# EFFICACY OF GIBBERELLIC ACID IN CONTROLLING PATHOLOGICAL DECAY AND MAINTAINING POST-HARVEST QUALITY OF PASSION FRUITS (*Passiflora edulis* var. *edulis* Sims.)

BY

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DEPARTMENT OF HORTICULTURE

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## DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

George Mwangi Ngugi (signed) — 16th October 2001

**GEORGE MWANGI NGUGI** — **DATE**

This thesis has been submitted with our approval as University supervisors

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**ABSTRACT**

Pathogens cause deterioration of passion fruits and resist pesticides applied post-harvest to control them. Pesticides, unlike most plant growth regulators, may pose a health hazard to consumers. This research aimed at determining the effect of gibberellic acid ($GA_3$) and timing of application on the post-harvest quality of passion fruits inoculated with *Penicillium expansum* and stored in a room maintained at 17 to 22° C and over 85 % relative humidity for 48 hours. Fruits were sprayed with 0, 50, 100, or 250 ppm $GA_3$, packed in clear polythene bags and kept at room temperature. Repeated data were recorded at 2-days interval for 20 days post-treatment.

Early application of $GA_3$ significantly maintained soluble solids, colour, firmness, chlorophyll, and weight, and slowed disease incidence and severity, fruit loss, and ethylene evolution. Application of $GA_3$ at 0 days post-harvest (dph) was more effective in delaying deterioration than application at 5, 10, or 15 dph.

Gibberellic acid significantly inhibited disease incidence, fruit loss, and ethylene evolution frequently, whereas it significantly affected colour, soluble solids, chlorophyll, and firmness occasionally. Weight and disease severity were least affected by $GA_3$. The effects of 50, 100, and 250 ppm $GA_3$ were similar, but greater than of 0 ppm $GA_3$.

In a related experiment, passion fruits were stored for 0, 5, 10, or 15 dph before fungi were isolated on a potato dextrose agar medium to determine the effect of storage time on prevalence. *P. expansum*, *Fusarium oxysporum*, *Alternaria passiflorae*, *Septoria* sp., *Cladosporium* sp., *Aspergillus flavus*, and *Rhizopus stolonifer* were isolated. Prevalence increased with storage time.

This research revealed that passion fruits should be treated with preservatives soon after harvest, because delaying renders the fruits susceptible to post-harvest pathogens.

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Last but not least, a lot of appreciation goes to my classmates, staff mates, wife and daughter, whose assistance in one way or another enabled this research to be completed. Thank you all.

## DEDICATION

Emillianah Wanjiku Wanjama (wife) and daughter, Njeri.

## BIOGRAPHICAL SKETCH

George Mwangi Ngugi was born on 24th January 1971 in Karinga Village Kamahula Location, Muranga District by the late Bernard Ngugi and Rakael Njeri. He was baptised on 3rd April 1983 in Thaara Catholic Church. He attended Thaara Primary School from 1978 to 1985. He joined Githembe Secondary School from 1986 to 1989.

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In mid 1996, he was employed as a teaching assistant in the Department of Horticulture at Egerton University. He married Emillianah W. Wanjama in 1997, with whom he was blessed with a baby girl Rakael U. Njeri in Jan. 2000. In 1998, he enrolled for a M.Sc. degree in Horticulture at Egerton University.

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# CHAPTER ONE

## INTRODUCTION

Passion fruit belongs to the genus *Passiflora*. It is a perennial vine, native to N. and S. America (Snowwdon, 1984). *P. edulis* var. *edulis* (purple passion fruit) thrives in the subtropics and tropical highlands of Brazil (Medina et al., 1980), S. Africa, Kenya and Uganda (Emechebe and Mukiibi, 1975). The golden-yellow passion fruit (*P. edulis* var. *flavicarpa*) is better adapted to tropical lowlands, and is mostly grown in the Caribbean countries such as Hawaii and the S. Pacific Islands (Fullerton, 1982).

Purple passion fruits are picked once their colour changes from green to purple, whereas yellow passion fruits are gathered once they drop on the ground (Medina et al., 1980). Passion fruits are a source of vitamins and minerals. They are exported, processed into juice, pulp, and syrup for flavouring, or consumed fresh.

Passion fruits, like other fruits, continue respiring post-harvest. Respiration involves a series of processes such as oxygen uptake, carbohydrate breakdown, and evolution of carbon dioxide, water vapour and heat energy (Watada et al., 1984, Sherman, 1987). Respiration rate depends on the stage of maturity and temperature, and determines perishing of passion fruits (Berg and Lentz, 1972; Pantastico, 1975). It is generally increased by pathogens, which accelerate carbohydrate use and subsequent cell breakdown (Millerd and Scott, 1962).

Increased respiration is associated with the ripening process, which signals the beginning of senescence (Biale, 1950), and leads to faster weight loss, chlorophyll

breakdown, colour change, acidity change, and an ultimate deterioration of fruits. Therefore, post-harvest environments should be designed to exclude disease and reduce respiration rate to the minimum required to maintain vital fruit processes such as protein synthesis, enzyme activation, cell division and growth and defense reactions (Sommer, 1985 and 1992). Low-temperature storage, controlled atmosphere storage, packaging and chemical preservatives are some manipulations aimed at increasing storage life (Sommer, 1985 and 1992; Conway et al., 1994a). Plant growth regulators have been applied both pre-harvest and post-harvest to retard senescent breakdown and pathological decay of harvested fruits. Positive results have been observed in bananas and lemons treated with 2,4-dichlorophenoxy acetic acid (Haard, 1973), and in lemons treated with $GA_3$ (Coggins, et al. 1992).

Water loss through transpiration is another important physiological process of harvested fruits (Weichmann, 1987). Harvested produce is deprived of water replenishment, hence is prone to weight loss, wilting and shriveling (Burton, 1982). Substantial weight loss can occur before visible symptoms appear, making moisture loss a major post-harvest concern. The rate of water loss depends on the nature of surface tissues, stage of maturity, composition of cuticle tissues (Lownds et al., 1993 and 1994), and the rate of water diffusion through the cuticle (Salisbury and Ross, 1985). However, holding factors such as maturity and harvesting procedures constant, infection by pathogens raises the respiration rate, which increases water loss from tissues (Weichmann, 1987).

Passion fruits are susceptible to several diseases such as brown spot (*Alternaria passiflorae*), aspergillus rot (*Aspergillus niger*), cladosporium rot (*Cladosporium* sp.), gray mould (*Rhizopus stolonifer*), penicillium rot (*P. expansum*) and septoria leaf spot (*Septoria* spp.) (Pruthi et al., 1958; Ondiek, 1973; Inch, 1978; Rao and Ravishankar, 1982). Pathogens worsen deterioration, causing economic loss to growers and high consumer prices (Agrios, 1988). The fungal species attacking fruits vary depending on the region in which the fruits are grown. Therefore, selection of an effective control treatment depends on knowledge of what fungus is attacking fruits.

### 1.1. Statement of the Problem

Passion fruits are susceptible to a number of pre-harvest and post-harvest diseases. Some of the most important include brown spot (*Alternaria passiflorae*), Septoria leaf spot, Aspergillus rot (*Aspergillus niger*), Gray mould (*Rhizopus stolonifer*), Cladosporium rot (*Cladosporium* sp.), and *Penicillium expansum* rot (Pruthi et al., 1958; Ondiek, 1973; Inch, 1978; Rao and Ravishankar, 1982). These diseases constraint passion fruit production and utilization right from the field to traders' shelves. With good cultural practices, pre-harvest pathogens can be suppressed only to manifest themselves post-harvest. In Kenya, too much emphasis has been placed on the control of pre-harvest diseases such as brown spot, woodiness, and Fusarium wilt (Ondiek, 1973; Anonymous, 1978), but little has been done in the control of post-harvest diseases that reduce quality, storage life and usable fruits.

Passion fruits exhibit a typical climacteric respiratory pattern with unusually large quantities of ethylene (370 µl/kg/hr) under ordinary temperature (25$^{o}$ C) conditions (Akamine et al., 1957). Unless this increased rate of ethylene evolution is halted it accelerates the rate of ripening and senescence, which encompass colour and biochemical changes as well as cellular component breakdown. Several studies have been carried out to investigate the effect of respiration and ethylene evolution on the ripening process (Akamine et al., 1957; Pratt and Workman, 1962; Shiomi et al., 1996), but the effect of evolved ethylene on storage duration, weight loss, disease development and tissue firmness have not been reported.

Treatment of harvested fruits with plant growth regulators has been reported to retard the ripening process and to control post-harvest diseases. Desai and Deshpande (1978) reported retarded ripening of bananas treated with gibberellic acid. Coggins et al. (1992) reported that treating citrus with $GA_3$ controlled post-harvest peel diseases. Treatment of passion fruits with plant growth regulators such as $GA_3$ to prevent post-harvest pathogens, particularly *Penicillium expansum*, from spreading and affecting quality has not been reported.

### 1.2. Research Justification

Passion fruits require proper post-harvest handling to maintain their nutritive value of minerals and vitamins, prolong shelf life, and increase marketable fruits. Gibberellins have been used to manage post-harvest decay in fruits such as banana

(Desai and Deshpande, 1978), citrus (Coggins et al., 1992; Ben-Yehoshua, 1995) and persimmons (Perez et al., 1995). Gibberellins have been reported to suppress the expression of microorganisms, action of ethylene, and rate of respiration (DeWolfe et al., 1959; Coggins et al., 1969). Gibberellins stimulate the action of "juvenility factors" in desensitizing fruits to exogenous or endogenous ethylene (Abdel-Gaward and Romani, 1967; Dostal and Leopold, 1967; Hsieh, 1967). This desensitizing increases storage life of fruits by arresting catabolism of juvenile hormones.

The use of plant growth regulators to control post-harvest diseases has been proposed, because pathogens develop resistance to fungicides commonly used to control them (Cohen and Shauli, 1983); and because pesticide residues on fruits pose a health hazard. The use of $GA_3$ to control post-harvest pathogens and rapid senescence, which occur during ripening, can positively benefit the passion fruit industry, which includes fruit growers, shippers, sellers and consumers. The benefits can include an increase in fruit shelf life, prolonged marketing period, and minimal rind diseases; they will have a net effect of increasing income and human nutrition.

### 1.3. Hypotheses

a) Gibberellic acid can confer tolerance against pathological decay caused by *P. expansum*, thereby maintain the post-harvest quality of passion fruits.

b) Timing influences the fungal species isolated on passion fruits.

c) Timing determines the percentage of passion fruits attacked by fungal species.

## 1.4. Objectives

1.3.1. The general objective of this research involved maintaining post-harvest quality of passion fruits, by using $GA_3$ to control physiological processes and pathological decay that result in post-harvest deterioration.

1.3.2. The specific objectives involved:

a) Determining the level of $GA_3$ that is most effective in maintaining post-harvest quality of passion fruits.

b) Determining the timing of $GA_3$ application that is most effective in maintaining post-harvest quality of passion fruits.

c) Determining the combination of $GA_3$ level and timing of $GA_3$ application that is most effective in maintaining post-harvest quality of passion fruits.

d) Study effects of storage duration on post harvest attributes.

e) Identify fungi attacking passion fruits at four different timings of isolations.

f) Determine whether timing of isolation has a bearing on the number and the types of fungal species isolated.

g) Determine whether timing of isolation affects the percentage of fruits attacked by the fungi.

# CHAPTER TWO

# LITERATURE REVIEW

## 2.1. Effects of Pathogenic Infections on Fruits

Infection by fungi and bacteria may occur during the growing season, harvest, handling, storage, transport and marketing, or even after purchase of fruits (Dennis, 1983). Disease development starts with infection followed by symptom manifestation, either immediately or after a period of time (Dennis, 1983; Agrios, 1988). Various interactions among microorganisms, plants and the environment determine whether particular microorganisms succeed in attacking plant tissues, and the amount of damage caused (Wood, 1967; Schiffmann et al., 1986).

Some molecules produced by microbes can kill healthy fruit tissues through their hypersensitive effects; for instance, glycoproteins isolated from cell walls of the fungal pathogen *Cladosporium fulvum* caused host cell necrosis in tomatoes (DeWit and Kodde, 1981). Pectic-degrading enzymes cause cell death by initiating release of cell wall polysaccharide fragments in the host plant (Bateman and Basham, 1976).

Plants or plant parts, by virtue of their genetic make up and morphology, are resistant or tolerant to attack by the majority of microorganisms. Plant tissues may be too acidic to permit survival and development of microorganisms; pathogens may be inhibited by plant substances already present or produced in response to injury or infection; and the water content of cells may be too high or too low to permit infection (Wood, 1967). But, some of these defense factors change with time such

that if microorganisms remain viable, then invasion and complete colonization may eventually take place (Wheeler, 1975).

Pathogenic infection often causes hormonal imbalance in plants and brings about abnormal growth responses, which are incompatible with healthy development of plants. Pathogens can cause disease through secretion of growth regulators, or their effects on growth regulatory systems of infected plants (Sequeira, 1973). Stall and Hall (1984) observed that yellowing and premature ripening of pepper fruits treated with *Xanthomonas campestris* (Bacterial spot) was due to an increase in ethylene evolution. Biale (1948) subjected lemon fruits to emanations of common green mould (*P. digitatum*) at different times during storage and observed increased respiration rate of 60% to 100% and accelerated perishing. In addition, storage-life of lemons (indicated by colour change, onset of *Alternaria* rot and shedding of stem-ends) was greatly reduced as a result of the exposure to fungal emanations.

## 2.2. Effects of Ethylene on Fruit Shelf Life and Pathogen Infection

Passion fruits, being climacteric fruits, undergo a large increase in respiration accompanied by a marked change in composition and texture during ripening. Akamine et al. (1957) reported ethylene evolution rate of 370 µl/kg/hr, which coincided with commencement of autocatalytic activities and exhibition of a respiratory peak (Shiomi et al., 1996).

Ethylene plays a major role in plant senescence through its direct and indirect effects in regulation of metabolism. The known physical and biochemical effects of ethylene on fruits include increased respiration, cellular-compartment permeability and loss, and activity of enzymes such as polygalacturonase, peroxidase, lipoxidase, α-amylase, polyphenol oxidase, and phenylanine ammonialyase (Pratt and Workman, 1962; Pratt and Goeschl, 1969). These effects promote senescence, accelerate deterioration and abbreviate shelf life.

Ethylene induces loss of tissue firmness, accelerates degradation of chlorophyll and yellowing of green tissues, thereby reducing market quality of leafy, floral, and immature-fruit vegetables (Pratt and Workman, 1962). Ponicke et al. (1977) observed acceleration in cucumber, subjected to 0.1 to 10 ppm of ethylene. Exposing watermelons to 5, 30, or 60 ppm of ethylene reduced firmness and rind thickness, accelerated deterioration and reduced acceptability after three days at 18$^{o}$ C (Risse and Hatton, 1982). Shimokawa (1973) reported increased activity of pectinase, cellulase, esterase, polyphenol oxidase, and tissue maceration in watermelons treated with ethylene. Climacteric fruits such as bananas ripen and senesce much faster when subjected to ethylene than when not (Burg and Burg, 1965; Pratt and Goeschl, 1969).

Removal of ethylene from apples slowed softening and delayed its onset (Johnson et al., 1993). However, maintenance of ethylene in the storage room of ripening kiwifruits accelerated softening, which was accompanied with an increase in phenol, acid and water-soluble pectin fractions, and a decrease in the amount of cell wall

materials (Wegrzyn and MacRae). Redgwell et al. (1991) observed swelling of the cell wall and loss of galactose from pectic polymers, de-esterification of pectins, and some breakdown of middle lamella pectic polymers, when they subjected ripening kiwi fruits to ethylene.

Initiation of ripening occurs when ethylene attains a threshold in cells. The threshold is reached naturally during maturation, or if a fruit undergoes stress through attack by a pest, or exposure to exogenous ethylene (Rhodes, 1980). Introduction of any ethylene inhibitor such as auxins, cytokinins, and $GA_3$ into the fruit atmosphere delays or halts ripening. This anti-ethylene effect makes young seeds and developing fruits, which have high levels of growth promoting hormones, less sensitive to ethylene; their sensitivity increases only upon harvesting (Rhodes, 1980).

Looney (1975) reported that succinic acid 2,2-dimethyl hydrazide (SADH), an inhibitor of endogenous ethylene, retarded the rate of ripening in 'McIntosh' apples. There was also reduced activity of β-galactosidase, and prolonged storage of treated fruits. This effect proves the involvement of ethylene in regulation of pigmentation and cellular component breakdown of both non-climacteric fruits such as cucumber, and climacteric fruits such as apples.

Ethylene has been shown to stimulate progression of various pathogenic infections. Barmore and Brown (1976) and Brown and Barmore (1977) observed ethylene stimulate development of rot such as diplodia stem end rot (*Diplodia natalensis*) of citrus fruits, and accelerate growth of the fungus and/or increase

susceptibility of fruit tissues to hyphal penetration. This stimulatory effect of ethylene was further confirmed by El-Kazzaz et al. (1983), who subjected stored strawberries to 20 ppm ethylene and observed enhanced growth of *Botrytis cinerea* and development of disease. The use of ethylene to degree tangerines stimulated appresoria of *Colletotrichum gloeosporioides* to form infection hyphae, which later accounted for most of the decay (Brown and Barmore, 1977).

Contrary to these findings, ethylene has been observed to induce resistance to certain pathogens in some harvested plant organs. Sweet potato slices exposed to 8 ppm of ethylene for two days became more resistant to infection by *Ceratocystis fimbriata*; their resistance was accompanied by the induction of enzymatic activities possibly related to defense (Stahmann et al., 1960). Lockart et al. (1968) exposed 'Robinson' tangerines to ethylene and observed inhibition of apple rot (*Gloeosporium album*) and *Colletotrichum gloeosporioides*.

## 2.3. Effects of Gibberellic Acid on Pathogens

The use of hormones to control post-harvest diseases has been proposed due to resistance of pathogens to fungicides (Cohen and Shauli, 1983). Application of various growth regulating chemicals has been shown to confer disease resistance, achieve uniform maturity, or suppress post-harvest sprouting (Sharples, 1984). Treatment of citrus fruits with 2,4-D and gibberellins suppressed expression of microbes, and reduced production and action of ethylene (DeWolfe et al., 1959;

Coggins et al., 1969). In these studies, gibberellins and 2,4-D reduced the rate of deterioration, which is a function of respiration rate, disease infection, and transpiration rate. DeWolfe et al. (1959) observed that the presence of 2,4-D delayed senescence of lemon buttons infected with *Alternaria*, and reduced infection of oranges and grapes by *Diplodia* rot and *Alternaria* stem-end rot, respectively. Cohen and Shauli (1983) also reported that subjecting citrus fruits to 2,4-D and thiabendazole delayed fungal invasion.

