE).

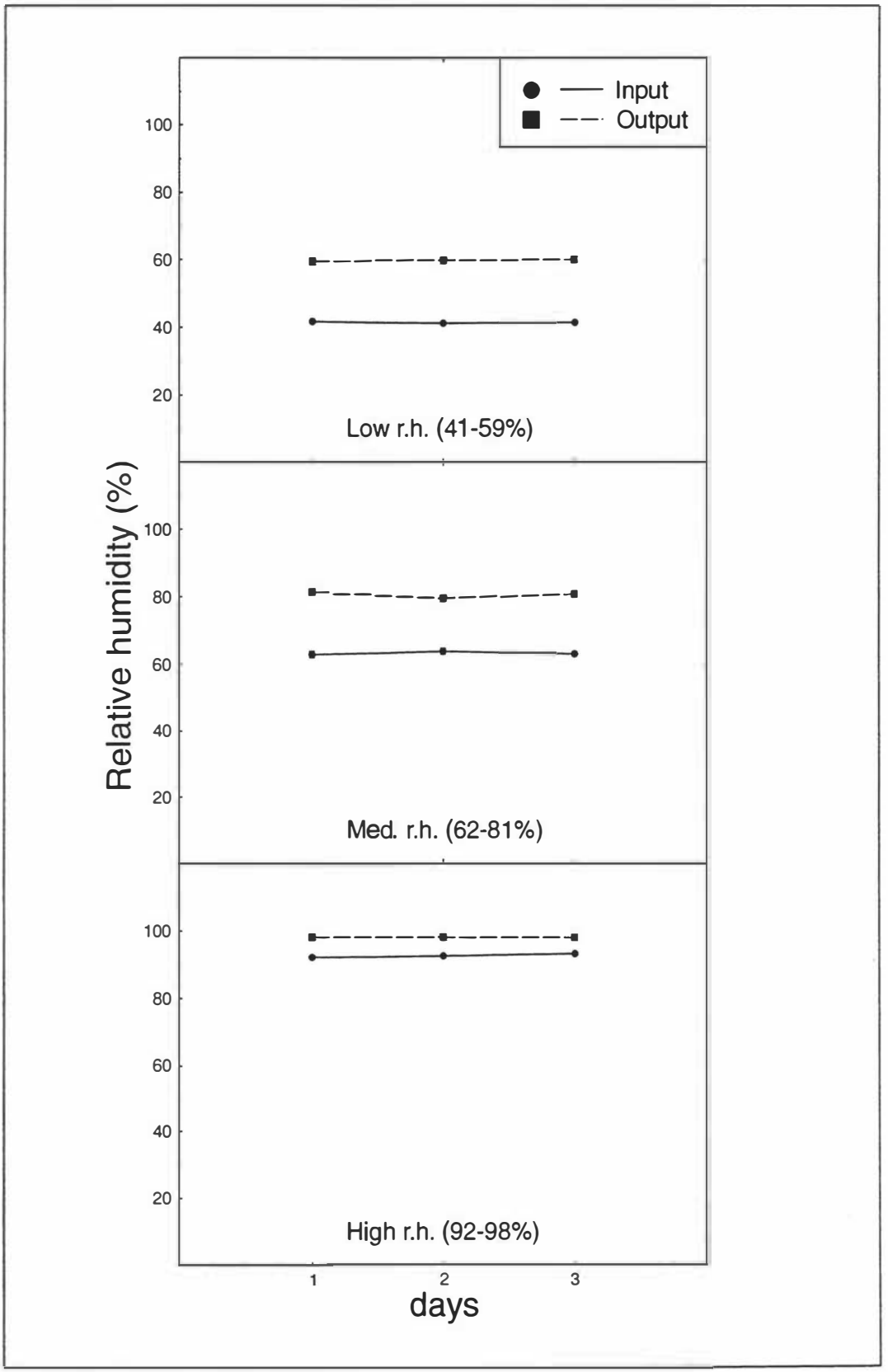


Figure 5-5. 1993: Second harvest relative humidity during a three day incubation at 10°C (Mean ± SE).

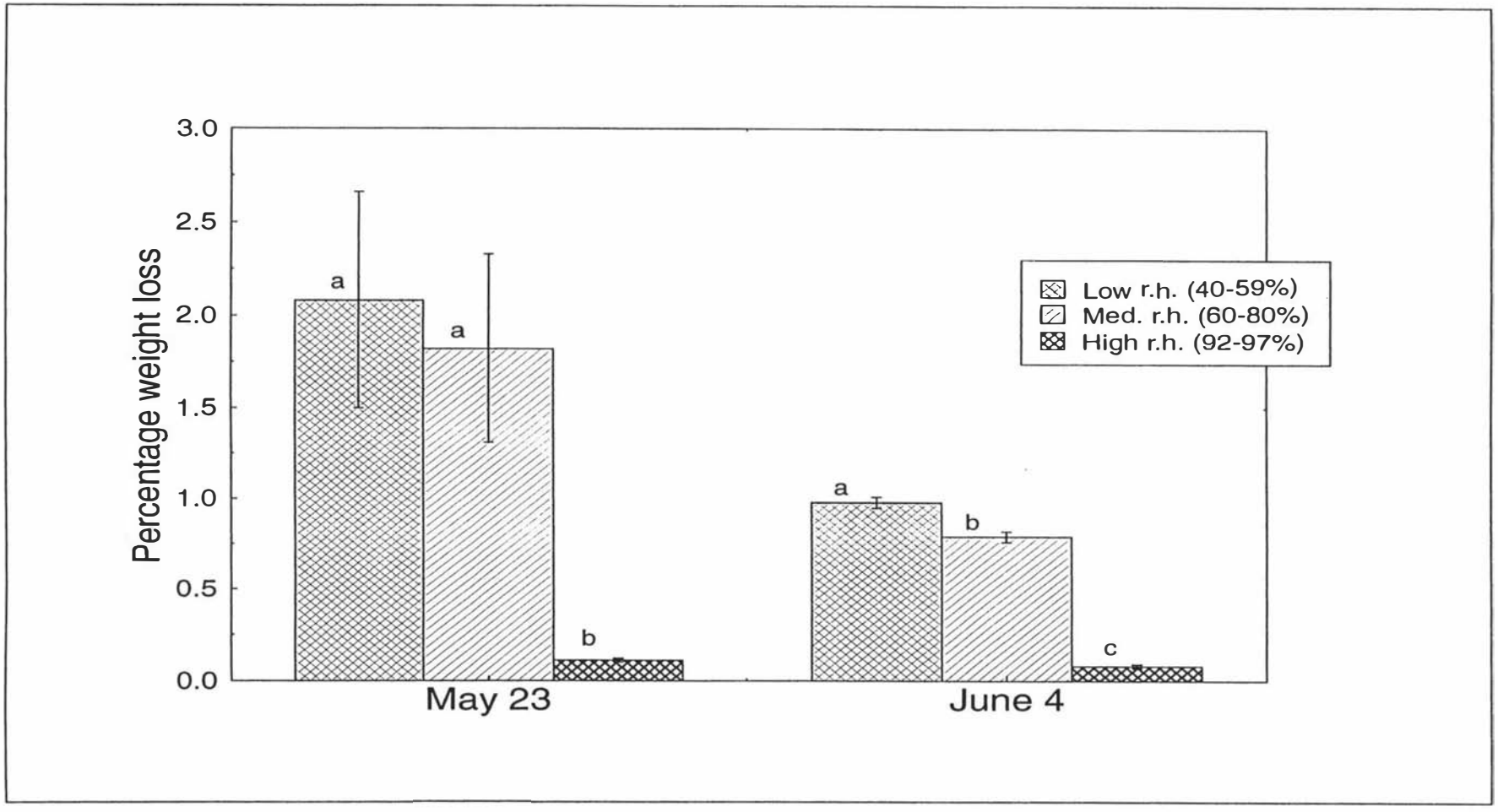


Figure 5-6. 1993: Weight loss of inoculated kiwifruit after curing for three days at 10°C and one of three relative humidities. Letters a, b & c refer to Duncan's test ($P < 0.05$). Vertical bars indicate SEM.

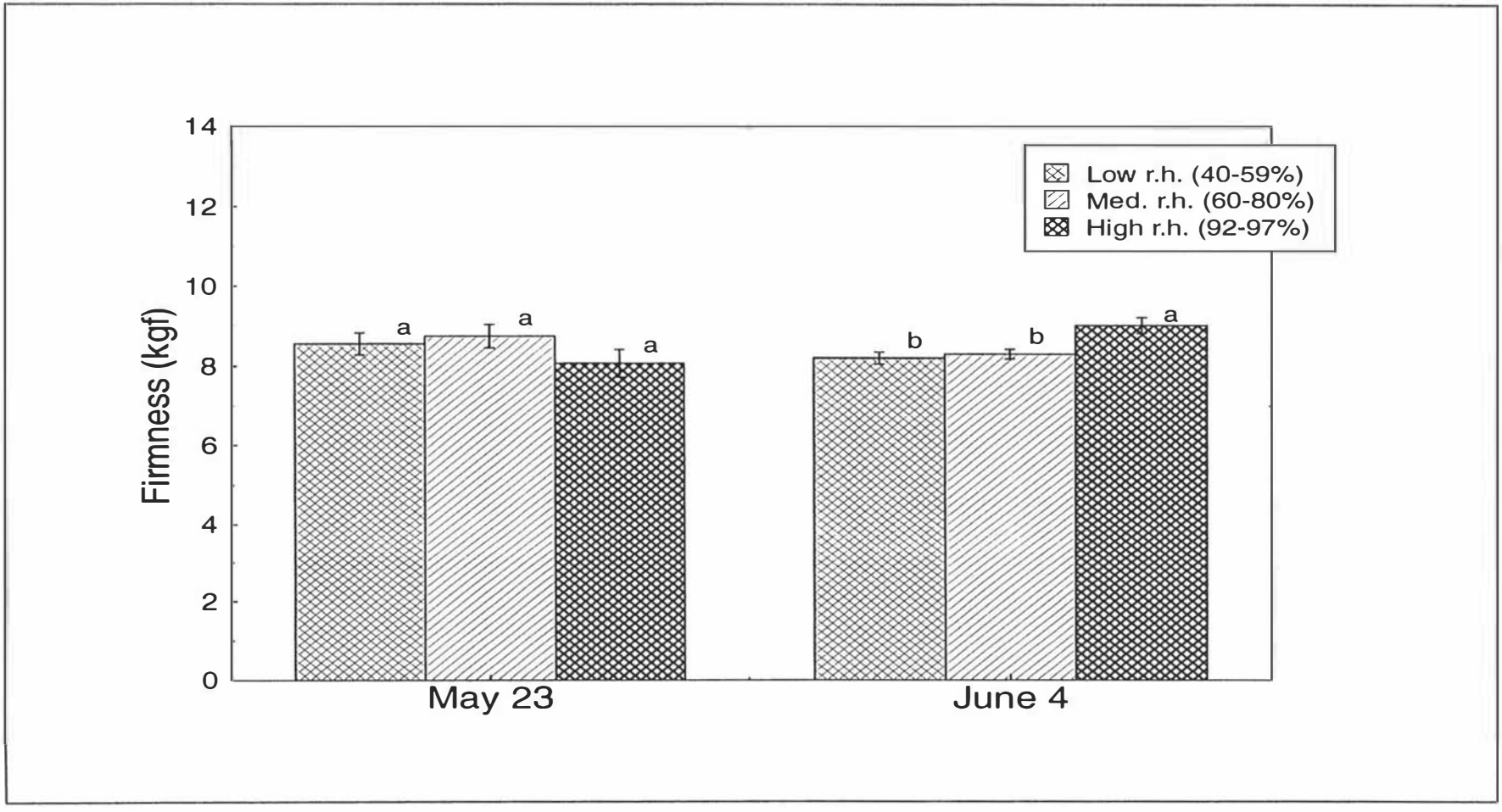


Figure 5-7. 1993: Firmness of inoculated kiwifruit after curing for three days at 10°C and one of three relative humidities. Letters a & b refer to Duncan's test ($P < 0.05$). Vertical bars indicate SEM.

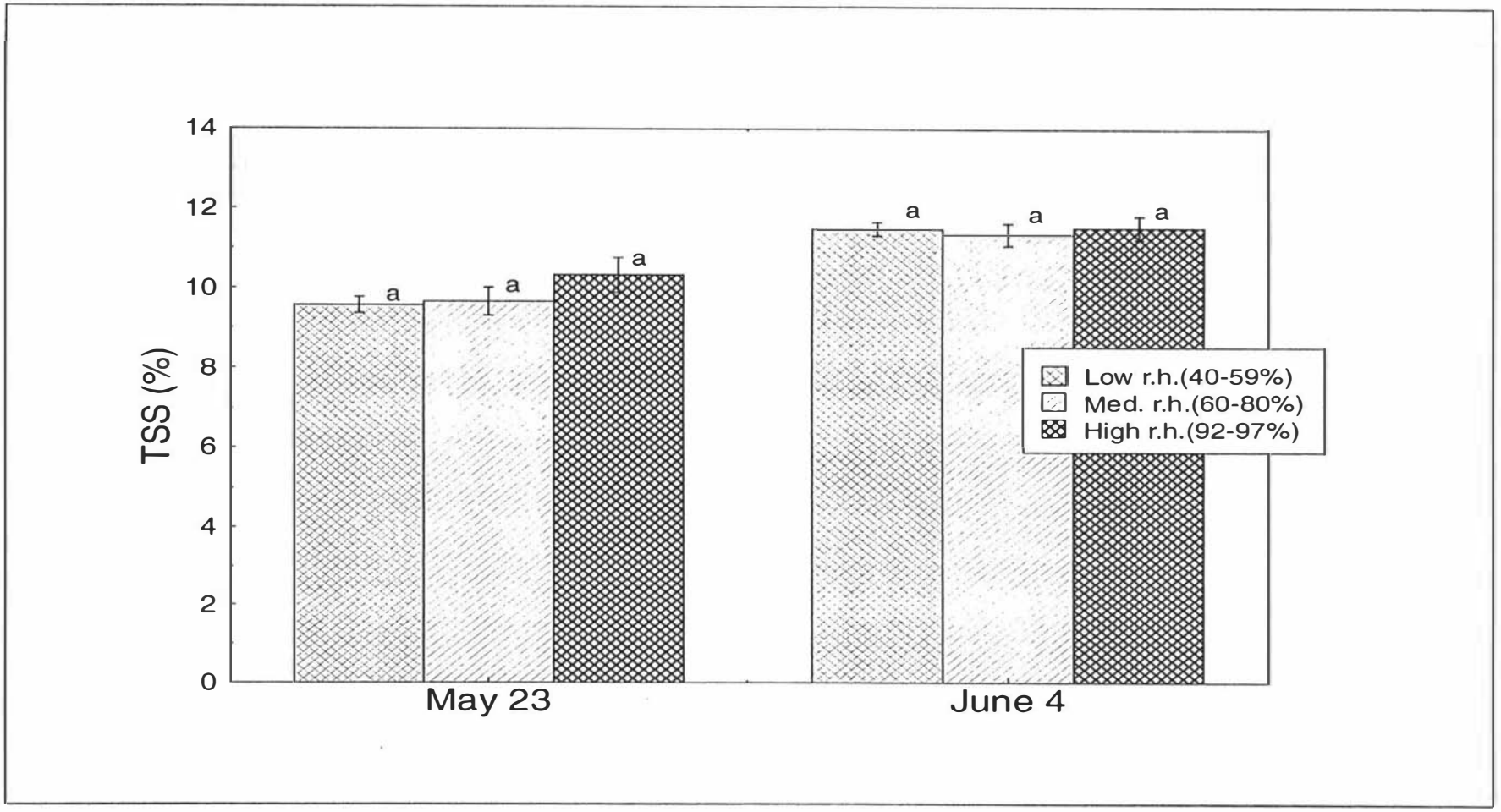


Figure 5-8. 1993: TSS of inoculated kiwifruit after curing for three days at 10°C and one of three relative humidities. Letter a refers to Duncan's test ($P < 0.05$). Vertical bars indicate SEM.

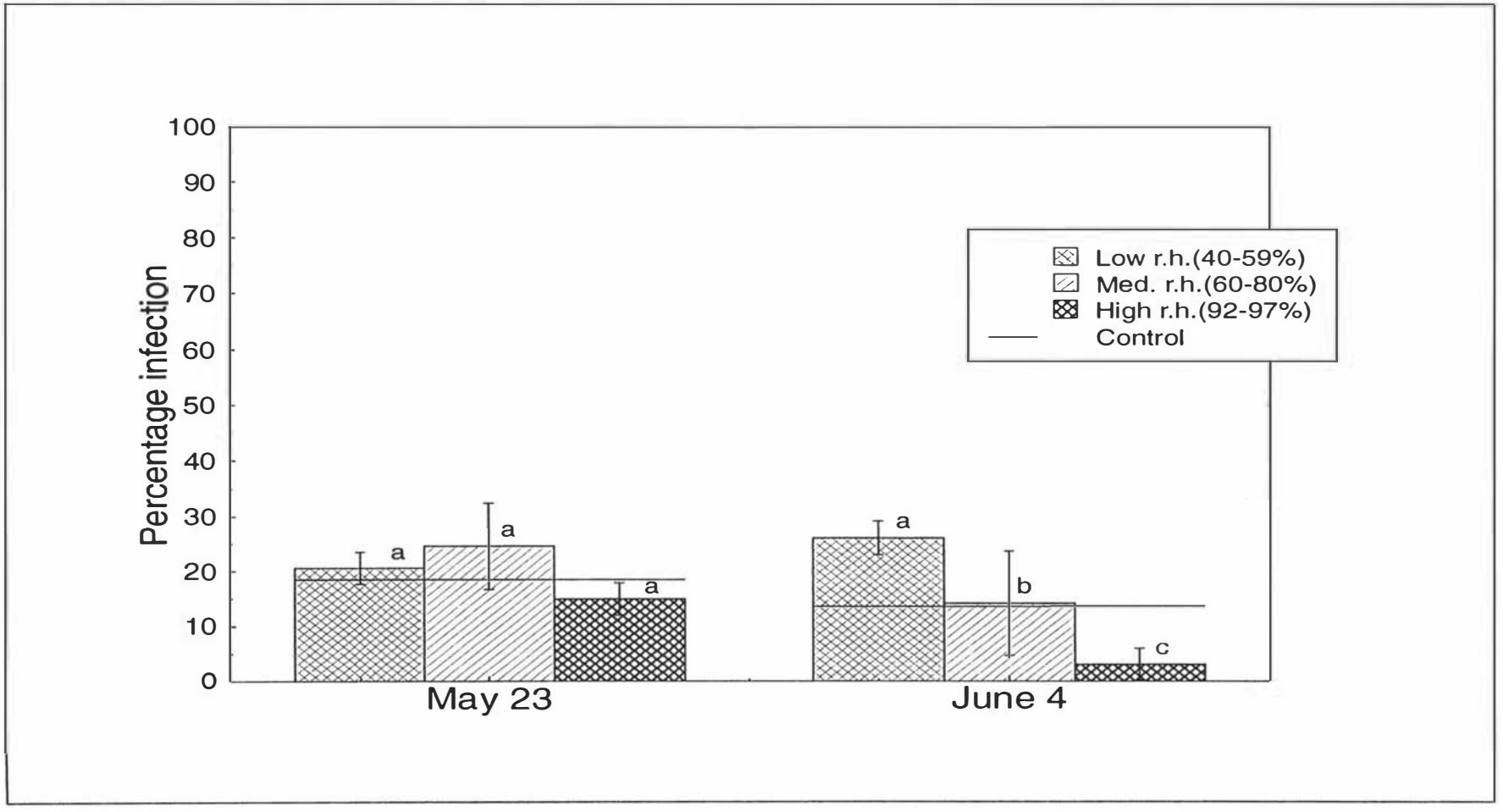


Figure 5-9. 1993: Effect of curing at 10°C on incidence of *B. cinerea* infection of kiwifruit after six weeks of coolstorage. Letters a, b & c refer to Duncan's test ($P < 0.05$). Vertical bars indicate SEM.

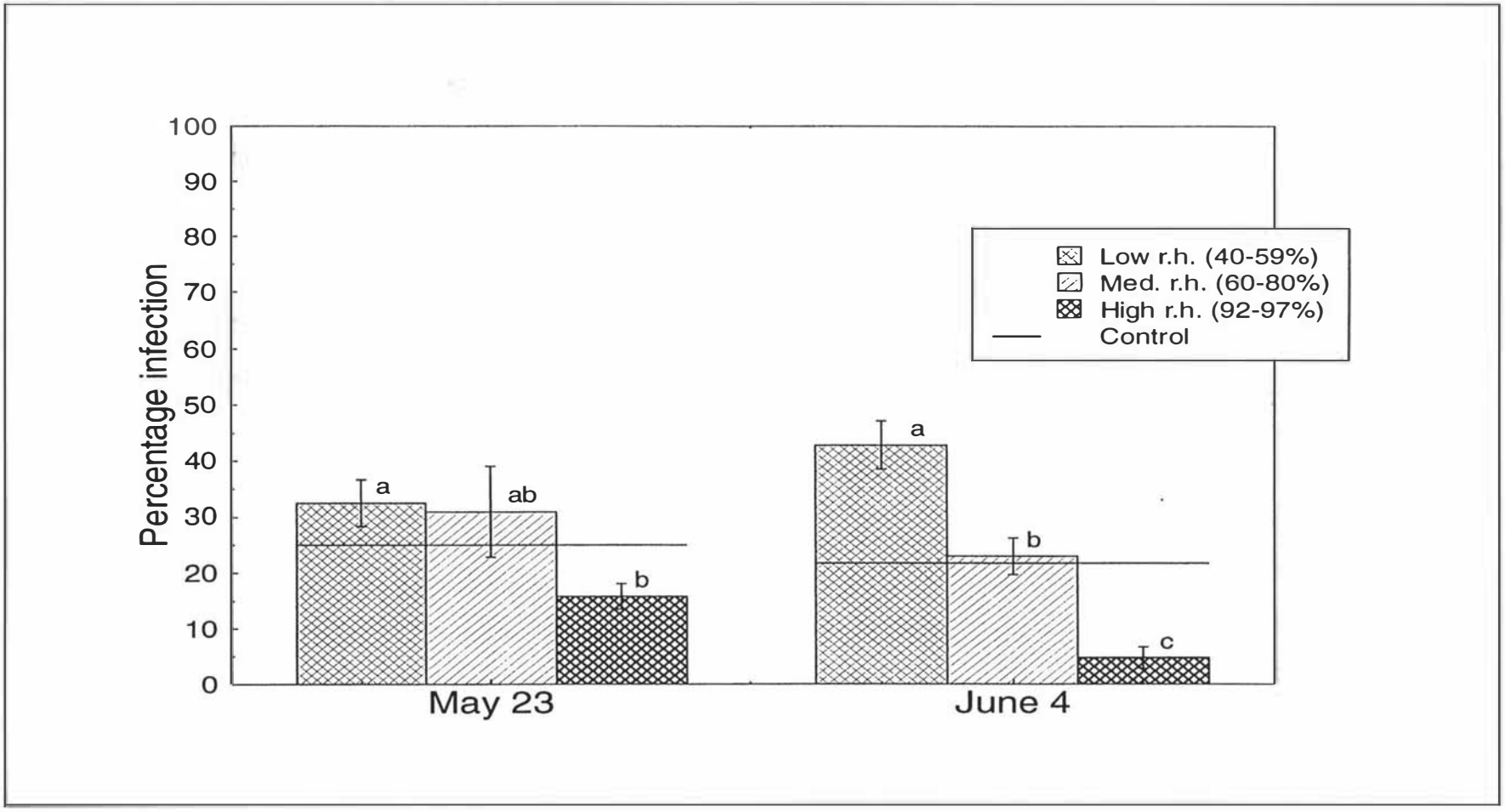


Figure 5-10. 1993: Effect of curing at 10°C on incidence of *B. cinerea* infection of kiwifruit after 12 weeks of coolstorage. Letters a, b & c refer to Duncan's test ($P < 0.05$). Vertical bars indicate SEM.

5-10). Differences in disease incidence were not significant after six weeks of incubation of fruit from the first harvest but the final incidence as assessed at 12 weeks showed that there were significantly more infections ($P < 0.05$) in the fruit cured at the low than at the highest relative humidity (Fig.5-10). At the second harvest there was the same pattern of results but this time the differences between humidity treatments were already significant at the first assessment.

Experiment No. 4

Relative humidity behaviour during curing in 1994:

With the exception of the lowest relative humidity range, the overall relative humidity during curing of fruit from the four harvests remained constant (Fig. 5-11). At the lowest relative humidity range the input was 50% while the output was initially 70% but increased during the curing period to 80%. The medium relative humidity range input remained constant at 80% and the output increased from 93% to 95%. The high range input was 95% relative humidity and the output 98-100% relative humidity.

Fruit quality after the curing period

Overall weight loss, firmness and total solid solubles were affected by fruit maturity at harvest with significant differences at $P < 0.001$ (Table 5-16). The highest significant ($P < 0.05$) weight loss was observed at the first and second harvest, while the lowest was in fruit from fourth harvest.

Firmness fluctuated from harvest to harvest but TSS increased progressively with each successive harvest (Table 5-16).

Relative humidity conditions during curing significantly affected weight loss (low weight loss at high relative humidity) but did not affect firmness or TSS (Table 5-16).

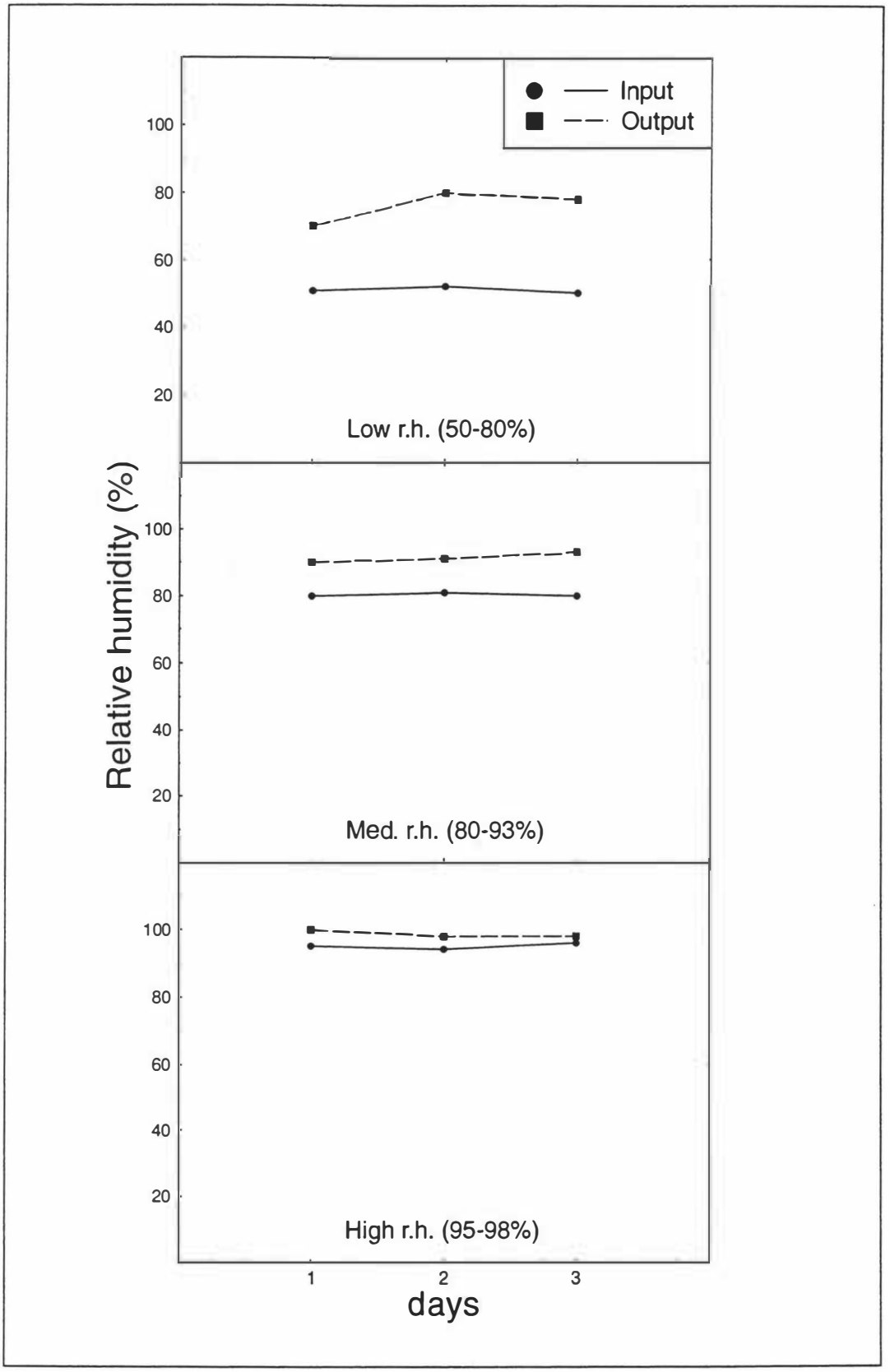


Figure 5-11. 1994: Range of relative humidities attained during three days incubation at 10°C for each of four harvests.

Table 5-16. 1994: WEIGHT LOSS, FIRMNESS AND TOTAL SOLUBLE SOLIDS OF INOCULATED KIWIFRUIT HARVESTED AT DIFFERENT MATURITIES AND CURED FOR THREE DAYS AT 10°C AND ONE OF THREE RELATIVE HUMIDITIES.

Source	Level	Mean weight loss (%)[x]	Mean firmness (Kg/f)	Mean TSS (%)
Main effects				
Maturity		*P < 0.001*	*P < 0.001*	*P < 0.001*
	1st. harvest	0.58[a] (0.05)	8.6[a] (0.1)	7.5[c] (0.1)
	2nd. harvest	0.69[a] (0.05)	7.5[c] (0.1)	8.5[b] 0.2)
	3rd. harvest	0.54[b] (0.05)	8.0[b] (0.1)	9.7[a] (0.2)
	4th. harvest	0.47[c] (0.05)	7.3[c] (0.1)	10.1[a] (0.1)
Relative humidity (RH)		*P < 0.001*	*NS*	*NS*
	Low	0.93[a] (0.02)	7.7[a] (0.1)	8.9[a] (0.2)
	Medium	0.66[b] (0.01)	7.9[a] (0.1)	9.0[a] (0.2)
	High	0.12[c] (0.03)	8.0[a] (0.1)	9.0[a] (0.1)
Type Inoculum (Inoc)		*NS*	*NS*	*NS*
	Spore suspension	0.55[a] (0.03)	7.8[a] (0.1)	9.0[a] (0.1)
	Dry conidia	0.58[a] (0.03)	8.0[a] (0.1)	8.9[a] (0.1)
Interactions				
Maturity * RH		*NS*	*P < 0.01*	*P < 0.001*
Maturity * Inoc		*NS*	*NS*	*NS*
RH * Inoc		*NS*	*NS*	*NS*

[x] *P* values after square root transformation.
Values enclosed in parenthesis indicate mean standard error (SEM).
NS = not significant.

