1-MCP as a tool to protect broccoli from ethylene exposure in the supply chain

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Abstract

In New Zealand, the annual broccoli (*Brassica oleracea* L. var. *italic* Plenck) harvest is approximately 30,000 tonnes, sourced from 2,080 hectares since 2014, with retail prices ranging from $3.57 to $13.95 per kilogram over that period (Figure NZ Trust, 2025). However, as a highly perishable vegetable, broccoli has a limited shelf life. Broccoli shelf life is primarily limited by yellowing, which is accelerated by higher than optimal temperatures (e.g., > 4 °C) and ethylene exposure. Ethylene contamination in the supply chain can hence promote broccoli senescence and induce yellowing. A potential solution to reduce the risk of yellowing as a result of exposure to ethylene is to provide a protective application of 1-MCP. Previously, postharvest treatment with 1-MCP has been shown to reduce weight loss and maintain chlorophyll content and h° values of broccoli at suboptimal storage temperatures.

In this thesis, a preharvest spray of 1-MCP was evaluated for efficacy in preserving the postharvest quality of 'Nobel' and 'Iron' broccoli during storage at 1 °C and 4 °C under air and ethylene exposure conditions through two trials: In Trial 1, 1-MCP was sprayed four days prior to harvest with the control being untreated. During storage at 1 °C for 29 days, 'Nobel' broccoli heads from both 1-MCP treated and control blocks were exposed to either a continuous flow of 1 $\mu L \cdot L^{-1}$ ethylene or clean air (< 0.001 $nL \cdot L^{-1}$ ethylene) in a flow-through system. After storage, all broccoli heads, including those from untreated field blocks exposed to ethylene during storage, remained a marketable green colour. These results possibly suggest that the storage duration was too short or storage temperature was too low to observe any potential differences. Therefore, in a second trial, the benefits of a preharvest 1-MCP application were tested in the context of postharvest storage temperature not being optimal (e.g., 1 °C) and more typical for a mixed vegetables supply chain or home storage (4 °C). In Trial 2, 1-MCP was sprayed three days before harvest and two storage temperatures for 'Iron' broccoli were employed: storage at 1 °C for 28 days and 4 °C for 18 days. As in Trial 1, broccoli heads stored at 1 °C stayed a marketable green colour. For those heads exposed to ethylene during storage at 4 °C, 1-MCP treatment in field was observed to significantly lower weight loss, and result in greener appearance (measured as h° closer to 110°), demonstrating a benefit of 1-MCP treatment in maintaining the quality of broccoli under ethylene exposure conditions. These findings suggested that preharvest 1-MCP application could be a potential tool to protect broccoli from ethylene exposure in the supply chain.

Keywords

Preharvest, yellowing, storage, postharvest quality, respiration rate, ethylene production, weight loss, firmness

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List of Abbreviations

1-MCP	1-methylcyclopropene
ACC	1-aminocyclopropane-1- carboxylic acid
ACO	1-aminocyclopropane-1-carboxylic acid oxidase
ACS	1-aminocyclopropane-1-carboxylic acid synthase
AVG	aminoethoxyvinylglycine
ANOVA	analysis of variance
CA	controlled atmosphere
CAT	catalyser
C*	chroma
C_2H_4	ethylene
CCP	mitochondrial electron transport with cytochrome pathway
Chlase	chlorophyllase
CIE lab colour space	International Commission on Illumination lab colour space
CO_2	carbon dioxide
CTR1	CONSTITUTIVE TRIPLE RESPONSE1
EILs	ETHYLENE INSENSITIVE 3 LIKEs
EIN3	ETHYLENE INSENSITIVE 3
EMS	environmental monitoring systems
ER	endoplasmic reticulum
ERF1	ETHTLENE RESPONSE FACTOR 1
ES	expansion stage
ETR1	ETHYLENE RESPONSE 1
FM	floral meristem
FS	formation stage
FSSI	Foodstuffs South Island
FSNI	Foodstuffs North Island
GLM	general linear model
GLs	glucosinolates

h°	hue angle
HMP	hexose monophosphate pathway
HSD	honest significant difference
IM	inflorescence meristem
L*	lightness from black to white in CIELab colour space
LDPE	low density polyethylene
LOX	lipoxygenase
K	potassium
MAP	modified atmosphere packaging
MDA	malondialdehyde
MS	maturation stage
N_2	dinitrogen
N	nitrogen
NO	nitric oxide
O_2	oxygen
P	phosphorus
PEREs	primary ethylene response elements
PG	polygalacturonase
PHGA	portable ethylene postharvest gas analyser
POD	peroxidase
PPO	polyphenol oxidase
RAN1	RESISTANT TO ANTAGONIST1
RH	relative humidity (%)
SAM	shoot apical meristem
SD	standard deviation
SDA	sensory descriptive analysis
SEM	scanning electron microscope
TCA	tricarboxylic-acid-cycle
TCR	temperature control room
VC	valve controller
WL	weight loss

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Chapter 1. Introduction

1-MCP has been a useful tool for horticultural products since commoditisation, especially for fruits and cut flowers. For vegetables, initial works have mainly focused on broccoli due to its sensitivity to ethylene and high values. Ethylene exposure accelerated the rate of senescence, particularly yellowing and decay, in postharvest broccoli.

1-MCP use on vegetables has received relatively less research and commercial attention than that on fruits and cut flowers. Three reasons were mentioned by Thompson and Catwell (2011): 1) Most vegetables are consumed quickly after harvest rather than being stored for extended life. Ethylene is not always treated as the determining factor affecting postharvest vegetable quality because the effects of ethylene do not have enough time to develop before the customers consume the vegetables. 2) Vegetable products, like leafy green vegetables, are not chilling sensitive and thus can be stored at 0 °C. In this circumstance, even relatively high ethylene concentrations have a negligible impact on vegetable quality. 3) Many vegetables have relatively low value and thus receive little postharvest treatment.

Despite 0 °C being optimal, broccoli commonly faces temperature and ethylene stresses in the supply chain. Broccoli is not stored separately, but in mixed loads with other products. If broccoli is mixed with high ethylene producers, a high ethylene concentration would be accumulated in its storage atmosphere. When broccoli is in a mixed load with other chilling sensitive products, the storage temperature must be set up at ≥ 5 °C, resulting in temperature stress to the broccoli (Thompson & Catwell, 2011). Under both circumstances, postharvest broccoli deteriorates rapidly and thus 1-MCP could be a promising tool in practice.

To the author's knowledge, no published paper has looked at the preharvest application of 1-MCP on broccoli. One possible advantage of preharvest application is improved uniformity at maturity; this reduces harvest times for broccoli and thus labour costs for harvesting. Another advantage is that broccoli might grow bigger heads without over-maturing, and this would benefit growers with higher yields and income. This study is therefore designed to evaluate the preharvest application of 1-MCP on the postharvest storage performance of 'Nobel' and 'Iron' broccoli through two trials.

In Trial 1, 1-MCP was sprayed four days prior to harvest to evaluate its impact on the quality of 'Nobel' broccoli during storage at 1 °C for 29 days under clean air and ethylene conditions. At-harvest quality results showed that broccoli heads had an average diameter of 127 ± 10 mm, weighed 334 ± 51 g, and exhibited colour values of 123.4 ± 3.1 hue angle (h°),

36.6 ± 1.1 for lightness (L*) and 9.4 ± 1.4 for chroma (C*). And stalk compression stress was measured at an average of 0.06 ± 0.02 $N \cdot mm^{-2}$, and head floret flexibility was 1.68 ± 0.46 N. The broccoli diameter in block 1 was significant smaller than that of block 2, indicating less maturity. The average respiration rate of central branchlets was 1645 ± 203 $nmol \cdot kg^{-1} \cdot s^{-1}$ and the ethylene production was increased and then decreased during 3 days at 20 °C.

After storage, all broccoli heads, including those from untreated field blocks exposed to ethylene during storage, remained a marketable green colour – average 120.0 ± 2.5 for h°, 37.1 ± 1.8 for L* and 10.9 ± 1.9 for C*, indicating that broccoli still in phase 1 of colour change (h° ≥ 110° and C* ≤ 20) (Vasconcelos & Almeida, 2003). Broccoli had an average weight loss of 3.3 ± 0.4%. Stalk compression stress averaged 0.05 ± 0.02 $N \cdot mm^{-2}$, head floret flexibility averaged 1.70 ± 0.53 N and head firmness averaged 1.35 ± 0.38 $N \cdot mm^{-1}$. These results possibly suggest that the storage duration was too short or storage temperature was too low to observe any potential differences. Cultivars, planting seasons and at-harvest maturity may also contribute to these results. Therefore, in a second trial, the effects of a preharvest 1-MCP application were tested on the summer broccoli 'Iron' in terms of postharvest storage temperature not being optimal (e.g., 1 °C) and more typical for a mixed vegetables supply chain or home storage (4 °C).

In Trial 2, 1-MCP was sprayed three days prior to harvest to evaluate its impact on the quality of 'Iron' broccoli during storage at 1 °C for 28 days and at 4 °C for 18 days under clean air and ethylene conditions. The result showed that application of 1-MCP three days before harvest did not affect the growth of broccoli heads, as indicated by both diameter and surface area. At harvest, broccoli heads had an average diameter of 129 ± 14 mm, weight 347 ± 66 g, and exhibited colour values of 115.8 ± 3.0 (h°), 41.6 ± 1.3 for L* and 8.5 ± 1.3 for C*. And head firmness was measured at an average of 2.24 ± 0.49 $N \cdot mm^{-1}$. Both respiration rate and ethylene production of central branchlets were decreased and then slightly stabilised at 60–70% of the initial rate during 11 days at 5.5 °C.

As in Trial 1, broccoli heads stored at 1.9 °C stayed a marketable green colour – average weight loss of 2.8 ± 0.9%, and exhibited colour values of 114.8 ± 2.5 for h°, 41.6 ± 1.2 for L* and 10.3 ± 1.2 for C* and head firmness averaged 1.77 ± 0.57 $N \cdot mm^{-1}$, indicating that all broccoli heads were still in phase 1 of colour change, as hypothesised by Vasconcelos and Almeida (2003). After being stored at 6.7 °C for 18 days, most of the broccoli heads were no longer marketable due to yellowing, especially for those heads exposed to ethylene. Compared

to the at-harvest value, the L* and C* increased (by 10.3% and 81.2%, respectively) while the h° value decreased by 13.2%. For those heads exposed to ethylene, 1-MCP treatment in the field significantly reduced weight loss, and maintained a higher h°, especially in block 1 (less mature heads). These results demonstrated a benefit of 1-MCP treatment in maintaining the quality of broccoli under ethylene exposure and non-optimal temperature conditions.

In summary, all broccoli stayed marketable green when stored at a temperature close to the optimal temperature (0 °C), even for the heads from field control and exposed to ethylene. When stored at non-optimal temperatures and exposed to ethylene, preharvest application of 1-MCP resulted in a marketable green colour, especially for less mature heads. These findings suggested that preharvest 1-MCP application could be a potential tool to protect broccoli from ethylene exposure in the supply chain, especially at non-optimal temperature conditions.

1.1 Objectives

This study builds on previous studies that primarily focused on the postharvest application of 1-MCP by investigating its impacts on the broccoli quality during storage under ethylene exposure. This study repeated the continuous 1.0 $\mu L \cdot L^{-1}$ ethylene exposure reported by Fan and Mattheis (2000). Additionally, preharvest application of 1-MCP is effective in broccoli, with good quality results reported in both California and Western Australia; however, work has not yet been done in New Zealand (M. Punter, personal communication, August 2, 2024). Thus, this study investigated the impacts of preharvest application of 1-MCP on the harvest quality and postharvest quality of 'Nobel' and 'Iron' broccoli through two experiments in two trials (Figure 2.12):

1. Field experiment: to investigate the effect of preharvest 1-MCP application on the growth and quality of broccoli at harvest.

2. Storage experiment: to evaluate the effect of preharvest application of 1-MCP on preserving the postharvest quality of broccoli during low-temperature storage under ethylene exposure.

This thesis begins with a brief introduction to both experiments and thesis. Chapter 2 identifies research gaps in the literature. The materials and methods are covered in Chapter 3. Next, the impacts of preharvest application of 1-MCP on 'Nobel' broccoli at 1 °C are researched and discussed in Chapter 4. The impacts of preharvest application of 1-MCP on 'Iron' broccoli when stored at 1 and 4 °C are discussed in Chapter 5. An overall discussion, conclusion, and recommendations are found in Chapter 6.

Chapter 2. Literature Review

This literature review briefly introduces broccoli, focusing on the development and growth of the broccoli head. It then examines the preharvest factors critical to the commercial production of broccoli, including cultivars and postharvest physiology. Next, the discussion will shift to the at-harvest quality (head and stalk) of broccoli. The thesis will then explore postharvest techniques, such as temperature, relative humidity (RH), and ethylene management, as essential factors in maintaining broccoli quality. The thesis will analyse how postharvest quality is influenced by at-harvest quality and the postharvest techniques applied. Specifically, the use of 1-MCP as a tool to manage ethylene exposure will be addressed. Finally, the research opportunities are identified.

2.1 Broccoli

The genus *Brassica* covers many vegetables, such as broccoli, cauliflower, cabbage, turnip, and mustard. These are grown for their edible inflorescence, leaves, fleshy stems, roots, and the oils extracted from the seeds (Buck, 1956). Broccoli (also known as broccoli heads) is a plant derived from *Brassica oleracea* L. var. *italic* Plenck. It is thought to have originated in the eastern Mediterranean, and been introduced into Italy, where significant diversification occurred, and then grown in Europe and America (Gray, 1982; Latté et al., 2011).

Gary (1982) mentioned that the history and evolution of broccoli (*Brassica oleracea* L. var. *italic* Plenck) are considered in relation to cauliflower (*Brassica oleracea* L. var. *botrytis* L), particularly in the development of both annual and biennial types. Broccoli can be classified into two main categories: coloured-heading types and sprouting types. Pinera (1995) noted that the structure of the broccoli head is morphologically similar to that of cauliflower. However, broccoli develops a head with long, slender floret stalks that bear fertile flower buds (Figure 2.1). In contrast, cauliflower forms a single, compact white curd, with its fertile flower buds typically emerging only after the usual harvest stage.

Ilahy et al. (2020) point out that broccoli typically develops an upright, branching and strong green stalk and grows to a height of 60 to 90 cm. Its rosette basal leaves are leathery, oblong, and grey-blue green. Additionally, the surfaces of these leaves are coated with an epicuticular wax layer, making them hydrophobic (Song et al., 2021). Broccoli features thick, green-coloured edible clusters of flower buds (known as florets) at the tips of the central axis and the branches (known as stems). These florets eventually bloom into yellow flowers,

producing silique fruits if left in the field. The broccoli head (inflorescence) is the commonly consumed part, including the florets and stems (Figure 2.1).

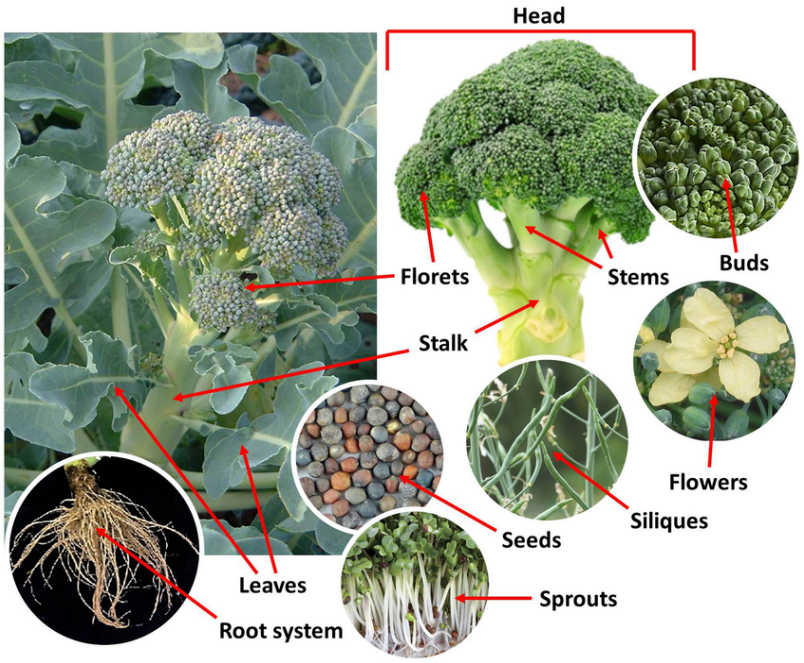

Figure 2.1 Schematic illustration of the different parts of broccoli. Image taken from Ilahy et al. (2020) with permission.

2.1.1 Plant development and growth of broccoli

Tallei et al. (2024) identified five stages in the broccoli plant, from sprouting to flowering (Figure 2.2). For the broccoli head, the two developmental and growth stages are head initiation and head growth (Pinera, 1995). Head initiation is the first stage and occurs from transplanting until the onset of head formation. Given the appropriate stimulus, the meristem shifts from a vegetative to a reproductive state during this stage. Head growth is the second stage, which extends from the start of head formation until harvest (Siomos et al., 2022).

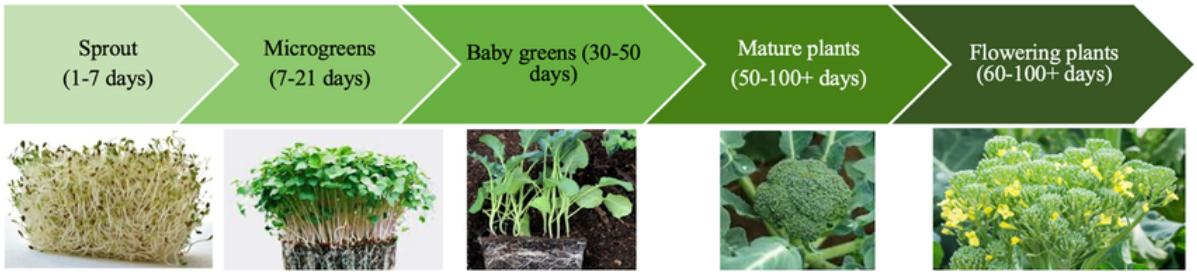

Figure 2.2 Five growing stages of broccoli plant development. Image taken from Tallei et al. (2024) with permission from Oxford University Press.

2.1.1.1 Head initiation

Buck (1956) states that broccoli flowers grow on a faceted floral shoot, terminating the plant's axis. This faceted floral shoot has meristem (shoot apical meristem, SAM) that are physiologically highly active. The inflorescence or "head", described as a corymb or a modified racemose panicle, includes functional floral buds, stems, perfect flowers, and bracts. Zhu et al. (2024) described that formation stage (FS) – SAM differentiated into the inflorescence meristem (IM), primarily through cell division. The head initiation occurred between 4 and 6 weeks of the plant's development (Grabowska et al., 2013).

Temperature strongly affects the duration of the vegetative period, head initiation, head growth, and harvest time (Pinera, 1995; Kałużewicz et al., 2010). Grabowska et al. (2013) found that chilling at 2 °C of 8 and 10-week-old seedlings negatively impacted the head initiation and quality (Figure 2.3). Temperature significantly explained the differences in dry matter accumulation, leaf production rate, head initiation, maturity time, and head growth rate across various sowing dates (Diputado, 1989). Siomos et al. (2022) highlighted that a minimum of 13 to 31 leaves is needed for head initiation. At this critical stage, roughly half of these leaves are visible to the naked eye.

2.1.1.2 Head growth

After initiation, Zhu et al. (2024) described that broccoli head expansion stage (ES, size/diameter increases rapidly) involved both continued cell division (especially in secondary inflorescence meristems) and cell expansion, contributing to head enlargement and growth.

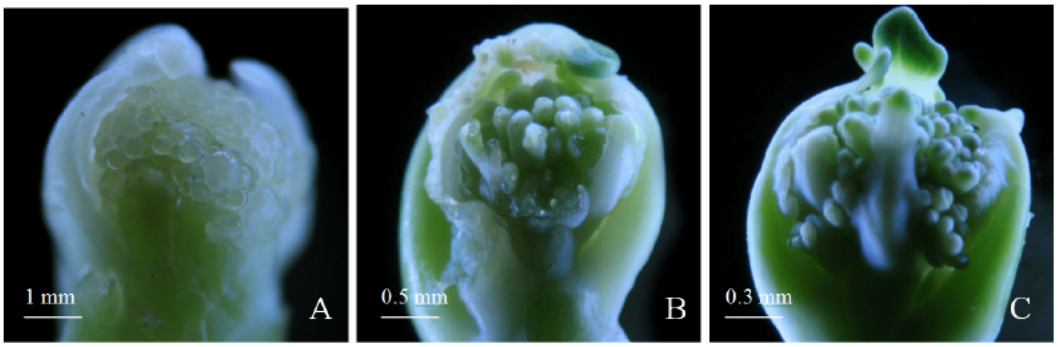

Figure 2.3 Head initiation in 10-week-old seedlings before chilling (A), after 2 weeks of chilling at 2 °C (B), and after two weeks of optimal temperature of 16 °C (C). Image taken from Grabowska et al. (2013) with permission.

Head growth is significantly affected by temperature and accumulated solar radiation, especially when the broccoli is planted at high densities (Grevsen, 1998). Temperature,

including maximum and minimum temperatures, and accumulated degree days (with a base), are commonly used to assess growth (Siomos et al., 2022). Grevsen (1998) recommended a minimum temperature of 0 °C and a maximum of 17 °C for the commercial cultivars 'Emperor', 'Caravel', and 'Shotgun'. A link between the temperature sum from the end of vernalisation and the dry matter fraction allocated to the broccoli head was identified by Lindemann-Zutz et al. (2016b).

2.1.1.3 Ethylene roles in flower initiation, development, and senescence

As a multifunctional plant hormone, reviewed by Iqbal et al. (2017), ethylene affects flower initiation (transition from vegetative to reproductive growth), development, and senescence (Figure 2.4). In *Arabidopsis thaliana* (from the same family – Brassicaceae), after comparing the development of the wild type and ethylene-related mutants (*etr1*, *eto 1*, *ein2-1* and *ein3-1*), Ogawara et al. (2003) concluded that ethylene promoted flowering via a signal transduction pathway: When plants cannot detect or respond to ethylene, they grow more leaves and flower later. Dolan (1997), Iqbal et al. (2017), and Dubois et al. (2018) reviewed the ethylene-induced changes in plants, including organ size, cell expansion, growth rate, and senescence. Ethylene accelerates flower senescence by activating genes for chlorophyll degradation, protein breakdown, and eventually, programmed cell death (Mattoo & Handa, 2004).

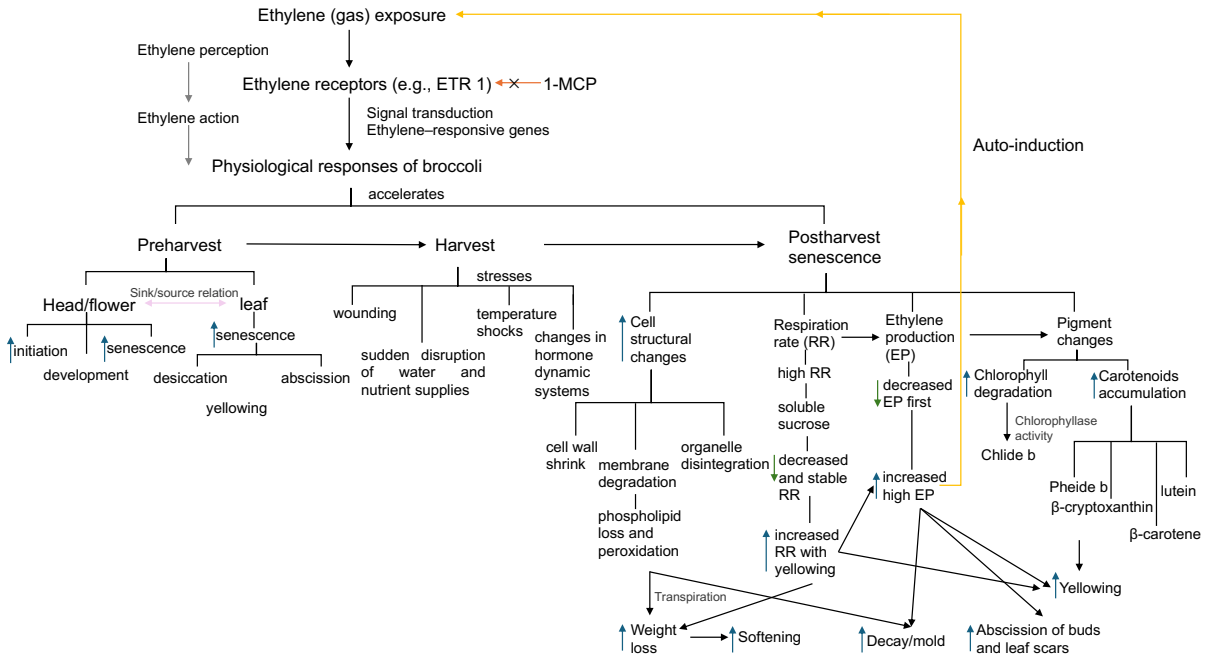

Figure 2.4 Schematic diagram of broccoli's physiological responses to ethylene exposure across preharvest, harvest, and postharvest stages.

Dolan (1997) and Iqbal et al. (2017) stated that ethylene accelerates leaf senescence, leading to leaf desiccation, yellowing, and abscission. The source-sink relations play significant roles in the size of reproductive/sink organs (e.g., flowers). In chrysanthemum flowers, Carvalho et al. (2006) demonstrated that increased source activities or reduced sinks for assimilates potentially increased flower size. Thus, in broccoli, the increased activities of leaves may increase head size.

2.1.2 Commercial production of broccoli

2.1.2.1 Production and seasons

Diputado (1989) emphasised the determination of yield and quality to the success of broccoli commercial production, whether for processing or the fresh market. While overall market returns are primarily driven by crop yield, head quality is equally important as it directly influences the marketability and value of the crop. In New Zealand, the annual broccoli harvest is approximately 30,000 tonnes, from 2,080 hectares, with the average retail price being $7.2 NZ per kg since 2014 (FAO, 2024a; Figure NZ Trust, 2025).

The planting and harvesting of broccoli strongly depend on the local positions and climate conditions. The primary harvest season in the U.S is from mid-October to December, while coastal valleys can produce year-round (Le Strange et al., 2010). In New Zealand, regions like Manawatu and northern areas can grow broccoli year-round, with Otago and Canterbury as prominent southern regions (Horticulture™ New Zealand, 2017; Fresh Facts, 2023). Broccoli is planted via direct seeding or transplanting, typically at 102 thousand plants per hectare. The planting season and density affect head weight, especially during summer for cultivars 'Shogun', 'Greenbelt', and 'Marathon' (Pinera, 1995; Le Strange et al., 2010).

2.1.2.2 Cultivars

Over 100 commercial hybrid cultivars of broccoli have been developed from landraces or open-pollinated varieties, with regional variation reported by Le Strange et al. (2010) and Fahey (2015). Huang et al. (2021) and Siomos et al. (2022) stated that modern F1 hybrids like 'Marathon' and 'Greenbelt' exhibit strong apical dominance and minimal lateral shoot growth. These hybrids are bred for high yield, bioactive content, efficient nutrient use, resistance to diseases and pests, and optimised crop duration. Although broccoli originated in temperate zones, heat-tolerant cultivars like 'Ching-Long 45' have been developed, particularly in Taiwan

(Yang et al., 1998). While F1 genotypes vary in head initiation requirements, these differences remain poorly understood.

Wichrowska et al. (2021) demonstrated that, regarding the quality traits after harvest and during storage, cultivars differed significantly. Cultivars also responded differently to 1-MCP treatment at 4 °C.

2.1.2.3 Climate and soil

Broccoli requires a long-day photoperiod and an extended period of exposure to low temperatures (vernalisation) for proper development because broccoli originated in temperate zones with temperatures ranging from 5 to 25 °C (Lin et al., 2018). Therefore, broccoli has been dramatically impacted by climate conditions. Le Strange et al. (2010) stated that optimal growth occurs when monthly air temperature averages from 16 to 18 °C. Production of broccoli is highly dependent on stable climate conditions. For instance, the March/April 2017 North Island flooding of New Zealand directly impacted crop supply, leading to a nationwide shortage and significantly increased prices, as about 50% of New Zealand's broccoli is grown there (Horticulture™ New Zealand, 2017).

According to Ludong (2008), broccoli typically performs best in well-drained soil. However, it can also be cultivated in various soil types, such as muck, heavy, and light soils, with suitable cultivars. Additionally, broccoli has greater sensitivity to salt than most other common vegetables.

2.1.2.4 Irrigation and fertilisation

Ludong (2008) and Le Strange et al. (2010) stated that broccoli requires plenty of water to maximise quality and yield, especially during head formation. However, overwatering can lead to hollow stems, loose heads, and an increased risk of root diseases. Broccoli is irrigated with sprinklers or surface irrigation like furrows and drip irrigation. Drip irrigation produced a slightly higher marketable yield of primary flowers and improved irrigation production efficiency compared to the micro-sprinkler irrigation method (Himanshu et al., 2013). Drip irrigation can save between 21.2% and 52.7% of water compared to surface irrigation, while allowing for an additional 17.1% to 53.3% increase in the area irrigated (Patra et al., 2022).

The quantity and timing of irrigation are determined by soil type, crop area, weather conditions, local water sources, and maturity stage (Ludong, 2008; Le Strange et al., 2010).

Moreover, water quality is essential for broccoli in terms of food safety (Horticulture™ New Zealand, 2017).

As a highly nutrient-demanding crop, broccoli requires N, P, and K based on soil tests (Le Strange et al., 2010). Organic and inorganic fertilisers impact the growth and yield of broccoli. The optimal yield of broccoli (40 $t \cdot ha^{-1}$), chlorophyll content and head diameter were obtained by combining 60 kg of inorganic fertiliser and 60 tonnes of organic manure per hectare (Ouda & Mahadeen, 2008).

The timing and amount of irrigation impact fertilisation efficiency. Over 130 $kg \cdot ha^{-1}$ N losses were observed in autumn broccoli planted in the alluvial loam, when broccoli was frequently irrigated at a threshold value of 75% available soil water. Moreover, this irrigation strategy decreased the total mass of broccoli (Gutezeit, 2004). Proper water deficit can improve the efficiency of nitrogen use and help achieve economic efficiency (Erdem et al., 2010). Thus, optimised irrigation and fertilisation determine the optimal yield and quality of broccoli.

2.1.2.5 Pests and diseases

UC Statewide IPM Program (2020) stated that a monitoring-based and year-round integrated pest management (IPM) system was an effective tool to manage pests and diseases of broccoli, minimising the negative influence of pesticides and herbicides on human health and the environment. In New Zealand, IPM broccoli can reduce insecticide usage by at least 50%, compared to traditional crops (Walker et al., 2001).

Berry et al. (2000) and Le Strange et al. (2010) reported common pests like wireworms (*Elateridae*, damaging to seedlings) and cabbage aphid (*Brevicoryne brassicae*, contaminating heads). Diseases affect the immature flower buds, leading to discolouration and rot. The broccoli head is prone to various diseases, including bacterial head rot and fungal diseases. Foliar damage and leaf spots are obvious symptoms of the young seedlings of broccoli affected by downy mildew (caused by *Peronospora parasitica*). Biological control using antagonistic bacteria plays a key role in IPM of broccoli (Nagai et al., 2017).

Brown bud and hollow stem are common physiological disorders. Nutritional imbalances or deficiencies likely cause brown bud, which resembles head rots caused by pathogens (Le Strange et al., 2010). Hollow stem refers to an open area in the stalk at the cut surface, which can discolour and decay (Cantwell & Suslow, 2002).

2.1.2.6 Harvesting and postharvest

Based on the appearance and size of heads, horticultural maturity (also known as commercial maturity) determines timing and frequency for broccoli harvest. In New Zealand, the marketable broccoli heads should have a diameter of at least 110 mm and be tight with un-opened buds based on Food Stuff standards (FSSI, n.d.; FSNI, 2024). Cantwell and Suslow (2002) stated that mature broccoli has well developed and unopen buds and firm heads; immature heads are very firm with tiny buds, while overmature heads are looser with opening buds. Well-developed buds consist of immature flowers enclosed within chlorophyll-rich sepals (Page et al., 2001). Over-maturity, evidenced by buds flowering, causes yellowing and ends commercial marketability. This is a common economic issue for retailers and growers. Therefore, although different to predict, a proper harvest timing ensures broccoli's quality and marketability (Lindemann-Zutz et al., 2016b).

Broccoli heads grow heterogeneously in the field, possibly due to the variability of head initiation, especially for transplanted broccoli (Lindemann-Zutz et al., 2016a). Thus, selective hand-harvesting occurs between three to seven times across two weeks (Figure 2.7). Harvest percentages range from 30% to 50% for the first cut across different seasons. Besides head size, market prices and supply drive the harvest decision (A. Cruz, personal communication, September 17, 2024).

As the standard cooling method for field-packed broccoli, liquid icing is followed by refrigerated storage. Hydro-cooling and forced-air cooling require more precise temperature management during distribution than iced broccoli (Le Strange et al., 2010). However, in New Zealand, without pre-cooling, field-packed broccoli is stored in a storage room within 2 h of harvest (Figure 2.7). Cantwell and Suslow (2002) indicated that holding optimal 0 °C and over 95% RH maintained the shelf life and quality of broccoli postharvest.

At harvest, broccoli heads undergo multiple stresses, including wounding, sudden disruption of water and nutrient supplies, temperature shocks and changes in hormone dynamic systems (Figure 2.4). Downs et al. (1997) divided the postharvest physiology of broccoli into early and late events (Figure 5.20). According to Phan (1987), Deschene et al. (1991), Downs et al. (1997), and Page et al. (2001), harvesting causes mechanical injury (wounding) due to cutting the stalk and leaves, causing rupture of water supply, and consequent loss of turgidity, resulting in biochemical damages: The degradation of membrane lipids (phospholipid loss and peroxidation, as indicated by MDA and LOX activity) and proteins. Consequently, broccoli

cannot lower its temperatures via transpiration, and less active superficial cells are vulnerable to pathogens. The atmosphere of intercellular spaces shifts from low O_2/high CO_2 to high O_2/low CO_2, facilitating respiration processes. Simultaneously, internal ethylene escapes through the wound, but ethylene concentration increases again because of the rapid respiration rate. Soluble sucrose concentration dropped to < 50% of the at-harvest concentration within 6 h of harvest due to extremely high respiration rate. For example, at 20 °C, 1894–2525 nmol·kg^{-1}·s^{-1} (300–400 mg CO_2 kg^{-1}·h^{-1}) for 'Shogun' broccoli from King and Morris (1994) (Table 2.3). Broccoli heads are sensitive to this mechanical injury, as stated by Zsom et al. (2020), leading to high mass loss – by water loss through transpiration, the loss of respired CO_2 and volatile compounds (e.g., ethylene) – and thus causing softening/wilting, decay and mould development.

Page et al. (2001) stated that postharvest deterioration process of broccoli head is similar to natural leaf senescence: chlorophyll degradation, loss in protein and membranes lipid and eventually programmed cell death. Further physiological changes occur as broccoli nears the end of shelf life – sepals yellowing, mainly caused by rapid degradation of chlorophyll and reduced integrity of thylakoid membranes, especially storage at ≥ 10 °C (Deschene et al., 1991; Gómez-Lobato et al., 2014; Reyes Jara et al., 2021). Although broccoli is classified as a non-climacteric vegetable by some authors (Li et al., 2017; Grzegorzewska et al., 2023), some studies have described its postharvest physiology as climacteric-like due to respiration rate and ethylene production (Makhlouf et al., 1989; Tian et al., 1994; Ma et al., 2009). The climacteric status of broccoli was confirmed by Tian et al. (1994) with 0.5% propylene. Exogenous ethylene stimulated ethylene production and respiration rate and accelerated yellowing, resulting in a 16% greater decline in h°. Paul et al. (2012) also categorised broccoli into climacteric groups, showing auto-induction of ethylene biosynthesis (Figure 2.4). The next section will detail the yellowing processes and causes.

2.1.3 Quality attributes of broccoli

Le Strange et al. (2010) noted that desirable broccoli features small, uniform blue-green to green flower buds, tight dome-shaped heads, and elevated growth for easy harvesting. Undesirable traits include hollow stems, rot, discolouration, uneven buds, and excessive bracts.

2.1.3.1 Sensory quality

Jacobsson et al. (2004b) evaluated broccoli quality based on individual attributes, including colour, texture, and off-odour development. Sensory descriptive analysis (SDA) is

less frequently used, with all sensory traits (smell, taste, texture, flavour, and appearance) to preserve market and eating quality. Paradis et al. (1996) used a rating scale (1-5) to comprehensively evaluate the appearance of broccoli, including colour, turgor, and decay. Head size, evenness, and compactness also contributed to the broccoli's appearance.

2.1.3.1.1 Head colour

Colour determines consumer acceptability of horticulture produce and the perception of overall appearance and sweetness (Lu, 2020). Visual colour is the primary quality indicator in broccoli, while yellowing during storage is undesirable and causes commercial quality loss.

Cai et al. (2019), Luo et al. (2019), and Zhu et al. (2025) evaluated the reasons for buds yellowing (without flowering), including ethylene alteration, pigment shifts (from chlorophyll to carotenoids), structural and cellular changes, signalling and molecular pathways, and protein decline. Phan (1987) noted that chlorophyllase breaks down chlorophyll into chlorophyllide and phytol. Chlorophyll a/b-binding proteins and chlorophyll-metabolising enzymes combine to affect the yellowing of broccoli. Changes in protein phosphorylation, such as with the transcription factor like ethylene insensitive protein 2 (EIN2), may indirectly affect this process. Cell wall loosening reduces barriers and enhances defence responses, inhibiting DNA replication and mRNA synthesis, and contributing to the degradation of transcripts, collectively contributing to broccoli heads' yellowing (Zhu et al., 2025).

Besides the above factors, buds flowering and bacterial infections also contribute to the yellowing of broccoli. Buds will turn yellow from green as they transition from unopened to fully opened. When storing broccoli without external ethylene, Wichrowska et al. (2021) noted that flowering/blooming was one of the causes of yellowing. Yellowing occurred from the bottom/base to the top of chlorophyll-rich sepals (Clarke et al., 1994; Cai et al., 2019; Luo et al., 2019). Once the head becomes loose, the faded sepals will become visible, further contributing to the yellowing. Bacterial infection, especially by *Pseudomonas* spp. (Cui et al., 2022), promoted the yellowing, possibly because the flowers exhibit a stress response to infection and produce ethylene.

According to Lu (2020), CIELab colour space is usually used to objectively evaluate broccoli's colour change. The L* value of the colour space defines lightness, ranging from 0 (black/darkness) to 100 (white/light) and the chroma (C*) value means colour from grey to pure. Ku and Wills (1999) stated that the hue angle (h°) value was calculated using below

Equation 2.1 (where a is the red/green and b is the yellow/blue colour coordinates), with a value of 180° indicating totally green and 90° indicating totally yellow.

$$h^{\circ} = tan^{-1}(b/a) \qquad \textit{Equation 2.1}$$

Vasconcelos and Almeida (2003) hypothesised two phases of colour change (Figure 2.5): The colour remains unchanged during phase 1 (h° ≥ 110° and C* ≤ 20), which occurs immediately after harvest. Phase 2 follows, during which h° declines while L* and C* increase. Similarly, Ma et al. (2009) also divided the yellowing process into two stages: No visual yellowing during phase 1, while the appearance of yellowing starts in phase 2. Vasconcelos and Almeida (2003) demonstrated that storage temperature affected the duration of phase 1 and the rate of colour change during phase 2: lower temperature extended phase 1 duration and slowed the rate of colour change in phase 2. However, 1-MCP only increased the duration of phase 1, but did not impact the rate of colour change of phase 2. As discussed in 2.1.2.6, given that some studies demonstrated that broccoli head had a climacteric-like pattern, it is plausible to suggest that during phase 1 of colour change, broccoli potentially in system 1 of ethylene production, while during phase 2, it shifts from system 1 to system 2 (Section 2.2.1).

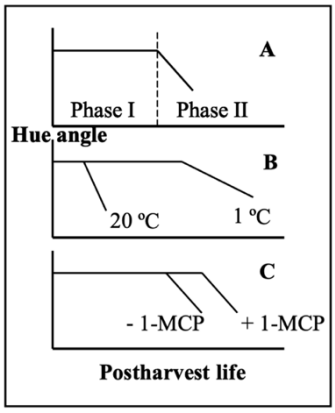

Figure 2.5 Schematic diagram of colour change in postharvest broccoli heads. A: Two phases of colour change: The colour remains unchanged immediately after harvest in phase 1. During phase 2, the h° declines while L and C* increase. B: The effects of temperature on duration of phase 1 and the rate of colour change during phase 2. C: The influence of 1-MCP application on duration of phase 1. Image taken from Vasconcelos and Almeida (2003) with permission.*

There are two principal evaluation methods for the colour quality of postharvest broccoli heads. A 5-point scale is the primary subjective measurement for broccoli yellowing (Table 2.1 and Figure 2.6). Compared to the other studies, Ku and Wills (1999) and Ma et al. (2009) used an opposite rating scale. This study used a 5-point scale from Cantwell and Suslow (2002).

Table 2.1 Example of five point scales for evaluating broccoli yellowing

Scale	Ku and Wills (1999)	Ma et al. (2009)	Rangkadilok et al. (2002); Guirao et al. (2024)
1	> 50% yellowing	> 80% yellowing	Dark green and closed buds
2	50% yellowing	60% yellowing	Trace yellow (10% yellow) and initial opening bud
3	30 % yellow	40% yellowing	Slightly yellow (25% yellow)
4	10 % yellow	20% yellowing	Medium yellow (50% yellow)
5	Green	Green	Completely yellow (100%) and completely open buds

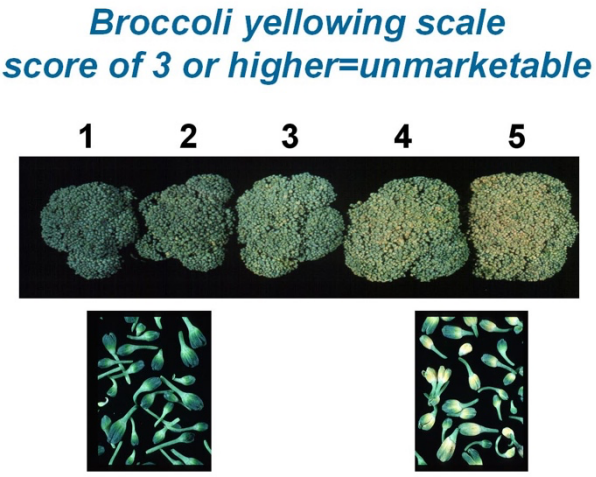

Figure 2.6 Broccoli yellowing scale from Cantwell and Suslow (2002) with permission. Scores 1 and 2 represent marketable broccoli heads with unopen buds, while scores 3, 4 and 5 mean unmarketable broccoli heads with buds opening.

The CIELab values can be used separately or in combination with each other for broccoli colour evaluation. The h° value assesses the colour change during the postharvest shelf life of broccoli heads, with a value of < 110° indicating unacceptable yellowing (Vasconcelos & Almeida, 2003). Able et al. (2002) reported a h° of 116° after 12 $\mu L \cdot L^{-1}$ 1-MCP treated florets and stored at 10 °C for 8 days, compared to untreated florets (108°). C* and h° were used to assess broccoli's yellowing in an ethylene and 1-MCP experiment previously (Fan & Mattheis, 2000). An increase in L* and C* value, or a decrease in h° value (Table 2.3), indicates the yellowing of broccoli, which is associated with reduced chlorophyll content (Fan & Mattheis, 2000; Wu et al., 2019; Lu, 2020).

2.1.3.1.2 Head compactness/firmness

The compactness of broccoli heads was defined as the "denseness of broccoli florets" by Jacobsson et al. (2004b). Guirao et al. (2024) noted that as a vital external quality parameter

of broccoli, firmness is assessed based on compactness. Cantwell (2011) demonstrated that a weight loss of 4% resulted in a 30% decrease in firmness, which is likely the point at which consumers would consider the head soft. Weight loss is one of the significant problems for stored broccoli heads, affecting their marketability (Serrano et al., 2006).

Subjective methods can assess the firmness change during broccoli storage time. A scale from 5 to 0 with 0.5 graduations (where 5 means very firm and 0 represents totally flaccid) was used to assess the firmness (Gillies & Toivonen, 1995). Wichrowska et al. (2021) scored the compactness of broccoli florets using a 5-point scale (Table 2.2). Grzegorzewska et al. (2023) used a 10-point scale to score the compactness of crown.

Table 2.2 Example of five point scales for assessing the compactness of florets

Scale	Wichrowska et al. (2021)
1	Very loose
2	Medium loose
3	Visible loss of compactness
4	Slight loss if compactness
5	Very compact florets

Nowadays, a few studies have used a texture analyser as a non-destructive method to quantify the head firmness change during broccoli storage (Table 2.4). Fernández-León et al. (2013) and Paulsen et al. (2022) did not mention the compression speed, while Guirao et al. (2024) used a compression speed of 20 $ml \cdot min^{-1}$. Paulsen et al. (2022) and Guirao et al. (2024) expressed the result of head firmness in $N \cdot mm^{-1}$ and found similar results. Therefore, this study used the same unit ($N \cdot mm^{-1}$) to express head firmness.

Table 2.3 The CIELab values, respiration rate in CO_2 production, ethylene production, and weight loss of broccoli heads were reported from previous studies. "NA" means "data not available" in the referenced study. Values in the form of "x–y" represent the range from the initial value (x) to the final value at the end of shelf life (y) for broccoli heads. All values provided by the original authors or estimated from figures in the cited publication, units converted to the same units used in the thesis, as needed, and the same with colour scores.

h°/colour score	L*	C*	Respiration rate (CO_2 production) ($nmol \cdot kg^{-1} \cdot s^{-1}$)	Ethylene production ($pmol \cdot kg^{-1} \cdot s^{-1}$)	Weight loss (%)	Treatment and storage	Reference
130–110	NA	NA	6929–4619	1–10	NA	Air + 20 °C for 72h in dark	Tian et al. (1994)
130–95			9238–6929	6–35		0.5% propylene + 20 °C for 72h in dark	
126–90	NA	NA	1894–2525	1–11	NA	'Shogun' head at 20 °C for 100 h	King & Morris (1994)
130–105	NA	NA	758–505	5–7	NA	10 °C for 14 days	Izumi et al. (1996)
130–127	NA	NA	442–284	1–2	NA	5 °C for 14 days	
127–120	NA	NA	NA	NA	NA	≥ 1 $\mu L \cdot L^{-1}$ 1-MCP + 20 °C + 0.1 C_2H_4	Ku and Wills (1999)
123–90			NA	NA	NA	Air + 20 °C + 0.1 C_2H_4	
123–120	NA	13–15	900–1000	NA	NA	12 h 1 $\mu L \cdot L^{-1}$ 1-MCP + C_2H_4 + 10 °C for 18 d	Fan and Mattheis (2000)
123–90		13–26	900–500	NA	NA	1 $\mu L \cdot L^{-1}$ C_2H_4 continuous +10 °C for 18 d	
125–110	35–37	11–19	NA	NA	0.03 d^{-1}	1 °C for 75.2 d	Vasconcelos and Almeida (2003)
	35–45	11–20	NA	NA	0.23 d^{-1}	Untreated + 10 °C for 15.1 d	
	35–41		NA	NA		6 h 2 $\mu L \cdot L^{-1}$ 1-MCP + 10°C for 22.9 d	
	35–45		NA	NA	1.82 d^{-1}	20 °C for 2.6 d	
130–100	NA	NA	1068–1781	12–24	NA	Untreated + 12 °C for 5 d	Forney et al. (2003)
130–110	NA	NA	1068–1543	12–12	NA	14 h 1 $\mu L \cdot L^{-1}$ 1-MCP + 12°C for 5 d	
134–93	NA	NA	NA	NA	NA	Control + 20 °C for 3 d	Gong and Mattheis (2003)
134–113	NA	NA	NA	NA	NA	14 h 1 $\mu L \cdot L^{-1}$ 1-MCP + 20 °C for 3 d	
134–102	NA	NA	NA	NA	NA	1-MCP + 5 h 1000 $\mu L \cdot L^{-1}$ C_2H_4 + 20 °C 3 d	
1–4.5	NA	NA	915–448	0–25	NA	Air/control + 20 °C for 48 h	Ma et al. (2009)
1–1.3	NA	NA	915–379	0–125	NA	12 h 2.5 $\mu L \cdot L^{-1}$ 1-MCP + 20 °C for 48 h	

1–1.5	NA	NA	915–410	0–94	NA	1-MCP+ 12 h 20 μL·L^{-1} C_2H_4 + 20 °C	
114–103	35–40	NA	NA	NA	39.5 ± 4.4	Control + 1–2 °C 85-90% RH for 27 d	Fernández-León et al. (2013)
114–110	35–40	NA	NA	NA	37.6 ± 3.7	24h 0.6 μL·L^{-1} 1-MCP + 1–2 °C for 27 d	
114–112	35–37	NA	NA	NA	5.6 ± 2.1	CA (10% O_2, 5% CO_2) + 1–2 °C for 27 d	
130–106	15–38	NA	NA	NA	NA	Control + 15 °C 95% RH for 5 d	Xu et al. (2013)
130–125	15–33	NA	NA	NA	NA	6 h 2.5 μL·L^{-1} 1-MCP at 20 °C + 15 °C 5 d	
127–112	NA	NA	3298–1221	13.4–7.3	NA	Control + 4 °C for 30 d	Ku et al. (2013)
127–123	NA	NA	3298–1099	13.4–12.2	NA	24 h 0.5 μL·L^{-1} 1-MCP at 20 °C + 4 °C 30 d	
NA	NA	NA	NA	NA	4.4	Control + 4 °C for 30 d	Wichrowska et al. (2021)
NA	NA	NA	NA	NA	3.0	20 h 1.0 μL·L^{-1} 1-MCP + 4 °C for 30 d	
132–116	NA	NA	697–755	0.3–13	0.76	Control + LDPE 0 °C 55% RH for 50 d	Phuong et al. (2022)
132–120	NA	NA	697–643	0.3–10	0.68	15 h 15 °C 1.0 μL·L^{-1} 1-MCP + LDPE 0 °C 55% RH for 50 d	
132–98	NA	NA	697–643	0.3–13	0.55	Control + LDPE 10 °C 55% RH for 20 d	
132–117	NA	NA	697–570	0.3–8	0.44	15 h 15 °C 1.0 μL·L^{-1} 1-MCP + LDPE 10 °C 55% RH for 20 d	
112–101	44–51	NA	1097–925	2–12	NA	Control + LDPE 15 °C 55% RH for 10 d	Phuong et al. (2023)
112–112	44–46	NA	1097–823	2–6	NA	15 h 15 °C 1.0 μL·L^{-1} 1-MCP + LDPE 15 °C 55% RH for 10 d	
NA	NA	NA	611–428	1–6	6.5	Control + 4 °C 90–95% RH for 18 d	Ghimire et al. (2024)
NA	NA	NA	513–305	1–1	6.5	24 h 20°C 1.0 μL·L^{-1} 1-MCP + 4 °C for 18 d	
NA	NA	NA	733–1160	12–6	12	Control + 10 °C 90–95% RH for 18 d	
NA	NA	NA	513–1038	1–6	12	24 h 20°C 1.0 μL·L^{-1} 1-MCP + 10 °C for 18 d	

Table 2.4 A texture analyser used to measure the head firmness of broccoli.