Treating lemons with 50 and 100 mg/L $GA_3$ in the pack house significantly reduced the incidence of *Geotrichum candidum* (Coggins et al., 1992). These treatments reduced decay incidences and increased shelf- and shipping-life of lemons. Ben-Yehoshua (1995) observed that applying the same $GA_3$ levels on stored lemons inhibited development of decay, caused by *P. digitatum*, and slowed decline of citral content and anti-fungal activity in the flavedo.

To further support the effect of $GA_3$ in controlling post-harvest diseases, Perez et al. (1995) reported that spraying persimmons with 20 µg/ml $GA_3$ at 30, 20, and 10 days before harvest reduced the rate of decay. Persimmons stored well for three months and became less covered with black spot disease (*Alternaria alternata*). Effectiveness, evidenced by reduced percentage of unmarketable fruits, increased when they combined $GA_3$ with iprodione.

Lewis et al. (1967) observed that application of $GA_3$ maintains firmer rinds that halt unfavourable effects occurring during maturation and senescence of navel

oranges. These effects include vacuolation and enlargement of albedo and flavedo cells that lead to water spotting, puffiness, increased susceptibility to decay pathogens, and accumulation of sticky exudates. These changes could lead to increased manifestation of rot and accelerated fruit losses. Firmer rinds also reduce the amount of decay by increasing resistance to entry of microbes and by reversing rind changes that make it a better medium for microbial growth. The reduced growth of microorganisms is accompanied by a low rate of sugar accumulation in the treated rind (Lewis et al., 1967). Therefore, using $GA_3$ either alone or in combination with other plant hormones can confer resistance to plants when in the field, and/or after harvest, thereby reducing post-harvest losses.

### 2.4. Effects of Gibberellic Acid on Fruit Shelf Life

The shelf life of any fruit depends on the rate of ripening and extent to which ripening can be retarded. Pronounced changes in texture, pigments, aroma, carbohydrates, and other tissue constituents mark fruit ripening. During ripening, climacteric fruits undergo an upsurge in respiration and ethylene evolution. It is also accompanied by breakdown of cell organization (Brian and Mercer, 1964; Sacher, 1967); and is a direct process requiring coupled respiration, energy-linked functions and enzyme synthesis (Frenkel et al., 1968; Haard and Hultin, 1970).

Researchers have indicated that growth-regulating substances affect cell wall-degrading enzymes activities, especially those involved in the life of plants and plant

organs (Coggins and Hield, 1968; Datko and Maclachlan, 1968; Tanimoto and Masuda, 1968). The effects of plant growth regulators on the activities of polygalacturonase, pectin esterase, cellulase, β-1-glucanase and amylase in plant growth control have been reported (Datko and Maclachlan, 1968; Tanimoto and Masuda, 1968). Most significant ones include respiration, softening and lycopene synthesis, which affect colour in tomato fruit. Abdel-Kader et al. (1966) observed that treatment with $GA_3$ and IAA greatly retarded normal-colour development and quality of tomatoes. However, the retarded ripening of tomato fruits by $GA_3$ was overcome by applying ethylene (Dostal and Leopold, 1967).

Respiration rate of tomatoes treated with $GA_3$ was lower than of control tomatoes and the climacteric rise delayed for 2 to 3 days (Babbit et al., 1973). Colouring of tomatoes treated with $GA_3$ was delayed and altered; lycopene synthesis and polygalacturonase activities were suppressed, but chlorophyll degradation continued (Babbit et al., 1973).

Applied plant growth regulators such as auxins (Gortner, 1969; Vendrell 1969; Frenkel and Dyck, 1973), cytokinins (Van Overbeek, 1961; Doughty et al., 1967), and gibberellins (Abdel-Gawad and Romani 1967; Dostal and Leopold, 1967; Hsieh et al., 1967; Coggins and Hield, 1968; Coggins et al., 1969) can stimulate the action of "juvenility factors" in desensitizing fruits to exogenous or endogenous ethylene. This desensitizing prolongs storage-life of fruits by arresting catabolism of juvenile hormones. Frenkel and Dyck (1973) observed that treating pear fruits with IAA and

2,4-D in 0.4 M mannitol carrier retarded the ripening rate, measured as chlorophyll degradation and texture softening, although ethylene evolution increased. The two hormones and a variety of other auxin-type growth regulators such as naphthoxy acetic acid, ethyl-3-indoleacetate, and 3-indole-propionate delayed ripening of mature-green bananas (Haard, 1973). Coggins and Hield (1968) observed delayed senescence of citrus rind tissue after applying $GA_3$, which reduced rind disorders such as water spots, creasing, staining and softening. Pre-harvest spraying of citrus fruits with $GA_3$ increased storage-life (Coggins et al., 1969; Monselise, 1979).

Colour change from green to yellow, purple, orange, or red involves the degradation of chlorophyll followed by enhanced carotenoid and anthocyanin synthesis (Goldschimdt, 1980). The effect of gibberellins in delaying colour development during fruit maturation has been observed in citrus (Coggins and Lewis, 1962; Lewis et al., 1967; Goldschimdt and Eilati, 1970), tomatoes (Abdel-Kader et al., 1966; Dostal and Leopold, 1967, Babbit et al., 1973), apples (Abdel-Gawad and Romani, 1974), and bananas (Vendrell, 1969). El-Zeftawi and Garret (1978) observed that gibberellic acid inhibited carotenoids and increased chlorophyll content at the re-greening stage, whereas ethephon reduced chlorophyll and increased starch content in plastids of 'Valencia' oranges.

Ben-Arie et al. (1996) observed that pre-harvest spraying of persimmons with $GA_3$ delayed fruit ripening and reduced the rate of softening post-harvest. The treated fruits exhibited a delay in cell wall changes such as dissolution of the middle lamella,

separation of the plasmalemma from the cell wall and solubilization of pectic polymers that accompany fruit softening. Martinez et al. (1994) reported that $GA_3$ inhibited strawberry fruit ripening, as evidenced by decreased respiratory activity, and delayed anthocyanin synthesis and chlorophyll degradation. However, the effectiveness of $GA_3$ depended on the concentration.

Desai and Deshpande (1978) reported that dipping of bananas in gibberellic acid and kinetin delayed ripening. Murthy and Rao (1982) observed that dipping 'Alphonso' mangoes in 250 ppm $GA_3$ for 5 minutes, followed by storage at 28$^{o}$ C and 40% to 60% RH delayed ripening, as was evidenced by prolonged firmness and maintenance of chlorophyll. In contrast, Lakshminarayana (1973) subjected 'Alphonso' mangoes to a natural ripening environment and observed increased respiratory climacteric within 6 to 10 days, followed by tissue deterioration.

Application of $GA_3$ to fruits helps antagonize ethylene-mediated catabolic processes that reduce the post-harvest keeping quality of fruits (Goldschmidt and Galily, 1974; Goldschmidt, et al., 1977). Lewis et al. (1967) observed that ethylene reduced pigment change and carbohydrate metabolism, which accelerated senescence in Navel oranges.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1. Preparation of *Penicillium expansum* Cultures

*Penicillium expansum* was chosen as a pathogen of study based on a preliminary study, which identified this fungus as a serious post-harvest threat to passion fruits in the Nakuru municipal market. Also, the same fungus has been reported as a major cause of post-harvest losses in fruits (Agrios, 1988).

The fungus was isolated from rotting fruits obtained from the municipal market in Nakuru, Kenya. The fruits were kept in the laboratory for some days to allow *P. expansum* and other fungi to grow. Infected fruit peels were cut and cultured on a potato dextrose agar (PDA) medium at room temperature. Pure strains of *P. expansum* were identified using a microscope (model XSZ-D2, China) and its green colour as out-lined in Agrios (1988) and Snowdon (1990). Pure spores of *P. expansum* were then isolated from the fungal mixture, using a sterile needle and cultured onto a fresh PDA medium. Isolation was carried out under sterile conditions in a laminar air-flow-chamber. The pure *P. expansum* spores were multiplied by culturing further on fresh PDA medium for 10 days in an incubator maintained at $22^{o}$ C, after which spores of the fungus were harvested.

The spores were mixed with 50 ml sterilized distilled water. A concentration of $1.6 \times 10^{6}$ spores/ml was made by micro-pipetting a drop of the suspension into the grooves of a haemacytometer (cell counter). A drop of lactophenol/cotton blue ink

was mixed with the spore suspension to facilitate spore counting under a microscope (model XSZ-D2, China). The suspension was diluted to four spores per division of the haemacytometer. The concentration of $1.6 \times 10^6$ spores/ml was based on an average count of 24 random squares. The average count (four spores) was multiplied by the constant 400,000 to achieve the concentration of $1.6 \times 10^6$ spores per ml.

### 3.2. Fruit Harvest and Treatment

Fresh purple passion fruits were harvested at the mature-green stage from a farm in Bahati division, Nakuru district (Kenya). The fruits were picked early in the day, kept under shade to avoid field heat accumulation, and transported to the laboratory at Egerton University, Njoro, where the experiments were conducted. Fruits were sorted and only those of uniform colour and size were used in the experiments.

On the same day of harvest, all fruits were washed under running tap water to remove surface contaminants followed by surface sterilization in 5 % sodium hypochlorite solution for 5 minutes (Arjona and Metta, 1991). Surface disinfection ensured that the effect of $GA_3$ on *P. expansum* was not influenced by other pathogens, and that surface contaminants, which might affect the absorption and activity of the growth regulator, had been removed. After surface disinfection, fruits were rinsed in sterilized distilled water and air-dried on a laboratory bench.

Each fruit was pierced using a sterile needle to a 1-mm depth to facilitate infection by *P. expansum* (Ben-Yehoshua et al., 1995). A drop (0.1 ml) of the *P. expansum* suspension, containing $1.6 \times 10^6$ spores/ml was injected into each wound.

The inoculated fruits were divided into four groups. One group was sprayed with $GA_3$ at zero days post-harvest (dph). The other three groups were sprayed similarly after 5, 10, and 15 dph. The levels of $GA_3$ were zero (distilled water), 50, 100, and 250 ppm (Murthy and Rao, 1982; Coggins et al., 1992). Treated fruits were then packed in low-density polythene bags, sealed using an electric plastic sealer. Fruits for the 5, 10, and 15 dph were stored in ventilated crates, covered with a thin polythene film. Fruits for each level of time were drawn from the crates and sprayed with $GA_3$.

### 3.3. Experimental Room

The room was maintained at 17 to 22° C and above 85% relative humidity for the first 48 hours to facilitate sporulation of *P. expansum* (Ben-Yehoshua et al., 1995). Thus, the room was lit at night, misted and flooded with warm water. Ambient room conditions were maintained for the rest of the experimental period.

### 3.4. Experimental Design

This experiment was laid out as a 4 x 4 factorial in a split-plot design. Timing of $GA_3$ application formed main plots and $GA_3$ levels formed sub-plots. Treatments were replicated thrice, and the experiment was repeated once.

### 3.5. Isolation of Pathogenic Fungi

Fruits for this experiment were obtained from the same field where those used for the $GA_3$ experiment were obtained. Mature-green fruits of a uniform size were selected from the crates and stored in open polythene bags at room temperature. Fungi were isolated at 0, 5, 10, and 15 days post-harvest. Thus, four groups of fruits (isolation times) were used for this experiment. Five fruits made a replication and each isolation timing had 15 fruits. This experiment was set up in a completely randomized design, with timing of pathogen isolation as the factor of study.

### 3.6. Variables Assessed

#### 3.6.1. Rind firmness, sugar content, and chlorophyll

Ninety fruits (90) were used per treatment for these variables. In the first trial, 30 fruits were packed in each clear, low-density polythene bag, and sealed using an electric plastic sealer. The 30 fruits formed a replication. From each bag, two fruits were randomly sampled and destructively analyzed for sugar, rind firmness, and chlorophyll content every two days for a total of 20 days. In the second trial, 30 fruits were packed in 10 separate bags, each containing three fruits. The change was implemented to avoid the effect of variable fruit number per bag, once sub-sampling had begun. Two fruits were randomly sampled from each bag and analyzed, as in the first trial. The fruit remaining in each bag was discarded.

A fruit peeler was used to cut a 1-mm thick slice of rind from each fruit. An 8-mm probe (plunger) was fitted into a hand penetrometer (model FT 037, England) and then driven through the peeled part of the rind (Kader, 1992). The pressure exerted to puncture the fruit rind was recorded in kilogrammes force. Puncturing was done on two opposite sides of the fruit. Readings of the two fruits were averaged, resulting in values that were analyzed.

Sugar content was determined by squeezing some fruit juice from the same fruits used in assessing rind firmness onto a hand refractometer and then reading the brix value (or percent sucrose) (Kader, 1992). Four readings from the two fruits were averaged and their mean value used for data analysis.

Pieces of the rind, measuring one gram were cut from the two punctured fruits and blended, using a mortar and a pestle. The paste was mixed with distilled water to make a 10-ml suspension. About 0.5 ml were pipetted and mixed with 4.5 ml of 80 % acetone to make 5 ml. The 5-ml suspension was placed in a centrifuge tube and loaded into a centrifuge (model LDZ5-2, China). Tubes were centrifuged at 3,000 rpm for 10 minutes. About 3.5 ml of the supernatant were transferred into a cuvette. The optical density (OD) was determined using a spectrophotometer (model MC-752, China) at wavelengths 645 nm and 663 nm. The readings at the two wavelengths were fitted into a formula for calculating chlorophyll content (Jayaraman and Das-Gupta, 1992). Thus, total chlorophyll (mg/L) = $0.0202OD_{645} + 0.00802OD_{663}$, where 0.0202 and 0.00802 are constants.

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### 3.6.2. Weight loss, disease incidence and purple colour development

Fifty-four fruits were used per treatment for these variables. Each treatment had 18 fruits packed together in a clear polythene bag and replicated three times.

Each bag was weighed immediately the fruits were packed, resulting in the base weight upon which percent weight loss was calculated. With disease incidence, all fruits showing germination and growth of *P. expansum* were counted and recorded every two days. The infected fruits were expressed as a percentage of the total fruits in the bag. Purple colour development was scored according to the index developed for this experiment. Eight fruits were sub-sampled from each bag, tagged and used for repeated scoring of colour throughout the experimental period. The colour scale ranged from 1 to 7, with 1, 2, 3, 4, 5, 6, and 7 indicating: 0% to 10%; 11% to 20%; 21% to 30%; 31% to 40%; 41% to 60%; 61% to 80%, and 81% to 100% purple colour, respectively (Shiomi et al., 1996). The average score of each eight fruits was subjected to the analysis of variance.

### 3.6.3. Disease severity

Ten fruits per treatment were packed together in each polythene bag and replicated three times. Six fruits in each bag were tagged and used to measure disease severity throughout the experimental period. The remaining four fruits in each bag were discarded. Lesion diameter resulting after inoculation with *P. expansum* was measured every two days, using a scale.

### 3.6.4. Fruit loss

A total of 72 fruits were used per treatment to determine fruit loss. Twenty-four fruits were packed in a clear polythene bag. All the fruits showing more than 5% attack by *Penicillium expansum* were counted at 24 dph, when most fruits had started rotting, and expressed as a percentage of the total fruits in each bag.

### 3.6.5. Ethylene gas

Fruits used were based on weight rather than on number to avoid the variation caused by different fruit weights during gas evolution. Half a kilogramme of fruits were placed in each clear polythene bag, fitted with a rubber stopper. Fruits were incubated for two days at room temperature, after which ethylene was determined for a period of two weeks at a 48-h interval. Ethylene was determined by drawing 1 ml of air and injecting into a flame ionization gas chromatograph (Model Shimadzu GC-9A, Japan), fitted with alumina column. Values were printed out by a chramatopac 6-CRA printer. Gas chromatograph conditions were 80$^{o}$ C column temperature, 150$^{o}$ C injection temperature, 150$^{o}$ C detector temperature, 0.5 kg/cm$^{2}$ air pressure, 0.85 kg/cm$^{2}$ hydrogen gas pressure, and 1.02 kg/cm$^{2}$ nitrogen gas pressure.

### 3.6.6. Isolation of pathogenic fungi

Each fruit was washed using tap water before the peel was cut into pieces with a scalpel. The pieces were dipped in 95 % alcohol for three minutes and rinsed in

sterilized distilled water to remove surface contaminants (Agrios, 1988). Five pieces were sampled randomly and cultured on a PDA medium under aseptic conditions; the petri dishes were incubated at 22$^{o}$ C. After 10 days, petri dishes were removed and fungal species growing identified using a microscope (model XSZ-D2, China) and colour plates (Agrios, 1988; Snowdon, 1990).

A drop of lactophenol/cotton blue ink was placed on a slide, on which spores were firmly spread using cellotape impression technique (Agrios, 1988). The number of fruits that showed growth of a particular fungus were counted and expressed as a percentage, using the formula: Percentage fruits attacked = number of fruits attacked divide by total number of fruits per treatment multiply by 100.

### 3.7. Data Analysis

Analysis of variance was performed using the MSTAT statistical programme and Duncan's Multiple Range Test was used to separate significantly different means at $P \leq 0.05$ (Nissen, 1991). Data for the two trials were pooled, because the room conditions under which the two trials were conducted were similar and also there was no significant variation in the data for the two trials.

# CHAPTER FOUR

# RESULTS AND DISCUSSION

## 4.1. Total Soluble Solids Content

### 4.1.1. Main effect of timing of $GA_3$ application

The main effect of timing of $GA_3$ application on total soluble solids was significant, when analyzed at 4, 6, 12, 14, 16 and 20 days post-treatment (Table 1, $P \leq 0.05$). Fruits treated at 0 days post-harvest (dph) had the highest soluble solids for most of the 20 days post-treatment, while those treated at 10 dph had the lowest soluble solids, except for the last 4 days post-treatment, when those treated at 15 dph had the lowest soluble solids (Table 1, $P \leq 0.05$). Diversion of some sugar to the juice fraction could account for the observed higher soluble solids content in the fruits treated at 15 dph, rather than the expected drastic reduction (Table 1).