The type of inoculum (spore suspension or dry conidia) did not affect weight loss, firmness or TSS (Table 5-16).

There was a significant interaction between maturity and relative humidity which affected firmness and TSS but not weight loss. The highest and medium relative humidity ranges gave a higher firmness than the low relative humidity at the first harvest date but the position were reversed at the fourth harvest (Fig.5-12). The relative TSS of the high and medium relative humidity treated fruit alternated at each successive harvest hence the significant interaction.

Infection levels during coolstorage:

Eighty three percent of the infections which ultimately developed were detected after six weeks of storage (Table 5-17). At both assessments, overall significant differences in infection levels were found between harvest maturities and type of inoculum (Table 5-17). There were progressively lower levels of disease over the 15 days from the first to the fourth harvests. Fruit treated at the highest relative humidity had the lowest percentage infection after six and 12 weeks coolstorage while those treated at the lowest relative humidity range had most disease. Fruit treated in the medium relative humidity range had a level of disease intermediate between that of the other two relative humidity ranges. Application of dry conidia caused nearly double the disease incidence of the spore suspension.

There were significant interactions between maturity and relative humidity, maturity and type of inoculum and between relative humidity and type of inoculum (Table 5-17). Fruit cured at the low and medium relative humidity showed a consistent drop in infection levels at each successive harvest but this pattern was not found in fruit cured at the high relative humidity where fruit from the first harvest had a low disease incidence (Fig.5-13).

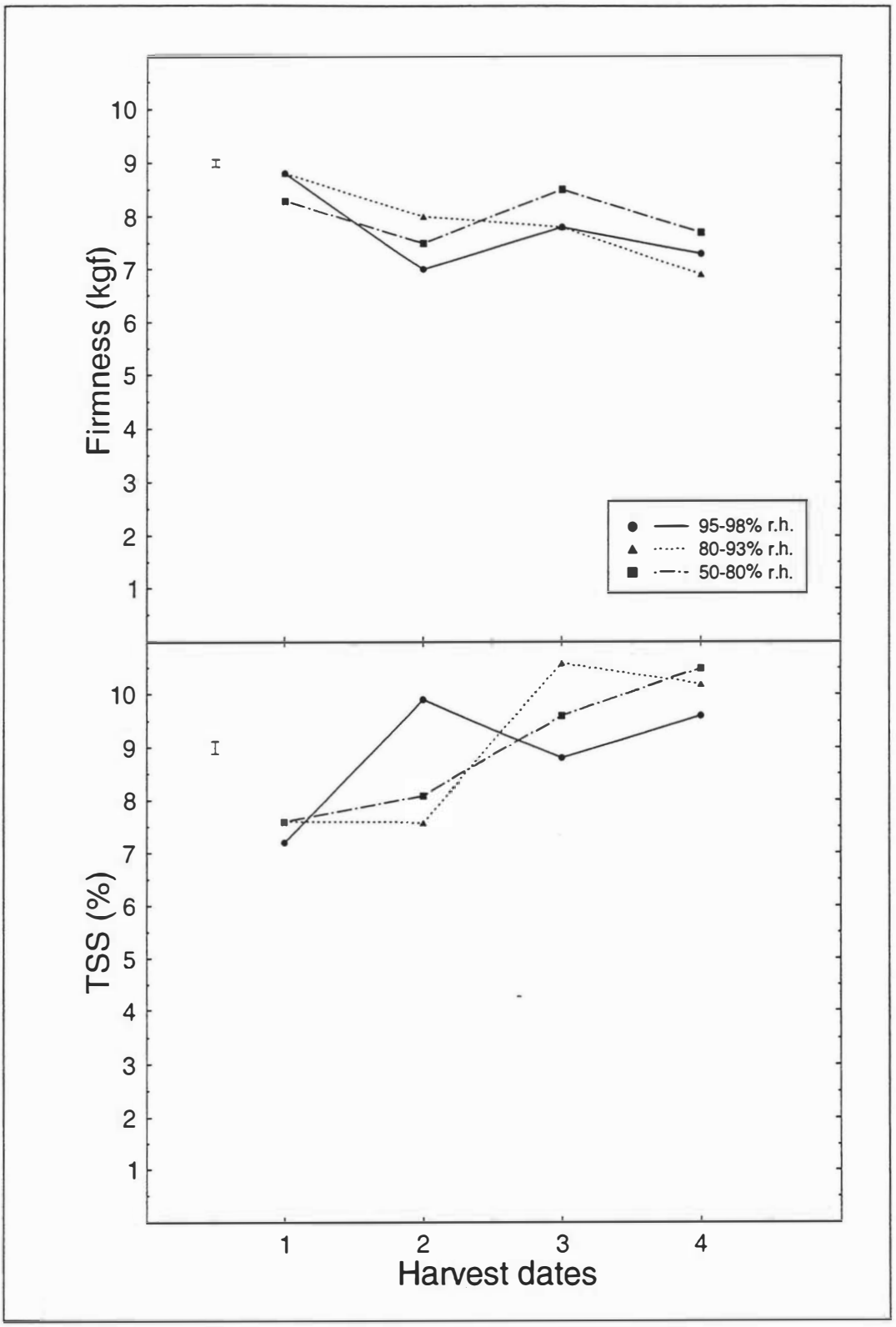


Figure 5-12. 1994: Interaction between maturity and relative humidity on firmness and total soluble solids of inoculated kiwifruit cured at one of three rh's and four harvest maturities. Vertical bars indicate overall standard error of the mean (SEM).

Table 5-17. 1994: INFECTION LEVELS OF *B. CINEREA* DEVELOPED DURING COOLSTORAGE OF KIWIFRUIT INOCULATED WITH SPORE SUSPENSION OR DRY CONIDIA BEFORE CURING AT 10°C AND A RANGE OF RELATIVE HUMIDITIES.

Source	Level	Mean infection 6 weeks (%)	Mean infection 12 weeks (%)
Main effects			
Maturity		*P < 0.001*	*P < 0.001*
	1st. harvest	46.5[a] (2.7)	57.8[a] (3.3)
	2nd. harvest	35.7[b] (3.5)	39.9[b] (3.6)
	3rd. harvest	25.3[c] (3.9)	29.6[c] (4.0)
	4th. harvest	17.9[d] (3.1)	22.4[d] (3.5)
← Relative humidity (RH)		*P < 0.001*	*P < 0.001*
	Low	42.4[a] (3.8)	50.5[a] (4.2)
	Medium	32.7[b] (3.4)	39.8[a] (3.5)
	High	19.0[c] (2.4)	22.0[b] (2.8)
Inoculum (Inoc)		*P < 0.001*	*P < 0.001*
	Spore suspension	21.4[a] (2.5)	27.1[a] (2.8)
	Dry conidia	41.3[b] (2.2)	47.8[b] (2.5)
Interactions			
Maturity * RH		*P < 0.01*	*P < 0.05*
Maturity * Inoc		*P < 0.001*	*P < 0.001*
RH * Inoc		*P < 0.001*	*P < 0.01*

Overall means of inoculated control: 6 weeks storage, 32.8 ± 4.4; 12 weeks storage, 38.1 ± 4.4.
Values enclosed in parenthesis indicate mean standard error (SEM).
P values after square root transformation.
NS = not significant.

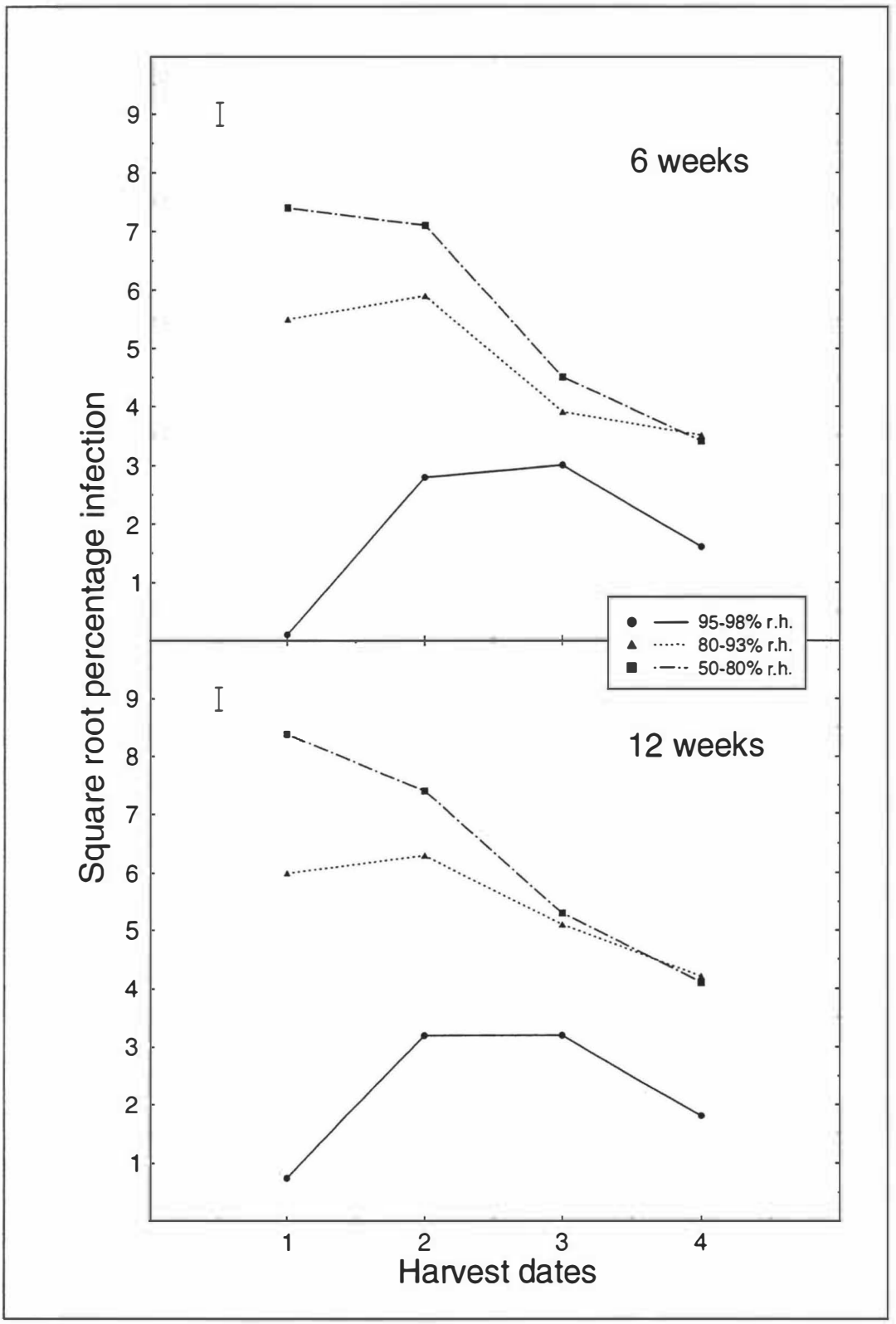


Figure 5-13. 1994: Interaction between maturity and relative humidity on *B. cinerea* storage rot incidence of kiwifruit cured at 10°C. Vertical bars indicate overall standad error of the mean (SEM).

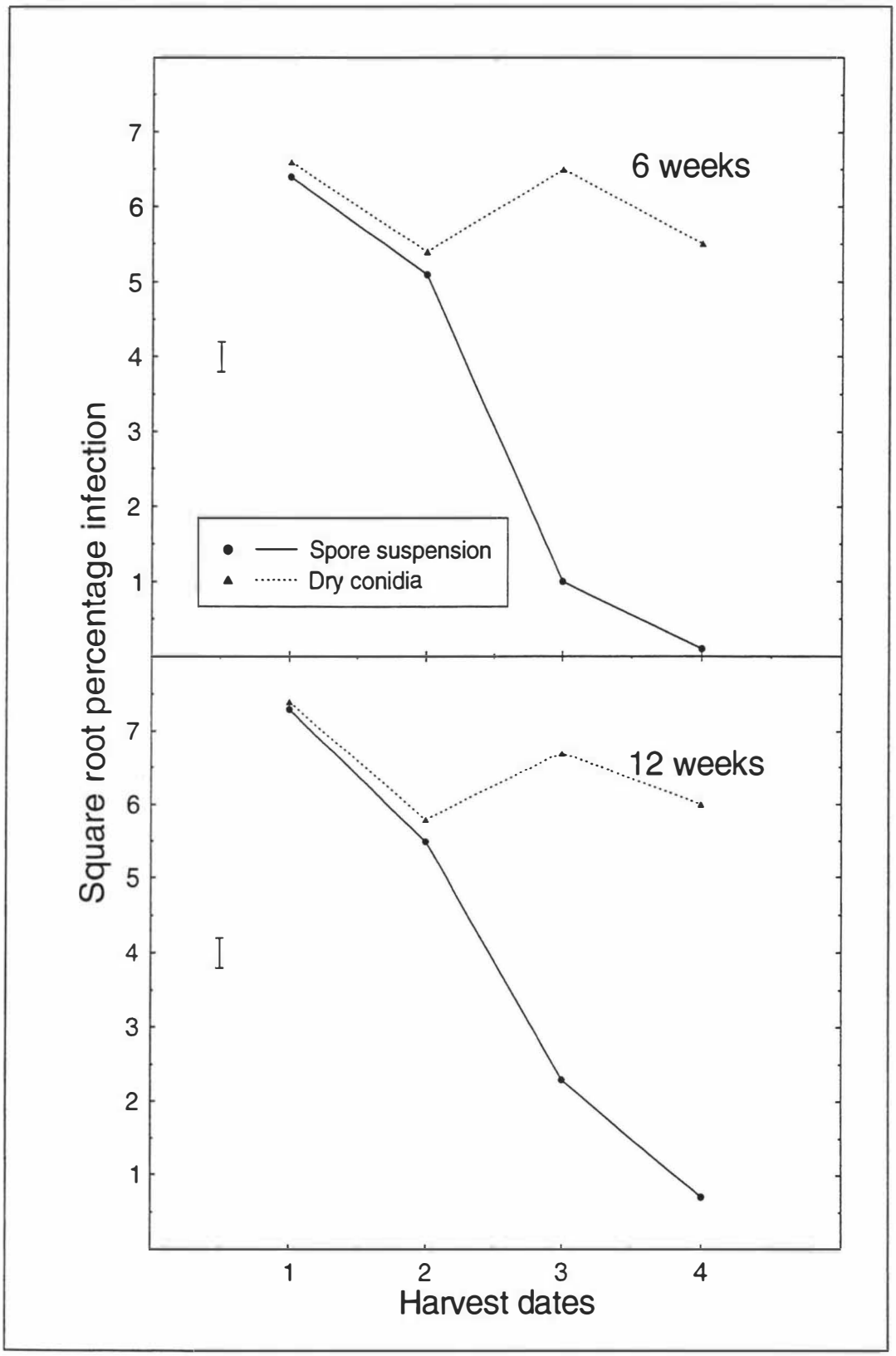


Figure 5-14. 1994: Interaction between maturity and type of inoculum on *B. cinerea* storage rot incidence of kiwifruit cured at 10°C. Vertical bars indicate overall standard error of the mean (SEM).

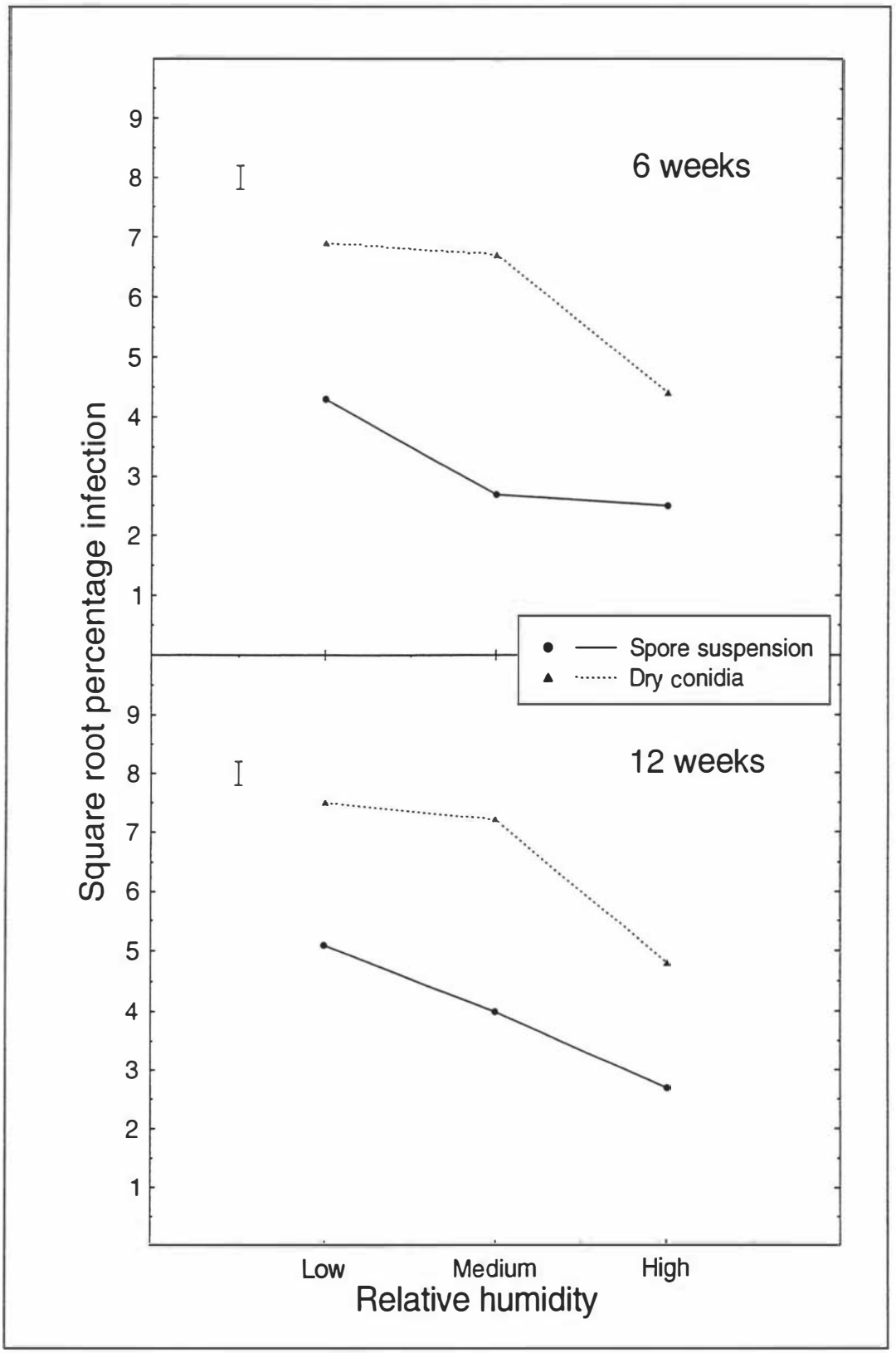


Figure 5-15. 1994: Interaction between relative humidity and type of inoculum on *B. cinerea* storage rot incidence of kiwifruit cured at 10°C. Vertical bars indicate overall standard error of the mean (SEM).

Spore suspensions resulted in fewer infected fruit with each successive harvest but dry conidial inoculation did not continue this trend (Fig.5-14) after harvest two.

The higher significant level of interaction between inoculum type and relative humidity at six than at 12 weeks coolstorage was caused by the greater divergence of disease levels in the medium relative humidity at six weeks coolstorage (Fig.5-15).

DISCUSSION

Physiological response during curing and coolstorage periods:
In the present study, temperature, relative humidity and length of curing influenced fruit weight loss during both the curing process and subsequent coolstorage. In general, the percentage weight loss observed in kiwifruit from all experiments increased at higher curing temperatures and with longer curing periods (*Experiments No.1 & 2*), and decreased with increased relative humidity during curing (*Experiments No.3 & 4*). The effect of curing on weight loss has been widely studied in tuber crops such as sweetpotatoes (Kushman 1975; Delate *et al.* 1985; Walter *et al.* 1989), potatoes (Picha 1986b; Kitinoja 1987; Morris *et al.* 1989), yams (Passam *et al.* 1976) caladium and *Zantedeschia* tubers (Marousky & Harbaugh 1976; Funnell 1988) and also in crops such as citrus (Ben-Yehoshua *et al.* 1987a).

There is general agreement from these studies with the current work that the curing environment, especially temperature and relative humidity, influence weight loss both during the curing process and in subsequent coolstorage. In the present study, high weight loss was found after two days of incubation at temperatures of 10°C and above. Similar commercially adverse effects on fruit quality by high temperatures have been reported in citrus (Ben-Yehoshua *et al.* 1987a), although in that study an additional

practice such as sealing played an important role in fruit weight loss since they reported 18% of weight loss during storage at 17°C over a period of 32 days of cured and not sealed citrus, compared with 3.1% in cured and sealed fruit. Kushman (1975) reported that weight loss of artificially injured and cured sweetpotatoes at 28°C for nine days decreased with increasing relative humidities from 80, 90, 97 and 100% but the weight loss recorded during the subsequent storage of five months at 15°C, 85% relative humidity was similar to fruit from all treatments.

In this present study, weight loss after the three days of curing for all temperatures and harvest dates, was less compared with weight loss values recorded after six or 12 weeks storage at 0°C. However daily percentage weight loss for the first curing day, for all temperatures (second and third harvests) was higher compared with the second and third curing days. Similar findings to this every day weight loss behaviour have been reported by Walter *et al.* (1989). They reported that in general the highest percentage daily weight loss of sweet potatoes was recorded during the first days of curing. Funnell & MacKay (1988) reported similar weight loss behaviour during the first days of curing (30°C) regardless of relative humidity (80-84% or 40-44%). In this present study with exception of the first harvest (*Experiment No.3*) differences in weight loss between the medium and low relative humidities were clear.

Although in other experiments carried out in vegetable crops such as peppers (Capsicum annuum L) (Lownds *et al.* 1994) and fruit crops such as guava (Vazquez-Ochoa & Colinas-Leon 1990), apples (Lidster & Porrit 1978; Lidster 1990) durians (*Durio zibethinus* M.) (Ketsa & Pangkool 1994), carambolas (*Averrhoa carambola* L.) (Miller *et al.* 1990); yellow passion fruit (*Passiflora edulis* f. *flavicarpa* D.) (Arjona *et al.* 1992) and papaya (*Carica papaya* L.) (An & Paull 1990), the effects of temperature and/or relative humidity as a curing practice were not evaluated on weight loss, similar tendencies to those found in the current study were reported ie. weight loss

increased at higher temperature and at lower relative humidities.

Changes in firmness of cured fruit is another attribute of fruit quality to consider during curing and subsequent coolstorage of fruit. In 1992 (*Experiment No.1*), inoculated, cured kiwifruit, cured at any temperature were firmer than the corresponding inoculated, uncured fruit during the curing period but the differences had disappeared by the end of coolstorage. Similar results were obtained in 1994 (*Experiment No.2*) although in this case the fruit cured at 20°C from the second and third harvests and at 10°C from the second harvest were still significantly firmer than uncured fruit after three months coolstorage. Curing appears to have had a beneficial rather than adverse effects on fruit firmness except when fruit was cured at 30°C. At this temperature there was a loss of firmness and other adverse effects (e.g. increased rots). Clearly 30°C is a temperature which is marginal for fruit treatment. In this respect kiwifruit would appear unable to tolerate the temperatures to which various cultivars of apples can be exposed since there are reports of increased firmness with increased temperature from 21 to 48°C ((Liu 1978; Porrit & Lidster 1978; Klein & Lurie 1990; Lurie & Klein 1990; Klein & Lurie 1992). The effect of temperature during long-term storage may be quite different from that during curing. Vazquez-Ochoa & Colinas Leon (1990) found that guavas kept at ambient temperature (20°C) had a lower firmness than those stored at 3, 4, 7 or 11°C. Commercially, kiwifruit are always stored at 0°C.

Other studies carried out in apples showed retention in fruit firmness regardless of relative humidities (Lidster & Porrit 1978). Further studies, found that firmness in apples gradually decreases with decrease in relative humidity after storage at 3°C for various months (Lidster 1990). In the present study, with exception of fruit from the second harvest (*Experiment No.3*) fruit firmness after three days curing was not influenced by relative humidity (*Experiments No.3 & 4*). As with the current study, no significant difference was reported in fruit firmness of durians when stored at three

different relative humidity levels 75, 83 or 93% at 30°C for six days (Ketsa & Pangkool 1994). However other studies carried out in guavas, reported significantly higher firmness on guavas stored at 80 or 88% relative humidity compared with 55% ambient relative humidity (Vazquez-Ochoa & Colinas-Leon 1990)

In the current study changes in fruit firmness during storage from one season to another were observed (*Experiments No.1 & 2*). Results of this study showed that harvest date probably had more influence over fruit firmness during storage than curing environment. For example, in *Experiment No.2* although there were significant differences among treatments, the low firmness recorded during storage in fruit from all curing temperatures could be explained not only by the curing period but also by the initial low firmness (6.6 kgf) recorded at harvest as well. Kiwifruit (*Experiment No.2*) during coolstorage showed the typical curve of firmness loss reported by Reid & Harris (1977) and Crisosto *et al.* (1984). In the present study the combination of those ambient factors maturity and relative humidity influenced fruit firmness. Although fruit firmness was affected by harvest date (*Experiment No.4*) a consistent decrease of firmness with harvest maturity was not apparent in this study either at harvest or after the curing period. Differences in yearly firmness in kiwifruit at harvest have been already explained by Reid & Harris (1977).

In this study the effects of curing temperature/incubation time were evident on ethylene production of kiwifruit (*Experiment No.1*) since higher curing temperatures increased ethylene production in kiwifruit. Porrit & Lidster (1978) reported that ethylene production in apples gradually increased with incubation time (10 days) at temperatures at 21°C while apples incubated at 38°C had lower ethylene production. They reported that at 38°C ethylene tended to decrease during the 10 days incubation. Similar inhibition of ethylene production with high temperatures was reported by Klein (1988) & Klein & Lurie (1990). However they found that with high temperatures

(38°C) apples showed low ethylene production, when they were stored at 0°C but when returned to ambient temperature (20°C) ethylene production was reactivated. In the present study, with the exception of fruit from the inoculated control during subsequent coolstorage, ethylene production did not follow any specific pattern according to the previous temperature/incubation period. Apparently in the current study, ethylene produced during curing did not affect ethylene production of fruit during storage. For example in fruit cured for four days at 10°C there was ethylene production (0.29 µl), however fruit from this treatment coolstoraged for one month did not produce ethylene.

In the current study, except for fruit cured for six days at 10°C, the rate of respiration in kiwifruit increased proportionately to curing time but incubated kiwifruit showed a decline in respiration rate with increased temperatures. Carbon dioxide production during coolstorage did not show any specific trend respect to the curing temperature/time of incubation. Probably the lack of a climacteric respiratory peak observed in this study can be explained by differences in the climacteric rise behaviour typical of this fruit as reported by Reid *et al.* (1982) and Mitchell (1990).

Apparently there was no relationship between ethylene concentration and carbon dioxide production as has been reported in other fruit crops such as pears (Maxie *et al.* 1974). In their study, respiration rates of pears exposed to 100 ppm ethylene were increased. Similarly in other studies, sweetpotatoes cured at 30°C with ethylene for three days increased carbon dioxide production by 30% more than the control during curing while no effect between ethylene and rate of respiration was reported during storage at 15°C for eight days (Kitinoja 1987). In the present study, respiration rates of fruit incubated at 0, 10 or 20°C for two days did not correspond to the zero ethylene concentration observed in these treatments.

In the current study the pattern of ethylene production did not correspond

with the pattern of loss of firmness (*Experiment No.1*). For example kiwifruit firmness from the inoculated control decreased from the second to the fourth incubation day, while there was no ethylene production at either of these two incubation periods.

Chemical composition of fruit

In general neither curing temperature nor relative humidity affected the total solid solubles concentration of kiwifruit. There were significant differences in TSS in fruit from different curing treatments in subsequent storage periods (*Experiment No.1*) but in general those values were high among all treatments. Similar results to this present study have been reported in other fruit crops such as apples, passion fruits and durians (Porrit and Lidster 1978; Klein & Lurie 1990; Lidster 1990; Lurie & Klein 1990; Arjona *et al.* 1992; Ketsa & Pangkool 1994). In those studies, any significant changes in TSS levels were reported during heat or relative humidity treatments and subsequent storage. Clearly in this current study, those changes observed in TSS were more related to the harvest date and fruit maturity than temperature and/or relative humidity combinations. In this present study TSS increased with fruit maturity (*Experiments No.3 & 4*). The high TSS values in 1991 (*Experiment No.1)* during curing and storage periods can be explained by the initial high values at harvest (10.8%) in spite of the harvesting date (11th May). These values differed from those recommended for export purposes. According to Lallu (1989a); Mitchell (1990) and Hopkirk (1992) the more suitable TSS at harvest in New Zealand to avoid postharvest problems during storage should be 6.2%, although a range between 7 to 10% is acceptable. In the present study, as reported by others (Hopkirk *et al.* 1986), seasonal differences were also observed in fruit at harvest from 1992 to 1994. Fruit TSS at harvest in the three years of experimentation varied from 10.8% the first year, 8.8 to 10.6% the second and 6.8 to 9.2% in the third year.

In the current study the content of sugars during fruit coolstorage varied

regardless of the previous curing temperature. In general glucose and fructose increased with storage period. Studies on kiwifruit carried out by Nicolas *et al.* (1988) reported that the amount of reducing sugars stored at 0°C for a period of 26 weeks increased during the first five to 10 weeks of storage but remained constant for the remaining storage period. Similar findings were reported by Mac Rae *et al.* (1989a; 1989b) in fruit from different regions and maturities or in fruit stored at 4 or 0°C for up to 12 weeks. In both studies they found glucose was the main individual sugar, followed by fructose and sucrose. With the exception of the glucose content of fruit from the inoculated control after three months storage, the amount of sugars (sucrose, glucose and fructose) in fruit from the inoculated control was higher than the treated fruit. In general agreement with these results, Heatherbell (1975); Okuse & Ryugo (1981); Reid *et al.* (1982); Ben-Arie *et al.* (1982); Matsumoto *et al.* (1983) and MacRae *et al.* (1989b) concluded that glucose was the main individual sugar of kiwifruit, followed by fructose and in lesser amount, sucrose during fruit ripening, fully mature fruit or in fruit previously incubated at low temperatures at 4 or 0°C for a period of time. Similarly Pesis *et al.* (1991), reported that the amount of glucose was higher in the non-infected tissue zone (by *B. cinerea*) of kiwifruit stored at 0°C for a period of 15 to 20 weeks, compared with lower amounts of the invaded or infected zone of the fruit. In the present study kiwifruit were artificially inoculated but only fruit showing no symptoms of diseases were taken for chemical analysis.