Reference	Instrument	Probe	Deformation	Method description	Result expression	Result
Fernández-León et al. (2013)	TA-XT2i texture analyser	100 mm aluminium plate	2%	Compression test on whole heads	Maximum force (N)	54.41 ± 1.88 N (0 d); after stored at 1–2 °C and 85–90% RH for 27 d: 1.37 ± 0.10 N (Control); 17.10 ± 1.64 N (CA: 10% O_2, 5% CO_2); 0.89 ± 0.12 N (1-MCP)
Paulsen et al. (2022)	TA.XT plus texture analyser	100 mm compaction plate (P/100)	5%	Two measurements per head at 90° rotation to capture data from two axes	Maximum force/ displacement ($N \cdot mm^{-1}$)	2.75 $N \cdot mm^{-1}$ (0 d); after stored at 2 °C for 28 d: 0.25 $N \cdot mm^{-1}$ (no film); 0.75 $N \cdot mm^{-1}$ (Nature Fresh®); 1.5 $N \cdot mm^{-1}$ (Low density polyethylene)
Guirao et al. (2024)	TX-XT2i texture analyser	100 mm flat steel probe (P/100)	5%	Lateral placement; probe moved at 20 $mm \cdot min^{-1}$ until 5% deformation was achieved	Force/displacement ($N \cdot mm^{-1}$)	3.1 $N \cdot mm^{-1}$ (0 d), after stored at 2 °C for 7 d: 0.6 $N \cdot mm^{-1}$ (Ethylene); 0.9 $N \cdot mm^{-1}$ (Control); 1.4 $N \cdot mm^{-1}$ (Ethylene scrubber); After stored at 2 °C for 21 d: < 0.1 $N \cdot mm^{-1}$ (Ethylene) 0.3 $N \cdot mm^{-1}$ (Control) 0.5 $N \cdot mm^{-1}$ (Ethylene scrubber)

2.1.3.1.3 Flavour/volatile/aroma

Jones et al. (2006) described that broccoli's flavour is often characterised as mildly bitter, earthy, and vegetal, with a touch of sweetness that becomes more noticeable after cooked. Many phytochemicals (glucosinolates, phenols, and flavonoids) have a bitter taste, creating a challenge for the food industry as it seeks to increase the levels of these compounds in products to enhance their perceived health benefits. Three major glucosinolates (sinigrin, progoitrin, and glucobrassicin) may contribute to its bitter flavour in broccoli.

Fresh broccoli heads have a "smell of fresh newly cut green grass" (Jacobsson et al., 2004b). Flavour and aroma are key indicators of broccoli and should be monitored throughout the shelf life. In broccoli, sulphurous aroma compounds are prominent volatile components. Methanethiol, dimethyl disulfide, and dimethyl trisulfide are the primary volatile sulphur compounds responsible for the strong off-odours produced by broccoli (Chen et al., 2019).

Instrumental analysis, such as gas chromatography (GC) and electronic nose (e-nose), are commonly used methods for determining aroma profiles (Chen et al., 2019; Ezhilan et al., 2019). Sensory analysis requires a trained sensory panel, which involves high costs for training participants, limiting its widespread application (Chen et al., 2019).

2.1.3.1.4 Stalk quality

Stalk quality, such as leaf scars of cutting and texture, are also crucial broccoli quality attributes. Cantwell and Suslow (2002) provides a 5-point scale standard for leaf scars (Table 2.5). The broccoli stalk texture is usually destructively measured by instrumental techniques like a texture analyser and an instron universal testing machine (Jacobsson et al., 2004a; Serrano et al., 2006; Zhao et al., 2022). Zhao et al. (2022) used radial compression tests on stalk samples of 25 mm with a compression displacement of 0, 6.5, 13, and 19.5 mm to measure stress and mechanical properties.

Table 2.5 Five scales for the quality of leaf scar

Score	Description
1	No discolouration
2	Slight discolouration
3	Moderate discolouration
4	Moderately severe discolouration
5	Severe discolouration

2.1.3.2 Nutritional value

Broccoli is also one of the ten essential vegetables in the Kiwi diet in New Zealand (Fresh Facts, 2023). Ilahy et al. (2020) highlighted that broccoli is a rich source of essential minerals (such as calcium, phosphorus, potassium, and sodium) and vitamins. Broccoli is high in dietary fibre and contains a range of health-promoting compounds, including carotenoids like β-carotene and lutein, flavonoids like kaempferol, and glucosinolates (GLs). GLs are the primary health-promoting compounds in broccoli. Regular consumption of GLs has been linked to a lower risk of several types of cancer (including lung and breast cancers), a reduced risk of degenerative diseases, such as Alzheimer's, and a decreased incidence of cardiovascular conditions. Li et al. (2022) stated that consumers favour broccoli for its rich content of fibres, vitamin C, and beneficial nutraceutical compounds. However, health-beneficial compounds concentration declined as the broccoli heads started to deteriorate (Rangkadilok et al., 2002).

2.1.4 Postharvest preservation techniques for broccoli

Except when stored at close to optimal temperature (0 °C) and > 95% RH (Cantwell and Suslow, 2002), broccoli deteriorates and senesces rapidly after harvest due to its high respiration rate (at 20 °C, ~ 4500 $nmol \cdot kg^{-1} \cdot s^{-1}$, 400 $\mu L \cdot g^{-1} \cdot h^{-1}$ from Tian et al. (1994) and ~ 1800 $nmol \cdot kg^{-1} \cdot s^{-1}$, 160 $mL \cdot kg^{-1} \cdot h^{-1}$ from Cantwell and Suslow (2002)) and extreme sensitivity to ethylene, even at a < 0.02 $\mu L \cdot L^{-1}$ concentration (Vasconcelos & Almeida, 2003; Martínez-Romero et al., 2007; Phuong et al., 2022). Although broccoli has a low ethylene production (< 12 $pmol \cdot kg^{-1} \cdot s^{-1}$ at 20 °C, 1 $nL \cdot g^{-1} \cdot h^{-1}$ from Tian et al. (1994)), environmental stresses, such as physical damage, diseases, ethylene exposure, and dehydration, can trigger ethylene production, leading to yellowing and abscission of leaves and florets (Tian et al., 1994; Kato et al., 2002). Therefore, preservation techniques, such as temperature and ethylene management, can maintain broccoli quality and extend shelf life.

The remainder of the section will talk about temperature, RH, storage duration, and atmosphere (CA and MAP), except for ethylene management, as Section 2.3 will focus on the ethylene management for broccoli.

2.1.4.1 Temperature, RH and storage time

Phan (1987) and Jones et al. (2006) noted that placing broccoli immediately after harvest in a temperature of close to 0 °C can slow down the chlorophyll breakdown and the loss of GLs. Cantwell and Suslow (2002) highlighted that maintaining a low temperature is crucial for extending the shelf life of broccoli. Storing at 0 °C can preserve broccoli for 21-28 days, while

10 °C reduces shelf life to about 5 days. Able et al. (2002) extended the shelf life of broccoli to over 50 days at 2 °C from under 3 days at 20 °C – the rate of h° decline (per day) was 12 times greater. Variety influenced the response to temperature: 'Diplomat' showed more sensory changes at higher temperatures, while only the colour of 'Emerald' was negatively affected by temperature increases (Pellegrino et al., 2019). Storage temperature determines the declines in: 1) integrity (structurally and functionally) of chloroplast membranes; 2) membrane fatty acids and phospholipid phosphate, suggesting enzymatically mediated deteriorative reactions in broccoli postharvest (Deschene et al., 1991). Thus, storage at or close to 0 °C extends shelf life and maintains the quality of broccoli.

As storage time passes, negative changes in appearance, taste, flavour, and texture occur. When stored for 2 days or less, there was no significant difference in the visual quality of broccoli whether kept at 5 or 21 °C (Zsom et al., 2020). Lowering storage temperature and selecting a more resilient variety might slow or prevent many changes (Pellegrino et al., 2019).

Optimal RH for broccoli head storage is over 95% RH (Cantwell & Suslow, 2002). Jones et al. (2006) advised an RH of 98–100% to maintain the postharvest quality of broccoli by preserving the weight of broccoli heads, thereby maintaining head compactness.

However, in practice, broccoli is not always stored and transported under optimal temperature and RH conditions. In the Australian supply chain, Ekman (2006, 2010 and 2017) noted that domestic broccoli is typically transported and distributed at ≥ 5 °C, while for exported broccoli the temperature is approximately 0 °C. Temperatures range from 8 °C (refrigerated) to 20 °C (ambient) during retail display. The author did not talk about the timeline of the supply chain and RH. Commercial confidentiality may have caused this omission, which is similar to the situation in New Zealand.

In New Zealand, year-round broccoli is transported domestically within each island. It may take approximately two to four weeks from broccoli production to consumption (Figure 2.7): First, multiple harvests may occur across one to two weeks; then unpackaged broccoli heads are delivered (within 2 h of harvest) and stored at the growers' cool rooms (1 °C with high RH (approximately 100%), and are likely in mixed loads with other vegetables) for one to two weeks. Broccoli is then transported via truck to distribution centres (chiller room 4.5 °C and ambient area 10–15 °C) at one to three weeks. Consumers purchase broccoli (typically, without any packaging) in retail shops (chiller room at 4–5 °C and shelf area at 12–20 °C, ≥ 5 days of shelf life based on the Foodstuffs standard) at two to four weeks. Commonly, consumers

keep broccoli unpackaged and mixed with food in home refrigerators (~ 4 °C) or at an ambient temperature (~ 20 °C) if they do not consume it immediately after purchase (Lu, 2020; A. Cruz, personal communication, September 17, 2024).

Suboptimal and high temperatures are encountered during the transport and retail marketing of broccoli (Jones et al., 2006; Winkler et al., 2007). Broccoli is typically stored and transported in mixed loads with other products. If ethylene producers – ripening fruits, especially tropical ones – are present, then the concentration of ethylene may increase to > 1 $\mu L \cdot L^{-1}$ and the temperature should be ≥ 5 °C. The deterioration of broccoli quality is rapid in these scenarios (Nath et al., 2011). Therefore, multiple temperature and ethylene stresses occur in the supply chain, significantly affecting the postharvest quality of broccoli.

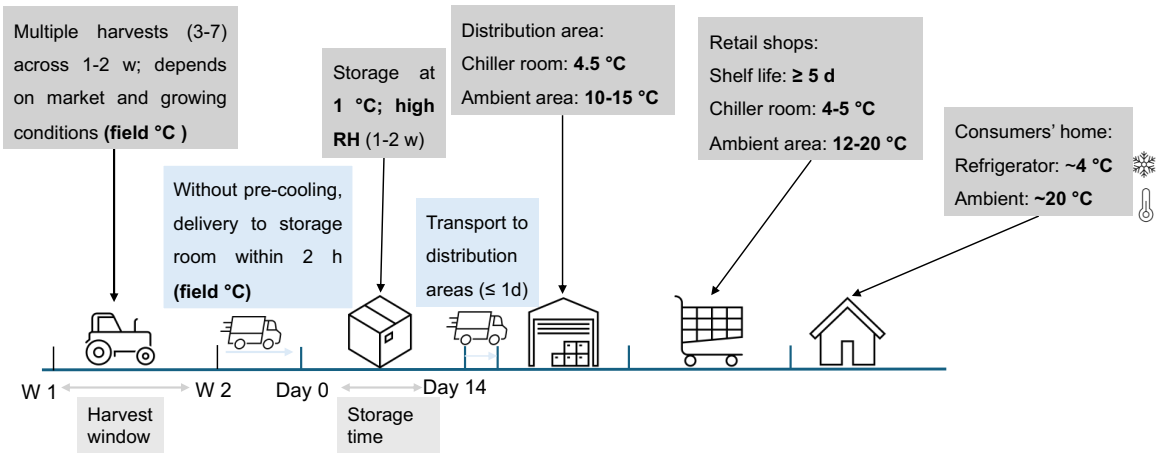

Figure 2.7 Supply chain of broccoli from field to table in New Zealand. Without pre-cooling, year-around broccoli is delivered to farmers' storage room within 2 h after harvest , with temperatures change from field temperature to 1 °C. Broccoli may be transported to the distribution area after storage for 1–2 weeks. It may be stored in the chiller room at the distribution area for 1 week. It is then required that at least 5 days of shelf life for retail shops, which means that broccoli may be purchased after 2–4 weeks.

2.1.4.2 CA storage and MAP

Gross et al. (2016) defined controlled atmosphere (CA) storage as adjusted the atmospheric composition to differ from standard air, which contains about 78% N_2, 21% O_2, and 0.03% CO_2. Oxygen levels are maintained below 8% and CO_2 is kept above 1% in CA.

Modified-atmosphere packaging (MAP) involves sealing produce in polymeric film packages to adjust the levels of O_2 and CO_2 in the enclosed atmosphere (Jones et al., 2006).

Broccoli benefits from being stored in an atmosphere with 1–2% O_2 and 5–10% CO_2 within a temperature range of 0–5 °C (Cantwell & Suslow, 2002). Some uses of CA and MAP

for broccoli are summarised in Table 2.6. High CO_2 and low O_2 reduce the weight loss and respiration rate, maintain the green colour and metabolic substrates for broccoli. Thus, CA and MAP delay the senescence and extend the shelf life. Li et al. (2016) evaluated the respiratory pathway in response to CA, and found inhibited ATP reduction, mitochondrial electron transport with cytochrome pathway (CCP) and tricarboxylic-acid-cycle (TCA) proportion, enhanced hexose monophosphate pathway (HMP) proportion and decreased consumption of metabolic substrates. However, injury and off-odours may occur in MAP and CA storage for broccoli. For example, Kasmire et al. (1974) noted that low O_2 levels and poor air exchange caused shipping odours of broccoli. Therefore, the gas levels should be controlled carefully to keep broccoli fresh without causing off-odours and injury to the broccoli.

2.2 Ethylene

While ethylene plays significant roles during broccoli head initiation, growth, and natural senescence (Section 2.1.1.3), the postharvest effects of ethylene are different – often accelerating deterioration and senescence (Ghimire et al., 2023). This section will focus on ethylene's impacts and management on broccoli during postharvest storage and the supply chain.

2.2.1 Ethylene biosynthesis and action

Wills & Golding (2016) summarised the overview of ethylene biosynthesis pathway and its action (Figure 2.8). Endogenous ethylene is produced from methionine via Yang cycle using S-adenosyl-methionine (SAM, or AdoMet) and 1-aminocyclopropane-1-carboxylic acid (ACC) as intermediates (Adams & Yang, 1979). Bleecker and Kende (2000) and Wills and Golding (2016) highlighted two enzymes in ethylene production: The enzyme ACC synthase (ACS, located in the cytoplasm) converts the SAM to ACC, limiting the rate of ethylene biosynthesis; the enzyme ACC oxidase (ACO) converts ACC to ethylene gas, requiring O_2 due to its liability. Two systems sequentially regulate ethylene production (McMurchie et al., 1972): During growth and development of vegetative tissues and unripe fruit, system 1 regulates the basal level of ethylene production in an auto-inhibitory manner – means that any further ethylene biosynthesis is inhibited by exogenous ethylene; during fruit ripening and floral senescence, system 2 auto-inductively regulates the large amounts of ethylene – means that any further ethylene biosynthesis is stimulated and triggered by exogenous ethylene.

Table 2.6 CA and MAP are used in broccoli

Aspect	CA	MAP
O_2 level	1–2%	3–10%
CO_2 level	5–10%	7–10%
Temperature	Optimal at 0–5 °C	Commonly used at 0–10 °C
Benefits	Reduces respiration rate and loss of bioactive compounds, increases storage life	Reduces weight loss; extends shelf life; slows respiration
Risks	O_2 < 1% may cause off-odours due to sulphur volatiles; requires good air exchange	O_2 < 0.5% and CO_2 > 15% may cause injury and off-odours
Application	Commercial storage and shipping containers (requires sealed systems and monitoring equipment)	Retail and transport packaging (bags or trays with film wrap)
Example studies	Storage at 5 °C in a CA (5% CO_2, 3% O_2, 92% N_2) maintained chlorophyll for 60 days and inhibited membrane lipid degradation for 40 days (Deschene et al., 1991). CA (2% O_2, 6% CO_2) at 4 °C resulted in a longer shelf life and greener colour (although faster stem turgor loss) for broccoli, when compared with these stored in the air (Paradis et al., 1996). 0.5% O_2 and 10% CO_2 at 0 or 5 °C and 1% O_2 and 10% CO_2 at 10 °C storage maintained the colour and supressed rot and browning of broccoli, without developing off-odour (Izumi et al., 1996). 1–2°C, 5% CO_2, 10% O_2 and 85–90% RH preserved broccoli quality and health-promoting compounds (Fernández-León et al., 2013); At 10 °C, 50% O_2 + 50% CO_2 treatment delayed senescence and extended storage life of broccoli from 12 d to 31 d by reducing the respiration rate, inhibiting ATP reduction, energy charge level, CCP and TCA proportion, enhancing HMP proportion and decreasing consumption of metabolic substrates (Li et al., 2016).	Broccoli in MAP had lower weight loss (3% vs. 16.5%) and higher ascorbic acid content (55 vs. 35 $mg \cdot 100g^{-1}$) after 12h at 20 °C and then placed in 0 °C for 7 d (Sabir, 2012). MAP treatment maintained h° (120° vs. 101°), chlorophyll content (0.21 vs. 0.08 $mg \cdot g^{-1}$FW tissue) and glucose (13 vs. 6 $mg \cdot g^{-1}$) in control broccoli, after being stored at 20 °C for 4 d (Hasperué et al., 2014). MAP broccoli had < 5% weight loss vs. 23.49% in controls after 6 d at 10°C (Singh et al., 2018). Broccoli with MAP (5% CO_2 and 10% O_2) had lower weight loss (0.75% vs. 3.36%), lower loss rate of total phenol and intact GLs (20 and 23% vs. 48 and 57%) after being stored for 12 d at 5 °C (Fernández-León et al., 2013a). 2.0% O_2 and 8.2% CO_2 in MAP maintained texture and reduced the loss of green colour in broccoli (He & Xiao, 2018).

At the endoplasmic reticulum (ER) membrane, ethylene is perceived by binding to a family of receptors (such as ETR1, requiring a copper cofactor, delivered by RAN1) in the plant. As a negative regulator of the ethylene response pathway, CTR1 is activated by these receptors. The ethylene signal is transduced to EIN3 via CTR1 and EIN2 (from ER to nucleus). Ethylene-responsive genes are activated by EIN3/EILs (EIN3-LIKEs) binding to primary ethylene response elements (PEREs), eventually leading to physiological responses, such as ripening and abscission. As the immediate target of EIN3, ERF1 acts downstream of all components in the ethylene signaling pathway. See more from the review by Paul et al. (2012) and book by Wills and Golding (2016).

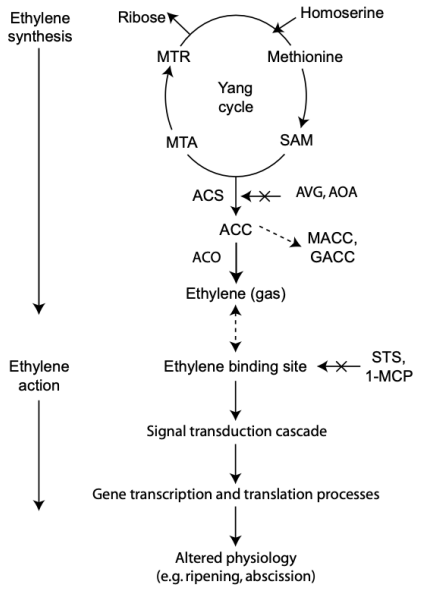

Figure 2.8 Ethylene biosynthesis pathway and its action. Image taken from Wills and Golding (2016) with permission from CABI.

2.2.2 Ethylene sensitivity of broccoli

Botanically, broccoli heads are ethylene-sensitive flowers, with extreme sensitivity to ethylene, even at < 0.02 $\mu L \cdot L^{-1}$ (Serek et al., 2006; Martínez-Romero et al., 2007). Ethylene accelerates yellowing, weight loss, and flowering and reduces head compactness in broccoli (Cefola et al., 2010; Guirao et al., 2024). Tian et al. (1994) reported that ethylene increased broccoli's respiration rate and ethylene production and accelerated the decline of h°. Exposure to ethylene hastens senescence and the decay of broccoli heads (Thompson & Cantwell, 2001; Lu, 2020).

These symptoms are associated with ethylene-accelerated senescence processes, including cell structural changes and protein degradation, respiration rate and ethylene

production changes, pigment changes (shift from chlorophyll to carotenoids) and abscission of buds (Figure 2.4). For example, after broccoli was stored at 10 °C for 8 days, Cai et al. (2019) compared with cells from control sepals, ethylene treatment resulted in shrunk and disordered cells as indicated by SEM images. Ethylene exposure also increased pigment contents, including chlorophyllide b (Chlide b), and several carotenoids, such as lutein, β-carotene, pheophorbide b (Pheide b), and β-cryptoxanthin.

Serek et al. (2006) and Wills and Golding (2016) discussed that the environmental factors determine the horticultural produce's response to ethylene: The sensitivity of produce to ethylene is diminished by increasing the concentration of CO_2, decreasing the concentration of O_2 and storing it at low temperature. The biochemical mechanism of these effects remains largely unknown, as reviewed by Serek et al. (2006). High CO_2 inhibits ethylene effects, likely by altering ACS activity and ethylene perception; Low O_2 suppresses ACO activity and ethylene perception requires O_2. At lower temperatures, ethylene production generally declines, ethylene responses are slowed, and the time required to observe an effect increases. These factors explain the extreme effects of temperature on ethylene sensitivity and imply that, once produce is stored at sufficiently low temperatures, additional ways to block ethylene effects may be unnecessary.

Broccoli is less sensitive to ethylene concentration and less likely to yellow when it is stored at optimal temperature. When the broccoli was exposed to the same ethylene concentration (1.0 $\mu L \cdot L^{-1}$), its shelf life was significantly dependent on the storage temperature. While broccoli could be stored at 0 °C for nearly 40 days, when at 5 °C, its storage life decreased to about 10 days (Li et al., 2017). Thus, the interaction of storage temperature and ethylene concentration determines the sensitivity of broccoli to ethylene.

2.2.3 Ethylene exposure in the supply chain of broccoli

The typical supply chain of broccoli in New Zealand was discussed in Section 2.1.4.1 (Figure 2.7). Broccoli is potentially exposed to an environment with ethylene throughout the entire supply chain. At harvest, ethylene is continuously released from the intercellular space and induced by wounding and other stresses. Ethylene concentration may be accumulated during transportation, storage, distribution, retail display, and in consumers' refrigerators.

Typically, ethylene concentrations in transport vehicles and packages can be 10–100 times higher than those in ambient air, especially when ethylene producers (e.g., apples) were present (Keller et al., 2013; Warton et al., 2000). After ethylene concentration in the whole

supply chain of horticultural products was measured over 700 times, Warton et al. (2000) concluded that industries should diminish ethylene exposure, as even low concentrations (0.015–0.1 $\mu L \cdot L^{-1}$) could result in 10-30% more postharvest losses.

In New Zealand, the ethylene concentration could be relatively low (< 8 $nL \cdot L^{-1}$, unpublished data from a local vegetable grower's storage room) because of the high RH spraying and frequent opening and closing of the door. Lu (2020) reported the highest ethylene concentrations in the distribution centres of horticultural produce (13–3678 $nL \cdot L^{-1}$), the lowest (< 10 $nL \cdot L^{-1}$) in the flower distribution centres, and < 400 $nL \cdot L^{-1}$ in supermarkets.

2.3 Ethylene management techniques

Managing ethylene supports maintaining postharvest quality and shelf life of horticultural produce. A numbers of management techniques can minimise ethylene impact on postharvest quality: Some methods inhibit ethylene synthesis, while others inhibit ethylene action, and other remove the exogenous ethylene from the atmosphere (Figure 2.9) (Wills and Golding, 1989; Martínez-Romero et al., 2007; Keller et al., 2013). The following section provides a range of techniques.

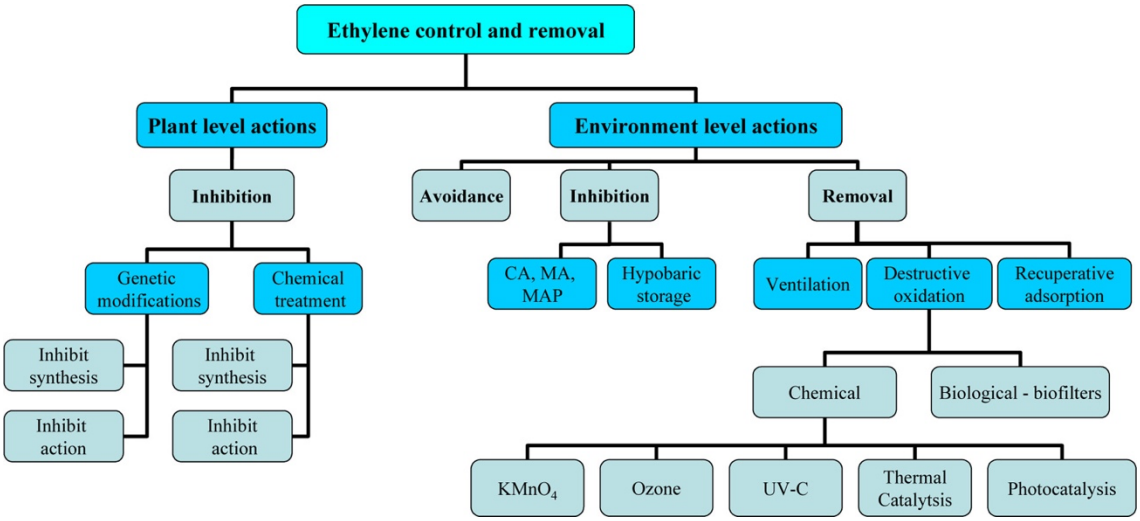

Figure 2.9 Main techniques for managing ethylene. Image taken from Keller et al. (2013) with the permission.

2.3.1 Synthesis inhibitors

Ethylene synthesis inhibitors, such as aminoethoxyvinylglycine (AVG) and aminooxyacetic acid (AOA) (Figure 2.8), block the synthesis pathway of ethylene and thus reducing its production. AOA and AVG inhibit competitively ACS enzyme activity (Scariot et al., 2014; Wills & Golding, 2016). ReTain™ (Valent BioSciences Corporation, USA) is a

commercial AVG (15% w/w) product, which is effectively used pre- and postharvest on many fruits, including apples, pears, and tomatoes (Martínez-Romero et al., 2007). For example, the ethylene production of mature-green tomato (*Lycopersicon esculentum* Mill., cv. Castlemart) was decreased by 89% with the application of 20 µL 10 mM AVG (Saltveit, 2005). In broccoli, Suzuki et al. (2004) reported that the ethylene production of ethanol vapour-treated broccoli was reduced to one-quarter of that in the control broccoli at 20 °C.

2.3.2 Action inhibitors

Ethylene action inhibitors, such as silver ions and 1-methylcyclopropene (1-MCP) (Figure 2.8), hinder the horticultural produce's ability to perceive or respond to ethylene. The remainder of the section will discuss STS, while Section 2.4 will focus on 1-MCP.

Martínez-Romero et al. (2007) and Wills and Golding (2016) noted that silver ions are an ethylene inhibitor at the receptor level by binding extremely tightly to the enzyme receptor site. Silver can be commercially applied as silver thiosulfate (STS) and silver nitrate ($AgNO_3$) in non-food systems to prevent various ethylene-induced responses, such as senescence and abscission. However, silver ions may only be applied to ornamentals due to their toxicity and potential environmental concern.

2.3.3 Removal of the exogenous ethylene from the atmosphere

Keller et al. (2013) summarised ethylene management at the environmental level, including removing the external ethylene (e.g., ventilation and scrubbers) and avoiding ethylene exposure. Guirao et al. (2024) reported that when the broccoli was stored at 2 °C, the ethylene was eliminated by an ethylene scrubber.

2.4 1-methylcyclopropene

1-MCP competes with ethylene for binding to the ethylene receptor (ETRs) (Figure 2.10) for extended periods, thus effectively blocking the receptor (the binding affinity of 1-MCP for the receptor is about 10 times greater than that of ethylene) and preventing ethylene perception (Sisler & Serek, 1997). Compared to the other inhibitors, 1-MCP is considered an environmentally friendly, non-toxic, and safe postharvest chemical worldwide. Additionally, 1-MCP is highly effective and active even at a low 2.5 $nL \cdot L^{-1}$ to $1\mu L \cdot L^{-1}$ concentration (Blankenship & Dole, 2003; Wills & Golding, 2016).

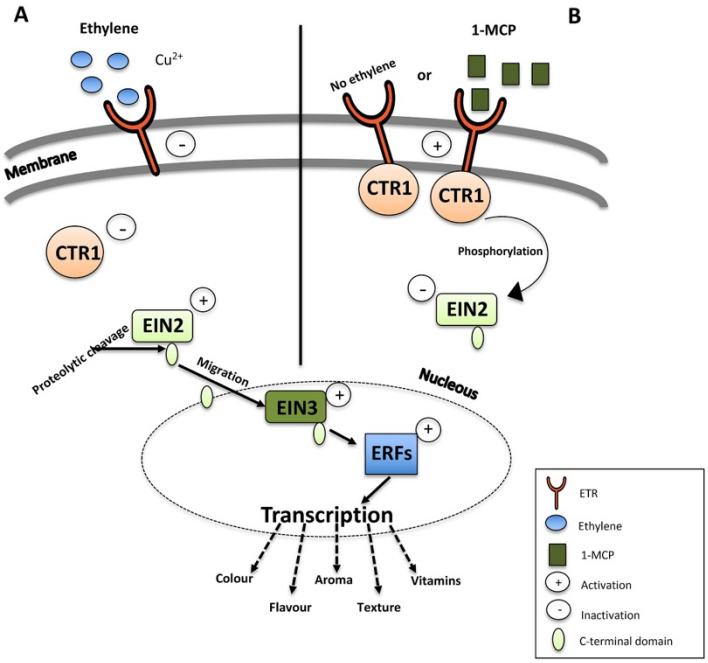

Figure 2.10 Ethylene signalling pathway. Image taken from Dias et al. (2021) with permission.

2.4.1 Commercial products of 1-MCP

Sisler and Serek (2003) and Wills and Golding (2016) stated that 1-MCP is strongly unstable in the liquid phase but is very stable when it is complexed with α-cyclodextrin. In 1999, 1-MCP was first commercialised for ornamental and floriculture products under the name EthylBloc™ by the company FloraLife Inc. AgroFresh Inc. (Rohm and Hass) took the rights for the continuation of the development of 1-MCP in the same year. AgroFresh Inc. then commercialised 1-MCP for the vegetable and fruit market under the name SmartFresh™, which is mainly used in the postharvest industry. Harvista™, the preharvest application of formulations of 1-MCP, was developed and registered by AgroFresh Inc. in New Zealand, the United States, Chile, and South Africa by 2014.

2.4.1.1 EthylBloc™

As the first commercial product of 1-MCP, EthylBloc™ protects ornamental plants and flowers from ethylene exposure to reduce ethylene-induced responses, including flowering, abscission of flowers, buds, leaves, and petals, and wilting (AgroFresh, 2025a). For example, Uthaichay et al. (2007) reported that 100% of the flowers and buds of *Dendrobium* 'Karen' orchid inflorescences abscised within 3 days when exposed to 1 $\mu L \cdot L^{-1}$ ethylene for 3 days at 25 °C. Conversely, when the inflorescences were treated with 500 $\mu L \cdot L^{-1}$ EthylBloc™ (Floralife, SC, USA) for 3 h at 25 °C prior to the ethylene treatment, the abscission of flowers and buds was < 30% after 15 days.

2.4.1.2 SmartFresh™

SmartFresh™ contains 0.14% 1-MCP, which is complexed with cyclodextrin in powder formulation. The powder releases the active ingredient into the air when it is dissolved in water (Sisler & Serek, 2003). Based on your operation needs, AgroFresh (2025b) offers different convenient application options of SmartFresh™, such as SmartFresh™ Quality System (for larger operations) and Inbox for shipping and distribution. Wills and Golding (2016) summarised the beneficials of SmartFresh™ on horticultural commodities (such as apple and European pear), including decreasing the respiration rate and ethylene production and maintaining the acidity and firmness of apples.

2.4.1.3 Harvista™

Harvista™ is a multipurpose near-harvest technology that controls ethylene response to expand the harvest window for optimising crop yield, colour, and firmness. Applications include in the crops of apples, pears, berries, and cherries (AgroFresh, 2025c). Wills and Golding (2016) mentioned that Harvista™ benefits both pre- and postharvest phases by delaying premature fruit drop and lowering the ethylene production of pear and apple trees (preharvest); slowing softening, acidity loss and development of soft and superficial scald (postharvest) in apples. However, the effects of Harvista™ on maturity are sometimes inconsistent. Moreover, the influences of Harvista™ on fruit responses depend on the concentration and the application time before harvest. Vilhena et al. (2023) reviewed the main findings of preharvest Harvista™ on different fruits.

2.4.2 The use of 1-MCP on horticultural produce

Since commercialisation, 1-MCP has been registered for use with a variety of horticultural products, including apples, avocados, bananas, kiwifruits, stone fruits, broccoli, cucumbers, and ornamental plants (AgroFresh, 2025). However, the specific products registered in each country can vary significantly, reflecting the relative importance of each crop in that region. 1-MCP can be used singly or in combination with other techniques, such as MAP and CA. Blankenship and Dole (2003) and Watkins (2016) both review the conditions and effects of 1-MCP on climacteric and non-climacteric fruits and vegetables. This information is briefly summarised in the following sections.

2.4.2.1 1-MCP used in fruits

Watkins (2016) described the differential responses to 1-MCP and outlined the potential advantages and disadvantages associated with commercialising 1-MCP products. While most 1-MCP applications occur postharvest, it is essential to note that each fruit has distinct requirements regarding postharvest quality. For instance, in contrast to other climacteric fruits such as banana and avocado, apples require inhibiting ripening processes, as maintaining a crisp texture is a key desirable attribute for this fruit.

2.4.2.2 1-MCP used in vegetables

Generally, vegetables tend to have a faster retail market turnover than fruits due to their limited shelf life. Blankenship and Dole (2003) concluded that 1-MCP can delay the yellowing, inhibit abscission and extend the shelf life of brassicas, carrots, and leafy vegetables (e.g., coriander and garland chrysanthemum) when exposed to exogenous ethylene. Able et al. (2003) demonstrated that 1 $\mu L \cdot L^{-1}$ ethylene significantly reduced the shelf life of six leafy Asian vegetables, primarily due to yellowing. Without ethylene exposure, overnight 12 $\mu L \cdot L^{-1}$ 1-MCP treatment only significantly increased mizuna and tatsoi's shelf life. In contrast, under ethylene exposure, 1-MCP significantly protected garland chrysanthemum, Chinese mustard, choy sum, and tatsoi. Therefore, they concluded that 1-MCP may be ideal for use on leafy vegetables for protection from ethylene exposure (e.g., when stored or sold alongside ethylene-producing commodities).

2.4.2.3 1-MCP used in flowers

Blankenship and Dole (2003) concluded that 1-MCP generally prevents ethylene-induced senescence in floricultural crops. However, without ethylene exposure, 1-MCP has a limited impact on normal senescence for flowers. Serek et al. (1995a) and Jones et al. (2001) demonstrated that 1-MCP extended the vase life and slowed petal abscission in cut flowers exposed to ethylene. Cameron and Reid (2001) found that the duration of the inhibitory effect of 1-MCP on ethylene was strongly dependent on storage temperature. For example, the inhibitory effect lasted only 1 day at 25 °C but 3–4 days at 12 °C for *Pelargonium peltatum* (L.). For *Dendrobium* orchids, Uthaichay et al. (2007) demonstrated that 1-MCP prevented flower and bud abscission, blocked ethylene action, and inhibited ethylene production by lowering ACS and ACO activity in open flowers and floral buds, respectively. Collectively, these findings demonstrate that the effectiveness of 1-MCP in extending flower longevity is strongly affected by both ethylene exposure and storage conditions, such as temperature.

2.4.2.4 Factors influencing the efficacy of 1-MCP

The effectiveness of 1-MCP depends on its concentration until all the ethylene binding sites are occupied. Vegetables need the inhibition to last for a long time, but climacteric fruits must recover from their impact to ripen properly. The longevity and extent of 1-MCP on horticultural produce are affected by cultivars, species, tissue, maturity, treatment temperature, duration, and concentration, mode of ethylene production (system 1 or 2), and the duration between preharvest 1-MCP treatment and harvest, and the delays between harvest and 1-MCP treatment (Blankenship & Dole, 2003; Watkins, 2016). Ku and Wills (1999) and Able et al. (2002) reported opposing effects of treatment temperature on the shelf life of broccoli, but used different treatment conditions. Ku and Wills (1999) demonstrated that treating broccoli with 1.0 $\mu L \cdot L^{-1}$ 1-MCP for 6 h at 20 °C led to a 61.4-day shelf life, compared to 49.9 days at 5 °C. In contrast, Able et al. (2002) found that 12 $\mu L \cdot L^{-1}$ 1-MCP at 2 °C for 16 h gave 14.1 days shelf life, which decreased to 11 days at 20 °C. Scariot et al. (2014) also noted that three factors contributed to challenges and the effectiveness of postharvest using 1-MCP: 1) the requirement for an enclosed area; 2) the concentrations of 1-MCP and lighting conditions, may result in only transitory impacts; and 3) treatment temperature (0–5 °C) and ethylene exposure strongly reduced the effictiveness of the commercial formulation of 1-MCP.

2.4.3 Physiological and biochemical responses of horticultural produce to 1-MCP

Blankenship and Dole (2003) and Watkins (2006) reviewed the physiological and biochemical responses of horticultural produce to 1-MCP, including ethylene metabolism (perception and production), respiration, pigment metabolism, and quality changes. Key information is briefly summarised in the following sections.

2.4.3.1 Ethylene metabolism

The ethylene perception is typically inhibited by 1-MCP treatment, although the duration of inhibition can vary. However, ethylene production of horticultural products is not always inhibited by 1-MCP treatments, which depends on the systems (1 or 2) of ethylene production (Watkins, 2006; Paul et al., 2012). Porter et al. (2005) demonstrated that treatment with 1.0 $\mu L \cdot L^{-1}$ 1-MCP for 12 h at 22 °C increased the ethylene production of Chinese cabbage 'Yuki' at first, and then that ethylene production decreased when the cabbage was stored at 3 °C for 9 weeks.

2.4.3.2 Respiration

In general, the respiration rates of 1-MCP treated produce were decreased or delayed, particularly in climacteric products, where increases in respiration rate are closely associated with increased ethylene production (Blankenship & Dole, 2003; Watkins, 2006). Interestingly, 1-MCP treatment resulted in an equal or greater respiration rate than that of the control in coriander (Jiang et al., 2002). Grzegorzewska et al. (2023) found that 1-MCP treatment did not affect the respiration rate of broccoli heads.

2.4.3.3 Pigment metabolism

Watkins (2006) listed the 1-MCP impacts on ethylene-regulated pigment metabolism in horticultural produce, including chlorophyll degradation, anthocyanin accumulation, and lycopene accumulation. Typically, 1-MCP treatment inhibits yellowing caused by chlorophyll degradation (Blankenship and Dole, 2003; Watkins, 2006). For example, Gong and Mattheis (2003) reported that 1-MCP treatment reduced the chlorophyllase activity and thus inhibiting yellowing in broccoli.

Sometimes, horticultural products only respond significantly to 1-MCP treatment under ethylene exposure. Able et al. (2003) reported that in the presence of 1.0 $\mu L \cdot L^{-1}$ ethylene, 1-MCP delayed the yellowing of leaf Asian vegetables, such as tatsoi and choy sum. 1-MCP treatment reduced the yellowing of broccoli under ethylene exposure (Fan & Mattheis, 2000). However, Ghimire et al. (2024) reported inconsistent results in the chlorophyll fluorescence in broccoli when stored at the same temperature (10 °C) without ethylene exposure.

2.4.3.4 Quality changes (firmness/flavour/volatile/sugars/acidity)

In general, the effects of 1-MCP on horticultural product quality, such as firmness, flavour, volatiles, sugars, and acidity, are mixed, with some produce being affected and others not. For instance, typically, 1-MCP maintained the firmness of most fruits. 1-MCP delayed the softening of banana and avocado by lowering the activities of cell wall enzymes, such as polygalacturonase (PG) and cellulase (Feng et al., 2000; Lohani et al., 2004), while Tian et al. (2000) reported that 1-MCP did not affect the firmness of strawberry. Further details of quality changes to 1-MCP are reviewed by Blankenship and Dole (2003) and Watkins (2006).

2.5 Managing broccoli quality change with 1-MCP

Ethylene plays an important role during harvest, and in the pre- and postharvest physiology of broccoli (Figure 2.4). 1-MCP blocks ethylene receptors and thus prevents

broccoli cells from detecting ethylene (Section 2.4). Consequently, typical ethylene-induced physiological responses, including chlorophyll degradation and senescence, are suppressed or delayed. Thus, 1-MCP could potentially be useful for managing broccoli quality change from ethylene exposure.

Twenty-three publications related to postharvest 1-MCP application are numbered and summarised in Table 2.7. A collective graph in terms of positive and null results is then provided in Figure 2.11.

Previous studies reported positive effects of postharvest application of 1-MCP on broccoli quality, including maintaining the h° and reducing respiration rate, ethylene production, and weight loss under both air and ethylene storage conditions (Table 2.7 and Figure 2.11). Three studies of 23 (13%) found that 1-MCP did not affect the weight loss, respiration rate, and ethylene production of broccoli without ethylene exposure. However, positive publication bias, as discussed by Mlinarić et al. (2017), was resulted from that studies with positive results were published much more than those with null results, which may in turn stimulated the misinterpretation for data to intentionally obtain "positive" results.

A number of studies showed that postharvest 1-MCP treatment extended the shelf life of broccoli: After comparing different concentrations of 1-MCP, Ku and Wills (1999) and Yuan et al. (2010) demonstrated that treatment with 1.0 $\mu L \cdot L^{-1}$ 1-MCP at 20 °C for 6 h increased the shelf life of broccoli by ≥ 60%. Ekman et al. (2019) described that 1-MCP treatment combined with RipeLock™ film had the longest storage life (24 days) at 5 °C. During storage at 10 °C, Able et al. (2002) demonstrated that 12 $\mu L \cdot L^{-1}$ 1-MCP extended the shelf life of broccoli branchlets by 22–49% across a range of treatment temperatures (2, 10, 15, 20 °C). Vasconcelos and Almeida (2003) showed that 1-MCP extended the shelf life by 13, 67 and 50% when the broccoli was stored at 1, 10 and 20 °C, respectively. Vasconcelos and Almeida (2003) also demonstrated the longest storage life was 86 days at 1 °C, when 12 $\mu L \cdot L^{-1}$ 1-MCP was applied immediately after harvest to the broccoli in the field.

1-MCP treatments can inhibit the yellowing of broccoli heads caused by ethylene action (Ghimire et al., 2024). The application of postharvest 1-MCP inhibited the yellowing of broccoli heads when moved from 0–1 °C after 30 days to 15 °C conditions for 6 days. Moreover, 1-MCP retarded the discolouration and senescence of broccoli stalk scars (Grzegorzewska et al., 2023).

For the postharvest application, Able et al. (2002) demonstrated that 1-MCP is more effective on broccoli than pak choy. After harvest, 1-MCP should be applied as soon as possible to achieve maximal positive effectiveness on brassicas. However, Ekman et al. (2019) noted that delaying the application of 1-MCP on broccoli heads by 24 h after harvest had a negligible impact on its effectiveness.

Across different cultivars, treatments (1-MCP formulations and concentrations, treatment temperatures, and duration), and storage conditions (temperatures, RH, ethylene, and duration), studies of postharvest 1-MCP application on broccoli generally report maintained green colour (especially h°) and extended shelf life, but results are variable. The inconsistencies highlighted that the magnitude of the effect may depend strongly on cultivars, treatments (such as concentration and exposure duration), storage temperature and duration, and the presence of ethylene (Table 2.7). Overall, the literature supports postharvest 1-MCP as a useful tool for quality maintenance in broccoli, but optimal application parameters and the magnitude of its effects may remain cultivar- and condition-specific.