It is likely that at zero days post-harvest $GA_3$ reduced respiration, resulting in a low rate of sugar depletion through the glycolytic and Krebs cycles (Babbit et al., 1973). In contrast, increased fungal growth and tissue degeneration could have made absorption and activity of $GA_3$ less effective in retarding total soluble solids depletion in fruits treated at 10 and 15 dph. Also, increased metabolism of rind carbohydrates could have been stimulated to sustain fungal and fruit cellular activities, thereby reducing soluble solids in fruits treated at 10 and 15 dph (Agrios, 1988). It has been reported that during storage fungal and fruit respiration increase carbon dioxide in the package, resulting in inhibition of mitochondria oxidation, and diversion of pyruvate

Table 1. Main effect of timing of $GA_3$ application on total soluble solids (%) of passion fruits

| | Timing of $GA_3$ application (dph)[z] | | | | | |
|---|---|---|---|---|---|---|
| Days post-treatment | 0 | 5 | 10 | 15 | LSD[y] | %CV[x] |
| 2 | 12.34 | 11.80 | 11.53 | 12.04 | NS | 10.58 |
| 4 | 12.73a | 12.14ab | 11.23b | 11.51b | 0.88 | 11.76 |
| 6 | 12.45a | 11.17bc | 10.73c | 11.68b | 0.62 | 10.61 |
| 8 | 11.70 | 11.48 | 10.93 | 11.57 | NS | 11.21 |
| 10 | 11.13 | 11.65 | 11.43 | 11.51 | NS | 9.61 |
| 12 | 12.58a | 12.17a | 10.61b | 10.75b | 0.94 | 7.25 |
| 14 | 11.61a | 11.73a | 10.67b | 10.68b | 0.98 | 8.59 |
| 16 | 12.07a | 11.01b | 10.15bc | 9.70c | 0.64 | 7.52 |
| 18 | 12.08 | 11.01 | 10.15 | 9.70 | NS | 10.02 |
| 20 | 10.83a | 11.14a | 9.72b | 8.85c | 0.63 | 8.84 |
| Slope | -0.13 | -0.37 | -0.16 | -0.07 | | |
| $R^2$ | 0.37 | 0.24 | 0.73 | 0.85 | | |

[z]Means followed by the same letter or no letter within each row are not significantly different at $P \leq 0.05$, according to the Duncan's multiple range test (DMRT).

[y]Least significant difference. NS = not significant.

[x]Coefficient of variation.

to formation of acetaldehyde and ethanol (Agrios, 1988). These processes could have also reduced total soluble solids of fruits treated at 10 and 15 days post-harvest.

The soluble solids of fruits treated within 5 dph decreased gradually across the 20 days post-treatment with temporal increases (Table 1). Arjona and Metta (1991) observed that application of $GA_3$ to passion fruits at an early (mature-green) stage had no significant effect on soluble solids, although it enhanced pigmentation. This observation contradicted that of Hill and Ap-Rees (1995), who reported reduced conversion of starch to sugar and low synthesis of sugar following $GA_3$ treatment that reduced respiration. The temporal increase in soluble solids, especially between the $10^{th}$ and $12^{th}$ days post-treatment probably resulted from the increased ethylene in the package. Thus, ethylene increased respiration and antagonized $GA_3$ effect.

As fruits stored longer (for 10 or 15 dph), oxygen rapidly decreased and carbon dioxide increased in the polythene bags. The increased carbon dioxide reduced respiration, sugar breakdown and/or sugar synthesis, resulting in ultimate tissue senescence (Hill and Ap-Rees, 1995). Consequently, fruits treated at 10 and 15 dph had a decreasing trend in soluble solids content across the 20 days post-treatment. Thus, deterioration had already started by the time of treatment and any $GA_3$ applied did not significantly maintain soluble solids content.

The last 8 days post-treatment exhibited accelerated decrease in soluble solids probably due to increased starch hydrolysis and also increased use of sugars, especially glucose by the fruit and the fungus for metabolic activity (Lizada, 1985). In

other studies, an increase in sugar content as fruits begin to degenerate has been reported (Farkas, 1978). It has also been reported that increased respiratory activity provides substrates and low molecular weight building blocks of macromolecules to infected areas (Farkas, 1978).

### 4.1.2. Main effect of $GA_3$

Total soluble solids were significantly affected by $GA_3$ at 10, 14, and 20 days post-treatment (Table 2; $P \leq 0.05$). Soluble solids content generally decreased gradually for the four $GA_3$ levels across the 20 days post-treatment (Table 2). Thus, there were minor fluctuations in the soluble solids obtained over the 20 days post-treatment for all the $GA_3$ levels. Shiomi et al. (1996) found that upon harvesting fruits sugar levels rise during the first five days, followed by a general decrease, with temporal peaks as ripening progresses. Therefore, $GA_3$ only reduced the rate of sugar depletion before tissue-breakdown set in.

Between 0 and 20 days post-treatment, fruits treated with 100 ppm $GA_3$ exhibited the highest decrease (2.35%) in total soluble solids. Control fruits with 1.76% decrease followed them, while those treated with 50 and 250 ppm $GA_3$ had 1.34% and 1.36% decrease in soluble solids, respectively (Table 2). The relatively high rate of depletion observed for fruits treated with 100 ppm $GA_3$ could be supported by El-Zeftawi and Garett (1978), who reported an accelerated loss of starch in citrus and a decrease in sugar over time.

Table 2. Main effect of $GA_3$ on total soluble solids (%) of passion fruits

| | $GA_3$ (ppm)[z] | | | | | |
|---|---|---|---|---|---|---|
| Days post- treatment | 0 | 50 | 100 | 250 | LSD[y] | %CV[x] |
| 2 | 12.02 | 11.95 | 12.22 | 11.53 | NS | 10.58 |
| 4 | 12.01 | 11.93 | 11.93 | 11.73 | NS | 11.76 |
| 6 | 11.78 | 11.36 | 11.77 | 11.12 | NS | 10.61 |
| 8 | 11.40 | 10.98 | 11.62 | 11.69 | NS | 11.21 |
| 10 | 12.09a | 11.20ab | 11.01b | 11.42ab | 0.93 | 9.61 |
| 12 | 11.89 | 11.31 | 11.40 | 11.50 | NS | 7.25 |
| 14 | 10.49b | 11.37ab | 11.53a | 10.98ab | 0.80 | 8.59 |
| 16 | 11.38 | 11.07 | 11.24 | 11.00 | NS | 7.52 |
| 18 | 10.83 | 10.36 | 11.02 | 10.73 | NS | 10.02 |
| 20 | 10.26ab | 10.56a | 9.73b | 9.92b | 0.76 | 8.84 |
| Slope | -0.18 | -0.14 | -0.13 | -0.19 | | |
| $R^2$ | 0.63 | 0.73 | 0.37 | 0.70 | | |

[z]Means followed by the same letter or no letter within each row are not significantly different at $P \leq 0.05$, according to the DMRT.

[y]Least significant difference. NS = not significant.

[x]Coefficient of variation.

The relatively low rates of depletion observed in fruits treated with 50 and 250 ppm $GA_3$ revealed that 100 ppm $GA_3$ was the optimum level at which starch hydrolysis occurred, below and above which the rate of hydrolysis decreased. This $GA_3$ level probably activated enzymes such as catalases and pectin methylesterase, which have been observed to dominate the juice of ripe passion fruits (Salunkhe and Desai, 1984). Lower enzyme activity probably occurred in the control fruits, because their rate of sugar depletion was lower than that of fruits treated with 100 ppm $GA_3$.

### 4.1.3. Effect of interaction between timing of $GA_3$ application and $GA_3$ level

There was no significant effect of interaction on total soluble solids (Table 2.1, $P \leq 0.05$). Generally, slopes were negative, indicating that total soluble solids decreased over time even after the fruits had been sprayed with $GA_3$ (Table 2.1A). The sugar synthesis and/or degradation in fruits treated at 0 and 5 dph had a weak linear relationship with storage time as evidenced by the relatively low $R^2$ values (Table 2.1B). The total soluble solids depletion in fruits treated with $GA_3$ at 15 dph had a relatively stronger linear relationship with storage time than the others. Gibberellic acid did not substantially retard loss of total soluble solids, which were already low at 15 dph, when these fruits received $GA_3$. The low total soluble solids in fruits treated at 15 dph could be attributed to increased rate of carbohydrate metabolism and starch depletion as fungal growth and fruit-ripening cellular activities progressed (Agrios, 1988; Lizada, 1985).

Table 2.1. Slopes (A) and $R^2$ values (B) from the plot of total soluble solids versus time post-treatment as influenced by timing of $GA_3$ application and $GA_3$ level

| **A (slopes)** | Timing of $GA_3$ application (dph) | | | |
|---|---|---|---|---|
| $GA_3$ (ppm) | 0 | 5 | 10 | 15 |
| 0 | -0.06 | -0.14 | -0.16 | -0.35 |
| 50 | -0.17 | 0.09 | -0.19 | -0.30 |
| 100 | -0.17 | -0.44 | -0.11 | -0.33 |
| 250 | -0.11 | -0.07 | -0.17 | -0.22 |

| **B ($R^2$ values)** | Timing of $GA_3$ application (dph) | | | |
|---|---|---|---|---|
| $GA_3$ (ppm) | 0 | 5 | 10 | 15 |
| 0 | 0.06 | 0.49 | 0.42 | 0.64 |
| 50 | 0.35 | 0.18 | 0.68 | 0.66 |
| 100 | 0.25 | 0.29 | 0.25 | 0.66 |
| 250 | 0.24 | 0.06 | 0.54 | 0.67 |

Total soluble solids for the sixteen treatments had gentle slopes of less than -0.5, but those for 0, 50, 100, and 250 ppm $GA_3$ applied at 15 dph had relatively steeper slopes of -0.35, -0.30, -0.33, and -0.22, respectively. The gentle slopes indicated that the rates of synthesis and/or depletion of soluble solids were low, but similar among treatments. In addition, growth of *P. expansum* probably changed soluble solids in the juice at low, but similar rates for all treatments.

## 4.2. Chlorophyll Content

### 4.2.1. Main effect of timing of $GA_3$ application

The effect of timing of $GA_3$ application on chlorophyll content was significant (Table 3, $P \leq 0.05$). Fruits treated at 0 and 5 dph had similar and higher chlorophyll contents across the 20 dpt than fruits treated at 10 and 15 dph (Table 3). Fruits treated at 0 and 5 dph had a drastic decrease in chlorophyll content during the first six days post-treatment, followed by chlorophyll increase (Table 3). Thus, the synthesis of enzymes responsible for chlorophyll breakdown probably set in within the first few days post-treatment. Subsequently, $GA_3$ and increased $CO_2$ probably reversed pigment breakdown from the $6^{th}$ to $12^{th}$ day post-treatment.

Fruits treated at 10 and 15 dph probably had increased enzymatic activity that significantly reduced chlorophyll content before $GA_3$ was applied; thus, due to normal senescence process. The low chlorophyll content probably resulted from the increase in fruit tissues and environment of ethylene, which accelerated senescence.

Table 3. Main effect of timing of $GA_3$ application on chlorophyll content (mg/L) of passion fruits

| | Timing of $GA_3$ application (dph)[z] | | | | | |
|---|---|---|---|---|---|---|
| Days post-treatment | 0 | 5 | 10 | 15 | LSD[y] | %CV[x] |
| 2 | 2.08a | 1.92a | 1.35b | 1.00c | 0.287 | 19.65 |
| 4 | 1.75b | 2.24a | 1.60b | 0.79c | 0.286 | 16.15 |
| 6 | 1.05b | 1.47a | 1.24ab | 0.73c | 0.287 | 19.28 |
| 8 | 1.35ab | 1.49a | 1.20b | 0.72c | 0.191 | 17.42 |
| 10 | 1.57a | 1.06b | 0.84c | 0.53d | 0.174 | 16.47 |
| 12 | 1.42a | 1.23a | 0.73b | 0.60b | 0.206 | 18.04 |
| 14 | 0.86a | 0.71a | 0.68a | 0.36b | 0.168 | 21.86 |
| 16 | 0.67a | 0.76b | 0.58b | 0.57c | 0.119 | 22.09 |
| 18 | 0.54b | 0.69a | 0.76a | 0.38c | 0.111 | 21.14 |
| 20 | 0.58ab | 0.66a | 0.46b | 0.27c | 0.160 | 30.41 |
| Slope | -0.15 | -0.17 | -0.11 | -0.07 | | |
| $R^2$ | 077 | 0.87 | 0.85 | 0.93 | | |

[z]Means followed by the same letter or no letter within each row are not significantly different at $P \leq 0.05$, according to the DMRT.

[y]Least significant difference

[x]Coefficient of variation.

Ethylene initiates loss of inner membranes in chloroplasts through the degrading enzymes it activates (Burg and Burg, 1965; Burg, 1968). Dissolution of chlorophyll matrix in the rind reduces chlorophyll (Burg and Burg, 1965; Barmore, 1975). Fungal activities also increase respiration, which breaks down cell wall polymers (Biale, 1948). These observations show that the $GA_3$ applied after endogenous ethylene in fruit tissues has reached high levels is not able to retard chlorophyll breakdown. In addition, when $GA_3$ is applied late its absorption and activity are reduced and overwhelmed by the detrimental effects of fungi (Biale, 1948).

Nevertheless, the relatively low rate (gentle slope) of chlorophyll breakdown in fruits treated at 10 and 15 dph probably occurred not due to the $GA_3$ effect, but as a result of increased $CO_2$, which lowered synthesis of proteins and enzymes responsible for chlorophyll breakdown (Barmore, 1975: Wang, 1977; Kader, 1985), or because chlorophyll content was already low by the time fruits received $GA_3$.

### 4.2.2. Main effect of $GA_3$

A significant main effect of $GA_3$ on chlorophyll was observed at 6, 8, 16, 18, and 20 days post-treatment (Table 4, $P \leq 0.05$). Generally and despite the appearance of senescence, $GA_3$-treated fruits had high chlorophyll than control fruits, although the degradation rate for all fruits was constant. $GA_3$ probably slowed chlorophyllase, and maintained high chlorophyll levels (Frenkel and Dyck, 1973). Also, $GA_3$ probably maintained the chlorophyll ultrastructure, delayed disappearance of the grana

Table 4. Main effect of $GA_3$ on chlorophyll content (mg/L) of passion fruits

| Days post-treatment | $GA_3$ (ppm)[z] 0 | 50 | 100 | 250 | LSD[y] | %CV[x] |
|---|---|---|---|---|---|---|
| 2 | 1.42 | 1.64 | 1.63 | 1.67 | NS | 19.65 |
| 4 | 1.50 | 1.54 | 1.62 | 1.72 | NS | 16.15 |
| 6 | 0.96b | 1.24a | 1.24ab | 1.18a | 0.183 | 19.28 |
| 8 | 1.14ab | 1.21ab | 1.31a | 1.10b | 0.175 | 17.42 |
| 10 | 0.98 | 1.03 | 0.96 | 1.04 | NS | 16.47 |
| 12 | 0.93 | 1.04 | 0.99 | 0.99 | NS | 18.04 |
| 14 | 0.70 | 0.78 | 0.75 | 0.81 | NS | 21.86 |
| 16 | 0.67ab | 0.76ab | 0.58a | 0.57b | 0.119 | 22.09 |
| 18 | 0.51b | 0.64a | 0.53b | 0.69a | 0.092 | 21.14 |
| 20 | 0.42b | 0.52ab | 0.59b | 0.270a | 0.125 | 30.41 |
| Slope | -0.15 | -0.15 | -0.15 | -0.15 | | |
| $R^2$ | 0.77 | 0.87 | 0.85 | 0.93 | | |

[z]Means followed by the same letter or no letter within each row are not significantly different at $P \leq 0.05$, according to the DMRT.

[y]Least significant difference. NS = not significant.

[x]Coefficient of variation.

fretwork (Purvis, 1980), and reduced ethylene activity, which triggers chlorophyllase to breakdown chlorophyll moieties (Barmore, 1975; Shimokawa et al., 1978).

Another hypothesis on how $GA_3$ delays ripening was advanced by Coggins and Lewis (1962), who observed a linkage between the low rate of chlorophyll loss and the promotion of chlorophyll synthesis. According to Goldschimdt et al. (1977), a delay in chloroplast-chromoplast transformation might occur following $GA_3$ application. The low rate of chlorophyll loss can be attributed to slow formation of pigments indicative of fruit senescence, and maintenance of peel tissue viability.

Yi and Ji (1988) reported a low chlorophyll level in control cabbage tissues relative to $GA_3$-treated ones. This observation further supports the efficacy of $GA_3$ in maintaining chlorophyll structure. The low chlorophyll content was attributed to the high abscissic acid (ABA) content in the control cabbage, in which absence of $GA_3$ that inhibits ABA could have stimulated ribonuclease activity and increased enzyme synthesis, especially chlorophyllase that degrades chlorophyll (Yi and Ji, 1988).

### 4.2.3. Effect o f interaction between timing of $GA_3$ application and $GA_3$ level

A significant interaction between timing of $GA_3$ application and $GA_3$ level occurred at 6, 14, and 18 days post-treatment (Table 5, $P \leq 0.05$). Fruits treated at 5 dph with 100 and 250 ppm $GA_3$ had higher chlorophyll levels than the rest. Probably $GA_3$ delayed chlorophyll degradation through its action in delaying transformation of chloroplasts to chromoplasts (Goldschimdt et al., 1977), or it probably promoted

Table 5. Effect of timing of $GA_3$ application and $GA_3$ level on chlorophyll content (mg/L) of passion fruits

| Days post-treatment | $GA_3$ (ppm) | Timing of $GA_3$ application (dph)[z] | | | |
|---|---|---|---|---|---|
| | | 0 | 5 | 10 | 15 |
| 6 | 0 | 0.89def | 1.13cde | 1.39bc | 0.49f |
| | 50 | 1.14cde | 1.58ab | 1.34bc | 0.88def |
| | 100 | 1.00cde | 1.32bc | 1.34bc | 1.34bc |
| | 250 | 1.20bcd | 1.83a | 0.90de | 0.79def |
| 14 | 0 | 0.79bcd | 0.70cde | 0.91abcd | 0.40e |
| | 50 | 0.95abc | 0.79bcd | 0.93abcd | 0.44e |
| | 100 | 0.86abcd | 0.87abcd | 0.87bcd | 0.46e |
| | 250 | 1.05ab | 1.16a | 0.61de | 0.40e |
| 18 | 0 | 0.47ef | 0.58cdef | 0.77abc | 0.22a |
| | 50 | 0.54def | 0.75abcd | 0.88a | 0.38fg |
| | 100 | 0.51ef | 0.62bcde | 0.61cde | 0.38fg |
| | 250 | 0.65bcde | 0.82ab | 0.76abc | 0.54ef |

[z]Means followed by the same letter within each sampling period are not significantly different at $P \leq 0.05$, according to the DMRT. Least significant difference for 6, 14 and 18 days post-treatment is 0.37, 0.28 and 0.19, respectively.

chlorophyll synthesis (Coggins and Lewis, 1962). This result proved the positive effect of the 100 and 250 ppm $GA_3$ in retarding ripening when applied within 5 dph.