In the present study pH decreased and acidity increased with time during fruit coolstorage. There were significant differences in pH levels of fruit during the coolstorage but in general the range of pH was between 3.3 to 4.0. Ben-Arie *et al.* (1982) reported that stored kiwifruit cv *Bruno* at -1°C cv pH values significantly increased for about 7% (3.7 to 4.0) and percentage titratable acidity decreased about 40% (1.01 to 0.5%). Matsumoto *et al.* (1983) found similar pH ranges in kiwifruit harvested at mature green stage and kept at 20°C for 20 days for ripening. In that study non-treated fruit had

a pH of about 3.3 and 1.4% of total acidity. However Pesis *et al.* (1991) reported a similar pH (3.5) in fruit from infected tissue compared with 4.1 from the non-infected tissue. In that study, acidity levels were lower than those found in the current study.

Infection levels during coolstorage

In this study curing conditions influenced percentage disease of kiwifruit during coolstorage. In 1991 (*Experiment No.1*) a curing temperature of 10°C for two to six days reduced *B. cinerea* infection of fruit during storage, compared with those held at 0, 20 or 30°C. In 1994 (*Experiment No.2*) the same temperature of 10°C reduce *B. cinerea* in fruit from the first harvest, but higher curing temperatures at 15°C or 20°C also reduced *B. cinerea* in fruit harvested at the second and third harvests. In both 1993 and 1994 (*Experiments No.3 & 4*) fruit cured at the lowest relative humidity ranges gave the highest infection levels during fruit coolstorage. Although in other studies the effect of temperature was not studied as a curing practice, similar findings were reported by Hyre (1972) on incubated geranium leaves (*Pelargonium hortorum*) infected by *B. cinerea*. This researcher observed smallers lesions (1.5 cm) when incubated at 10°C for three to seven days, compared with 25°C (8.5 cm). Mridha & Wheeler (1993) found significantly lower infection by *A. brassicae* on old leaves of oilseed rape incubated at 10°C compared with temperatures of 15, 20, 25 and 29°C for 8h. They observed the greatest number of spore penetrations at 25°C and the highest number of lesions at 20°C with a 24h period of wetness. In studies on kiwifruit in which fruit were cured for two or seven days at ambient temperature, Sharrock & Hallet (1991; 1992) observed lower spore germination and germ tube development on the stem scar surface of those fruit cured for seven days than when cured for only two days. In the present study less percentage infection was not observed when incubation time increased since higher infection levels were recorded in fruit incubated for four or six days at 20°C or 30°C compared with levels of infection after two days curing. Other studies carried out by Pennycook & Manning (1992)

reported that in kiwifruit held, at 14°C ambient temperature for one, two, four and seven days, *B. cinerea* infection were gradually reduced with an increase in the duration of curing. In the present study the higher infection levels that developed after curing at temperatures higher than 10°C and the rapid onset of symptoms in fruit cured at 20°C for six days or at 30°C for up to two days, may be associated with heat damage to the fruit which would facilitate *B. cinerea* infections. Bartz *et al.* (1991) found that bacterial soft rot on inoculated tomatoes began earlier and progressed more rapidly in fruit stored at 30 or 35°C for 24h than in fruit stored at 15 or 25°C. After 72h most of the fruit stored at 25, 30 or 35°C showed bacterial disease though those at 15°C were relatively disease free. Ben-Yehoshua (1987b) suggested that the faster growth of *P. digitatum* on pummelos and lemons cured at 30°C for one day compared with the non-cured fruit can be associated with the curing conditions that affect the fruit membranes in a manner that made nutrients more available to the pathogen which could then grow faster.

Alderman & Nutter (1994) reported that in order to increase leaf spot disease (*Cercosporidium personatum* Berk. & M.A. Curtis) of peanuts (*Arachis hypogaea*) it was necessary to incubate peanut plants in an additional treatment at 50-60% relative humidity and 16h/day fluorescence light photoperiod. However in other reports, it was observed complete inhibition of sporulation of *Helminthosporium maydis* sporulation on corn plants (*Zea mays*) when incubated at relative humidities lower than 93% (Hyre 1974). Probably in the current study, temperatures of 0°C and 5°C and the lowest humidity ranges, suppressed the development of resistance mechanisms which suppress *B. cinerea* in kiwifruit as reported by Poole *et al.* (1993). Mechanisms such as enzyme activity, production of lignin and changes in tissue chemical composition were slowed down compared with those in fruit held at higher temperatures and higher relative humidities. For example, in those studies, high enzymatic activity such as lipoxygenase, phenylanaline ammonia lyase and peroxidase were observed immediately

after harvest and during curing. Another explanation for the infection levels recorded in the current study could be related to the distribution of *B. cinerea* toxins and kiwifruit phenolic compounds produced during the infection process as has been reported by Harrison (1980). This researcher studied the influence of relative humidity on the diffusion of toxins produced by *B. fabae* on leaves of broad beans. He suggested that toxins produced by *B. fabae* at 100% relative humidity diffused throughout the leaf while at lower relative humidities (75%), they concentrated in certain areas of the leaf, hence the highest disease severity was found at the highest relative humidity.

In the present study, the highest infection levels were at the lowest relative humidity and could be associated with the highest weight loss of fruit held at these relative humidities, hence enhancing infection levels. In other studies, a positive correlation between weight loss and infection levels during sweetpotatoe storage has been reported (Kushman 1975). In that study, curing and storage conditions at 26°C for seven days and later at 15°C for five months respectively, showed that percentage decay increased with weight loss increased (above 6%).

Conversely to the present results, Marois *et al.* (1988) reported an overall linear and inverse correlation with vapour pressure deficits between the susceptibility of greenhouse grown rosa flowers *(Rosa hybrida*) to *B. cinerea*. They reported higher susceptibility with a VPD of 0.7 Kpa compared with 1.05 Kpa. In the present study in both, *Experiments No.3 & 4*, infection levels significantly increased with VPD increase: the highest infection levels were recorded in these VPD ranges of 0.70 - 0.24 Kpa compared with the lowest infection in fruit held at a VPD of 0.09 - 0.02 Kpa. The difference in infection levels between Marois *et al.* (1988) study and the present results, can also be accounted for by the environmental temperature. For example, Marois *et al.* (1988) reported, that greenhouse temperatures during the two day treatment fluctuated between 15 to 22°C,

while the temperature used in this study was 10°C for three days.

Another important environmental factor such as dew period or period of wetness on onion leaves, inoculated with *B. squamosa,* has been extensively investigated by Shoemaker & Lorbeer (1971; 1977); Alderman & Lacy (1983) and Alderman *et al.* (1985). They reported that the longer the wet periods the greater the infection levels regardless of the type of inoculum (spore suspension or dry conidia). Although in those studies the relative humidity of the dew chamber was not controlled it was in general high. Similarly, Mansfield & Deverall (1974) reported that infections of *B. cinerea* on leaves of broad beans only took place if droplets of water were present to permit establishment of a spreading disease. They reported higher infection levels (75%) after six days at 17 ± 2°C when droplets of *B. cinerea* suspension persisted throughout the first days compared with a lower infection (17%) in sites where droplets were absent after the first day of inoculation. In the present study, there was no constant wet period during the curing treatments, but during the subsequent kiwifruit coolstorage, there was some condensation of water, since fruit was wrapped in polyethylene liners. This condensation was on the liners and may not have affected the infection process since curing was completed at this stage.

In the current study, contrary to the general belief (Sharrock & Hallet 1991) infection levels were higher at both storage periods (93% and 76% respectively) when dry conidia were applied to the stem scar compared with those recorded from inoculations with spore suspensions. Snow (1949) and Jarvis (1962) reported that although spore germination of *B. cinerea* on strawberries was faster in the presence of a droplet of water compared with germination in a saturated atmosphere, eventually, dry conidia could germinate.

As with other studies on kiwifruit (Beever 1979; Pennycook 1981), in this study, in general, fruit maturity influenced infection levels during fruit

coolstorage. For example in *Experiment No.4* °brix levels increased from 6.8% to 9.2% (first and fourth harvests respectively) while infection levels after 12 weeks coolstorage decreased from 57.8% to 22.4%.

Throughout this study, it was also observed that infection levels varied from year to year, for example in 1991 fruit from the inoculated control showed 7.9 percentage infection while percentage infection of fruit from the 1994 season was 38.1%. Similar seasonal differences in infection levels of kiwifruit during coolstorage have been reported in kiwifruit (Brook 1990a; Hopkirk *et al.* 1990b) and in other commodities such as potatoes (Malcomson & Gray 1968; Adams & Griffith 1978; Stewart *et al.* 1983; Hide & Boorer 1991; Merida & Loria 1994). In general agreement, these authors mentioned weather conditions in the orchard and pre and postharvest handling operations as the main reasons for these fluctuations.

CHAPTER SIX

ANATOMICAL AND HISTOCHEMICAL STUDY OF INOCULATED KIWIFRUIT STEM SCARS DURING CURING

INTRODUCTION

Curing and wound healing

It is well established that in many diseases of fruits and vegetables the pathogens gain entrance through wounds. According to Davies (1987) wounding includes physical damage to the plant tissue and cells, especially mechanical disruption of cell membranes, that eventually lead to loss of compartmentation of ions. It is well known that many of these wounds have the ability to heal by a number of different mechanisms (Bostock & Stermer 1989). One of the main objectives of curing is to encourage the repair of wounded areas as soon as possible thereby rebuilding the mechanisms of defense such as structural barriers and/or production of antifungal substances near to the damaged site. The more rapid the curing process the more chance of avoiding pathogen attack or arresting further invasion from incipient infections.

Structural changes have been reported as an important aspects involved in wound repair as a mechanism of defence against plant pathogens (Ride 1975; 1978; Passam *et al.* 1976; Cline 1983; Biggs 1984; 1986; Rittinger *et al.* 1987; Larson 1994). The process of wound healing in plants has been classified by Bostock & Stermer (1989) according to the cell activity involved. They considered that three types of tissue repair can exist: In one type there is an autolysis of the infected dead cells as occurs in carrots; In another type there is both regeneration of cells and cellular depositions as in kohlrabi tubers and in the third there is a more complex mechanism of

wound repair that includes these two cellular mechanisms plus redifferentiation of parenchyma cell walls to form a suberized periderm layer in the damaged site, as occurs in potatoes.

Anatomical and/or histochemical responses during wound healing

The induction of structural barriers is one of the most common processes that occur in response to pathogen invasion and during wound healing. For example, the reported response to infection by different pathogens such as *Phytophthora* spp, *Verticillium* spp or *Ceratocystis ulmi*, include: alterations in the vascular system e.g. (as gum duct formation in *Citrus* trees), cell wall multilayering and tyloses development e.g.(in american elm, *Ulmus americana* L.) and in avocado rootstocks, (*Persea americana* M.) and vessel coatings and plug formation in leaves of various vegetables e.g. (snapdragons, *Anthirrhinun majus* L., eggplants, *Solanum melogena* L. and potatoes) and in shoots and branches of trees e.g. (hop plants, *Humulus lupulus* L., sycamore maple, *Acer pseudoplatanis* L. and hedge maple, *Acer campestris* L.). (Ouellette 1978; 1980; 1981, Robb *et al.* 1979; 1982, Phillips *et al.* 1987).

Periderm development and cellular suberization and lignification have been reported as a response to damage by mechanical activities and to infection by pathogens such as *Fusarium roseum* f.sp. *sambucinum*, *Cladosporium cucumerinum*, *Colletotrichum lagenarium* in various vegetables such as potatoes, yams, sweet potatoes and leaves and seedlings of cucumbers, (Passam *et al.* 1976; Hammershmidt & Kuc 1982; Hammershmidt 1984; Dean & Kuc 1987). In kiwifruit, preliminary anatomical studies on stem scars of fruit infected by *B. cinerea* and incubated at various temperatures showed no apparent evidence of structural changes of the vasculature and surrounding tissues (Poole & McLeod 1991; Sharrock & Hallet 1991). Similar studies in kiwifruit suggested the presence of antifungal compounds such as tannins, phenolics and alkyl aldehydes as a mechanism of defense against *B. cinerea* in the stem-end of the fruit (Poole & McLeod 1991).

Additional histochemical studies in onions (Moon *et al.* 1984), carrots (Garrod *et al.* 1982), in a range of avocado rootstocks (Phillips 1993), cucumber (Walter *et al.* 1990) and in some wheat varieties (Ride 1975) have demonstrated the activation of suberin, lignin or phenolic compounds, when plants were artificially wounded and/or inoculated by various pathogens. On inoculated apples, peaches and almond bark similar histochemical components were observed to develop in response to infection by various pathogens such as *Leucostoma cincta*, *L. persooni*, *Phytophthora* spp, *Botryosphaeria obtusa*, *B. dothidea* (Biggs 1986; Doster & Bostock 1988; Biggs & Britton 1988).

Factors affecting response to wound healing

The process of wound repair is closely linked to external factors such as temperature, relative humidity, light, oxygen, carbon dioxide production and harvesting period. Biggs (1993) reported that in general wound healing is more rapid at 20 to 25°C with relative humidities between 70% and 100% and at 10°C between 80% and 100% relative humidity. He also suggested that the type of anatomical changes developed were determined by the relative humidity.

Biggs (1993) also considered that temperature is one of the most important environmental factors to influence the rate of wound healing in woody plant species and fruit trees. He found a reduction in wound repair time (i.e. in development of ligno-suberized layers) in potted plants with an increase in temperature.

Studies on uninoculated, wounded apples, have shown that meristematic cell division can vary according to harvest date and temperature. Apples harvested at the beginning of the picking season showed complete healing, but healing was delayed on those harvested late and no healing was observed in those kept at 3°C after harvest (Skene 1981). Further studies in mature apple fruit showed that development of resistance to *B. cinerea*

and to *P. expansum* occurred in those fruit incubated for 38 days at 5°C or for 14 days at 20°C (Lakshminarayana *et al.* 1987).

In potato tubers the most suitable environmental conditions for the wound repair process may be different. For example, Wigginton (1974) observed a stimulation of cell division and inhibition of suberization in cut potato tubers held at relative humidities closest to 100% and also a similar inhibition of suberization at low relative humidity. This researcher considered the most appropriate range of relative humidity and temperature for the process of suberization was 70%-100% at 20°C. Meijers (1987), found that rates of wound healing on potatoes can vary according to the temperature and time of curing. Results of this study showed that the highest temperature (30°C) and 80% relative humidity accelerated the healing process. Morris *et al.* (1989) reported a temperature/relative humidity combination of 25°C and 98% relative humidity as optimum for wound healing to induce resistance against *Fusarium oxysporium* and *Erwinia carotovora* pv *carotovora*. They also found that temperature was a more important factor for wound repair than relative humidity.

OBJECTIVE

To ascertain whether physical barriers and/or antifungal substances develop on inoculated kiwifruit stem scars during the process of wound healing.

MATERIALS AND METHODS

Fruit samples

Five samples of healthy fruit from *Experiment No.1, Chapter 5* were incubated for two, four and six days at 0 and 10°C, for two and four days at 20°C and for two days at 30°C to carry out the anatomical and histochemical study. Since fruit from the 20 and 30°C incubation treatments for six and two days respectively showed early symptoms of softening and

of disease, and since sectioning of fresh or paraplast embedded stem scars showing mycelial growth was difficult because of disintegration of the tissue, these treatments were not included in the present study.

Tissue preparation for anatomical and histochemical study

Stem scar tissue samples of each fruit were longitudinally hand sectioned to blocks about 1 to 2 mm (Plate 6-1) and fixed by vacuum infiltration overnight in a solution of formalin: acetic acid: and alcohol (FAA). Samples were washed with water 4-5 times to remove excess fixative before dehydration. By the modified procedure of Feder & O'Brien (1968), samples were then dehydrated and infiltrated with Paraplast. They were sectioned at 5-8µm thickness with a Jung Rotary microtome.

Anatomical and Histochemical staining

After removal of the paraffin by xylene and passage of slides through an ethanol series (Bautista-Baños 1989) the following two staining procedures were used as a general staining procedure to highlight different tissues: a double stain of 1% methyl violet and 1% eosin and a safranin-aniline blue combination (Johansen 1940). Specific tissue stains were a) Phloroglucinol-HCl and Toluidine Blue (Peacock & Bradbury 1973) for lignin; b) Safranin and Fast Green (Bautista-Baños 1989) for lignin and cellulose respectively; c) Sudan IV (Johansen 1940) and Sudan Black B (Peacock & Bradbury 1973) for suberin and cutin respectively and d) Glycerine-ferricyanide (Sherwood & Vance 1976) for reducing compounds. Slides for histochemical studies were mounted in a 2% $CaCl_2$ and glycerine solution (Herr 1992) and specimens for anatomical studies were mounted in D.P.X. Light micrographs were taken with a Nikon fx-35wa camera mounted on a Reichert Diapan phase contrast microscope and on an Olympus dissecting microscope.

Details of fixative and stain preparation and procedures are given in the Appendix.

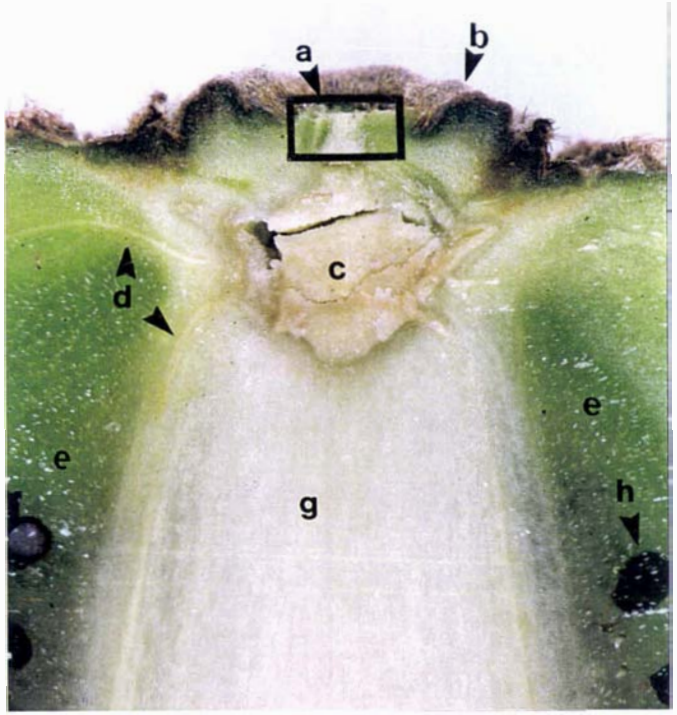


Plate 6-1

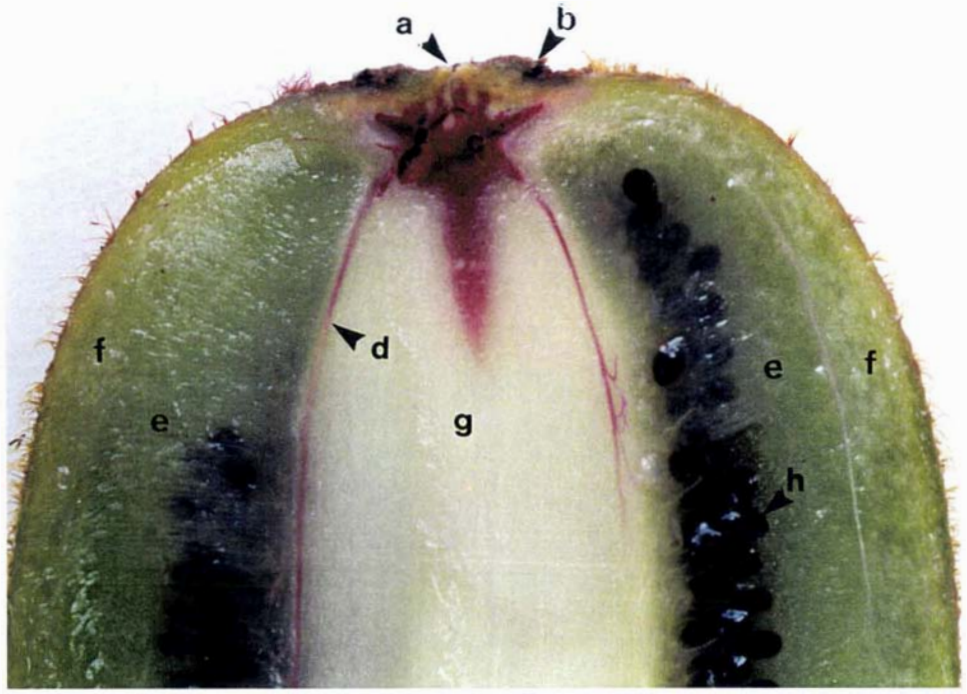


Plate 6-2

Longitudinal section of a fresh kiwifruit. (Plate 6-2 stained with Phloroglucinol-HCl. mg. x1.8).

a) stem scar surface (Plates 6-9 - 6-14)
b) suberized shoulder
c) sclerified plug
d) vascular strands
e) inner pericarp
f) outer pericarp
g) core
h) seeds

RESULTS

Anatomical components of stem scar tissues

The kiwifruit stem scar is a circular area of broken tissue about 3-4 mm diameter surrounded by a raised shoulder of suberised skin (Plate 6-1). The surface consists of parenchyma cells with a small number of vascular bundles arranged in a circular pattern. These vascular bundles contain lignified xylem vessels that spread out over a woody, sclerified plug (Plate 6-2). The main body of the kiwifruit is composed of a central core, an inner pericarp where the seeds are formed, an outer pericarp and hairy skin (Plates 6-1 & 6-2).

The pedicel attachment is very narrow at the point where it is snapped off the fruit (Plate 6-3). The central vascular core of the pedicel diverges into three vascular bundles just above this point (Plate 6-4) and these in turn are further subdivided to give five or six at the level of the tip of the sclerified plug (Plate 6-5). The upper surface of the sclerified plug is not smooth but is formed by a number of irregular ridges (Plate 6-6). The vascular tissues now consist of a very large number of small units. Part way down the plug, the vasculature is intimately associated with the stem plug but branches spread out and ramify between the inner and outer pericarp (Plate 6-6 & 6-7). After this point, the vascular strands separate from the plug and continue through the fruit between the core and the inner pericarp. The developing seeds are supplied by side branches of these vascular strands (Plate 6-8).

The kiwifruit stem scar above the sclerified plug consists of two main tissue systems: Fundamental or ground tissue and vascular tissue. The ground tissue consists of parenchyma, collenchyma and sclerified cells (Plate 6-9). Idioblasts containing depositions of elongated calcium oxalate crystals (raphides) were particularly distributed among the parenchyma cells and along and parallel to the xylem vessels (Plate 6-10).

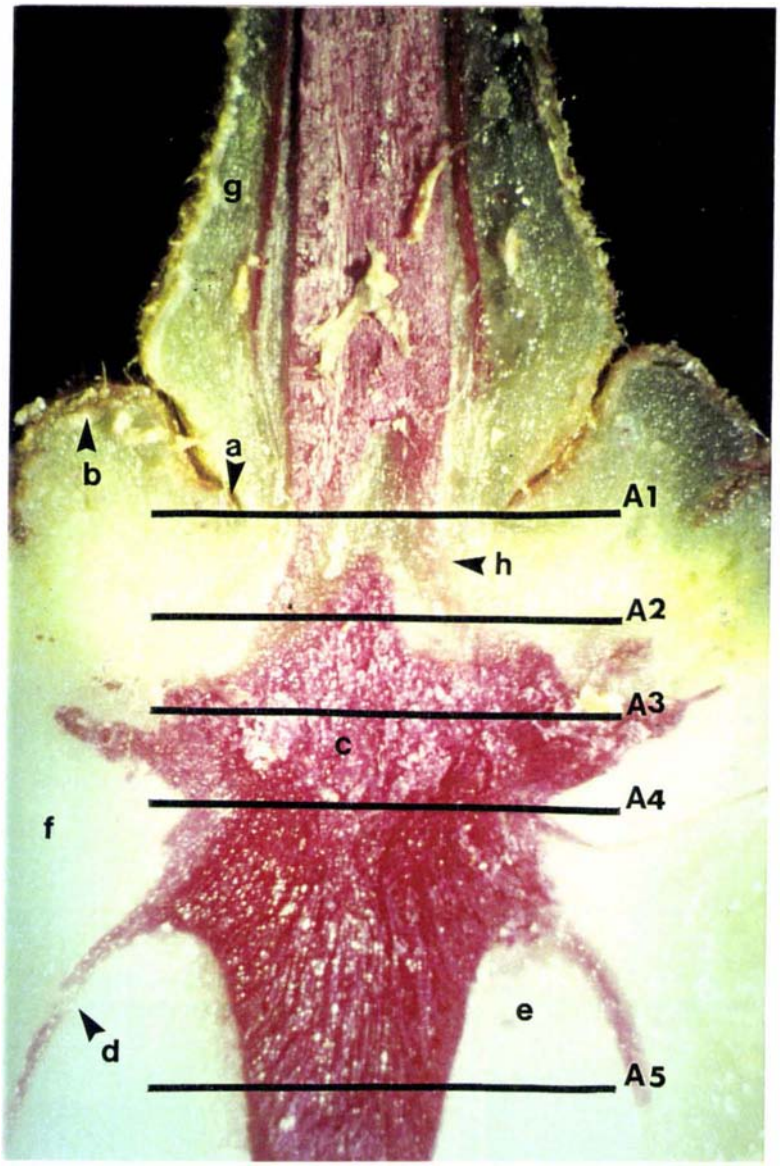


Plate 6-3. Longitudinal section of a fresh kiwifruit with pedicel attached stained with Phloroglucinol-HCl.(mg. x0.7). Positions of the cross-sections shown in Plates 6-4 -6-7 are indicated with horizontal lines. Section A1 (Plate 6-4), section A2 (Plate 6-5), section A3 (Plate 6-6), section A4 (Plate 6-7) and section A5 (Plate 6-7).

a) stem scar surface
b) suberized shoulder
c) sclerified plug
d) vascular strands
e) inner pericarp
f) outer pericarp
g) pedicel
h) point at which pedicel vascular tissue divides into three strands.

Plate 6-4. Cross section (A1) of a fresh kiwifruit stem scar stained with Phloroglucinol-HCl (mg. x4.0). At the union between pedicel and fruit, there is a circular arrangement of three vascular bundles (v) surrounded by, parenchyma and the suberized tissue from the fruit shoulder (b).

Plate 6-5. Cross section (A2) of a fresh kiwifruit stem scar stained with Phloroglucinol-HCl (mg. x1.8). The original three vascular bundles (v) have divided to form five. The upper most point of the sclerified plug (p) can be seen in the centre.

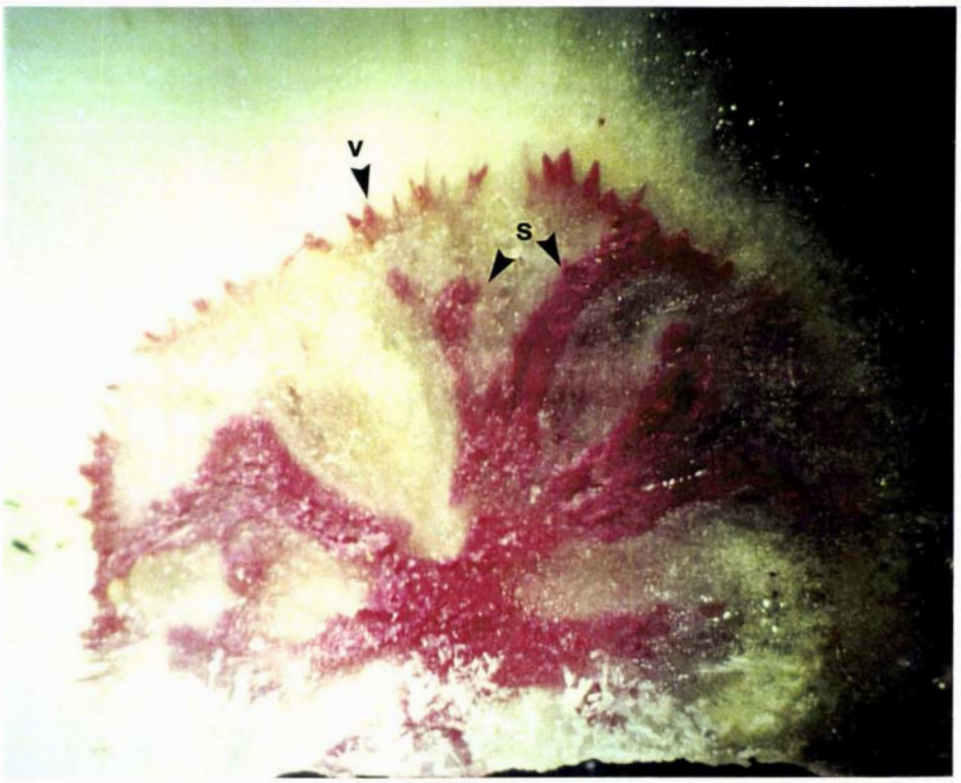


Plate 6-6. Cross section (A3) of a fresh kiwifruit stained with Phloroglucinol-HCl (mg. x0.7), showing radial development of sclerified tissue (s) on the upper surface of the plug and vascular bundles (v).

Plate 6-7. Cross section (A4) of a fresh kiwifruit stained with Phloroglucinol-HCl (mg. x0.7). The sclerified plug (p) is well defined and vascular bundles (v) are diverging between the inner and outer pericarp.

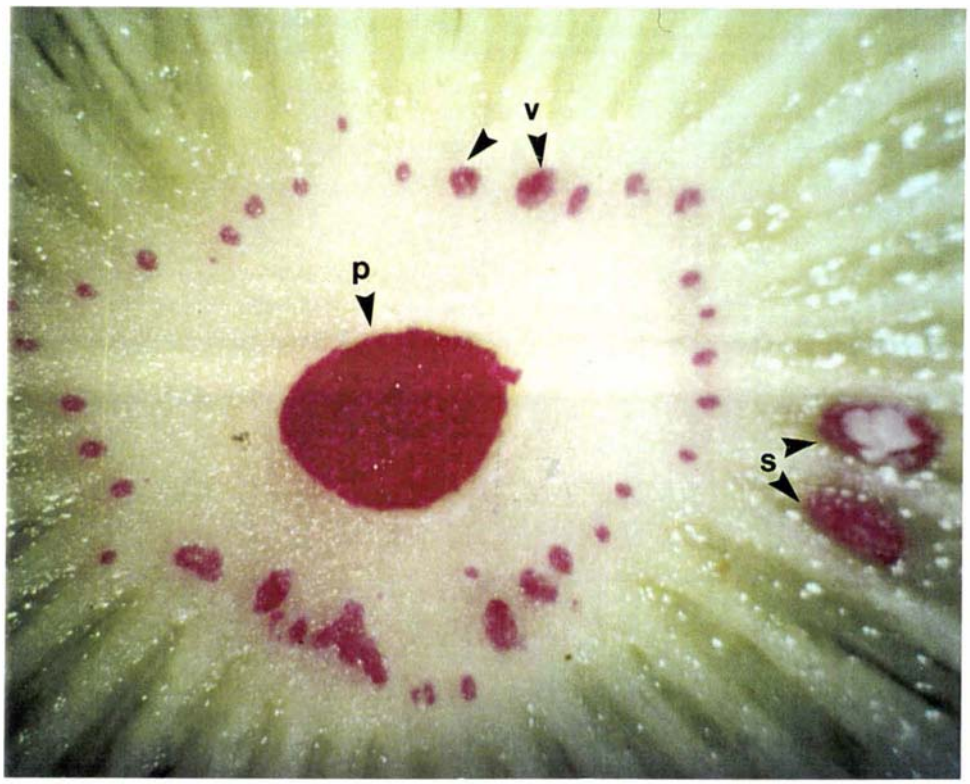


Plate 6-8. Cross section (A5) of a fresh kiwifruit stained with Phloroglucinol-HCl (mg. x0.7). The lower, compacted region of the sclerified plug (p) is surrounded by well defined vascular bundles (v) and seeds (s).