Table 2.7 Previous studies of 1-MCP treatment effects on broccoli

No.	Cultivars	Treatments	Storage conditions	Effects	References
1	'Green Belt'	0, 0.02, 0.1, 1.0, 10 and 50 $\mu L \cdot L^{-1}$ 1-MCP for 6 h at 20 °C or 5 °C	20 °C or 5 °C with air containing 0.1 $\mu L \cdot L^{-1}$ ethylene until the end of shelf life	The effects of 1-MCP on broccoli florets were dependent on concentration and treatment time. Overall, 1.0 $\mu L \cdot L^{-1}$ 1-MCP for 6 hours at 20 °C was more effective in shelf life extension when storage at either 20 °C or 5 °C.	Ku and Wills (1999)
2	'Windsor'	0, 0.01, 0.1 and 1.0 $\mu L \cdot L^{-1}$ 1-MCP for 12 h at 10 °C	10 °C in air and 1.0 $\mu L \cdot L^{-1}$ ethylene conditions for 12 and 18 days	Reduced the yellowing and respiration rate of broccoli branchlets and negated the impact of ethylene. The inhibiting of 1-MCP on respiration rate under ethylene exposure was concentration-dependent, whereas its impact on yellowing was not.	Fan and Mattheis (2000)
3	'Maverick', '886' 'Green Belt'	12 $\mu L \cdot L^{-1}$ 1-MCP for 16 h at 10 °C; multiple treatment: daily 1-MCP application for 20 h	2, 10, 15, 20 °C to determined shelf life; 0.1 or 1 $\mu L \cdot L^{-1}$ ethylene storage treatments	Increased the shelf life of broccoli heads by greater than 20%. Multiple application of 1-MCP did not further impact the broccoli and 1-MCP should be applied as soon as possible after harvest.	Able et al. (2002)
4	'Marathon'	Untreated or treated with 2.0 $\mu L \cdot L^{-1}$ 1-MCP for 6 h immediately after harvest	In the dark at 1, 10 and 20 °C until the end of shelf life	For untreated branchlets, the potential shelf life were 2, 12 and 76 days while 1-MCP treatment increased them to 3, 20 and 86 days respectively, when stored at 20, 10 and 1 °C. 1-MCP had minimal practical benefit when applying at 20 °C and 1 °C, but at 10 °C (a temperature representative of retail conditions), it helped maintain the postharvest quality of broccoli. 1-MCP only affected duration of phase 1 in colour change while temperature impacted both duration of phase 1 and change rate of phase 2.	Vasconcelos and Almeida (2003)
5	'Everest' 'Regal'	Untreated or treated with 1.0 $\mu L \cdot L^{-1}$ 1-MCP for 14 h at 10 °C	12 ± 0.5 °C and 95–98% RH for up to 12 days	1-MCP treated broccoli had a lower decline rate of h° and higher chlorophyll florescence.	Forney et al. (2003)
6	'Windsor'	At 20 °C, (1) air (control), (2) 1 $\mu L \cdot L^{-1}$ 1-MCP for 14 h, (3) 1000 $\mu L \cdot L^{-1}$ ethylene for 5 h, or (4) 1-MCP followed by ethylene	20 °C for 3 days in darkness	1-MCP treated broccoli branchlets had significantly higher h° values and total chlorophyll content. It delayed the yellowing possibly by decreasing the activities of peroxidase (POD) and chlorophyllase (Chlase).	Gong and Mattheis (2003)

7	'Montop'	At 20 °C, 1) air (control), 2) 2.5 μL·L^{-1} 1-MCP for 12 h, 3) 20 μL·L^{-1} ethylene for 12 h, or 4) 1-MCP followed by ethylene, both for 12 h	20 °C and 80–85% RH for 60 hours (< 3 days)	Reduced the yellowing of broccoli florets "Inhibited the activities of 1-aminocyclopropane-1-carboxylate acid (ACC) oxidase (ACO), and delayed the peaks in the ACC synthase (ACS) activity and ACC concentration". Additionally, ethylene treatment did not accelerate the yellowing of the florets when pretreated with 1-MCP.	Ma et al. (2009)
8	'Lvxiong'	Air (control), 0.1, 1.0, 2.5, 5.0 and 10 μL·L^{-1} 1-MCP for 6 h at 20 °C	20 °C and 95% RH for up to 5 days	1-MCP treated broccoli had the longest shelf life of 5 days at 20 °C. It inhibited the activities of polyphenol oxidase (PPO) and lipoxygenase (LOX) and the increase of malondialdehyde (MDA) content. It also maintained the ascorbic acid, total carotenoids and glucosinolates.	Yuan et al. (2010)
9	'Cicco'	With or without 1 μL·L^{-1} 1-MCP for 16 h at 22 °C; 100 mg·kg^{-1} ethephon with 0.1% DMSO or 0.1% DMSO	20 °C for 120 h in darkness; 22 °C for 0, 72 and 120 h	1-MCP had the highest chlorophyll content and h° values. It also inhibited expression of *BoSGR* (*Stay-Green* ("a chloroplast-located protein") encoding gene), demonstrating an opposite effect to ethylene. Postharvest 1-MCP inhibited selective "genes encoding enzymes related chlorophyll catabolism".	Gómez-Lobato et al. (2012)
10	'Parthenon'	Treated with 0.6 μL·L^{-1} 1-MCP for 24 h at 1 °C and untreated broccoli headss	1–2 °C and 85–90% RH for 2, 6, 13, 20 and 27 days; followed by 2 or 4 days of shelf life at 20 °C	Reduced the degreening of broccoli as indicated by chlorophyll contents. However, the results of weight loss, colour (L*, a* and b*) and firmness showed the inconsistent responses to 1-MCP.	Fernández-León et al. (2013)
11	'Chaoda No 1'	0 (control) and 2.5 μL·L^{-1} 1-MCP for 6 h at 20 °C	15 °C and 95% RH for 5 days	1-MCP treated broccoli heads had lower L* value, higher h° value, glucosinolate and chlorophyll content than that of control.	Xu et al. (2013)
12	'Green Magic'	Untreated and 500 nL·L^{-1} 1-MCP for 24 h at 20 °C	4 °C for 10, 20 and 30 days	1-MCP treated branchlets had significantly higher chlorophyll and h° since stored for 20 days.	Ku et al. (2013)

13	'Cicco'	With or without 1 μL·L^{-1} 1-MCP for 16 h at 22 °C; 100 mg·kg^{-1} Ethephon with 0.1% DMSO or 0.1% DMSO	22 °C for 120 h in the dark	1-MCP treatment resulted in a h° of 117.25 ± 4.86°, compared to that of control (102.63 ± 8.09°). "Delayed the increment of *BoSGR* expression whereas ethylene accelerated the process".	Gómez-Lobato et al. (2014)
14	'Parthenon'	With or without 1-MCP packaged in Ripelock MA bags; then treated with 5 μL·L^{-1} ethylene for 6 h at 0 °C	0, 7.5 and 12 °C for 42, 22 and 18 days, respectively. A shelf life was then obtained at 10 °C for 3 days	1-MCP significantly reduced yellowing at 7.5 °C and 12 °C, maintaining retail quality during storage and shelf-life. At 7.5 °C + 3 d at 10 °C, 1-MCP exhibited 7 days longer shelf life than untreated or ethylene-exposed broccoli. At 12 °C, it allowed up to 8 days of storage. No differences were found at 0 °C, where all treatments retained < 20% yellowing for 42 days.	De Beer and Crouch (2015)
15	'Chaoda No 1'	Untreated and 2.5 μL·L^{-1} 1-MCP for 6 h at 20 °C	15 °C and 95% RH for 4 days in the dark	1-MCP treated broccoli had a significant higher chlorophyll content (0.5 mg·g^{-1}) and h° (126°), and a lower L* (32) than those of untreated broccoli heads (0.2 mg·g^{-1}; 108 ° and 30)	Xu et al. (2016)
16	'Marathon', 'Aurora', 'Ironman'	0, 2, 4 and 8 SmartFresh™ In-Box sachets were applied immediately after harvest and 24 h after harvest with 2 liners	5 °C for 30 days, 7 and 16 °C until end of shelf life, 7 °C for 21 days and then 20 °C until no longer marketable	1-MCP maintained the visual quality of broccoli like green colour and increased the percentage of marketable heads from 0% to 72-100%.	Ekman et al. (2019)
17	Broccoli	625 nL·L^{-1} 1-MCP, 2 μL·L^{-1} ethylene for 24 h at 5 °C and 21 °C and 1-MCP followed by ethylene	5 °C for 9 days after treatments; 21 °C for 5 days after treatments	Reduced the weight loss of broccoli at 5 °C and 21 °C. Ethylene treatments induced the degradation of chlorophyll fluorescence, thus yellowing of broccoli heads. 1-MCP treated broccoli had the highest chlorophyll florescence, preserving the green colour of broccoli, especially when stored at 5 °C.	Zsom et al. (2020)
18	'Legacy'	Untreated and 1.0 μL·L^{-1} 1-MCP for 15 h at 20 °C in darkness; immersed in 100 mg·kg^{-1} Ethephon with 0.1% DMSO for 10 minutes	20 °C for 120 hours in darkness	Maintained the h° and total chlorophylls in broccoli heads. 1-MCP treatments showed a lower expressions of *BoNOL* and higher expression of *BoHCAR* while ethylene treatment showed a lower expression of *BoHCAR*.	Reyes Jara et al. (2021)

19	‘Bay Meadows’, ‘Monaco’, ‘Naxos’ and ‘Vicario’	Air and 1.0 $\mu L \cdot L^{-1}$ 1-MCP for 20 h at 4 °C	4 °C and 98–99% RH for 10, 20 and 30 days	Maintained the commercial values by decreasing the weight loss, maintaining the totals sugar and vitamin C content of broccoli cultivars. All cultivars responded differently to 1-MCP treatments. 1-MCP treated ‘Monaco’ broccoli had the lowest weight loss, the highest content of dry weight, vitamin C and total sugars.	Wichrowska et al. (2021)
20	'MKS-B107'	Air and 1.0 $\mu L \cdot L^{-1}$ 1-MCP for 15 h at 15 °C	Packed in LDPE, storage at 10 °C for 25 days and 0 °C for 50 days with 55% RH	Reduced the ethylene production, respiration rate and weight loss. Maintained the content of chlorophylls, ascorbic acid and total phenolic compounds.	Phuong et al. (2022)
21	‘Grandome’	0.0, 0.5, 1.0, 1.5, 2.5 and 5.0 $\mu L \cdot L^{-1}$ 1-MCP at 15 ℃ for 15h	Packed in LDPE, storage at 15 °C and 55% RH for 10 days	Broccoli responded to 1-MCP concentration. 2.5 $\mu L \cdot L^{-1}$ 1-MCP inhibited the respiration rate and ethylene production, and lowered weight loss of broccoli.	Phuong et al. (2023)
22	‘Parthenon F1’	0, 1.0 or 3.0 $cm^3 \cdot m^{-3}$ 1-MCP at 5 °C for 20 h	0–1 °C for 30 days (and then transferred to 15 °C for 6 days) or 60 days	1-MCP maintained fleshy stems and better quality of the crowns and inhibited the senescence of the residual petiole fragments. It did not affect the ethylene production and respiration rate	Grzegorzewska et al. (2023)
23	Broccoli	1.0 $\mu L \cdot L^{-1}$ 1-MCP for 24 h at 20 °C and untreated control	90–95% RH, 4 °C for 28 days and 10 °C for 18 days	Reduced the respiration rate of broccoli at 4 °C and 10 °C. However, 1-MCP did not impact the weight loss.	Ghimire et al. (2024)

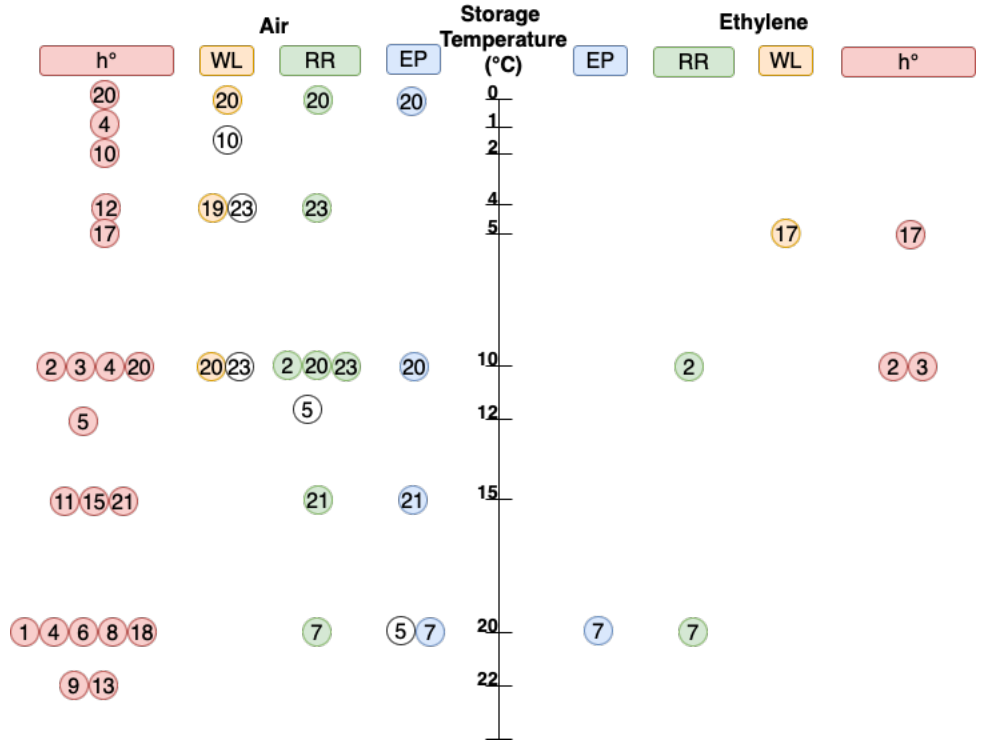

Figure 2.11 Positive and null results from previous studies for postharvest 1-MCP application during storage in the air and ethylene conditions. Storage temperature ranges from 0 to 22 °C. Each number (colourful numbers = positive results, while the white circle numbers = null results) represents one article from Table 2.7.

2.5.1 Physiological and quality responses of broccoli to 1-MCP

Previous studies found mixed physiological responses of broccoli to postharvest application of 1-MCP. For instance, Fan and Mattheis (2000), Phuong et al. (2022, 2023), and Ghimire et al. (2024) demonstrated that 1-MCP reduced the respiration rate of broccoli, whereas Forney et al. (2003) and Grzegorzewska et al. (2023) reported no effects.

Phuong et al. (2022 and 2023) demonstrated that 1-MCP decreased the ethylene production of broccoli, but no significant effects were reported by Forney et al. (2003) and Ghimire et al. (2024). Grzegorzewska et al. (2023) found that 1-MCP treated broccoli heads tended to have a higher ethylene production than that of untreated broccoli.

Zsom et al. (2020), Wichrowska et al. (2021), and Phuong et al. (2022) demonstrated that 1-MCP reduced the weight loss of broccoli heads, whereas Fernández-León et al. (2013) and Ghimire et al. (2024) found no effects.

Many researchers found that 1-MCP maintained the h° and total chlorophyll content (Forney et al., 2003; Gong & Mattheis, 2003; Gómez-Lobato et al., 2012; Ku et al., 2013; Xu

et al., 2013; Reyes Jara et al., 2021). However, Phuong et al. (2022), Grzegorzewska et al. (2023), and Ghimire et al. (2024) found inconsistent effects of 1-MCP on the colour of broccoli; at times it maintained the colour, while at other times it did not.

Fernández-León et al. (2013) reported that 1-MCP did not affect the firmness of broccoli heads.

The effectiveness of 1-MCP on broccoli was strongly affected by storage temperature. De Beer and Crouch (2015) noted that 1-MCP reduced the yellowing of broccoli at 7.5 °C and 12 °C, but not at 0 °C. Vasconcelos and Almeida (2003) demonstrated that compared to 1 °C and 20 °C, 1-MCP helped maintain the postharvest quality of broccoli when applied at 10 °C, a temperature representative of retail conditions.

Fan and Mattheis (2000) reported that the impact of 1-MCP on yellowing was not concentration-dependent. In contrast, Ku and Wills (1999) found that yellowing of broccoli was dependent on 1-MCP concentration and treatment duration.

Wichrowska et al. (2021) demonstrated that cultivars responded differently to 1-MCP. Therefore, it can be concluded that the effects of 1-MCP on broccoli are not fully understood yet.

2.6 Research gaps and conclusions

As discussed previously, the effectiveness of 1-MCP on broccoli varied, potentially impacted by treatment temperatures, storage temperatures, application duration and concentrations, and cultivars. Section 2.2.3 explained that ethylene exposure induced the decline of h°, resulting in the advancement of yellowing of broccoli heads, especially at suboptimal temperature (e.g., > 4 °C) (Tian et al., 1994; Zsom et al., 2020; Guirao et al., 2024; Ghimire et al., 2024). Mixed responses of broccoli to 1-MCP by ethylene concentration and storage temperature were reported in Figure 2.11 (Fan & Mattheis, 2000; Able et al., 2002; Gong & Mattheis, 2003; Ma et al., 2009; Zsom et al., 2020; Reyes Jara et al., 2021).

To the author's knowledge, there are no publicly published studies that examine the effects of preharvest application of 1-MCP on broccoli. As discussed in 2.1.2.6, some studies have described the postharvest physiology of broccoli as a climacteric-like pattern due to respiration rate and ethylene production (Makhlouf et al., 1989; Tian et al., 1994; Ma et al., 2009; Paul et al., 2012). Given this similarity, it is reasonable to cautiously compare with climacteric fruits like apples when evaluating ethylene and 1-MCP effects. Previously, Elfving et al. (2007), Nock et al. (2009), and Sakaldas and Gundogdu (2015) demonstrated that multiple

benefits of preharvest applications of 1-MCP for the apple growers and marketers, including reduced fruit drop and ethylene production, delayed ripening, and maturity on apple trees. Preharvest applications of 1-MCP maintained the postharvest firmness in apples. These benefits depended on cultivars (for example, preharvest applications of 1-MCP worked well on 'Golden Delicious', 'Cameo', and 'McIntosh'), maturity, time between application and harvest, and storage atmosphere. These findings indicate that preharvest applications of 1-MCP may also provide benefits for broccoli, especially in reducing postharvest deterioration (Figure 2.4) and managing harvest timing, and thus providing potential benefits for growers, marketers, and consumers.

In practice, preharvest application of 1-MCP may delay commercial harvest, reduce the multiple harvests, and thus reduce harvest-labour use (Elfving et al., 2007). Compared to postharvest application, preharvest application of 1-MCP does not require a sealed environment because of its formulation. Preharvest application of 1-MCP may allow broccoli head growth to a bigger size without over-maturing due to its inhibition of ethylene receptors and thus delaying maturity and senescence. Specifically, growers can earn more money for a higher yield. Preharvest application of 1-MCP may benefit marketers and consumers by preserving broccoli from ethylene and temperature stresses in the supply chain.

As no published research to date has examined the preharvest application of 1-MCP on broccoli, and there are potential benefits based on studies in apples, a preharvest spray of 1-MCP was therefore evaluated for its efficacy in preserving the postharvest quality of broccoli under varying storage temperature and air and ethylene exposure conditions in this study (Figure 2.12). This study aimed to ask these questions: Does preharvest application of 1-MCP affect the head growth of broccoli? Does preharvest application of 1-MCP affect the at-harvest quality of broccoli? Does preharvest application of 1-MCP affect the postharvest quality of broccoli under both air and ethylene exposure at different storage temperatures? The research objectives are provided in Section 1.1. Two different trials assessed the responses of two cultivars ('Nobel' and 'Iron') to preharvest application of 1-MCP

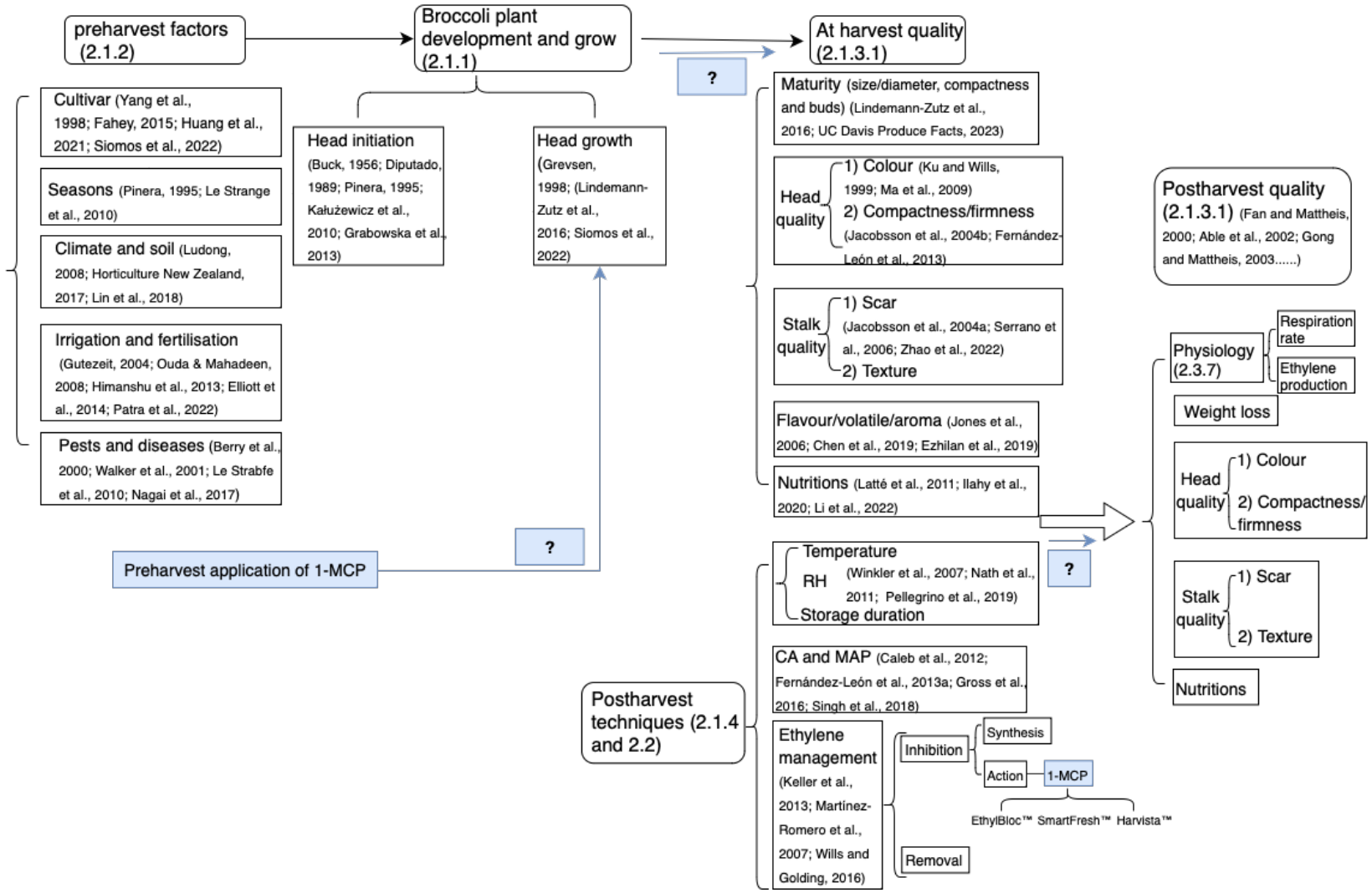

Figure 2.12 A diagram illustrating the structure of the literature review and research gap in this area. The light blue colour indicates the research gap.

Chapter 3. Materials and Methods

3.1 Plant materials

Winter cultivar 'Nobel' and summer cultivar 'Iron' broccoli were cultivated commercially at Woodhaven Garden (40.64° S, 175.24° E), Levin, New Zealand. Two trials were conducted in two different seasons – winter (June to October) of 2024 and summer (December to February) of 2025. 'Nobel' broccoli was planted on 18th June 2024 and harvested on 8th October 2024, with a total growing period of 113 days from seedling. 'Iron' broccoli was planted on 10th December 2024 and harvested on 7th February 2025, resulting in a total growing period of 60 days.

3.2 Experimental design

The experiment included two compounds: Preharvest spraying of 1-MCP in the field experiment on broccoli and a subsequent storage experiment at 1 °C or 4 °C, with ethylene exposure or in clean air. Exposure to ethylene allows testing of the risk reduction effect that a 1-MCP application may provide. A 1 $\mu L \cdot L^{-1}$ ethylene exposure condition was chosen as this concentration was occasionally in the distribution centres of four New Zealand supermarkets (Lu, 2020).

An experiment was conducted for each of the seasons. The treatment allocation for the two trials is provided (Table 3.1). At each storage temperature, there were 12 combined treatments (including block). Trial 2 repeated Trial 1 with an additional storage temperature of 4 °C, but doubled in size.

Table 3.1 Treatment layout for Trial 1 and Trial 2

Treatments and heads	Trial 1	Trial 2
Field treatments (±1-MCP)	2	2
Field blocks	3	3
Storage treatments (1 $\mu L \cdot L^{-1}$ ethylene & air)	2	2
Storage temperatures	1 (1 °C)	2 (1 & 4 °C)
Head per combined treatment	10	10
Total	120	240

The timeline and general flow the experiment is provided in Figure 3.1. 1-MCP was sprayed on Day 0. In Trial 2, the initial diameter of broccoli heads was also measured. The

broccoli heads were harvested on Day 4 and Day 3 for Trials 1 and 2, respectively, which was also day 0 for the storage experiment. Broccoli heads were graded, grouped and labelled before the measurements. On the same day, the at-harvest quality was measured, and the broccoli heads were then placed in barrels of a flow through system for storage. At the same time, respiration rate and ethylene production of central branchlets from 18 broccoli heads were measured. The remainder of this chapter details each of these processes.

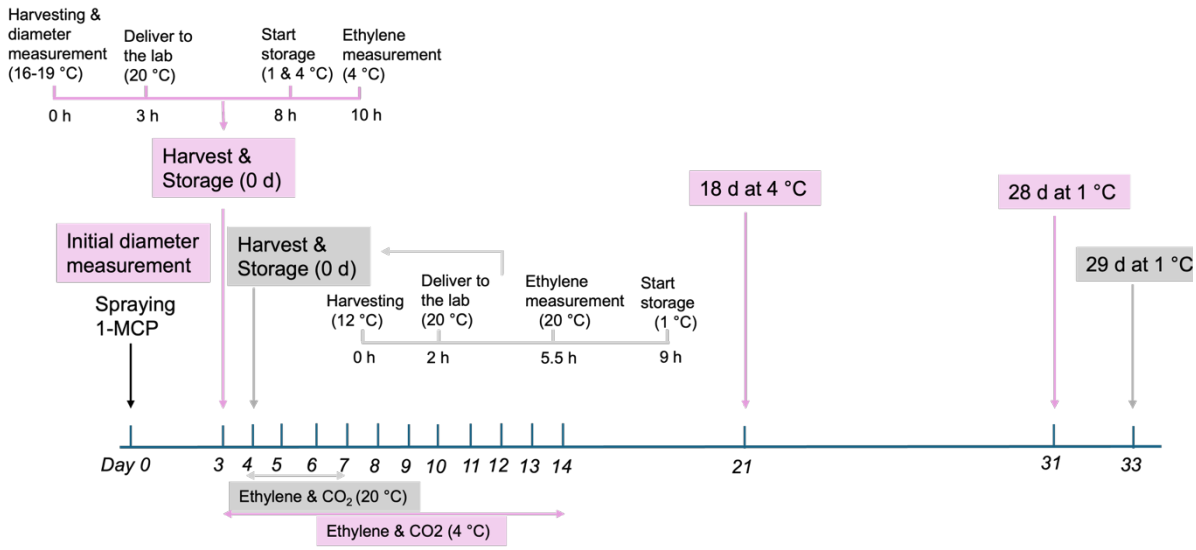

Figure 3.1 Timeline of the experiment. The grey colour represents the timeline in Trial 1, while the plum colour represents the timeline in Trial 2.

For Trial 1, the initial weight and diameter of 120 broccoli heads were determined at harvest. The at-harvest colour, head floret flexibility, and stalk compression stress of 18 broccoli heads were also measured. After 29 days, broccoli heads were taken from barrels to conduct the final measurements of weight for weight loss at 1 °C. Afterwards, colour, head firmness, head floret flexibility, and stalk compression stress were measured at 20 °C.

For Trial 2, the at-harvest weight and diameter of 240 broccoli heads were determined on day 0 at 20 °C. The colour of 39 broccoli heads and the head firmness of 18 broccoli heads were also measured. After 18 days of storage at 4 °C, 120 broccoli heads were taken from barrels to conduct the final measurements for weight (at 4 °C), followed by colour and head firmness measurements at 20 °C. Similarly, after 28 days at 1 °C, 120 broccoli heads were taken from barrels to conduct the final measurements for weight (at 1 °C), followed by colour and head firmness measurements at 20 °C.

3.3 Experimental procedures

3.3.1 Field experimental

The field experiment was conducted on broccoli plants as two treatments, each represented by three block replicates (Figure 3.2). Broccoli plants were spaced within a row at 0.42 m and between-row at 0.82 m, with two rows per planting block. A gap of ten plants separated the 1-MCP applied area from the field control area.

For Trial 1, a preharvest of 1-MCP was sprayed at a rate of 1.0 $mL \cdot m^{-2}$ four days prior to commercial harvest. For Trial 2, 1-MCP (1.0 $mL \cdot m^{-2}$) was applied three days prior to commercial harvest. The weather information, including temperature, rain, and wind, between application and harvest was recorded. The details of how the field experiment was executed are provided in the remainder of this section.

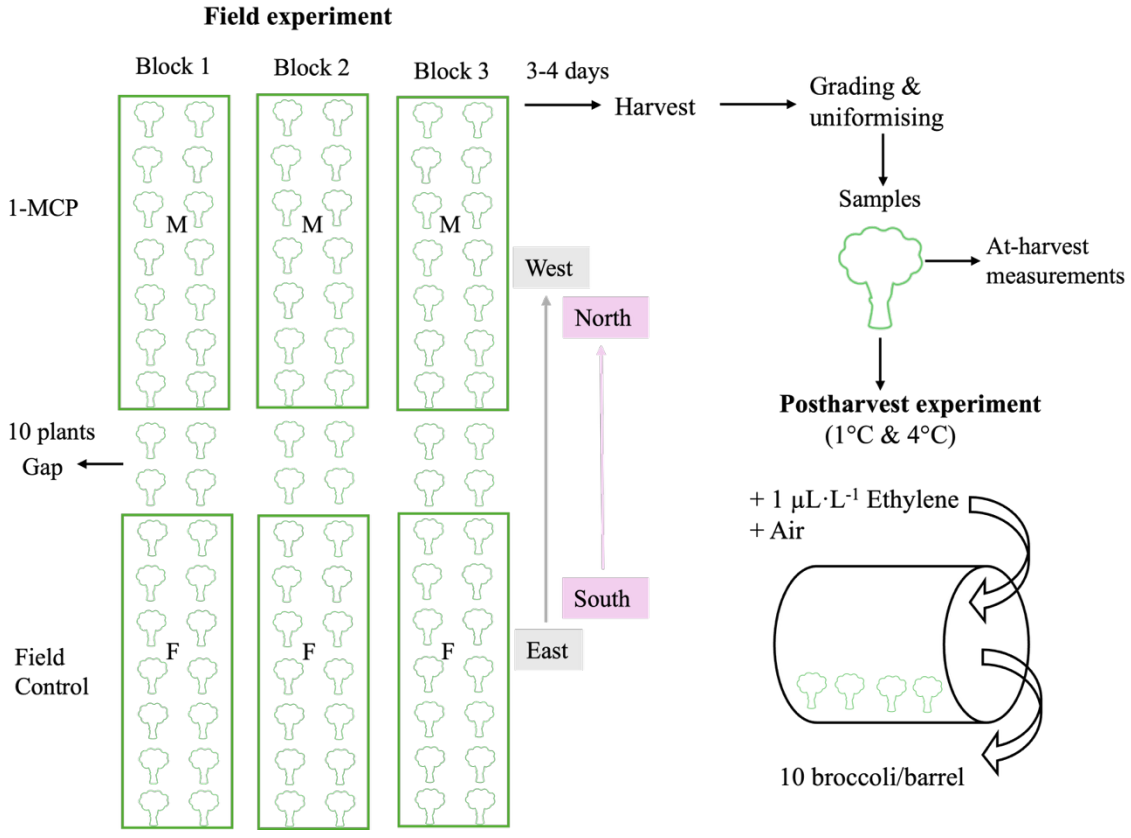

Figure 3.2 Experimental design diagram. The grey and plum colours represent the field (vertically oriented) directions in Trials 1 and 2, respectively.

3.3.1.1 Block identification and 1-MCP application

Three adjacent blocks were chosen and identified, while no boundary blocks were included to avoid edge effects. Preharvest 1-MCP was applied according to the protocol (Table

3.2). For the broccoli in the field control area, nothing was applied. Alternatively, the same amount of water as that used for the 1-MCP area could be sprayed as the control.

For Trial 1, three blocks of 60 ‘Nobel’ broccoli plants were designated as the 1-MCP area (width: 4.5 m, length: 12.5 m). On the morning of October 4, 2024 (8:20–8:40 am), 1-MCP (1.0 $mL \cdot m^{-2}$) was added to 5 L of water and sprayed on the broccoli plants in the 1-MCP area. The solution was sprayed twice evenly. Conditions included a wind speed of 10 $km \cdot h^{-1}$, a temperature of around 11 °C, and it was not raining (8:00 am, October 4, 2024, Levin weather).

For Trial 2, three blocks of 100 ‘Iron’ broccoli plants were designated as the 1-MCP area (width: 4.5 m, length: 21 m). Ten broccoli plants from each treatment were marked for diameter measurement. The diameter was measured before 1-MCP application and before harvest to study the impact of preharvest 1-MCP application on head size. Photographs of selected broccoli heads were also taken in the field. On the morning of February 4, 2025 (9:30–9:40 am), 1-MCP (1.0 $mL \cdot m^{-2}$) was added to 4 L of water and sprayed on the broccoli plants in the 1-MCP area. The solution was finished with a first spray and a second, lighter spray. The temperature was around 15 °C, the wind was 4 $km \cdot h^{-1}$, and it was sunny (9:00 am, February 4, 2025, Levin weather).

3.3.1.2 Weather conditions between application and harvest

For Trial 1, from 4 October to 8 October 2024, Levin experienced typical spring weather with average daily high temperatures ranging from 14 °C to 16 °C and average daily low temperatures being around 10 °C. The region was overcast or mostly cloudy about 43% of the time, with occasional light rain. On 8 October 2024 (8:30–9:30 am), the at-harvest temperature was around 12 °C. There was light rain during harvest.

For Trial 2, from 4 February to 7 February 2025, Levin experienced typical summer weather with average daily high temperatures ranging from 22 °C to 26 °C and average daily low temperatures of around 11 °C. On 7 February 2025 (8:00–10:30 am), the at-harvest temperature ranged from 16 °C to 19 °C. No rain was recorded between the 1-MCP application and harvest.

Table 3.2 Field protocol for 1-MCP application

Step	Activity	Details
1	Marking the areas and calculating the plot size for 1-MCP spraying area	1-MCP spraying area: marked with pink tape. Field control area: marked with white tape. Plot size (length × width) for 1-MCP spraying area measured using a measuring tape and calculated in square meters.
2	Mixing 1-MCP ingredients and preparing the sprayer	1-MCP ingredients were thoroughly mixed for at least 2 minutes using a paint stirrer attached to a battery drill. 50 $mL \cdot m^{-2}$ of water added to knapsack sprayer based on plot size
3	Adding 1-MCP ingredients	A 60 mL syringe used to measure 1 $mL \cdot m^{-2}$ of 1-MCP. Then added to the water in the sprayer and thoroughly mixed.
4	Spraying 1-MCP solution	Spraying was completed within 20 minutes of mixing. Solution sprayed evenly over the plot. Any leftover solution was sprayed again to finish all the solution.

3.3.2 Head harvest and diameter measurement in the field

Commercial harvest of broccoli is determined by the size (diameter) and tightness of the broccoli head (Section 2.1.2.6). In New Zealand, the marketable head should have a diameter of at least 110 mm and be tight with unopened buds. Broccoli fields are typically harvested over multiple occasions, with suitable heads being selected at each time. Winter broccoli, like 'Nobel', may have three to seven harvests from the same field planting, whereas summer cultivars, like 'Iron', may produce three to five harvests. The broccoli used in this study were all harvested on a single commercial harvest day to control timing from 1-MCP treatment and simplify the postharvest storage work. Three steps were taken to control harvest (Table 3.3).

For Trial 1, approximately 200 'Nobel' broccoli heads were harvested four days after the field treatments. At least 25 heads from each treatment block were harvested. For Trial 2, approximately 450 'Iron' broccoli heads were harvested three days after the field treatments. At least 50 heads from each treatment block were harvested. The 'Iron' broccoli plants used for diameter measurement in the field were harvested separately. The diameter was measured by a calliper in the field before harvest.

Table 3.3 Steps taken for the harvest of broccoli heads

Step	Activity	Details
1	Labelling the crates	Crates were labelled for different treatments and Blocks: 1-MCP areas: M1, M2, M3 Field Control areas: F1, F2, F3
2	Harvesting the marketable heads	Big heads were cut first using a knife, then any heads around 110 mm were harvested. Stalks were cut to > 10 cm length from the lowest stem. All leaves were removed using a knife. Heads were placed into the appropriately labelled crates.
3	Harvesting extra heads	To ensure sufficient samples for experiment, additional undersized heads (unmarketable) were harvested and placed in labelled black bags.

All the harvested broccoli heads were transferred to the lab (a 1-hour drive) immediately without pre-cooling, the same as commercial harvest. In commercial harvest, broccoli heads are packed in the field and directly transferred to the cool room without pre-cooling within approximately 2 hours.

3.3.3 Postharvest experiment

3.3.3.1 Sample preparation

Broccoli heads were first graded by diameter, using the Foodstuffs South Island (FSSI, n.d.) categories (< 110 mm, 110–120 mm, 120–150 mm, > 150 mm). To facilitate grading, grading circles were created from plastic tubing with internal diameters of 110 mm, 120 mm and 150 mm, respectively (Figure 3.3a, b).

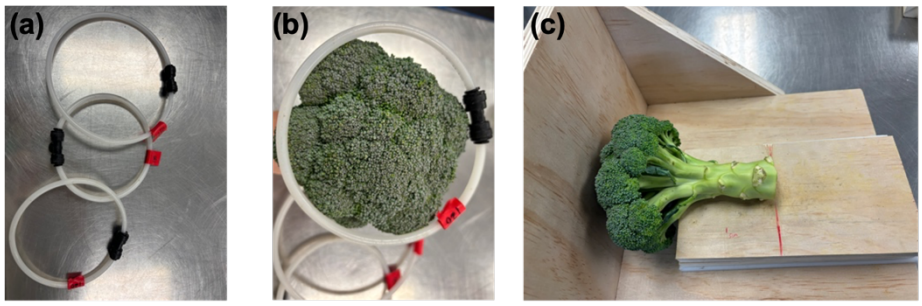

Figure 3.3 Grading and uniformising broccoli samples. (a) grading circles; (b) grouping heads with the grading circles; (c) uniformising heads with the specialised cutting board. The red line indicates the 10 cm stalk length from the lowest stem.

For each field treatment × block combination, samples were pre-selected to minimise the potential effects of head maturity/size on the experimental result. Ideally, all samples would have been from a single diameter category. However, despite planning efforts, after harvest, there were insufficient heads in any one category for them to be solely allocated to storage conditions. Consequently, samples were chosen to cover as many head size categories as possible, with the aim of using an equal proportion of heads from the diameter categories to ensure uniformity for storage treatments within field-treated blocks. However, practically, this outcome could not be perfectly achieved, which resulted in a slightly unequal proportion of head sizes from each diameter category, due to the limited number of heads available. The resulting outcome of these decisions is detailed in Table 4.1 for Trial 1, and Table 5.2 and Table 5.3 for storage at 1 and 4 °C in Trial 2, respectively.

After diameter size grading, the stalk length was made uniform, being 10 cm from the lowest part of the stem. To achieve this, a specialised broccoli cutting board was designed to ensure uniformity and efficiency (Figure 3.3c). The broccoli head was positioned against the left wall, and a movable plate was positioned against the lowest stem of the broccoli head. The stalk was then cut at the designated red line, ensuring a consistent length of 10 cm from the lowest part of the stem. After trimming the stalks, individual broccoli heads were then labelled using Tyvek waterproof colour-code tags. The tags were fixed to the lowest small stem at the base of the head. The heads were then weighed, and their initial weight recorded.

3.3.3.2 Storage

For Trial 1, a temperature-controlled room (TCR) was used to set up a flowthrough experiment with 12 barrels at 1 °C and 98% RH. Ten broccoli heads (representing a field treatment × block combination) were packaged in each barrel (60 Litres). Six barrels, one for

each field treatment × block combination, were continuously supplied with air. The other six barrels were supplied with 1 μL·L^{-1} ethylene in air for 29 days.

For Trial 2, 24 storage treatments were obtained by conducting the same experimental design in Trial 1, but at two different storage temperatures. Broccoli heads were stored at 98% RH and 1 °C (TCR 2) and 4 °C (TCR 5), for 28 and 18 days, respectively. As in Trial 1, ten broccoli heads (per field treatment × block combination) were packaged in one barrel. Gas flow conditions (1 μL·L^{-1} ethylene in air or clean air) were split evenly (as was done in Trial 1) for both storage temperatures. Gas conditions establishment and checking were done in the same way as Trial 1.

3.3.3.3 Flow-through system establishment

The ethylene flow was generated by using mass flow controllers (GSC-B9TABB23/21, Vögtlin instruments GmbH, Aesch, Switzerland) to mix in 100 μL·L^{-1} ethylene with air. Nominally, 100 μL·L^{-1} ethylene standards (94 ± 5 μL·L^{-1} C_2H_4 mixed with 20.7% ± 0.4% O_2 and balanced N_2; BOsC, NSW, Australia) were diluted with dry air to achieve 1 μL·L^{-1} ethylene concentration. Flows of dry air and ethylene standard were set with mass flow controllers first and then mixed using manifold units to create a total flow. Needle values were used to control the input flow rate to each of the barrels. Both treatments were humidified by bubbling through a 10% glycerol water solution to achieve a delivered 98% RH before flowing to the barrels (Forney & Brandl, 1992).

Upon sealing, approximately 4.5 kg of broccoli was in each barrel. According to Phuong et al. (2022), the respiration rate of broccoli at 1 °C is about 8 μmol·kg^{-1}·s^{-1} (119.68 mg CO_2 kg^{-1}·h^{-1} at 0 °C), resulting in a CO_2 production rate of 4.86 mL·min^{-1} from 4.5 kg broccoli (Equation 3.1). In order to limit the increase of CO_2 change inside the barrel to < 0.6%, a flow rate (*Fr*) of 810 mL·min^{-1} at 1 °C was set up (Equation 3.2). In Trial 2, TCR 2 (1 °C) and TCR 5 (4 °C) were separate rooms, which were supplied from a single flow-through manifold to standardise gas composition. Thus, ethylene concentration was maintained at 1 μL·L^{-1} in both rooms for ethylene-treated barrels, with per-barrel flow rate set at 810 L· min^{-1}.

$$R\,[CO_2] = \frac{r\,(CO_2)\,\times M}{C \times 60} \qquad \textit{Equation 3.1}$$

Where R [CO_2] (mL·min^{-1}) is 4.5 kg (M) broccoli produced CO_2. Respiration rate (mL·kg^{-1}·h^{-1}) is converted to r (CO_2) (mol·kg^{-1}·s^{-1}) using the factor $C = \frac{3.341\times10^{-11}\times P}{(T+273.15)}$, P (Pa) is

atmospheric pressure and T (°C) is the temperature (Banks et al., 1995). 60 converts hours to minutes. Fr is flow rate in mL·min^{-1}.

$$Fr = \frac{R\ [CO_2]\ \times 100}{0.6} \qquad \textit{Equation 3.2}$$

In order to prevent the broccoli heads from sitting in condensation during storage in the flow through experiment, the heads were placed on top of a layer of weave polypropylene mesh bags within the barrel. Nine mesh bags (cushions) were placed into one other mesh bag, with two of these cushions used in the bottom of each barrel.

Temperature and humidity within each barrel and TCR were monitored with iButton® temperature/humidity loggers (DS1923, Maxim Integrated, USA). For Trial 1, where the TCR was set at 1 °C, the resulting temperature and RH inside the barrels were recorded as 1.4 ± 0.1 °C and approximately 100% RH. For Trial 2, where two temperature-controlled rooms were set at 1 °C and 4 °C, respectively, the resulting conditions were 1.9 ± 0.4 °C and 6.7 ± 0.4 °C and approximately 100% RH (for nominally 4 °C).

The ethylene concentration of the output of barrels was checked twice weekly by using the ETD-300 ethylene detector with a VC-1 valve control box and a CAT-1 catalyser (Sense B·V., The Netherlands) or the MACView® portable ethylene postharvest gas analyser (PHGA) (environmental monitoring systems (EMS) B.V., Netherlands). Both types of equipment were used to improve the effectiveness and accuracy of the measurement (Figure 3.4). The monitoring results and ranges are provided in results Chapter 4 and Chapter 5.

In Trial 1, the ETD-300 ethylene detector and the PHGA were employed to measure the output of each barrel. Two cuvettes were set up (Figure 3.4): Cuvette 1 was used for barrel ethylene measurement and cuvette 2 served as a zero calibration as the flow recycled from the catalyser. Cuvette 2 measurements were designated after each barrel ethylene measurement. Both cuvettes had a continuous flow rate (FR) of 3 L·h^{-1} of hydrocarbon-free air flushing through them. Each cuvette was measured for six minutes to ensure a stable measurement was recorded. In Trial 2, the PHGA was used to check the ethylene concentration. The PHGA was calibrated at the beginning and end of the experiment by using two standard gases: 102 ± 5 µL·L^{-1} C_2H_4 mixed with 20.9% ± 0.4% O_2 and balanced with N_2 (BOC, NSW, Australia) and 0.534% ± 0.011% CO_2 mixed with 21.15% ± 0.10% O_2 and balanced N_2 (BOC, NSW, Australia). Four channels from the PHGA were connected to the outputs of barrels, with each measurement

taking 15 minutes (Figure 3.4) (Verschoor, 2017). The results of both C_2H_4 and CO_2 were recorded through the web-based portal (http://www.mymacview.com) of EMS.

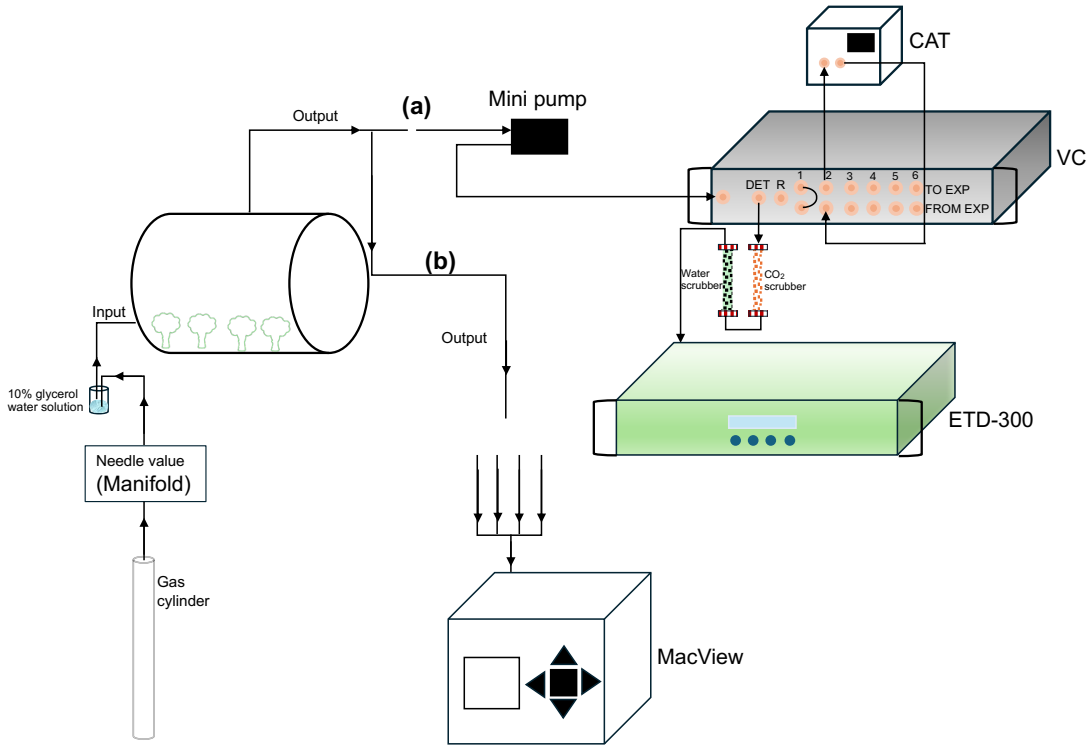

Figure 3.4 Schematic diagram of ethylene concentration checking for barrels with (a) ETD-300 ethylene detector and (b) MacView®. Image modified from Gwanpua et al. (2018) with permission. The outputs of barrel was directly connected to (b) four inputs of MacView®. For ETD-300 ethylene detector, a T-junction was connected to separate the output pipe from the barrels. A mini pump was connected to one of the output lines to draw gas, while a jar containing an ethylene scrubber was used to absorb excess ethylene and prevent its release into the room. The sampling gas was pumped from the barrel outlet to the valve controller (VC). Cuvette 1, which remained empty, was used to measure the sampling gas. Cuvette 2 was connected to the catalyser (CAT), and served as a zero calibration reference.

3.4 Physiology measurement

This study evaluated the potential impact of preharvest application of 1-MCP on the harvest physiology of the broccoli head. Specifically, the ethylene production and respiration rate of the broccoli branchlets were assessed.

3.4.1 Branchlets sample preparation

Central branchlets from three broccoli heads per field treated block were cut to fit in a 500 mL jar, which contained 57–84 g (with an average of 70 g) to ensure uniformity. In Trial 1, these samples' jars were kept at 20 °C for 3 days. In Trial 2, jars were stored at 4 °C for 11

days. Prior to the experiment, the weight of florets in each jar was determined by an electronic balance (3000D SCS, Precisa, Switzerland) at 0.001 g precision.

Branchlets were placed upright, with the cut stem facing down. Each jar had one input and one output pipe connected to a valve controller, which controlled the continuous gas flow rate and sampling to the ETD-300 ethylene detector (Sense B·V., Nijmegen, The Netherlands). Three value controllers provided 18 channels, matching the number of the sampled branchlets (3 × 6 blocks) used in this study.