The chlorophyll content of all the 16 treatments was negatively correlated with time of storage (Table 5.1). As fruits stayed in polythene bags loss of chlorophyll progressed, because $GA_3$ only reduced the rate of loss rather than eliminated it. This loss could have occurred under the action of polygalacturonase and chlorophyllase, and $GA_3$ only reduced the rate of action of these enzymes (Yi and Ji, 1988).

Gentle slopes (rate of chlorophyll decrease) observed in fruits treated with $GA_3$ at 10 and 15 dph probably occurred due to low initial levels of the chlorophyll at the time of treatment (Table 5.1). Thus, less time was taken for the loss of chlorophyll across the 20 dpt to be almost complete. The opposite result occurred for $GA_3$ applied between 0 and 5 dph, where fruits had high initial chlorophyll contents (Table 5.1). Therefore, 50, 100, and 250 ppm $GA_3$ applied at 0 and 5 dph proved to be the most effective treatments in delaying chlorophyll breakdown.

## 4.3. Rind Firmness

### 4.3.1. Main effect of timing of $GA_3$ application

Timing of $GA_3$ application had a significant effect on rind firmness (Table 6, $P \leq 0.05$). Generally, treatments at 0 and 5 dph were better in maintaining rind firmness compared to treatments at 10 and 15 dph, whose fruits had lower firmness (Table 6). Effectiveness of treating fruits within 5 dph could be attributed to rind freshness,

Table 5.1. Slopes (A) and $R^2$ values (B) from the plot of chlorophyll content (mg/L) versus time post-treatment as influenced by timing of $GA_3$ application and $GA_3$ level

| **A (slopes)** | Timing of $GA_3$ application (dph)[z] | | | |
|---|---|---|---|---|
| $GA_3$ (ppm)[z] | 0 | 5 | 10 | 15 |
| 0 | -0.13 | -0.15 | -0.10 | -0.07 |
| 50 | -0.14 | -0.15 | -0.10 | -0.09 |
| 100 | -0.17 | -0.20 | -0.12 | -0.07 |
| 250 | -0.15 | -0.17 | -0.12 | -0.06 |

| **B ($R^2$ values)** | Timing of $GA_3$ application (dph)[z] | | | |
|---|---|---|---|---|
| $GA_3$ (ppm)[z] | 0 | 5 | 10 | 15 |
| 0 | 0.62 | 0.73 | 0.76 | 0.89 |
| 50 | 0.72 | 0.86 | 0.72 | 0.88 |
| 100 | 0.81 | 0.86 | 0.89 | 0.87 |
| 250 | 0.80 | 0.86 | 0.68 | 0.84 |

Table 6. Main effect of timing of $GA_3$ application on firmness (kg) of passion fruits

| Days post-treatment | Timing of $GA_3$ application (dph)[z] | | | | LSD[y] | %CV[x] |
|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | | |
| 2 | 8.39a | 7.03b | 7.05b | 6.43b | 0.66 | 14.75 |
| 4 | 8.76a | 6.71c | 7.53b | 7.42b | 0.45 | 13.98 |
| 6 | 6.67 | 6.85 | 7.16 | 6.51 | NS | 13.49 |
| 8 | 6.10a | 7.41a | 6.97a | 5.50b | 0.97 | 18.33 |
| 10 | 7.01b | 7.17a | 6.33a | 5.87b | 0.80 | 21.49 |
| 12 | 7.01a | 7.17a | 6.33ab | 5.87b | 1.08 | 15.54 |
| 14 | 6.87ab | 7.31a | 5.97bc | 5.23c | 1.09 | 14.78 |
| 16 | 6.58a | 5.82ab | 5.50bc | 4.75c | 0.98 | 14.33 |
| 18 | 6.39a | 5.80b | 5.89b | 3.28c | 0.44 | 19.97 |
| 20 | 5.93a | 5.75a | 5.22a | 2.41b | 0.83 | 21.53 |
| Slope | -0.24 | -0.13 | -0.23 | -0.45 | | |
| $R^2$ | 0.59 | 0.39 | 0.84 | 0.84 | | |

[z]Means followed by the same letter or no letter within each row are not significantly different at $P \leq 0.05$, according to the DMRT.

[y]Least significant difference. NS = not significant.

[x]Coefficient of variation.

which improved $GA_3$ absorption and activity. Also, the lag phase needed to activate ethylene relative to the onset of $CO_2$ activity in packages probably led to increased firmness early in the experiment (Barmore, 1975; Wang, 1977).

Firmness was high early in the experiment, especially for fruits treated within 5 dph. As storage progressed, respiration increased in the remaining healthy fruit tissues, in a bid to sustain *P. expansum* (Farkas, 1978). The increased respiration probably led to a drastic decline in pressure as rind tissues disintegrated.

There was a general decrease in rind firmness across the 20 days post-treatment (Table 6). Fruits treated within 10 days post-harvest exhibited low rates of decrease in rind firmness of -0.24, -0.13 and -0.23, while those treated at 15 dph had a slightly higher rate of -0.45 (Table 6). The high $R^2$ value of 0.84 revealed the existence of a strong linear relationship between rind softening and storage time for fruits treated at 10 and 15 dph (Table 6).

Generally, the decreasing rind pressure was probably due to loss of water, which made it easy for the penetrometer to puncture the rind. The drastic decrease in firmness for 15 dph was probably due to the severe loss of moisture and the senescing rind tissues. In addition, increased enzymatic activity as aging progressed probably led to dissolution of cell wall polymers, resulting in decreased firmness. It has been shown that cellulase and polygalacturonase increase as fruit firmness decreases (Wegrzyn and MacRae, 1992). When under the influence of exogenous and/or endogenous ethylene, these enzymes cause breakdown of pectic polymers, resulting in loss and de-

esterification of pectins and some breakdown of middle lamella pectic polymers (Redgwell, 1991). All these changes accelerate senescence. Wegrzyn and MacRae (1992) observed an increase in pectin esterase and polygalacturonase in ripening Kiwi fruits, whose softening and senescent enzymes increased simultaneously. Bartley (1974) reported an increase in pectinesterase, β-galactosidase, and the content of polyuronide soluble fraction as a result of cleavage of some interpolymer bonds in ripening apples. Cleavage causes a change in texture, and contributes significantly to tissue softening (Proctor and Peng, 1989).

In our research, enzyme activity probably varied with $GA_3$ application time. Delaying fruit treatment probably resulted in autocatalytic evolution of ethylene, which promoted enzymes involved in deploymerization of starch and soluble pectins. The increased enzyme activity probably reduced chances of the modified atmosphere in reversing rind softening to a significant degree (Sommer, 1985 and 1992).

### 4.3.2. Main effect of $GA_3$

A significant $GA_3$ effect on rind firmness was observed only at the $4^{th}$ and $18^{th}$ days post-treatment; $GA_3$ levels seemed to have a similar effect (Table 7, $P \leq 0.05$). Nevertheless, a negative linear relationship was observed between rind firmness and storage time (Table 7). The firmness of fruits treated with 50 ppm $GA_3$ had a gentle slope of -0.22 compared to -0.28 and -0.31 for those treated with 100 and 250 ppm $GA_3$, respectively. Thus, as $GA_3$ level increased above 50 ppm, the effectiveness of

Table 7. Main effect of $GA_3$ on firmness (kg) of passion fruits

| | $GA_3$ (ppm)[z] | | | | | |
|---|---|---|---|---|---|---|
| Days post-treatment | 0 | 50 | 100 | 250 | LSD[y] | %CV[x] |
| 2 | 6.68 | 7.53 | 7.29 | 7.38 | NS | 14.75 |
| 4 | 6.94b | 7.62ab | 7.98a | 7.88ab | 0.895 | 13.98 |
| 6 | 7.19 | 6.75 | 6.72 | 6.53 | NS | 13.49 |
| 8 | 6.86 | 6.27 | 6.06 | 6.38 | NS | 18.33 |
| 10 | 6.69 | 6.29 | 6.28 | 6.73 | NS | 21.49 |
| 12 | 6.60 | 6.37 | 6.45 | 6.46 | NS | 15.54 |
| 14 | 6.27 | 6.15 | 6.88 | 6.07 | NS | 14.78 |
| 16 | 5.71 | 5.68 | 5.91 | 5.35 | NS | 14.33 |
| 18 | 4.95b | 6.08a | 5.13b | 5.21ab | 0.898 | 19.97 |
| 20 | 4.86 | 5.27 | 4.57 | 4.61 | NS | 21.53 |
| Slope | -0.24 | -0.22 | -0.28 | -0.31 | | |
| $R^2$ | 0.77 | 0.84 | 0.72 | 0.88 | | |

[z]Means followed by the same letter or no letter within each row are not significantly different at $P \leq 0.05$, according to the DMRT.

[y]Least significant difference. NS = not significant.

[x]Coefficient of variation.

$GA_3$ slightly decreased (Table 7). The 50-ppm $GA_3$ probably increased resistance to damage by antagonizing production of ethylene, which accelerates enzymatic breakdown of cell wall polymers in the rind. This observation agreed with that of Babbit et al. (1973), who observed a reduced rate of tomato fruit softening following $GA_3$ application. They observed a temporal slow down in cellulase and polygalacturonase activity, which then accelerated later after $GA_3$ application. Softening resulted from the action of cellulases on the interwoven cellulase fibrils of the cell walls, thereby enabling the pectin enzymes to penetrate into the middle lamella. A subsequent breakdown of the pectin adhesive that binds cells together followed, causing a rapid change in texture. Ben-Arie et al. (1996) expressed similar views and attributed softening during ripening of persimmons to dissolution of the middle lamella, separation of the plasmalemma from the cell wall, and solubilization of pectic polymers. Thus, despite treatment with $GA_3$, rind softening resulted later due to the activities of enzymes.

Monselise et al. (1976) also observed that spraying Kaki fruits with $GA_3$ maintained rind firmness. The $GA_3$ probably rendered the tissues more compact, thereby reducing incorporation of amino acids into proteins connected with pectin degradation, and delaying senescence. Similar positive effects of $GA_3$ in delaying tissue softening were reported by Nolte et al. (1990). These researchers associated the reduced electrolyte leakage and increased protoplast viability in pummelo-

mesocarp with decreased or no efflux of potassium ions and elevated protoplast survival. Ultimately, a firmer rind for a longer period relative to the control resulted.

In contrast, Saure (1996) observed reduced calcium activity after applying $GA_3$ on apples. Saure (1996) speculated that $GA_3$ activity led to loss of cell wall stability. Binding of polygalacturonate chains with calcium normally brings about stability (Knee, 1978; Conway et al., 1994b). The binding makes fruit cell walls less accessible to enzymes produced by fungal pathogens.

### 4.3.3. Effect of interaction between timing of $GA_3$ application and $GA_3$ level

No significant interaction was observed between timing of $GA_3$ application and $GA_3$ concentration. Nevertheless, based on the regression analysis, fruits treated with the four levels of $GA_3$ at 0, 5, and 10 dph had relatively similar slopes of less than 0.3, whereas application at 15 dph produced steeper slopes of up to 0.52 (Table 7.1). Thus, fruits treated after 0, 5 and 10 dph had relatively low rates of tissue softening compared to those treated after 15 dph (Table 7.1).

Although firmness for fruits treated at 0 and 5 dph had weak linear relationships with storage time (low $R^2$ values), the relatively gentle slopes indicated a low rate of tissue softening. At these timing levels fruits were fresh enough to permit $GA_3$ penetration and activity that probably lowered the rate of tissue softening. $GA_3$ could have rendered rind tissues more compact by reducing incorporation of amino acids into proteins connected with pectin degradation (Kitagawa, 1966).

Table 7.1. Slopes (A) and $R^2$ values (B) from the plot of firmness (kg) versus time post-treatment as influenced by timing of $GA_3$ application and $GA_3$ level.

| **A (slopes)** | Timing of $GA_3$ application (dph) | | | |
|---|---|---|---|---|
| $GA_3$ (ppm) | 0 | 5 | 10 | 15 |
| 0 | -0.15 | -0.15 | -0.25 | -0.42 |
| 50 | -0.21 | -0.13 | -0.15 | -0.41 |
| 100 | -0.25 | -0.15 | -0.26 | -0.45 |
| 250 | -0.33 | -0.10 | -0.28 | -0.52 |

| **B ($R^2$ values)** | Timing of $GA_3$ application (dph) | | | |
|---|---|---|---|---|
| $GA_3$ (ppm) | 0 | 5 | 10 | 15 |
| 0 | 0.37 | 0.34 | 0.38 | 0.82 |
| 50 | 0.38 | 0.43 | 0.56 | 0.85 |
| 100 | 0.38 | 0.20 | 0.71 | 0.63 |
| 250 | 0.57 | 0.25 | 0.67 | 0.76 |

Therefore, $GA_3$ effect in retarding fruit softening was relatively higher when applied soon after harvest (Table 7.1). In some cases, shriveling made it hard for the penetrometer to puncture the rind, resulting also in low rates of tissue softening.

The lack of a significant difference among the sixteen treatments led to a conclusion that no treatment was superior to the other. Therefore, higher levels of $GA_3$ than the ones used in this research should be investigated to determine the most effective level for retarding softening of passion fruits.

## 4.4. Colour Development

### 4.4.1. Main effect of timing of $GA_3$ application

The main effect of timing of $GA_3$ application on colour was significant across the 20 days post-treatment (Table 8, $P \leq 0.05$). Purple colour development had a positive and strong linear trend across the 20 dpt, with $R^2$ values ranging from 0.89 to 0.97 (Table 8). Fruits treated at 0 and 5 dph exhibited constantly increasing purple colour, with gradients of 0.48 and 0.44, respectively.

Fruits treated at 10 and 15 dph had an initial high score of 3.5 and 4.8 purple colour, respectively (Table 8). The fruits treated at 15 dph displayed increasing purple colour that stabilized at 5.69 from the $14^{th}$ day post-treatment (Table 8).

Application of $GA_3$ soon after harvest was more effective in reducing purple colour development, because $GA_3$ penetrated the succulent tissues readily to curtail rapid climacteric rise between day 2 and 8 post-treatment.

Table 8. Main effect of timing of $GA_3$ application on purple colour of passion fruits

| Days post-treatment | Timing of $GA_3$ application (dph)[z] | | | | LSD[y] | %CV[x] |
|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | | |
| 2 | 1.00c | 1.13c | 3.49b | 4.84a | 0.152 | 10.19 |
| 4 | 1.23d | 1.86c | 3.80b | 4.98a | 0.074 | 13.49 |
| 6 | 1.53a | 3.05c | 4.55bc | 5.16b | 0.471 | 7.83 |
| 8 | 1.73a | 3.47c | 5.01c | 5.23b | 0.471 | 8.11 |
| 10 | 2.88c | 3.98b | 5.09a | 5.28a | 0.322 | 11.83 |
| 12 | 3.36c | 4.24b | 5.24a | 5.66a | 0.517 | 12.69 |
| 14 | 3.80c | 4.65b | 5.46a | 5.69a | 0.683 | 12.69 |
| 16 | 4.40b | 4.83b | 5.81a | 5.69a | 0.657 | 11.55 |
| 18 | 4.61c | 5.08bc | 5.99a | 5.69ab | 0.676 | 10.49 |
| 20 | 4.80c | 5.22bc | 6.00a | 5.69ab | 0.592 | 8.93 |
| Slope | 0.48 | 0.44 | 0.28 | 0.10 | | |
| $R^2$ | 0.97 | 0.91 | 0.93 | 0.89 | | |

[z]Means followed by the same letter within each row are not significantly different at $P \leq 0.05$, according to the DMRT.

[y]Least significant difference.

[x]Coefficient of variation.

Fruits treated at 5 dph exhibited rapid purple colour development during the first 8 days post-treatment. It appears that at 5 dph $GA_3$ absorption was limited to some extent by the shriveling rind. As a result, the activity of $GA_3$ in curtailing degradation of chlorophyll by enzymes was lowered.

Delayed treatment, especially at 15 dph, did not retard purple colour development, because by this time most fruits had already turned purple, whereas others had wilted. In addition, the shriveling hindered physiological changes such as respiration and enzymatic activity, which in turn affected most biochemical reactions involved in purple colour development.

### 4.4.2. Main effect of $GA_3$

A significant main effect of $GA_3$ on purple colour development was observed at 6, 8, 10 and 20 days post-treatment (Table 9). The 50 ppm $GA_3$ was more effective in retarding purple pigmentation, although some overlap existed with the 100 ppm $GA_3$ (Table 9). Similar positive effects were reported by Hicklenton (1991), who observed maintenance of colour intensity, and reduced or eliminated foliage yellowing in cut-alstromeria, pulsed with $GA_3$ and 6-benzyl amino purine. $GA_3$ can delay colour development through its effects on polygalacturonase and chlorophyllase (Lewis et al., 1967; Babbit et al., 1993), antagonize ethylene production, renew chloroplast formation, or delay anthocyanin synthesis and chlorophyll degradation (Lewis et al., 1964; Dostal and Leopold, 1967; Goldschimdt et al., 1974; Martinez et al., 1994).

Table 9. Main effect of $GA_3$ on purple colour development in passion fruits

| | $GA_3$ (ppm)[z] | | | | | |
|---|---|---|---|---|---|---|
| Days post-treatment | 0 | 50 | 100 | 250 | p-value[y] | %CV[x] |
| 2 | 2.70 | 2.60 | 2.63 | 2.53 | NS | 10.19 |
| 4 | 3.13 | 2.88 | 2.92 | 2.93 | NS | 13.49 |
| 6 | 3.90a | 3.34c | 3.47bc | 3.58b | 0.00 | 7.83 |
| 8 | 4.23a | 3.64c | 3.70c | 3.88b | 0.00 | 8.11 |
| 10 | 4.51a | 4.28ab | 4.04b | 4.39ab | 0.00 | 11.83 |
| 12 | 4.73 | 4.48 | 4.78 | 4.81 | NS | 12.69 |
| 14 | 4.94 | 4.78 | 4.82 | 5.06 | NS | 12.69 |
| 16 | 5.18 | 5.12 | 5.13 | 5.30 | NS | 11.55 |
| 18 | 5.30 | 5.34 | 5.26 | 5.48 | NS | 10.49 |
| 20 | 4.80c | 5.22bc | 6.00a | 5.69ab | 0.00 | 8.93 |
| Slope | 0.30 | 0.33 | 0.32 | 0.35 | | |
| $R^2$ | 0.93 | 0.97 | 0.98 | 0.96 | | |

[z]Means followed by the same letter or no letter within each row are not significantly different at $P \leq 0.05$, according to the DMRT.