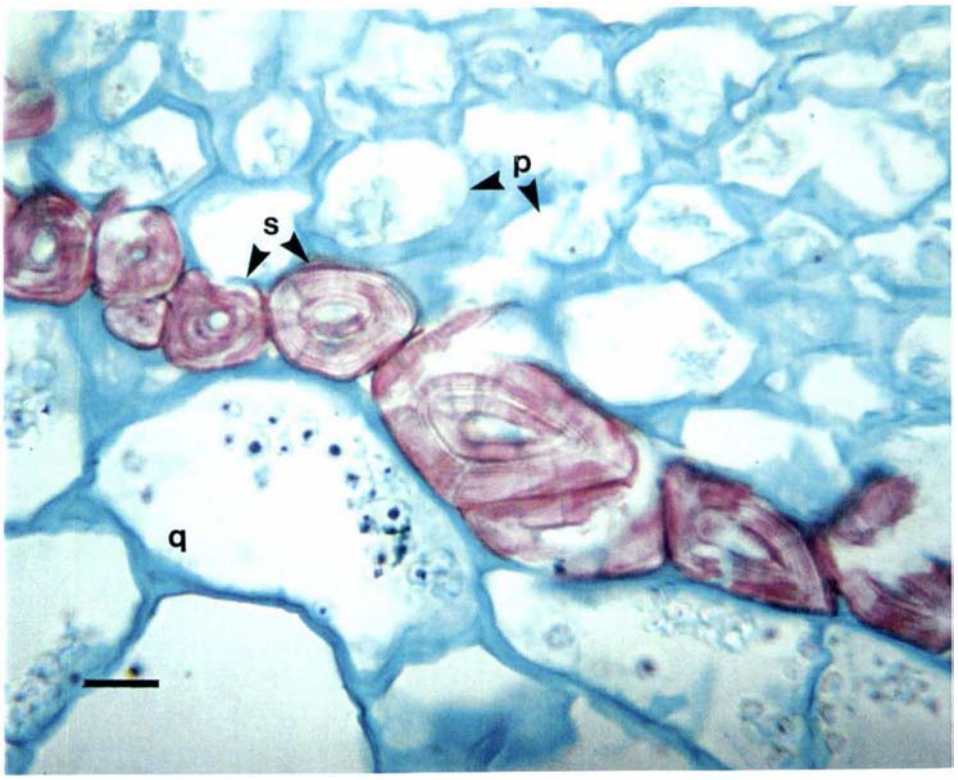


Plate 6-9. Longitudinal section of a kiwifruit stem scar stained with safranin-fast green showing parenchyma (p), sclereids (s) and collenchyma (q) cells. Bar indicates 0.2 mm.

In general, the vascular tissue system was composed of xylem (with helical thickening) (Plate 6-11), phloem and cambium.

At 20 and at 30°C spore germination and penetration of hyphae of *B. cinerea* through the xylem vessels of kiwifruit were observed (95%) after 2, 4 and 6 days of incubation (Plate 6-12). By contrast, after 2 days (Plate 6-13) at 0 or 10°C most of the spores scattered on the surface of the xylem vessels and parenchyma tissue had not germinated (75%) and of those spores that had germinated little hyphal growth had occurred. After 4 and 6 days at 10°C germination was still rare but was common at 0°C. There was no development of tyloses from neighbouring parenchyma cells at any temperature. At 10°C there was evidence of cellular thickenings of the parenchyma cell walls where they were in contact with the conidia of *B. cinerea* which had commenced germination (Plate 6-14).

Histochemical tests

Histochemical tests showed some differences between fruit incubated at 0, 20 or 30°C and those incubated at 10°C but the sole difference between times of incubation was with the test for reducing compounds (Table 6-1).

a) Lignin. The Phloroglucinol and Toluidine Blue tests gave a deeper colour reaction in the xylem vessels and in the parenchyma cell wall thickenings of the 10°C samples and a faint colour in the axial parenchyma of the vascular system. The double staining procedure with Safranin and Fast-Green stained lignified tissue in xylem vessels and scleroids red and cellulose in parenchyma and phloem cells, green but with no distinction between treatments.

b) Suberin. A positive reaction was found in the cell walls of the vascular vessels in the stem scar of fruit from all temperatures using Sudan IV, but Sudan Black B indicated the presence of suberin only in samples held at 10°C, where it was concentrated in the vessel walls and in thickenings where the parenchyma cell walls were in contact with *Botrytis* hyphae or spores.

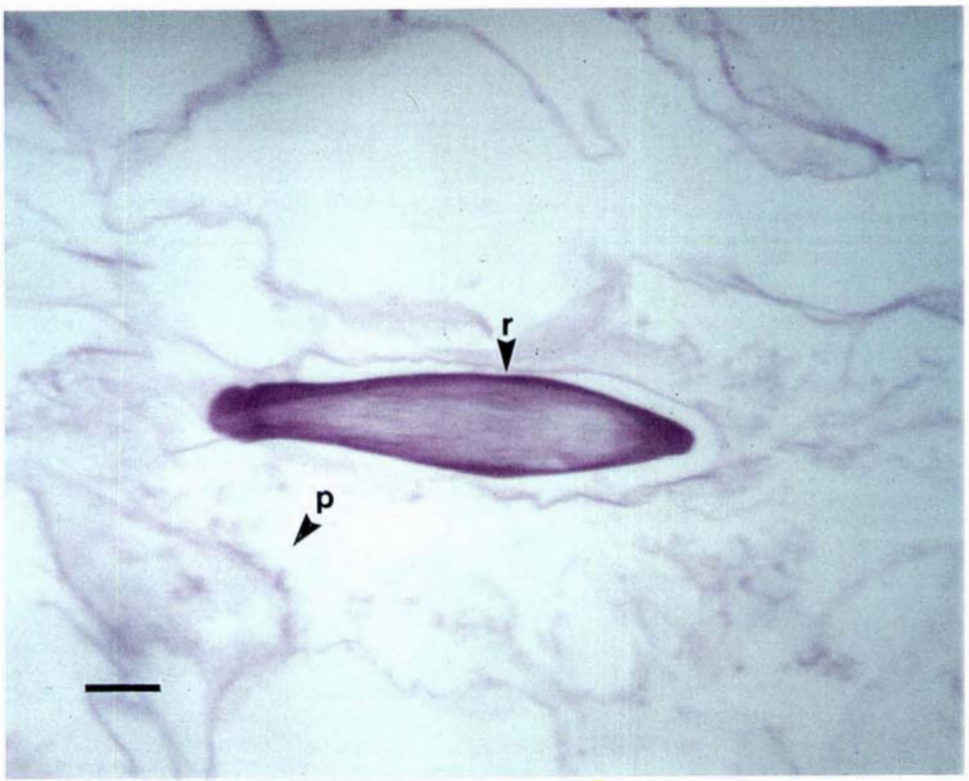


Plate 6-10. Longitudinal section of a kiwifruit stem scar stained with methyl violet eosin showing idioblast containing calcium oxalate crystals (raphides) (r) and parenchyma (p) cells. Bar indicates 0.2 mm.

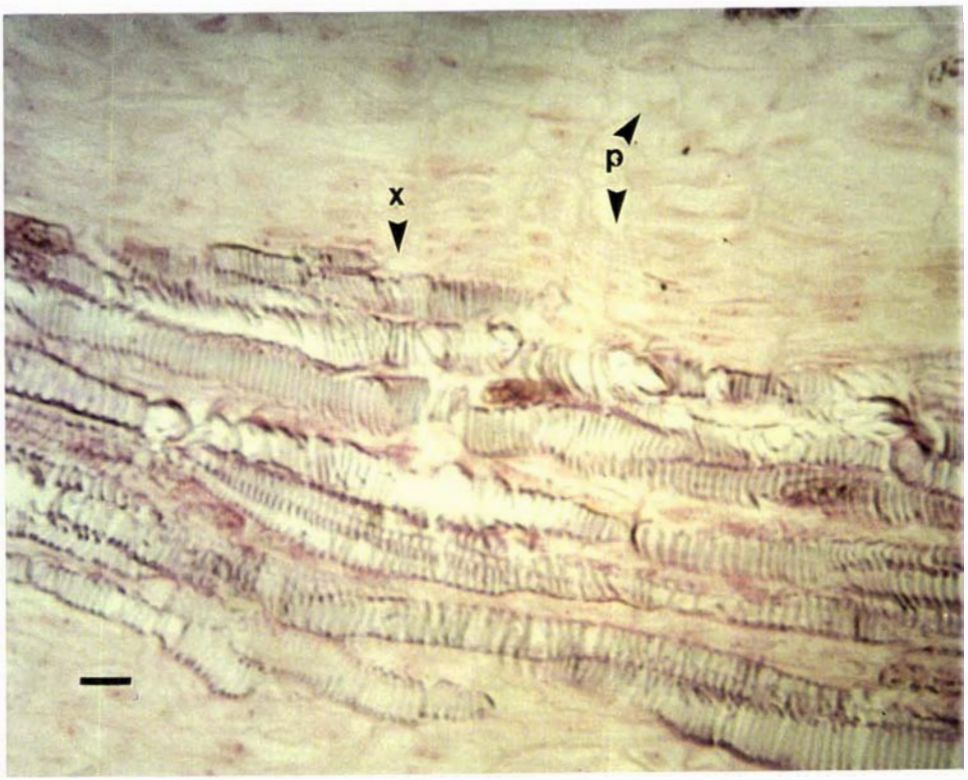


Plate 6-11. Longitudinal section of a kiwifruit stem scar stained with methyl violet eosin showing xylem vessels (x) with helicoidal secondary wall thickening and parenchyma (p) cells. Bar indicates 0.02 mm.

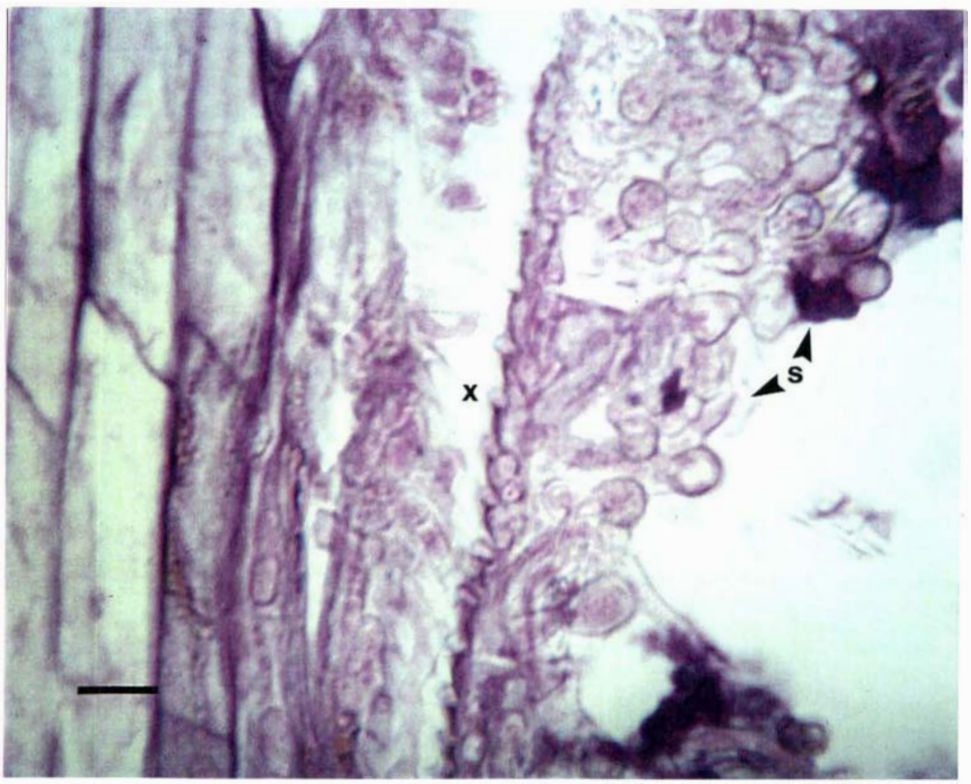


Plate 6-12. Longitudinal section of a kiwifruit stem scar stained with methyl violet eosin after two days curing at 20 or 30°C. Most of the spores (s) scattered on the surface of the xylem vessels (x) have germinated. Bar indicates 0.2 mm.

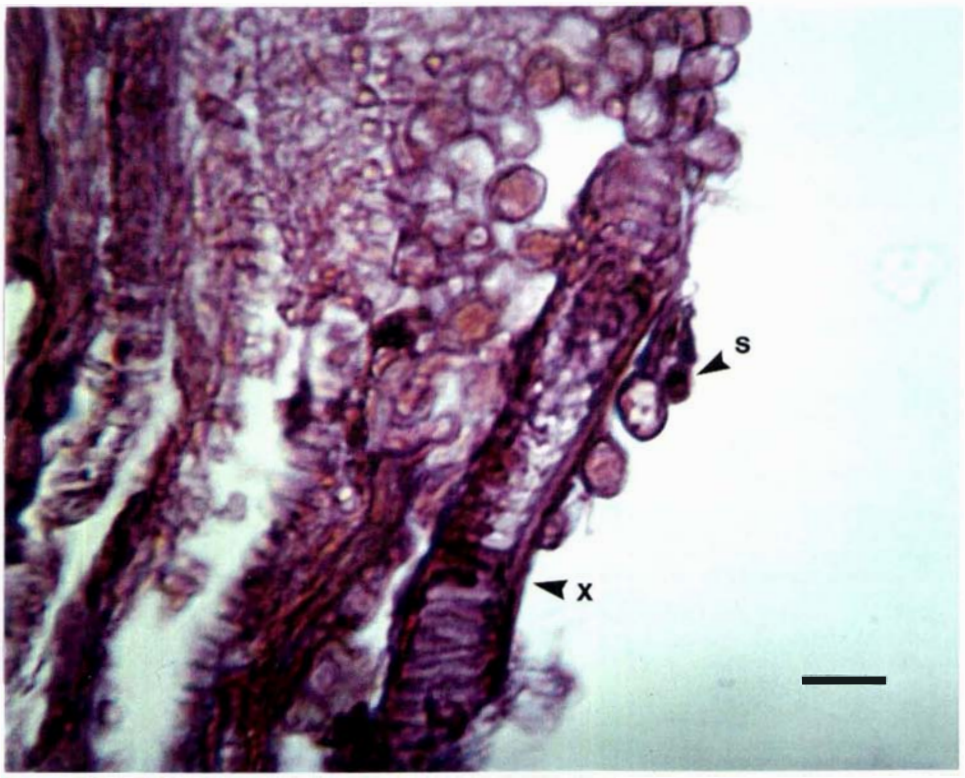


Plate 6-13. Longitudinal section of a kiwifruit stem scar stained with methyl violet eosin after two days curing at 10°C. Most of the spores (s) scattered on the surface of the xylem vessels (x) have not germinated. Bar indicates 0.2 mm.

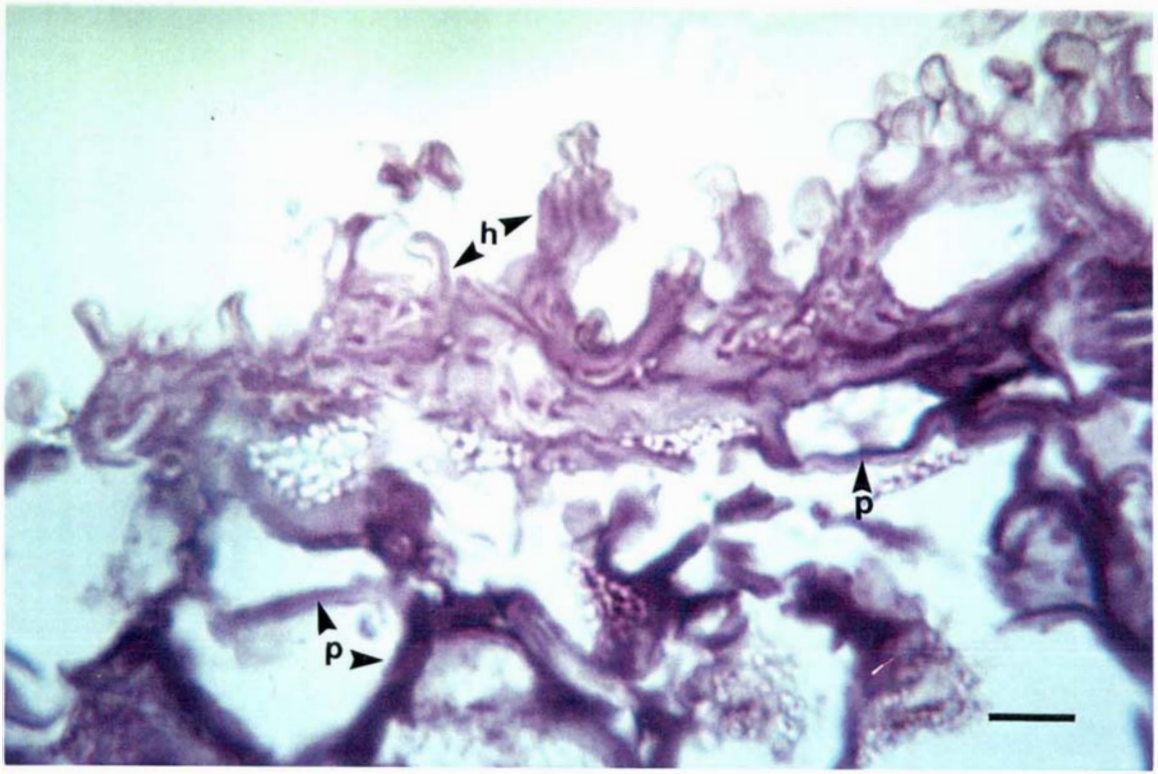


Plate 6-14. Longitudinal section of a kiwifruit stem scar cured at either 0 or 10°C for two days stained with methyl violet eosin. Parenchyma cell walls (p) in contact with hyphae (h) are thicker than normal. Bar indicates 0.2 mm.

Table 6-1. HISTOCHEMICAL TEST OF KIWIFRUIT STEM SCARS INOCULATED WITH *B. cinerea* AND INCUBATED AT VARIOUS TEMPERATURES FOR UP TO SIX DAYS.

Stain	Test	Tissue	Temperature (°C)								
			0			10			20		30
			Days								
			2	4	6	2	4	6	2	4	2
Phloroglucinol-HCl	Lignin	Xylem	R	R	R	DR	DR	DR	R	R	R
Toluidine Blue O	Lignin	Xylem	BP	BP	BP	DBP	DBP	DBP	BP	BP	BP
Safranin & Fast-Green	Lignin & Cellulose	Xylem, parenchyma, collenchyma	GR	GR	GR	GR	GR	GR	GR	GR	GR
Sudan IV	Suberin	Xylem	R	R	R	R	R	R	R	R	R
Sudan Black B	Suberin	Xylem	-	-	-	B	R	R	-	-	-
Glycerine Ferri-cyanide	Reducing Compounds	Xylem, parenchyma	GY	GY	GY	GY	GY	DGY	GY	GY	GY

Colour code: R = red; DR = deep red; BP = blue & purple; DBP = deep blue purple; GR = green & red; B = black; GY = green and yellow; DGY = deep green yellow.

c) Reducing compounds. The glycerine ferricyanide test gave a light staining in all tissues from all curing temperatures, until 6 days when a deep green colour developed in vascular vessels of the fruit incubated at 10°C.

DISCUSSION

The stem scar cellular components of the two tissue systems i.e fundamental and vascular were as described by Esau (1965) for plants in general. In the present study the vessels and cells in the area where the stem scar was snapped from the pedicel were irregular with broken cells as previously reported by Poole & McLeod (1991). The anatomical descriptions of fruit development in kiwifruit (Hopping 1976) and of the vascular system (Habart 1974) showed similar cellular components of fruit tissue and similar arrangement of parenchyma cells, xylem vessels, scleroids and vascular bundles. The basic parenchyma tissue above the sclerified plug was composed of small, rectangular cells - *the densely packed tissue* of Hallet *et al.* (1991). The vascular strands by-passed the sclerified plug 2-3 mm below the surface of the stem scar and branches repeatedly divided over a small distance to spread out into the inner and outer pericarp.

In the present study there were no major anatomical changes in the vascular system of fruit infected by *B. cinerea.* However the presence of cellular thickening in the parenchyma cell walls in contact with hyphae indicate that the fungus influenced the structure of the host cell walls with which it came into contact. This is contrary to another study of inoculated kiwifruit using scanning electron microscopy where no cellular modification of stem scar tissue was observed in fruit incubated for 0, 2, 4, 7, 14, 21, and 42 days for up to 7 days at 0 or 7°C (Sharrock & Hallet 1991). In other commodities, similar results to this present study were reported. For example, in apples modifications of the cells of fruit incubated at 5°C and 20°C after infection by *Botrytis* were found although in that study the cellular modifications consisted of the presence of periderm formation as a wound

repair response (Skene 1981). Tissue response in apricots to latent infections of *Monilinia fructicola* consisted of periderm formation and suberization of living cells which surround the infection site together with accumulation of phenolic compounds (Wade & Cruickshank 1992a; 1992b). In seedlings of arabica coffee (*Coffea arabica* L.) inoculated with *Colletotrichum coffeanum* development of suberized phellogen tissue was observed below the site of infection only in those varieties classified as resistant (Masaba & Van den Vossen 1982). In roots of tobacco plants and soybean hypocotyl, formation of an epidermal and outer cortical cells were observed in response to penetration of *P. parasitica var. nicotiana* or *P. megasperma var. sojae* (Phillips 1993).

The use of histochemical staining on sections of plant material to detect deposition of new materials is a fairly straightforward procedure but the use of such techniques to quantify the density of materials is a more subjective exercise. Caution must be taken in interpretation of results because of variability in batches of stain, slight differences in staining procedures or times and simply variability in plant material. Nevertheless there are many examples in the literature where histochemical staining procedures have been used successfully to elucidate the nature of host-pathogen interaction.

In the present study the positive reaction of samples incubated at 10°C to Sudan B suggested a process of lignification and/or suberization in the kiwifruit stem scar tissues in response to infection by *B. cinerea.* Although the Sudan IV test showed a positive reaction of stem scar tissue of fruit held at all temperatures, the Sudan Black B test appeared more sensitive since it was detected extensive suberization in the xylem vessels of samples held at 10°C. Synthesis of suberin (a complex heteropolymer consisting of aliphatic and phenolic components in association with waxes) is widely known as a barrier which protects plant tissue against pathogens (Kolattukudy & Dean 1974; Kolattukudy 1981; 1985). Thus histochemical studies of various *Prunus* species using a residual autofluorescence test to

detect depositions of suberin after inoculation with *Leucostoma cincta* and *L. persoonii*, have shown the presence of ligno-suberized cells as a response to mechanical injury and to infection. The effects of temperature on these processes was not studied but suberin deposition in periderm, lignified cells and xylem parenchyma occurred within 24 to 48h after wounding (Biggs 1987). Nielsen & Johnson (1974) reported differences in the rate of suberization on wounded sweetpotato roots after exposure to various temperatures (4.5, 38 and 45°C for 2h) and a curing period of 29°C for seven days. They found that a temperature of 38°C gave the highest suberization index in samples excised three or six days after wounding. Sukumaran *et al.* (1990) reported that 25°C and 90% relative humidity for seven days gave the maximum suberization deposition on several potato cultivars compared with a low suberization at 20, 30 and 35°C. The effect of temperature and time of incubation on suberin deposition was also studied in carrots inoculated with *Fusarium culmorum* or *Mycocentrospora acerina.* In that study deposition of suberin (detected with gentian violet) was first observed in the wall region of cells adjacent to the surface after 6h at 25°C and at 15°C suberization was complete in 48h, while very little suberization occurred at 3.5°C (Garrod *et al.* 1981). The optimum environmental conditions to induce suberization and periderm formation in potato have also been studied by Smith & Smart (1955) who found that the best temperatures to induce resistance to *Erwinia atroseoptica* were 21.1 and 26.6°C for one to two days incubation compared with 4.4, 10 or 15.5°C. They found that barrier formation increased (suberin and periderm) as the length of the curing period was increased to four days. Morris *et al.* (1989) reported an increase in the number of suberized cells (detected with Sudan III and IV) when potato tubers were inoculated with *Erwinia carotovora* pv *carotovora* or with *Fusarium oxysporum* and incubated at temperatures between 10 and 25°C or relative humidities of 50 and 90%. When temperature and relative humidity were studied together they found that the best combination was 25 and 30°C with 90 and 98% relative humidity. In the present study incubation time for 2, 4 or 6 days did not have any effect

on suberization while a temperature at 10°C was better than 20 or 30°C.

Another possible explanation for the staining difference between Sudan IV and Sudan B could be the different light absorption spectrum of each dye as reported by Conn's & Lillie (1969). The colour density of Sudan Black B has a wavelength of 596-605 nm while for Sudan IV it is between 520-523 nm. Perhaps in the present study in samples incubated at 10°C there were more newly synthesized suberin components detected only by Sudan B colorant. Although no chemical analysis was carried out to distinguish the chemical nature of the suberin depositions, structural and quantitative differences in suberin composition in healthy wood and that decayed by *Stereum gausapatum* have been reported in the vascular system of oaks (*Quercus robur* L.) by Pearce & Holloway (1984).

Except for the Sudan B reaction, there was no apparent difference between treatments in the histochemical staining reactions found in the stem scar tissues. It seems unlikely that the large difference in infection rates at different temperatures was caused by physical barriers developed during the curing period. This is in contrast to the extensive studies on potato and sweetpotato reviewed above where development of a physical barrier lignin/suberin barrier appears to be a major response to wound healing during curing. The detection of an extra staining reaction with Sudan B in the xylem vessels of fruit cured at 10°C indicates that an extra physical barrier could play some role in defense. Hallet *et al.* (1991) considered that conidia of *B. cinerea* could enter the stem scar through the broken xylem vessels and any extra barriers in the vessels would help prevent infection.

Another approach to this problem would have been to use a range of solvents to separate materials extracted from the stem scar. At the start of this study it was felt that location of physical barriers could be more important than absolute quantities of materials and time constraints

prevented a dual project on extraction of antifungal materials which would have involved a considerable amount of biochemical work. Nevertheless the results with the Sudan Black B in the xylem and the increased intensity of glycerin ferricyanide stain of fruit incubated for six days at 10°C indicates that future work in this area could produce an advance in our understanding of the mechanisms of resistance in the kiwifruit stem scar to *B. cinerea.*

Phenolic compounds (an aromatic ring with substituent groups: hydroxyl, carboxyl, methoxyl groups or non aromatic ring structures) have been implicated as resistance factors in many host-parasite combinations (Rohringer & Samborsky 1967; Ismail & Brown 1975). For example, total phenolic acids such as *p*-coumaric and ferulic acid increased in young tomato fruits during the first three days after inoculation with *B. cinerea* conidia (Glazener 1982). Studies of the effect of temperature and relative humidity on the production of phenolic compounds (free and conjugated) in injured *Valencia* oranges, showed that the concentration of free phenolic constituents increased more than two-fold in oranges incubated for 48h at 30°C and 96 to 98% relative humidity and that they declined at temperatures of 5°C. In that study no differences were found between phenolics in oranges artificially injured or inoculated with *P. digitatum* (Ismail & Brown 1975). Another study in *Valencia* oranges showed the presence of several compounds with antifungal activity when fruit were artificially wounded and inoculated by *P. digitatum* (Stange *et al.* 1993). Phenolics such as quinic acid, chlorogenic acid, esterified *p*-coumaric and ferulic acids were found to increase in response to injury in potato tubers left uninoculated or inoculated with *Phytophthora* and *Phoma* (Cottle & Kolattukudy 1982; McLauchlin 1983; Ampomah & Friend 1988).

In the current study the lower proportion of germinated spores on the stem scar of kiwifruit cured at 10°C compared with those at 0, 20 or at 30°C, combined with the positive reaction to Sudan B and the more intense colour observed in the test for phenolics indicated that host defense based on

antifungal materials is also more active at 10°C. Although analytical tests for reducing compounds were not carried out in this study, previous reports of antifungal compounds in kiwifruit have shown the presence of such materials in water, 70% methanol-water and petroleum ether extracts of fruit infected by *B. cinerea* (Poole & McLeod 1991). Increases in enzyme activity thought to be involved in plant defense process, e.g. phenylalanine-ammonia lyase and endochitinase increased in kiwifruit at varying times (2h to 168h) after harvest and inoculation by *B. cinerea* (McLeod & Poole 1994). These authors considered that the enzyme activity probably affected the establishment phase of infection since they found many germinated conidia in the inoculum drop. The current state of knowledge of the resistance of kiwifruit stem scars to infection by *B. cinerea* indicates that physical barriers to infection are probably not the major resistance mechanism and that there are many potentially anti-fungal materials in the stem scar. The relative importance of each of these materials is highly speculative at present and a considerable amount of research will be required before there is an accurate picture of the dynamics of infection in and on the kiwifruit stem scar.

CHAPTER SEVEN

INITIAL COOLSTORAGE RELATIVE HUMIDITY OF KIWIFRUIT AND INFECTION BY *BOTRYTIS CINEREA*

INTRODUCTION

One of the main features of postharvest storage systems for most commodities is an adequate storage capability for short or long-term storage. The storage system should be able to reduce or minimize postharvest losses due to the normal physiological ageing processes of the fruit and to infection by microorganisms.

The principal objectives of storage procedures are to slow down commodity metabolism, minimize moisture loss and reduce growth and spread of microorganism (Harvey *et al.* 1983; Knee *et al.* 1983; Robertson *et al.* 1990). Davis (1980), considered that for long term storage the produce should be kept at lower temperatures than those used for shorter periods. Once the fruit or vegetable is separated from the plant, their postharvest life is short at ambient temperature, hence, low temperatures will help to extend storage life of the commodity by suppressing disease and extending host resistance (Sommer 1985; 1989). From the economical point of view the best storage system will not only provide an extension of the time over which produce is marketable but will allow the produce to be held until better prices can be obtained (Thompson 1992).

Several physiological parameters of the fruit can be positively influenced by a combination of low temperature and high relative humidity during storage. The combination of both these environmental factors reduces water loss and avoids rapid loss of firmness. Softening, shrivelling and wilting have been reported as the major symptoms of high weight loss in fruits and

vegetables during storage (Krahn 1974; Kitinoja & Kader 1994). In several climacteric fruits high weight loss enhances to some extent the process of ripening (Littmann 1972; Mitchell 1990). Van den Berg & Lentz (1966; 1973a) showed that high moisture loss during refrigerated storage reduced the quality of some vegetables such as carrots and cabbages. Similarly, Goodliffe & Heale (1977) showed that a percentage weight loss of 5-10% reduced the ability of carrots to resist *B. cinerea* invasion during storage at 5°C.