3.4.2 Ethylene production measurement

Real-time ethylene concentration in the outflow from the cuvette was measured using the ETD-300 ethylene detector. Cuvettes had a continuous flow rate (*FR*) of 2 $L \cdot h^{-1}$ of hydrocarbon-free air flushing through them. Each sample cuvette was measured for eight minutes with the ethylene concentration in the final two minutes used as the measurement. The results were converted from ethylene concentration to ethylene production ($pmol \cdot kg^{-1} \cdot s^{-1}$) with the use of Equation 3.3 and Equation 3.4 (Gwanpua et al., 2018).

A zero-calibration value was obtained using an empty cuvette and recorded at the beginning and end of the experiment. Moreover, a baseline reading ($[C_2H_4]_b$, $nL \cdot L^{-1}$) obtained using the supplied air was recorded at the beginning and end of the experiment. The corrected real-time ethylene concentration ($[C_2H_4]$, $nL \cdot L^{-1}$) was calculated by using the raw reading of samples ($[C_2H_4]_r$, $nL \cdot L^{-1}$) minus the baseline reading.

$$[C_2H_4] = [C_2H_4]_r - [C_2H_4]_b \qquad \textit{Equation 3.3}$$

Where $[C_2H_4]$ is the corrected real-time ethylene concentration in $nL \cdot L^{-1}$, $[C_2H_4]_r$ is the actual raw reading of samples in $nL \cdot L^{-1}$ and $[C_2H_4]_b$ is the averaged baseline reading.

$$r[C_2H_4] = \frac{[C_2H_4] \times FR \times P \times 10^3}{m \times 3600 \times R \times T} \qquad \textit{Equation 3.4}$$

Where $r[C_2H_4]$ = ethylene production in $pmol \cdot kg^{-1} \cdot s^{-1}$; $[C_2H_4]$ = corrected real-time ethylene concentration in $nL \cdot L^{-1}$; FR = flow rate in $L \cdot h^{-1}$; P = atmospheric pressure in Pa; m = the mass of central florets in kg; R = molar gas constant (8314 $L \cdot Pa \cdot mol^{-1} \cdot K^{-1}$); T =

temperature in K; 10^3 = unit conversion from nmol to pmol; 3600 = unit conversion from seconds to hours.

3.4.3 CO_2 production measurement

Respiration rate was measured as CO_2 production (nmol·kg^{-1}·s^{-1}), which was calculated based on the CO_2 concentration established at steady state in the flow through cuvettes. The CO_2 concentration was quantified daily using O_2/CO_2 analyser with a CO_2 transducer (Analytical Development Company, Hoddesdon, UK), which was interfaced to an integrator (HP3396A, Hewlett Packard, USA). A 1 mL syringe was used to take a sample gas from each jar while it was connected to the valve controller to ETD-300 in flow through mode. The real-time flow rate (FR) for each jar was recorded from the ETD-300 mass flow controller system. In addition, the input CO_2 concentration $CO_{2\ in}$ was measured by sampling 1 mL of the air from the supply (input) with the syringe.

The CO_2 concentration produced by the central branchlet of individual broccoli heads was calculated by Equation 3.5, where 3600 converts hours to seconds and 10^9 converts moles to nanomoles.

$$rCO_2 = \frac{P \times FR \times (CO_{2\ out} - CO_{2\ in}) \times 10^9}{m \times 3600 \times R \times T} \qquad \textit{Equation 3.5}$$

Where: rCO_2 = respiration rate expressed in CO_2 production in nmol·kg^{-1}·s^{-1}; P = atmospheric pressure in Pa; FR = flow rate in L·h^{-1}; $CO_{2\ out}$ = CO_2 concentration of central florets in %; $CO_{2\ in}$ = CO_2 concentration of supplied air in %; m = the mass of central florets in kg; R = molar gas constant (8314 L·Pa·mol^{-1}·K^{-1}); T = temperature in K; Unit is converted from mol·kg^{-1}·s^{-1} to nmol·kg^{-1}·s^{-1} by multiplying 10^9.

3.5 Quality assessments

3.5.1 Weight loss

The weight of individual broccoli heads was determined via electronic balance (3000D SCS, Precisa, Switzerland) with a precision of 0.001 g. Weight loss was expressed as a percentage (%) of the initial weight, recorded on the day of harvest (Fernández-León et al., 2013; Wichrowska et al., 2021). Final weights were measured in the storage TCR in order to avoid condensation formation that influence the measurement. Weight loss was determined by application of Equation 3.6.

$$WL(\%) = \frac{initial\ weight - final\ weight}{initial\ weight} \times 100\% \qquad \textit{Equation 3.6}$$

3.5.2 Diameter

The diameter of each broccoli head at harvest and in the field was measured using a digital calliper (IP67, Mitutoyo, Japan). Acrylic extension boards were added to the lower jaws of the calliper to improve the ability to measure the broccoli heads (Figure 3.5). Both the largest diameter (D_1, determined by eye) and the diameter at 90° to the largest (D_2) were recorded and collectively averaged. The surface area (A_s, mm^2) was calculated with the Equation 3.7 to estimate of the 2D size of broccoli head.

$$A_s = \frac{\pi \times D_1 \times D_2}{4} \qquad \textit{Equation 3.7}$$

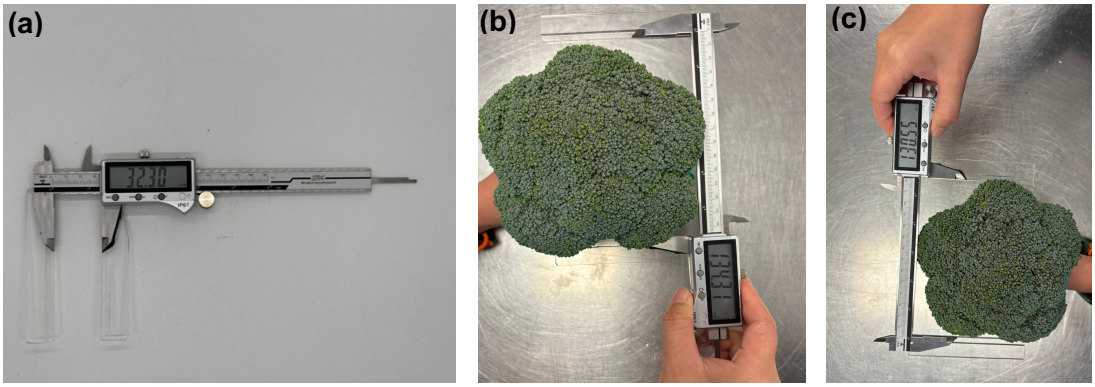

Figure 3.5 The digital calliper for broccoli diameter measurement. (a) The digital calliper with acrylic extension boards; (b) measuring the largest diameter; (c) measuring the diameter at 90° to the largest.

3.5.3 Colour measurement

Broccoli colour was measured with a spectrophotometer (CM-2600d, Konica Minolta, Japan) at 20 °C (Lu, 2020). Four locations of the broccoli head were measured and averaged. Two peripheral florets and two centre floret locations were used as individual sampling points (Figure 3.6). Gaps between different florets were avoided.

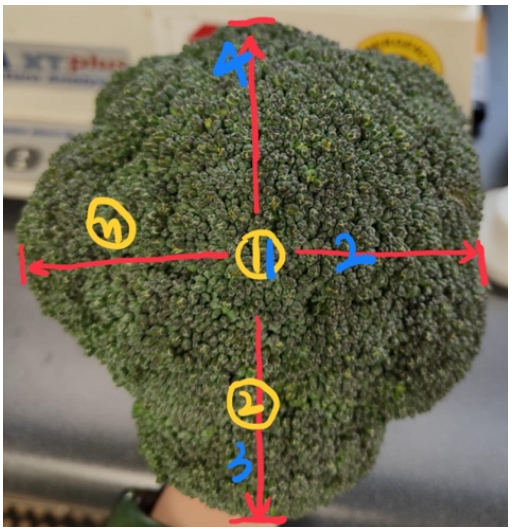

Figure 3.6. Sampling points for head diameter, colour measurement (blue numbers), and head floret flexibility (yellow numbers). Red lines indicate the diameter measurement. Blue numbers suggest the sequence and location for colour measurement. Yellow numbers suggest the sequence and location of head floret flexibility measurement: 1) The top points of broccoli head; 2) the junction points among florets and below the top point; 3) the junction points among florets and 90° to the second point.

In addition to the instrumental measurement, five (of 10) broccoli from each barrel were photographed. Five broccoli heads were selected based on diameter grades for photography: one of the smallest (< 110 mm), three medium-sized heads (110–150 mm), and one of the largest (> 150 mm). All pictures were taken using a Canon EOS 600D with an exposure time of 1/160 seconds and an ISO speed of 400, with the broccoli arranged in the same order in a lightbox fitted with D65 lighting for consistent illumination. The colour of individual broccoli heads was also scored on a scale of 1 to 5 based on the broccoli yellowing scale from Cantwell and Suslow (2002) (Figure 2.6). The rot development of broccoli was recorded descriptively.

3.5.4 Head firmness

After considering the methods from Fernández-León et al. (2013), Paulsen et al. (2022), and Guirao et al. (2024), a modified method for head firmness measurement was used in this study. Head firmness was defined as the compression force to achieve a 5% deformation of the broccoli head. Head firmness was evaluated with a stable micro systems texture analyser TA-XT2i (Aname, Madrid, Spain) using a compression test on whole broccoli heads. A 51-mm diameter flat aluminium plate was employed to apply force until a 5% deformation was performed at a speed of 2 $mm \cdot s^{-1}$. The maximum force (N) was selected, and the maximum displacement was recorded. Each broccoli head was tested twice, with a 90° rotation between

measurements in order to capture firmness data from two axes: the largest diameter and the diameter perpendicular to it. The broccoli head was positioned perpendicular to the surface of the flat plate by hand (Figure 3.7). Firmness values were averaged and expressed as maximum force recorded divided by the maximum displacement ($N \cdot mm^{-1}$). Additionally, the head firmness was also scored on a scale of 0 to 5 as developed by Gillies and Toivonen (1995) (Table 2.2).

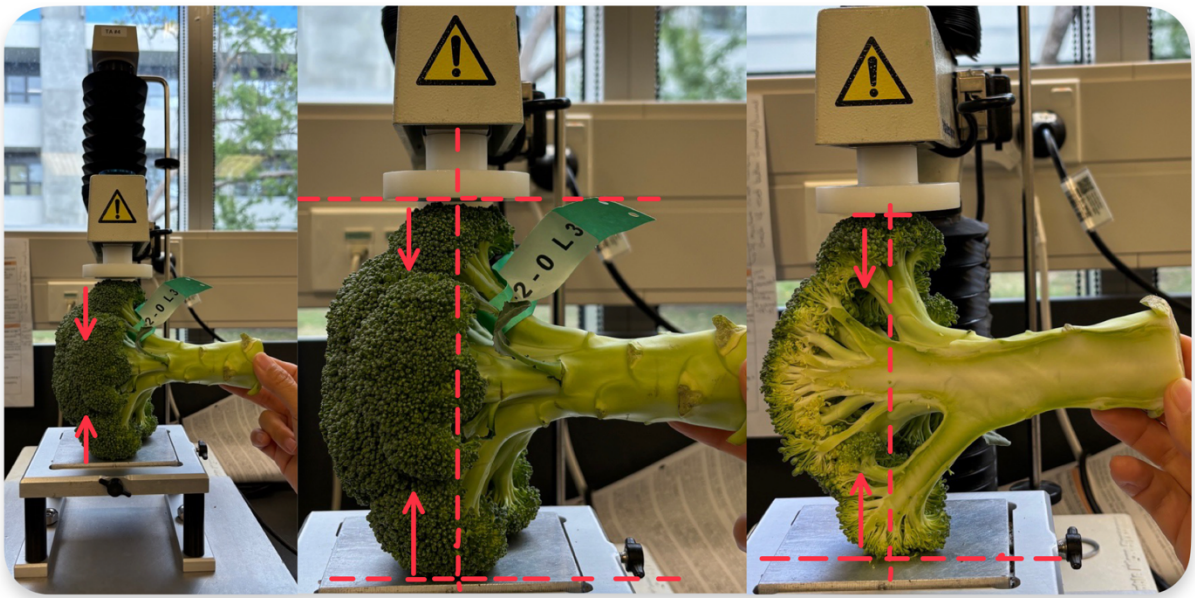

Figure 3.7 Head firmness measurement of broccoli. (a) The probe was positioned perpendicular to the surface of the broccoli head; (b) a 5% deformation of the head; (c) head was compressed by the probe. The red arrows in the figures indicate the direction and movement of the applied force.

3.5.5 Head floret flexibility

The denseness of broccoli florets was defined as compactness, which is considered a critical quality trait in broccoli. A few studies have involved the measurement of head firmness to determine the compactness (Fernández-León et al., 2013; Paulsen et al., 2022; Guirao et al., 2024) (Section 2.1.3.1.2). However, when the head firmness was measured, not only the florets but also the stems were compressed during the measurement process.

A broccoli head floret flexibility method was utilised in an attempt to measure how easily the florets can be separated from each other (Figure 3.8). A P/45c probe (stable micro systems) was used to test the force to part two florets using a stable micro systems texture analyser TA-XT2i (Spain). Settings used were a trigger force of 5 g, a distance of 5 mm (try to part the florets not include the stems below), a speed of 2 $mm \cdot s^{-1}$, and a pre-test and post-test

speed at 10 mm·s^{-1}. Three measurements were taken for each broccoli head (Figure 3.6). The results were expressed as the peak force (N).

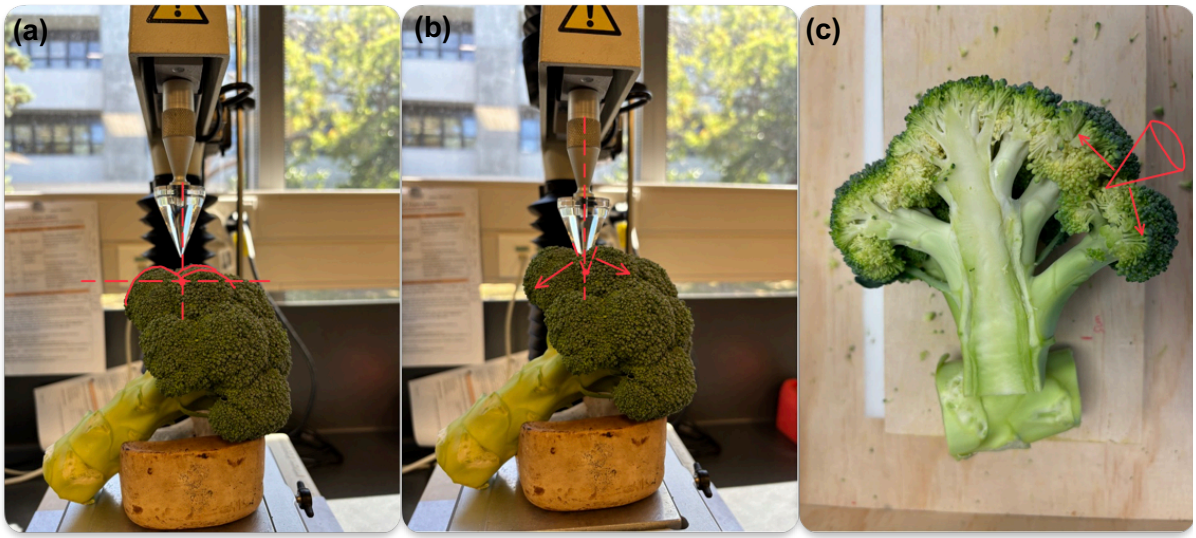

Figure 3.8 The measurement of head floret flexibility of broccoli head. (a) The probe was positioned perpendicular to the surface of the head; (b) 5 mm distance of penetration between the florets; (c) florets were separated by the probe. The red lines in the figures indicated the direction and movement of the applied force.

The floret flexibility results of two tested samples were shown in Figure 3.9. The fresh and green broccoli head required higher peak forces to separate the adjacement florets than that of the yellow and loose broccoli head. Thus, the peak force (N) may reflect the compactness of broccoli heads. A higher peak force means that the florets are tightly packed (compactness of the broccoli), suggesting that the broccoli heads are fresher. Conversely, looser broccoli heads showed a smaller peak force when the force was applied to separate the florets.

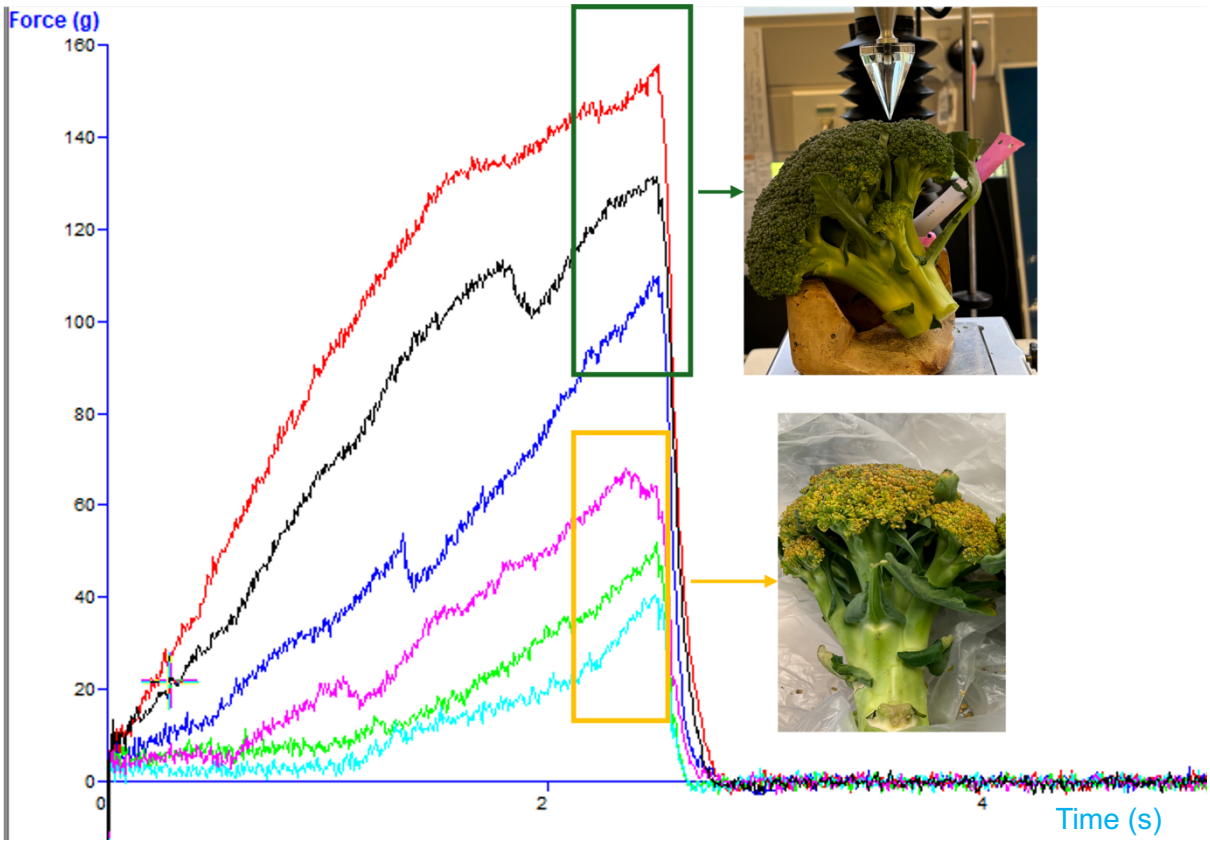

Figure 3.9 Sample data for floret flexibility in two broccoli heads. The x-axis represents the seconds used when the related force was applied to separate the adjacent florets. A green and fresh broccoli head required higher force to separate florets and thus higher peak force may represent tighter broccoli florets.

3.5.6 Stalk compression stress

After considering the methods for head firmness compression and the methods for stalk compression from Zhao et al. (2022), Ben-Fadhel et al. (2018), and Singh et al. (2018), a modified method was used in this study. The sample for the stalk compression test was taken 5 cm away from the stalk of the lowest floret and cut into 15 mm width (w) with a mandolin slicer (Figure 3.10a, b). Stalk compression strength was determined by a stable micro systems texture analyser TA-XT2i (Aname, Madrid, Spain). The sample was placed on a metal plate and compressed using a 51-mm diameter flat probe to 5% strain at a speed of 2 mm·s^{-1} with a trigger force of 5 g. Each sample was tested twice, with a 90° rotation between measurements to capture data from two axes. These dimensions were the direction with the largest diameter and the direction perpendicular to it. The maximum distance (h) of compression was recorded, allowing the whole diameter of the stem (d) to be calculated:

$$d = \frac{h}{0.05}$$ *Equation 3.8*

The compression stress (σ, N·mm^{-2}) was calculated from the maximum force (F, N) divided by the cross-sectional area of the broccoli stalk at the plane (A, mm^2) (Zhao et al., 2022).

$$\sigma = \frac{F}{A}$$ *Equation 3.9*

The cross-sectional area of the broccoli stalk at 5% strain compression can be calculated as follow from the website of Pierce (2023).

$$A = 2\sqrt{r^2 - (r-h)^2 \times w}$$ *Equation 3.10*

$$r = {}^{d}/_{2}$$ *Equation 3.11*

Where h is the maximum distance recorded by the software; w is the width of the cutting samples (Figure 3.11).

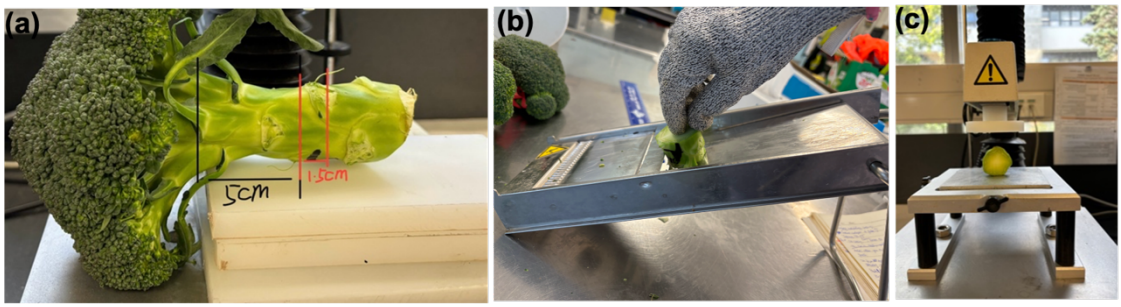

Figure 3.10 Stalk sample and stalk compression stress measurement. (a) Sampled location of broccoli stalk; (b) sample was cut into 15 mm width with a mandolin slicer; (c) sample was placed on a metal plate and compressed using the flat probe.

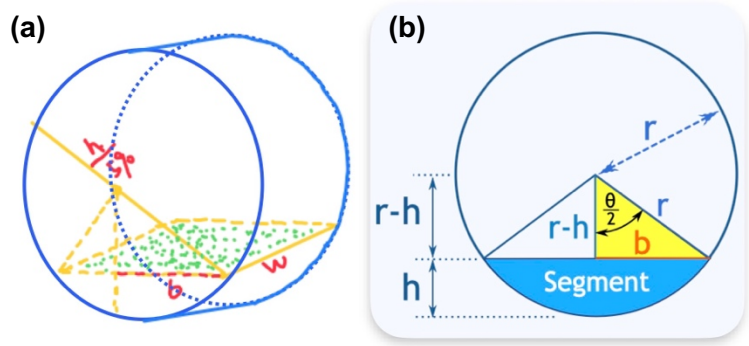

Figure 3.11 Compression stress of the stalk model with 5% deformation. Image (b) was taken from the website of Pierce (2023) with permission. (a) the area defined by the yellow line (with green dots) is the cross-sectional area of the broccoli stalk when the stalk is compressed until a 5% deformation is achieved; (b) cross-sectional area diagram.

3.6 Statistical analysis

The data were expressed as the mean of the independent broccoli heads ± standard deviation (SD). Differences in means were identified using a two-way (field treatments ("Field control" and "1-MCP") and blocks) analysis of variance (ANOVA) when studying effects at harvest. A general linear model (GLM) was used to analyse the effects of field treatments ("Field control" and "1-MCP"), storage treatments ("Air" and "1 $\mu L \cdot L^{-1}$ ethylene") and blocks on broccoli quality after storage. The model included main effects and interactions among the three factors. Tukey's Honestly Significant Difference (HSD) test was used for means comparisons if ANOVA or GLM identified significant differences between the mean values. The statistical analysis was performed using RStudio 4.3.3 software (Posit, Boston, MA). All graphs were generated with RStudio by using the package ggplot2.

A series of logic steps were used to identify and present the data that was significant and interesting. For the quality attribute measures of broccoli (both at harvest and after storage), those factors that had significant p-values were identified first. The experimental factors, including field treatment ("Field control" and "1-MCP") and storage treatment ("Air" and "1 $\mu L \cdot L^{-1}$ ethylene"), that contributed to differences in quality attributes, were then identified. Only significant factors were subsequently plotted, and where possible, when multiple factors influenced a single quality attribute, these were shown adjacent to one another in a single figure. For clarity, field treatments ("Field control" and "1-MCP") and storage treatments ("Air" and "1 $\mu L \cdot L^{-1}$ ethylene") are referred to in abbreviated form as "1-MCP" and "ethylene", respectively, in the following two chapters. The term "block" is used consistently throughout to refer to the blocking of replicates.

For physiological measurements, data were averaged using three broccoli branchlets. For ethylene production of broccoli branchlets, the data were averaged from three broccoli branchlets every 4 hours after harvest. For example, “4d 4h” means the average of 3 broccoli branchlets between 0 and 6 hours after harvest (4d); “4d 16h” = the average of between 14 and 18 hours after harvest.

Chapter 4. Impacts of Preharvest Application of 1-MCP on 'Nobel' Broccoli Quality During Low-temperature Storage

4.1 Introduction

In the literature review, ethylene was shown to accelerate the postharvest senescence of broccoli, resulting in yellowing, even at relatively low concentrations (< 0.02 $\mu L \cdot L^{-1}$) (Martínez-Romero et al., 2007). However, the sensitivity of broccoli to ethylene can be diminished by storing at low temperature (e.g., 0–1 °C) (Wills & Golding, 2016). Alternatively, 1-MCP (as a non-toxic commercial ethylene response inhibitor) can further reduce ethylene responses, including ethylene production, respiration rate, and chlorophyll degradation in broccoli heads (Section 2.4). Fan and Mattheis (2000) demonstrated that 1-MCP treatment reduced broccoli branchlets' yellowing and respiration rate and negated ethylene's impact at 10 °C for 12 days at continuous ethylene exposure (1.0 $\mu L \cdot L^{-1}$). Thus, 1-MCP might be a useful tool to protect broccoli from ethylene exposure in the supply chain. However, inconsistent effects of 1-MCP were found by Forney et al. (2003), Grzegorzewska et al. (2023), and Ghimire et al. (2024), suggesting that more research is needed.

The literature review has identified that no published works have investigated the effects of preharvest application of 1-MCP on broccoli. There were commercial trials of preharvest application of 1-MCP on broccoli in California and Western Australia, with good quality results reported from those trials. However, no work has yet been done in New Zealand (M. Punter, personal communication, August 2, 2024).

In this study, broccoli heads came from transplanted plants and had already passed the juvenile phase, thus excluding the variation of this phase (Lindemann-Zutz, 2015). Broccoli exhibits a significant heterogeneity of head size in the field, likely caused by strong variability in the timing of head initiation (Lindemann-Zutz et al., 2016a). Temperature and accumulated solar radiation strongly explained the variation in head initiation (Section 2.1.1). Thus, multiple harvests occurred by selective hand in commercial harvests. However, broccoli used as experimental materials was harvested on a single commercial harvest day to control timing from 1-MCP treatment and simplify the postharvest storage work (Section 3.3.2).

As discussed in Section 2.6, based on the results of preharvest application of 1-MCP on apples, in the field, one possible advantage of preharvest application of 1-MCP is improved uniformity at maturity; this reduces harvest times for broccoli and thus labour costs for

harvesting. Another advantage is that broccoli might grow bigger heads without over-maturing and this would benefit growers with higher yields and income.

Our hypothesis was that preharvest application of 1-MCP would result in less mature broccoli heads at harvest compared with untreated broccoli, and that treated heads would maintain colour, weight, and texture better during storage under ethylene exposure. The experiment was conducted with winter-growing broccoli 'Nobel', and some methods developments, such as head floret flexibility, were designed and tested in Section 3.5.4. This study used continuous ethylene exposure (1.0 $\mu L \cdot L^{-1}$) as a storage condition at 1 °C, the same ethylene concentration but at a different temperature from Fan and Mattheis (2000). This chapter provides the results and discussion for Trial 1. Associated methods are provided in Chapter 3.

4.2 Preharvest 1-MCP treatment and its possible physiological impacts

Before the preharvest application of 1-MCP, broccoli heads were observed to have grown heterogeneously. Block 2 seemed to have larger heads than blocks 1 and 3. Field control group generally seemed to have bigger heads than the 1-MCP application area.

When 1-MCP applied in the field, I assumed that all broccoli plants already completed the transition from vegetable to reproduction growth (head imitation, primarily cell division) and be in head growth stage (both cell division and expansion) (Section 2.1.1 and Figure 6.2): In source-sink relation, mature leaves were primary source via photosynthesis, actively providing nutrients and energy to head – a strong and primary sink at this stage.

1-MCP is dissolved in water for spraying, but most of the 1-MCP gas remains in a bound complex during tank mixing and spraying operations due to high concentration. Once spray droplets are on leaves and heads (Figure 4.1) and exposed to ambient moisture in the field, gaseous 1-MCP gradually releases into the microenvironment. It then reaches these leaves and heads, diffuses in, binds ethylene receptors (ETRs) (Figure 2.10) and blocks ethylene action, thereby delaying senescence. However, it is possible that new ethylene receptors may synthesise after 1-MCP blocked the current receptors, and plants and tissues may thus restore ethylene sensitivity (Dias et al., 2021). Cell division continues and 1-MCP may not block the ethylene action in new cells.

Figure 4.1 The droplets on leaves and heads after 1-MCP spraying.

After spraying of 1-MCP, 1-MCP solution was mostly applied on leaves, with some on heads, leading to the blockage of ethylene roles in leaves and flowers (Section 2.1.1.3). 1-MCP (may similar to *etr1* mutation) blocked the ethylene receptors, and thus the heads and leaves could not detect ethylene, the senescence of heads and leaves would be delayed – the leaves could active longer and remain green, supplying more photosynthates to the developing broccoli heads – result from less breakdown of chloroplast and degradation of plastid constituents (Krupinska & Humbeck, 2004). Delaying leaves senescence could potentially increase head growth, size, and yield. If the blockage of ethylene receptors by 1-MCP could last during and after harvest, 1-MCP treated broccoli could potentially lower senescence rate, and thus maintain storage quality and extend shelf life in the supply chain.

4.3 At-harvest quality

As described in Section 3.3.3.1, for each preharvest treated or untreated block × postharvest treatment combination, samples were pre-selected to minimise the potential effects of head maturity/size on the experimental result. Ideally, all samples would have been from a single diameter category. However, despite planning efforts, after harvest, there were insufficient heads in any one category for them to be solely allocated to storage conditions. Consequently, samples were chosen to cover as many head size categories as possible, with the aim of using an equal proportion of heads from the diameter categories to ensure uniformity for storage treatments within field-treated blocks. However, practically, this outcome could not be perfectly achieved, which resulted in a slightly unequal proportion of head sizes from each diameter category, due to the limited number of heads available. The resulting outcome of these decisions is detailed in Table 4.1. All field-treated blocks had the same allocation of diameter categories for broccoli heads, except for F1.

Table 4.1 The allocation of diameter categories for broccoli heads storage treatment in each block. Where: F = field control, M = 1-MCP. Different letters show significant differences ($p < 0.05$, $n = 40$). Data represent mean ± standard deviation (SD).

Block	Storage treatment	110–120 mm	120–150 mm	> 150 mm	Diameter range (mm)
F1	Air	3	5	2	123 ± 10 b
	Ethylene	3	5	2	126 ± 12 b
M1	Air	1	8	1	125 ± 10 b
	Ethylene	1	8	1	124 ± 11 b
F2	Air	1	8	1	126 ± 9 a
	Ethylene	1	8	1	129 ± 8 a
M2	Air	1	8	1	128 ± 11 a
	Ethylene	1	8	1	134 ± 10 a
F3	Air	1	8	1	130 ± 13 ab
	Ethylene	1	8	1	126 ± 12 ab
M3	Air	1	8	1	126 ± 11 ab
	Ethylene	1	8	1	130 ± 14 ab

At harvest, broccoli heads had an average diameter of 127 ± 10 mm, weighed 334 ± 51 g, and exhibited colour values of 123.4 ± 3.1 hue angle (h°), 36.6 ± 1.1 for lightness (L*) and 9.4 ± 1.4 for chroma (C*). Stalk compression stress was measured at an average of 0.06 ± 0.02 $N \cdot mm^{-2}$, and head floret flexibility was 1.68 ± 0.46 N. Preharvest application of 1-MCP was not found to significantly impact any of the at harvest quality attributes, except for at-harvest weight ($p > 0.05$, Table 4.2). 1-MCP treated broccoli had a significant smaller weight of 326 ± 54 g than that of broccoli from the field control group (345 ± 47 g), which was consistent with the observation before 1-MCP application.

Although the three blocks were adjacent and assumed to have a uniform growth pattern, data analysis revealed that planting block significantly impacted the diameter at harvest (Table 4.2 and Figure 4.2), consistent with observation. Heads from block 2 had the largest diameter of 130 ± 9 mm, larger than that of broccoli in block 1 (124 ± 10 mm). This indicated that block 1 possibly had less mature broccoli heads (Table 4.1). The diameter of broccoli heads varied within block 3 (128 ± 12 mm) more than that of blocks 1 and 2. However, most broccoli diameters (86.2%) were within the 110–140 mm range in all blocks.

Table 4.2 P-value table for broccoli at harvest quality

Factor	Diameter (mm)	Weight (g)	Colour			Floret WL (%)	Head floret flexibility (N)	Stalk compression stress ($N \cdot mm^{-2}$)
			L*	C*	h°			
1-MCP	0.360	0.044	0.678	0.478	0.830	0.278	0.610	0.138
Sum of squares (%)		3.36						
n	*69*	*60*	*9*	*9*	*9*	*9*	*9*	*9*
Block	0.028	0.168	0.988	0.560	0.947	0.447	0.434	0.391
Sum of squares (%)	5.21							
n	46	40	6	6	6	6	6	6
1-MCP × Block	0.833	0.428	0.477	0.905	0.894	0.344	0.066	0.760
n	*23*	*20*	*3*	*3*	*3*	*3*	*3*	*3*

The following image shows the at-harvest diameters by blocks. Broccoli heads from block 2 had a significantly bigger diameter than those from block 1.

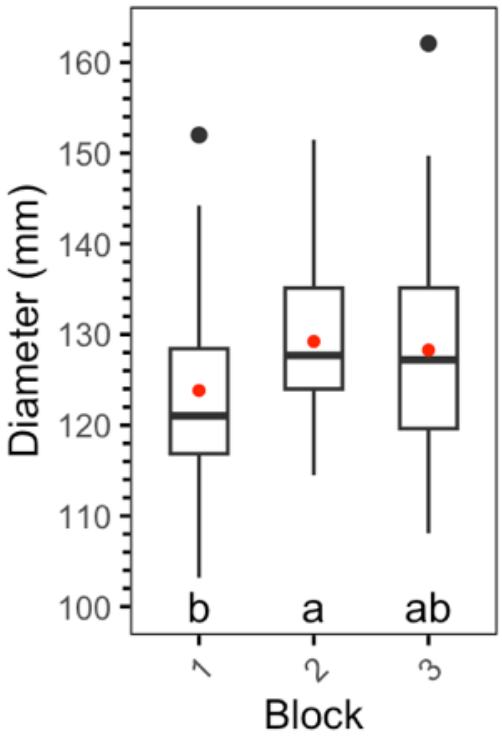

Figure 4.2 The block significantly impacted the diameter of broccoli. Different letters show significant differences ($p < 0.05$, $n = 46$). The line inside the box represents the median of the population, and the red point represents the mean value of the population.

4.4 Physiological measurement at 20 °C

The plan was to take three broccoli heads (representing three diameter categories) for physiology measurement from each of the six blocks. In practice, for blocks 2 and 3, only heads

from 120–150 mm were used (Table 4.3). For F1, three broccoli heads from three categorized diameter groups were used, while two categorized groups were used in M1 (Table 4.3).

During 3 days at 20 °C , the average weight loss of florets was 6.38 ± 1.76% for field control and 7.75 ± 2.95% for 1-MCP treated broccoli. Average respiration rate (CO_2 production) of broccoli branchlets 5 to 7 days after 1-MCP treatments is listed in Table 4.4. There was no significant difference caused by 1-MCP treatments (5 days after harvest) on any day.

Table 4.3 The allocation of diameter categories of broccoli heads for ethylene production and CO_2 measurement in each block

Block	< 110 mm	110–120 mm	120–150 mm	> 150 mm
F1	1	1	1	0
M1	0	1	2	0
F2	0	0	3	0
M2	0	0	3	0
F3	0	0	3	0
M3	0	0	3	0

Table 4.4 Respiration rate of broccoli branchlets at 20 °C for 3 days. Data represent mean ± standard deviation (SD), n = 3 (because there are only 18 channels for ETD-300 ethylene detector, 3 replications × 3 blocks × 2 preharvest treatments). Broccoli was sprayed with 1-MCP on Day 0 and harvested on Day 4 as indicated by the timeline in Chapter 3.

Day after treatments	Respiration rate in CO_2 production ($nmol \cdot kg^{-1} \cdot s^{-1}$)	
	1-MCP	Field control
5	1647 ± 199	1563 ± 111
6	1726 ± 174	1648 ± 109
7	1692 ± 333	1574 ± 196

Ethylene production of broccoli branchlets increased and then decreased during 3 days at 20 °C (Figure 4.3). Planting block significantly affected the ethylene production of broccoli florets ($p < 0.001$), with block 1 seemingly having a more dynamic response than blocks 2 and 3, while treatment difference was not evident for block 3. When ethylene production differences were evident, 1-MCP treated broccoli florets had a significantly higher ethylene production than that of untreated broccoli florets ($p < 0.001$). The ethylene production of 1-MCP treated broccoli had a delayed peak (4d 16h) compared to untreated broccoli (4d 12h).

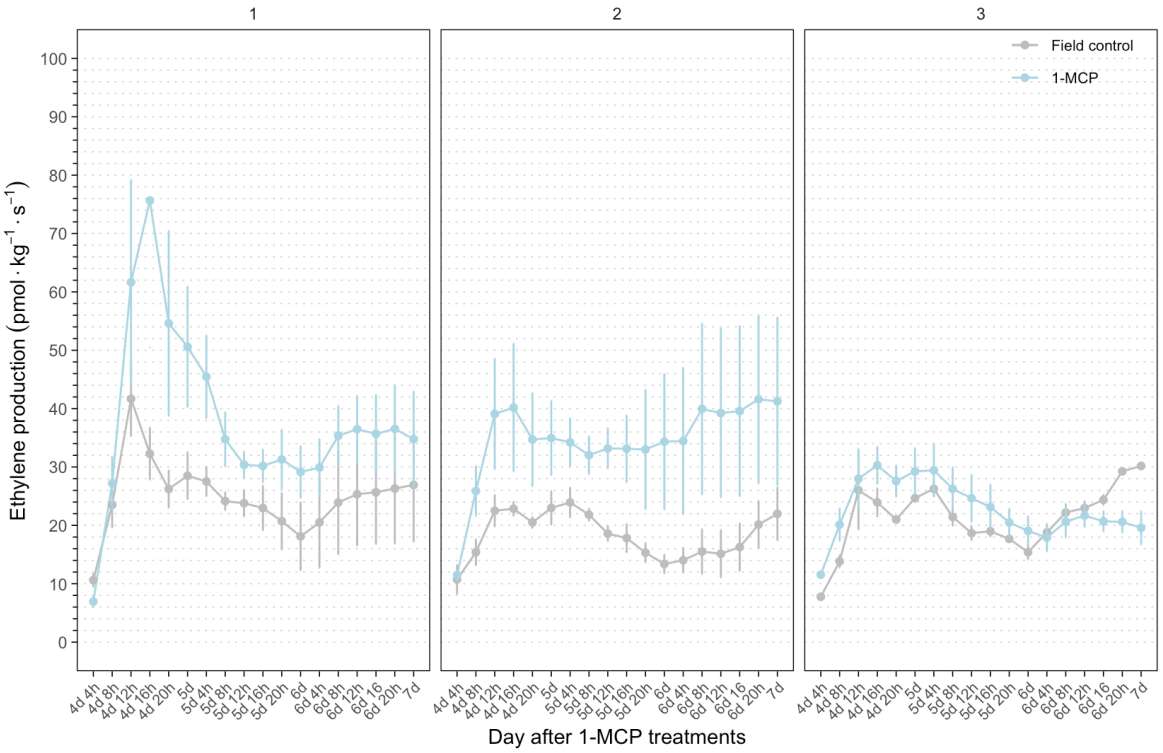

Figure 4.3 Ethylene production of the central branchlets over 3 days at 20 °C. Data from each block is presented separately. Each point represents the average of three samples over a 240-minute period. 1-MCP was sprayed in the 1-MCP applied area on day 0. The broccoli heads were harvested on day 4 (4d). Broccoli was harvested at around 12 °C (4d) and then transported to a 20 °C laboratory after 2 h. Ethylene measurement started 5.5 h after harvest (4d 4h in the graph) (Figure 3.1). For example, "4d 4h" means the average of 3 broccoli branchlets between 0 and 6 hours after harvest (4d); "4d 16h" = the average of between 14 and 18 hours after harvest. The error bars are standard errors.

4.5 Storage conditions and observation

The average temperature and RH for the storage room were 1.1 ± 0.1 °C and 94.0 ± 0.1%. Inside the barrels where the broccoli was held, the average temperature and RH were 1.4 ± 0.1 °C and approximately 100%, respectively. Condensation was observed at the bottom of the barrels, not on the broccoli, due to the mesh bags. Ethylene concentrations in the broccoli holding barrels under continuously flow system were stable, with an average of 1044.6 ± 27.0 $nL \cdot L^{-1}$ for ethylene-treated barrels and 9.8 ± 14.0 $nL \cdot L^{-1}$ for air-treated barrels. The accumulated CO_2 concentrations were 0.13 ± 0.03% and 0.11 ± 0.02% for ethylene and air-treated barrels, respectively.

4.6 After storage quality

After being stored, the broccoli heads were still marketable without any visually noticeable differences between the treatment and control groups as influenced by either 1-MCP

or ethylene. Broccoli heads maintained a deep green colour and great compactness, without any opening buds (Figure 4.4). After storage, broccoli had an average weight loss of 3.3 ± 0.4%, and exhibited colour values of 120.0 ± 2.53 for h°, 37.1 ± 1.8 for lightness (L*) and 10.9 ± 1.9 for chroma (C*). Stalk compression stress averaged 0.05 ± 0.02 $N \cdot mm^{-2}$, head floret flexibility averaged 1.70 ± 0.53 N and head firmness averaged 1.35 ± 0.38 $N \cdot mm^{-1}$.

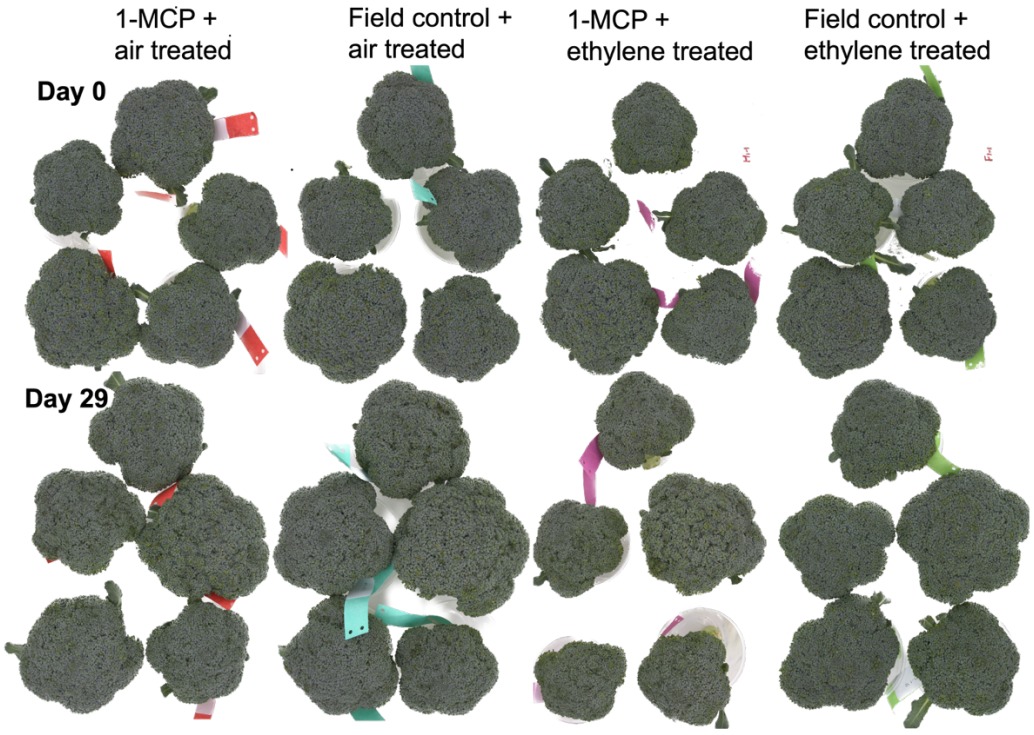

Figure 4.4 Visual quality of representative broccoli heads after being continuously exposed to ethylene and air for 29 days at 1.4 °C.

Compared to the at-harvest value, the L* and C* values slightly increased, while h° values slightly decreased. Stalk compression stress was slightly reduced, but head floret flexibility did not change after harvest.

Each of the post-storage attribute data was analysed for the effects of each factor (preharvest 1-MCP, ethylene in storage or growing block) and the interactions of all factors using a full ANOVA (Section 3.6). In order to identify the significant results, a p-value table demonstrating the effects on the quality outcomes is provided (Table 4.5).

The following sections detail each factor that influenced each quality attribute. The data has been organised to detail how each quality attribute responds to the influence of each factor and the combination of the factors.

None of the three factors significantly influenced head firmness (1.35 ± 0.38 $N \cdot mm^{-1}$) and stalk compression stress (0.05 ± 0.02 $N \cdot mm^{-2}$) (data not shown). Likewise, none of the three factors significantly affected weight loss (7.1 ± 2.5%) or respiration rate (1645 ± 203 $nmol \cdot kg^{-1} \cdot s^{-1}$) of the central branchlets. Given that no effects were significant, no results were presented in this chapter.

Table 4.5 P-value table for broccoli quality as influenced by preharvest 1-MCP treatment, in storage ethylene treatment and in-field block after storage at 1.4 °C and 100% RH for 29 days

Factor	Weight loss (%)	Colour			Head floret flexibility (N)	Head firmness (N)	Stalk compression stress ($N \cdot mm^{-2}$)
		L*	C*	h°			
1-MCP *(n=60)*	0.348	0.002	0.970	0.717	0.049	0.052	0.322
Sum of squares (%)		5.90			2.86		
Ethylene *(n=60)*	0.032	0.202	0.873	0.334	0.144	0.487	0.924
Block *(n=40)*	0.720	< 0.001	0.010	0.008	0.262	0.993	0.765
Sum of squares (%)		25.20	6.94	8.28			
1-MCP × Ethylene *(n=30)*	0.014	0.271	0.792	0.397	0.200	0.219	0.275
Sum of squares (%)	3.86						
1-MCP × Block *(n=20)*	0.008	0.470	0.087	0.399	0.006	0.161	0.540
Sum of squares (%)					7.86		
Ethylene × Block *(n=20)*	< 0.001	0.731	0.003	0.604	0.018	0.759	0.371
Sum of squares (%)	17.22		9.12		6.05		
1-MCP × Ethylene × Block (n=10)	0.154	0.317	0.201	0.729	0.837	0.205	0.654

4.6.1 Weight loss

For broccoli not treated with 1-MCP, ethylene treatment resulted in a higher weight loss than air-treated broccoli (Figure 4.5a), suggesting that these may be potential benefits of applying 1-MCP on weight loss. Promisingly, preharvest application of 1-MCP resulted in no differences in weight loss as influenced by storage atmosphere. However, both 1-MCP treated broccoli groups were not different from either of the field control groups, meaning that protection from ethylene by 1-MCP application was not conclusively observed.