[y]Level of significance. NS = not significant.

[x]Coefficient of variation

Ethylene plays a major role in colour development (Barmore, 1974). It has also been hypothesized that $GA_3$ inhibits chlorophyllases, oxidative systems, and leakage of organic acids from the vacuole, all of which otherwise bring about loss of green colour (Brady, 1987).

As $GA_3$ level increased, the rate of purple colour development increased slightly. Thus, the 250 ppm $GA_3$ slightly increased the rate of colour development compared to the 50 and 100 ppm $GA_3$; the slopes were 0.35 compared to 0.33 and 0.32, respectively (Table 9). Control fruits had the lowest rate of purple colour development of 0.30 (Table 9). As fruits ripened, a constant or a minimal change resulted in purple colour development (Table 9). The frequent lack of a significant difference in purple colour probably resulted because of the visual scoring used to assess this variable. Visual assessment made it difficult to detect changes in colour for most days post-treatment, especially the later ones.

### 4.4.3. Effect of interaction between timing of $GA_3$ application and $GA_3$ level

A significant interaction between timing of $GA_3$ application and $GA_3$ level was observed at 2, 6, 8, 10, 12, and 18 days post-treatment (Table 10). The $GA_3$ applied at 0 and 5 dph was most effective with a mean score of 1.00 at 2 days post-treatment. It appears that at 2 days post-treatment, $GA_3$ activity was at its peak in antagonizing ethylene production and activity on purple colour development. At 0 dph, the 50 ppm $GA_3$ was most effective with a mean score of 1.00 at 6 and 8 days post-treatment,

Table 10. Effect of the interaction between timing of $GA_3$ application and $GA_3$ concentration on development of purple colour in passion fruits

| Days post-treatment | $GA_3$ (ppm) | Timing of $GA_3$ application (dph)[z] | | | |
|---|---|---|---|---|---|
| | | 0 | 5 | 10 | 15 |
| 2 | 0 | 1.00d | 1.00d | 3.77b | 5.03a |
| | 50 | 1.00d | 1.00d | 3.50b | 4.90a |
| | 100 | 1.00d | 1.17d | 3.67b | 4.67a |
| | 250 | 1.00d | 1.33d | 3.03c | 4.77a |
| 6 | 0 | 2.40d | 3.00c | 5.07a | 5.13a |
| | 50 | 1.00f | 2.93c | 4.40b | 5.03a |
| | 100 | 1.20ef | 3.13c | 4.40b | 5.13a |
| | 250 | 1.53e | 3.13c | 3.13c | 5.33a |
| 8 | 0 | 2.97c | 3.47bc | 5.23a | 5.20a |
| | 50 | 1.00e | 3.40bc | 4.97a | 5.20a |
| | 100 | 1.37de | 3.37bc | 4.87a | 5.20a |
| | 250 | 1.53e | 3.13c | 3.13c | 5.33a |
| 10 | 0 | 3.70cd | 3.83cd | 5.30a | 5.20a |
| | 50 | 3.13de | 3.87cd | 4.93ab | 5.20a |
| | 100 | 1.80f | 3.87cd | 5.13ab | 5.37a |
| | 250 | 2.90e | 4.33bc | 5.00ab | 5.33a |
| 12 | 0 | 4.13de | 4.07def | 5.37ab | 5.37ab |
| | 50 | 3.53efg | 3.97de | 5.03bc | 5.40ab |
| | 100 | 2.50g | 4.40cd | 5.33abc | 5.67ab |
| | 250 | 3.27fg | 4.53cd | 5.23abc | 6.20a |
| 18 | 0 | 5.07cde | 4.83def | 5.90abcd | 5.40abc |
| | 50 | 5.03cde | 5.17bcdef | 5.67abcd | 5.5abcde |
| | 100 | 3.87f | 5.20bcde | 6.30a | 5.67abcd |
| | 250 | 4.47ef | 5.13bcde | 6.10a | 6.20ab |

[z]Means followed by the same letter within each sampling period are not significantly different at $P \leq 0.05$, according to the DMRT. Least significant difference for 2, 6, 8, 10, 12 and 18 dpt is 0.47, 0.47, 0.74, 0.99 and 0.95, respectively.

whereas 100 ppm $GA_3$ proved to be most effective with mean scores of 1.8, 2.5, and 3.87 at 10, 12, and 18 days post-treatment, respectively (Table 10).

Fruits treated at 0 and 5 dph had high rates of purple colour development compared to those treated at 10 and 15 dph (Fig. 1). These results showed that the purple colouration process for 0 and 5 dph fruits was just beginning, while for fruits treated at 10 and 15 dph the process had already advanced by the time $GA_3$ was applied, hence the low colouration rate. The relatively similar slopes for fruits treated at 0 and 5 dph and for those treated at 10 and 15 dph showed that these groups of fruits had almost similar colouration behaviour. The 50-ppm $GA_3$ applied 0 dph had the steepest slope of 0.56 compared to other treatments at 0 and 5 dph.

Purple colour development for all treatment combinations had a positive and strong linear relationship with storage time, as evidenced by $R^2$ values of above 0.85 (Fig. 1). Thus, purple colour development did not depend on treatment.

Nevertheless, 50 and 100 ppm $GA_3$ applied at 0 dph appeared to be the most effective treatments, especially at the early days post-treatment, whereas 100 ppm $GA_3$ applied at this timing level dominated as the best treatment at later days post-treatment. Fruits receiving no $GA_3$ at the four timing levels generally had highest purple colour scores. However, due to the overlap in purple colour scores for 50, 100, and 250 ppm $GA_3$ applied at 0 and 5 dph, no single treatment combination can be termed as the best. Therefore, more research on $GA_3$ is needed to establish the level that effectively delays purple colour development.

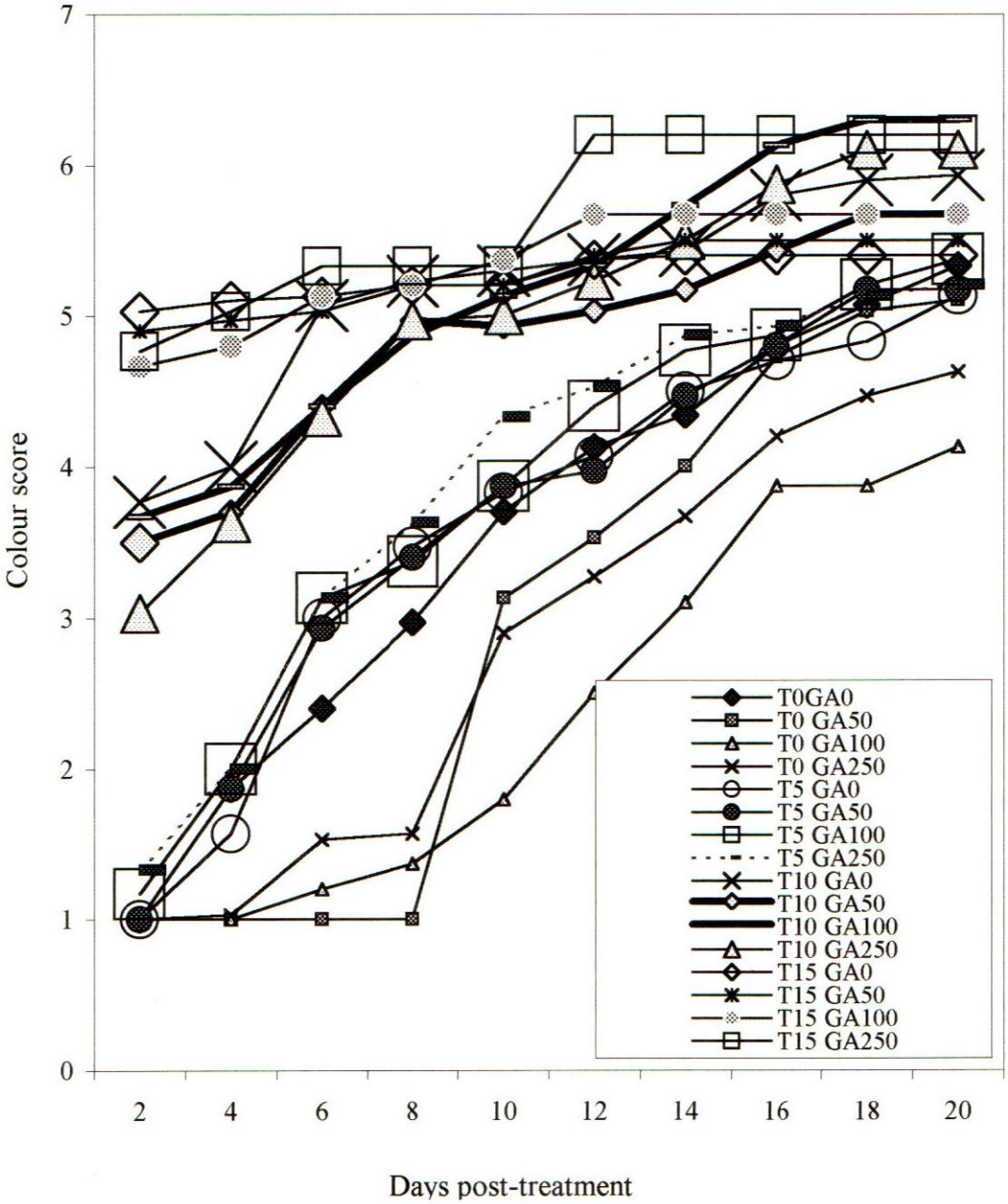



Figure 1. Effect of interaction between time of GA3 application and GA3 level on purple colour development in passion fruits. The slope and R-squared value for T0GA0 through T15GA250 are: 0.47, 0.97; 0.56, 0.91; 0.41, 0.95; 0.47, 0.96; 0.44, 0.90; 0.44, 0.93; 0.45, 0.93; 0.42, 0.88; 0.22, 0.84; 0.24, 0.90; 0.32, 0.98; 0.33, 0.93; 0.05, 0.90; 0.08, 0.91; 0.12, 0.85; 0.18, 0.85, respectively. T refers to time and GA refers to GA3.

## 4.5. Disease Severity

### 4.5.1. Main effect of timing of $GA_3$ application

The main effect of timing of $GA_3$ application on disease severity was significant at all the 20 days post-treatment (Table 11, $P \leq 0.05$). The disease severity trend was constant throughout the experimental period. The lesion diameter for fruits treated at 15 dph was greater than that for fruits treated at 10 dph followed by that for fruits treated at 5 and 0 dph (Table 11). Nevertheless, a positive and strong linear relationship existed between lesion diameter and storage time, for each timing of $GA_3$ application. The increase in lesion diameter with storage time was gradual for fruits treated at 0 and 5 dph (Table 11). Fruits treated at 10 dph showed a gentle increase followed by a rapid increase, while fruits treated at 15 dph had a rapid rise right from the start of the experiment (Table 11). The rate of lesion growth, therefore, depended on the timing of $GA_3$ application.

Fruits treated at 0 and 5 dph developed lesions slower than fruits treated at 10 and 15 dph, because immediately after harvest fruits were fresh and could resist germination of *P. expansum*; the polythene bags (modified atmosphere) also assisted fruits increase resistance to fungal attack (El-Goorani and Sommer, 1981; Sommer, 1985 and 1992). Bateman and Basham (1976) and Sams and Conway (1987) reported that modified atmosphere storage reduced decay caused by *P. expansum*, increased firmness, and reduced endogenous ethylene production in apples.

Table 11. Main effect of timing of $GA_3$ application on lesion diameter (mm) in passion fruits inoculated with *P. expansum*

| | Timing of $GA_3$ application (dph)[z] | | | | | |
|---|---|---|---|---|---|---|
| Days post-treatment | 0 | 5 | 10 | 15 | LSD[y] | %CV[x] |
| 2 | 0.71d | 1.37c | 2.38b | 2.88a | 0.428 | 14.16 |
| 4 | 0.89d | 1.78c | 2.57b | 3.28a | 0.500 | 18.47 |
| 6 | 1.18d | 2.15c | 2.80b | 4.07a | 0.487 | 9.31 |
| 8 | 1.54d | 2.42c | 3.00b | 4.99a | 0.385 | 15.98 |
| 10 | 1.67d | 2.61c | 3.37b | 5.65a | 0.404 | 15.74 |
| 12 | 1.82d | 2.80c | 3.79b | 6.93a | 0.878 | 18.38 |
| 14 | 2.04d | 3.06c | 4.08b | 8.93a | 0.623 | 13.77 |
| 16 | 2.17d | 3.25c | 5.44b | 10.63a | 0.586 | 7.55 |
| 18 | 2.32d | 3.65c | 6.82b | 10.74a | 0.753 | 8.13 |
| 20 | 2.55d | 4.28c | 7.98b | 10.83a | 0.438 | 11.13 |
| Slope | 0.20 | 0.28 | 0.59 | 1.03 | | |
| $R^2$ | 0.98 | 0.98 | 0.87 | 0.96 | | |

[z]Means followed by the same letter within each row are not significantly different at $P \leq 0.05$, according to the DMRT. Data were square root-transformed for analysis.
[y]Least significant difference.
[x]Coefficient of variation.

Growth, sporulation, and enzyme activity of *P. expansum* were reduced, and the improved physiological condition of apples enabled them to resist further decay. Introducing fruits into bags at 0 dph created a modified atmosphere early enough, thereby reducing the amount of oxygen available to the germinating *P. expansum*. In contrast, fruits treated at 5 to 15 dph had a lot of atmospheric oxygen facilitating germination of *P. expansum* before they were placed into the polythene bags.

In addition, the presence of juvenile factors, such as growth hormones in fresh fruits, together with the applied $GA_3$ increased resistance of rind tissues to attack, besides negating fungal activity (Abdel-Gawad and Romani, 1967; Dostal and Leopold, 1967). However, as fruits delayed receiving $GA_3$, inoculum density of *P. expansum* increased and sporulated much faster in the polythene bags. The increased fungal growth probably resulted due to the reduced rind firmness and the leakage of solutes through the wounded tissues (Mangas et al., 1992; Knee, 1973). Mangas et al. (1992) observed a decrease of insoluble pectins in apples coincide with increased fruit deterioration, which in turn favoured penetration of pathogens into the fruits.

Death of infected tissues increased with time, especially for fruits treated at 10 and 15 dph, where there was an ultimate rapid increase in lesion diameter (Table 11). This trend agreed with Farkas (1978), who observed a high metabolic rate of the host-parasite complex at the infection courts trigger increased respiration in non-infected tissues that supplied respiratory substrates to infected areas, resulting in accelerated senescence of healthy tissues. Agrios (1988) attributed the faster rate of

death with increasing time to ATP consumption at the infection sites, especially during the sporulation phase. High levels of peroxidase, polyphenol oxidase and ascorbic acid oxidase ensued, resulting in autocatalytic acceleration of senescence and lesion development.

*Penicillium expansum* produces ethylene, which increases the respiration rate and accelerates senescence of harvested produce (Agrios, 1988). Later, patulin or amycotoxin production sets in, resulting in accelerated lesion development in rind tissues. Therefore, increased inoculum density in the fruits treated at 10 and 15 dph could have caused the high rate of lesion development, because disease severity increases as more of the rind tissue is attacked by the sporulating *P. expansum*.

### 4.5.2. Main effect of $GA_3$

The main effect of $GA_3$ on lesion growth was significant at 6 and 16 days post-treatment (Table 12). Fruits treated with 100 and 250 ppm $GA_3$ had smaller lesions compared to those treated with 0 and 50 ppm $GA_3$ at each day post-treatment.

The 100 ppm $GA_3$ probably delayed wax-ultrastructure changes (El-Otmani and Coggins, Jr., 1985), thereby limiting the enzymatic breakdown of carbohydrates, especially pectins, which ultimately favour penetration and sporulation of pathogens (Mangas et al., 1992). Another reason could be that $GA_3$ either altered the calcium

Table 12. Main effect of $GA_3$ on lesion diameter (mm) of passion fruits inoculated with *P. expansum*

| | $GA_3$ (ppm)[z] | | | | | |
|---|---|---|---|---|---|---|
| Days post-treatment | 0 | 50 | 100 | 250 | LSD[y] | %CV[x] |
| 2 | 1.83 | 1.92 | 1.79 | 1.80 | NS | 14.16 |
| 4 | 2.12 | 2.25 | 2.13 | 2.01 | NS | 18.47 |
| 6 | 2.53ab | 2.72a | 2.47b | 2.47b | 0.199 | 9.311 |
| 8 | 3.08 | 3.12 | 2.94 | 2.80 | NS | 15.98 |
| 10 | 3.55 | 3.40 | 3.24 | 3.11 | NS | 15.74 |
| 12 | 4.12 | 3.88 | 3.68 | 3.66 | NS | 18.38 |
| 14 | 4.51 | 4.66 | 4.44 | 4.51 | NS | 13.77 |
| 16 | 5.61a | 5.43ab | 5.13b | 5.33ab | 0.342 | 7.55 |
| 18 | 6.05 | 5.88 | 5.72 | 5.87 | NS | 8.13 |
| 20 | 6.44 | 6.24 | 6.41 | 6.54 | NS | 11.13 |
| Slope | 0.54 | 0.50 | 0.51 | 0.54 | | |
| $R^2$ | 0.99 | 0.98 | 0.98 | 0.97 | | |

[z]Means followed by the same letter or no letter within each row are not significantly different at $P \leq 0.05$, according to the DMRT. Data were square root-transformed.
[y]Least significant difference. NS = not significant.
[x]Coefficient of variation.

activity in maintaining protein synthesis, or reduced formation and activity of cell wall degrading enzymes. These effects improve firmness and maintain rigidity of epicuticular wax cell walls, which are the avenues for pathogenic attack (Faust and Shear, 1972; Sams and Conway, 1987; Kader, 1992; Saure, 1996).

Lesion development had a positive and strong linear relationship with storage time (Table 12). Gibberellic acid levels had similar rates of lesion growth (Table 12). Thus, the $GA_3$ levels randomized within each timing of $GA_3$ application, affected lesion growth less than timing of $GA_3$ application.

### 4.5.3. Effect of the interaction between timing of $GA_3$ application and $GA_3$ level

There was a significant interaction between timing of $GA_3$ application and $GA_3$ concentration on lesion diameter at 6 and 16 days post-treatment (Table 13, $P \leq 0.05$). $GA_3$ levels for each timing level were not significantly different from each other, except those for 10 and 15 days post-harvest (Table 13).