Storage temperature and relative humidity

Most studies on potatoes, sugar beets, onions, carrots and cabbage have reported temperature ranges between 0 and 4°C as the optimum for storage of these commodities (Ryall & Lipton 1980). Similarly, studies on kiwifruit (McDonald 1990; Mitchell 1990; Sawada 1992; Lallu *et al.* 1992) have reported 0°C as the best temperature to store this commodity in order to avoid a high incidence of disorders and high moisture loss. Differences in this recommended storage temperature are slight: Sale (1990) recommended temperature ranges between -0.5 to 0.5°C as the optimum for kiwifruit storage. It is unlikely that a more accurate control of temperature under commercial conditions could be achieved.

Although low temperatures can prevent the growth of pathogens such as *Colletotrichum gloeosporoides* Sommer (1985) observed that spores of *B. cinerea* can germinate and continue to grow when placed at 0°C temperatures and 90-95% relative humidity.

However, there is not universal agreement about the best humidity range in which vegetable crops should be stored. Thus Mann (1954) and Thompson (1992) considered that for most of the perishable commodities, the relative humidity should be maintained at 90-95%, and that higher levels of relative humidity in the storage room would encourage high levels of decay.

This range of relative humidities (90-95%) has also been reported for storage of potatoes, sugar beets, carrots and cabbages (Ryall & Lipton 1980). However, other studies carried out on carrots showed that lower or equal levels of infection occurred when they were stored at relative humidity ranges of 98-100% compared with storage at 92-96% (Van den Berg & Lentz 1966; 1973a; 1974). Further research (Van den Berg & Lentz 1977b; 1978) with parsnips, rutabagas, carrots, celery and other vegetable crops also showed that relative humidities between 98-100% reduced decay levels during storage compared with lower relative humidities.

For kiwifruit McDonald (1990) considered 95% relative humidity or higher as the most suitable levels of moisture to extend the storage life of the kiwifruit for 4 to 6 months. A similar conclusion was reached by Sale (1990) who recommended a storage relative humidity of 95% for bin-stored fruit and a relative humidity at 85% for packed fruit.

OBJECTIVE

To evaluate the effect of initial relative humidity during coolstorage at 0°C on physiological changes and infection levels by *B. cinerea* during coolstorage of kiwifruit.

MATERIALS AND METHODS

Experiment No.1

Title: Relative humidity at 0°C, physiological changes and incidence of *B. cinerea* stem-end rot during coolstorage in 1992.

Fruit harvesting

Fruit were harvested on 4 May 1992 from the Massey University Fruit Crops Unit. The orchard temperature and relative humidity were 13°C and 80%. The initial TTS and firmness were 6.9% and 9.9 kgf respectively.

Inoculum

Inoculum was applied to kiwifruit stem scars as a 17µl droplet of water containing 5000 spores (equivalent to 2.9 x 10^5 spores/ ml).

Preparation of treatments

The experiment was carried out at 0°C in the University Plant Growth Unit coolstore. Three relative humidity ranges were assessed: 100%, 75-90% and 34-80% corresponding to zero, 0.19-0.05 and 0.39-0.12 Kpa respectively. The procedure to develop the required relative humidity is given in the *General Materials and Methods Chapter Two.* During the initial relative humidity/incubation time, fruit were kept in specific relative humidity chambers as illustrated in Fig.7-1. The number and arrangement of trays within these chambers, was similar to that illustrated in *Chapter Five, Experiment No.1.*

Assessments

Ethylene and carbon dioxide production, TSS and firmness were evaluated at harvest and during the initial relative humidity storage time for 1, 4 or 6 weeks. Percentage weight loss were also evaluated during this period. Once the incubation period was completed trays of fruit were enclosed in the usual polyethylene liners and kept at 0°C to evaluate firmness after 3, 4 or 6 months storage and to assess levels of infection after 12 weeks coolstorage.

Statistical Analysis

Incubation period data were analyzed by weeks with separation of means by Duncan t-test, while firmness data from the storage period were analyzed as a nested design. Before analysis of variance log transformation for ethylene production and square and log transformations for rate of respiration were carried out for data after four and six weeks incubation

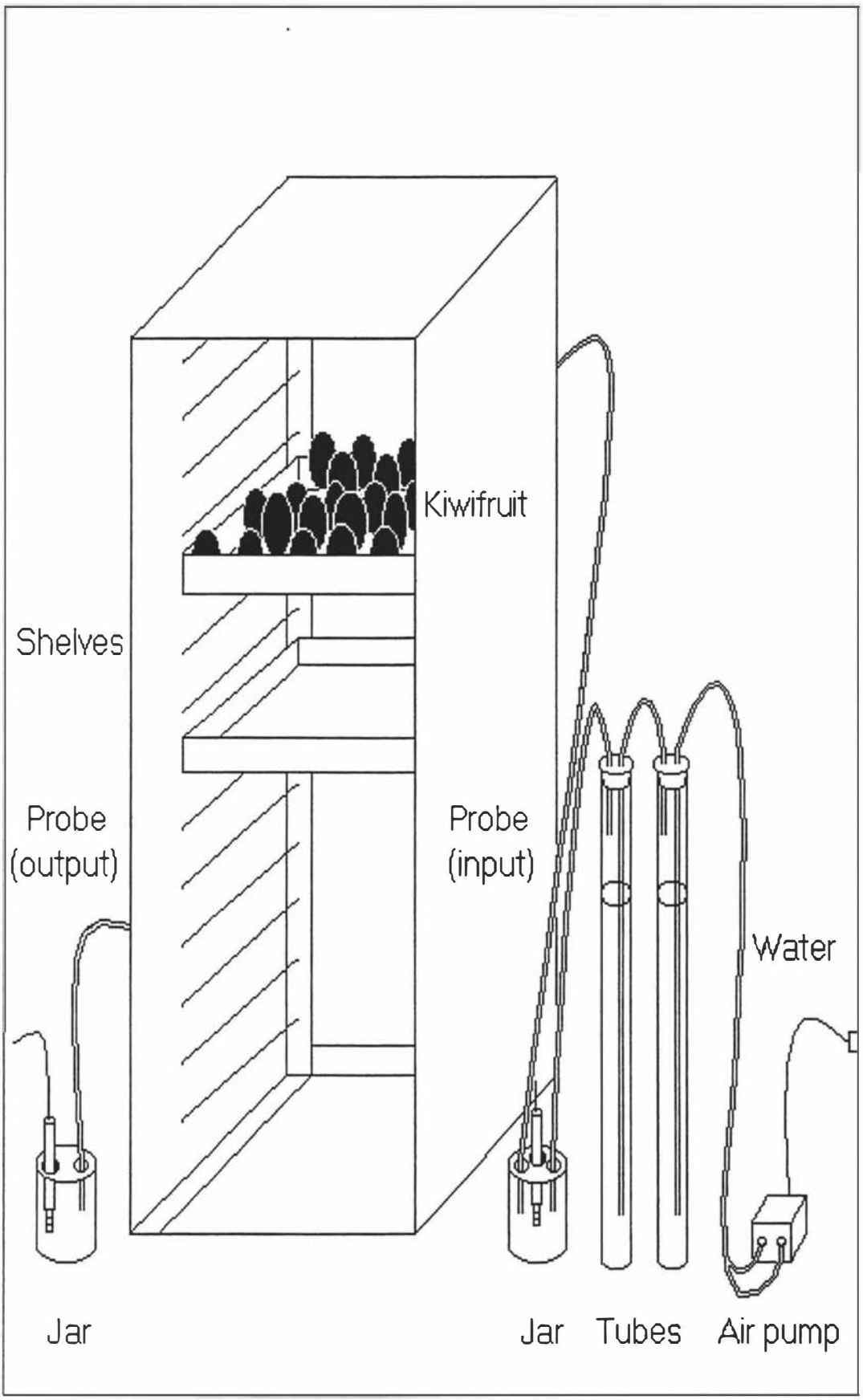


Figure 7-1. Relative humidity system.

period respectively. As percentage infection data were pooled only means were evaluated.

Experiment No.2

Title: Effect of initial coolstorage relative humidity and fruit maturity on fruit quality and infection of kiwifruit by *B. cinerea* in 1993.

Fruit Harvesting

The experiment was repeated on three different dates in 1993. Orchard temperature and relative humidity at each harvest were 15, 10 and 14°C and 85, 76 and 87% respectively. Fruit from the first and second dates were harvested from the same orchard while fruit from the third date were harvested from the orchard next door. The initial total soluble solids and firmness from the early 12th May, middle 27th May and late harvest 1st June were 6.6 11.2 and 12.8% and 9.0, 9.5 and 6.8 kg/f respectively.

Inoculum

The inoculum applied to each stem scar was 25000 spores/17 µl droplet of water (equivalent to 1.5x10^{6} spores/ml).

Preparation of treatments

At each harvest date, the initial incubation at 0°C was for a period of seven days. The overall relative humidity ranges assessed for the three harvest dates were 40-59%, 65-80% and 92-97% corresponding to vapour pressure deficits (VPD) of 0.35-0.24, 0.21-0.12 and 0.04-0.01 Kpa respectively. Inoculated control fruit were packed and coolstored as commercial practice.

Assessments

Weight loss, firmness and TTS were measured at the end of the incubation period. After the incubation period fruit were packed in commercial kiwifruit single layer trays and stored at 0°C to evaluate infection levels after 6 and 12 weeks coolstorage.

Statistical Analysis

The experiment was analyzed as a completely randomize design. At each harvest, the three relative humidities were repeated seven times to evaluate infection levels. Fruit from a further three relative humidity tubes, one tube per humidity were used to evaluate the physiological parameters. Before analysis of variance log transformation was carried out for percentage weight loss at each harvest and for firmness from the third harvest data. Square root transformation was carried out for firmness and TSS in data from the first and second harvest respectively.

RESULTS

Experiment No. 1

Relative humidity behaviour during treatment.

The input/output relative humidity ranges obtained remained constant throughout the initial incubation phase of this experiment (Fig.7-2) with the highest relative humidity constant at 100% the medium relative humidity varying from an input of 75% to an output of 90% and the low varying from 34% (input) to 80% (output). The 5% overlap between the medium and low ranges was minimal and unlikely to blur the distinction between the medium and low humidity ranges.

Fruit quality and physiological changes during incubation and storage periods.

Weight loss of fruit increased with time but in all treatments it significantly exceeded that in the two controls (Fig.7-3). Inoculated fruit lost significantly ($P < 0.05$) more weight than uninoculated fruit. Weight loss of fruit in the lowest relative humidity was higher than that in the high and medium humidities during the three incubation periods.

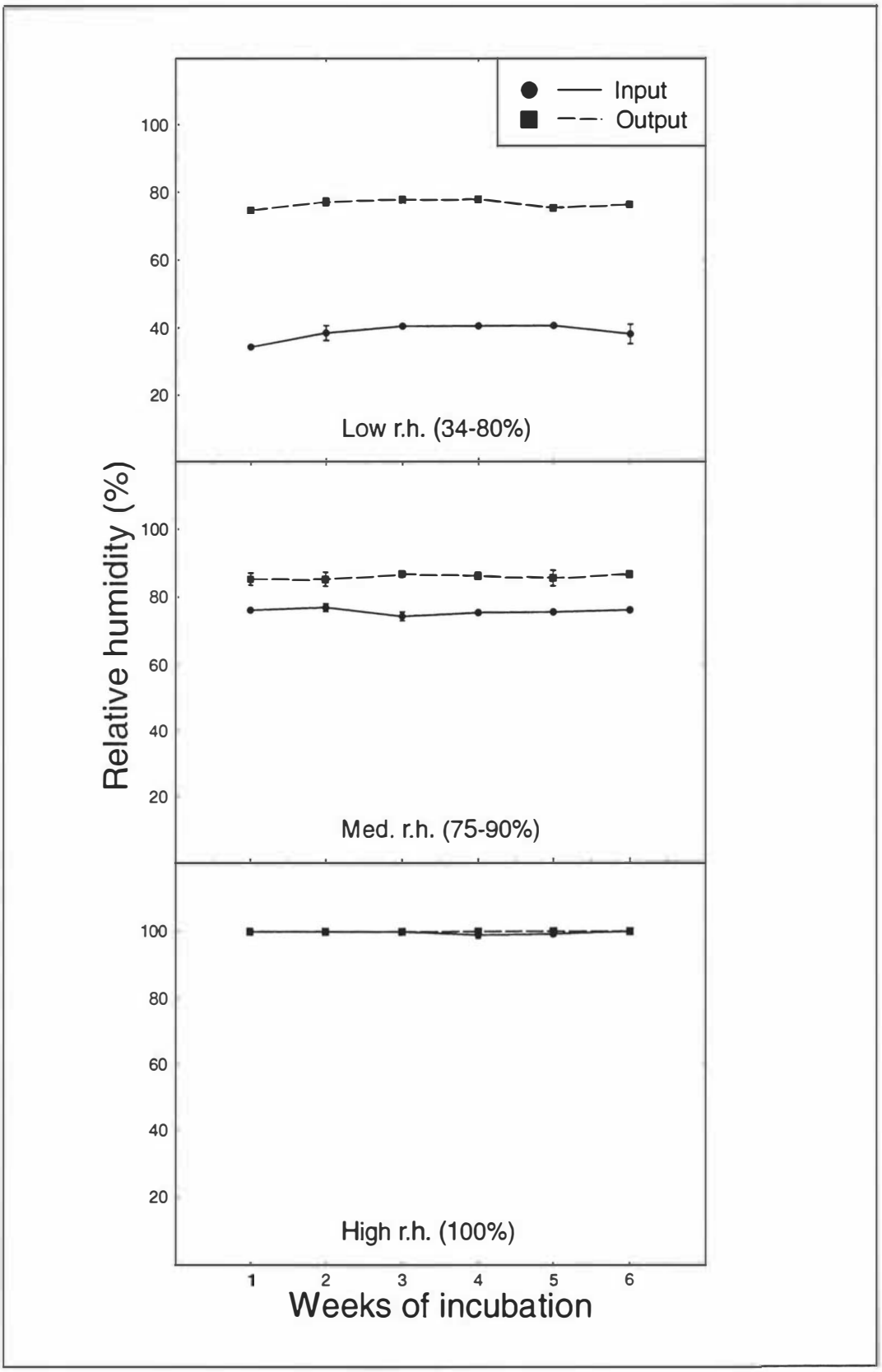


Figure 7-2. 1992: Input and output relative humidity over a six week period at 0°C.

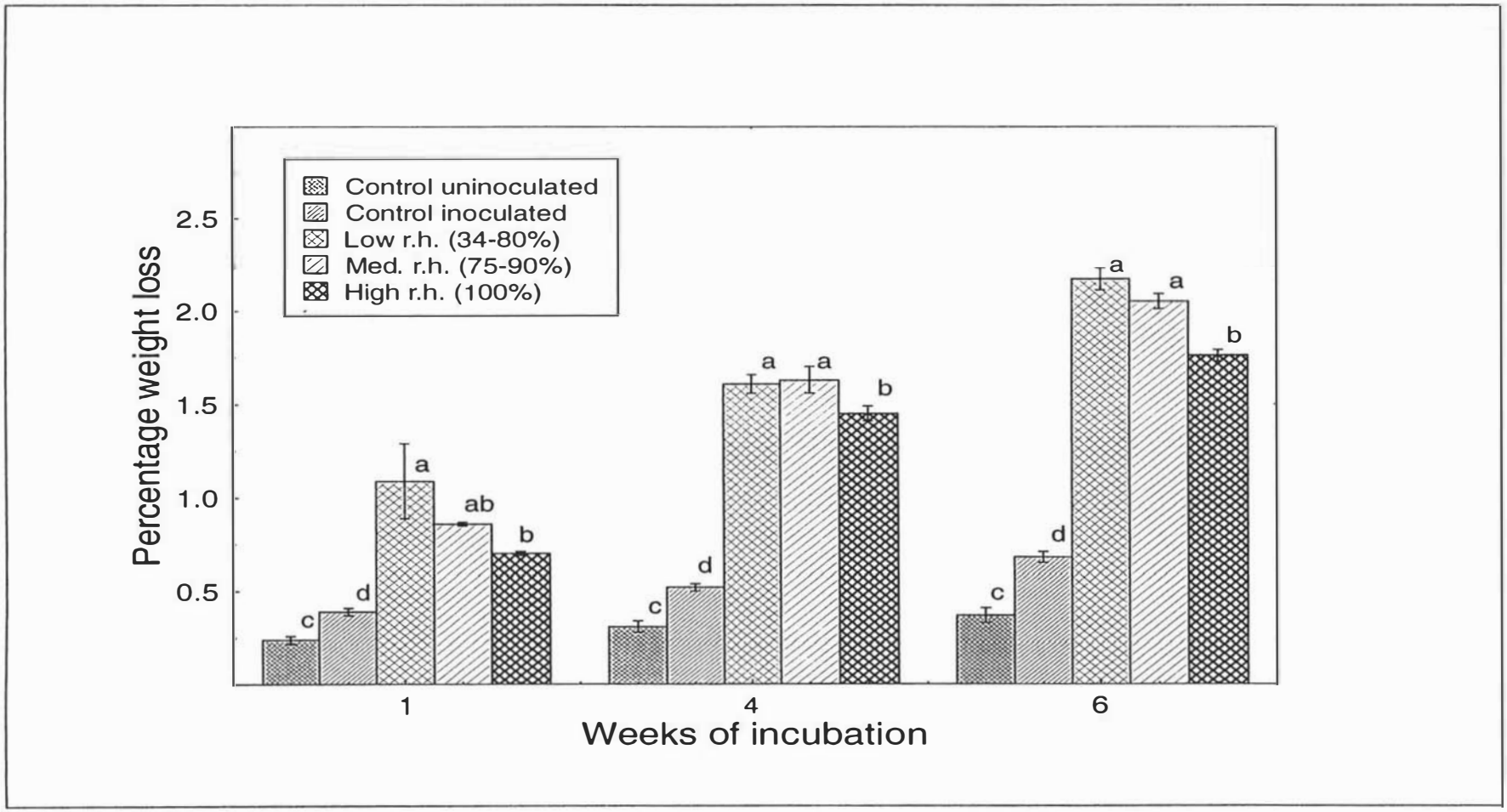


Figure 7-3. 1992: Percentage weight loss of inoculated kiwifruit over a six week period at 0°C and one of three relative humidities. Letters a, b, c & d refer to Duncan's test ($P < 0.05$). Vertical bars indicate SEM.

Fruit firmness was maintained after one week of incubation for all treatments and for both controls but as the incubation period increased to 4 and 6 weeks, firmness in all fruit decreased markedly to 2-4.5 kgf (Fig.7-4). The effect of relative humidity/incubation time/storage time, showed high significant differences ($P < 0.001$). By three months of coolstorage firmness had decreased to 2-2.5 kg/f where it remained for the second three month period of incubation (Fig.7-5).

The total soluble solids of fruit in all treatments and controls increased during the one to six week incubation period with most of the increase taking place in the one to four week period (Fig.7-6).

Ethylene production was not detected at harvest on uninoculated control fruit at any time during the experiment (Fig.7-7). Inoculated control fruit produced more ethylene after four weeks incubation than those in any of the treatments. Ethylene production by fruit in the low relative humidity treatment was not detected after one week incubation, it was low after four weeks incubation but rose dramatically after six weeks incubation when production by some fruit only was extremely high contributing to both a high average production and a large standard error. In the medium relative humidity treatment, there was some ethylene detected after one and four weeks incubation and this had doubled after six weeks. In the high humidity treatment there was some ethylene production by fruit after one week incubation, a considerable amount after four weeks but one after six weeks.

There was no definite pattem to changes in respiration rate as measured by CO_2 production and there appeared to be little overall differences between one and six weeks incubation (Fig.7-8). At harvest The control inoculated fruit had the highest respiration rate after four weeks incubation.

Infection levels during coolstorage

Fruit from the uninoculated control developed less than 5% incidence of *B.*

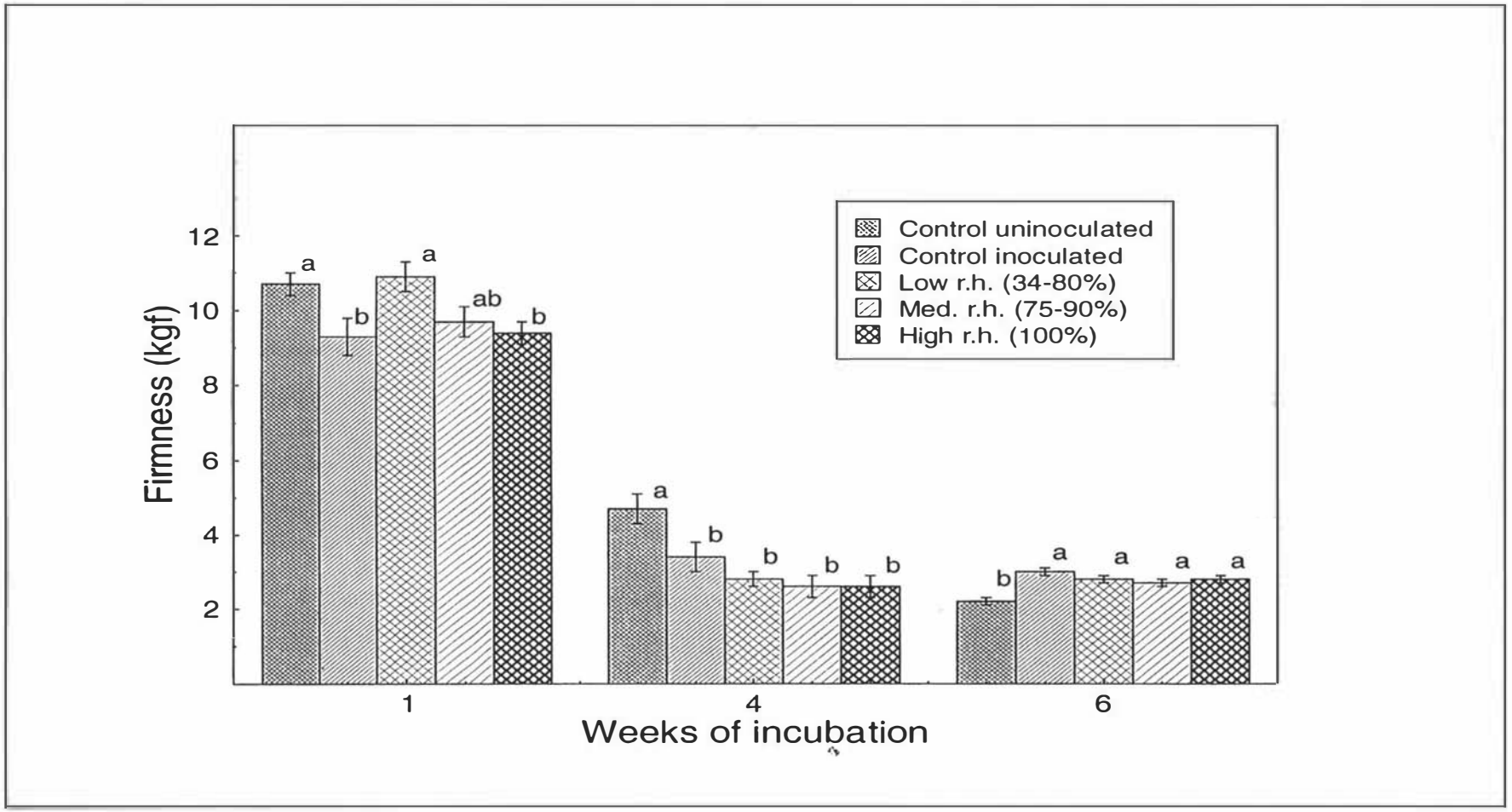


Figure 7-4. 1992: Firmness of inoculated kiwifruit over a six week period at 0°C and one of three relative humidities. Letters a & b refer to Duncan's test (*P < 0.05)*. Vertical bars indicate SEM. Firmness at harvest = 9.9.

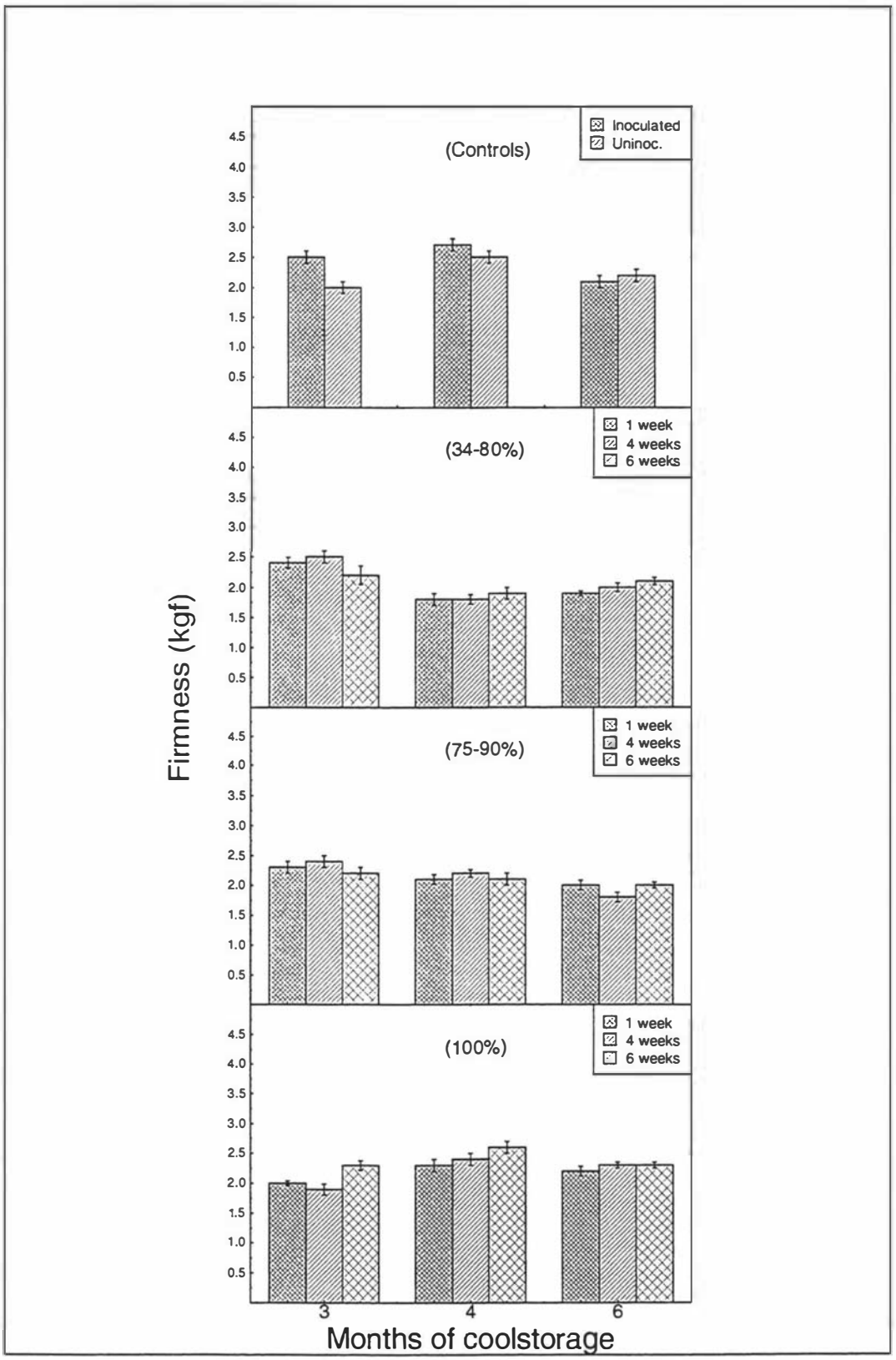


Figure 7-5. 1992: Mean (± SE) firmness of inoculated kiwifruit stored at 0°C after incubation at different relative humidities for up to six weeks. (Overall *P < 0.001*).

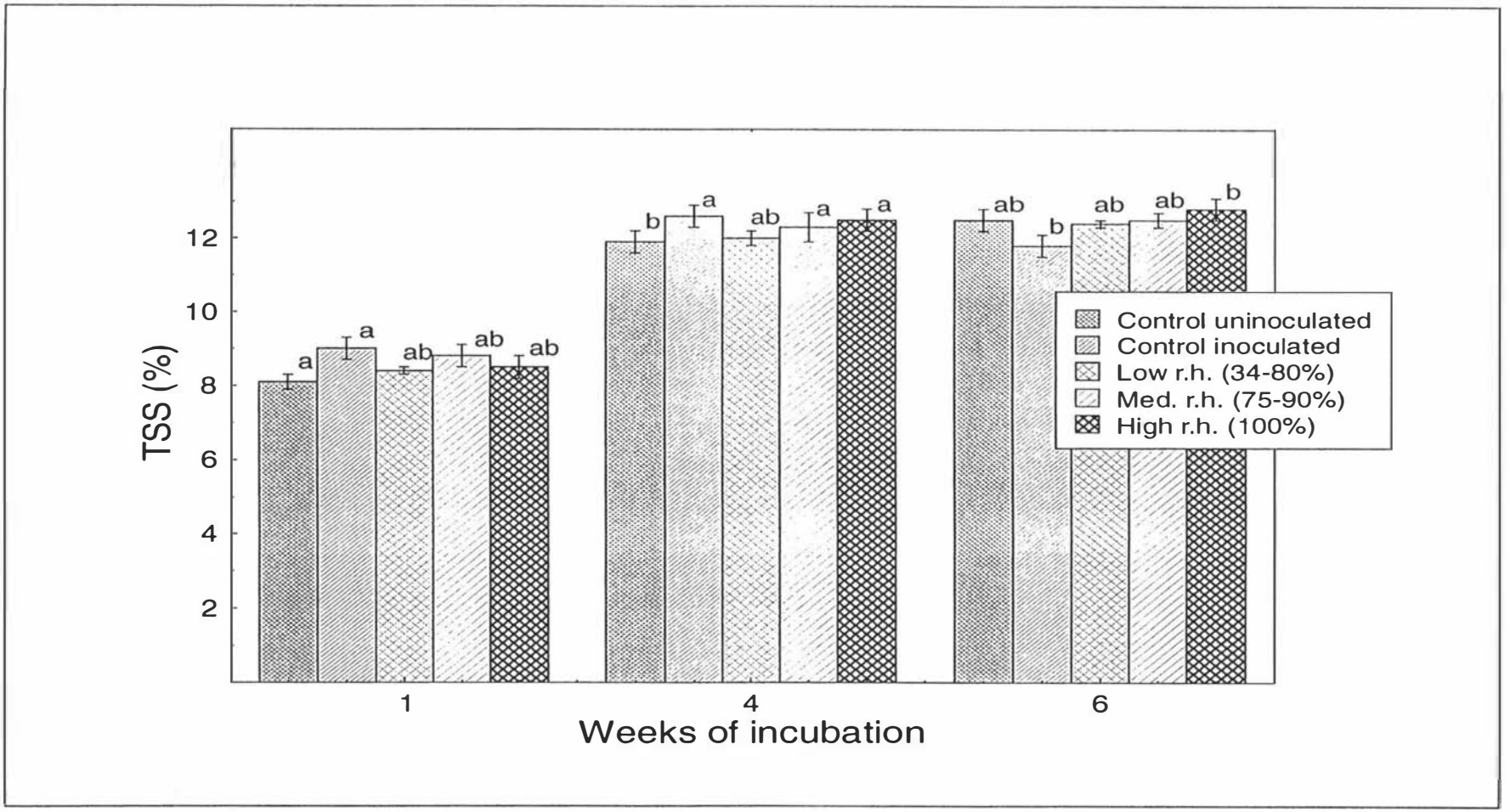


Figure 7-6. 1992: Total soluble solids of inoculated kiwifruit over a six week period at 0°C and one of three relative humidities. Letters a & b refer to Duncan's test ($P < 0.05$). Vertical bars indicate SEM. TSS at harvest = 6.9%.