The application of ethylene effect on weight loss was observed to be variable by the harvest block (Figure 4.5b). Ethylene-treated broccoli in block 1 had the highest weight loss (3.6 ± 0.4%), and was significantly different from air-treated broccoli (3.1 ± 0.4%) from the same block (Figure 4.5b). However, in the other two blocks, weight loss of broccoli heads was not influenced by ethylene treatment. Field block 1 differed from the other blocks in that the

field control broccoli heads used in storage were generally smaller (Table 4.1, Table 4.2 and Figure 4.2). This suggests that ethylene effects on broccoli weight loss may be influenced by maturity, with smaller heads (earlier maturity) being more sensitive to ethylene. Additionally, broccoli heads from block 1 had the highest ethylene peak (Figure 4.3), which may be a result of less maturity. However, this also suggests that 1-MCP may have a more effective effect.

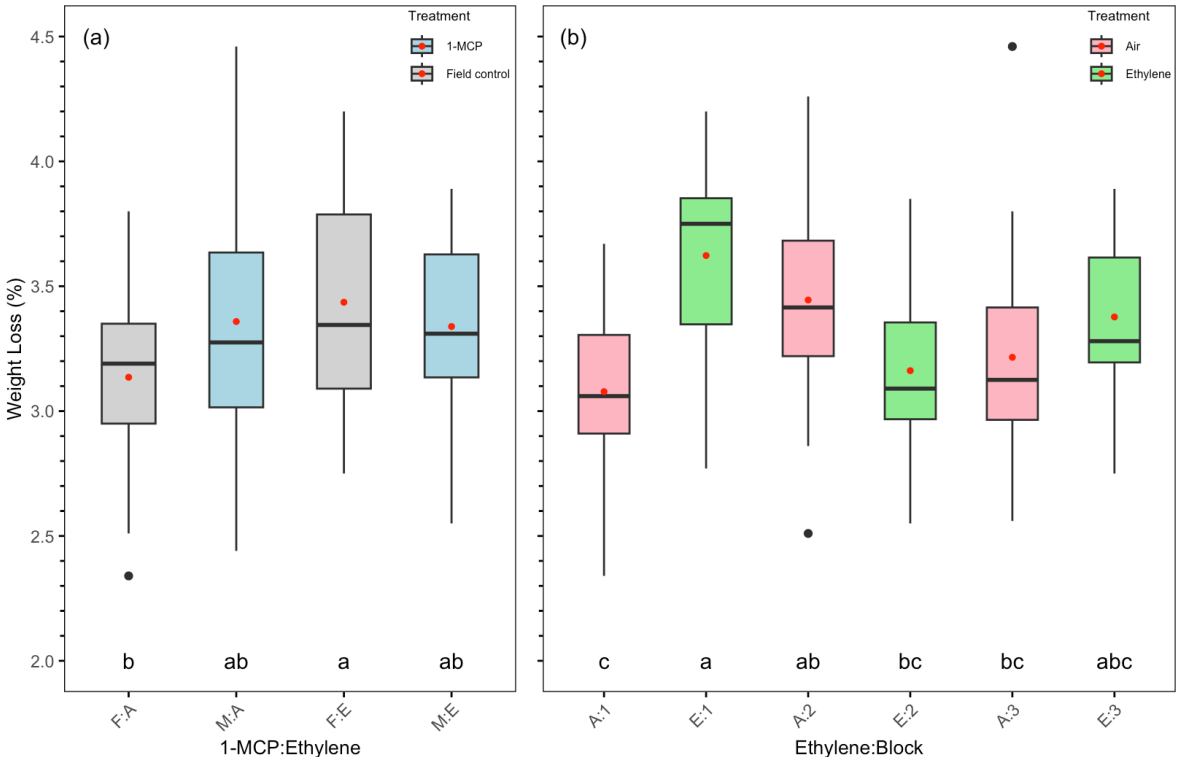

Figure 4.5 Weight loss as influenced by (a) the combination of ethylene treatment (A = air, E = ethylene) and preharvest 1-MCP treatment (F = field control, M = 1-MCP) (n = 30) and (b) ethylene treatment and growing block (1, 2, 3) (n = 20) during storage at 1.4 °C and 100% RH for 29 days. Different letters above the x-axis indicate significant differences in each sub-plot ($p < 0.05$). The line inside the box represents the median of the population, and the red point represents the mean value of the population.

4.6.2 Colour

After storage, broccoli still stayed marketable green (Figure 4.4). There were no visual colour differences between treatments, with an average score of 1.02 ± 0.10 on a 5-point scale (Figure 2.6, high quality, data not shown). However, 1-MCP treated broccoli had a significantly higher L* value of 37.5 ± 1.8, compared to the field control group (36.7 ± 1.7) (Figure 4.6a). Statistically, 1-MCP seemed to result in brighter heads. However, giving that field control broccoli heads used in storage were generally smaller than 1-MCP treated broccoli (Table 4.1, Table 4.2 and Figure 4.2), the result may also indicate L* value related to harvest maturity.

Block 1 had the smallest L* value at 35.8 ± 2.0, significantly lower than blocks 2 and 3, which consistent with diameter results – block 1 had the smallest broccoli heads (Figure 4.2). There was no significant difference between blocks 2 (37.9 ± 1.3) and 3 (37.6 ± 1.4) (Figure 4.6b). Considering that block 1 had more small heads (Table 4.1), suggesting that L* may be related to maturity. Less maturity may result in a darker head, with a smaller L*.

In block 2, broccoli had the highest h° value of 120.3 ± 2.5, significantly different from the h° value (119.0 ± 2.5) of the broccoli in block 3 (Figure 4.6c), indicating that block 2 had greener heads than those in block 3. However, visual assessment can only distinguish yellow from green when h° < 110° in the broccoli head.

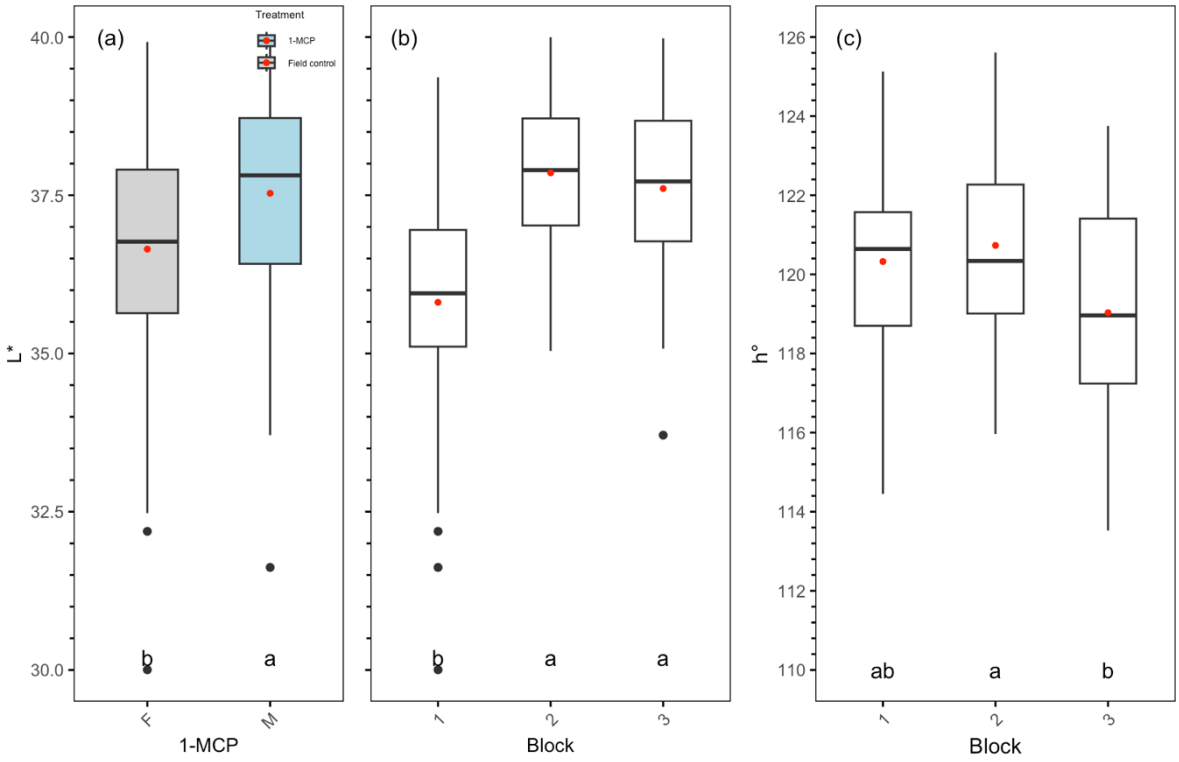

Figure 4.6 The L value of broccoli as influenced by (a) preharvest 1-MCP treatment (F = field control, M = 1-MCP) (n = 60), and the L* (b) and h° (c) values as influenced by growing block (1, 2, 3) (n = 40) during storage at 1.4 °C and 100% RH for 29 days. Different letters above the x-axis indicate significant differences in each sub-plot (p < 0.05). The line inside the box represents the median of the population, and the red point represents the mean value of the population.*

The application of ethylene had a variable effect on C* by harvest block (Figure 4.7a). In block 1, air-treated broccoli heads had a lower C* value than ethylene-treated ones, while in block 3, air-treated broccoli had a higher C* value than ethylene-treated broccoli. Considering that block 1 usually had smaller heads than block 3, indicating that maturity may influence the colour response (C*) to ethylene. Furthermore, block 3 broccoli exhibited significantly higher

C* values than blocks 1 and 2 (Figure 4.7b), suggesting that field variation may have influenced C* values.

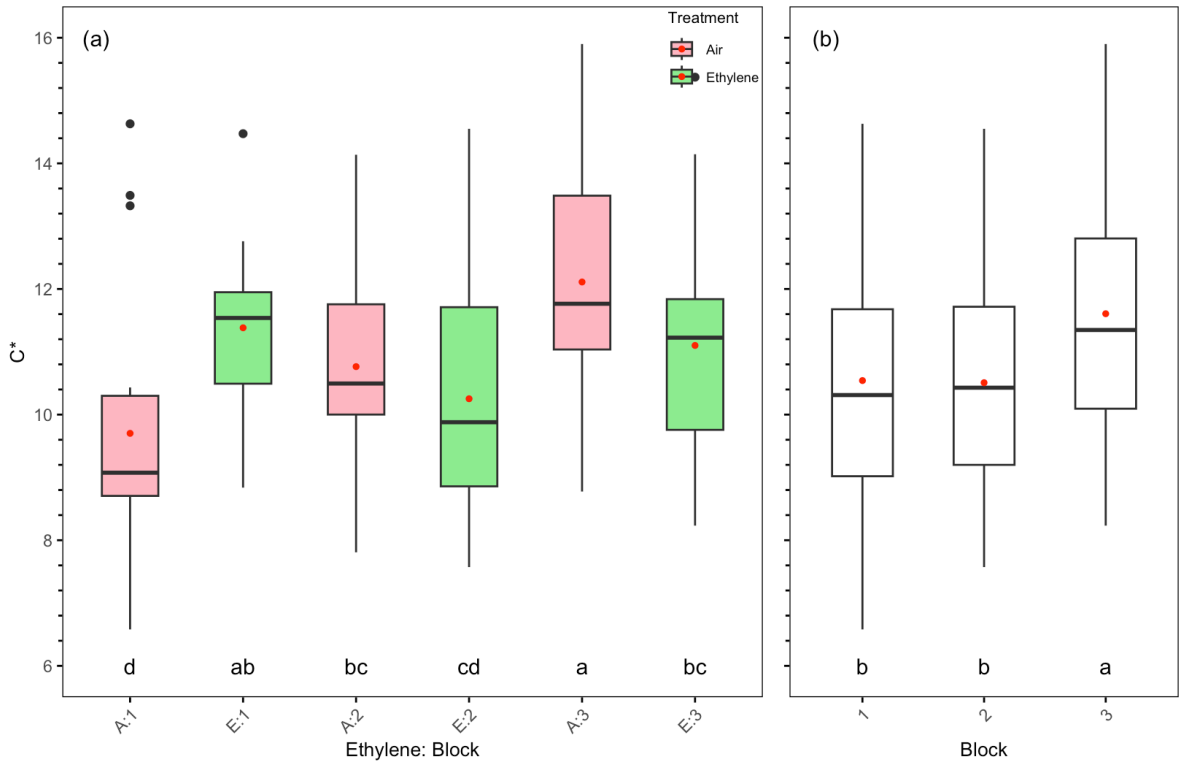

Figure 4.7 The C value of broccoli as influenced by (a) the combination of ethylene treatment (A = air, E = ethylene) and growing block (1, 2, 3) (n = 20) and (b) growing block (n = 40) during storage at 1.4 °C and 100% RH for 29 days. Different letters above the x-axis indicate significant differences in each sub-plot (p < 0.05). The line inside the box represents the median of the population, and the red point represents the mean value of the population.*

4.6.3 Head floret flexibility

The preharvest application of 1-MCP appeared to result in reduced floret flexibility, as the broccoli in the control group displayed significantly higher flexibility at 1.79 ± 0.52 N compared to the 1-MCP treated group (1.61 ± 0.53 N) (Figure 4.8a). Additionally, only in block 1, the broccoli from the field control group had a significantly higher flexibility than that of 1-MCP treated broccoli (Figure 4.8b). Considering block 1 generally had small heads and 1-MCP treated broccoli had larger heads than that of the field control group (Table 4.1), these indicate that maturity presumably influences floret flexibility, with earlier maturity resulting in higher flexibility.

In block 1, ethylene treatment reduced the flexibility compared with untreated broccoli. However, these patterns were not observed in blocks 2 and 3 (Figure 4.8c). Correspondingly,

the effects of ethylene on floret flexibility may be influenced by maturity, as florets with earlier maturity seem more sensitive to ethylene (Table 4.1).

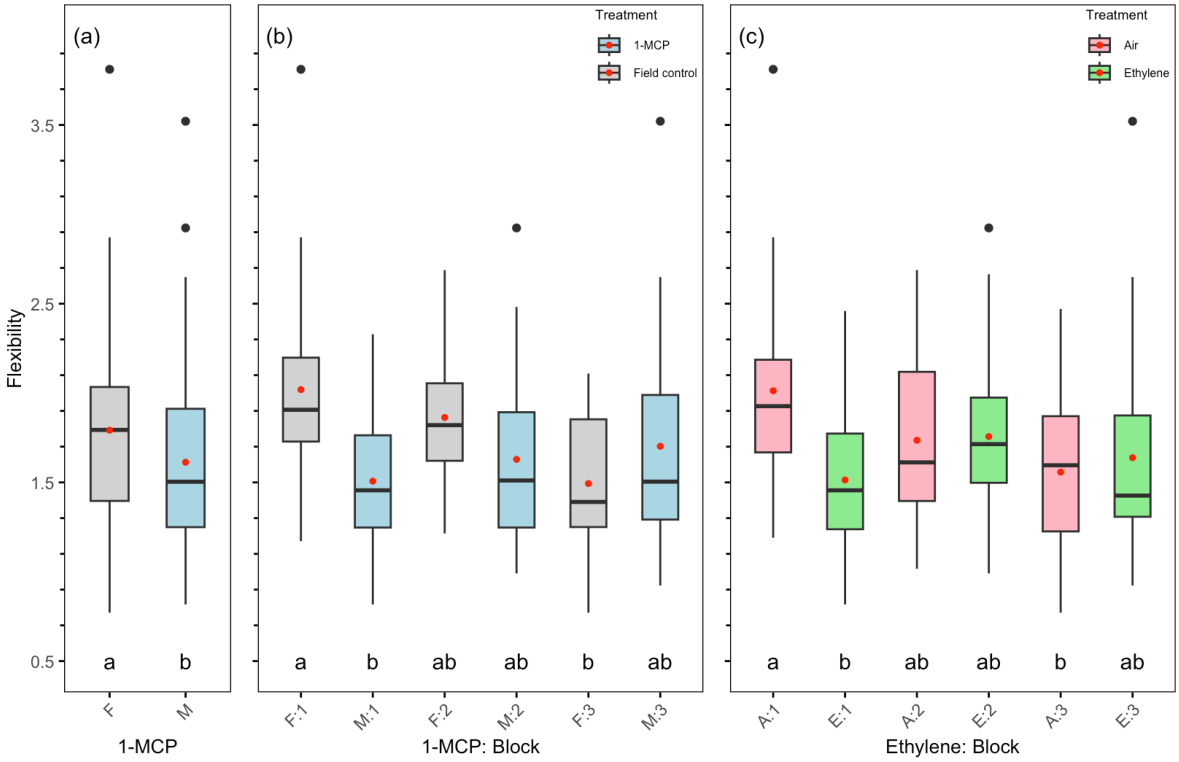

Figure 4.8 Floret flexibility of broccoli as influenced by (a) preharvest 1-MCP treatment (F = field control, M = 1-MCP) (n = 60), (b) the interaction between preharvest 1-MCP treatment and growing block (1, 2, 3) (n = 20), and (c) the interaction between ethylene treatment (A = air, E = ethylene) and growing block (n = 20) during storage at 1.4 °C and 100% RH for 29 days. Different letters above the x-axis indicate significant differences in each sub-plot ($p < 0.05$). The line inside the box represents the median of the population, and the red point represents the mean value of the population.

4.7 Discussion

This experiment was conducted to evaluate the application of preharvest 1-MCP treatment on broccoli and its impact on postharvest storage performance under continuously ethylene exposure and air conditions. As discussed in Section 2.1.2.6, commercial maturity determines harvest (both timing and frequency) and postharvest quality, as well as marketability. However, commercial maturity of broccoli is challenging to quantify, as it depends on a combination of traits, including head diameter, compactness, buds, and colour (Cantwell & Suslow, 2002). Moreover, in the field, broccoli exhibits substantial heterogeneity in head size, which further complicates the assessment of maturity. This variability challenges growers, as harvest decisions must balance labour costs against economic returns. Consequently, at-harvest maturity directly influence not only postharvest quality but also the

profitability and efficiency of commercial harvests. However, among all previous studies of 1-MCP treatment on broccoli (Table 2.7), only three papers provided diameter in method part to indicate maturity: Yuan et al. (2010) used 'Lvxiong' broccoli, with 150–200 mm diameter; a diameter of 200–250 mm (121 growing days) of 'Parthenon' broccoli was used by Fernández-León et al. (2013), and Wichrowska et al. (2021) harvested four broccoli cultivars when their diameter was 100–150 mm. None of them analysed maturity data in their results and discussion. It is challenging to compare them due to different cultivars and growing climates.

In this study, head diameter was used as an indicator of maturity: Block 1 had significant smaller heads than that in block 2. At-harvest weight was also used to indicate the maturity. To ensure uniformity, all broccoli heads were trimmed to a stalk length of 10 cm (Figure 3.3). Therefore, differences in weight may have primarily reflected head size and maturity, although variation in stalk diameter also contributed to the overall weight – this may have limited its usefulness as a maturity indicator. At-harvest weight did not show clear relationships with any other contributes ($R^2 < 0.2$, data did not show) after storage; only significant difference was at harvest between 1-MCP treated and untreated broccoli: 1-MCP untreated broccoli had a significant larger weight of 345 ± 47 g than that of treated broccoli (326 ± 54 g) (Table 4.2), which was consistent with the observation before 1-MCP application.

Overall, after 'Nobel' broccoli was stored at 1.4 °C for 29 days, broccoli quality did not respond to either preharvest 1-MCP application nor postharvest ethylene exposure – none of the combined treatments changed the quality of broccoli, broccoli heads stayed a marketable green colour – the low temperature and high RH might contribute to this outcome. As discussed in Sections 2.1.2.6 and 2.1.4.1, the change in lipid peroxidation was the early postharvest senescence event of broccoli (Zhuang et al., 1995), while temperature directly affected lipid peroxidation and deterioration (Zhuang et al., 1997). Deschene et al. (1991) demonstrated that deteriorative reactions are enzymatically mediated and membrane lipid degradation is temperature-dependent. Li et al. (2017) demonstrated that storage at low temperature (0 °C) suppressed the sensitivity of broccoli heads to ethylene. Therefore, in this experiment, storage temperature (1.4 °C) may have had a stronger influence on broccoli quality than preharvest 1-MCP treatment and postharvest ethylene exposure.

Vasconcelos and Almeida (2003) stated the two-phase hypotheses regarding colour changes in broccoli heads: Immediately following harvest, the colour remained visual green and unchanged during phase I ($h° \geq 110°$ and $C^* \leq 20$), whereas in subsequent phase II, the h° declined while C* and L* increased. In this study, the colour data supported their hypotheses

that the colour of the broccoli head was in phase I when stored at 1 °C for 29 days, which was over 70 days at 1 °C.

4.7.1 Physiology

Kato et al. (2002) noted that stalk cut caused a significant wound for broccoli, and wound-induced ACC and ethylene were synthesised in response to harvesting. Interestingly, different patterns of ethylene production were observed in the various tissue samples, such as florets and the basal portion of curds, at 20 °C after harvest. The peak of ethylene production in florets was 27.8 pmol·kg^{-1}·s^{-1} (0.1 nmol·g^{-1}·h^{-1}) at 24 h after harvest.

In this study, the ethylene production results exhibited a similar pattern to that of florets in the study of Kato et al. (2002) and King and Morris (1994), although in this case, the samples were centre branchlets (consisting of florets and stems, Figure 2.1). The data confirmed the hypothesis that the florets, not the other vegetative tissues, produced the majority of ethylene, as concluded by Aharoni et al. (1985, as cited in King and Morris, 1994).

The peak ethylene production was detected around 20–24 hours after harvest: 1-MCP treated broccoli branchlets produced a significantly higher ethylene production (48.7 ± 31.6 pmol·kg^{-1}·s^{-1}) than that of untreated branchlets (26.7 ± 6.5 pmol·kg^{-1}·s^{-1}). Ma et al. (2009) also demonstrated 1-MCP treatment resulted in higher ethylene production (125 pmol·kg^{-1}·s^{-1}, 0.45 nmol·g^{-1}·h^{-1}) than that of control (25 pmol·kg^{-1}·s^{-1}, 0.09 nmol·g^{-1}·h^{-1}) at 20 °C 48 h after harvest, as a result of high ACS activity and ACC concentration.

The temperature stress (from 12 °C to 20 °C) and wound stress (from harvesting and a second wound stress by cutting the branchlets from the head and stalk) possibly contributed to the initial increase in ethylene production. Interestingly, ethylene production in florets was increased again at around 48 h after harvest in both this study and King and Morris (1994). However, the possible reasons for this second increase were not stated. It is possibly caused by the yellowing of branchlets (Figure 4.3), but more studies are needed to confirm it. The different diameters of the sampled broccoli heads indicated different maturity levels – broccoli from block 1 was likely less mature than that of blocks 2 and 3, which might contribute to the differences in ethylene production among the three blocks (Table 4.3).

In this study, CO_2 production results (1645 ± 203 nmol·kg^{-1}·s^{-1}) agreed with the results of broccoli heads at 20 °C from Cantwell and Suslow (2002) (1617–1848 nmol·kg^{-1}·s^{-1}, 140–160 mL·kg^{-1}·h^{-1}). The results were consistent with those obtained 48 to 96 h after harvest in the

study by King and Morris (1994) (Table 2.3). They stated that CO_2 production was stabilised after 24 h after harvest, which was supported by our data. Downs et al. (1997) explained that this stabilisation in CO_2 production may have caused by rapid respiration rate and sucrose loss for respiration.

4.7.2 Impacts of preharvest application of 1-MCP on broccoli quality

In this trial, applying 1.0 $mL \cdot m^{-2}$ 1-MCP four days before harvest did not significantly affect the at-harvest broccoli heads' colour, head flexibility, and stalk compression stress (Table 4.2).

The florets from 1-MCP treated broccoli heads tended to have a higher ethylene production than those of the untreated broccoli (Figure 4.3). This finding agrees with the results of Grzegorzewska et al. (2023), especially for shelf life at 10 °C.

This study did not find that the preharvest application of 1-MCP inhibited the respiration rate of broccoli florets. Grzegorzewska et al. (2023) also found that treatment with 1.0 and 3.0 $cm^3 \cdot m^{-3}$ 1-MCP at 5 °C for 20 hours did not affect the respiration rate of broccoli heads during shelf life (5 or 10 °C for 6 days) after storage for 30 days at 0–1 °C.

After storage, 1-MCP did not affect the weight loss of broccoli heads, which is consistent with the result from Ghimire et al. (2024) when broccoli heads were treated with 1.0 $\mu L \cdot L^{-1}$ 1-MCP for 24 hours at 20 °C and then storage at 4 °C for 28 days and 10 °C for 18 days (Table 2.3). The broccoli heads experienced a slightly higher weight loss than those of Vasconcelos and Almeida (2003), which may be attributed to the barrels having a higher gas exchange rate (approximately 900 $ml \cdot min^{-1}$) compared to the plastic bags in their experiment.

Interestingly, broccoli treated with 1-MCP exhibited a lighter or brighter appearance (Figure 4.6). Moreover, compared to field control broccoli, a significantly smaller head floret flexibility for 1-MCP treated broccoli was observed. These suggest maturity may have contributed to results of C* value and head floret flexibility, as 1-MCP treated broccoli seemed to be smaller than field control broccoli (Table 4.1). Blankenship and Dole (2003) stated that 1-MCP responds differently to different maturity. Broccoli responded more to 1-MCP than leaf brassica, which was also very likely due to the different maturity between leaf and flower (Able et al., 2002).

As discussed in Section 2.4.2.4, the concentration and application time of 1-MCP on fruits before harvest also influence the effectiveness of 1-MCP, except for plant maturity. This

study sprayed 1-MCP four days before harvest; different results might be observed if different application methods and times were used.

For weight loss and head floret flexibility, block 1 had different patterns with blocks 2 and 3. This difference may have reflected the application order in the field, as block 1 was applied first, because the solution was continuously stirred during application with the knapsack sprayer, the earlier treatment may have resulted in a more effective dose.

4.7.3 Impacts of postharvest ethylene on broccoli quality

Fan and Mattheis (2000) concluded that continuous ethylene (1 $\mu L \cdot L^{-1}$ for 12 days) accelerated yellowing of broccoli florets at 10 °C. Gong and Mattheis (2003) also found that broccoli florets treated with 1000 $\mu L \cdot L^{-1}$ ethylene for 5 h significantly accelerated the decline of h° when stored at 20 °C for three days. Ethylene (1 $\mu L \cdot L^{-1}$) also accelerated the yellowing of broccoli heads at 4 °C for 14 days in the study by Lu (2020).

However, in this study, 1 $\mu L \cdot L^{-1}$ ethylene treatment did not independently impact any quality indices of broccoli heads when stored at 1.4 °C for 29 days. These results possibly suggest that the storage duration was too short or storage temperature was too low to observe any potential effects of ethylene treatment on broccoli. This is supported by the theory that storing broccoli at low temperatures suppresses its sensitivity to ethylene (Wills & Golding, 2016). The potential impact on industry is that this study suggests that low temperature is the key control for shelf life quality. When exposed to the same ethylene concentration, the higher the temperature, the shorter the shelf life of broccoli was (Li et al., 2017). Thus, a second trial was decided to investigate the effects of temperature on broccoli by storing it at 1 °C and 4 °C (non-optimal temperature).

4.7.4 Interactions between preharvest 1-MCP and postharvest ethylene

Preharvest application of 1-MCP resulted in no differences in weight loss as influenced by continuously ethylene exposure for 29 days at 1.4 °C (Figure 4.5a), while for broccoli not treated with 1-MCP, ethylene treatment resulted in a higher weight loss than air-treated broccoli (Figure 4.5a). This suggests that there may be potential benefits of applying 1-MCP on weight loss.

Zsom et al. (2020) found that 24 hours of 0.625 $\mu L \cdot L^{-1}$ 1-MCP followed by 24 h of 2 $\mu L \cdot L^{-1}$ ethylene at 5 °C and 21 °C reduced the weight loss of broccoli heads. However, this

study did not find a similar pattern, which was possibly due to low temperature and/or ethylene concentration.

4.7.5 Impacts of planting block and other preharvest factors on broccoli quality

This study treated the planting block as a factor due to uneven growth of broccoli heads and other possible preharvest factors. At harvest, the diameter differed in planting blocks (Figure 4.2), suggesting maturity differences across blocks. Moreover, physiological data revealed that planting block significantly affected the ethylene production of broccoli branchlets (Figure 4.3), further indicating that block 1 had less mature heads than blocks 2 and 3. These increased the challenges to interpret the results after storage.

After storage, planting blocks individually or in combination with 1-MCP or ethylene treatments significantly affected weight loss, colour, and floret flexibility, possibly indicating the impacts of preharvest factors on broccoli and/or at-harvest maturity influence. As discussed in Section 2.1.2, many preharvest factors affect the quality of broccoli heads, such as planting seasons, cultivars, climate conditions, management, and local positions.

The weight loss data revealed that less mature heads may have been more sensitive to ethylene exposure (Figure 4.5b) because their tissues are still actively developing, with higher metabolic activity, as indicated by ethylene production (Figure 4.3). Such immature tissues generally have weaker structural and antioxidant defences, making them more prone to ethylene-induced senescence (including weight loss) compared with more mature heads.

The significant influence of planting block on colour suggests variability in colour development of broccoli, which may be attributed to preharvest factors like environmental and management differences and/or at-harvest maturity. A lower L*, C* value and higher h° value of broccoli suggests less yellowing and better visual freshness, as these values are typically associated with discolouration and deterioration due to chlorophyll breakdown. Generally speaking, h° value has a positive linear relationship with chlorophyll fluorescence. However, Toivonen and DeEll (1998) demonstrated that the measurements of chlorophyll fluorescence are independent of broccoli head maturity. In our study, h° data affected by block supported this conclusion – block 1 had smaller heads than block 2 (Figure 4.2); but after storage, h° of broccoli from block 1 did not significant differ from that of block 2 (Figure 4.6).

Broccoli heads from block 1 responded differently to 1-MCP and ethylene treatment in terms of head floret flexibility (Figure 4.8b, c), indicating that maturity affected the responses of broccoli heads to 1-MCP and ethylene treatments (Section 2.4.2.4).

4.7.6 How to measure the compactness of broccoli heads with a texture analyser

The compactness of broccoli heads is one of the essential sensory quality indices (Jacobsson et al., 2004b). A texture analyser is a good instrumental method for quantifying compactness, except for subjective scores. Guirao et al. (2024) noted that head firmness is generally assessed based on compactness. Zhao et al. (2022) proposed that stalk compression stress can assess the structure and mechanical properties of the stalk. Considering that compactness is actually how tightly packed the florets are, the new method of floret flexibility (Section 3.5.5), created for the purpose of this thesis, was used to measure the compactness of broccoli heads.

Compared to stalk, consumers tend to focus more on the quality of head florets. The results of head floret flexibility and stem compression stress (Table 4.2, Table 4.5 and Figure 4.8) indicated that the measurement of stalk compression stress and head floret flexibility provided no useful information and hence could be stopped. Previously, Zhao et al. (2022) treated stalk compression stress as a mechanical property to measure maturity of broccoli at harvest, and not as an indicator of postharvest storage quality. In contrast, head firmness should remain a key quality indicator because: 1) Compared to head floret flexibility, head firmness assessment is a more standard method, and 2) Fernández-León et al. (2013), Paulsen et al. (2022), and Guirao et al. (2024) (Table 2.4) used head firmness as the indicator of head compactness (firmness to pressure by hand), which is one of the firmness characteristics used by consumers in purchase decision making. Thus, it is suggested that only head firmness should be chosen as the TA.XT analyser measurement in future research.

In this study, after the broccoli was stored at 1.4 °C for 29 days, the average head firmness (1.35 ± 0.38 $N \cdot mm^{-1}$) was larger than that from Paulsen et al. (2022) (after stored at 2 °C for 28 d for no film 0.25 $N \cdot mm^{-1}$) and Guirao et al. (2024) (0.9 $N \cdot mm^{-1}$ for control, 0.6 $N \cdot mm^{-1}$ for ethylene treated broccoli and 1.4 $N \cdot mm^{-1}$ for ethylene scrubber after stored at 2 °C for 7 d) (Table 2.4) – several factors may contribute to these differences, including storage conditions (temperature and RH), compression speed, and cultivars.

4.8 Conclusion

In conclusion, after broccoli was stored at 1.4 °C for 29 days, broccoli quality did not respond to either preharvest 1-MCP application nor postharvest ethylene exposure – broccoli heads stayed a marketable green colour.

The preharvest application of 1-MCP four days before harvesting did not significantly affect the quality of broccoli at harvest and after storage. The influence of the planting block on broccoli quality highlights the impact of preharvest environmental and management factors and at-harvest maturity, which may further influence broccoli's response to 1-MCP and ethylene exposure. Thus, a second trial should be conducted to gain a deeper understanding of preharvest factors, such as seasons and cultivars.

Compared to previous studies, broccoli heads did not respond to ethylene exposure. Based on the two-phase hypotheses regarding colour changes in broccoli heads (Vasconcelos & Almeida, 2003), colour data from this study supported their hypotheses that the colour of the broccoli head was in phase I (marketable green) when stored at 1 °C for 29 days, which was over 70 days at 1 °C. These findings indicate that storage duration may not have been long enough or the storage temperature was too low, to reveal any possible quality changes. Consequently, a higher and non-optimal temperature (≥ 4 °C) was applied during storage in the second trial. For Trial 2, the effects of preharvest application of 1-MCP on 'Iron' broccoli quality during storage at 1 °C and 4 °C were investigated. The results and discussion of Trial 2 will be presented in the following chapter.

Chapter 5. Impacts of Preharvest Application of 1-MCP on 'Iron' Broccoli Quality During Storage at 1 °C and 4 °C Under Ethylene Exposure

5.1 Introduction

Generally speaking, yellowing is the major limitation in broccoli storage at ambient temperatures (e.g., 20 °C), while the onset of decay is the primary determinant of storage life at 1 °C (Pogson & Morris, 1997). In the literature review, a number of authors have previously provided evidence that storage temperature influences the colour change of broccoli, and sensitivity to ethylene (Sections 2.2.2 and Figure 2.5). Li et al. (2017) demonstrated that storage at low temperature (0 °C) suppressed the sensitivity of broccoli heads to ethylene. When continuously exposed to 1.0 $\mu L \cdot L^{-1}$ ethylene, broccoli stored at 0 °C had a postharvest life of 39.3 days, representing increases of 94%, 84%, and 75% compared with storage at 20, 10, and 5 °C, respectively.

The previous experiment (Chapter 4) demonstrated that:

1) Application of 1-MCP four days before harvest did not significantly affect any of the at-harvest broccoli quality attributes (Table 4.2);
2) Preharvest application of 1-MCP delayed the peak of ethylene production but did not inhibit the respiration rate of broccoli branchlets (Figure 4.2 and Table 4.4);
3) There was no response from the field control group to 1.0 $\mu L \cdot L^{-1}$ ethylene exposure when stored at 1.4 °C for 29 days – the broccoli stayed marketable and green (Table 4.5 and Figure 4.3);
4) Statistically, inconsistent results were obtained for weight loss, colour change, and floret flexibility. In block 1, ethylene exposure resulted in a higher weight loss and C* value and lower flexibility than those of air control (Figure 4.4b, Figure 4.6a and Figure 4.7c). The other two blocks did not find the same patterns. The 1-MCP treated broccoli had a lower flexibility than untreated broccoli in field block 1 (Figure 4.7b). In block 3, air-treated broccoli had a higher C* value than ethylene-treated broccoli (Figure 4.6a).

In contrast to these results, Lu (2020) observed significant yellowing when the broccoli heads were stored at 4 °C and 98% RH under exposure to 1.0 $\mu L \cdot L^{-1}$ ethylene. Likewise, Fan & Mattheis (2000) demonstrated that 1-MCP inhibited respiration rate (by 50%) and yellowing (by 25%) of broccoli when exposed to 1.0 $\mu L \cdot L^{-1}$ ethylene at 10 °C. Able et al. (2002) described

that 1-MCP reduced the yellowing (115° > 108°, control) of broccoli when stored at 10 °C. When broccoli was stored at 5 °C, Zsom et al. (2020) also described that 1-MCP inhibited the yellowing of broccoli head under exposure to 2.0 $\mu L \cdot L^{-1}$ ethylene. This combined evidence suggests that, while at 1 °C, preharvest 1-MCP may not assist in the preservation of broccoli, as at higher temperatures, there remains potential for a beneficial storage effect.

Cultivar differences potentially contributed to variation in Trial 1, as discussed in Section 2.1.2.2. Broccoli cultivars differ significantly in terms of quality traits and hence could be explained to respond differently to 1-MCP treatment (Wichrowska et al., 2021).

Seasonal effects may have also affected the results of Trial 1, as temperature and accumulated solar radiation strongly affect head initiation and growth, at-harvest maturity and quality and thus postharvest responses (Section 2.1.2.1). These seasonal variations may have contributed to differences in quality and shelf life observed in Trial 1.

Given all these potential effects on the results of Trial 1, a second experiment was conducted with adjustments to investigating the effects of preharvest application of 1-MCP on postharvest broccoli quality. This included conducting the experiment in summer meaning that a different cultivar was also grown. A second storage temperature (4 °C) was also used (in addition to 1 °C) as was continuous postharvest ethylene treatment and storage in air. This chapter presents the results from Trial 2 with separation of the two different storage temperature conditions. The discussion for the two temperature conditions is then provided. All associated methods have already been provided in Chapter 3.

5.2 At harvest quality

Before preharvest application of 1-MCP, similar size broccoli heads were selected as the sample for field diameter measurement to investigate the possible effect of 1-MCP on head growing rate. The size of the broccoli heads was gauged by visual assessment and the use of a grading circle with an internal diameter of 110 mm (Section 3.3.3.1). Ten broccoli heads from each of six treated blocks were measured.

The resulting average diameters of identified broccoli heads in the field control (100 ± 6 mm) was significant smaller than that of the 1-MCP area (106 ± 9 mm). Before harvest, three days later, the same broccoli heads were measured again, with the average diameters of field control area being 129 ± 8 mm – significant smaller than that of 1-MCP area (135 ±10 mm). The detailed diameters of broccoli head before 1-MCP spraying and before harvest from each of six treated blocks were listed in Table 5.1. All blocks remained the same trend as that before

spraying, resulting in an approximate growth rate of 28%. The surface area of the broccoli head was also calculated based on the Equation 3.7. The absolute surface area growths for broccoli heads from field control and 1-MCP were 5100 ± 900 mm^2 and 5400 ± 800 mm^2, respectively. Three days before harvest, application of 1-MCP did not affect the growth of broccoli heads (Table 5.1), as indicated by both diameter and surface area.

Table 5.1 The diameters of broccoli head before 1-MCP spraying (Day 0) and before harvest (Day 3) from each of six treated blocks (n = 10). Different letters indicate significant differences within each block (p < 0.05). Student's t-tests were conducted within each block to compare the diameters of broccoli before 1-MCP spraying (Day 0) and before harvest (Day 3). Data represent mean ± standard deviation (SD).

Block	Field treatment	Day 0 diameter (mm)	Day 3 diameter (mm)	Head growth rate (%)
1	Field control	98 ± 4 a	127 ± 7 a	30
	1-MCP	99 ± 5 a	128 ± 7 a	30
2	Field control	100 ± 3 b	128 ± 4 b	28
	1-MCP	108 ± 8 a	136 ± 9 a	26
3	Field control	103 ± 5 b	132 ± 5 b	28
	1-MCP	111 ± 4 a	140 ± 3 a	26

As in Trial 1, for each preharvest treated or untreated block × postharvest treatment combination, samples were pre-selected to minimise the potential effects of head maturity/size on the experimental result (Section 3.3.3.1). Ideally, all samples would have been from a single diameter category. However, despite planning efforts, after harvest, there were insufficient heads in any one category for them to be solely allocated to storage conditions. Consequently, samples were chosen to cover as many head size categories as possible, with the aim of using an equal proportion of heads from the diameter categories to ensure uniformity for storage treatments within field-treated blocks. However, practically, this outcome could not be perfectly achieved, which resulted in a slightly unequal proportion of head sizes from each diameter category, due to the limited number of heads available. The resulting outcome of these decisions is detailed in Table 5.2 and Table 5.3 for storage at 1 and 4 °C, respectively.

Table 5.2 The allocation of diameter categories of broccoli heads to storage treatment at 1 °C in each block. Where: F = field control, M = 1-MCP. Data represent mean ± standard deviation (SD).

Block	Storage treatment	< 110 mm	110–120 mm	120–150 mm	> 150 mm	Diameter range mm
F1	Air	1	2	5	2	128 ± 16
	Ethylene	1	2	5	2	127 ± 14
M1	Air	1	2	5	2	130 ± 16
	Ethylene	1	2	4	3	130 ± 17
F2	Air	1	2	5	2	132 ± 12
	Ethylene	1	2	5	2	127 ± 14
M2	Air	1	1	6	2	131 ± 12
	Ethylene	1	2	6	1	128 ± 12
F3	Air	2	1	6	1	129 ± 11
	Ethylene	1	2	6	1	127 ± 11
M3	Air	2	2	4	2	127 ± 18
	Ethylene	1	2	6	1	131 ± 15

Table 5.3 The allocation of diameter categories of broccoli heads to storage treatment at 4 °C in each block. Where: F = field control, M = 1-MCP. Data represent mean ± standard deviation (SD).

Block	Storage treatment	< 110 mm	110–120 mm	120–150 mm	> 150 mm	Diameter range mm
F1	Air	1	2	5	2	129 ± 17
	Ethylene	1	2	5	2	129 ± 16
M1	Air	1	2	4	3	130 ± 17
	Ethylene	1	2	4	3	126 ± 19
F2	Air	1	2	6	1	127 ± 15
	Ethylene	1	2	5	2	130 ± 13
M2	Air	2	1	6	1	130 ± 13
	Ethylene	1	2	5	2	135 ± 12
F3	Air	1	1	7	1	132 ± 12
	Ethylene	1	1	7	1	129 ± 10
M3	Air	2	2	4	2	128 ± 16
	Ethylene	1	3	5	1	130 ± 16

At 1 and 4 °C, block F1 had the same allocation of diameter categories and similar diameter ranges for postharvest broccoli heads. However, data analysis suggested that neither the planting block nor the 1-MCP treatment affected the growth of broccoli heads, as indicated by the diameter and average surface area (Table 5.4 and Table 5.5). Student's t-tests were conducted within each treated block and storage temperature to compare the diameters of broccoli heads assigned to air (A) or ethylene (E) treatments. No significant differences were found in any block ($p > 0.25$), statistically indicating that diameter was evenly distributed across storage treatments for each temperature at the beginning of storage.

At harvest, broccoli heads had an average diameter of 129 ± 14 mm, weight 347 ± 66 g, and exhibited colour values of 115.8 ± 3.0 hue (h°), 41.6 ± 1.3 for lightness (L*) and 8.5 ± 1.3 for chroma (C*). Head firmness was measured at an average of 2.24 ± 0.49 $N \cdot mm^{-1}$.

Preharvest application of 1-MCP did not significantly impact any of the at-harvest quality attributes ($p > 0.05$), except for the L* value of broccoli heads ($p < 0.05$, Table 5.4). For each block of broccoli head, the average surface area (mm^2) was calculated in the storage treatment to estimate the 2D size of broccoli heads (Table 5.5).

Table 5.4 P-value table for broccoli at harvest quality

Factor	Diameter (mm)	Colour			Floret WL (%)	Head Firmness ($N \cdot mm^{-1}$)
		L*	C*	h°		
1-MCP	0.631	0.029	0.310	0.902	0.080	0.967
Sum of squares (%)		5.70				
n	*120*	*39*	*39*	*39*	*9*	*9*
Block	0.773	0.233	0.799	0.580	0.692	0.406
n	80	26	26	26	26	26
1-MCP × Block	0.889	0.770	0.179	0.040	0.453	0.901
n	*40*	*13*	*13*	*13*	*3*	*3*

Table 5.5 Average surface area (mm^2) table for each block of broccoli head used in the storage treatment. The surface area was calculated using Equation (8). The data were expressed as the average ± standard deviation (SD). The light grey colour highlighted the broccoli with a visual difference between treatments.

Block	Storage treatment	1 °C	4 °C
F1	Air	13000 ± 3200	13100 ± 3600
	Ethylene	12700 ± 2900	13200 ± 3300
M1	Air	13300 ± 3500	13400 ± 3600
	Ethylene	13500 ± 3500	12700 ± 3900
F2	Air	13800 ± 2500	12700 ± 3000
	Ethylene	12700 ± 2800	13400 ± 2600
M2	Air	13400 ± 2400	13300 ± 2500
	Ethylene	12900 ± 2300	14400 ± 2400
F3	Air	13000 ± 2200	13700 ± 2400
	Ethylene	12700 ± 2100	13100 ± 2000
M3	Air	12900 ± 3600	12900 ± 3200
	Ethylene	13500 ± 2900	13400 ± 3300

5.2.1 L value – colour*

At harvest, broccoli from the preharvest application of the 1-MCP group had a L* value of 41.3 ± 1.6, significantly lower than that of broccoli from the field control (41.9 ± 0.8) (Figure

5.1). The data from the spectrophotometer revealed that the broccoli heads treated with 1-MCP before harvest were slightly darker, for at least a proportion of the population. Although the result is statistically significant, the magnitude of change is insufficient to have commercial relevance because consumers would not detect the visual difference.

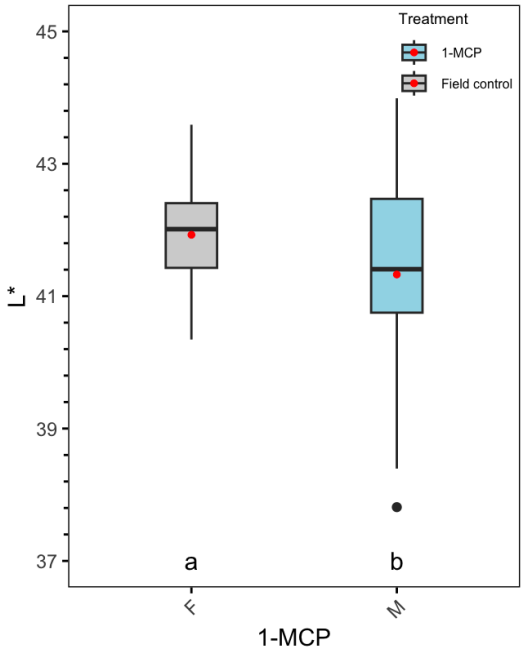

Figure 5.1 1-MCP treatment (F = field control, M = 1-MCP) (n = 39) significantly affected the L value of broccoli at harvest. Different letters above the x-axis indicate significant differences ($p < 0.05$). The line inside the box represents the median of the population, and the red point represents the mean value of the population.*

5.2.2 Postharvest physiological measurement

At the same time, the plan was to take three broccoli heads in the three diameter categories for physiology measurement from each of the six blocks. If the head number was insufficient for all samples, the extra heads (immature unmarketable heads, as discussed in Sections 3.3.2 and 3.3.3.1) would be used to cover the difference. In practice, for blocks F1 and M3, only extra heads were used. For all other blocks, three broccoli heads from the categorized diameter groups were used (Table 5.6).

During 11 days at 5.5 °C, while exposed to the flow through mode in the ETD-300 ethylene detector, the weight loss of florets was $5.6 \pm 0.6\%$. Average respiration rates expressed at CO_2 production between days 4 and 14 after preharvest 1-MCP treatment are illustrated in Figure 5.2. The respiration rate of broccoli florets reduced and then slightly stabilised at 60% of the initial rate during 11 days at 5.5 °C. There was no significant difference between 1-MCP treatments and the control on each and every day.