For all the sixteen treatments, lesion development had a positive and strong linear relationship with storage time, as evidenced by the high $R^2$ values of above 0.8. This showed that lesion development increased with storage time, irrespective of treatment combination. The rate of increase depended more on the timing of $GA_3$ application than on $GA_3$ concentration (Fig. 2).

Fruits treated with $GA_3$ at 0 and 5 dph had gentle slopes of about 0.2 to 0.3, compared to those treated with $GA_3$ at 10 and 15 dph, which had steeper slopes

Table 13. Effects of the interaction between timing of $GA_3$ application and $GA_3$ level on lesion diameter (mm) of passion fruits

| Days post-treatment | $GA_3$ (ppm) | Timing of $GA_3$ application (dph)[z] | | | |
|---|---|---|---|---|---|
| | | 0 | 5 | 10 | 15 |
| 6 | 0 | 1.34f | 2.23de | 3.00c | 3.82b |
| | 50 | 1.08f | 2.18de | 2.75c | 4.58a |
| | 100 | 1.27f | 2.05e | 2.65cd | 3.91b |
| | 250 | 2.15de | 2.82c | 3.82b | 3.95b |
| 16 | 0 | 2.52ef | 3.27d | 5.72b | 10.98a |
| | 50 | 1.97f | 3.43d | 5.88b | 10.43a |
| | 100 | 2.00f | 2.98de | 4.92c | 10.60a |
| | 250 | 2.20f | 3.34d | 5.25bc | 10.52a |

[z]Means followed by the same letter within each sampling period are not significantly different at $P \leq 0.05$, according to the DMRT. Data were square root-transformed for analysis. Least significant difference for 6 and 16 dpt is 0.48 and 0.69, respectively.

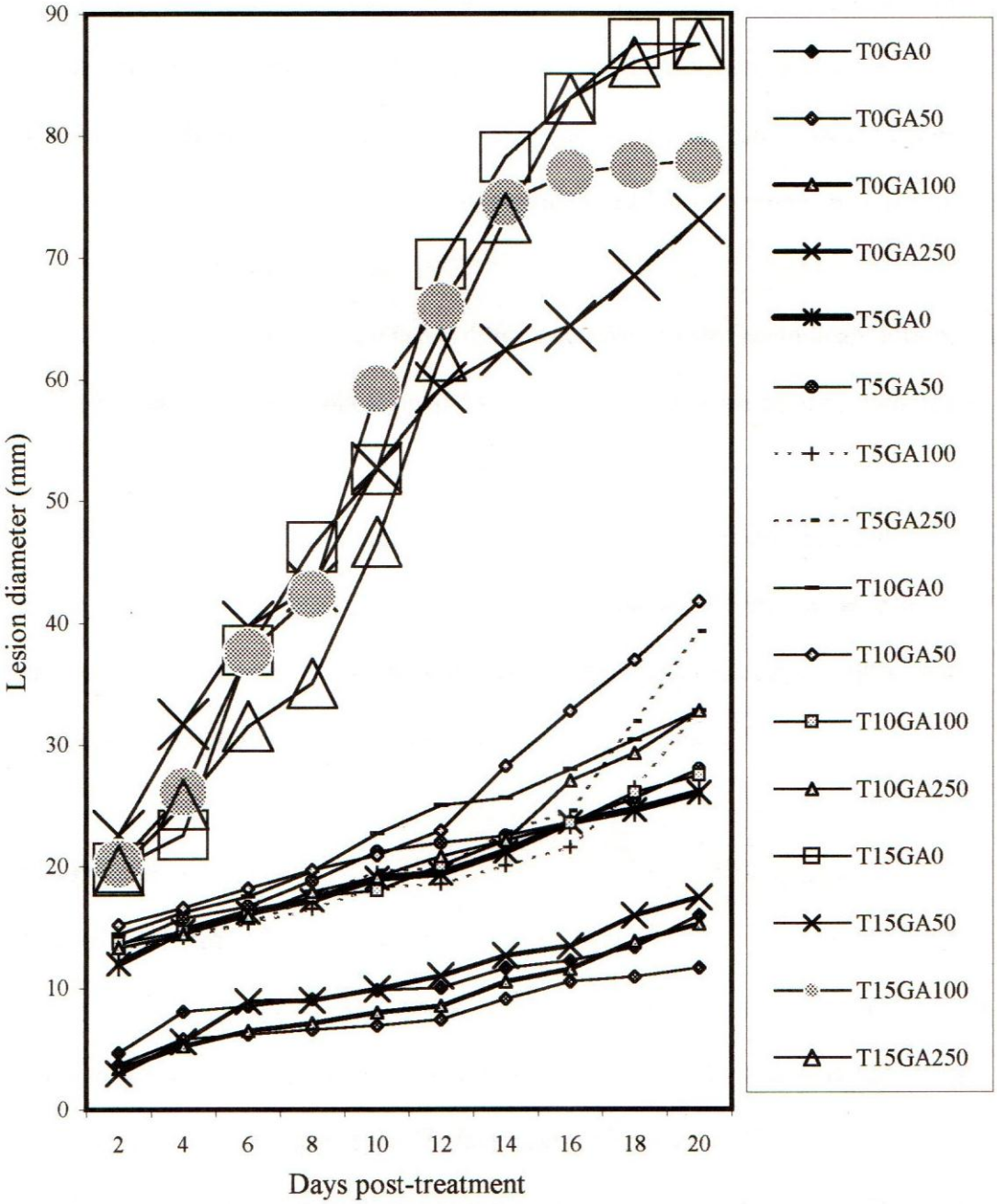



Figure 2. Effect of timing of GA3 application and GA3 level on lesion diameter of passion fruits inoculated with *P. expansum* . The slope and R-squared values for T0 GA0 thorugh T15 GA250 are: 0.23, 0.98; 0.18, 0.99; 0.19, 0.99; 0.21, 0.97; 0.29, 0.98; 0.29, 0.98; 0.25, 0.95; 0.31, 0.97; 0.39, 0.90; 0.58, 0.88; 0.59, 0.78; 0.59, 0.87; 1.05, 0.96; 0.96, 0.96; 1.04, 0.96; 1.06, 0.94, respectively. T refers to time and GA refers to GA3.

ranging from 0.6 to 1.1 (Fig. 2). $GA_3$ application at 0 and 5 dph had low rates of lesion development, probably because $GA_3$ delayed ultra-structural changes in wax. The delay limits the enzymatic breakdown of carbohydrates, especially the pectins, which ultimately favour penetration and sporulation of *P. expansum* (El-Otmani and Coggins Jr., 1985; Mangas et al., 1992).

The frequent lack of a significant difference revealed the insignificant interaction between timing of $GA_3$ application and $GA_3$ level. For the days where a significant interaction was observed, no single treatment combination was more effective than the other, although fruits treated with 250 ppm $GA_3$ at 0 dph had a relatively smaller lesion diameter. Therefore, $GA_3$ levels above 250 ppm should be evaluated within 5 dph to establish the best treatment combination, which can effectively curtail lesion development once *P. expansum* has attacked passion fruits.

### 4.6. Disease Incidence

#### 4.6.1. Main effect of timing of $GA_3$ application

A significant main effect of timing of $GA_3$ application on disease incidence was observed (Table 14, $P \leq 0.05$). Fruits treated at 0 dph had the lowest incidence of attack by *P. expansum* across the 20 days post-treatment (Table 14).

Disease incidence gradually increased with storage time (Table 14). Thus, a positive and strong linear relationship of above 0.8 existed between disease incidence and storage time for the four times of $GA_3$ application (Table 14).

Table 14. Percentage of diseased passion fruits as influenced by timing of $GA_3$ application and $GA_3$ level

| Time post-treatment (days) | $GA_3$ (ppm) | Timing of $GA_3$ application[z] | | | |
|---|---|---|---|---|---|
| | | 0 | 5 | 10 | 15 |
| 2 | 0 | 26ef | 34de | 46bc | 50b |
| | 50 | 11g | 27ef | 47bc | 62a |
| | 100 | 11g | 21f | 47bc | 62a |
| | 250 | 9g | 26ef | 40cd | 64a |
| 4 | 0 | 33fg | 42def | 52bc | 58b |
| | 50 | 11h | 40defg | 48bcd | 69a |
| | 100 | 12h | 31g | 52bc | 71a |
| | 250 | 9h | 36efg | 43cde | 71a |
| 6 | 0 | 80a | 47bcde | 53b | 74a |
| | 50 | 19f | 44cde | 50bcd | 72a |
| | 100 | 14f | 39e | 52bc | 80a |
| | 250 | 12f | 41e | 43de | 77a |
| 8 | 0 | 59b | 49bc | 54b | 77a |
| | 50 | 23d | 47bc | 56bcd | 74a |
| | 100 | 24d | 44c | 56b | 81a |
| | 250 | 19d | 44c | 44c | 83a |
| 10 | 0 | 59bc | 50cde | 58bcd | 78a |
| | 50 | 25f | 50cde | 56bcd | 78a |
| | 100 | 29f | 47de | 62b | 84a |
| | 250 | 26f | 46e | 48de | 86a |
| 12 | 0 | 62bc | 54bcd | 61bc | 85a |
| | 50 | 27e | 50cd | 58bcd | 81a |
| | 100 | 33e | 49cd | 64b | 86a |
| | 250 | 23e | 46d | 49d | 86a |
| 14 | 0 | 67b | 55bc | 64b | 86a |
| | 50 | 28d | 57bc | 59bc | 82a |
| | 100 | 33d | 49cd | 65b | 86a |
| | 250 | 30d | 49c | 55bc | 86a |
| 16 | 0 | 73b | 57cd | 66bc | 86a |
| | 50 | 33e | 58cd | 67bc | 86a |
| | 100 | 34e | 50d | 66bc | 86a |
| | 250 | 31e | 51d | 57cd | 86a |
| 18 | 0 | 73b | 63bcd | 71b | 86a |
| | 50 | 33e | 65bc | 69bc | 86a |
| | 100 | 34e | 53d | 66bc | 86a |
| | 250 | 31e | 54d | 59cd | 86a |
| 20 | 0 | 73bc | 72bcd | 78ab | 86a |
| | 50 | 36g | 66cde | 69bcd | 86a |
| | 100 | 39g | 58ef | 70bc | 86a |
| | 250 | 35g | 54f | 59def | 86a |

[z]Means followed by the same letter within each sampling period are not significantly different at $P \leq 0.05$, according to the DMRT. Data were angular-transformed for analysis. LSD for 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20 days is 9, 9, 8, 9, 9, 11, 16, 11, 9 and 9, respectively.

The gentle rise in disease incidence for the fruits treated at 10 and 15 days post-harvest occurred due to the high initial number of fruits infested with *P. expansum*. With fruits treated at 10 and 15 dph, it took a few days for almost all their fruits to be infected, hence the gentle increase in disease incidence.

Treating fruits at 0 dph was the most effective time, because it resulted in the lowest infection percentage. The low number of fruits attacked by *P. expansum* when treated at 0 dph agreed with reports that early packaging allows low oxygen and increased carbon dioxide in the polythene bags to reduce infection (suppress rotting) (El-Goorani and Sommer, 1981), curtail ethylene production and reduce tissue sensitivity to ethylene (Kader, 1985). In addition, the fresh state of the fruits treated at 0 dph probably improved $GA_3$ activity, and provided anti-fungal factors, especially anthocyanins and tannins, that limited fungal growth (Macheix et al., 1990). This observation agreed with El-Otmani and Coggins (1985) and Ben-Yehoshua et al. (1995), who reported that applying $GA_3$ and 2,4-D on stored citrus fruits inhibited development of decay caused by *P. digitatum* and slowed decline in citral content and anti-fungal activity in the flavedo. Increased anti-fungal activity was also reported by Afek and Carmeli (1995), who observed reduced decay after dipping celery in gibberellic acid. In their study, $GA_3$ slowed down the conversion of mermesin to psoralen; mermesin is an active anti-fungal compound in celery.

Fungal spores on the fruits treated at 5, 10, and 15 dph germinated, using oxygen from the open atmosphere. This happened as the fruits waited for $GA_3$ application in

readiness for packaging in polythene bags. Also, delaying $GA_3$ application allowed growth of *P. expansum* to be manifested more easily, but depending on the duration of the delay (Table 14). The easy manifestation could be due to the ripening process, which weakens tissues, through increased respiration and activation of softening enzymes, leading to fungal spore proliferation and lesion development (Bateman and Basham, 1976; El-Goorani and Sommer, 1981; Sams and Conway, 1987).

Another argument for the increased infection of fruits treated at 10 and 15 dph could be the increased inoculum, which attacked adjacent fruits and raised the level of disease incidence. El-Kazzaz et al. (1983) observed enhanced development of disease as growth of *Botrytis cinerea* on stored strawberries increased.

### 4.6.2. Main effect of $GA_3$

A significant main effect of $GA_3$ on the incidence of disease was observed for 20 days post-treatment (Table 14, $P \leq 0.05$). The 50 to 250 ppm $GA_3$ clearly deviated from the 0 ppm $GA_3$, proving the effectiveness of $GA_3$ in retarding disease spread. This result agreed with that of Sommer and Rissler (1981), who sprayed several cut-flower cultivars with $GA_3$ and ethephon and observed $GA_3$ inhibit endogenous ethylene, which contributes to symptom development in many plant-diseases. Ethephon increased the rate of disease spread. Also, $GA_3$ probably boosted endogenous disease resistance of passion fruits by modulating changes in preformed anti-fungal materials such as anthocyanins and tannins (Macheix et al., 1990).

Nolte et al. (1990) reported that $GA_3$ reduced efflux of solutes and potassium from pumello mesocarp, and enhanced protoplast survival. These effects resulted in maintenance of membrane integrity and sustenance of peel viability for a longer time. Reduced solute leakage denied fungal spores nutrients needed for germination and sporulation, whereas the viable peel made it difficult for the spores to penetrate the rind tissues (Knee, 1973; Faust and Shear, 1972). The same effects of $GA_3$ could have occurred in passion fruits used in this research, resulting in disease suppression.

### 4.6.3. Effect of the interaction between timing of $GA_3$ application and $GA_3$ level

A significant effect of the interaction on disease incidence was observed (Table 14, $P \leq 0.05$). At 0 dph, fruits receiving 250 ppm $GA_3$ had the lowest disease incidence, whereas those receiving 0 ppm $GA_3$ had the highest disease incidence. Nevertheless, disease incidences for fruits treated with 50, 100 and 250 ppm $GA_3$ at 0 dph were not significantly different across the 20 days post-treatment. The 100 ppm $GA_3$ applied at 5 dph was more effective in retarding disease spread at 2, 4, 6 and 8 days post-treatment, whereas 250 ppm $GA_3$ applied at the same time was more effective at 10, 12, 14 and 16 days post-treatment. Treating fruits at 10 and 15 dph with 250 ppm $GA_3$ resulted in the lowest disease incidence for most days post-treatment, although it was not significantly different from that of fruits treated at the same time with 0, 50, and 100 ppm $GA_3$.

Fruits treated with $GA_3$ at 0 dph had the lowest disease incidence across the 20 days post-treatment, probably because at this timing the fresh state of the fruits improved $GA_3$ absorption and activity, which together with anti-fungal factors such as anthocyanins and tannins limited fungal growth (Macheix et al., 1990).

There was a positive and strong linear relationship between disease incidence and storage time, as evidenced by high $R^2$ values (> 0.7) for the 16 treatments (Table 14.1). This showed that disease spread increased linearly with storage time.

Fruits for each timing of $GA_3$ application had almost similar gradients, except at 0 dph, where the slope (5.33) of the control fruits was steeper than slopes of fruits receiving the other $GA_3$ levels (Table 14.1). Nevertheless, the control fruits had steeper slopes at all times of $GA_3$ application (Table 14.1).

Therefore, 250 ppm $GA_3$ applied at 0 dph seemed to be superior to 0, 50, and 100 ppm $GA_3$ applied at 0, 5, 10, and 15 dph in suppressing disease spread. However, lack of a significant difference in 50, 100, and 250 ppm $GA_3$ at 0 dph made no treatment combination more effective in retarding the spread of penicillium rot.

## 4.7. Weight loss

### 4.7.1. Main effect of timing of $GA_3$ application

A significant main effect of timing of $GA_3$ application on weight loss occurred across the 20 days post-treatment (Table 15, $P \leq 0.05$). Fruits treated at 15 dph exhibited the highest weight loss across the 20 days post-treatment, whereas those

Table 14.1. Slopes (A) and $R^2$ values (B) from the plot of disease incidence versus time post-treatment as influenced by timing of $GA_3$ application and $GA_3$ level

| **A (slopes)** | Timing of $GA_3$ application (dph) | | | |
|---|---|---|---|---|
| $GA_3$ (ppm) | 0 | 5 | 10 | 15 |
| 0 | 5.33 | 3.37 | 3.07 | 3.71 |
| 50 | 2.71 | 3.71 | 2.68 | 3.33 |
| 100 | 3.21 | 3.33 | 2.49 | 2.67 |
| 250 | 3.13 | 2.67 | 2.31 | 2.15 |

| **B ($R^2$ values)** | Timing of $GA_3$ application (dph) | | | |
|---|---|---|---|---|
| $GA_3$ (ppm) | 0 | 5 | 10 | 15 |
| 0 | 0.89 | 0.94 | 0.97 | 0.77 |
| 50 | 0.95 | 0.94 | 0.95 | 0.94 |
| 100 | 0.90 | 0.86 | 0.94 | 0.70 |
| 250 | 0.91 | 0.88 | 0.96 | 0.70 |

Table 15. Main effect of timing of $GA_3$ application on weight loss (%) by passion fruits

| Days post-treatment | Timing of $GA_3$ application (dph)[z] | | | | LSD[y] | %CV[x] |
|---|---|---|---|---|---|---|
| | 0 | 5 | 10 | 15 | | |
| 2 | 5.18ab | 3.34b | 4.39b | 6.71a | 1.788 | 50.91 |
| 4 | 8.956a | 8.85a | 3.63b | 7.50a | 1.559 | 26.89 |
| 6 | 6.92b | 8.15b | 4.74c | 10.02a | 1.451 | 38.55 |
| 8 | 9.29b | 8.82a | 6.05b | 10.34a | 1.570 | 30.78 |
| 10 | 8.45ab | 9.51a | 7.24b | 10.02a | 1.560 | 26.54 |
| 12 | 8.29b | 9.73ab | 9.96a | 10.25a | 1.539 | 19.28 |
| 14 | 7.60c | 10.71a | 9.47b | 10.97a | 1.112 | 20.18 |
| 16 | 8.05b | 12.23a | 10.95a | 12.23a | 1.735 | 16.30 |
| 18 | 8.78c | 12.34a | 10.40b | 12.51a | 1.397 | 12.43 |
| 20 | 9.86b | 13.84a | 11.07b | 13.04a | 1.545 | 15.62 |
| Slope | 0.25 | 0.88 | 0.92 | 0.64 | | |
| $R^2$ | 0.32 | 0.84 | 0.91 | 0.90 | | |

[z]Means followed by the same letter within each row are not significantly different at $P \leq 0.05$, according to the DMRT. Data were angular-transformed for analysis.

