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THE ROLE OF ETHYLENE IN KIWIFRUIT SOFTENING

HYUN OK KIM

1999

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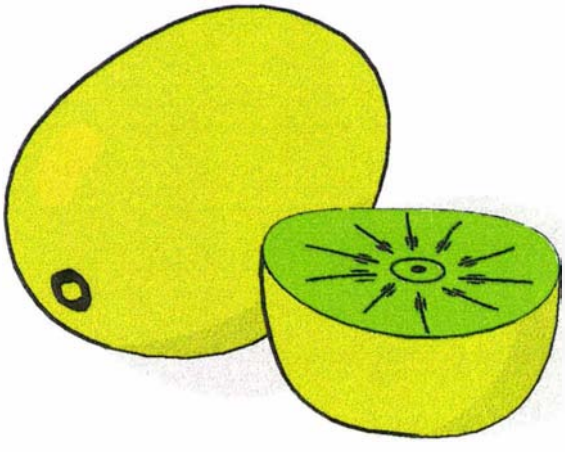

A thesis presented in partial fulfilment

of the requirements for the degree of

Doctor of Philosophy in

Plant Science

at

Massey University

Hyun Ok Kim

1999

ABSTRACT

Premature fruit softening during storage at 0°C is a serious and costly problem for the New Zealand kiwifruit industry. Ethylene gas (C_2H_4) is a potent promoter of fruit softening; it is involved in regulation of fruit ripening and influences a number of processes, including ethylene production, respiration rate and fruit softening. Exogenous ethylene increases softening rate in kiwifruit at 20°C and at 0°C; a concentration of 0.01µl/l will enhance softening at 0°C. The precise relationship between kiwifruit softening and endogenous ethylene concentration is not known. The influences of low temperature, an ethylene synthesis inhibitor (aminovinylglycine (AVG)), an inhibitor of ethylene action (1-methylcyclopropene (1-MCP)) and application of ethylene at different maturities were investigated in an attempt to elucidate ethylene's role in initiating kiwifruit softening.

Exposing fruit to 0°C for more than 52 days hastened ethylene production upon removal to 20°C compared with fruit maintained at 20°C continuously after harvest; this enhanced ethylene was associated with increased 1-aminocyclopropane-1-carboxylic acid (ACC) concentration and ACC oxidase (ACO) activity.

Kiwifruit softening at 0°C occurred even though ethylene production was low and constant between 0.02 to 0.06µl/kg/h (corresponding to internal ethylene concentrations (IEC) 0.2 to 0.6µl/l). This softening was associated with low ACC concentrations varying between 0.2 to 0.5nmole/g and ACO activity varying between 0.01 to 0.66nl/g/h. These results indicate that very low concentrations of ethylene may play an essential role in kiwifruit softening.

In kiwifruit treated with AVG (500ppm) 4 weeks before harvest, ethylene biosynthesis, manifested as reduced ACC concentration, ACO activity and ethylene production, was significantly inhibited both at 20°C and after storage at 0°C. AVG application resulted in a slower softening rate and firmer fruit than in untreated controls. Rates of softening

were 0.4N/day and 1.9N/day in the AVG treated fruit and control fruit respectively during 14 days at 20°C. However, the AVG effect was reduced after storage at 0°C for 14 days, indicating that the AVG effect was only temporary and may not be sufficient to warrant possible commercial use for longer storage.
Application of 250ppm AVG 2 or 4 weeks before harvest, and 500ppm AVG 2 weeks before harvest had no effect on ethylene production or fruit firmness.

Fruit infected with *B. cinerea* produced more ethylene than non-infected fruit at 0°, 4°, 10° and 20°C. An increase in ethylene production, induced by *B. cinerea* infection, occurred in tissue slices from the invasion zone (the infection front containing both infected tissue and sound tissue immediately ahead of the infection front) and adjacent zone (sound tissue ahead of the invasion zone) of kiwifruit. The increased ethylene in infected fruit was associated with increased ACO activity in tissue from adjacent and distal (sound tissue at the distal end of the fruit) zones.

Application of 500ppm AVG to kiwifruit vines before harvest reduced ACC concentration and ACO activity and ethylene production induced by *B. cinerea* at 20°C. However this AVG reduced ethylene production from *B. cinerea* infected fruit after 4 weeks at 0°C was insufficient to prevent rapid growth of *B. cinerea*. Therefore, such an AVG treatment can not be used to reduce *B. cinerea* infection during storage at 0°C.

As kiwifruit matured, softening was enhanced increasingly by exogenous ethylene, indicating that tissue sensitivity to this growth regulator increased with time. The sensitivity of kiwifruit was reduced by treatment with 1-MCP, an inhibitor of ethylene action. When applied to kiwifruit at harvest, 1-MCP reduced ethylene production and respiration rate, resulting in a slower fruit softening and firmer fruit during storage at both 0°C and 20°C. Kiwifruit treated with 1-MCP plus ethylene at harvest, remained firmer than fruit exposed to ethylene alone for 4 days at 20°C and 8 days at 0°C, after which this 1-MCP effect disappeared with firmness being the same for both ethylene plus 1-MCP and ethylene treated fruit at the two temperatures. When both 1-MCP plus

ethylene were applied after storage at 0°C, 1-MCP negated the ethylene-induced softening with treated fruit having a softening rate 4 times less than for fruit treated with ethylene alone. Softening rate of control and 1-MCP treated fruit was the same as those treated with 1-MCP plus ethylene (approximately 0.06 ('Kiwifirm'unit)/day) compared with 0.2 ('Kiwifirm'unit)/day for fruit treated with ethylene alone. Since 1-MCP binds to the ethylene receptor sites irreversibly, it is suggested that kiwifruit can synthesis new ethylene receptors with time during storage at 0°C and 20°C, making kiwifruit increasingly sensitive to endogenous ethylene.