Table 5.6 The allocation of diameter categories of broccoli heads for ethylene production and CO_2 measurement in each block. (F = field control, M = 1-MCP)

Block	Extra heads (unmarketable)	< 110 mm	110–120 mm	120–150 mm
F1	3	0	0	0
M1	0	0	1	2
F2	0	1	1	1
M2	0	3	0	0
F3	0	3	0	0
M3	3	0	0	0

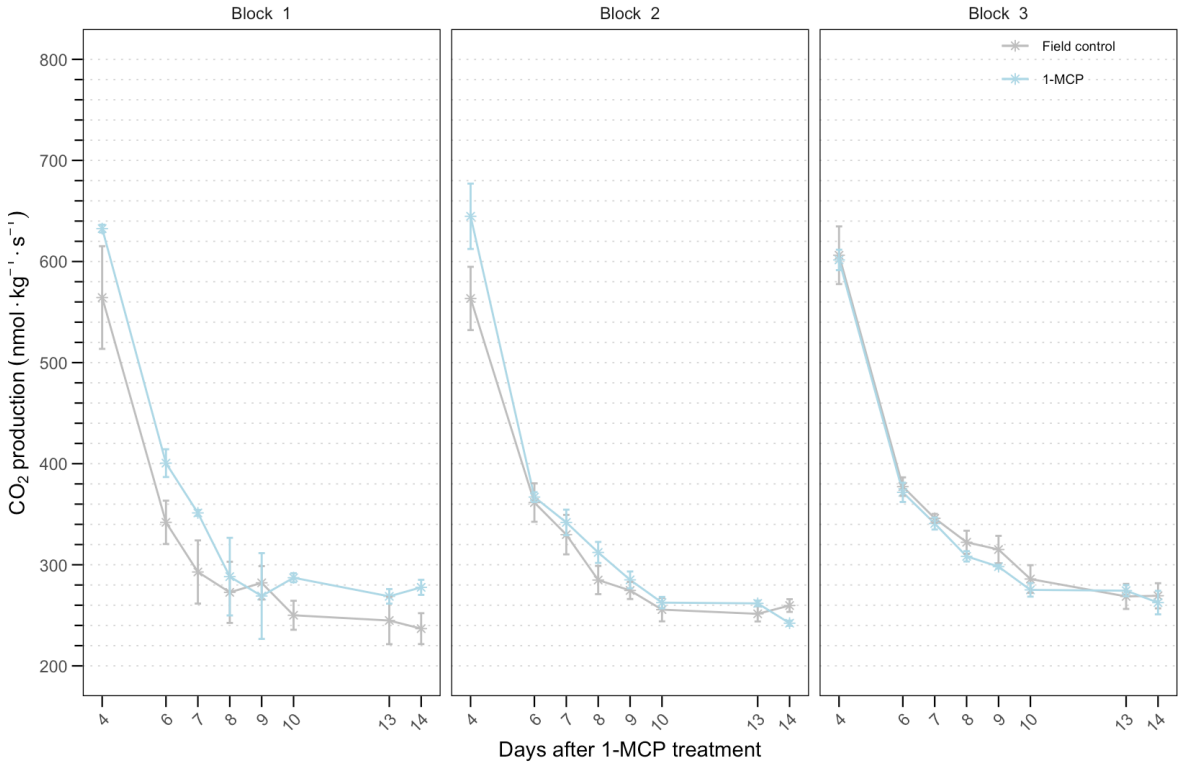

Figure 5.2 Respiration rate of broccoli branchlet at 5.5 °C for 11 days. Data represent mean ± standard deviation (SD), n = 3. Broccoli was sprayed with 1-MCP on Day 0 and harvested on Day 3 as indicated by the timeline in Chapter 3.

Ethylene production of broccoli florets decreased and then slightly stabilised at 70% of the initial rate during 11 days at 5.5 °C (Figure 5.3). 1-MCP treated broccoli florets had a significantly higher ethylene production than that of untreated broccoli florets ($p < 0.001$). After the 1-MCP treatments, there was a significant difference in the ethylene production of the broccoli florets from day to day ($p < 0.001$).

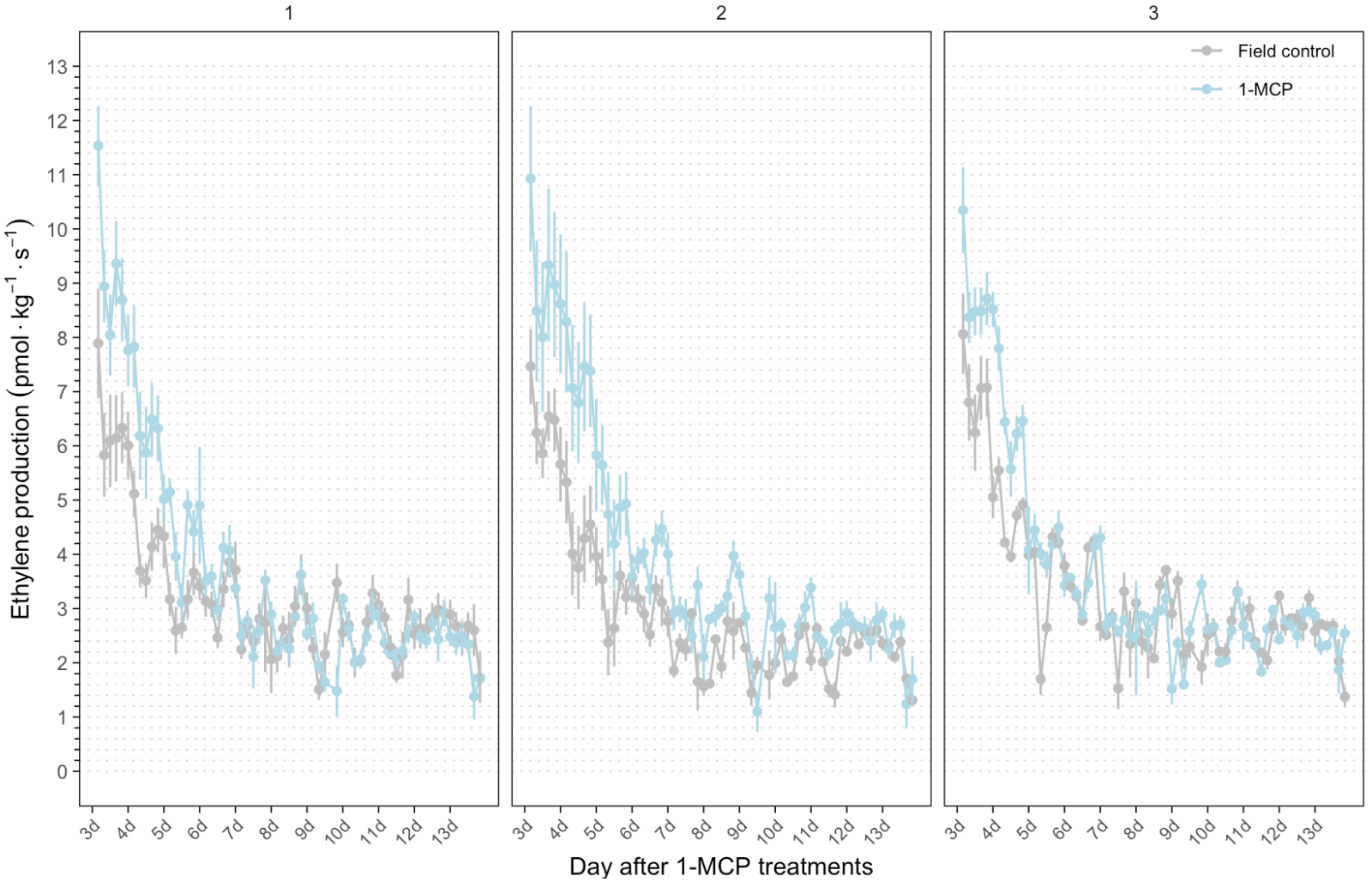

Figure 5.3 Ethylene production of the broccoli branchlet over 11 days at 5.5 °C. Data from each block is presented separately. Each point represents the average of three samples over a 4 h period. 1-MCP was sprayed in the 1-MCP applied area on day 0. The broccoli heads were harvested on day 3 (3d). Broccoli was harvested at 16–19 °C (3d) and then transported to a 20 °C laboratory after 3 h. Ethylene measurement started 10 h after harvest (Figure 3.1).

5.3 Storage conditions and observation from 1.9 °C storage

The average temperature and RH for the storage room were 1.7 °C and 96%. Inside the barrels where the broccoli was stored, the average temperature and RH were 1.9 ± 0.4 °C and approximately 100%, respectively. As in Trial 1, condensation was not observed on the broccoli. Generally, the ethylene concentrations within the barrels were stable, with an average of 977.4 ± 104.9 $nL \cdot L^{-1}$ for ethylene-treated barrels and 27.7 ± 20.7 $nL \cdot L^{-1}$ for the air-treated barrels. The average measured CO_2 concentrations were 0.12 ± 0.02% for both ethylene and air-treated barrels.

All heads stored in the clean air remained a marketable green colour. Only one broccoli head (≈ 2%) out of 60 had a slight mould. Some of the scars on the broccoli stalk started to turn grey but showed no sign of yellowing. In contrast, some of the scars on the stalk turned yellow and dropped off easily for those heads exposed to ethylene during storage (Figure 5.4). Only one broccoli head (≈ 2%) out of 60 had some dark and brown buds.

Figure 5.4 Visual quality of representative broccoli (heads, stalks and leaf scars) after being continuously exposed to ethylene (green arrow) and air (pink arrow) for 28 days at 1.9 °C

5.4 Quality change in broccoli stored in barrels at 1.9 °C for 28 days

As in Trial 1, after being stored, the broccoli heads remained a marketable green, with no visually noticeable differences between the treatment and control groups. After storage, the broccoli had an average weight loss of 2.8 ± 0.9%, and exhibited colour values of 114.8 ± 2.5 for h°, 41.6 ± 1.2 for lightness (L*) and 10.3 ± 1.2 for chroma (C*). Head firmness averaged 1.77 ± 0.57 $N \cdot mm^{-1}$.

Compared to the harvest value, the L* value remained unchanged, while the C* values increased slightly, and the h° values decreased slightly. Head firmness was slightly reduced.

As in Trial 1, each of the post-storage attribute data sets was analysed for the effects of each factor (preharvest 1-MCP treatment, ethylene in storage, or growing block) and the interactions of all factors using a full ANOVA (Section 3.6). To identify the significant results, a p-value table showing the effects on quality outcomes is provided (Table 5.7). The following sections detail each factor that influenced each quality attribute. The data has been organised to detail how each quality attribute responds to the influence of each factor and the combination of the factors. The effects of 1-MCP treatment were prioritised within each quality attribute, as this is the objective of this research.

Table 5.7 P-value table for broccoli quality as influenced by preharvest 1-MCP treatment, in storage ethylene treatment and in-field block after storage at 1.9 °C for 28 days

Factor	Weight loss (%)	Colour score	Firmness score	Colour			Head firmness ($N \cdot mm^{-1}$)
				L*	C*	h°	
1-MCP *(n=60)*	0.011	0.045	0.205	0.252	0.345	0.357	0.744
Sum of squares (%)	2.99	3.17					
Block *(n=40)*	< 0.001	0.086	0.575	0.191	0.060	0.002	0.001
Sum of squares (%)	17.15					10.08	10.59
Ethylene *(n=60)*	0.035	0.613	0.468	0.083	0.637	0.216	0.296
Sum of squares (%)	2.05						
1-MCP × Block *(n=20)*	0.056	0.016	0.774	0.002	0.071	0.046	0.057
Sum of squares (%)		6.65		9.51		4.83	
1-MCP × Ethylene *(n=30)*	< 0.001	0.613	0.364	0.061	0.913	0.372	0.487
Sum of squares (%)	9.14						
Ethylene × Block (n=20)	0.001	0.639	0.667	0.389	0.452	0.859	0.264
Sum of squares (%)	7.09						
1-MCP × Ethylene × Block *(n=10)*	< 0.001	0.299	0.667	0.401	0.279	0.980	0.958
Sum of squares (%)	10.70						

5.4.1 Weight loss

Preharvest 1-MCP treated broccoli had higher weight loss than that of the field control broccoli (Figure 5.5a). Interestingly, air-treated broccoli also exhibited a higher weight loss compared to ethylene-treated broccoli (Figure 5.5b). Consequently, preharvest 1-MCP treated broccoli without ethylene exposure had a significantly higher weight loss than all three other groups (Figure 5.5c).

There is no obvious mechanism by which ethylene exposure could reduce weight loss. The differences in surface area to volume ratio may explain the differences in weight loss. The rate of weight loss from a product increases with the rise in surface area. Statistically, there was no significant difference in the diameter and surface area of broccoli regardless of either preharvest 1-MCP treatment or growing block (Table 5.4 and Table 5.5).

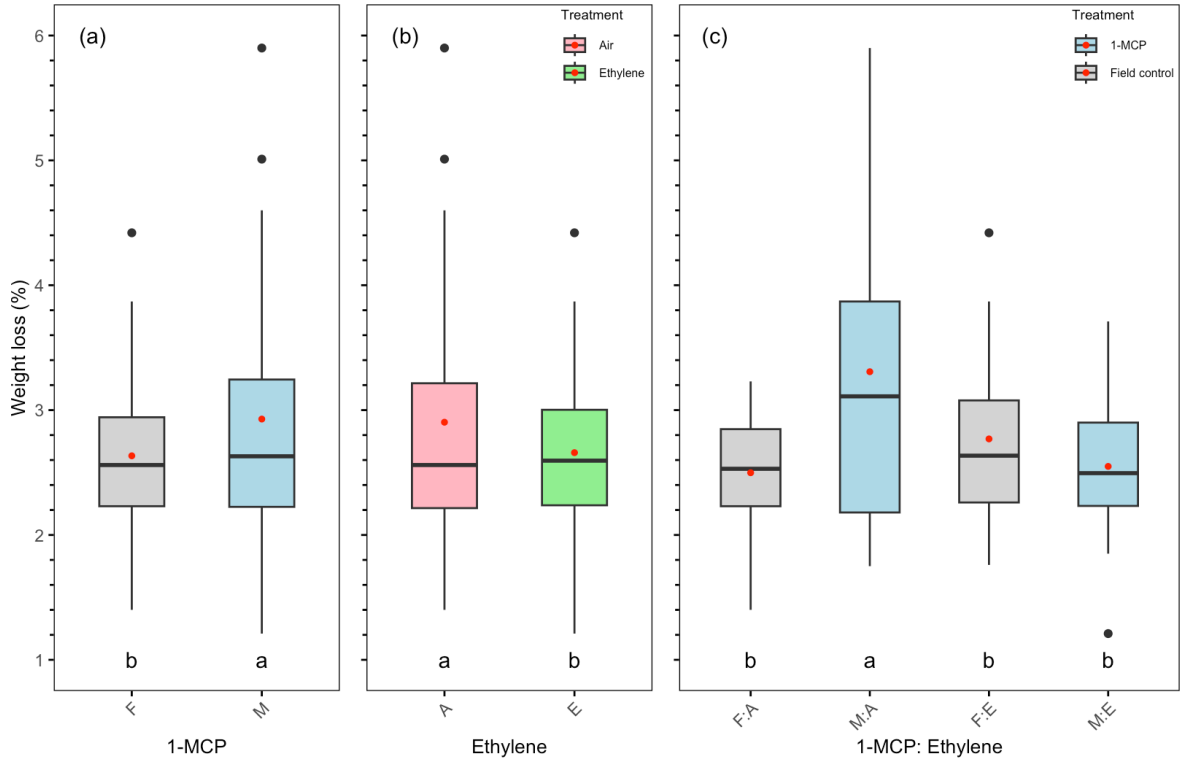

Figure 5.5 Weight loss as influenced by (a) preharvest 1-MCP treatment (F = field control, M = 1-MCP) (n = 60), (b) ethylene treatment (A = air, E = ethylene) and (c) the combination of preharvest 1-MCP and postharvest ethylene treatment (n = 30) during storage at 1.9 °C and 100% RH for 28 days. Different letters above the x-axis indicate significant differences in each sub-plot ($p < 0.05$). The line inside the box represents the median of the population, and the red point represents the mean value of the population.

In block 3, broccoli had a significantly higher weight loss compared to that in blocks 1 and 2 (Figure 5.6a), which may indicate that some unknown stress occurred in block 3.

The effect of ethylene application on weight loss varied by harvest block (Figure 5.6b). Air-treated broccoli in block 1 had a higher weight loss than that of ethylene-treated broccoli, suggesting that there may be some unknown stress that suppressed the effect of ethylene. However, in the other two blocks, the weight loss of the broccoli heads was not impacted by ethylene treatment.

The interaction among the three factors was evident only in block 3 under air treatment, where 1-MCP treated broccoli had a higher and widespread weight loss than that of untreated broccoli (Figure 5.6c). This suggests that the size and maturity of the broccoli heads varied considerably within block M3 (Table 5.2). However, the differences in weight loss may partly reflect variation in independent flow rate across barrels in the ethylene flow-through system, rather than ethylene and air treatment effects alone.

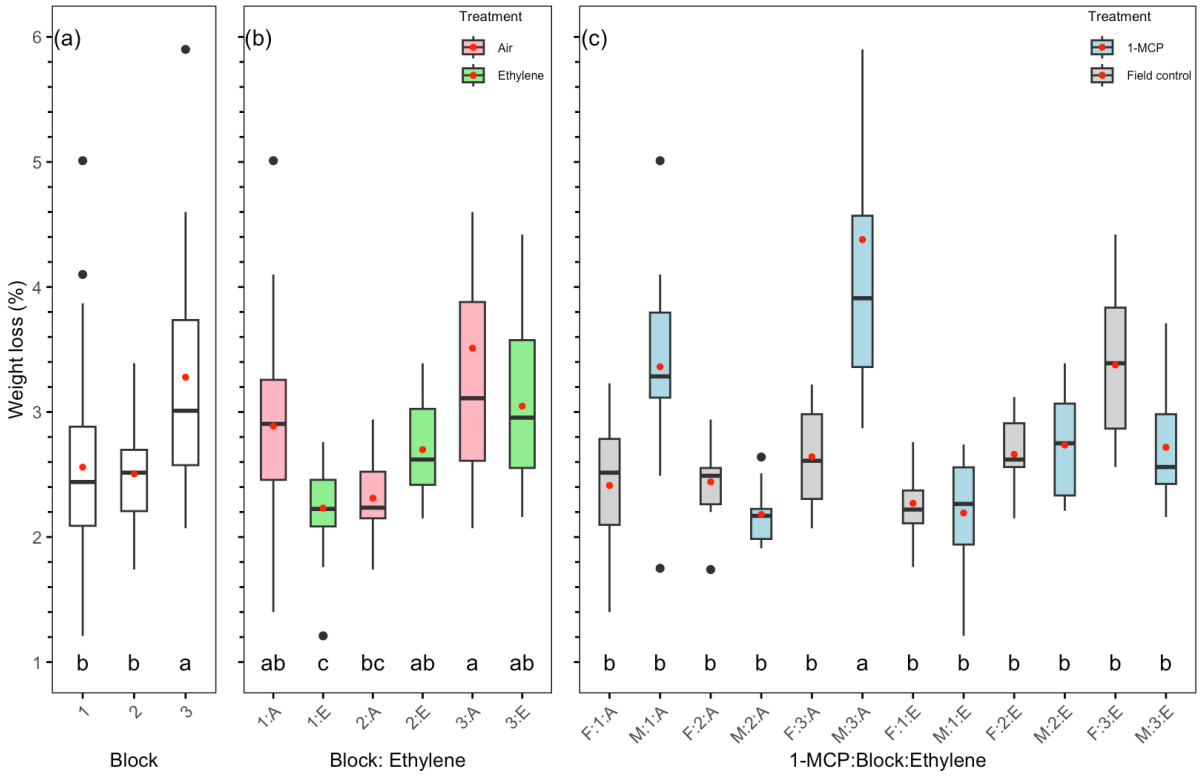

Figure 5.6 Weight loss as influenced by (a) growing block (1, 2, 3) (n = 60), (b) the combination of ethylene treatment (A = air, E = ethylene) and growing block (n = 20) and (c) the interaction among preharvest 1-MCP treatment (F = field control, M = 1-MCP), ethylene treatment and growing block (n = 10) during storage at 1.9 °C and 100% RH for 28 days. Different letters above the x-axis indicate significant differences in each sub-plot ($p < 0.05$). The line inside the box represents the median of the population, and the red point represents the mean value of the population.

5.4.2 Colour

After storage, broccoli heads were marketable with a green colour – no visual head quality changes occurred from the at-harvest measurement. Broccoli exhibited colour values of 114.8 ± 2.5 for h°, 41.6 ± 1.2 for L* and 10.3 ± 1.2 for C* – only C* value increased from 8.5 ± 1.3. There were no visual colour differences between treatments, with an average score of

1.05 ± 0.19 on the 5-point scale (high quality, Figure 2.6, data not shown). Although 1-MCP treated broccoli heads had a lower L* value at harvest, the effect of preharvest 1-MCP treatment on L* varied by the harvest block after storage (Figure 5.7). In block 2, 1-MCP treated broccoli had a lower L* value than that of field control broccoli, resulting in a darker colour. This may suggest potential benefits of applying 1-MCP. However, this trend is not observed in blocks 1 and 3.

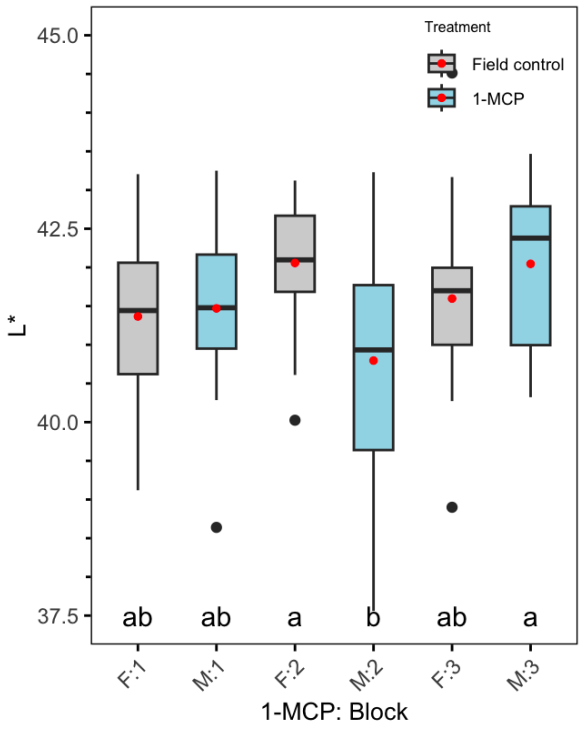

Figure 5.7 The L value as influenced by the combination of postharvest ethylene treatment (A = air, E = ethylene) and preharvest 1-MCP treatment (F = field control, M = 1-MCP) (n = 30) during storage at 1.9 °C and 100% RH for 28 days. Different letters above the x-axis indicate significant differences in sub-plot ($p < 0.05$). The line inside the box represents the median of the population, and the red point represents the mean value of the population.*

The influence of preharvest 1-MCP treatment on h° after storage was observed to vary by harvest block (Figure 5.8a). 1-MCP treated broccoli in block 1 had a higher h° than that of untreated broccoli, suggesting the potential benefits of applying 1-MCP. Considering that block 1 may have had less mature heads (Table 5.2 and Table 5.5), the effect of preharvest 1-MCP treatment on h° may have been influenced by maturity, with earlier maturity being more sensitive to 1-MCP. The broccoli head from block 2 had a higher h° value than that from block 3 (Figure 5.8b).

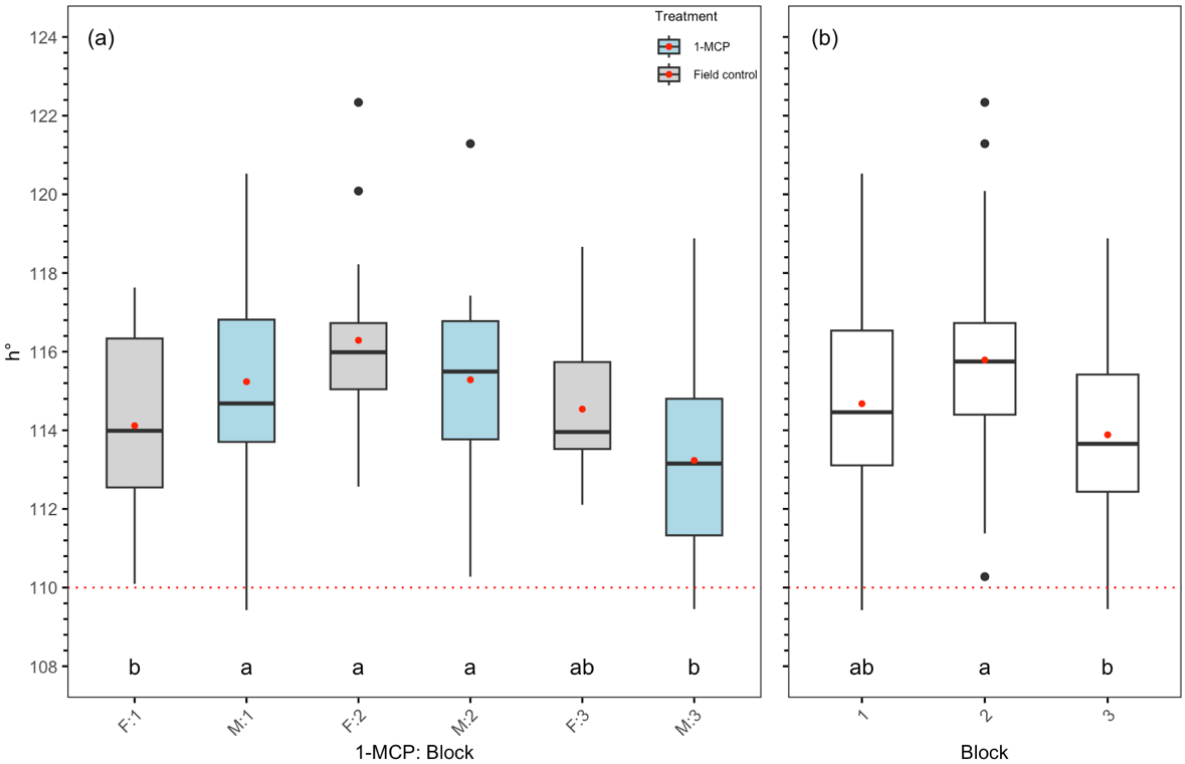

Figure 5.8 The h° value as influenced by (a) the combination of preharvest 1-MCP treatment (F = field control, M = 1-MCP) and growing block (1, 2, 3) (n = 20) and (n) growing block (n = 40) during storage at 1.9 °C and 100% RH for 28 days. Different letters above the x-axis indicate significant differences in each sub-plot ($p < 0.05$). The line inside the box represents the median of the population, and the red point represents the mean value of the population. The red dotted line represents the threshold of unacceptable yellowing (h° < 110 (Vasconcelos & Almeida, 2003)).

5.4.3 Firmness

The broccoli from block 1 had a significantly higher head firmness than that of block 3 (Figure 5.9), suggesting that broccoli had tighter heads with better compactness. This data may also indicate the maturity difference between blocks. The broccoli heads from block 1 may have been less mature than those from block 3.

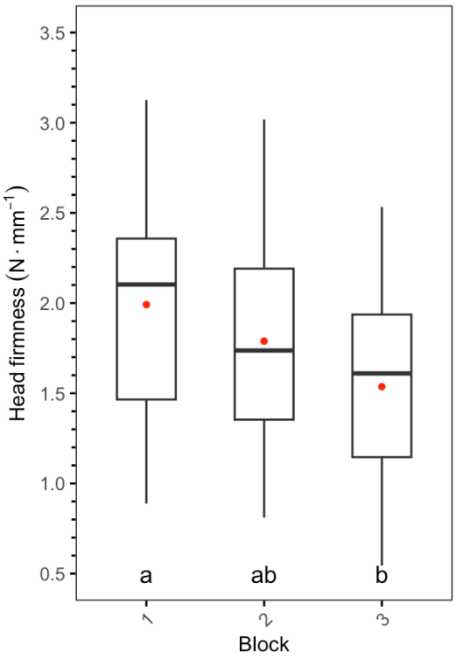

Figure 5.9 The head firmness as influenced by the growing block (1, 2, 3) (n = 40) during storage at 1.9 °C and 100% RH for 28 days. Different letters above the x-axis indicate significant differences in sub-plot ($p < 0.05$). The line inside the box represents the median of the population, and the red point represents the mean value of the population.

5.5 Quality change in broccoli stored in barrels at 6.7 °C for 18 days

5.5.1 Storage conditions and observation

The average temperature and RH during storage were 5.5 °C and 84%. For the barrels, the average temperature and RH were 6.7 ± 0.4 °C and approximately 100%, respectively. Similar to Trial 1, condensation was only observed at the bottom of the barrels.

For the heads stored in the air, smaller heads were observed to be marketable green, while the bigger heads started to exhibit yellowing (Figure 5.10). Low incidences of mould (3%) was observed. Leaf bases on the stalk showed < 50% yellowing and could easily be picked off. In contrast, for the heads that were exposed to ethylene during the storage, only occasional marketable green heads existed (≈ 7%, 4 out of 60) (Figure 5.10). Rot or mould was observed in approximately 23% heads (14 out of 60), with 4 of these showing severe mould and off-odour. Leaf bases on the stalk showed 100% yellowing and were easily detached, leaving distinct leaf scars.

The ethylene concentrations were stable, with an average of 1037.8 ± 48.3 nL·L^{-1} for ethylene-treated barrels and 36.4 ± 24.2 nL·L^{-1} for air-treated barrels. The measured CO_2

concentrations were 0.20 ± 0.02% and 0.17 ± 0.03% for ethylene and air-treated barrels, respectively.

5.5.2 After storage quality

Unlike in Trial 1 and the storage experiment at 1.9 °C in Trial 2, after being stored at 6.7 °C for 18 days, most of the broccoli heads were no longer marketable due to the development of yellowing (with an average score of 3.26 ± 1.55 on the 5-point scale (Figure 2.6)), especially for the heads exposed to ethylene (Figure 5.10). Additionally, some samples exhibited noticeable differences between the field treated (1-MCP) and control groups. Yellowing and darkening were observed in the leaf scar areas of the broccoli stalks (Figure 5.11). This same observation was made by Grzegorzewska et al. (2023), with broccoli treated with 1-MCP having fresher remaining petiole fragments, which were not easily detached by hand. This suggests that 1-MCP treatment effectively inhibited the senescence of the remaining petiole fragments.

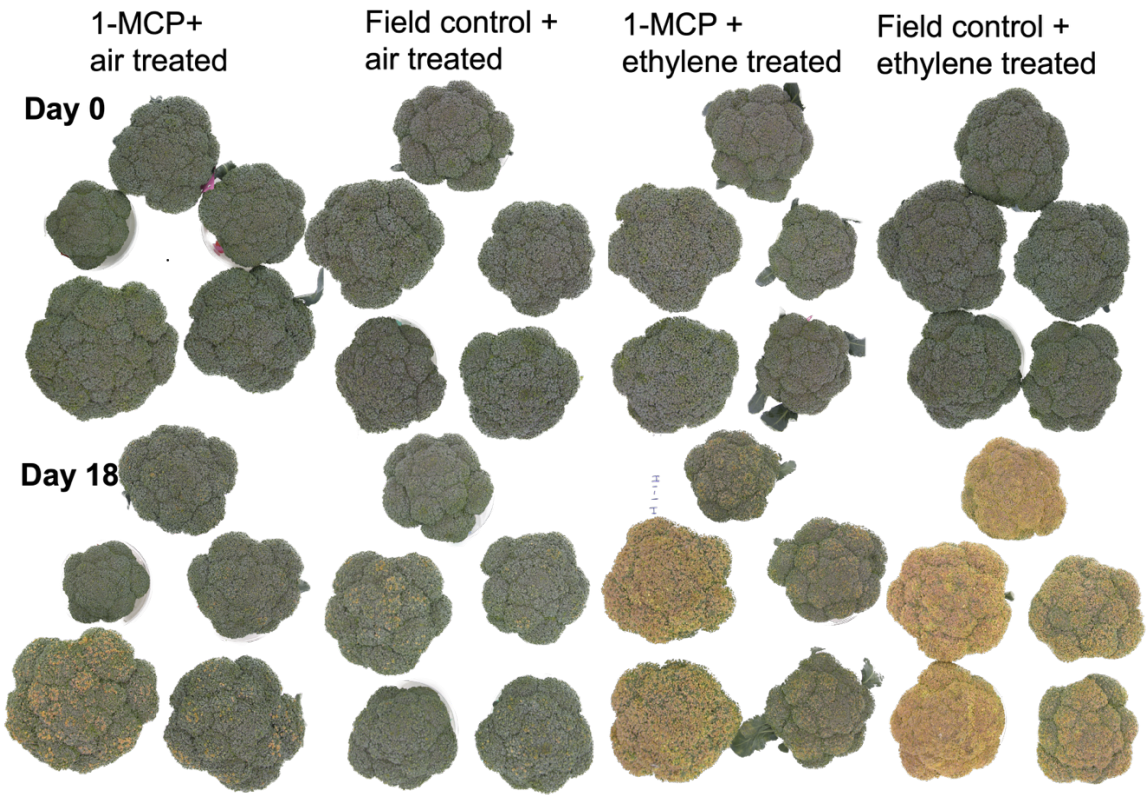

Figure 5.10 Visual quality change of representative broccoli heads after they had been continuously exposed to 1.0 μL·L^{-1} ethylene or air for 18 days at 6.7 °C

Figure 5.11 Visual quality of representative broccoli heads and leaf scars after they had been continuously exposed to ethylene for 18 days at 6.7 °C. The red arrows point to yellowing and darkening leaf scars.

After storage, broccoli had an average weight loss of 3.8 ± 1.1%, and exhibited colour values of 100.5 ± 12.7 for h°, 45.9 ± 4.1 for lightness (L*) and 15.4 ± 4.7 for chroma (C*). Head firmness averaged 2.12 ± 0.70 $N \cdot mm^{-1}$. Compared to the harvest value, the L* and C* increased (by 10.3% and 81.2%, respectively) while the h° value decreased by 13.2%. Interestingly, the average head firmness remained almost unchanged.

The methods for analysing and organising data were the same as those in Sections 4.6 and 5.4. Each of the post-storage attribute data sets was analysed for the effects of each factor (preharvest 1-MCP treatment, ethylene in storage, or growing block) and the interactions of all factors using a full ANOVA (as discussed in Section 3.6). To identify the significant results, a p-value table showing the effects on quality outcomes is provided (Table 5.8).

None of the three factors significantly impacted head firmness. Likewise, none of the three factors significantly affected weight loss (5.6 ± 0.6%) or respiration rate (335 ± 112 $nmol \cdot kg^{-1} \cdot s^{-}1$) of the central branchlets. Given that no effects were significant, no results were presented in this chapter.

Table 5.8 P-value table for broccoli quality as influenced by preharvest 1-MCP treatment, in storage ethylene treatment and in-field block after storage at 6.7 °C for 18 days.

Factor	Weight loss (%)	Colour score	Firmness score	Colour			Head firmness ($N \cdot mm^{-1}$)
				L*	C*	h°	
1-MCP *(n=60)*	0.162	0.015	0.718	< 0.001	0.002	0.001	0.475
Sum of squares (%)		1.61		3.85	3.03	3.42	
Block *(n=40)*	0.076	0.604	0.158	0.960	0.876	0.450	0.834
Ethylene *(n=60)*	0.003	< 0.001	0.856	< 0.001	< 0.001	< 0.001	0.052
Sum of squares (%)	6.32	61.69		60.31	56.95	59.44	
1-MCP × Block *(n=20)*	0.121	0.004	0.537	0.320	0.013	0.002	0.152
Sum of squares (%)		3.11			2.72	3.53	
1-MCP × Ethylene *(n=30)*	0.005	0.043	0.856	0.034	0.041	0.029	0.857
Sum of squares (%)	5.60	1.11		1.31	1.28	1.31	
Ethylene × Block *(n=20)*	0.043	0.013	0.087	0.525	0.575	0.233	0.625
Sum of squares (%)	4.52	2.40					
1-MCP × Ethylene × Block *(n=10)*	0.778	0.079	0.592	0.010	0.006	0.014	0.066
Sum of squares (%)				2.76	3.23	2.36	

5.5.2.1 Weight loss

Broccoli had an average weight loss of 3.8 ± 1.1% after storage. Ethylene treatment accelerated the weight loss of broccoli heads, resulting in ethylene-treated broccoli having a higher weight loss than that of air-treated broccoli (Figure 5.12a). The effects of ethylene treatment on weight loss varied by harvest blocks (Figure 5.12c). Ethylene-treated broccoli had the highest weight loss and was significantly different from air-treated broccoli in block 1. Nevertheless, this effect was not observed in blocks 2 and 3.

Similar to the results in Trial 1, for broccoli not treated with 1-MCP, ethylene treatment resulted in a higher weight loss than that for air-treated broccoli (Figure 5.12b), suggesting that there may be potential benefits to applying 1-MCP. Notably, the preharvest application of 1-MCP resulted in no difference in weight loss as influenced by the storage atmosphere. Importantly, when broccoli was continuously exposed to ethylene during storage, 1-MCP treatment resulted in significantly lower weight loss compared to that of untreated broccoli (Figure 5.12b). These findings suggested that 1-MCP could be a valuable tool for protecting broccoli from excessive weight loss when exposed to ethylene and non-optimal temperature conditions.

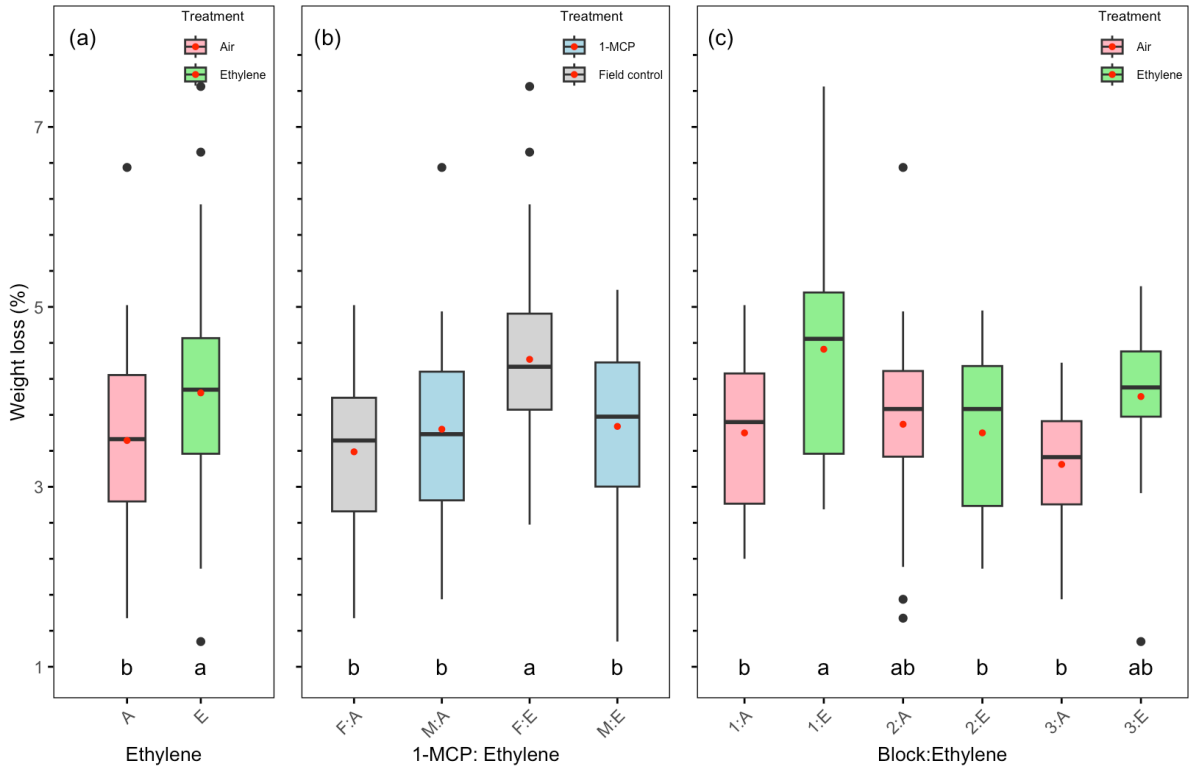

Figure 5.12 Weight loss as influenced by (a) ethylene treatment (A = air, E = ethylene) (n = 60), (b) the interaction of ethylene and preharvest 1-MCP treatment (F = field control, M = 1-MCP) (n = 30) and (c) the interaction of growing block (1, 2, 3) and ethylene treatment (n = 20) during storage at 6.7 °C and 100% RH for 18 days. Different letters above the x-axis indicate significant differences in each sub-plot ($p < 0.05$). The line inside the box represents the median of the population, and the red point represents the mean value of the population.

5.5.2.2 Colour

Compared to the at-harvest values, after storage, the L* and C* increased (by 10.3% and 81.2%, respectively), while the h° value decreased by 13.2%. Ethylene exposure in the postharvest environment dominated the colour results. As expected, ethylene treatment accelerated the yellowing of broccoli heads, resulting in a significantly greater yellowing (higher colour score) than air-treated broccoli (Figure 5.13b). With this observed effect, on average, preharvest 1-MCP treated broccoli heads had a lower colour score than untreated broccoli, indicating a positive effect of 1-MCP on broccoli colour (Figure 5.13a). While 1-MCP treated broccoli resulted in a lower colour score than untreated broccoli when exposed to ethylene, most ethylene-treated broccoli had a higher colour score than most air-treated broccoli, regardless of whether the broccoli heads were treated with 1-MCP or not (Figure 5.13b-c). This suggests that while 1-MCP may have a potential positive effect in maintaining broccoli colour, limiting exposure to higher concentrations of ethylene (1 $\mu L \cdot L^{-1}$) may be a better strategy to preserve broccoli colour.

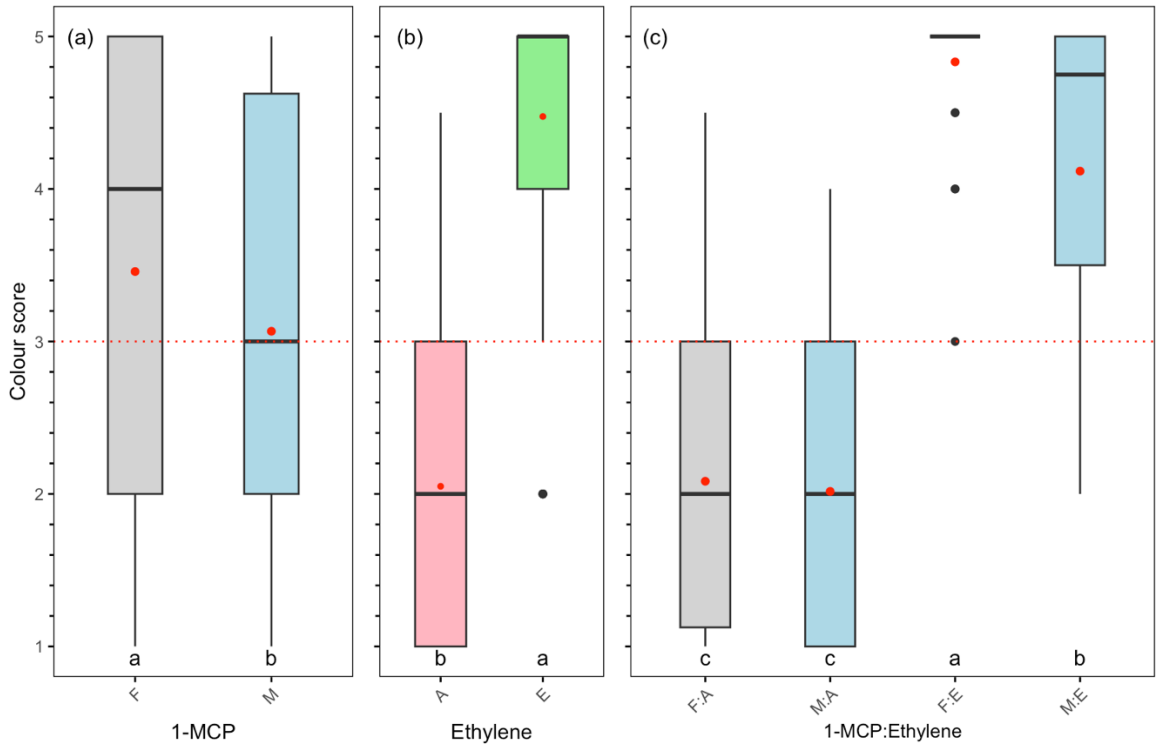

Figure 5.13 Colour score as impacted by (a) preharvest 1-MCP treatment (F = field control, M = 1-MCP) (n = 60), (b) ethylene treatment (A = air, E = ethylene) (n = 60), (c) the interaction of preharvest 1-MCP treatment (F = field control, M = 1-MCP) and postharvest ethylene treatment (n = 20) during storage at 6.7 °C and 100% RH for 18 days. The red dotted line represents the limit of marketability (colour scores ≥ 3 means unmarketable (Cantwell & Suslow, 2002)). Different letters above the x-axis indicate significant differences in each sub-plot ($p < 0.05$). The line inside the box represents the median of the population, and the red point represents the mean value of the population.

The effects of preharvest 1-MCP treatment or postharvest ethylene treatment on broccoli colour scores varied by harvest blocks (Figure 5.14). In block 1, 1-MCP treated broccoli had a significantly lower colour score than that of untreated broccoli, indicating that 1-MCP could maintain the colour of broccoli heads (Figure 5.14a). However, in the other two blocks, no significant colour score difference was observed for 1-MCP treated and untreated broccoli. Ethylene-treated broccoli had a higher colour score than air-treated broccoli in each block (Figure 5.14b), indicating that ethylene treatment accelerated the colour change in broccoli heads.

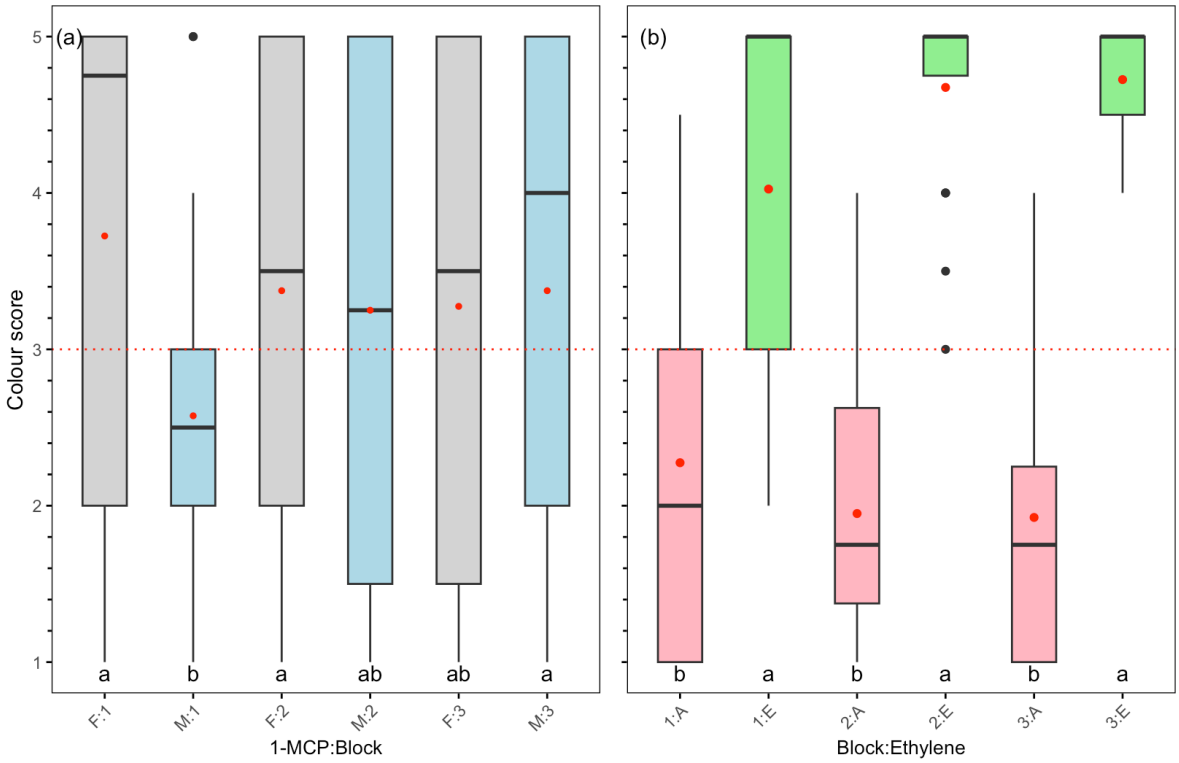

Figure 5.14 Colour score as impacted by (a) the interaction of 1-MCP treatment (F = field control, M = 1-MCP) and growing block (1, 2, 3) (n = 20) and (b) the interaction of block and ethylene (n = 20) during storage at 6.7 °C and 100% RH for 18 days. The red dotted line represents the limit of marketability (colour scores ≥ 3 means unmarketable (Cantwell & Suslow, 2002)). Different letters above the x-axis indicate significant differences in each sub-plot ($p < 0.05$). The line inside the box represents the median of the population, and the red point represents the mean value of the population.

Preharvest 1-MCP treated broccoli heads exhibited a lower L* than untreated broccoli, indicating potential beneficial effects of 1-MCP in inhibiting the increase of L* value (Figure 5.15a). This result mimics the observed difference in the L* at harvest (Figure 5.1). Given that the magnitude is approximately the same (from average 42 vs 41 to 46 vs 45), this difference in L* may have occurred in the field with it being conserved over the storage period.

Ethylene-treated broccoli had a significantly higher L* value than that of air-treated broccoli (Figure 5.15b). Treatment of broccoli with 1-MCP resulted in a lower L* than that for untreated broccoli when both were exposed to ethylene (Figure 5.15c). However, ethylene-treated broccoli had a higher L* value than air-treated broccoli, regardless of whether the broccoli heads were treated with 1-MCP or not (Figure 5.15c). These results may suggest that 1-MCP has the potential for slowing down the lightening of broccoli postharvest when exposed to ethylene. However, in this case, the adverse effects of ethylene treatment may suppress the positive impacts of 1-MCP.