[y]Least significant difference.

[x]Coefficient of variation.

treated at 10 dph had the lowest weight loss for the first 10 days post-treatment, after which those treated at 0 dph exhibited the lowest weight loss (Table 15).

The slope (0.25) of fruits treated at 0 dph was gentler than slopes of fruits treated at other days post-harvest. However, the low $R^2$ value of 0.32 revealed the insignificant linear relationship between weight loss and storage time when fruits were treated at 0 days post-harvest. Modified atmosphere storage could have contributed to weight loss fluctuation, because fruits treated at 0 dph were fresh and actively metabolizing (Sommer, 1985 and 1992). Thus, upon packaging in polythene bags, fruits continued respiring at a moderate rate before oxygen decreased and carbon dioxide increased, creating a modified environment. As a result, water loss (weight loss) was high in the first few days through the respiration process. In contrast, fruits treated at 5, 10, and 15 dph after most of the water had been lost continued losing water (weight) at higher rates, depending on the extent at which $GA_3$ inhibited the activity of the fungus, and on the benefit of modified atmosphere packaging.

Weight loss for fruits treated at 0 and 5 dph was not significantly different for the first 12 days post-treatment (Table 15, $P \leq 0.05$). Fruits treated at 0 dph had an initial higher weight loss compared to those treated at 5 and 10 dph (Table 15, $P \leq 0.05$). The higher weight loss could be attributed to the fact that $GA_3$ maintained rind tissues fresh and turgid, and cuticle cells open. The open cuticle cells increased loss of water to the atmosphere as was observed by Salisbury and Ross (1985).

In contrast, fruits treated at 10 dph, recorded low initial weight loss probably due to shriveled rind tissues, which were exposed to the atmosphere before treating with $GA_3$ and packaging. Thus, excessive water loss before the fruits were packaged reduced the initial weight, upon which successive losses were based, but at the same time accelerated deterioration (Mitra, 1997). Shriveling probably also reduced $GA_3$ absorption and activity. Fungal effects could have also increased weight loss, especially for the fruits treated at 10 and 15 dph. With these fruits, fungal growth had increased such that tissues died and collapsed, resulting in loss of cell compartments, leakage of solutes, and weight loss (FAO, 1989).

### 4.7.2. Main effect of $GA_3$

$GA_3$ had no significant main effect on weight loss (Table 16, $P \leq 0.05$). This result showed that $GA_3$ had only slight beneficial effects in controlling weight loss. Fruits treated with the four $GA_3$ levels had weight loss increasing with increasing storage time, and relatively similar slopes and $R^2$ values (Table 16).

The increase in weight loss, even after $GA_3$ had been applied, could have resulted from $GA_3$ partially increasing sensitivity of fruit cells to post-harvest water loss, or reducing the ability of abscissic acid in effecting closure of fruit-rind stomata (Saure, 1996). Reduced abscissic acid (ABA) increases rather than decreases transpiration.

Table 16. Main effect of $GA_3$ on weight loss (%) by passion fruits

| Days post-treatment | $GA_3$ (ppm) 0 | 50 | 100 | 250 | p-value[y] | %CV[x] |
|---|---|---|---|---|---|---|
| 2 | 4.52 | 5.13 | 4.85 | 5.12 | NS | 50.91 |
| 4 | 6.93 | 7.35 | 7.42 | 7.23 | NS | 26.89 |
| 6 | 7.56 | 7.47 | 7.16 | 7.64 | NS | 38.55 |
| 8 | 8.85 | 8.65 | 8.52 | 8.49 | NS | 30.78 |
| 10 | 8.46 | 8.90 | 8.29 | 9.57 | NS | 26.54 |
| 12 | 9.45 | 9.32 | 9.37 | 10.10 | NS | 19.28 |
| 14 | 9.53 | 9.27 | 9.59 | 10.36 | NS | 20.18 |
| 16 | 11.14 | 10.70 | 10.45 | 11.17 | NS | 16.30 |
| 18 | 11.07 | 10.90 | 10.85 | 11.21 | NS | 12.43 |
| 20 | 11.99 | 12.03 | 12.10 | 11.68 | NS | 15.62 |
| Slope | 0.71 | 0.64 | 0.71 | 0.67 | | |
| $R^2$ | 0.92 | 0.93 | 0.90 | 0.93 | | |

[z]Means followed by no letter within each row are not significantly different at $P \leq 0.05$, according to the DMRT. Data were angular-transformed for analysis.

[y]Level of significance. NS = not significant.

[x]Coefficient of variation.

This trend is the opposite of what increased ABA content does upon fruit detachment (Terry, 1990). This observation contradicted that of Mohammed and Wickham (1995), who reported that $GA_3$ reduced weight loss in shado-benni plants by maintaining freshness, turgidity and decay-free conditions. Probably, $GA_3$ did not affect the performance of abscissic acid in regulating the opening and closing of the stomata to appreciable levels in the shado-benni plants.

Rahman et al. (1995) reported that weight loss in ripening jackfruits could be attributed to low starch and cell wall contents, through starch hydrolysis and cell wall maceration. Also, enzymatic activity of polygalacturonase, glucanase and pectin esterase increased several-fold, resulting in tissue maceration, homogalacturonan loss, solute leakage, and water loss. Farkas (1978) attributed these changes to increased ethylene activity as a result of increased growth of *P. expansum*. Thus, the increased weight loss at the later days post-treatment could be explained by similar effects, which can nullify any positive effects of $GA_3$

### 4.7.3. Effect of the interaction between timing of $GA_3$ application and $GA_3$ level

No significant effect of interaction between timing of $GA_3$ application and $GA_3$ concentration on weight loss was observed across the 20 days post-treatment (Table 16.1, $P \leq 0.05$). The four treatment combinations in each timing of $GA_3$ application had similar slopes (Table 16.1). Fruits treated at 0 dph had very gentle slopes (rate of weight loss) of less than 0.34, compared to those treated at 5, 10, and 15 dph, which

Table 16.1. Slopes (A) and $R^2$ values (B) from the plot of weight loss (%) versus time post-treatment as influenced by timing of $GA_3$ application and $GA_3$ level

| **A (slopes)** | Timing of $GA_3$ application (dph) | | | |
|---|---|---|---|---|
| $GA_3$ (ppm) | 0 | 5 | 10 | 15 |
| 0 | 0.34 | 0.93 | 0.96 | 0.58 |
| 50 | 0.11 | 0.80 | 1.00 | 0.65 |
| 100 | 0.27 | 0.96 | 0.62 | 0.82 |
| 250 | 0.25 | 0.83 | 1.09 | 0.50 |

| **B ($R^2$ values)** | Timing of $GA_3$ application (dph) | | | |
|---|---|---|---|---|
| $GA_3$ (ppm) | 0 | 5 | 10 | 15 |
| 0 | 0.38 | 0.81 | 0.84 | 0.75 |
| 50 | 0.04 | 0.87 | 0.93 | 0.73 |
| 100 | 0.22 | 0.86 | 0.76 | 0.97 |
| 250 | 0.48 | 0.75 | 0.89 | 0.89 |

had steeper slopes of above 0.6 (Table 16.1). Thus, the rate of weight loss increased with increasing time before $GA_3$ was applied. The slight effectiveness of $GA_3$ applied at 0 dph probably resulted from reduced fungal activity, which lowered carbohydrate loss (Redgwell, 1991).

Nevertheless, the low $R^2$ values for 0 dph revealed the weak linear relationship between weight loss and storage time (Table 16.1). The opposite result was observed when fruits were treated at 5, 10, and 15 dph, whereby weight loss was significantly influenced by storage time (Table 16.1). Thus, $GA_3$ application at 0 dph was more effective probably by lowering the sensitivity of fruits to senescent factors that culminate in weight loss (Rahman et al., 1995). However, lack of a significant difference in all $GA_3$ levels tested suggests researching with higher levels within 5 dph to establish the treatment that can effectively delay weight loss in passion fruits.

### 4.8. Fruit loss

#### 4.8.1. Main effect of timing of $GA_3$ application

There was a significant main effect of timing of $GA_3$ application on fruit loss (Table 17, $P \leq 0.05$). Fruits treated at 0 dph exhibited significant differences across the $GA_3$ levels, while those treated at 5, 10, and 15 days post-harvest had slightly significant differences in their fruit loss (Table 17, $P \leq 0.05$). At the later dates of $GA_3$ application, the shriveled rind tissues probably reduced $GA_3$ absorption and activity.

Table 17. Effect of the interaction between timing of $GA_3$ application and $GA_3$ concentration on loss (%) of passion fruits

| $GA_3$ (ppm) | Timing of $GA_3$ application (dph) | | | |
|---|---|---|---|---|
| | 0 | 5 | 10 | 15 |
| 0 | 56.11c | 49.15d | 47.91d | 63.64ab |
| 50 | 25.76f | 46.92d | 49.34d | 59.25bc |
| 100 | 20.67fg | 39.72c | 47.73d | 67.57a |
| 250 | 18.77g | 43.45de | 44.51de | 66.13a |

[z]Means followed by the same letter are not significantly different at $P \leq 0.05$, according to the DMRT. Data were angular-transformed for analysis. Least significant difference = 6.01. Coefficient of variation = 7.64%.

Also loss of water from the fruits increased fruit loss. Mayak (1977) also observed increased cell death as a result of water stress in netted muskmelons. Water stress adversely affected cell membrane integrity, culminating in death.

Exhaustion of food reserves and water from fruits leads to death, decay and physiological deterioration, which accelerate post-harvest loss of fruits (Salunkhe and Desai, 1984; FAO, 1989). Loss of membrane integrity determines the extent of tissue damage and post-harvest loss of stored fruits (Mitra, 1997). This could have caused the high loss of passion fruits treated at 10 and 15 dph. With these fruits, high incidence of tissue breakdown resulted from increased fungal growth, which in turn lowered fruit vitality and keeping quality (Biale, 1948). Thus, a high percentage of fruits treated at 10 and 15 dph were infected by the time fruit loss was assessed.

### 4.8.3. Main effect of $GA_3$

$GA_3$ levels exhibited significant differences, while influencing fruit loss across the four times of $GA_3$ application (Table 17, $P \leq 0.05$). However, fruit loss of control fruits was similar to that of fruits treated with 50,100, and 250 ppm $GA_3$, across the various times of $GA_3$ application, except at the 0 dph (Table 17). This behaviour probably resulted because of the condition of fruits at the time of $GA_3$ application. At 0 dph the inoculated spores of *P. expansum* had not started growing by the time $GA_3$ was applied, which then prevented fruit loss. Fruits were also green and fresh enough to resist fungal germination and sporulation (Knee, 1973; Faust and Shear, 1972).

However, as fruits stayed longer before $GA_3$ was applied, ripening initiated and the fungus started growing in the infection courts. This growth increased spore population in the wounds, leading to a faster rate of infection of the intact fruits, hence the increase in fruit loss with increasing timing of $GA_3$ application.

### 4.8.3. Effect of the interaction between timing of $GA_3$ application and $GA_3$ level

There was a significant effect of interaction between timing of $GA_3$ application and $GA_3$ level on fruit loss (Table 17, $P \leq 0.05$). Fruits receiving 250 ppm $GA_3$ at 0 dph had the lowest fruit loss of 18.77%. Those receiving 100 and 50 ppm $GA_3$, with mean losses of 20.67% and 25.76%, respectively followed them. The 100-ppm $GA_3$ applied at 5 dph was superior to 0, 50, and 250 ppm $GA_3$ (Table 17, $P \leq 0.05$). Fruits treated with 250 and 50 ppm $GA_3$ at 10 and 15 dph had mean losses of 44.51 and 59.25%, respectively (Table 17, $P \leq 0.05$). The low fruit loss observed in fruits treated at 0 dph could have resulted from increased $GA_3$ absorption and activity. Exhaustion of fruit food reserves, which accelerates physiological deterioration, death, and decay, probably resulted slowly in fruits treated at 0 dph (Salunkhe and Desai, 1984; FAO, 1989; Mitra, 1997).

Ultimately, treating passion fruits at 0 dph with 250 ppm $GA_3$ was more effective than the other treatments. However, the treatment was not significantly more effective than 50 and 100 ppm $GA_3$ applied at the same time. This made the three $GA_3$ levels at 0 dph equally effective in controlling post-harvest fruit loss.

## 4.9. Ethylene Evolution

### 4.9.1. Main effect of timing of $GA_3$ application

There was a significant main effect of timing of $GA_3$ application on ethylene evolution across the 14 days post-treatment (Table 18, $P \leq 0.05$). Ethylene exhibited an increasing trend in fruits treated at 0 and 5 dph, an increasing then a declining trend in fruits treated at 10 dph, and a declining trend in fruits treated at 15 dph across the 14 days post-treatment (Table 18). These trends agreed with Akamine et al. (1957), who reported an increase, followed by a decrease in internal ethylene, as guiana fruits ripened and senesced. Fruits treated at 10 and 15 dph had low production rates (slopes) of 17 and 38, relative to high rates of 86 and 127 exhibited by the fruits treated at 0 and 5 dph, respectively (Fig. 3). Fruits treated at 0 dph had a low *P. expansum* activity, besides the effects of $GA_3$ and the anti-fungal substances. As a result, the ripening process progressed relatively slowly, attaining a climacteric peak at the 12$^{th}$ day post-treatment (Table 18). This happened unlike in fruits treated at 5 dph, where the initial concentration of ethylene was high, to an extent of initiating ripening and fungal growth. Fungal and fruit respiration increased the upsurge in ethylene, to a level of not attaining a climacteric peak within the experimental period of 14 days. The rapidly declining rate of ethylene evolution in fruits treated at 15 dph showed that once the ripening process had initiated ethylene production decreased, probably due to senescence and reduced fungal activity as respiratory substrates diminished (Farkas, 1978; Agrios, 1988).

Table 18. Effect of timing of $GA_3$ application and $GA_3$ concentration on ethylene evolution (µl/500 g of passion fruits)

| Days post-treatment (dpt) | $GA_3$ (ppm) | Timing of $GA_3$ application (dph)[z] | | | |
|---|---|---|---|---|---|
| | | 0 | 5 | 10 | 15 |
| 2 | 0 | 19e | 69e | 356a | 263cd |
| | 50 | 20e | 76e | 378a | 242d |
| | 100 | 15e | 50e | 318abc | 328ab |
| | 250 | 14e | 50e | 331ab | 276bcd |
| 4 | 0 | 0f | 113e | 390ab | 246d |
| | 50 | 0f | 79e | 407a | 271d |
| | 100 | 0f | 94e | 344bc | 350abc |
| | 250 | 0f | 73e | 375ab | 294cd |
| 6 | 0 | 92g | 247ef | 403abc | 241fd |
| | 50 | 84g | 407abc | 462a | 320cde |
| | 100 | 132g | 295def | 369abc | 369bcd |
| | 250 | 130g | 371bcd | 467ab | 321def |
| 8 | 0 | 132de | 507ab | 451b | 225c |
| | 50 | 104e | 573a | 505ab | 289c |
| | 100 | 203cd | 418b | 436b | 294c |
| | 250 | 219c | 497ab | 476b | 285c |
| 10 | 0 | 251fg | 644a | 510bcd | 278g |
| | 50 | 240fg | 646a | 548ab | 211ge |
| | 100 | 319ef | 440cd | 495bcd | 202ge |
| | 250 | 410de | 618a | 577bc | 298g |
| 12 | 0 | 285de | 618ab | 475de | 102f |
| | 50 | 239e | 687a | 464de | 131f |
| | 100 | 512cd | 540bcd | 449de | 129f |
| | 250 | 590abc | 679a | 530bcd | 111f |
| 14 | 0 | 377de | 821ab | 415de | 68f |
| | 50 | 306e | 902a | 415cde | 88f |
| | 100 | 522c | 708bd | 405cde | 68f |
| | 250 | 486cde | 767b | 398b | 79f |

[z]Means followed by the same letter within each sampling period are not significantly different at $P \leq 0.05$, according to the DMRT. Least significant difference is 59, 56, 80, 83, 95, 94 and 116 for 2, 4, 6, 8, 10, 12 and 14 dpt, respectively.