Over several different experiments in 3 seasons, kiwifruit softened from ≈ 90N to 10~19N while endogenous ethylene production remained low (below 0.1~0.2 μl/kg/h) and constant at 20°C. Increased ethylene production only occurred as fruit softened from 10N~19N to eating ripe (6~8N).

These results have led to a model being proposed for ethylene action during ripening of kiwifruit. Kiwifruit softening (phases 1 and 2) occurs even though ethylene production is low as is ACS and ACO activity. It is possible that this basal level of ethylene corresponds to System 1 ethylene production which is thought be associated with basal metabolic maintenance; this ethylene probably binds to System 1 ethylene receptors. The changing ability of fruit to soften with increased maturity is due to increasing sensitivity to such low ethylene concentration resulting from the progressive formation of new ethylene receptors in the fruit. Application of AVG and 1-MCP reduced ethylene production, leading to a delay in ethylene-induced softening. It is possible that low endogenous ethylene (System 1 ethylene) is sufficient to induce starch degradation and solubilization of pectins in cell walls caused by hydrolase enzymes such as amylase, β-galactosidase and xyloglucan endotransglycosylase early in ripening. Alternatively oligomers derived from cell wall breakdown may induce ethylene production even though such oligomer elicitors have not been reported to exist in kiwifruit. Maximal cell wall swelling, depolymerization of the solubilized pectin by polygalacturonase and breakdown

of the middle lamella only occur when kiwifruit already have softened to <20N (phase 3 of softening), and these events are associated with or co-ordinated by System 2 autocatalytic ethylene. This autocatalytic ethylene is associated with high ACS and ACO activity and may bind to System 2 ethylene receptors, leading to ethylene dependent responses such as PG activation which results in ready-to eat fruit with a firmness of 6~8N.

In conclusion, kiwifruit softening from 90N to 10~19N occurs with low and constant ethylene production. Because new ethylene receptors of kiwifruit can be formed with time at both 0°C and 20°C, it appears that kiwifruit sensitivity to low ethylene concentration also increases with time in storage. It is possible that different cultivars of kiwifruit with different softening rates, may have different amounts or rates of formation of new ethylene receptors. By comparing physiological, biochemical and molecular attributes of Hayward and other kiwifruit cultivars and selections, it should be possible to provide information on their responsiveness and sensitivity to ethylene. This will allow plant breeders to create new cultivars that have both low ethylene production and low sensitivity to ethylene that would provide a range of commercial cultivars with prolonged and different storage lives for the international kiwifruit market.

Application of inhibitors of ethylene biosynthesis (AVG) and ethylene action (1-MCP) reduced ethylene production and softening rate of kiwifruit with 1-MCP being more effective for longer than AVG. Although 1-MCP showed promise as a tool to delay softening during storage at 0°C and shelf life at 20°C, further research is required to determine optimum concentration, time and frequency of application, and efficiency when applied at 0°C in order to derive treatments that may have significant commercial applications.

Keywords: *Actinidia deliciosa*, kiwifruit, softening, maturity, ethylene, ethylene receptors, sensitivity, low temperature, aminoethoxyvinylglycine (AVG), 1-methylcyclopropene (1-MCP), *Botrytis cinerea*.

ACKNOWLEDGEMENTS

I am very glad to fulfil my PhD thesis. Thanks a lot to many people !

Especially, my supervisor, Prof. Errol Hewett who gave me many chances to expand my ideas and advised and encouraged me throughout this thesis. His continuous and patient 'English Checking' is deeply and greatly appreciated.

I would like to thank Nagin Lallu at HortResearch Mt. Albert Research Centre, Auckland for valuable advice throughout my PhD studies. Special thanks to David R. Dilley from Michigan State University in USA who provided the chemical 1-MCP, and a constant source of advice and encouragement during his time in our laboratory.

I appreciate the encouragement I received from Professors at Seoul National University in South Korea, including Lee Seung Koo, Kim Ki Sun, Lee Byung Il, Ko Kwang Chul, Kim Byung Dong who visited New Zealand during my PhD.

I wish to thank to other staff in the Institute of Natural Resources at Massey University; Pamela Howell, Dr Peter Long, Stephen Lawes and Bruce MacKay for information about experiment; Lorraine Davis, Chris Rawlingson and Hugh Neilson for support in the laboratory; Matt Alexander for assistant and advice in preparing poster presentations; Ray Johnston for provided work environment at Plant Growth Unit; Mr. Shane Max who supported my field activities at the Fruit Crops Unit; Peter Jeffery and Sue Nicholson for the time spent helping solve problems that occurred during gas measurement in Postharvest research laboratory.

Special thanks to the Doctoral Scholarship from Massey University, Turners and Growers Research Grant and Johannes August Anderson PhD Scholarship for providing living expenses throughout all years. Thanks are also extended to Zespri International, Nufarm Ltd., Auckland and Massey University Research Fund for providing a research grant.

I extend my gratitude to Roberto who spent 2 years with me collaborating on the AVG experiment, and to the other colleagues including Ivan, Anna, Cassandro, Danilo for help during my time in PhD. Special thanks to Irma who give me much love and encouragement.

I always feel appreciation and love to Tae Hoon Kim who encouraged me and let me know life; he gave me many ideas and labour through all times. I appreciate the love from all my family including Tae Hoon's parents, my parents, Min Ju Kim (my youngest brother) and my other sisters and brothers in South Korea.

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