The interaction among three factors was evident only in block 1 under ethylene exposure, where 1-MCP treatment resulted in a significantly lower L* value than that for untreated broccoli (Figure 5.15d). This suggested that 1-MCP could be a useful tool when broccoli heads were stressed with ethylene.

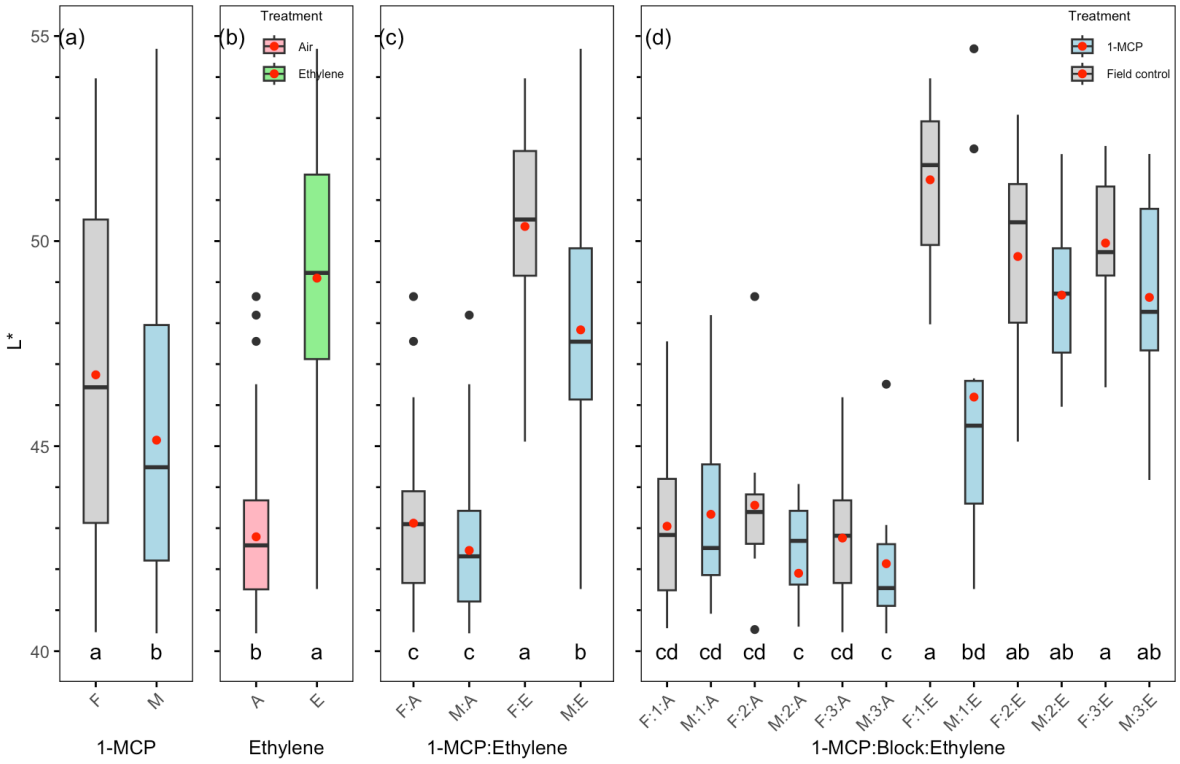

Figure 5.15 The L value as impacted by (a) preharvest 1-MCP treatment (F = field control, M = 1-MCP) (n = 60), (b) ethylene treatment (A = air, E = ethylene) (n = 60), (c) the interaction of preharvest 1-MCP and ethylene treatment (n = 30) and (d) the interaction among 1-MCP treatment, ethylene treatment and growing block (1, 2, 3) (n = 10) during storage at 6.7 °C and 100% RH for 18 days. Different letters above the x-axis indicate significant differences in each sub-plot ($p < 0.05$). The line inside the box represents the median of the population, and the red point represents the mean value of the population.*

The preharvest treatment of broccoli with 1-MCP resulted in lower C* than untreated broccoli (Figure 5.16a), suggesting the benefits of applying 1-MCP.

As expected, ethylene-treated broccoli had a significantly higher C* value than that of air-treated broccoli heads (Figure 5.16b), indicating that ethylene treatment accelerated the intensity of broccoli with a more yellowish colour.

The preharvest treatment of broccoli with 1-MCP resulted in a lower C* than that of untreated broccoli when both were exposed to ethylene (Figure 5.16c). However, ethylene-treated broccoli had a higher C* value than air-treated broccoli, regardless of whether the broccoli heads were treated with 1-MCP or not.

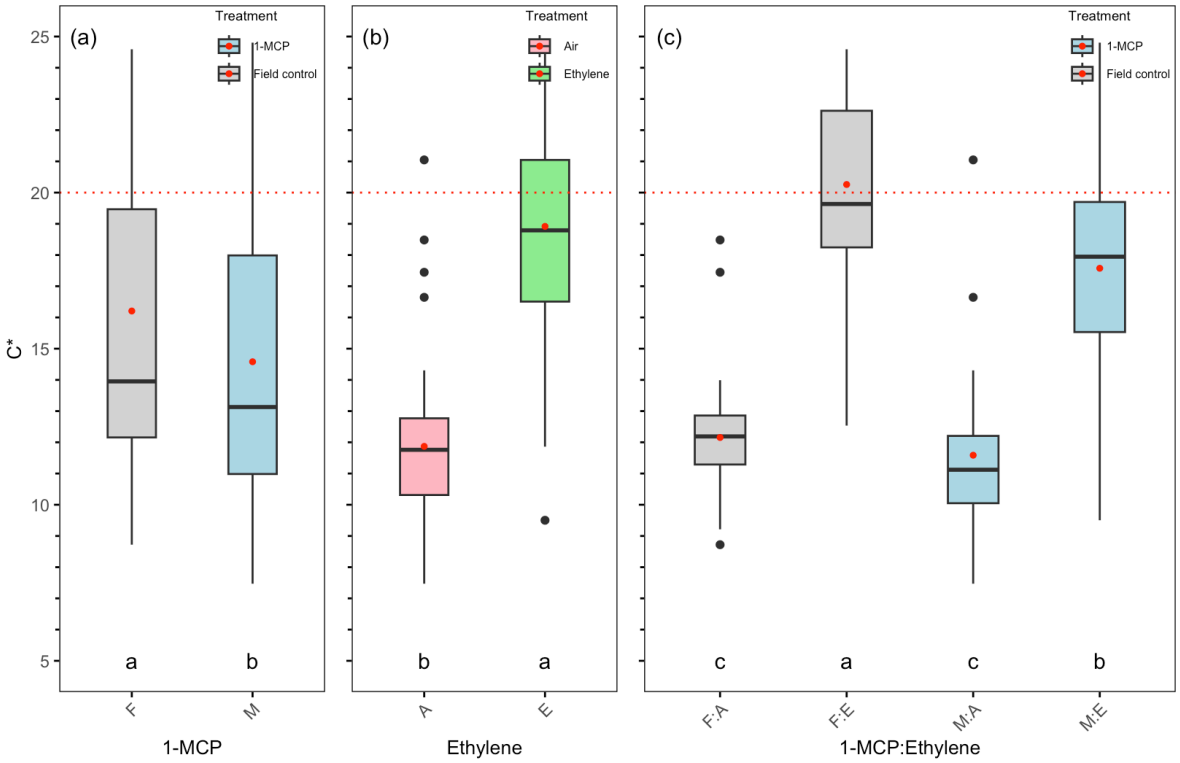

Figure 5.16 The C value as impacted by (a) preharvest 1-MCP treatment (F = field control, M = 1-MCP) (n = 60), (b) ethylene treatment (A = air, E = ethylene) (n = 60), (c) the interaction of preharvest 1-MCP and ethylene treatment (n = 30) during storage at 6.7 °C and 100% RH for 18 days. Different letters above the x-axis indicate significant differences in each sub-plot ($p < 0.05$). The line inside the box represents the median of the population, and the red point represents the mean value of the population. The red dotted line represents the threshold of unacceptable yellowing (C* ≥ 20 (Vasconcelos & Almeida, 2003)).*

The effects of 1-MCP treatment on the C* value varied by the harvest blocks (Figure 5.17a). 1-MCP treated broccoli heads had a significant lower C* value in block 1 than untreated broccoli. However, 1-MCP treatment did not show positive effects on C* value in blocks 2 and 3.

Similar to the L* value, the interaction among three factors was evident only in block 1 under ethylene exposure, where 1-MCP treatment resulted in a significantly lower C* value than that for untreated broccoli (Figure 5.17b). This suggested that 1-MCP could be a helpful tool when broccoli heads were stressed with ethylene.

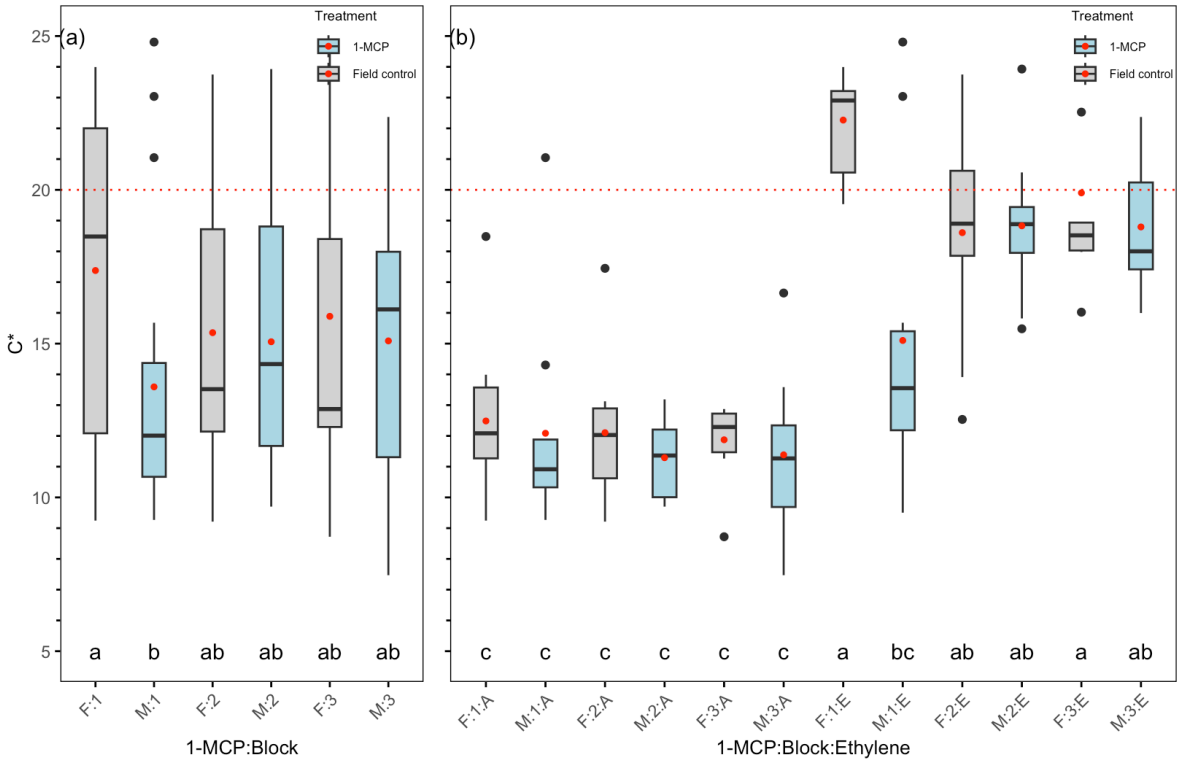

Figure 5.17 The C value as impacted by (a) the interaction of preharvest 1-MCP treatment (F = field control, M = 1-MCP) and growing block (1, 2, 3) (n = 20) and (b) the interaction among preharvest 1-MCP treatment, ethylene treatment (A = air, E = ethylene) and growing block (n = 10) during storage at 6.7 °C and 100% RH for 18 days. Different letters above the x-axis indicate significant differences in each sub-plot ($p < 0.05$). The line inside the box represents the median of the population, and the red point represents the mean value of the population. The red dotted line represents the threshold of unacceptable yellowing (C* ≥ 20 (Vasconcelos & Almeida, 2003)).*

The h° value directly reflects the green and yellow colour of broccoli heads. Preharvest 1-MCP treated broccoli heads had a significantly higher h° value than untreated broccoli (Figure 5.18a), suggesting that 1-MCP treatment resulted in a greener colour. Ethylene had the strongest effects on colour (Figure 5.18b) among all treatments, confirming its major role in accelerating chlorophyll degradation. 1-MCP treated broccoli resulted in a higher h° than untreated broccoli when exposed to ethylene (Figure 5.18c), suggesting the protection of 1-MCP on broccoli from ethylene exposure.

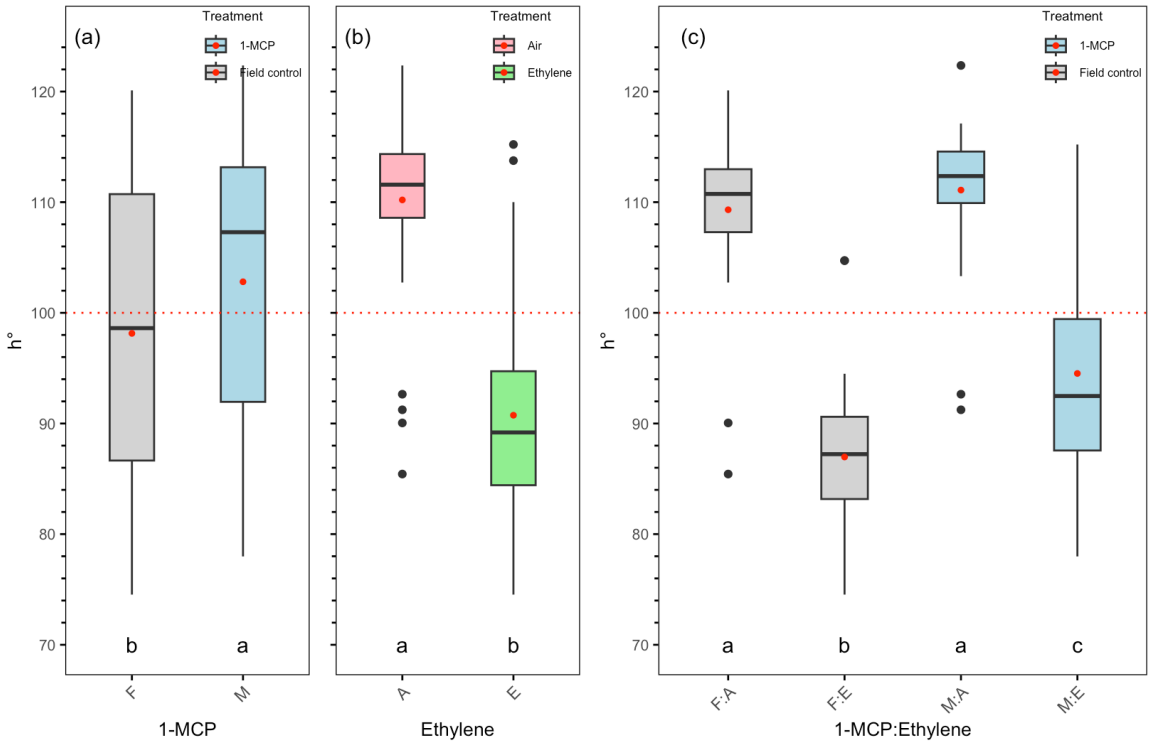

Figure 5.18 The h° value as impacted by (a) preharvest 1-MCP treatment (F = field control, M = 1-MCP) (n = 60), (b) ethylene treatment (A = air, E = ethylene) (n = 60), (c) the interaction of preharvest 1-MCP treatment and postharvest ethylene treatment (n = 30) during storage at 6.7 °C and 100% RH for 18 days. Different letters above the x-axis indicate significant differences in each sub-plot ($p < 0.05$). The line inside the box represents the median of the population, and the red point represents the mean value of the population. The red dotted line represents the threshold of unacceptable yellowing (h° < 110 (Vasconcelos & Almeida, 2003)).

The influence of preharvest 1-MCP on h° varied by the harvest blocks (Figure 5.19a). In block 1, 1-MCP treated broccoli had a higher h° value than untreated broccoli. However, this pattern was not observed in blocks 2 and 3.

The interaction among three factors was evident only in block 1 under ethylene exposure, where 1-MCP treatment resulted in a significantly higher h° value than that for untreated broccoli (Figure 5.19b). Temperature variation among barrels may have contributed to this difference. Under ethylene exposure, the average recorded temperature in the 1-MCP treated barrels was 6.2 °C, while that in the untreated barrels was 1.4 °C higher (7.6 °C).

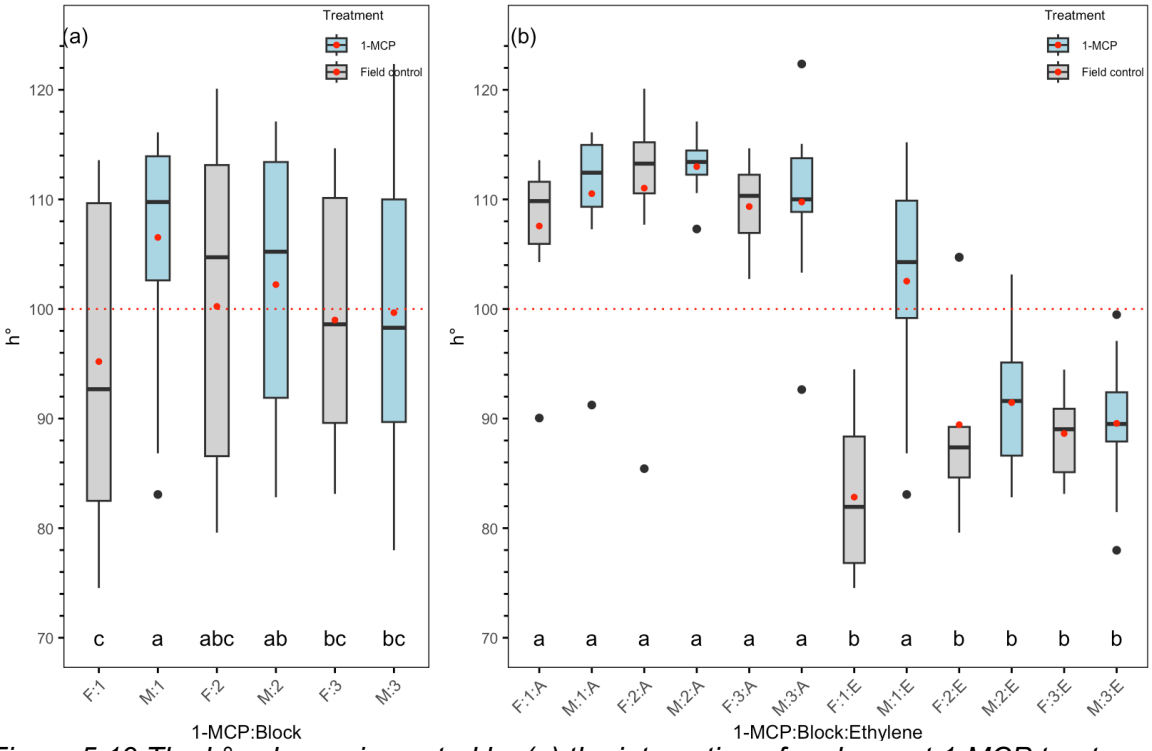

Figure 5.19 The h° value as impacted by (a) the interaction of preharvest 1-MCP treatment (F = field control, M = 1-MCP) and ethylene treatments (A = air, E = ethylene) (n = 20) and (b) the interaction among preharvest 1-MCP treatment, ethylene treatment and growing block (1, 2, 3) (n = 10) during storage at 6.7 °C and 100% RH for 18 days. Different letters above the x-axis indicate significant differences in each sub-plot ($p < 0.05$). The line inside the box represents the median of the population, and the red point represents the mean value of the population. The red dotted line represents the threshold of unacceptable yellowing (h° < 110 (Vasconcelos & Almeida, 2003)).

5.6 Discussion and conclusion

The objectives of this experiment were as follows:

1) To investigate the effects of preharvest 1-MCP application (three days prior to harvest) on the growth and development of broccoli heads (diameter) in the field.
2) To evaluate the impact of the preharvest 1-MCP on the at-harvest quality (colour and firmness) of broccoli.
3) To evaluate the influence of preharvest application of 1-MCP on postharvest storage performance of broccoli heads across a range of temperatures and when continuously exposed to ethylene stress.

Overall, after 'Iron' broccoli was stored at 1.9 °C for 28 days, broccoli quality did not respond to either preharvest 1-MCP application or postharvest ethylene exposure. None of the combined treatment changed the quality of broccoli; we could not observe any effects of 1-

MCP or ethylene exposure on broccoli. Broccoli heads stayed a marketable green colour (Figure 5.4).

When 'Iron' broccoli was stored at 6.7 °C for 18 days, mixed outcomes were obtained (Figure 5.10). Statistically, for weight loss, 1-MCP treatment only worked under ethylene exposure – broccoli preharvest untreated with 1-MCP and postharvest exposed to ethylene exhibited the greatest weight loss. In contrast, all other three combined treatments (broccoli untreated with 1-MCP and without ethylene exposure; broccoli preharvest treated with 1-MCP and with or without ethylene exposure) resulted in lower weight loss, with no significant differences among them (Figure 5.12b). For colour, an intermediate effect was observed (Figure 5.13c, Figure 5.15c, Figure 5.16c and Figure 5.18c), suggesting that preharvest 1-MCP treatment only partially protected the broccoli from continuous ethylene exposure when broccoli was stored at 6.7 °C for 18 days. The underlying cause of this partly effective response is unclear, but may be related to factors such as ethylene exposure, storage temperature and duration, 1-MCP concentration, possibly the development of additional receptors after treatment – these will be discussed in the remainder of this chapter and Chapter 6.

While these results demonstrate a statistically significant difference among preharvest 1-MCP and postharvest ethylene treatments, it is also important to consider whether such differences translate into commercial significance. A statistically significant difference – as affected by sample size, data variability, and experimental design – reflects measurable variation between treatments, but not whether the size of this difference matter commercially; statistical differences does not always correspond to a commercially meaningful outcome. For example, at-harvest L* value was statistically different between 1-MCP treatment and the field control (Figure 5.1), but human eyes cannot detect the difference, so there is no commercial significance. In New Zealand, broccoli is generally sold by the head in retail (not weight). However, weight loss indirectly affects growers, retailer, and consumers, as it reduces overall quality, including size, visual appearance, and eating quality. Commercially, broccoli heads are considered unmarketable once unacceptable yellowing occurs, typically defined as a colour score ≥ 3 or h° < 110° (Cantwell & Suslow, 2002; Vasconcelos & Almeida, 2003; Li et al., 2017). Therefore, although 1-MCP did partially protected broccoli from ethylene exposure, heads exposed to ethylene were unmarketable after being stored at 6.7 °C for 18 days, as a colour score ≥ 3 and h° <110°.

When the broccoli plants are growing, ethylene may initiate head production and promote the senescence of leaf and head, resulting in advanced maturity (Section 2.1.1.3).

Preharvest 1-MCP application has the potential to block the ethylene receptor, thereby blocking these responses of the broccoli plant to ethylene.

As noted, and not unusually, broccoli grew unevenly in the field. Based on observation, broccoli plants were assumed to already be in the head growth stage, involving both cell division and expansion (Section 2.1.1) at the time of 1-MCP application. As discussed in Trial 1 (Section 4.2), 1-MCP solution releases gaseous 1-MCP after spraying, which binds ethylene receptors in broccoli tissues. However, newly formed cells may not be fully protected as cell division continues. It is possible that new ethylene receptors may synthesise after 1-MCP blocked the current receptors, and plants and tissues may thus restore ethylene sensitivity (Dias et al., 2021).

5.6.1 Physiology

The ethylene sensitivity of broccoli changed after harvest. Immediately after harvest, broccoli was less sensitive to ethylene; it can be speculated that the broccoli is still in system 1 of ethylene production or dominated by harvest stress. While 24–36 h after harvest, the h° of broccoli started to decline, indicating a higher sensitivity (Tian et al., 1994). It could be hypothesised that broccoli shifted from system 1 to system 2 after harvest (Figure 6.2). Broccoli may firstly have responded to the harvest stress and then other stress like ethylene exposure. This wound-induced ethylene exposure could potentially advance the sensitivity of heads by upregulating ethylene receptors. Ethylene exposure in late events (that likely happen in system 2) rather than in the early events stage would be expected to trigger an auto-inductive response to ethylene perception and response in the broccoli heads.

During storage at 4 °C for 30 days, ethylene production of broccoli branchlets reduced from 13.4 pmol·kg^{-1}·s^{-1} (1.1 μL·kg^{-1}·h^{-1}) to 1.2 pmol·kg^{-1}·s^{-1} (0.1 μL·kg^{-1}·h^{-1}) and then increased to 7.3 pmol·kg^{-1}·s^{-1} (0.6 μL·kg^{-1}·h^{-1}) and 12.2 pmol·kg^{-1}·s^{-1} (1.0 μL·kg^{-1}·h^{-1}) for control and 1-MCP treatment, respectively (Ku et al., 2013). The pattern of ethylene production in this study was that ethylene production initially decreased from 9.4 ± 2.1 pmol·kg^{-1}·s^{-1} and then slightly stabilised at 2.7 ± 0.5 pmol·kg^{-1}·s^{-1}. The initial decrease in ethylene production is likely to be explained by the temperature response from 20 °C to 5.5 °C. Phuong et al. (2022) found that the ethylene production of broccoli was 8.06 and 1.46 pmol·kg^{-1}·s^{-1}(0.66 and 0.12 μL·kg^{-1}·h^{-1}) after 10 days of storage at 10 and 0 °C, respectively. Although the ethylene induced by wounds in broccoli heads occurs after harvest, it is possible that the temperature response to ethylene production dominated as the measurement of ethylene production began 10 h after harvest

(Figure 5.3). Another possible reason could be that broccoli was still in system 1 and this wound-induced ethylene had not yet inducted a climacteric response (Figure 6.2).

As discussed in Section 4.7.1, Ma et al. (2009) demonstrated that postharvest 1-MCP treatment resulted in higher ethylene production (125 pmol·kg^{-1}·s^{-1}, 0.45 nmol·g^{-1}·h^{-1}) than that of control (25 pmol·kg^{-1}·s^{-1}, 0.09 nmol·g^{-1}·h^{-1}) at 20 °C 48 h after harvest, as a result of high ACS activity and ACC concentration.

Ku et al. (2013) demonstrated that postharvest application of 1-MCP did not affect the respiration rate of broccoli branchlets, which is in accordance with the results in the present study. The respiration rate in our study supported what Cantwell and Suslow (2002) described, that the respiration rates of florets are more than twice that of the intact heads (194.7-219.1 nmol·kg^{-1}·s^{-1} at 5 °C). However, Fan and Mattheis (2000), Ma et al. (2009), Phuong et al. (2022, 2023), and Ghimire et al. (2024) all found that the postharvest application of 1-MCP reduced the respiration rate of broccoli heads. The different applications of 1-MCP may cause different results.

In this study, physiology measurement started ~ 10 h after harvest, whereas Downs et al. (1997) showed that sucrose in broccoli florets at 20 °C declined to less than half within 6 h. As the broccoli in my study was also held at 20 °C during preparation for storage experiments, the rapid early changes were likely missed. The possible new dividing cell after 1-MCP application and physiological changes that occurred during harvesting may also contribute to the inefficiency of 1-MCP. In addition, cultivars and storage temperature might also be the causes of these different results; for example, Ku et al. (2013) used 'Green Magic' cultivar stored at 4 °C, while Fan and Mattheis (2000) used 'Winsor' stored at 10 °C, Ma et al. (2009) used 'Montop' cultivar stored at 20 °C, and Phuong et al. (2022) used 'MKS-B107' stored at 10 °C (Table 2.7).

5.6.2 Storage temperature

As highlighted in Section 2.1.4.1, storage temperature significantly influences the storage life and post-storage quality of broccoli. Vasconcelos and Almeida (2003) showed that 'Marathon' broccoli had a storage life of 76 days when stored at 1 °C, but only 2 days and 12 days at 20 °C and 10 °C, respectively. Green (125°) and yellow (98°) h° values were reported by Phuong et al. (2022), when the broccoli was stored at 0 °C and 10 °C, respectively, for 20 days. Able et al. (2002) demonstrated that storage at 2 °C significantly extended the shelf life of broccoli, and this influence is far greater than that induced by 1-MCP treatment. In this study,

broccoli heads stored at 6.7 °C for 18 days had a hue angle measurement of 100.5°, whereas those stored at 1.9 °C for 28 days had a hue angle of 114.8°.

As outlined in Section 2.1.3.1.1, a two-phase colour change hypothesis was introduced by Vasconcelos and Almeida (2003): Phase 1 begins immediately after harvest, with no visual change in colour (h° ≥ 110° and C* ≤ 20). During Phase 2, h° decreases while L* and C* increase, leading to visual yellowing. These two phases can be linked to early and late events categorised by Downs et al. (1997) (Figure 5.20). Vasconcelos and Almeida (2003) also demonstrated that the storage temperature affected both the duration of Phase 1 and the rate of colour change during Phase 2 (Figure 2.5). Higher storage temperature reduced the duration of Phase 1 and accelerated the speed of colour change in Phase 2. This corroborates the finding that broccoli heads exhibiting a greener colour when stored at 1.9 °C rather than 6.7 °C in this study, despite being stored for a further 10 days.

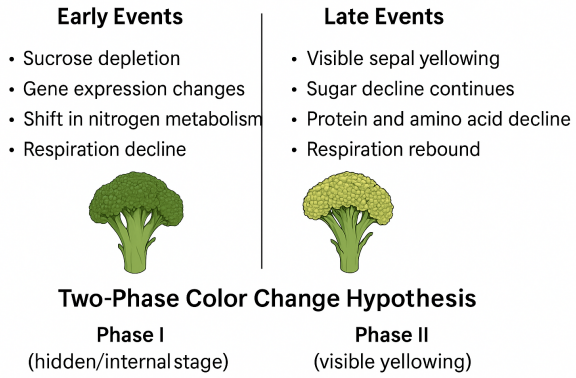

Figure 5.20 Conceptual alignment of postharvest broccoli physiology with the two-phase colour change hypothesis. Image is self-produced with AI assistance. According to Downs et al. (1997) and Vasconcelos and Almeida (2003), early events (such as sucrose depletion) occur during Phase I, when the florets remain green externally. Late events (such as visible sepal yellowing) correspond to Phase II, when senescence becomes visible.

For broccoli stored at 6.7 °C under ethylene exposure, the loss of quality was due to a combination of yellowing and the onset of rot. Izumi et al. (1996) mentioned the incidence of rot possibly due to differences in maturity and field conditions, and the contamination during handling. Importantly, ethylene exposure not only induced the yellowing of broccoli florets but also increased the decay of broccoli heads (Thompsom & Cantwell, 2001).

5.6.3 Impacts of preharvest application of 1-MCP on broccoli quality

In this trial, applying 1.0 $mL \cdot m^{-2}$ 1-MCP three days before harvest did not significantly affect any at-harvest quality of broccoli, except for the L* value (Table 5.2).

In this study, when the broccoli heads were continuously exposed to 1.0 $\mu L \cdot L^{-1}$ ethylene during storage at 6.7 °C, preharvest application of 1-MCP resulted in lower weight loss, L* and C*, and a higher h° value. Dehydration may occur during storage and transportation, which may elevating ethylene production and thus resulting in abscission of leaves and flowers (Serek et al., 1995). However, when the broccoli heads were stored at 1.9 °C, similar results were not observed. To the author's knowledge, no published articles have reported on the preharvest application of 1-MCP on broccoli. Hence the results are compared to previous postharvest application of 1-MCP studies.

The effects of postharvest 1-MCP application on broccoli quality have been mixed, as previously discussed (Section 2.4), with most cases of 1-MCP resulting in positive results, while others showed null results (Table 2.7 and Figure 2.11). Positive results were reported for when 1-MCP treated broccoli heads were exposed to ethylene during storage. For example, Ma et al. (2009) demonstrated that treating broccoli heads with 2.5 $\mu L \cdot L^{-1}$ 1-MCP for 12 h prior to 20 $\mu L \cdot L^{-1}$ ethylene exposure for 12 h reduced yellowing and maintained visual colour when stored at 20 °C.

Phuong et al. (2023) hypothesised that the postharvest application of 1-MCP was effective only when the treatment index (TI) $\geq$ 120 $\mu L \cdot L^{-1}$ °C h, where TI = 1-MCP concentration ($\mu L \cdot L^{-1}$) × treatment temperature (°C) × time (h). After calculation, 90% of the studies with positive results (1-MCP was effective) supported this hypothesis (Table 2.7 and Figure 2.11). For example, Able et al. (2002) demonstrated that 1-MCP treatment increased the shelf life by 22 to 48% when broccoli was treated at different temperatures.

The treatment index (TI) hypothesis does not consider storage temperature and duration. Phuong et al. (2022) demonstrated that storage temperature and duration affected the efficacy of 1-MCP. The h° values were only significantly different when broccoli was stored at 0 °C for 40 days or 10 °C for 20 days with the same treatment of 1-MCP (1.0 $\mu L \cdot L^{-1}$ at 15 °C for 15 h).

For details on the factors influencing 1-MCP efficacy, refer to Section 2.4.2.4. In this study, 1-MCP was applied by spraying on broccoli plants (Section 4.2), resulting in droplets on leaves and heads (Figure 4.1). 1-MCP was mixed with water just before spraying (Table 3.2) The release rate of 1-MCP was influenced by environmental factors, including humidity and temperature. During the 10–20 minutes of application, the concentration of active 1-MCP in the droplets may have decreased over time, as 1-MCP was gradually released while the spray was being deposited on plant surfaces.

In this study, postharvest ethylene treatment resulted in higher L* and C* and lower h°, particularly during storage at 6.7 °C. As shown in Sections 2.1.4.1 and 2.2.2, these colour changes are likely due to chlorophyll breakdown and declining chloroplast membranes integrity. Both effects are accelerated by ethylene exposure (Figure 2.4) and non-optimal temperatures (Deschene et al., 1991; Tian et al., 1994; Cefola et al., 2010; Guirao et al., 2024).

It can be inferred from the hypothesis first described by Vasconcelos and Almeida (2003) that ethylene could reduce the duration of the colour change Phase 1 but could potentially not affect the rate of colour change during Phase 2 (Figure 2.5 and Figure 5.20). The rate of h° decline per day from Able et al. (2002) and the rate of yellowing per day in the study of De Beer and Crouch (2015) also agree with this inference. The inference explains the colour difference between ethylene-treated broccoli heads and untreated broccoli stored at 6.7 °C. Ethylene treatment accelerated the onset of yellowing in broccoli heads, resulting in broccoli heads with a yellowish appearance.

There were no visual colour differences in broccoli heads among treatments when stored at 1.9 °C. The h° and C* values changed from 115.8 ± 3.0° and 8.5 ± 1.3 to 114.8 ± 2.5° and 10.3 ± 1.2, respectively. Probably, the colour of broccoli heads was still in Phase 1 (h° ≥ 110° (Figure 5.8) and C* ≤ 20). Vasconcelos and Almeida (2003) reported a h° of 115° and C* of 17 after the 'Marathon' broccoli was stored at 1 °C for 70 days. A h° value of 116 was also presented by Phuong et al. (2022) in 'MKS-B107' broccoli after storage at 0 °C for 50 days. Potentially, the storage duration was not long enough to see the effect of either ethylene or 1-MCP treatment on broccoli when it was stored at 1.9 °C for 28 days.

5.6.4 Impacts of planting block and other preharvest factors on broccoli quality

Similar to the results in Chapter 4 for Trial 1, the planting block, either individually or in combination with treatments, affected all the post-storage attributes at both storage temperatures. These indicated the contributions of preharvest factors to postharvest quality.

Wills and Golding (2016) emphasised the importance of maturity when considering the response to 1-MCP. However, the maturity of the broccoli head is challenging to measure. Cantwell and Suslow (2002) stated that head size (diameter), compactness, and unopened buds on all florets are the maturity indices for broccoli heads. In practice, the head diameter, which is typically estimated visually, is the most commonly used measurement for harvest selection.

In this study, head diameter was also used as a measure of broccoli maturity. The surface area was then introduced in this trial because generally, the broccoli head had an irregular shape.

The average diameter could not accurately represent the size, especially when heads varied in floret arrangement and compactness. Calculating surface area can provide a more comprehensive estimation of head size and maturity. Additionally, at-harvest weight was analysed as a potential maturity index. Since broccoli was standardised by trimming the stalks to the same length (10 cm), the whole weight was expected to reflect head size and maturity. However, data analysis did not identify any significant factors – none of the treatments before harvest significantly impacted the at-harvest weight. This meant that 1-MCP and planting block had similar at-harvest weights for broccoli ($p > 0.16$ and $p > 0.55$ for 1.9 and 6.7 °C, respectively).

In the present study, when stored at 1.9 °C, broccoli from planting block 3 had the highest weight loss (Figure 5.6a), as well as the lowest h° (Figure 5.8b) and firmness (Figure 5.9). Moreover, the decline in broccoli head firmness is associated with water loss. Cantwell (2011) demonstrated that a weight loss of 4% resulted in a 30% decrease in firmness, which is likely the point at which consumers would consider the head soft. These indicate that the broccoli heads from block 3 may have been under more stress, resulting in more weight loss, yellowing, and firmness loss. More specifically, broccoli heads from block 3 with preharvest 1-MCP treatment and postharvest air treatment were under some unknown stress, as indicated by weight loss (Figure 5.6c).

Considering maturity, heads from block 1 had the lowest maturity (Table 5.2). This probably results in firmer heads (Figure 5.9), as well as more sensitivity to ethylene treatment (as indicated by weight loss, Figure 5.6b) and preharvest 1-MCP treatment (as indicated by h°, Figure 5.8a) when stored at 1.9 °C. Similarly, broccoli from block 1 had the smaller diameters (less mature, Table 5.3), resulting in a higher sensitivity to ethylene and 1-MCP treatment (Figure 5.12c, Figure 5.14a, Figure 5.15d, Figure 5.17b, and Figure 5.19b) when stored at 6.7 °C. Another explanation for the differences observed between blocks may be the applied sequence of 1-MCP (Section 5.6.3). Block 1 was applied first, and hence, this may have indicated that 1-MCP was applied to block 1 at a higher concentration and may have been a more effective dose. Taken together, these factors may have explained why broccoli from block 1 responded differently to the 1-MCP and ethylene treatments than broccoli from blocks 2 and 3 during storage at both temperatures.

5.7 Conclusion

In conclusion, the response of broccoli to 1-MCP in mitigating ethylene sensitivity is strongly influenced by storage temperature, as little quality change occurs when the cool chain is kept optimal. However, the preharvest application of 1-MCP three days before harvest may reduce weight loss and maintain the colour of 'Iron' broccoli heads under possible industry and home conditions. These conditions include where broccoli heads are delivered without optimal temperature control and are sold alongside ethylene-producing commodities in commercial distribution systems, retail stores storage and display, and in home refrigerators.

Chapter 6. Overall discussion and conclusion

Previously, in most cases, the postharvest application of 1-MCP on broccoli quality was demonstrated to reduce ethylene production or delay the peak of ethylene production, reduce the respiration rate and maintain h° (Table 2.7 and Figure 2.11). In this work, a preharvest 1-MCP spray was evaluated for the consequent effects on broccoli head quality at harvest and during subsequent storage under ethylene stress conditions. 1-MCP was applied three or four days prior to harvest on broccoli plants in two trials on 'Nobel' and 'Iron' broccoli at two different times in the season.

Four types (A-D) of possible outcomes are diagrammatically depicted in Figure 6.1. Type A: Ethylene exposure was necessary, and 1-MCP worked (only the quality of broccoli untreated with 1-MCP and exposed to ethylene changed. The quality of all three other combined treatment did not change). Type B: Ethylene exposure was necessary,, and 1-MCP did not work (the quality of broccoli exposed to ethylene, either with or without 1-MCP treatment, changed, while without ethylene exposure, the quality did not change). Type C: Ethylene exposure was not necessary, and 1-MCP worked (no matter whether there had been exposure to ethylene or not, the quality of preharvest 1-MCP treated broccoli did not change while untreated broccoli changed). Type D: Ethylene exposure was not necessary, and 1-MCP did not work (none of the combined treatments changed the quality).

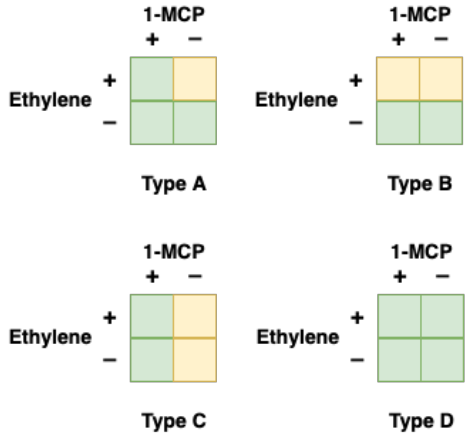

Figure 6.1 Four types (A-D) of possible outcomes. Green colour = quality did not change, while yellow colour = quality changed.

Type D outcome was obtained for 'Nobel' broccoli stored at 1.4 °C for 29 days (Figure 4.4) and for 'Iron' broccoli stored at 1.9 °C for 28 days (Figure 5.4). Broccoli did not respond to either preharvest 1-MCP treatment nor postharvest ethylene exposure. All heads stayed a marketable green colour, suggesting that broccoli was in phase 1 of colour change (Figure 2.5

and Figure 6.2). Since broccoli from the control exhibited minimal quality loss, the additional benefit of 1-MCP treatment was not evident, suggesting that under near-optimal conditions, its application may not be necessary. When broccoli was stored at 6.7 °C for 18 days, preharvest 1-MCP treatment exhibited an intermediate effect – more effective than type B but not reaching the full effectiveness of type A (Figure 6.1), although weight loss followed the type A pattern (Figure 5.12b). This suggested that 1-MCP treatment only partially protected the broccoli from continuous ethylene exposure under the storage conditions.

6.1 Major findings

To synthesise the outcome of this study in the context of existing literature and provide a framework for interpreting broccoli postharvest physiology, a conceptual model was developed (Figure 6.2). This model addresses (a) respiration and ethylene production patterns and (b) developmental stages of broccoli, collectively illustrating possible effects when 1-MCP was applied and the shifts in ethylene sensitivity in the postharvest physiology of broccoli.

High respiration rate during head initiation (shifts from vegetative growth to reproductive growth). At harvest, multiple stresses occur (Figure 2.4). Postharvest senescence primarily involves shifting from system 1 to system 2 for ethylene biosynthesis, changing from early events to late events, and colour changes from phase 1 (green) to phase 2 (yellow) (Figure 5.20) – all might happen simultaneously (Downs et al., 1997; Vasconcelos & Almeida, 2003). Commercially, broccoli is transplanted from seedlings. Zhu et al. (2024) categorised head initiation and development into four stages: the shoot apical meristem (SAM) stage, followed by the formation stage (FS), the expansion stage (ES), and the maturation stage (MS). The transition from SAM to FS occurs as the SAM differentiates into the inflorescence meristem (IM). FS transitions to ES when secondary IMs are generated; during ES, each secondary IM becomes a second-order SAM and starts differentiating into new secondary IMs, with curd diameter increasing rapidly. ES then progresses to MS, characterised by the IM resuming the ability to differentiate into floral meristem (FM) and continuing flower development. If broccoli heads are not harvested at MS, floral development continues, leading to flowering and subsequently to floral/pollen senescence. In this study, 1-MCP was applied at MS, where cell expansion (apical elongation) dominated cell division (lateral expansion).

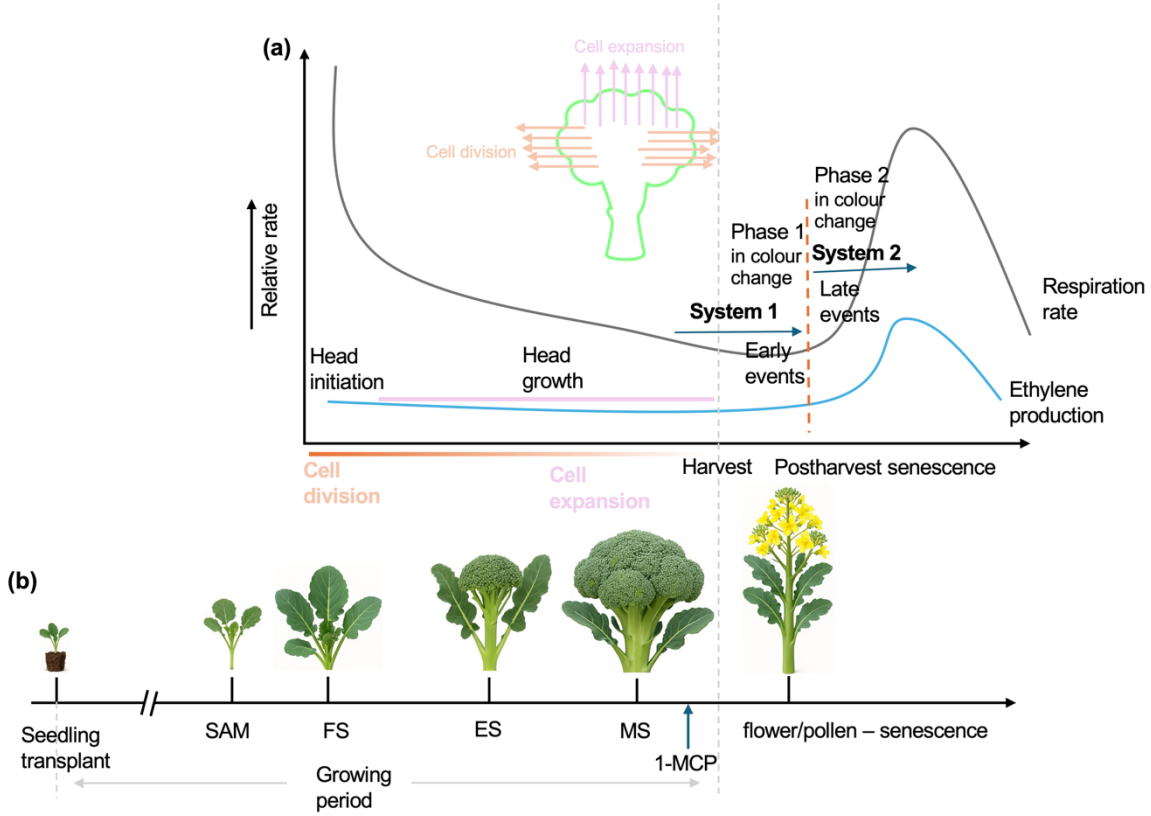

Figure 6.2 A conceptual model of broccoli development, respiration, and ethylene production patterns. (a) Respiration and ethylene production patterns across the broccoli head cycle life, and (b) Developmental stages from seedling transplant to flower senescence, which is self-produced with AI assistance.

Preharvest 1-MCP treatment was found to delay the peak of ethylene production from 4d 12h to 4d 16h when the broccoli branchlets were stored at 20 °C but did not reduce the respiration rate (Table 4.4 and Table 5.5). Possible benefits of preharvest application of 1-MCP were revealed, including decreasing weight loss (Figure 5.12b) and maintaining h° (Figure 5.18a, c and Figure 5.19), especially under ethylene exposure and suboptimal storage temperature conditions (e.g., > 5 °C). Given that h° (colour) is considered an important quality attribute for broccoli, the results of this study suggest that preharvest application of 1-MCP may protect broccoli from the deleterious effects of ethylene exposure in the supply chain, when at non-optimal temperature conditions.

Across both experiments, inconsistent results were observed, with differences in responses being associated with the field block. The potential explanation may be related to the effects of preharvest factors, such as maturity, spraying sequence, and unknown stresses. 'Nobel' and 'Iron' broccoli from block 1 had smaller heads, as indicated by the diameter (Table 4.1, Table 5.2, and Table 5.3). These heads exhibited higher sensitivity (Figure 6.3) to ethylene

exposure, as suggested by ethylene production (Figure 4.3), weight loss (Figure 4.5b, Figure 5.6b, Figure 5.12c), colour (Figure 5.8a, Figure 5.14a, Figure 5.15d, Figure 5.17, and Figure 5.19), and firmness (Figure 5.9). Furthermore, 1-MCP may have been applied to block 1 at a higher concentration and a more effective dose due to the spraying sequence and formulation release. The weight loss data (Figure 5.6c) revealed that broccoli heads from block 3 with preharvest 1-MCP treatment and postharvest air treatment may have been under some unknown stress. Thus, more research may be required to understand the mechanism that influences the consistency of results.