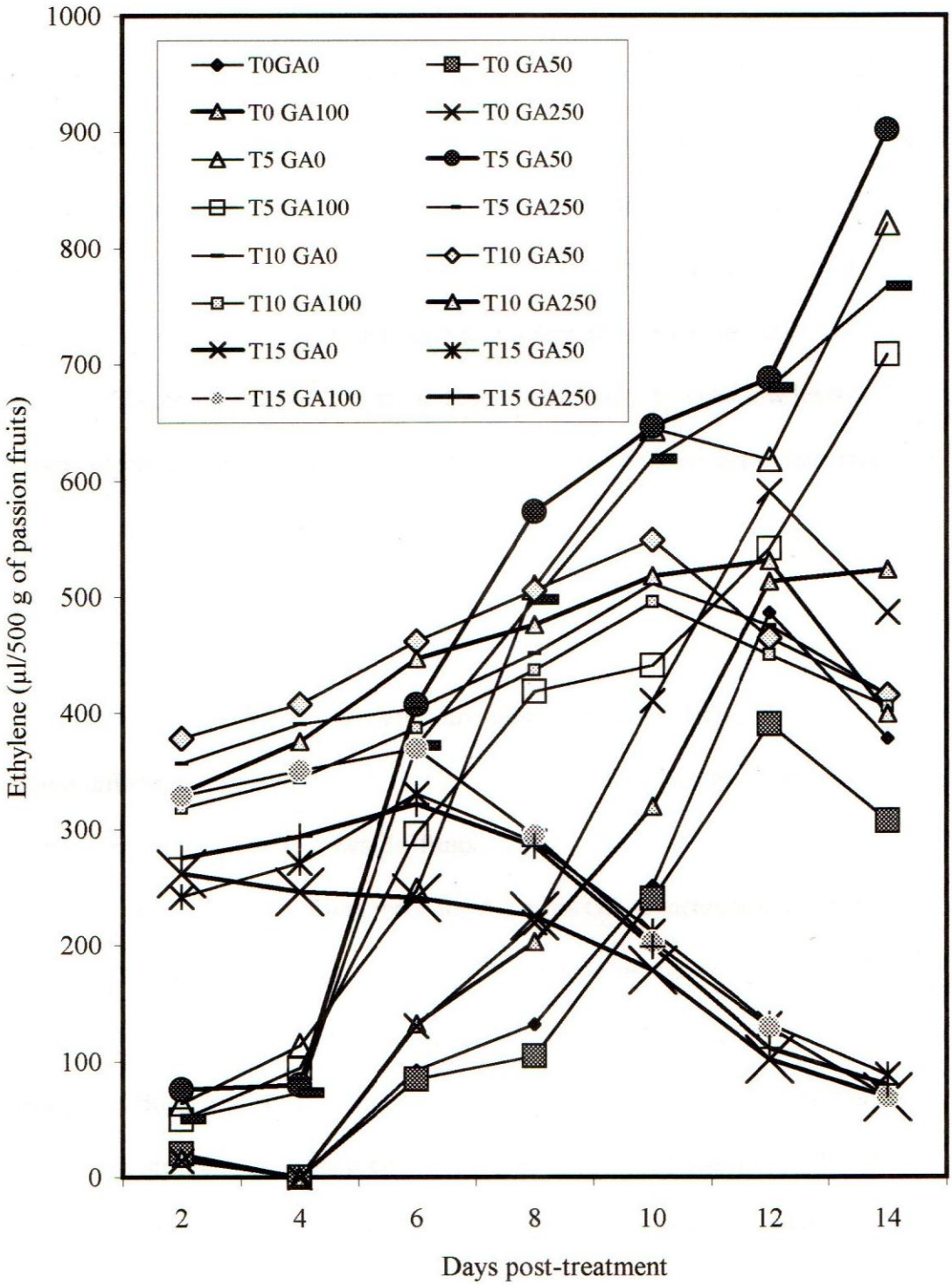



Figure 3. Effect of interaction between time of GA3 application and GA3 level on ethylene evolution by passion fruits. Slope and R-squared value for T0GA0 through T15GA250 are: 78.67, 0.85; 64.05, 0.85; 97.42, 0.90; 102.73, 0.90; 131.4, 0.95; 140.5, 0.94; 107.5, 0.96; 128.9, 0.95; 16.12, 0.43; 11.06, 0.16; 20.69, 0.53; 20.69, 0.56; - 33.3, 0.89; - 30.7, 0.58; - 49.6, 0.84; - 38.5, 0.75, respectively. T refers to time and GA refers to GA3.

The significant main effect of $GA_3$ on fruits treated at 0 dph started appearing at the eighth day post-treatment (Table 18, $P \leq 0.05$). This could be attributed to the fact that immediately after spraying, it took some time for $GA_3$ to alter the respiratory behaviour of tissues through its action on enzymes. Also, the fresh fruits had juvenile factors, both in their pulp and rind, such that before they could decrease to a minimal level, all $GA_3$ levels behaved the same. Fruits treated at 5 dph had low levels of juvenile factors; therefore, the effect of $GA_3$ depended on how fast it penetrated the shriveling rind tissues to retard ethylene evolution.

### 4.9.2. Main effect of $GA_3$

Ethylene production was significantly affected by $GA_3$ (Table 18, $P \leq 0.05$). Slight differences were observed among the $GA_3$ levels, which had almost similar slopes. From the sixth day onwards, fruits receiving 50 ppm $GA_3$ at 0 dph produced the lowest ethylene, while fruits receiving the same $GA_3$ concentration at 5 dph produced the highest ethylene (Table 18, $P \leq 0.05$). This showed that at 0 dph, $GA_3$ effectively retarded ripening through its antagonism to ethylene evolution and activity (Burg and Burg, 1965). However, at 5 dph, the same $GA_3$ concentration was not effective in stopping ethylene production. At this time, autocatalytic ethylene had already initiated ripening and $GA_3$ could not reverse the process. It is known that ripening is a sequential phase in the life of a fruit; once it has been initiated it proceeds

in a predetermined pattern (Kader, 1992). Thus, late application of $GA_3$ had minimal or no effect in stopping ripening.

As time progressed, a slight or no significant variation existed among effects of the $GA_3$ levels in each timing of $GA_3$ application. This was clearly evident in fruits treated at 15 dph, especially from the 8$^{th}$ day post-treatment, when fruits treated with $GA_3$ had no significant difference in the ethylene evolved (Table 18). Probably, internal ethylene had reached high levels such that $GA_3$ exhibited minimal effects.

The gradients observed among $GA_3$ levels indicated slight variations in $GA_3$ effects (Table 18, Fig. 3). The steep slope of 48 showed that 0 ppm $GA_3$ was ineffective in suppressing ethylene production compared to 50 and 100 ppm $GA_3$, whose fruits had production rates of 46 and 44, respectively. Ethylene level increased with time in storage, although at sometimes the control fruits produced less ethylene than the other $GA_3$-treated fruits. Probably late in storage, increased cell membrane permeability occurred as a result of the infused $GA_3$ (Coggins et al., 1992; Ben-Arie et al., 1996), causing leakage of solutes, which in turn favoured growth of *P. expansum*. An increase in respiration of the fruit tissues then ensued, resulting in increased oxidative enzymes and ethylene evolution (Farkas, 1978).

### 4.9.3. Effect of the interaction between timing of $GA_3$ application and $GA_3$ level

A significant interaction effect on ethylene evolution was observed from the 2nd to the 14$^{th}$ day post-treatment (Table 18, $P \leq 0.05$). Fruits treated at 0 dph had no

significant difference in ethylene produced at 2, 4, and 6 days post-treatment, while fruits treated at 15 dph exhibited no significant difference in ethylene evolved from the $6^{th}$ to the $14^{th}$ day post-treatment (Table 18, Fig. 3).

Fruits treated at 0 and 5 dph exhibited rising ethylene levels, while those treated at 10 and 15 dph had gentle slopes (Fig. 3). This result showed that fruits treated at 0 or 5 dph were still initiating the ripening process, while those treated at 10 or 15 dph had already initiated ripening and senescence when $GA_3$ was applied. This observation agreed with that of Kader (1992), who has reported that ethylene evolution increases with increasing respiration rate as ripening progresses. Therefore, $GA_3$ probably reduced the rate of ethylene evolution in fruits treated within 5 dph.

Ethylene had a strong linear relationship with storage time, as depicted by the high $R^2$ values, except for fruits treated at 10 days post-harvest (Fig. 3). The relationship was positive for fruits treated at 0, 5 and 10 dph, but negative for fruits treated at 15 dph (Fig. 3). These results revealed that the nature of the relationship depended on the timing of $GA_3$ application.

Therefore, ethylene level depended on timing of $GA_3$ application, level of $GA_3$ and the day of sampling. Application of $GA_3$ at 0 dph was better than application at 5 dph because fruits decayed slowly and exhibited prolonged quality. The low level of ethylene for fruits treated at 10 or 15 dph is not useful, because it was caused by total fruit senescence.

## 4.10. Pathogenic Fungi

The following pathogenic fungi were isolated from passion fruits: *P. expansum* (1), *Fusarium oxysporium* (2), *Cladosporium* sp. (3), *Alternaria passiflorae* (4), *Aspergillus flavus* (5), *Septoria* sp. (6), and *Rhizopus stolonifer* (7) (Table 19).

The percentage of fruits attacked in the first trial were significantly different across the four times of isolation for *Penicillium expansum*, and *Fusarium oxysporium*, while the other fungal species were similar in their isolation percentages across time (Table 19, $P \leq 0.05$). In the second trial, significant differences were observed in all the isolated fungi (Table 19, $P \leq 0.05$).

In the first trial, *Cladosporium* sp., *Aspergillus flavus*, *Septoria* sp. and *Rhizopus stolonifer* were isolated from fewer fruits, whereas *Penicillium expansum*, *Fusarium oxysporium*, and *Alternaria passiflorae* were isolated from more fruits (Table 19). In the second trial, *Rhizopus stolonifer* and *Cladosporium* sp. appeared on more fruits than in the first trial, when isolated at 10 and 15 dph (Table 19). This probably resulted due to either the two farms where fruits for the two trials were harvested, or the increasing inoculum density of these fungi as the experiment progressed from the first to the second trial.

In both trials, fresh fruits were more effective in suppressing germination and growth of post-harvest pathogenic fungi, especially *P. expansum*, *Fusarium oxysporium*, and *Alternaria passiflorae* (Table 19, $P \leq 0.05$). The three fungi had low

Table 19. Effect of time on passion fruits (%) infected by various fungi

| | First trial fungal species[z] | | | | | | |
|---|---|---|---|---|---|---|---|
| Time (dph) | 1 | 2 | 3 | 4 | 5 | 6 | Mean[y] |
| 0 | 12.92c | 12.92a | 12.92 | 12.92 | 12.92 | 12.92 | 12.92 |
| 5 | 43.08b | 26.24ab | 12.92 | 22.02 | 17.47 | 17.47 | 23.20 |
| 10 | 77.08a | 35.01ab | 17.47 | 30.46 | 12.92 | 17.47 | 31.74 |
| 15 | 77.08a | 38.86a | 17.47 | 26.24 | 12.92 | 12.92 | 30.92 |
| LSD[x] | 6.66 | 21.93 | NS | NS | NS | NS | |
| %CV | 6.34 | 38.85 | 39.61 | 46.20 | 28.03 | 39.61 | |

| | Second trial fungal species | | | | | |
|---|---|---|---|---|---|---|
| Time (dph) | 1 | 2 | 3 | 4 | 7 | Mean |
| 0 | 22.02b | 17.47b | 12.92c | 12.92b | 12.92b | 15.65 |
| 5 | 30.79b | 30.79b | 12.92c | 17.47b | 17.47b | 21.89 |
| 10 | 67.98a | 54.99a | 26.24b | 59.21a | 59.21a | 53.53 |
| 15 | 77.08a | 59.21a | 46.92a | 77.08a | 59.54a | 63.97 |
| LSD[x] | 14.78 | 15.08 | 12.88 | 12.39 | 14.48 | |
| %CV | 14.96 | 18.59 | 26.05 | 14.89 | 19.45 | |

[z]1: *P. expansum*, 2: *Fusarium oxysporium*, 3: *Cladosporium sp.*, 4: *Alternaria passiflorae* , 5: *Aspergillus flavus*, 6: *Septoria sp.* and 7: *Rhizopus stolonifer*.

[y]Means followed by the same letter or no letter within each column are not significantly different at $P \leq 0.05$, according to the DMRT. Data were angular-transformed for analysis.

[x]Least significant difference. NS = not significant. CV = Correlation of variation.

incidences when isolated at 0 dph, compared to their incidences when isolated at 10 and 15 dph (Table 19). The 0 and 5 dph fruits exhibited low incidences of the three post-harvest fungi, probably because of the higher resistance at this stage to fungal penetration and attack and/or to expression of the already existing fungal spores (Agrios, 1988). This occurred probably due to the freshness of the fruit rind tissues, and the presence of anti-fungal substances such as anthocyanins and tannins (Macheix et al., 1990). Isolation at 10 and 15 dph revealed increased fungal attack, probably due to loss of rind-tissues structural integrity, through physiological deterioration, and increased vulnerability to invading microbes (FAO, 1989).

In the first trial, *Penicillium expansum* and *Fusarium oxysporium* were more prevalent at 10 and 15 dph, because they were isolated from 77.08 % and 38.86 % of the total number of passion fruits, respectively (Table 19). These fungi were followed by *Alternaria passiflorae*, which had a low rate of infection of 30.4 %. However, the other fungal species, especially *Aspergillus flavus* and *Septoria* sp. had low infection incidences, which indicated their low prevalence. Limited spores in the atmosphere and/or limited affinity of these fungi to attack passion fruits could have brought about this low prevalence.

In the second trial, *P. expansum*, and *Alternaria passiflorae* were isolated from more fruits (67.98% and 59.21 %, respectively) at 10 and 15 dph. *Cladosporium* sp., *Fusarium oxysporium*, and *Rhizopus stolonifer* with isolation percentages of 26.24%, 54.99%, and 59.21 %, respectively followed these fungi (Table 19).

*Penicillium expansum* had the highest incidence probably due to its prevalence as a post-harvest fungus in harvested fruits (Agrios, 1988). *Fusarium oxysporium* and *A. passiflorae*, being pre-harvest fungi, followed *P. expansum* in prevalence, thereby revealing that their inoculum on the fruits from the field was high and ready to germinate the moment the fruits were harvested (Snowdon, 1984). This result showed that despite the prevalence of *P. expansum* in attacking harvested passion fruits, *F. oxysporium* spores, which land on fruits pre-harvest, have the capacity to germinate and sporulate rapidly the moment fruits initiate ripening and senescence.

In the second trial, *R. stolonifer* and *Cladosporium* sp. became prevalent only after fruits had been stored for some time (Table 19). This was evident from their low incidences when isolated at 0 and 5 dph (Table 19). Their increasing incidences, although delayed, could also be attributed to their relative aggressiveness, fast growth and sporulation (Jones and Aldwinkle, 1990).

The fruits attacked by the isolated fungi increased with increasing isolation timing (Table 19). This could be attributed to the breakdown of polymeric carbohydrates, especially pectin substances and hemicelluloses, leading to a change in rind texture (Knee, 1973; Brady, 1987). These researchers attributed the vulnerability to invading or existing microbes to the softening rind tissues.

# CHAPTER FIVE

# CONCLUSIONS AND RECOMMENDATIONS

## 5.1. Main effect of timing of $GA_3$ application

The main effect of timing of $GA_3$ application in maintaining post-harvest quality of passion fruits was significant for all variables assessed. Treating fruits with $GA_3$ at 0 and 5 dph was more effective than treating at 10 and 15 dph in retarding processes such as chlorophyll degradation, rind softening, weight loss, colour change, incidence and/or severity of disease, all of which are indicative of senescence. These timings of treatment helped maintain the more acceptable post-harvest qualities.

Application of $GA_3$ within a short span of time after harvest in combination with use of polythene bags for packaging helped maintain fruits for a longer time before full ripening and subsequent senescence followed. Thus, traders can sell their fruits for a longer time, while at the same time consumers can get a longer time to consume the fruits. However, treating fruits at 10 and 15 dph might only increase the costs of preservation through the purchase of $GA_3$, but not improve shelf life.

Due to lack of significant differences among $GA_3$ levels for some of the vital post-harvest quality attributes, timing of $GA_3$ application should be emphasized. $GA_3$ application at 0 dph proved to be most effective, although it did not differ significantly from application at 5 dph. This result implied that farmers and traders could treat passion fruits within 5 dph and still realize the benefits of $GA_3$. The result also

suggests applying $GA_3$ starting in the field followed by after harvest to determine whether $GA_3$ acts better in actively growing fruits than in mature fruits.

### 5.2. Main effect of $GA_3$

Chlorophyll degradation, rind firmness, total soluble solids, colour change, yields loss, disease incidence, and ethylene production were affected significantly by $GA_3$. Chlorophyll degradation, rind firmness, total soluble solids and purple colour development were significantly affected by $GA_3$ in some days post-treatment, whereas disease incidence, fruit loss and ethylene production were affected significantly in most days post-treatment. The main effect of $GA_3$ on disease severity and weight loss was not significant. However, slight variations appeared in rates of change over time for these variables.

The 50 and 250 ppm $GA_3$ proved to be the most effective levels in retarding chlorophyll loss, purple colour development, disease incidence, fruit loss, and ethylene production. However, for other variables 0 and/or 100 ppm $GA_3$ were more effective than the 50 and 250 ppm $GA_3$. Therefore, the most effective level to prolong post-harvest quality of passion fruits depends on the variable.

The lack of a significant difference among some of the $GA_3$ levels tested, in influencing some post-harvest quality variables, suggests testing higher levels of $GA_3$, probably above 500 ppm. Application method (spraying or dipping) also needs to be researched to determine the best method for delivering $GA_3$ so as to retard senescent

processes. Dipping duration in $GA_3$ solution also needs to be researched to determine whether it has any effect on the performance of $GA_3$.

### 5.3. Effects of interaction between timing of $GA_3$ application and $GA_3$ level

The two factors frequently interacted significantly, while influencing disease incidence, colour change, and ethylene production, whereas while influencing chlorophyll degradation and disease severity, a significant interaction resulted at only two or three days of the experimental period. No significant interaction was observed for soluble solids, rind firmness, and weight loss.

$GA_3$ applied at 0 and 5 dph proved to be more effective than $GA_3$ applied at 10 and 15 dph. The 50 and 250 ppm $GA_3$ applied at 0 and 5 dph were more effective in maintaining post-harvest quality, although at some days post-treatment the 0 and 100 ppm $GA_3$ performed better than the 50 and 250 ppm $GA_3$. Therefore, higher levels of $GA_3$ above 250 ppm need to be evaluated within five days after harvesting fruits to establish the best treatment combination in maintaining post-harvest quality.

### 5.4. Pathogenic fungi

Immediately after harvest, fruits were resistant to attack by the pathogenic fungi. Upon attack by the pathogenic fungi, the severity increased with time of storage. *Penicillium expansum* was relatively more prevalent post-harvest, because the percentage of fruits it attacked at 0 dph was lower than that at 5, 10, and 15 dph. The

increase in prevalence of *F. oxysporium* and *A. passiflorae* with isolation timing revealed their ability of becoming more aggressive as fruits ripen and senesce.

Relatively less prevalent fungi such as *Alternaria passiflorae*, *Fusarium oxysporium*, and *Septoria* sp. should be controlled pre-harvest either by use of chemicals or cultural methods to avoid their effects upon harvest. Prevalence of fungi such as *Cladosporium* sp., *Penicillium expansum,* and *Rhizopus stolonifer* revealed the need to effectively control them immediately after harvest. Therefore, use of pre-harvest and post-harvest fungicides and growth regulators is recommended for the control of losses resulting from these fungi. The increase in percentage of fruits attacked by the pathogenic fungi as fruits were kept from 0 to 15 days before isolation justifies the need to apply the control chemicals either at harvest or before harvesting. In addition, the freshness of the rind tissues that increase effectiveness of most chemicals justifies application at the pre-harvest stage.

The results of pathogen isolation at different times revealed that several fungi attack passion fruits pre-harvest and post-harvest. This fact makes it difficult to know which pathogens are or are not controlled by preservation treatments that may be administered to harvested fruits. This result then justifies the need to surface-sterilize and re-inoculate with a known pathogen or pathogens, so that conclusions can be made precisely about the pathogen that is or is not controlled by applied preservation treatments. This result also showed that cleaning passion fruits before storage can help reduce pathogenic inoculum and prolong quality.

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