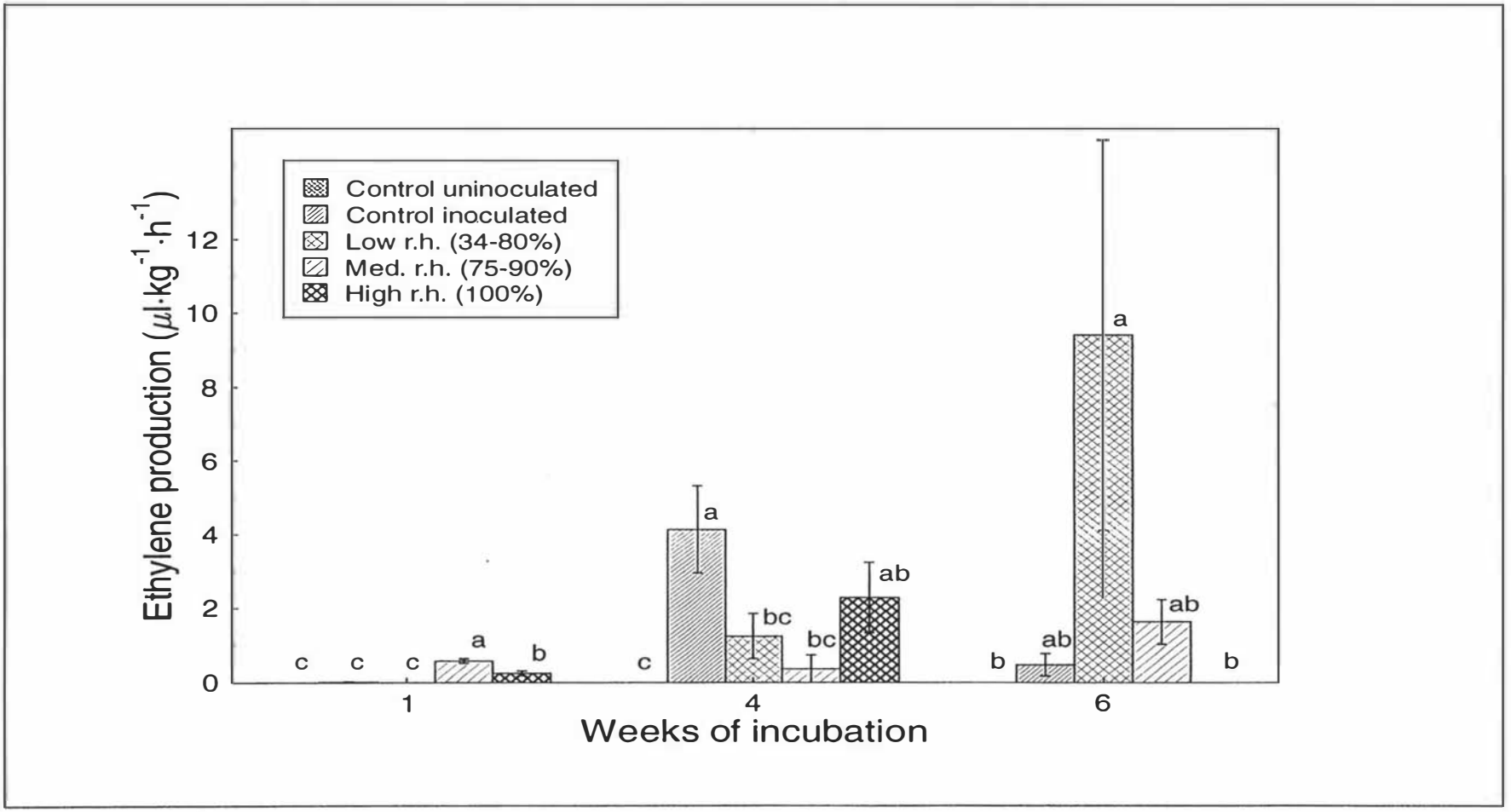


Figure 7-7. 1992: Ethylene production of inoculated kiwifruit over a six week period at 0°C and one of three relative humidities. Letters a, b & c refer to Duncan's test ($P < 0.05$). Vertical bars indicate SEM.

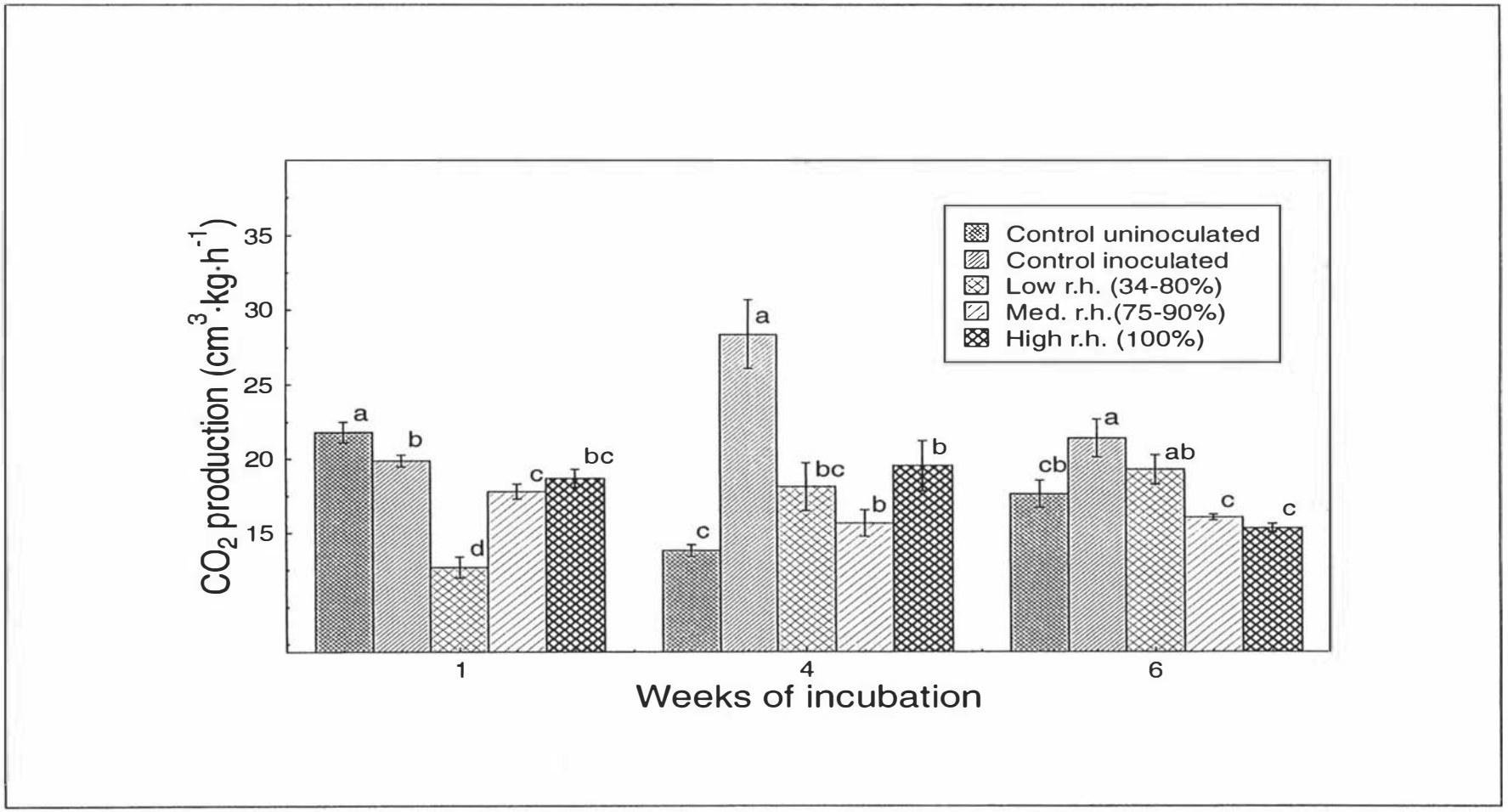


Figure 7-8. 1992: Rate of respiration of inoculated kiwifruit over a six week period at 0°C and one of three relative humidities. Letters a, b & c refer to Duncan's test ($P < 0.05$). Vertical bars indicate SEM.

cinerea infection while fruit from the inoculated control increased this to over 40%. The general pattern was for a reduction in disease incidence with increasing treatment time for all relative humidities. Compared with the control fruit, the lowest infection levels were recorded from the medium (one week) and lowest relative humidity (fourth and sixth weeks) (Fig.7-9).

Experiment No.2

Relative humidity behaviour

Except for the medium relative humidity during the third day of treatment at the first harvest and the lowest relative humidity during the first day of treatment at the second harvest, the input/output ranges of each relative humidity range obtained for each harvest date remained constant throughout the seven days treatment.

The lowest relative humidity from the first harvest date (Fig.7-10) varied from an input of 40 to 60%, that of the second maturity (Fig.7-11) from 42 to 57% and that of the third maturity (Fig.7-12) from 41 to 50%. The medium input relative humidity from the first harvest was slightly different (63%) compared with the remainder two harvests (65%) and the output varied from 79% (second harvest) to 81% (first and third harvest). The highest relative humidity input varied from 90% (first harvest), 94% (second harvest) and 93% (third harvest), with an output similar for all harvests of 97%.

Fruit quality after initial relative humidity coolstorage period

At the first harvest (May 12th), the weight loss of fruit incubated at the lowest relative humidity was significantly greater than that of fruit from the medium humidity which in turn was significantly greater ($P < 0.05$) than that in fruit incubated at the high relative humidity (Fig.7-13). This pattern of weight loss was repeated at both the second (May 27th) and third (June 10th) harvests although the differences between weight loss at the low and medium relative humidity were not significant at the third harvest.

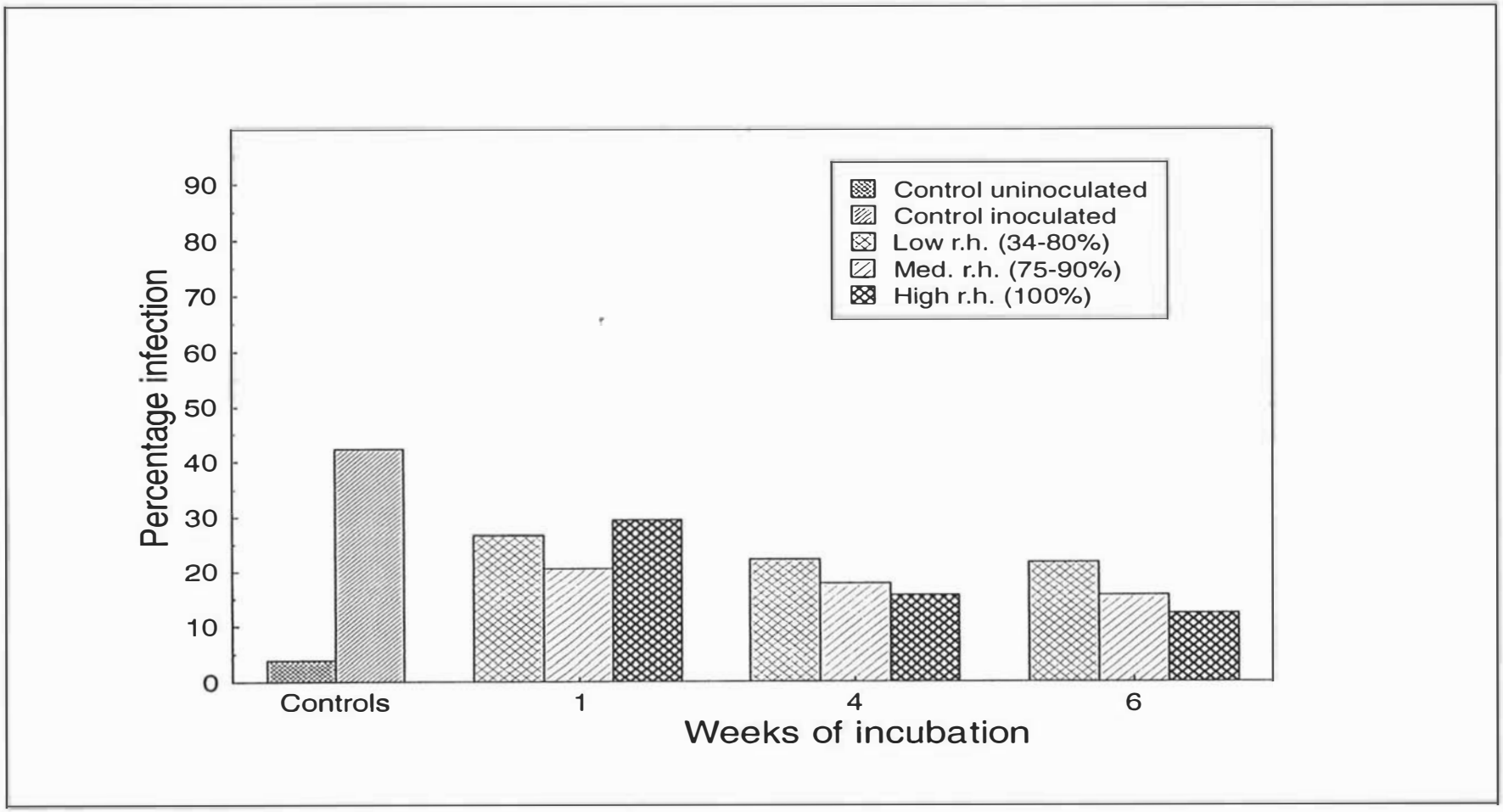


Figure 7-9. 1992: Mean percentage infection of inoculated kiwifruit over a six week period at 0°C and one of three relative humidities after 12 weeks coolstorage.

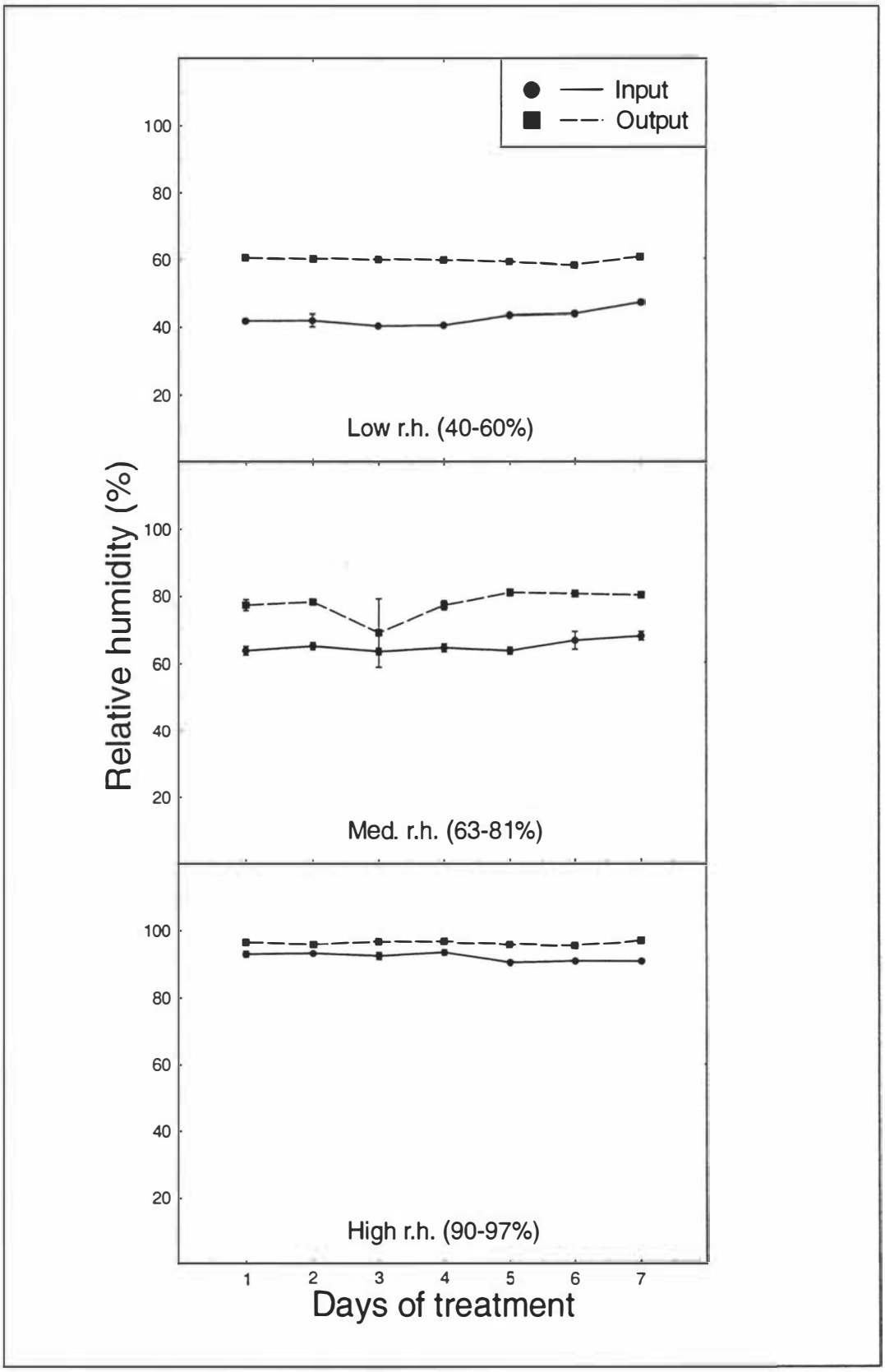


Figure 7-10. 1993: Input and output relative humidity for fruit from the first harvest over a seven day period at 0°C.

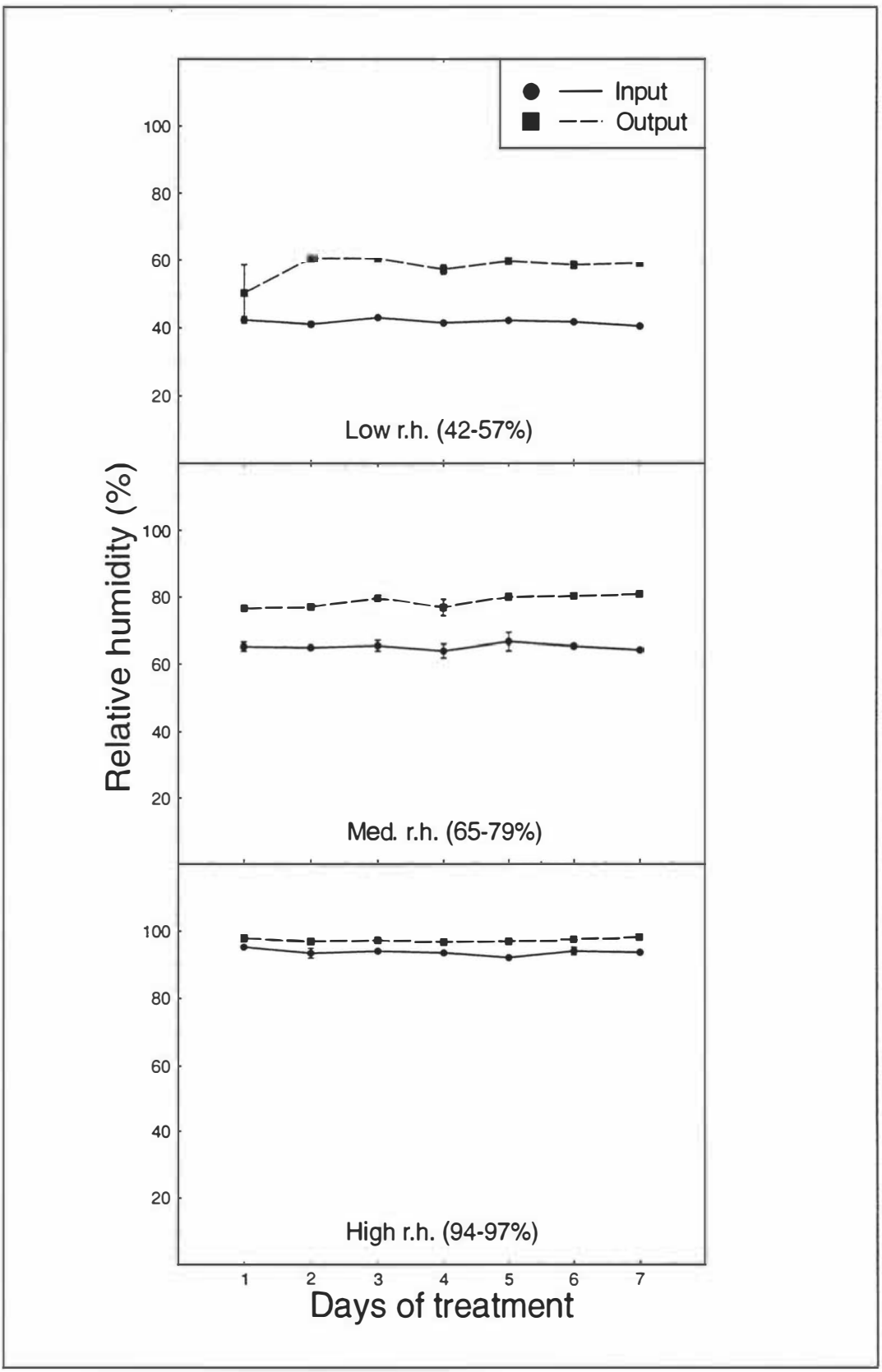


Figure 7-11. 1993: Input and output relative humidity for fruit from the second harvest over a seven day period at 0°C.

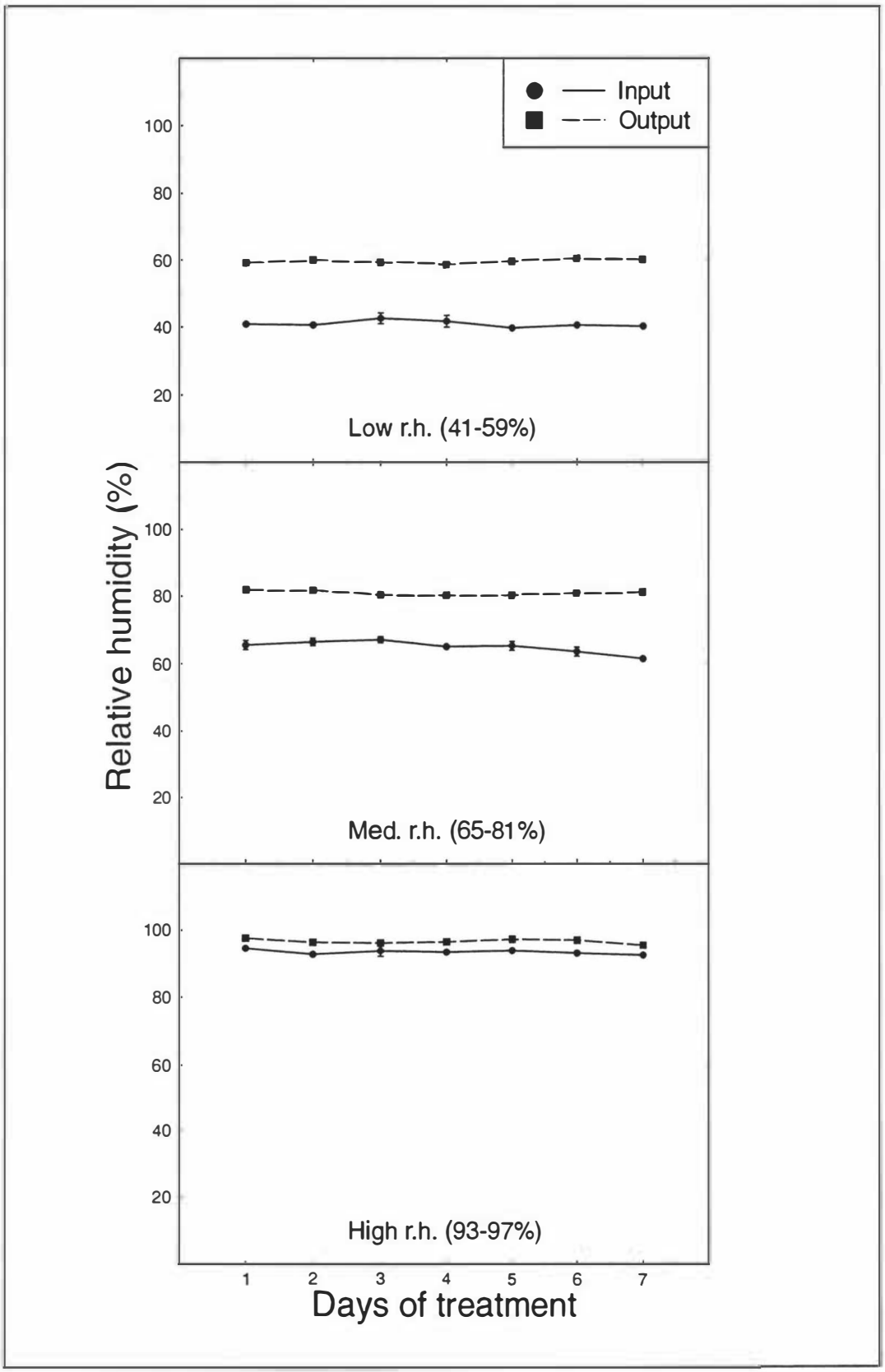


Figure 7-12. 1993: Input and output relative humidity for fruit from the third harvest over a seven day period at 0°C.

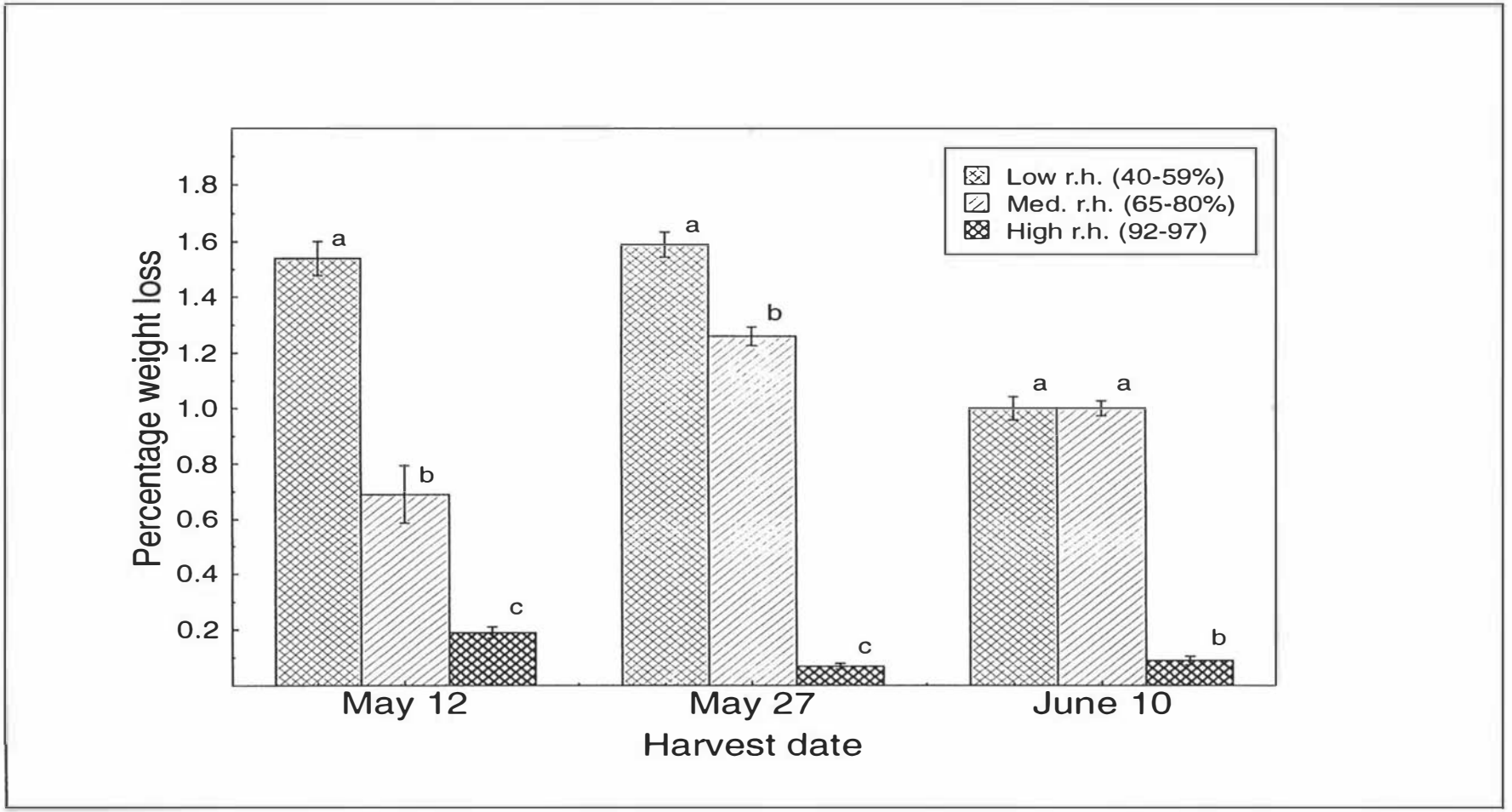


Figure 7-13. 1993: Percentage weight loss of inoculated kiwifruit incubated for seven days at 0°C and one of three relative humidities. Letters a, b & c refer to Duncan's test ($P < 0.05$). Vertial bars indicate SEM.

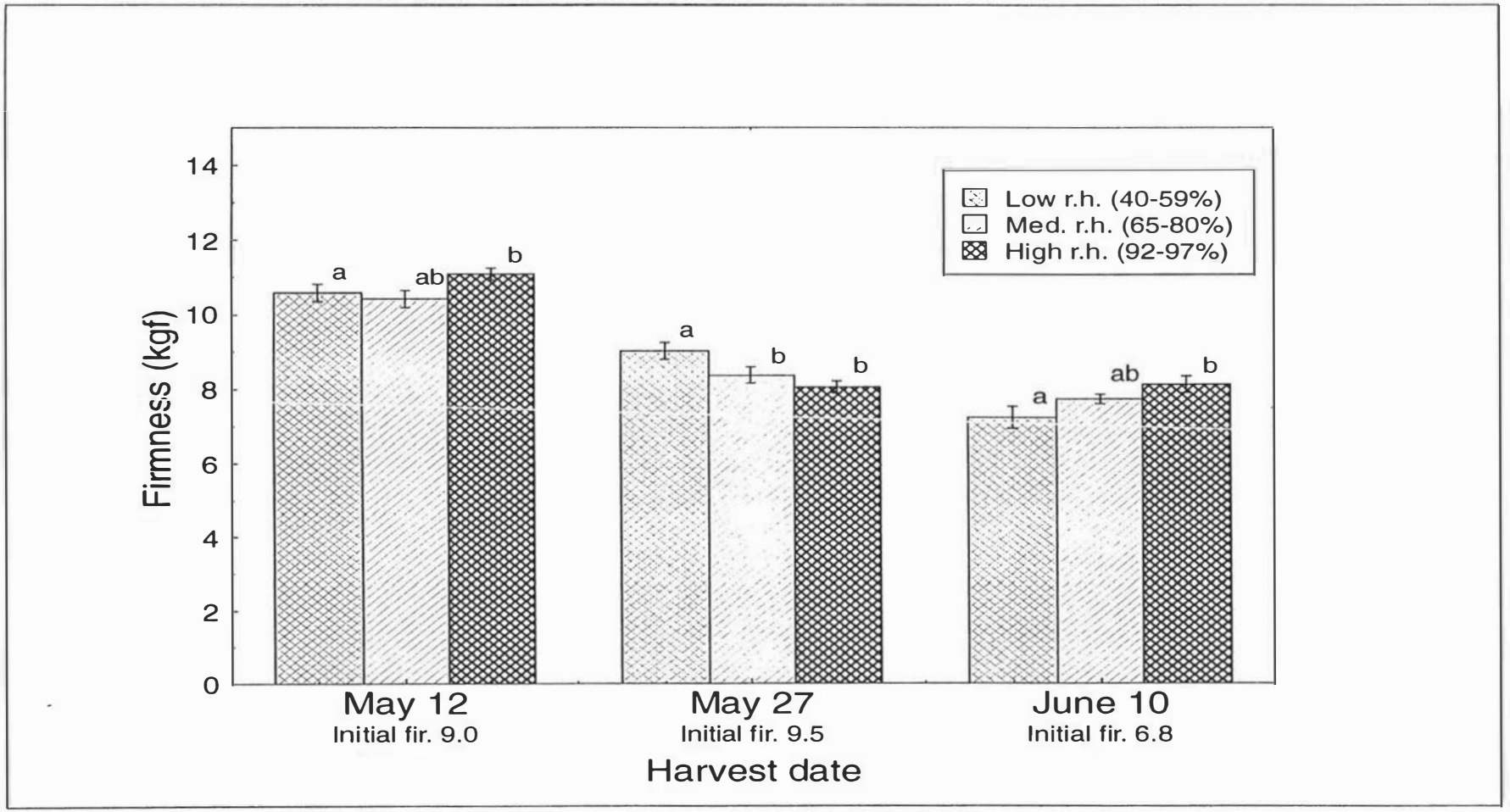


Figure 7-14. 1993: Firmness of inoculated kiwifruit incubated for seven days at 0°C and one of three relative humidities. Letters a & b refer to Duncan's test ($P < 0.05$). Vertical bars indicate SEM.

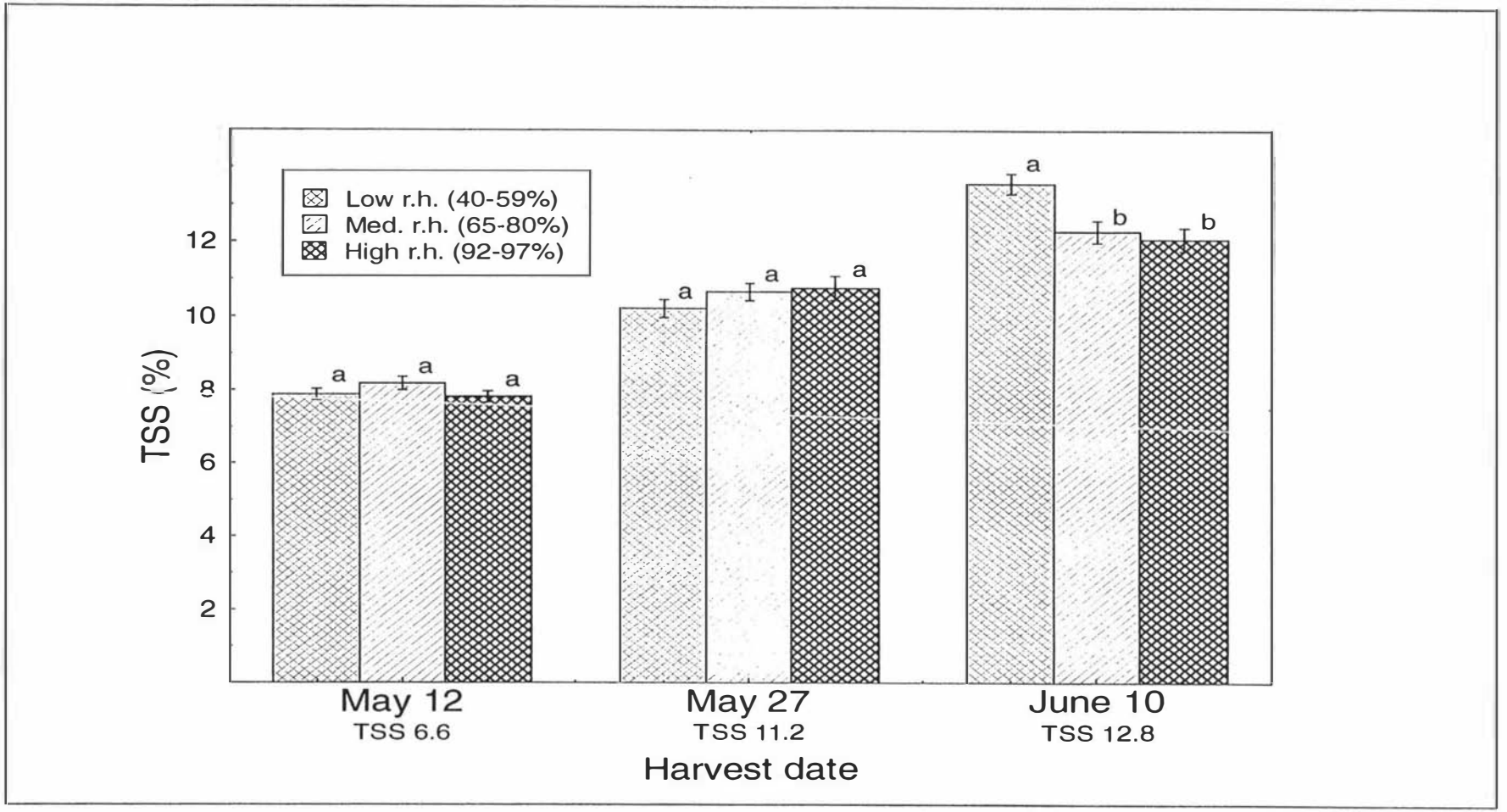


Figure 7-15. 1993: Total soluble solids of inoculated kiwifruit incubated for seven days at 0°C and one of three relative humidities. Letters a & b refer to Duncan's test ($P < 0.05$). Vertical bars indicate SEM.

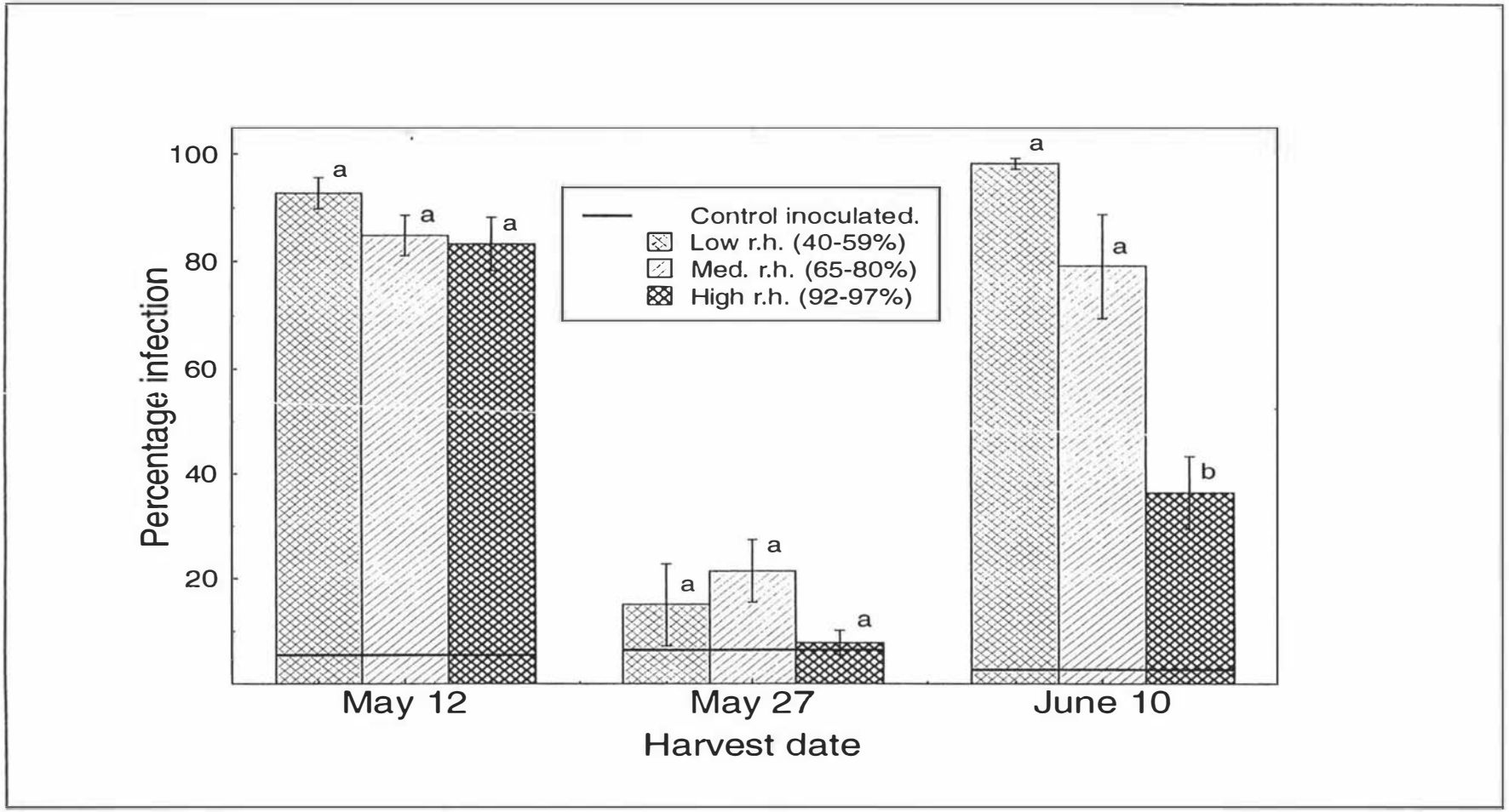


Figure 7-16. 1993: Percentage infection of inoculated kiwifruit incubated for seven days at 0°C and one of three relative humidities after six weeks coolstorage. Letters a & b refer to Duncan's test ($P < 0.05$). Vertical bars indicate SEM.

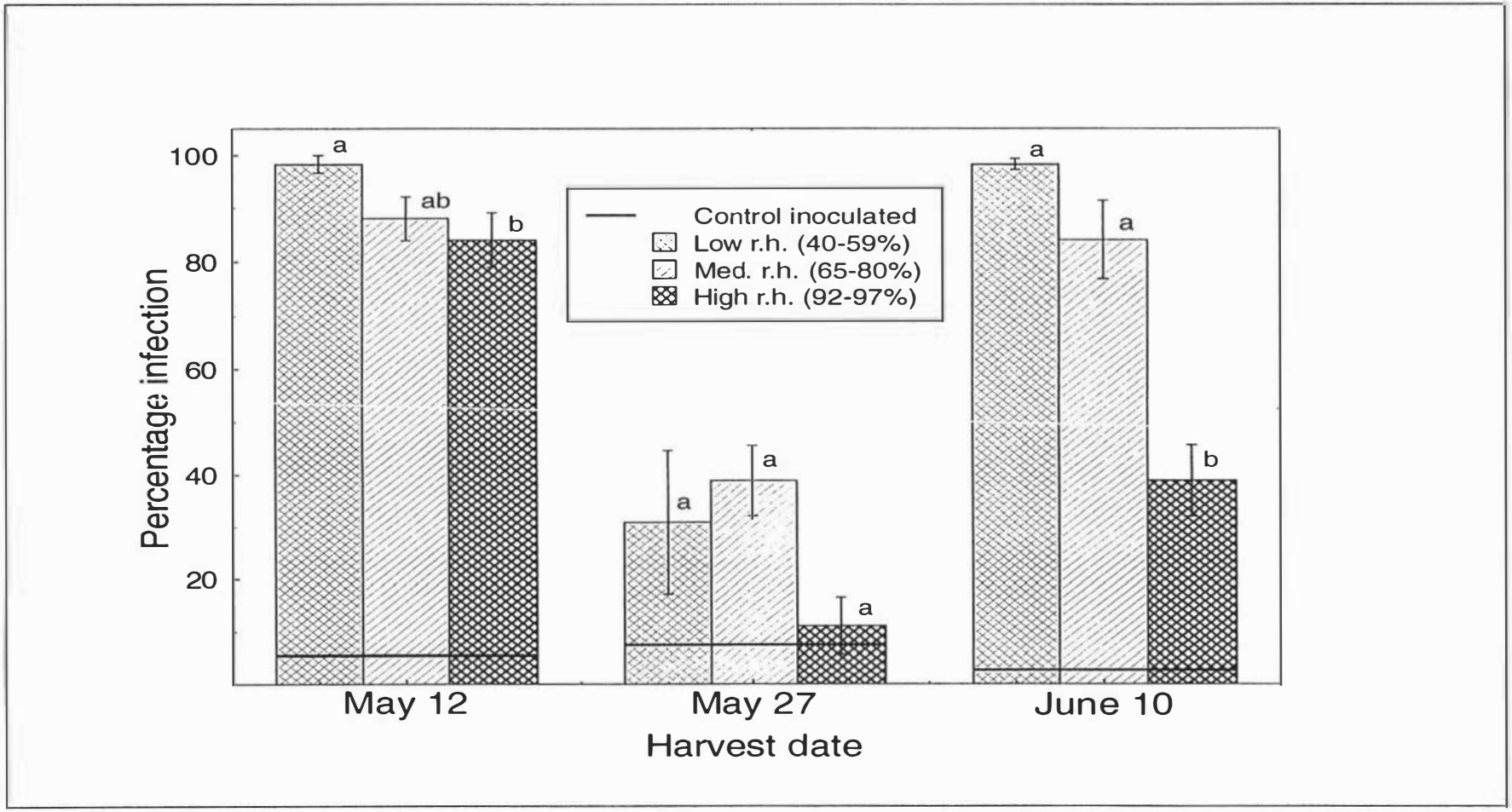


Figure 7-17. 1993: Percentage infection of inoculated kiwifruit incubated for seven days at 0°C and one of three relative humidities after 12 weeks coolstorage. Letters a & b refer to Duncan's test (*P < 0.05)*. Vertical bars indicate SEM.

Fruit firmness at the first and third harvests was significantly greater ($P < 0.05$) in the high humidity treatment than in the low relative humidity. The contrary was found at the second harvest where the low firmness was found at the highest relative humidity (Fig.7-14).

Brix levels (TSS) were not significantly affected by treatment except at the third harvest where TSS in fruit from the medium and high relative humidity treatments were significantly lower ($P < 0.05$) than fruit from the lowest relative humidity. The TSS increased with each successive harvest (Fig.7-15).

Infection levels during coolstorage

The percentage infection of fruit was similar after six and twelve weeks coolstorage although there were more infections in total after twelve weeks, particularly at the second harvest. Fruit from the first harvest incubated in high humidity had fewer infections after six weeks coolstorage than fruit incubated in the low relative humidity (Fig.7-16). By twelve weeks these differences significantly increased ($P < 0.05$). A similar pattern emerged at the second and third harvests although the differences were not significant at 6 or 12 weeks after the second harvest but were significant at both six and 12 weeks for the third harvest (Fig.7-17).

DISCUSSION

Fruit quality and physiological changes during incubation time

In this present study, weight loss from the first experiment and the third harvest from the second was not significantly different between the medium and low relative humidity ranges. In general, moisture loss measured during incubation of both experiments increased with decreased relative humidity and with storage time. A similar tendency among weight loss/relative humidity/storage time has been shown for various vegetable

and fruit commodities. In vegetables such as carrots cv. *Chantenay* and *Nantes*, rutabagas, brussels sprouts, celery, chinese cabbage, leeks and to some extent parsnips, weight loss decreased when ambient storage relative humidity was almost saturated (98-100%) at 0 to 3°C for a period of several months. In these studies the low relative humidity was within the range 90-95% and a higher moisture loss was recorded in those vegetables held at this relative humidity compared with the saturated atmosphere (Van den Berg & Lentz 1966; 1974; 1978). Although in other studies a relative humidity range was not given, a similar trend in weight loss to that in the present study was observed. For example, in apples and pears cv. *Bramley's seedling* and *Beurre Clairegeau* respectively, water loss measured as a rate of evaporation (at 3°C for a period of up to five for apples and two months for pears), was higher in fruit held at relative humidities of approximately 75% compared to those fruit held at 95% relative humidity. In both commodities evaporation tended to increase with storage time (Smith 1933). Similarly in grapes stored at 0°C and relative humidities of 95, 90 and 85% for six and 13 days incubation, weight loss from the lowest relative humidity was almost three times greater at the end of the first incubation period and three and a half times greater after the second incubation period compared with that at the highest relative humidity (Allen & Pentzer 1935).

In the first experiment of this present study, fruit firmness decreased with incubation time for both controls. Regardless of the relative humidity in which fruit were incubated firmness greatly decreased between the first incubation period (one week) and the second one (four weeks). Fruit firmness after four or six weeks of incubation at any relative humidity was reduced within the ranges of 4.7-2.6 kgf and 2.2-2.8 kgf respectively, remaining at these levels during the normal coolstorage period of 1, 3 or 6 months. Reports from commercial coolstores showed that firmness decreased, about 2.5 kgf after eight weeks of normal coolstorage with a further reduction to 0.5-1.0 kgf at the end of the storage period (Reid &

Harris 1977; Nicolas *et al.* 1988; McDonald 1990).

For the second experiment, fruit firmness after the seven days incubation period for all relative humidities was maintained in levels similar to those recorded in fruit normally coolstoraged at 0°C (McDonald 1990). Commercial storage of kiwifruit is recommended at a relative humidity between 90-95% for bin-stored fruit (Sale 1990), 80% (Reid & Harris 1977; Sale 1990) or 90-95% (McDonald 1990) for packed fruit to avoid high moisture loss and consequent reduction in firmness. In this present study, differences in fruit firmness according to relative humidity were more evident at the second experiment than the first one. For example, at the second experiment firmness in fruit incubated at the medium (60-80%, 0.80-0.12 VPD) and high (92-97%, 0.04-0.01 VPD) relative humidity ranges was significantly higher than that of fruit coolstored at the lowest relative humidity range (40-59%, 0.35-0.24 VPD). In other studies (Whitelock *et al.* 1994) fruit were not incubated at 0°C, but a similar pattern of results was reported on four different cultivars of peaches incubated at temperatures and relative humidities of 6 ± 1°C, 97%, 5.6 ± 0.1°C, 88% and 4.3°C, 75% respectively and two different air flow rates (0.7 to 4.0 and 0.2 to 1.5 $m.s^{-1}$). In that study firmness was reported to be reduced with the lowest relative humidity at both air flows and overall firmness increased as VPD decreased.

In this present study, fruit firmness of kiwifruit decreased with an increase in harvest maturity at the second experiment. Similar findings in kiwifruit and in other commodities such as apples, peaches and mangoes have been reported. In those studies of kiwifruit firmness decreased with harvest date (Crisosto *et al.* 1984) and with storage time (Kempler *et al.* 1992). Further studies of kiwifruit firmness stored in temperature ranges of -0.5 to 2.5 for 10, 20 or 24 weeks showed a similar pattern of firmness decrease with storage time (Lallu *et al.* 1992). Reports on peaches harvested at various dates, showed a firmness decrease with increase in maturity (Whitelock *et*

al. 1994). Similarly in other studies, although the storage temperature was not specified, in apple cultivars such as *Golden delicious* and *Pink lady*, it was reported that storage time had a significant effect on firmness. Mango harvested at three different maturity stages (different harvest dates) and stored at 12°C for 0, 14 and 21 days showed firmness reduction with storage time and with maturity stage for all harvest dates (Seymour *et al.* 1990).

In general, in both experiments, there was no effect of relative humidities on the TSS. Changes in brix levels during the incubation time and at each harvest date can be explained as the normal increase in maturity. Results of this present study, agreed with numerous investigations carried out not only with kiwifruit but with other fruit crops. A similar pattern of TSS increase with harvest and storage period has been reported in different cultivars of mangoes (Seymour *et al.* 1990) and apples (Chvyl & Tugwell (1993). Crisosto *et al.* (1984); Lawes & Sawanobori (1984); Nicolas *et al.* (1988); Lallu (1989b); Lallu *et al.* (1989) and MacRae *et al.* (1989b) reported an increase in TSS of kiwifruit (15% approximately) with harvesting season and 4-12 weeks coolstorage period. However it is important to mention that high levels of TSS were achieved at the second and third harvest (second experiment) after seven day incubation period compared with those levels recorded in fruit from the first experiment after a similar incubation time, a difference that was apparent in the values obtained from fruit at harvest.

In this study, differences among TSS in fruit from the first experiment at harvest (9.9%) compared with those obtained after the first week of incubation could be explained by the variation in TSS concentrations in kiwifruit at harvest such as canopy density, position of the fruit within vine etc.. reported by Hopkirk *et al.* (1986).

No specific pattern in ethylene production was observed in relative

humidity/incubation time combinations. In this study fruit from the uninoculated control at any incubation period and fruit from the highest and lowest relative humidity after a one or six weeks incubation period respectively did not produce ethylene. Studies on uninoculated kiwifruit stored at 0°C showed no autocatalytic ethylene production (Sfakiotakis *et al.* 1989). Similar findings were found in carnation flowers (*Dianthus caryophyllus*) stored at 2°C for up to five days. In that study ethylene production completely ceased after the third and fourth days storage, but after the fifth days there was an increase in ethylene production (Field & Barrowclough 1988). Differences in ethylene production in this present study can be explained by the marked differences during ripening of kiwifruit i.e. ethylene production among individual fruits already found by Pratt & Reid (1974) and Hasegawa & Yano (1990).

Fruit from the remainder of the inoculated controls and the other treatments produced higher ethylene levels than those considered as trace amounts (0.1 µl) (Pratt & Reid 1974). It has been widely reported that plants infected by pathogens such as bacteria or fungi can hasten ethylene production (Archer 1976; Archer & Hislop 1975; Elad 1990; Boller 1991). Similarly in *in vitro* studies, Ilag & Curtis (1968), El-Kazzaz *et al.* (1983b) and Kepczynska (1989) found that conidia of *B. cinerea* and 22 other fungi species produced ethylene. Kiwifruit inoculated with *B. cinerea* and stored at 0°C for more than three months, produced high amounts of ethylene compared with uninoculated fruit which produced a very low amount of ethylene after two months storage (Niklis *et al.* 1993). In other studies with kiwifruit, a close relation was found between soft rot extension caused by *Botryosphaeria* and *Phomopsis* spp. and ethylene production (Hasegawa & Yano 1990). Although in other studies the store temperatures were above 0°C, a similar effect induced by other pathogens was observed. In tomatoes inoculated with *Phytophthora infestans* ethylene production increased 36-40h after inoculation coinciding with visual symptoms of infection and disease and compared with small amounts of ethylene in uninoculated

tomatoes (Spanu & Boller 1988). In oranges var. *Valencia*, incubated at 30°C, 94-96 relative humidity for four weeks, an increase in susceptibility to *Ditiorella natalensis* was reported with an increase in exogenous applications of ethylene. Doses of 0, 1, 10, or 50 µl/l for 96 h increased infection about 4. 24, 47 or 65% respectively (El-Kazzaz *et al.* 1983a; Barmore 1985).

In this study, rate of respiration did not show a specific pattern according to relative humidity and incubation time. Only fruit from the inoculated control, and the high relative humidity showed a peak of respiration after the second incubation period declining after the third period of incubation. Fruit from the lowest relative humidity increases their respiration rate for all incubation times. Similarly, in other studies, carried out at 20°C, kiwifruit showed the typical respiratory pattern around five weeks after harvest (Pratt & Reid 1974). In this present study CO_2 values after harvest were higher than those reported by McDonald (1990). Although further details of treatments were not given, CO_2 production of kiwifruit was about 1.3 ml·kg·h at 0°C. Similar low CO_2 values for kiwifruit respiration were reported by Wright & Heatherbell (1967) who found that respiration of kiwifruit stored at 0°C was 2.0 ml·kg·h after six weeks. According to Kader (1992), kiwifruit stored at 5°C is classified as a fruit with a low respiration rate (5-10 mg CO/kg·h). Conversely, Turk (1989) studied half mature and immature figs stored at 1°C, 90% relative humidity and found a range of initial respiration rates between 39-27 ml CO_2/kg·h at harvest, with a climacteric peak after about two or four weeks storage for half mature and mature figs respectively. In other studies carried out on vegetable commodities such as cabbage, carrots, celery and others, the rate of respiration was measured on the basis of respiratory heat production (Q_{10}) (rates of heat production calculated from steady state rates of CO_2 production) at 0, 5 or 16°C. It was found that the heat of respiration was dependant on temperature, storage time and to a lesser extent cultivar. In that study lower Q_{10} values were reported in commodities incubated at 0°C

for longer periods (4-6 weeks) compared with higher temperatures of 5 or 16°C (Van den Berg & Lentz 1972). An explanation for the undefined pattern of CO_2 production during storage in some treatments of this present study can be related to individual differences in the ripening process already explained by Pratt and Reid (1974).

In this present study, ethylene production was not paralleled by increased flesh softening, the rise of respiration rate and soluble solids content as reported in other investigations in kiwifruit (Hyodo & Fukasawa 1985; Cheng *et al.* 1994). Contrary to other reports, the synergistic effect of rate of respiration versus ethylene production were not observed in this present study (Sfakiotakis *et al.* 1989). Likewise, related effects of relative humidity, water loss and ripening were not apparent in this study, as reported for other commodities (Gac 1956; Vaadia *et al.* 1961; Littmann 1972). In those studies although the storage time was not specified it was reported that weight loss hastened the ripening process i.e. the green life of pears were reduced five days when stored at 5°C, 57-86% relative humidity and four to ten days in avocados, bananas and pears when stored at 20°C, 13% relative humidity.

Infection levels during coolstorage

In the second experiment there was a more marked increase of infection levels with a low relative humidity. An analogous tendency was observed in the first experiment when fruit were incubated for one week. Similar findings have also been reported by Goodliffe & Heale (1977) who inoculated carrots with *B. cinerea* before incubation in a range of humidities at 5°C. They suggested that the ability of the secondary phloem parenchyma of the root to resist invasion by this pathogen was markedly reduced when the weight loss of carrots in storage was between 5 and 10%. Carrots kept at lower humidities lose weight more quickly and become susceptible to *Botrytis* attack sooner. The kiwifruit stem scar is wound tissue and not strictly comparable with the secondary phloem parenchyma of the

carrot root but the principle of the conditions being more favourable to the host defense mechanism than to the pathogen still apply. Thus at a low relative humidity the stem scar tissue could desiccate and die before active defense mechanisms are established thereby providing dead tissue for colonisation by necrotrophic pathogens such as *B. cinerea* in addition to weakening or inactivating the host defense. This argument is supported by the work of Van den Berg & Lentz (1974) who observed that decay in some vegetables was reduced when stored at 0-1°C at a relative humidity between 98-100% than when stored at a relative humidity of 90-95%. In the current study, infection levels from both experiments at the highest relative humidities (100% and 92-97%) were reduced compared with those at the lower relative humidities.

Conversely, Sharky & Peggie (1984) studied the influence of three relative humidity ranges on storage decay of cherries, lemons and peaches and found that in the first crop, percentage decay in fruit held at 90-94% and 95-99% relative humidities was similar. However, infections in lemons stored at 95-99% were fewer compared with those at other relative humidities while storage rots were low in peaches stored at any relative humidity range.

In this study, at the second experiment (1992), percentage infection also varied according to harvest maturity. High infection levels were recorded in fruit from the third harvest. On the other hand, infection levels in fruit from the inoculated control from the second experiment were lower compared with those infection levels recorded from the inoculated controls of the first year experiment. In epidemiology studies of *B. cinerea* in kiwifruit patterns of infection levels developing in coolstorage varied on a day-to-day basis of harvest and also year-to-year (Brook 1990a). He related these daily and seasonal variations to weather conditions to differences in the overall population of *B. cinerea* in the orchard and to numbers of mobile spores at the time of harvest. Brook (1990a) also reviewed the contradictions in

evidence for the concept that kiwifruit stem-end rot susceptibility is reduced with kiwifruit maturity. Data reported by others (Hopkirk *et al.* 1990a; 1990b) showed that increase in harvest date reduced *B. cinerea* rots from 14.9 to 1.3%.

CHAPTER EIGHT

GENERAL DISCUSSION AND FUTURE RESEARCH

Kiwifruit are one of New Zealand's major export crops and any constraints on production and export of high quality produce will have an adverse effect not only on the kiwifruit industry but on New Zealand as a whole. The New Zealand Kiwifruit Marketing Board is the sole New Zealand exporter of this commodity worldwide and has suffered severe economic losses from the *Botrytis* storage rot problem. These losses involve a direct loss from diseased fruit during coolstorage plus a loss of other fruit which have softened because of the ethylene produced by diseased fruit, cost of transporting diseased fruit overseas and the high cost of manual inspection and repackaging of fruit from infected lines. As recently as 1994 there were several reports highlighting the high levels of *Botrytis* infections observed in fruit sold to overseas markets (Anonymous 1994b; 1994c; Tapper 1994). Of greatest concern is the perception of New Zealand kiwifruit by our major markets. The New Zealand Kiwifruit Marketing Board makes great efforts to ensure that New Zealand fruit is of top quality and heavily promotes a top quality image in its advertising campaigns. The *Botrytis* storage rot problem has dented this image as evidenced by reference to it in some European countries such as Germany, Switzerland and Austria as the *New Zealand disease* (Tapper 1994).

Before commencing a study of the effect of humidity and temperature on infection of kiwifruit by *B. cinerea* it was important to know more about the variables which could affect the results and to develop a standardised procedure for inoculation. Work by Long and Wurms (1993) had already established that high inoculum levels were necessary to give a reasonable incidence of infection for experimental work. Other workers (Yoder & Whalen 1975) have found that at high concentrations the conidia of *B.*

cinerea have a low percentage germination. However, this self-inhibition found by those workers was not manifest in terms of percentage infection of kiwifruit in the work of Long and Wurms (1993). Disease incidence of *B. fabae* on beans has been shown to be proportional to inoculum concentration (Last & Hamley 1956) and aggressiveness as measured by lesion size has been shown to be influenced by spore concentration of *B. cinerea* (Louis 1963), *B. allii* (Segall & Newhall 1960) and *B. tulipae* (Price 1970). A range of inoculum levels were used in this work to ensure that a reasonable number of infections were obtained without overwhelming the host defenses.

Long & Wurms (1993) found that infection levels of *B. cinerea* on kiwifruit could be increased by adding nutrients to the spore suspension - a phenomenon already well documented for *B. cinerea* infections of other crops (Chu-Chou & Preece 1968; Fokkema 1971; Khöl & Fokkema 1994). This was not attempted here as the aim was to achieve a *natural* type of inoculum as far as possible. However there remains the question of whether the growing medium affects the nutrient status of the spores. The age of *B. cinerea* spores is known to affect both germination rate and infectivity, an effect that can be modified by nutrition, hence the initial trials focused on the question of substrate and colony age on which the inoculum was produced.

B. cinerea is a weak, facultative parasite which colonises wounds and dead tissues of a wide range of host plants. Despite this necrotrophic growth habit Williamson & Hargreaves (1981) and Williamson & Jennings (1986) have found that there is some host specialisation on raspberries. The contribution of inoculum from neighbouring fields or orchards of other crops such as grapes is not known but there are definite differences in percentage infection of kiwifruit by *B. cinerea* between orchards in different regions, within orchards and with fruit maturity at harvest (Hopkirk *et al.* 1990a; Manning & Pak 1993; Pyke *et al.* 1993). Such differences were found in the

present work where disease incidence of fruit harvested at Levin was greater than that of fruit at Palmerston North (Chapter 3). Similarly, conidia produced on autoclaved kiwifruit leaves caused more infections at Levin while those produced on MA caused more infections at Palmerston North. However, there was no evidence of any host specialisation of isolates obtained from kiwifruit compared with those from other crops.

At the conclusion of this first set of trials it was decided to use the K3 Massey University isolate since there was no apparent host specialisation, this isolate was known to be virulent on kiwifruit and it had already been used extensively on kiwifruit trials here. Inoculum was prepared from colonies grown on MA for 10-14 days since MA was more consistent and easier to prepare than autoclaved kiwifruit leaves and spore age within the 7-18 day range did not appear to be critical for this work.

Further work on host specificity would require the collection of a larger number of isolates than used here and from more crops with cross-inoculation of these isolates onto all the crops from which they were collected. This would give a more definitive answer to the question of host specialisation but was too big a project to be undertaken within the scope of this work. The development of new strains of *B. cinerea* through UV and chemical mutagenesis could also throw light on this problem. If host specialisation were detected isolates could be crossed by the method developed by Faretra & Antonacci (1987) to study the genetic control of such host specialisation.

The second step before starting the curing experiments was to establish the parameters of survival of *B. cinerea* conidia by *in vitro* exposure to a range of temperature/humidity combinations that could possibly be used for curing kiwifruit (Chapter 4). Some *B. cinerea* conidia survived in all the treatments tested but there was a reduction in survival rate at the higher temperatures. Of greater practical significance could be the reduction in speed of

germination after exposure to some treatment/humidity combinations. The speed with which a plants defence mechanisms are activated can determine the difference between susceptibility and resistance (Browder 1985; Goodman & Novacky 1994) and any factor which slows down pathogen germination and growth could alter the balance between successful and unsuccessful infection (Rotem *et al.* 1978; Harrison *et al.* 1994). The resistance of the kiwifruit stem scar has been shown to increase within a few hours of harvest (Poole & McLeod 1994) so a delay in germination from less than eight hours to more than eight but less than 24 hours could be critical.

The options for control of *B. cinerea* infections prior to and during coolstorage have already been discussed in the General Introduction (*Chapter One*) where it was pointed out that curing would appear to be the most promising non-pesticide control measure since significantly fewer *Botrytis* rots were recorded when fruit were cured compared with non-cured fruit (Beever 1991; 1992). However, the early investigations of curing kiwifruit were carried out at ambient temperatures and humidities, not under controlled conditions. Manning *et al.* (1990) did achieve a curing effect at 18°C, Poole *et al.* (1990) found that a packing delay (curing period) of 5-6 days at 14-16°C had no consistent effect on curing and Cheah *et al.* (1991). found that in one trial the process of curing actually increased the incidence of infections caused by *B. cinerea*: disease incidence was 27.3% when fruit were coolstored within three hours of harvest but was 42.4% when coolstorage was delayed three days. The optimum curing conditions in terms of temperature and humidity are known to vary according to the specific crop/pathogen combination (Kitinoja & Kader 1994). For example, Ben-Yehoshua *et al.* (1987a; 1987b; 1989) found that a range of temperatures from 21°C to 42°C were required to control different species of *Penicillium* in citrus. However, in tuber crops such as potato the curing temperatures may be much lower. Hide & Boorer (1991) reported that the best curing conditions to reduce potato decay caused by *Helminthosporium*

solani (Dur. & Mont.) were a temperature of 15°C and humidity of 75% for two weeks.

In the current study, the optimum temperature for curing kiwifruit was 10°C. At 0°C and at 5°C there was little curing effect after three days - in some trials the disease incidence was increased compared with that on the control fruit which were packed in the usual commercial manner. At temperatures above 20°C the curing effect was less marked. Temperatures in the North Island of New Zealand during the April/May period when kiwifruit are harvested would normally be in the 5-20°C range depending on locality and time of day.

An explanation for the results of Cheah *et al.* (1991) could be that if fruit were harvested and left at ambient temperatures on unusually hot or unusually cold days then the curing effect would be minimal or even reversed. Other data of Cheah *et al.* (1991) from controlled temperature experiments support this rationale. They obtained a curing effect at 5°C but disease incidence was almost doubled at 15°C and more than double at 25°C.

Previous approaches to investigations of the effect of humidity on kiwifruit have relied on relatively crude techniques such as packing fruit in trays with polyethylene liners to achieve a *high humidity* and leaving trays open for a *low humidity* (Pennycook & Manning 1992). In the current study, after overcoming problems such as salt blocking, adequate containers, etc. as explained in the *General Materials and Methods*, a well defined relative humidity system was achieved. With the attainment of this system of an established, reliable relative humidity range over individual fruit it was possible to obtain a more accurate picture of the effect of relative humidity on curing of kiwifruit. It was observed that highest relative humidity ranges decreased weight loss during curing and infection levels during coolstorage. Firmness and TSS were not affected by any relative humidity treatment.

No process can be *fine tuned* to achieve the optimum results if the underlying mechanisms are not understood. Curing of onions to prevent neck rot (Sanguansri & Gould 1990; Kasmire & Cantwell 1992) is carried out with the objective of desiccating the neck tissues. On the other hand, curing of most root crops is done to provide a warm, humid environment in which damaged tissue will remain alive and actively carry out repair work and stimulate the development of defense mechanisms. The later would seem to be the case with kiwifruit where curing was not effective at the low temperatures or low humidity that would have inactivated the cells at and near the stem scar surface. Although constraints in time did not permit extensive analytical work, histochemical reactions showed suberin deposition in the xylem vessels of fruit cured at 10°C for more than two days. In the present study there was an attempt to observe and to evaluate the anatomical and structural composition of the stem scar of kiwifruit. Since the arrangement of the vascular tissues in the stem scar is uneven, the first step would be to find a suitable technique that would permit an accurate study of the xylem tissue in the stem scar. It would be important that the study detailed specific aspects such as length of vessel and related them to incidence of *B. cinerea* rots as has been done by Zimmerman (1983) who associated diameter, length and number of xylem vessels with resistance to *Ceratocystis ulmi* (Busiman) C. Moreau on elms. This is an area where the potential for use of confocal laser scanning microscopy and nuclear magnetic resonance imaging could be investigated.

No combination of curing temperature and humidity will be acceptable by the kiwifruit industry if non-disease parameters of fruit quality are adversely affected. Ethylene is considered one of the most important physiological parameters because of its influence on fruit ripening. In the present study, no adverse effects of ethylene on fruit quality were detected during curing or during the subsequent coolstorage. However fruit cured at 20 or 30°C showed high ethylene production. Fruit weight loss increased with curing temperature, incubation time and the lowest relative humidity. Curing

temperatures between 10 or 20°C together with relative humidities higher than 92% for a curing period of no more than three days should provide an adequate disease control without excessive weight loss. Firmness dropped quickly during curing and coolstorage (2.5 - 1.5 kgf) but these values remained above the minimum (1.0 kgf) recommended by The New Zealand Kiwifruit Marketing Board for fruit export (McDonald 1990).

Curing treatments did not affect the chemical composition of fruit components such as TSS, sugars and pH.

Another important step in this study was the evaluation of the effect of initial coolstorage (0°C) relative humidity on fruit quality infection levels during the incubation period and the subsequent storage period. The literature review showed that a low temperature is required to maintain the quality of commodities for long-term storage (Wade 1990). However, references to coolstorage relative humidity vary from commodity to commodity (Allen & Pentzer 1935; Mann 1957; Grierson & Wardosky 1975; Van den Berg & Lentz 1977a; 1977b). These initial, defined humidities did not produce a consistent effect on ethylene and carbon dioxide production, but firmness rapidly decreased during the incubation period. However, the firmness values remained within the limits accepted by The New Zealand Kiwifruit Marketing Board. Infection levels fluctuated with respect to the relative humidity and to incubation time. The overall general pattern showed that with an increase in incubation time infection levels decreased. Fruit harvested in *1993* showed a similar response to humidity as those in the curing experiments at 5 - 30°C where the lowest relative humidity gave the highest infection levels during the subsequent coolstorage.

Under practical conditions even though *Botrytis* storage rots develops on kiwifruit at relative humidities of 95% (the recommended storage relative humidity), higher relative humidities should achieve the dual objectives of maintaining overall fruit quality as well as minimising storage rots. Further

work is necessary to relate this work to the specific environmental conditions found during postharvest management of fruit such as temperature and relative humidity in bins, during transportation, packing, coolstorage and export. In particular, the influence of a continuous air flow over the stem scar should be checked against still air under a combination of temperature and relative humidity conditions since mass air flow through bulk bins may be difficult to achieve.

This work has advanced the knowledge of infection of kiwifruit by the fungal pathogen *Botrytis cinerea*. Of particular importance is the development of an apparatus for ensuring a constant, uniform flow of air within a specified humidity range over a sufficient number of fruit to carry out physiological and infection studies. This has allowed a precise evaluation of the effect of humidity during the curing period and is a technique that should be applicable to many other host/pathogen combinations. The optimum temperature/humidity combination of 10°C and > 92% relative humidity provides a basis for larger scale, semi-commercial applications which should give greater confidence to the kiwifruit industry in the curing process.

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APPENDIX

Tissue Dehydration Procedure: (Feder & O'Brien 1968)

1.- Remove excess fixative in running water.

2.- Transfer the fixed tissue to methyl cellosolve at about 0°C.

3.- Two changes of methyl cellosolve (24h ea.).

4.- Transfer the tissue to 100% ethanol at 0°C (24h).

5.- Transfer the tissue to xylene at 0°C (24h).

Embedding Procedure: (Bautista-Baños 1989)

1.- Transfer dehydrated tissue to a mixture of xylene and paraffin in the following order: 75:25 (24h), 50:50 (12h) and paraffin (24h).

2.- Vacuum infiltrate overnight in Paraplast.

3.- Place infiltrated tissue in embedding moulds and fill with paraffin.

4.- Trim and section the tissue.

5.- Place sectioned tissue on glass slides.

6.- Keep microscope slides in oven at 35°C for 24 h.

Removal of paraffin from the tissue: (Bautista-Baños 1989)

1.- Place slides in two changes of xylene (5 min. ea.).

2.- Transfer to another solution of equal parts of xylene and absolute alcohol (5 min.).

3.- Transfer trough an ethanol series and xylene (100, 95, 75, 50 and 25%; 5 min. ea.).

4.- Transfer to distilled water.

5.- Stain.

Staining procedures:

a) Safranin-Fast Green: (Bautista-Baños 1989)

1.- Stain with safranin "O" (5 min.).

2.- Wash with distilled water.

3.- Transfer through an ethanol series: 25, 50, 75, 95 & 100% (5 min ea.).

4.- If overstained, transfer to acidulated alcohol (20 sec.).

5.- Stain with Fast-Green (20 sec.).

6.- Transfer to xylene (Two changes: 4 min ea.).

8.- Mount in D.P.X. and add coverslip.

b) Differential staining for fungus and host: (Johansen 1940)

Stain the slides for 2 minutes in 1% methyl violet in 50% ethyl alcohol, wash in 50% alcohol and stain for 45 minutes in 1% eosin. Transfer to two changes of xylene (5 min. ea.), mount in D.P.X. and add coverslip.

c) Glycerine-ferricyadine: (Sherwood and Vance 1976)

Place the tissue in glycerine solution (5 min.), rinse with water, dry and add one drop of 0.02 M FeCl3 solution and one drop of 0.02 M potassium ferricyanide solution.

Preparation of solvents and stains:

Formalin-Aceto-Alcohol (FAA): (Johansen 1940)

Absolute alcohol	500 ml
Acid Glacial Acetic	50 ml
Formaldehyde	100 ml
Distilled water	350 ml

Safranin "O": (Johansen 1940)

Dissolve 4g of safranin in 200 ml Methyl Cellosolve. Add 100ml each of 95% alcohol and distilled water.

Fast-Green: (Bautista-Baños 1989)

Saturated solution A

Fast Green 1 part

Alcohol absolute 1 part

Methyl Cellosolve 1 part

Solution B

Alcohol Absolute 25 parts

Clove oil 75 parts

Mix solution A and B (1:1)

Sudan IV: (Johansen 1940)

Saturated alcoholic solution. Stain for about 10 minutes and wash in alcohol

Sudan Black B: (Peacock & Bradbury 1973)

Sudan Black B	5 g
Alcohol (70%)	100 ml

Reflux for 20 minutes cool and filter

Phloroglucinol - HCl: (Peacock & Bradbury 1973)

Phloroglucinol	5 g
Alcohol (75%)	100 ml
HCL (concentrated)	1 drop

Toluidine Blue "O": (Peacock & Bradbury 1973)

Toluidine Blue	0.25 g
Alcohol (70%)	100 ml
HCl (concentrated)	0.5 ml

PUBLICATIONS

ABSTRACTS

6th International Congress of Plant Pathology

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RÉSUMÉS

6^{e} Congrès international de phytopathologie

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14.3.6

ETIOLOGY OF PREHARVEST INFECTIONS OF CHERRY FRUITS. F.M. Dugan and R.G. Roberts. USDA ARS Tree Fruit Research Lab, Wenatchee, Washington.

Samples of 100 symptomless Bing cherry fruits were collected from each of 3 orchards for 10-11 weeks after petal fall. Fruits were surface-disinfested and incubated. By the second week more than 10% of fruits contained infections; at harvest 90-100% of fruits contained one or more species of fungi. Most infected fruits yielded species of *Cladosporium, Alternaria* and/or *Aureobasidium*. Most infections originated at stylar or receptacular scars. Tests with representative isolates demonstrated pathogenicity to cherry fruits. Fungi in approximately 50 other genera were isolated, including *Penicillium, Ulocladium, Botrytis, Aspergillus, Phoma, Stemphylium, Geotrichum* and *Arthrinium*.

14.3.7

INOCULUM LEVEL OF *BOTRYTIS CINEREA* AND DISEASE DEVELOPMENT IN KIWIFRUIT DURING COOL STORAGE. [1]P.G.Long, [2]S.Ganeshanandam, [1]Department of Plant Science, [2]Department of Statistics, Massey University, Palmerston North, New Zealand.

Kiwifruit were harvested in May 1991 from a commercial orchard in Wanganui. Each kiwifruit was inoculated on the stem scar with one drop of a spore suspension adjusted to contain 0, 10, 100, 1,000, 10,000 or 100,000 spores of *B. cinerea* per drop. One batch of fruit was inoculated within 10 h of harvest. Others were held in ambient conditions for 24 h or for 4 days before inoculation and coolstorage. Fruit was assessed at weekly intervals from the first appearance of symptoms (week 4) to 12 weeks of cool storage.

Most infections appeared after 4-8 weeks of cool storage. There was a consistent relationship between inoculum level and disease incidence but even at the highest inoculum level fewer than 50% of the fruit became diseased. Once visible symptoms of infection appeared, the disease lesion spread at a constant rate of 0.8-1.00 mm/day.

14.3.8

KIWIFRUIT PICKING SCAR TREATMENT AND DEVELOPMENT OF *BOTRYTIS CINEREA* INFECTIONS DURING COOL STORAGE. [1]K.V.Wurms, [1]P.G.Long, [2]N.Pyke, [3]G.Tate, [4]S.Ganeshanandam, [1]Department of Plant Science, [4]Department of Statistics, Massey University, Palmerston North, [2]HortResearch, Riwaka Research Centre, [3]HortResearch, Hawkes Bay Research Centre, New Zealand.

The influence of age and condition of kiwifruit picking scars on susceptibility to infection by *B. cinerea* was examined on fruit harvested and coolstored at three geographically different locations (Wanganui/Massey University, Riwaka and Hawkes bay). There were four stem scar treatments. Inoculation of: 1) fresh picking wounds, created by harvesting fruit with pedicels attached and removing the pedicel immediately prior to inoculation, 2) old scars where the pedicel was removed at harvest, 3) the cut ends of pedicels and 4) stem scars with a standard wound made with a 5 mm drill bit. Batches of fruit were left for 0 h, 6 h, 24 h, or 6 wks before inoculation with one drop of a spore suspension containing 5000 spores of *B. cinerea* per drop. Percentage infection was assessed monthly.

Overall infection levels were different at each of the three sites but the same pattern of results was obtained. Differences between stem scar treatments and between inoculation times were highly significant. Disease incidence was not reduced by leaving fruit up to 24 hours before inoculation, but if cool stored for six weeks before inoculation then percentage infections were reduced in all treatments. Numbers of successful infections were significantly higher from inoculation of wounded stem scars or attached pedicel stumps than from inoculation of fresh or old stem scars. These results indicate that natural defense mechanisms operate at the stem scar and that these mechanisms are not activated unless the pedicel is removed. This information should aid the development of a standardized artificial inoculation technique that produces consistently high levels of infection.

14.3.9

EFFECT OF CULTURE AGE AND GROWTH MEDIA ON INFECTIVITY OF CONIDIA OF *BOTRYTIS CINEREA* ON KIWIFRUIT. [1]S. Bautista, [1]P.G.Long, [2]L.Cheah, [3]S.Ganeshanadam. [1]Department of Plant Science, [3]Department of Statistics, Massey University, Palmerston North, [2]Crop and Food Research, Levin, New Zealand.

Spore suspensions are frequently used to inoculate plant material with fungal plant pathogens. This work was carried out to determine whether the substrate on which the fungus is grown and the age of the cultures influence the infectivity of spore suspensions as of *B. cinerea*. The experiment was repeated in two locations: Massey University (using fruit from Wanganui) and Levin. *B. cinerea* was grown on Malt agar (MA), Potato Dextrose agar (PDA) and autoclaved kiwifruit leaves (Kwlv). Cultures 7, 18 and 28 days old were used to prepare concentrations of 1000, 5000 and 25000 spores per 17 µl drop of suspension. An additional treatment of 125000 spores per drop was used at Levin. Fruit was inoculated within a few hours of harvest by placing one drop of spore suspension on the stem scar. After 12 weeks coolstorage, disease incidence showed significant differences between the spore concentration treatments at both locations. High inoculum levels gave high levels of disease. At Levin, disease incidence was highest when autoclaved kiwifruit leaves were used to produce inoculum There was no significant effect of culture age. At Massey, there was a significant interaction between media and culture age. Spores from 18 day old colonies growing on MA gave the highest percentage infections after both 6 and 12 weeks of coolstorage.

14.3.10

ETIOLOGY AND POSTHARVEST CONTROL OF LEUCONOSTOC ROT OF TOMATO FRUIT. K.E. Conn, J.M. Ogawa, and J.E. Adaskaveg. Department of Plant Pathology, University of California, Davis, U.S.A.

A coccoid bacterium, *Leuconostoc mesenteroides* ssp. *mesenteroides* (*LMM*), was reported for the first time as a plant pathogen. This bacterium was isolated originally from harvested tomato fruit grown in fresh market production regions in California and Mexico. After inoculation, symptoms are visible within 24-48 hr on immature, mature, and ripe tomato fruit. Decayed portions of mature fruit are firm, water-soaked, and fail to turn red when fruit are ripened. All isolated strains of *LMM* were pathogenic and include strains from *LMM*-decayed tomato fruit and those affected with various fungal decays. Epiphytic populations were isolated from fruit and leaves of tomato, foliage of weeds, and soil from tomato fields, as well as from chlorinated water dump tanks, packing lines, and fruit in packed boxes. To examine the effect of chlorination on survival of *LMM*, laboratory tests were done by suspending strains grown on agar or in broth in sterile, glass-distilled water containing sodium hypochlorite. Strains were inactivated in 50 µg/ml free chlorine (Cl) after 1 min, but not consistently in 10 µg/ml. In similar laboratory tests, tomato fruit were sprayed with *LMM* (1 x 10^8 cfu/ml; 1 ml/fruit), air dried (24 hr), and agitated in free Cl (2 min, 50 µg/ml) to simulate dump tank conditions. Chlorinated water failed to completely inactivate the bacterium on wounded and non-wounded fruit surfaces. In packinghouse studies, non-wounded fruit sprayed with *LMM* (1 x 10^8 cfu/ml; 1 ml/fruit) and immersed (2 min) in a dump tank (100 µg/ml Cl, pH 7.5-8.3), showed symptoms of infection by *LMM* (up to 24% of fruit). Additionally, the bacterium was recovered from most of the Cl-treated fruit that showed no symptoms after 11 days at 20C.

14.3.11

IPRODIONE-WAX/OIL MIXTURES FOR CONTROL OF POSTHARVEST DECAYS OF FRUIT CROPS. J. E. Adaskaveg, J. M. Ogawa, and K. E. Conn, Dept. of Plant Pathology, Univ. of California, Davis, CA USA

Iprodione-containing fungicides mixed with specific wax/oil emulsions proved more effective than iprodione alone or other fungicides for control of postharvest decays of fruit. Major decays caused by *Alternaria alternata* (apple, pear, tomato), *Botrytis cinerea* (apple, cherry, pear, tomato), *Monilinia fructicola* (cherry, peach), *Penicillium expansum* (apple, pear), *P. digitatum* (orange), *Rhizoctonia solani* (tomato), and *Rhizopus stolonifer* (apple, cherry, peach, pear, tomato) were controlled effectively with iprodione-wax/oil mixtures as indicated by disease incidence and severity (lesion diameter). Efficacy of iprodione-wax/oil mixtures was similar to dichloran for control of Rhizopus rot. One wax/oil formulation mixed with iprodione also was effective for control of *Mucor racemosus* of pear. Quantitative analyses indicated that wax/oil emulsions increased the solubility of iprodione several hundred fold, whereas residual amounts on treated fruit were increased slightly. Optimal percentages of wax/oil emulsions added to iprodione were different for each crop. Quantitative studies on volume of fungicide mixtures applied (454 g a.i./378.5 L) indicated that for a specific crop and fruit size, a critical volume is necessary for effective decay control. In these tests, residual amounts of iprodione measured for whole fruit samples were similar. Thus, solubility and availability of iprodione, as well as coverage of fruit during postharvest fungicide applications are critical for improved efficacy of treatments and for effective decay control. In additional tests, a Rhone-Poulenc, experimental fungicide mixture was more effective than iprodione for control of blue mold of apple and orange fruit.

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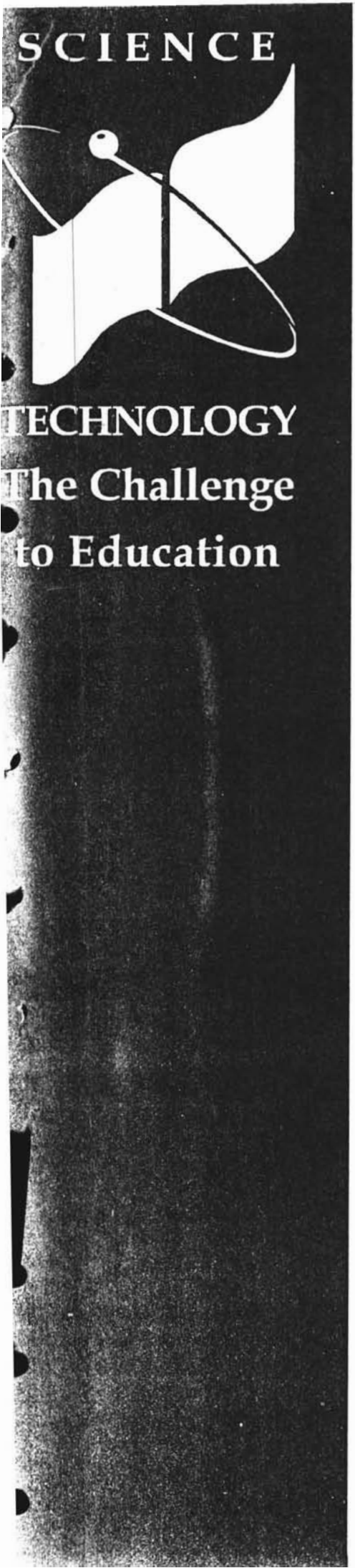

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SCIENCE TECHNOLOGY The Challenge to Education

Massey University, 22-25 August, 1994

Initial coolstorage relative humidity and infection by *Botrytis cinerea* of kiwifruit at three maturities.

Silvia Bautista-Baños[1], *Peter G. Long*[1], *Marion O. Harris*[1], and *S. Ganeshana*[2]
[1]Department of Plant Science,
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Massey University, Palmerston North.

Fruit were harvested from the Massey University Fruit Crops Unit. Palmerston North, New Zealand, on three different dates in 1993 to obtain a range of fruit maturities at harvest. The initial total solid solubles (%) and firmness (kgf) from the early, middle and late harvest were 6.6, 11.2 and 12.8% and 9.0, 9.5 and 6.8 kgf respectively. Before coolstorage, fruit were artificially inoculated with ca. 25,000 *Botrytis cinerea* spores in a 17µl droplet of water. At each harvest date, fruit were held at 0°C for 7 days at three different relative humidity (RH) ranges. Weight loss (%), firmness and total solid solubles were measured at the end of this period. Subsequently fruit was stored at 0°C to evaluate infection levels after 12 weeks. At the three harvest dates weight loss increased as relative humidity decreased and firmness was significantly higher at the highest RH. Total solid solubles were significantly higher at the late harvest and infection levels were significantly lower at the highest relative humidity for all three harvest dates.

Effect of mineral nitrogen on spring growth and nitrogen fixation of white clover lines.

H.B. Berthelsen[2], *J. Ford*[1], *J. Evans*[1] and *J.R. Caradus*[1]
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[2]Department of Agricultural Sciences, The Royal Veterinary and Agriculture University, Denmark.

White clover *(Trifolium repens* L.) can assimilate nitrogen either through biological nitrogen fixation or uptake of mineral nitrogen. These are not mutually exclusive however, white clover persistence in mixed species swards supplied with mineral nitrogen is however often poor due to aggressive grass growth. Several studies have compared the growth of different white clover cultivars in grass swards supplied with nitrogen. The aim here is to measure the impact of mineral nitrogen applied at 0, 100 and 200 kg N/ha, on the nitrogen fixation of a range of white clover cultivars and breeding lines. Measurements were made of acetylene reduction, nitrogen content and herbage yield at two weekly intervals during mid- to late-spring, when demand by grasses for nitrogen is often greatest. There were no nitrogen x breeding line interactions for any of the characters measured. However, nitrogen effects were significant for acetylene reduction, clover dry weight, clover percent nitrogen, grass percent nitrogen and total sward nitrogen. Date and breeding line differences were significant for most characters measured. There were significant nitrogen x date interactions for acetylene reduction, clover percent nitrogen, grass percent nitrogen, total sward nitrogen and proportion of clover to total sward nitrogen. Cultivar x date interactions were significant for proportion of clover in sward, and clover, grass and total sward dry weight.

Expression of the GUS gene in 11 *Pelargonium* cultivars transformed by *Agrobacterium tumefaciens.*

M.R. Boase and *J.L. Smith*
Crop & Food Research, Levin Research Centre, Private Bag 4005, Levin.

We intend to extend the colours and patterns in pelargonium flowers using genetic engineering. As part of this programme, we sought to increase the range of cultivars that can be transformed. We inoculated leaf discs of seven regal (*Pelargonium x domesticum*) and four zonal (*Pelargonium x hortorum*) cultivars with two strains of *Agrobacterium tumefaciens* (LBA4404 & A722), each containing either plasmid pKIWI110 or pLN3. The cultivars tested were the regals - Hazel Cherry, NC Eatse, Alaska, Her Majesty, White Chiffon, White Dawn, Mrs James Rodgers - and the zonals from F1 hybrid seed - Orange Appeal, White Multibloom, White Orbit and White Elite. We assayed the 14 mm diameter leaf discs for GUS expression at four to six days and nine to 14 days after inoculation. All 11 cultivars gave GUS positives with at least one strain/plasmid combination on one occasion. Fourteen out of 24 discs (58.3%) from eight cultivars tested four days after inoculation were GUS positive. At day nine, 17 of 27 discs (63%) from the same eight cultivars were GUS positive. We have developed leaf disc regeneration systems for three of these cultivars and intend to produce transgenic plants containing novel flavonoid genes.

Maximising shelf-life of retail packs of chopped lettuce.

D.W. Brash and *Liu XuFeng*
Crop & Food Research, Levin Research Centre,
Private Bag 4005, Levin.

Modified atmosphere packaging (MAP) is being used widely overseas for retail packs of chopped lettuce. Customers are looking for ready-to-use lettuce salads at the supermarket.

We compared the visual quality of chopped lettuce in a range of films and packaging methods at 5°C over a period of 120 hours. While a CA (controlled atmosphere) treatment was best the gas flushed MAP (modi-

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PHYSIOLOGICAL CHANGES OF INFECTED KIWIFRUIT DURING A CURING PERIOD

Bautista-Baños, S.[1]*, Long, P.G.[1] and Ganeshanandam, S.[2]

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[2] Department of Statistics, Massey University, Palmerston North, New Zealand.

Stem-end rot cause by the fungus *Botrytis cinerea* is the most important disease of stored kiwifruit *(Actinidia deliciosa)* in New Zealand. In this study harvested fruit stem scars were inoculated with B. cinerea and held for a pre-coolstorage curing period at 90-100% RH and temperatures of 0°C, 10°C, 20°C or 30°C. Fruit were incubated for 2, 4 or 6 days. During curing fruit samples were tested for ethylene production ($\mu l \cdot kg^{-1} \cdot hour^{-1}$), rate of respiration ($cm^3 \cdot CO_2 \cdot kg^{-1} \cdot hour^{-1}$), weight loss (%), and firmness (kg/f). Numbers of infected fruit (%) were evaluated after 12 weeks coolstorage at 0°C. Levels of ethylene, production of CO_2 and percentages of weight loss increased as the period of curing and temperature increased. Temperature and curing time did not affect firmness of fruit. Percentage infection of fruit was significantly less at the lower temperatures.

Co-authors

Family Name	Initials
LONG	P.G.
GANESHANANDAM	S.

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