The observed yellowing depends on storage temperature, head size/maturity, and ethylene exposure. Thus, a conceptual diagram was created to show the effects of these three on broccoli yellowing (Figure 6.3). Bigger heads have a higher baseline of senescence, while smaller heads have higher ethylene sensitivity. Lower temperatures (close to 0 °C) reduce both baseline senescence and ethylene-induced yellowing, which is supported by the data from 'Nobel' and 'Iron' broccoli when stored at < 2 °C for around 30 days.

Regarding the effects of postharvest application of 1-MCP on broccoli quality, mixed findings (Section 2.4) indicated potential unknown knowledge in this field, as seemingly similar experiments result in different results. These inconsistencies highlight the complexity of the subject. Able et al. (2003) concluded that 1-MCP may be ideal for use on leafy vegetables that may be exposed to ethylene, for example, in situations where they are stored or sold alongside ethylene-producing commodities.

When considering the results of this study, the full range of responses were observed. Specifically, without ethylene stress, null effects of 1-MCP on broccoli quality were observed. In contrast, under less optimal and ethylene stress conditions, the ability to maintain quality was associated with the preharvest 1-MCP treatment. Furthermore, our results demonstrate that the response of broccoli to 1-MCP in mitigating ethylene sensitivity is strongly influenced by storage temperature. Supporting this, Able et al. (2002) demonstrated that storage at 2 °C significantly extended broccoli shelf life, with a much stronger effect than 1-MCP treatment. Taken together, these findings indicated that at near-optimal temperatures, temperature plays a more decisive role in quality changes than ethylene (Figure 6.3).

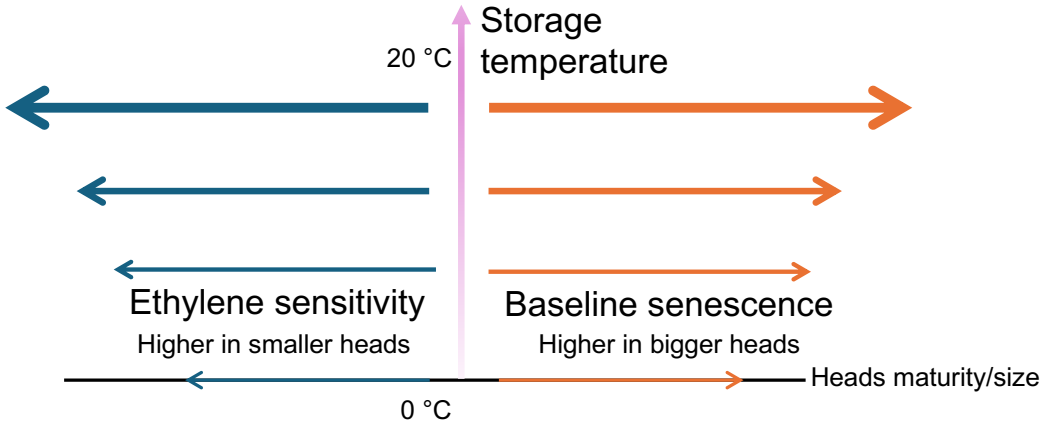

Figure 6.3 A conceptual diagram of head maturity, ethylene sensitivity and storage temperature effects on broccoli yellowing. The horizontal axis represents head maturity as described by head size (diameter). The orange arrow means that larger, more mature heads have higher baseline senescence (right), while the blue arrow represents that smaller, less mature heads are more sensitive to ethylene (left). Storage temperature is represented on the vertical axis, suggesting that lower temperatures (close to optimal temperature (0 °C) reduce both baseline senescence and ethylene-induced yellowing.

The remainder of this chapter examines preharvest factors affecting 1-MCP treatment, followed by the impact of storage conditions and ethylene exposure on broccoli quality. The potential advantages of preharvest 1-MCP application are discussed, along with study challenges. The chapter concludes with a summary and recommendations for future research.

6.2 The responses of preharvest factors to 1-MCP treatments

6.2.1 Effect of 1-MCP treatment on broccoli quality as affected by maturity

The maturity of broccoli is most commonly measured by head diameter. In this study, the diameter of broccoli heads (100–170 mm) was smaller than that of the heads used in the studies by Yuan et al. (2010) (150–200 mm) and Fernández-León et al. (2013) (200–250 mm), which may indicate a different maturity of broccoli heads. Based on data and observation, larger heads (more mature) already had higher baseline senescence, while smaller (less mature) heads were often more ethylene-sensitive (Figure 6.3). Since 1-MCP blocks ethylene perception, its effectiveness depends on ethylene sensitivity. Thus, 1-MCP treatment may be more effective on less mature heads because these heads are more responsive to ethylene. Conversely, the senescence process in larger heads may already proceed even without ethylene, limiting the effect of 1-MCP.

In the present study, other possible maturity indices, such as surface area and at-harvest weight, were also used. This was necessary due to broccoli's irregular shape and standardised

trim of the stalks. However, data analysis did not identify any significant factors for these two indices or their ratio. Regression analysis did not reveal any high correlation between possible maturity indices (diameter, surface area, and at-harvest weight) and other quality contributors (weight loss, colour, and firmness).

The difference in other preharvest factors, such as cultivars, growing period from seedling to harvest (Figure 6.2), seasons and location, would also affect the maturity and at-harvest quality of broccoli heads (Section 2.1.2). 'Parthenon' broccoli, used by Fernández-León et al. (2013), had a growing period of 121 days from seedling to harvest. In this study, the growing periods of 'Nobel' and 'Iron' broccoli were 113 and 60 days, respectively. The length of the growing period may indicate the accumulated nutrition in broccoli heads that can be used after harvest.

6.2.2 Application time, concentration, and treatment temperature of 1-MCP

Blankenship and Dole (2003) and Watkins (2016) concluded that generally, the effectiveness of preharvest application of 1-MCP decreased with longer intervals time between application and harvest (Section 2.4.2.4). In this study, 1-MCP (1.0 $mL \cdot m^{-2}$, 180 g ai/ha) was sprayed three and four days prior to harvest. In the trials conducted in California and Western Australia, 1-MCP was sprayed the same day as harvest (M. Punter, personal communication, August 2, 2024). Consequently, this difference in timing of 1-MCP application may influence the differences in effectiveness of quality preservation between these trials and our study. Furthermore, temperature fluctuations were employed in these trials in California and Western Australia to stimulate the practical temperature scenarios that occurred in the supply chain. However, no fluctuating temperatures were applied in the present study.

During storage at 10 °C, Able et al. (2002) demonstrated that treatment temperatures affected the efficacy of 1-MCP: 12 $\mu L \cdot L^{-1}$ 1-MCP extended the shelf life of broccoli branchlets by 22–49% across a range of treatment temperatures (2, 10, 15, 20 °C).

Previously, 90% of the studies (Table 2.7 and Figure 2.11) with positive results (1-MCP was effective) supported the hypothesis developed by Phuong et al. (2023) – the postharvest application of 1-MCP was effective only when the treatment index (TI = 1-MCP concentration ($\mu L \cdot L^{-1}$) × treatment temperature (°C) × time (h)) ≥ 120 $\mu L \cdot L^{-1}$ °C h. However, this hypothesis may not be suitable for the preharvest application of 1-MCP due to differences in formulation and method of application. Additionally, the concentration used in preharvest treatments cannot be calculated in the same units as those used for postharvest applications.

Fan and Mattheis (2000) reported that the inhibitory effect of postharvest 1-MCP application on the respiration rate of broccoli under ethylene exposure was concentration-dependent. In the present study, the applied concentration of 1-MCP (1.0 $mL \cdot m^{-2}$) may have been insufficient to affect the respiration rate of broccoli branchlets. Another two possible explanations may be that 1) ethylene exposure was also necessary for 1-MCP effects on respiration, or 2) the physiology measurement occurred too late after harvest, and the measurement might have missed the meaningful data. Preharvest 1-MCP treatment only partially protected the broccoli from continuous ethylene exposure when broccoli was stored at 6.7 °C for 18 days. Since both cell expansion and division were involved when 1-MCP was applied (Figure 6.1), a greater amount of 1-MCP might be required to effectively bind to newly synthesised receptors and suppress ethylene responses. However, there is limited information regarding the effective dose and duration of preharvest 1-MCP application.

The application methods may influence the effectiveness of preharvest application of 1-MCP by the variable applied results. For fruits, such as apples, figs and plums, preharvest of 1-MCP can be applied through sprays or fumigation on the tree (Watkins, 2016). In this study, spraying was used due to the irregular shape and uneven growth of broccoli plants. Visible solution droplets remained on the leaf and head surface following the application of 1-MCP (Figure 4.1). However, most of the droplets were found on the leaf surface rather than the head surface due to the difference in surface area. Our results demonstrated that block 1 responded to 1-MCP differently from both trials, which may also be related to the spraying sequence in the field.

6.3 Impact of storage temperature and duration, ethylene concentration, and exposure duration on broccoli quality

Storage temperature determines the sensitivity of broccoli to ethylene and 1-MCP (Figure 6.3). Most previous studies demonstrated the positive effects of postharvest 1-MCP on broccoli quality when the broccoli heads were stored at ≥ 4 °C. Although postharvest 1-MCP treatment contributed to an extended storage life of 86 days at 1 °C, its positive effect on the maintenance of h° was significant only after storage for 70 days without ethylene exposure (Vasconcelos & Almeida, 2003). Collectively, these studies indicated the significant role of storage temperature and duration in determining ethylene sensitivity of broccoli. In this study, for those broccoli heads stored at < 2 °C, the null results should be interpreted in the context of the control treatments. The absence of significant differences may be attributed to the effectiveness of near-optimal storage conditions in maintaining broccoli quality, rather than to

the ineffectiveness of 1-MCP. Since broccoli from the control exhibited minimal quality loss, the additional benefit of 1-MCP was not evident, suggesting that its use may be unnecessary when storage conditions are well controlled.

In the study, barrels containing 10 broccoli heads were stored in temperature-controlled rooms (TCRs). The actual average temperature recorded for barrels differed slightly from the designed setpoints in TCRs: the 1 °C setpoint resulted in 1.9 °C and 1.4 °C in the barrels for 'Iron' and 'Nobel' broccoli, while the 4 °C treatment was measured at 6.7 °C for 'Iron' broccoli barrels. Three possible explanations include: 1) temperature variation in different positions/areas; 2) door opening and frequency of operation, and 3) microenvironment inside the barrels. Liu et al. (2004) and James et al. (2017) demonstrated that both 1) and 2) resulted in temperature variation due to airflow patterns. Moreover, the CO_2 produced by branchlets was sampled from the 4 °C room at around defrosting time for 11 days, which resulted in a higher temperature. Thus, the air temperature measured at the microenvironment inside the barrels differed from the set points in the rooms.

The efficacy of 1-MCP on broccoli is also associated with the ethylene concentration and exposure time during storage. A few studies employed a continuous ethylene exposure for broccoli heads during storage (Fan & Mattheis, 2000; Able et al., 2002; Lu, 2020). For the continuous ethylene exposure, Li et al. (2017) demonstrated that storage temperature and ethylene concentration determined the postharvest life (determined by leaf yellowing) for broccoli. Figure 6.4 illustrates this relationship using data from Table 1 of Li et al. (2017). Previously, when the broccoli was continuously exposed to 1.0 $\mu L \cdot L^{-1}$ ethylene, Fan and Mattheis (2000) reported a h° just around 110° (a threshold for green and yellow) after storage at 10 °C for 8 days; Able et al. (2002) showed around 6 days of shelf life of broccoli at 10 °C; Lu (2020) demonstrated that broccoli heads exhibited yellow and became unmarketable after storage at 4 °C and 98% RH for 14 days.

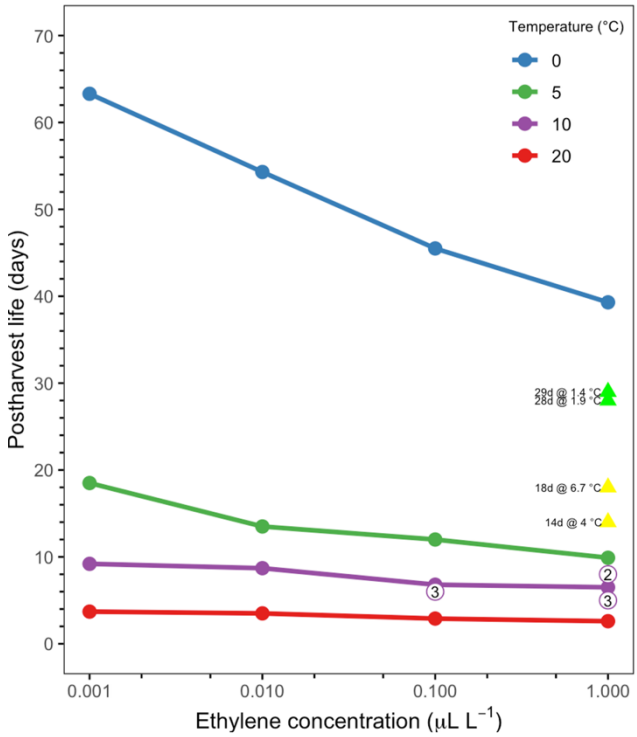

Figure 6.4 Broccoli postharvest life is affected by storage temperature and ethylene concentration under continuous exposure. Green triangles represent marketable broccoli, while yellow triangles mean yellowing broccoli. Purple numbers 2 and 3 indicate data from Fan and Mattheis (2000) and Able et al. (2002), respectively.

Li et al. (2017) reported a postharvest life of 10 days for broccoli stored at 5 °C under 1.0 µL·L^{-1} ethylene. In my study, storing broccoli at 6.7 °C under 1.0 µL·L^{-1} ethylene for 18 days resulted in unmarketable yellow heads (h° < 110°), likely because the storage period was too long. Li et al. (2017) also demonstrated a 40-day postharvest life when broccoli was stored at 0 °C. This aligns with the marketable, green broccoli observed in my study when stored at 1.9 °C for 28 days and at 1.4 °C for 28 days (Figure 6.4).

6.4 Potential advantages of preharvest application of 1-MCP

The data for 'Iron' broccoli revealed that preharvest application of 1-MCP partially protected broccoli heads from ethylene contamination produced by other horticultural products, such as apples. This protection was observed under the non-optimal storage conditions in the supply chain by reducing weight loss and maintaining colour. Although no significant effect on head growth rate was observed in this study (Table 5.1), previous studies on apples suggest that preharvest 1-MCP application may improve crop uniformity at maturity, which could reduce harvest times and thus labour costs for harvesting. Preharvest application of 1-MCP may allow broccoli head to grow to a bigger size without over maturing due to its inhibition of ethylene receptors and thus delay of senescence. Growers could earn more money for a higher yield.

Furthermore, the preharvest application of 1-MCP does not require a sealed environment because of its formulation.

6.5 The challenges of the study

6.5.1 Sample selection, preparation, and at-harvest maturity

Before postharvest storage, the broccoli heads were deliberately assigned to each postharvest treatment. This operation increased the complexity and made it challenging to interpret the post-storage attribute results.

There are variations in the maturity of commercially marketed broccoli, with different ranges of diameter categories based on the standards of Foodstuffs (FSNI, 2024; FSSI, n.d.). As discussed in Section 2.1.2.6, head diameter is a critical index of broccoli maturity. Maturity at harvest is the most significant factor that determines the duration and quality of storage life and shelf life of vegetables (Kader, 1997; Wichrowska et al., 2021). Consequently, the maturity of broccoli used in postharvest treatment was controlled through the allocation of size diameter categories (Section 3.3.3.1, Table 4.1 and Table 5.1). The allocation aimed to mitigate the potential effects of maturity and size on the postharvest study. In this study, the target was to obtain heads with a single diameter category (120–150 mm); however, at least 17% of 'Nobel' and 19% of 'Iron' heads in each field treated block fell within this category. Consequently, the total plot sizes used (approximately 350 'Nobel' plants and 550 'Iron' broccoli) were insufficient (Section 3.3.1.1). To secure sufficient samples in future experiments, substantially bigger plots would be required – approximately 720 'Nobel' plants and 1300 for 'Iron'.

6.5.2 1-MCP effective concentration assessment after spraying

In the study, 1-MCP may have been applied at a higher concentration to block 1, potentially resulting in a more effective dose due to the spraying sequence and formulation release in both trials. Regarding the preharvest application of 1-MCP, challenges included broccoli morphology variation, droplet distribution variability after spraying, and varied field conditions. As noted earlier, broccoli exhibits a significant heterogeneity of head size in the field. Unlike the stable conditions of an indoor controlled environment, the plant microenvironment in the field can vary considerably, thus influencing the actual exposure of broccoli heads and leaves to 1-MCP.

To the author's knowledge, there is no method to measure the concentration of preharvest applied 1-MCP. Typically, the efficacy of preharvest application of 1-MCP is

measured by physiological response (respiration rate and ethylene production), not by directly measuring gas concentration in the field. Visible droplets on broccoli heads and leaves can indicate that the 1-MCP formulation has been applied successfully. In the present study, broccoli from the control stayed untreated. In future experiments, water could be sprayed on the broccoli as a control.

6.5.3 Physiological measurement, flow rate measurements, and rot assessment

In this study, physiological measurements commenced approximately 10 h after harvest. By contrast, Downs (1997) reported that sucrose content in broccoli florets stored at 20 °C declined to less than half of the harvest level within the first 6 h. As the broccoli in my study was also held at 20 °C during preparation for other experiments, it is likely that the measurements missed the rapid physiological changes occurring immediately after harvest. Therefore, in future experiments, physiological measurements (respiration rate and ethylene production) could be conducted first. Moreover, samples for physiological measurements should be more representative.

Flow rate could have been measured daily in this study, because the differences in weight loss may have partly reflected variation in independent flow rate across barrels in the ethylene flow-through system, rather than ethylene and 1-MCP treatment effects alone. A possible explanation for the results may have been found by the flow rate data. However, the flow rate data had not been accurately recorded.

In the present study, the rot development of broccoli was recorded descriptively. In future experiments, taking more detailed photos could provide clearer records and comparisons. It is also recommended that the rot development be assessed based on the subjective score from Ekman et al. (2019), where 1 means no rots and 5 represents severe rots, > 10% rotten florets, because yellowing and the onset of rotting are the two biggest limitations of broccoli quality (Pogson & Morris, 1997). The post-storage broccoli photos, especially for those heads from block 1 exposed to ethylene conditions at 6.7 °C, indicated both rot development and yellowing on the broccoli heads. It was challenging to tell whether the difference between 1-MCP and control treatments was caused by 1-MCP's inhibiting of ethylene-induced yellowing or the differences in rot development.

6.5.4 Time limitations

Restricted time is another significant limitation because the longest research time for a master's student at Massey University is one year. Although the experiment was conducted

twice in two cultivars for two seasons to minimise the effects of the time constraint, there was still not enough time to conduct the experiment and analyse the results in more depth. For example, regression correlation among different quality indices was analysed roughly within each storage temperature, but no obvious relationship was found between possible maturity indices, weight loss, colour, and firmness. If there had been enough time, the potential relationships among these indices might have been found across multiple seasons and cultivars.

6.6 Conclusion

This work provides evidence that preharvest application of 1-MCP may have similar benefits as the postharvest application on broccoli by reducing weight loss and maintaining the green colour, especially under non-optimal storage conditions (suboptimal temperature and ethylene exposure). Therefore, 1-MCP can be a useful tool to protect broccoli from ethylene exposure in the supply chain. Practically, broccoli can be stored at suboptimal temperatures due to mixed load with chilling sensitive horticultural products or in consumers' homes. Broccoli could also be in mixed loads with ethylene producers, such as bananas and kiwifruits, in the supply chain and thus continuously exposed to ethylene. Therefore, 1-MCP should be taken into consideration to reduce the ethylene-induced yellowing, especially at suboptimal temperatures.

This study supports the hypothesis of the two-phase colour change in postharvest broccoli. The h° and C* values of broccoli after storage at low temperature (< 2 °C) suggested that the colour of broccoli was still in phase 1 (green), while high temperature (> 6 °C) and ethylene exposure resulted in phase 2 (yellow). There is potential for the industry to benefit from this study by lowering the storage temperature and ethylene concentration to maintain the quality and extend the shelf life of broccoli.

6.7 Recommendations

Preharvest application of 1-MCP could be recommended to delay yellowing and weight loss in postharvest broccoli when under ethylene exposure and non-optimal storage temperature, but its effectiveness seems strongly dependent on storage temperature and at-harvest maturity. Commercial businesses should therefore consider achieving optimal storage temperature firstly, and then applying 1-MCP, and further work is needed to assess maturity-specific responses.

The specific role of 1-MCP, whether applied before or after harvest, in modulating broccoli's sensitivity to ethylene and its effect on postharvest life remains unclear. Moreover,

given the significant implications of storage temperature in modulating the response of broccoli to 1-MCP, it would be valuable to conduct experiments under a range of suboptimal and fluctuating temperatures to further explore the relationship among 1-MCP, storage temperature, and ethylene exposure. In future experiments, temperature should be applied to mimic typical supply chain conditions (e.g., temperature changes from 5 to 10 or 15 °C). Bigger plots (approximately 720 'Nobel' plants and 1300 for 'Iron') would be required to secure sufficient samples from a single diameter category (120–150 mm) to make uniform the at-harvest maturity. This might assess maturity-specific responses.

The inconsistent responses of broccoli to 1-MCP and ethylene treatments may also be related to differences in the sequence of preharvest 1-MCP spraying. It would therefore be valuable to repeat this experiment using broccoli with varied spraying sequences. For example, under the same storage conditions, spraying 1-MCP across all three blocks or starting from a different block (2 or 3) would help researchers assess the potential influence of spraying sequences. Moreover, water could be sprayed as an additional water control, except for the untreated control, in future experiments. The effective dose and optimal applying time of preharvest application of 1-MCP remain unknown. It would have been interesting to see whether the application rate and timing affect the effectiveness of 1-MCP on broccoli.

This study confirmed the existence of two-phase of colour change, and hypothesised that broccoli might shift from system 1 to system 2 for ethylene production after harvest, simultaneously with the change from early events to late events, but the underlying mechanism still need to be clarified. More research, including direct measurement of physiological variables (such as ethylene production and respiration rate), may confirm this hypothesis of ethylene production shift. Moreover, the physiological variables should be measured directly after harvest, and data throughout whole postharvest life, especially after yellowing, should be tracked.

References

Able, A. J., Wong, L. S., Prasad, A., & O'Hare, T. J. (2002). 1-MCP is more effective on a floral brassica (*Brassica oleracea* var. *italica* L.) than a leafy brassica (*Brassica rapa* var. *chinensis*). *Postharvest Biology and Technology*, *26*(2), 147-155. https://doi.org/10.1016/S0925-5214(02)00011-X

Able, A. J., Wong, L. S., Prasad, A., & O'Hare, T. J. (2003). The effects of 1-methylcyclopropene on the shelf life of minimally processed leafy Asian vegetables. *Postharvest Biology and Technology*, *27*(2), 157-161. https://doi.org/10.1016/S0925-5214(02)00093-5

Adams, D., & Yang, S. (1979). Ethylene biosynthesis: Identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. *Proceedings of the National Academy of Sciences*, *76*(1), 170-174. https://www.pnas.org/doi/pdf/10.1073/pnas.76.1.170

AgroFresh. (2025a). *EthylBloc™ extends freshness of your flowers and plants*. https://www.agrofresh.com/solutions/ethylbloc/

AgroFresh. (2025b). *SmartFresh™ enables just-picked quality all the way to the consumer.* https://www.agrofresh.com/solutions/smartfresh/?cn-reloaded=1

AgroFresh. (2025c). *Harvista™ 1.3 SC helps your operation earn bigger, tastier returns*. https://www.agrofresh.com/solutions/harvista/

Banks, N. H., Cleland, D. J., Cameron, A. C., Beaudry, R. M., & Kader, A. A. (1995). Proposal for a rationalized system of units for postharvest research in gas exchange. *HortScience*, *30*(6), 1129-1131. https://journals.ashs.org/view/journals/hortsci/30/6/article-p1129.xml

Ben-Fadhel, Y., Ziane, N., Salmieri, S., & Lacroix, M. (2018). Combined post-harvest treatments for improving quality and extending shelf-life of minimally processed broccoli florets (*Brassica oleracea* var. *italica*). *Food and Bioprocess Technology*, *11*, 84-95. https://link-springer-com.ezproxy.massey.ac.nz/article/10.1007/s11947-017-1992-2

Berry, N., Cameron, P., & Walker, G. (2000). Integrated pest management for brassicas. *Crop & Food Research IPM Manual*, *11*, 1-71. http://agrilink.co.nz/wp-content/uploads/2007/12/updated_FSP-PDF-3.pdf

Blankenship, S. M., & Dole, J. M. (2003). 1-Methylcyclopropene: A review. *Postharvest Biology and Technology*, *28*(1), 1-25. https://doi.org/10.1016/S0925-5214(02)00246-6

Bleecker, A. B., & Kende, H. (2000). Ethylene: A gaseous signal molecule in plant. *Annual Review of Cell and Developmental Biology, 16*(1), 1-18. https://www-annualreviews-org.ezproxy.massey.ac.nz/docserver/fulltext/cellbio/16/1/annurev.cellbio.16.1.1.pdf?expires=1757393727&id=id&accname=ar-175835&checksum=D4ACFBAB48B892110A15CE3F77E00275

Buck, P. (1956). Origin and taxonomy of broccoli. *Economic Botany*, *10*(3), 250-253. https://www.jstor.org/stable/pdf/4287895.pdf?casa_token=LVg5kDl9v_IAAAAA:PlMcptWalMZoDzsYSXcfxQ2W1W9L5_-JHmwARbMmWvR0b-sV7QxY4X1QJIlTWGaFp0rvRo4fCvwY2xP7GOzZ9XZD5b5mjm15C0qrbMHhnYRZAp63Xp62

Cai, J. h., Cheng, S. c., Luo, F., Zhao, Y. b., Wei, B. d., Zhou, Q., Zhou, X., & Ji, S. j. (2019). Influence of ethylene on morphology and pigment changes in harvested broccoli. *Food and Bioprocess Technology*, *12*, 883-897. https://link-springer-com.ezproxy.massey.ac.nz/article/10.1007/s11947-019-02267-1

Cameron, A. C., & Reid, M. S. (2001). 1-MCP blocks ethylene-induced petal abscission of *Pelargonium peltatum* but the effect is transient. *Postharvest Biology and Technology*, *22*(2), 169-177.

Cantwell, M. (2011). *Vegetable meeting: Food safety and postharvest handling of vegetables* https://ucanr.edu/sites/default/files/2011-09/121449.pdf

Cantwell, M., & Suslow, T. (2002). *Broccoli: Recommendations for maintaining postharvest quality* [Fact sheet]. University of California, Davis, Postharvest Technology Center. Retrieved July 1, 2025 from https://postharvest.ucdavis.edu/produce-facts-sheets/broccoli

Carvalho, S. M., Heuvelink, E., Harbinson, J., & Van Kooten, O. (2006). Role of sink–source relationships in chrysanthemum flower size and total biomass production. *Physiologia Plantarum*, *128*(2), 263-273. https://onlinelibrary.wiley.com/doi/pdf/10.1111/j.1399-3054.2006.00733.x

Cefola, M., Amodio, M. L., Rinaldi, R., Vanadia, S., & Colelli, G. (2010). Exposure to 1-methylcyclopropene (1-MCP) delays the effects of ethylene on fresh-cut broccoli raab (*Brassica rapa* L.). *Postharvest Biology and Technology*, *58*(1), 29-35. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0925521410001031?via%3Dihub

Chen, H.-z., Zhang, M., & Guo, Z. (2019). Discrimination of fresh-cut broccoli freshness by volatiles using electronic nose and gas chromatography-mass spectrometry.

Postharvest Biology and Technology, *148*, 168-175. https://www.sciencedirect.com/science/article/pii/S0925521418307178?casa_token=VI690KbYLH8AAAAA:GbDyEvWPkRCdzpTasy3fLeszd3WST3LKjtlZi58pAhVNr_ZZJ6JE7bUUg-9YUBeeSV1sVWyIvg8

Clarke, S. F., Jameson, P. E., & Downs, C. (1994). The influence of 6-benzylaminopurine on post-harvest senescence of floral tissues of broccoli (*Brassica oleracea* var *italica*). *Plant Growth Regulation*, *14*(1), 21-27. https://link-springer-com.ezproxy.massey.ac.nz/article/10.1007/BF00024137

Cui, S., Yu, X., Chen, Y., Zhang, J., & Liu, G. (2022). Bacterial diversity and dominant spoilage microorganisms in fresh-cut broccoli. *Applied Sciences*, *12*(7), 3370. https://www.mdpi.com/2076-3417/12/7/3370

De Beer, T., & Crouch, E. M. (2015). Maintaining broccoli shelf-life during mixed load marketing with a novel in-box 1-methylcyclopropene and modified atmosphere packaging system. *Acta Horticulturae, 1071*, 607-613. https://www.actahort.org/books/1079/1079_19.htm

Deschene, A., Paliyath, G., Lougheed, E., Dumbroff, E., & Thompson, J. (1991). Membrane deterioration during postharvest senescence of broccoli florets: Modulation by temperature and controlled atmosphere storage. *Postharvest Biology and Technology*, *1*(1), 19-31. https://www.sciencedirect.com/science/article/abs/pii/0925521491900165

Dias, C., Ribeiro, T., Rodrigues, A. C., Ferrante, A., Vasconcelos, M. W., & Pintado, M. (2021). Improving the ripening process after 1-MCP application: Implications and strategies. *Trends in Food Science & Technology*, *113*, 382-396. https://www.sciencedirect.com/science/article/pii/S0924224421003332?casa_token=N-2AUqVAZDMAAAAA:eqM3M7eB-Wt2hZK3LuMx8v9s15nUNgPulH_KII1Wpb6t9H9Jn1h0ZCedLWu0iHJJkk7uYGuf3yY

Diputado, M. T. (1989). *Growth and development studies with broccoli (*Brassica oleraceae *var.* italica [unpublished doctoral dissertation]. Massey University. https://mro.massey.ac.nz/items/f4c9d9c3-0c1a-419c-8fe9-32ad08f4a0f2

Dolan, L. (1997). The role of ethylene in the development of plant form. *Journal of Experimental Botany*, *48*(2), 201-210. https://www-jstor-org.ezproxy.massey.ac.nz/stable/23695594

Downs, C. G., Somerfield, S. D., & Davey, M. C. (1997). Cytokinin treatment delays senescence but not sucrose loss in harvested broccoli. *Postharvest Biology and*

Technology, *11*(2), 93-100. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0925521497014191?via%3Dihub

Dubois, M., Van den Broeck, L., & Inzé, D. (2018). The pivotal role of ethylene in plant growth. *Trends in Plant Science*, *23*(4), 311-323. https://www.cell.com/trends/plant-science/pdf/S1360-1385(18)30015-3.pdf

Ekman, J. (2006). *Evaluation of new shipping technology for Australian vegetables* (Project No. VG04020 Final Report). Horticulture Australia Ltd. https://ausveg.com.au/app/data/technical-insights/docs/VG04020.pdf

Ekman, J. (2010). *Extension to project VG04020: Evaluation of new shipping technology for Australian vegetables* (Project No. VG06045 Final Report). Horticulture Australia Ltd. https://ausveg.com.au/app/data/technical-insights/docs/VG06045.pdf

Ekman, J. (2017). *Identifying and sharing postharvest best practice on-farm and online* (Project No. VG13083 Final Report). Horticulture Innovation Australia. https://ausveg.com.au/app/uploads/2024/09/VG13083.pdf

Ekman, J. H., Goldwater, A., Marques, J. R., Winley, E., Holford, P., & James, H. (2019). Preserving broccoli quality from harvest to retail: Use of 1-MCP. *Acta Horticulturae*, *1253*, 215–222. https://www.actahort.org/books/1256/1256_4.htm

Elfving, D. C., Drake, S. R., Reed, A. N., & Visser, D. B. (2007). Preharvest applications of sprayable 1-methylcyclopropene in the orchard for management of apple harvest and postharvest condition. *HortScience*, *42*(5), 1192-1199. https://journals.ashs.org/hort/hort/published/rest/pdf-watermark/v1/journals/hortsci/42/5/article-p1192.pdf/watermark-pdf/

Erdem, T., Arın, L., Erdem, Y., Polat, S., Deveci, M., Okursoy, H., & Gültaş, H. T. (2010). Yield and quality response of drip irrigated broccoli (*Brassica oleracea* L. var. *italica*) under different irrigation regimes, nitrogen applications and cultivation periods. *Agricultural Water Management*, *97*(5), 681-688. https://www.sciencedirect.com/science/article/pii/S0378377409003655?casa_token=dfTsxzWZSzsAAAAA:37ctvATfIo4wYM8cjdQh8fp55sVDNN4MjjwIScKm6wiPJHNecs9Qn4CA9q8MUru-wNW-wtlgiFU

Ezhilan, M., Nesakumar, N., Babu, K. J., Srinandan, C., & Rayappan, J. B. B. (2019). Freshness assessment of broccoli using electronic nose. *Measurement*, *145*, 735-743. https://www.sciencedirect.com/science/article/pii/S0263224119305718?casa_token=yfS7OUV9FrcAAAAA:IwzYuIfq7ygI6fnRqI-0O0z9O5geaCIMHsmo8cNNabEOdjV1epmoMQZZ-G51scMBMyIB3j8hD50

Fahey, J. W. (2015). Brassica: Characteristics and properties. In B. Caballero, P. M. Finglas, & F. Toldrá (Eds.), *Encyclopedia of food and health* (pp. 469-477). Elsevier Inc. https://pure.johnshopkins.edu/en/publications/brassica-characteristics-and-properties

Fan, X., & Mattheis, J. P. (2000). Yellowing of broccoli in storage is reduced by 1-methylcyclopropene. *HortScience*, *35*(5), 885-887. https://journals.ashs.org/hort/hort/published/rest/pdf-watermark/v1/journals/hortsci/35/5/article-p885.pdf/watermark-pdf/

Feng, X., Apelbaum, A., Sisler, E. C., & Goren, R. (2000). Control of ethylene responses in avocado fruit with 1-methylcyclopropene. *Postharvest Biology and Technology*, *20*(2), 143-150. https://doi.org/10.1016/S0925-5214(00)00126-5

Fernández-León, Fernández-León, A., Lozano, M., Ayuso, M., & González-Gómez, D. (2013). Different postharvest strategies to preserve broccoli quality during storage and shelf life: Controlled atmosphere and 1-MCP. *Food Chemistry*, *138*(1), 564-573.

Fernández-León, M., Fernández-León, A., Lozano, M., Ayuso, M., Amodio, M. L., Colelli, G., & González-Gómez, D. (2013a). Retention of quality and functional values of broccoli 'Parthenon'stored in modified atmosphere packaging. *Food Control*, *31*(2), 302-313.

Figure NZ Trust. (2025). *Broccoli harvested in New Zealand: Year ended June 2014–2022, hectares harvested* [Chart]. Figure.nz. https://figure.nz/chart/rB4FhLHwfjLKcjnl-OW7cfwMVQkMwMFP2

Food and Agriculture Organization of the United Nations (FAO). (2024a). *FAOSTAT. Crops and livestock products.* https://www.fao.org/faostat/en/#data/QCL

Food and Agriculture Organization of the United Nations (FAO). (2024b). *FAOSTAT. SDG indicators.* https://www.fao.org/faostat/en/#data/SDGB

Food and Agriculture Organization of the United Nations (FAO). (2024c). *Technical platform on the measurement and reduction of food loss and waste: Food loss and waste database*. https://www.fao.org/platform-food-loss-waste/flw-data/en/

Foodstuffs North Island (FSNI). (2024). *Produce specification: Broccoli.* https://www.foodstuffs.co.nz/partnering-with-us/Produce-Specifications

Foodstuffs South Island (FSSI). (n.d.). *Produce specification: Broccoli.* https://www.foodstuffs-exchange.co.nz/assets/documents/FSSI-docs/FSSI-Produce-Specifications/Specification-Broccoli-Version-1.pdf

Fresh Facts. (2023). *New Zealand's fresh fruit and vegetable industry*. https://unitedfresh.co.nz/assets/site/images/images/Fresh-Facts-–-December-2023.pdf

Forney, C. F., & Brandl, D. G. (1992). Control of humidity in small controlled-environment chambers using glycerol-water solutions. *HortTechnology*, *2*(1), 52-54. https://journals.ashs.org/view/journals/horttech/2/1/article-p52.xml

Forney, C. F., Song, J., Fan, L., Hildebrand, P. D., & Jordan, M. A. (2003). Ozone and 1-methylcyclopropene alter the postharvest quality of broccoli. *Journal of the American Society for Horticultural Science*, *128*(3), 403-408. https://journals.ashs.org/view/journals/jashs/128/3/article-p403.xml

Ghimire, U., Abeli, P., Brecht, J. K., Pliakoni, E., & Liu, T. (2024). Unique molecular mechanisms revealed for the effects of temperature, CA, ethylene exposure, and 1-MCP on postharvest senescence of broccoli. *Postharvest Biology and Technology*, *213*, 112919. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0925521424001649?via%3Dihub

Ghimire, U., Pliakoni, E., Yu, F., Brecht, J. K., & Liu, T. (2023). Identifying genes regulated during natural, on-plant senescence in broccoli (*Brassica oleracea*) in contrast to postharvest senescence. *Postharvest Biology and Technology*, *206*, 112535. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S092552142300296X?via%3Dihub

Gillies, S., & Toivonen, P. (1995). Cooling method influences the postharvest quality of broccoli. *HortScience*, *30*(2), 313-315. https://journals.ashs.org/view/journals/hortsci/30/2/article-p313.xml

Gómez-Lobato, M. E., Hasperué, J. H., Civello, P. M., Chaves, A. R., & Martínez, G. A. (2012). Effect of 1-MCP on the expression of chlorophyll degrading genes during senescence of broccoli (*Brassica oleracea* L.). *Scientia Horticulturae*, *144*, 208-211. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0304423812003445?via%3Dihub

Gómez-Lobato, M. E., Mansilla, S. A., Civello, P. M., & Martínez, G. A. (2014). Expression of Stay-Green encoding gene (*BoSGR*) during postharvest senescence of broccoli. *Postharvest Biology and Technology*, *95*, 88-94. https://doi.org/10.1016/j.postharvbio.2014.04.010

Gong, Y., & Mattheis, J. P. (2003). Effect of ethylene and 1-methylcyclopropene on chlorophyll catabolism of broccoli florets. *Plant Growth Regulation*, *40*(1), 33-38. https://doi.org/10.1023/A:1023058003002

Grabowska, A., Sękara, A., Bieniasz, M., Kunicki, E., & Kalisz, A. (2013). Dark-chilling of seedlings affects initiation and morphology of broccoli inflorescence. *Notulae*

Botanicae Horti Agrobotanici Cluj-Napoca, *41*(1), 213-218. https://www.notulaebotanicae.ro/index.php/nbha/article/view/8272

Gray, A. (1982). Taxonomy and evolution of broccoli (*Brassica oleracea* var. *italica*). *Economic Botany*, *36*(4), 397-410. https://www-jstor-org.ezproxy.massey.ac.nz/stable/4254428?seq=1

Grevsen, K. (1998). Effects of temperature on head growth of broccoli (*Brassica oleracea* L. var. *italica*): Parameter estimates for a predictive model. *The Journal of Horticultural Science and Biotechnology*, *73*(2), 235-244. https://www-tandfonline-com.ezproxy.massey.ac.nz/doi/abs/10.1080/14620316.1998.11510970

Gross, K. C., Wang, C. Y., & Saltveit, M. E. (2016). *The commercial storage of fruits, vegetables, and florist and nursery stocks*. (Agriculture Handbook No. 66). U.S. Department of Agriculture, Agricultural Research Service. https://www.ars.usda.gov/ARSUserFiles/oc/np/CommercialStorage/CommercialStorage.pdf

Grzegorzewska, M., Badełek, E., Ciecierska, A., Fabiszewski, K., & Rutkowski, K. P. (2023). The storage ability of broccoli after 1-methylcyclopropene treatment. *Journal of Horticultural Research*, *31*(1), 71-80. https://doi.org/10.2478/johr-2023-0004

Guirao, A., García-Pastor, E., Valverde, J. M., García-Ponsoda, P., Díaz-Mula, H. M., Valero, D., Serrano, M., Serradilla, M. J., & Martínez-Romero, D. (2024). A new ethylene scrubber reactor that also eliminate ozone: Practical application confirmed with broccoli heads. *Scientia Horticulturae*, *328*, 112939. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0304423824000992?via%3Dihub

Gutezeit, B. (2004). Yield and nitrogen balance of broccoli at different soil moisture levels. *Irrigation Science*, *23*, 21-27. https://link-springer-com.ezproxy.massey.ac.nz/article/10.1007/s00271-003-0089-z

Gwanpua, S. G., Jabbar, A., Tongonya, J., Nicholson, S., & East, A. R. (2018). Measuring ethylene in postharvest biology research using the laser-based ETD-300 ethylene detector. *Plant Methods*, *14*, 1-17. https://link.springer.com/content/pdf/10.1186/s13007-018-0372-x.pdf

Hasperué, J., Lemoine, M. L., Chaves, A., & Martínez, G. (2014). Effect of time of day for harvest and postharvest treatments on the sugar metabolism of broccoli (*Brassica oleracea* var. *italica*). *Agricultural and Food Science*, *23*(1), 48-59. https://doi.org/10.23986/afsci.8576

He, Q., & Xiao, K. (2018). Quality of broccoli (*Brassica oleracea* L. var. *italica*) in modified atmosphere packaging made by gas barrier-gas promoter blending materials. *Postharvest Biology and Technology*, *144*, 63-69. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0925521417308128?via%3Dihub

Himanshu, S., Singh, A., Kumar, S., & Kalura, P. (2013). Response of broccoli to irrigation scheduling and methods under drip, sprinkler and surface irrigation. *International Journal of Engineering and Advanced Technology*, *2*(4), 777-782. https://scholar.googleusercontent.com/scholar?q=cache:uiliowphJeIJ:scholar.google.com/+Response+of+broccoli+to+irrigation+scheduling+and+methods+under+drip,+sprinkler+and+surface+irrigation&hl=en&as_sdt=0,5

Horticulture Innovation Australia (HIA), Applied Horticultural Research Pty Ltd (AHR), & Western Sydney University (WSU). (n.d.). *Broccoli* (Brassica oleracea *var.* italica). https://www.postharvest.net.au/imagesDB/wysiwyg/4-Broccoli.pdf

Horticulture™ New Zealand. (2017). *New Zealand domestic vegetable production: The growing story*. https://www.hortnz.co.nz/assets/Environment/National-Env-Policy/JR-Reference-Documents-/KPMG-2017-NZ-domestic-vegeable-production-.pdf

Huang, J., Liu, Y., Han, F., Fang, Z., Yang, L., Zhuang, M., Zhang, Y., Lv, H., Wang, Y., & Ji, J. (2021). Genetic diversity and population structure analysis of 161 broccoli cultivars based on SNP markers. *Horticultural Plant Journal*, *7*(5), 423-433. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S2468014121000716?via%3Dihub

Ilahy, R., Tlili, I., Pék, Z., Montefusco, A., Siddiqui, M. W., Homa, F., Hdider, C., R'Him, T., Lajos, H., & Lenucci, M. S. (2020). Pre-and post-harvest factors affecting glucosinolate content in broccoli. *Frontiers in Nutrition*, *7*, 147. https://pmc-ncbi-nlm-nih-gov.ezproxy.massey.ac.nz/articles/pmid/33015121/

Iqbal, N., Khan, N. A., Ferrante, A., Trivellini, A., Francini, A., & Khan, M. (2017). Ethylene role in plant growth, development and senescence: Interaction with other phytohormones. *Frontiers in Plant Science*, *8*, 475. https://pmc-ncbi-nlm-nih-gov.ezproxy.massey.ac.nz/articles/pmid/28421102/

Izumi, H., Watada, A. E., & Douglas, W. (1996). Optimum O_2 or CO_2 atmosphere for storing broccoli florets at various temperatures. *Journal of the American Society for Horticultural Science*, *121*(1), 127-131. https://journals.ashs.org/view/journals/jashs/121/1/article-p127.xml

Jacobsson, A., Nielsen, T., & Sjöholm, I. (2004a). Effects of type of packaging material on shelf-life of fresh broccoli by means of changes in weight, colour and texture. *European Food Research and Technology*, *218*, 157-163. https://link-springer-com.ezproxy.massey.ac.nz/article/10.1007/s00217-003-0820-2

Jacobsson, A., Nielsen, T., Sjöholm, I., & Wendin, K. (2004b). Influence of packaging material and storage condition on the sensory quality of broccoli. *Food Quality and Preference*, *15*(4), 301-310. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0950329303000703?via%3Dihub

James, C., Onarinde, B. A., & James, S. J. (2017). The use and performance of household refrigerators: A review. *Comprehensive Reviews in Food Science and Food Safety*, *16*(1), 160-179. https://ift-onlinelibrary-wiley-com.ezproxy.massey.ac.nz/doi/full/10.1111/1541-4337.12242

Jiang, W., Sheng, Q., Zhou, X. J., Zhang, M.-J., & Liu, X. J. (2002). Regulation of detached coriander leaf senescence by 1-methylcyclopropene and ethylene. *Postharvest Biology and Technology*, *26*(3), 339-345. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0925521402000686?via%3Dihub

Jones, M. L., Kim, E.-S., & Newman, S. E. (2001). Role of ethylene and 1-MCP in flower development and petal abscission in zonal geraniums. *HortScience*, *36*(7), 1305-1309.

Jones, R. B., Faragher, J. D., & Winkler, S. (2006). A review of the influence of postharvest treatments on quality and glucosinolate content in broccoli (*Brassica oleracea* var. *italica*) heads. *Postharvest Biology and Technology*, *41*(1), 1-8. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0925521406000822?via%3Dihub

Kader, A. A. (1997). Fruit maturity, ripening, and quality relationships. *Acta Horticulturae*, *485*, 203–208. https://www.actahort.org/books/485/485_27.htm

Kałużewicz, A., Krzesiński, W., Knaflewski, M., Lisiecka, J., Spiżewski, T., & Frąszczak, B. (2010). The effect of temperature on the broccoli yield and length of the period from head initiation to harvest. *Acta Scientiarum Polonorum Hortorum Cultus*, *9*(3), 167-174. https://czasopisma.up.lublin.pl/asphc/article/view/3385/2340

Kasmire, R., Kader, A., & Klaustermeyer, J. (1974). Broccoli shipping odors caused by poor air circulation and low oxygen levels. *Calfornia Agriculture, 28*(9), 14–15. https://californiaagriculture.org/article/112085

Kato, M., Kamo, T., Wang, R., Nishikawa, F., Hyodo, H., Ikoma, Y., Sugiura, M., & Yano, M. (2002). Wound-induced ethylene synthesis in stem tissue of harvested broccoli and

its effect on senescence and ethylene synthesis in broccoli florets. *Postharvest Biology and Technology*, *24*(1), 69-78. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0925521401001119?via%3Dihub

Keller, N., Ducamp, M. N., Robert, D., & Keller, V. (2013). Ethylene removal and fresh product storage: A challenge at the frontiers of chemistry. Toward an approach by photocatalytic oxidation. *Chemical Reviews*, *113*(7), 5029-5070. https://pubs-acs-org.ezproxy.massey.ac.nz/doi/pdf/10.1021/cr900398v

King, G. A., & Morris, S. C. (1994). Physiological changes of broccoli during early postharvest senescence and through the preharvest-postharvest continuum. *Journal of the American Society for Horticultural Science*, *119*(2), 270-275. https://doi.org/10.1021/cr900398v

Krupinska, K., & Humbeck, K. (2004). Photosynthesis and chloroplast breakdown. In L. D. Noodén (Ed.), *Plant cell death processes* (pp. 169–187). Academic Press.

Ku, K. M., Choi, J. H., Kim, H. S., Kushad, M. M., Jeffery, E. H., & Juvik, J. A. (2013). Methyl jasmonate and 1-methylcyclopropene treatment effects on quinone reductase inducing activity and post-harvest quality of broccoli. *PLoS ONE*, *8*(10), Article e77127. https://doi.org/10.1371/journal.pone.0077127

Ku, V., & Wills, R. (1999). Effect of 1-methylcyclopropene on the storage life of broccoli. *Postharvest Biology and Technology*, *17*(2), 127-132. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0925521499000423?via%3Dihub

Latté, K. P., Appel, K. E., & Lampen, A. (2011). Health benefits and possible risks of broccoli–An overview. *Food and Chemical Toxicology*, *49*(12), 3287-3309. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0278691511004364?via%3Dihub

Le Strange, M., Cahn, M., Koike, S., & Smith, R. (2010). *Broccoli production in Caifornia*. University of California, Agriculture and Natural Resources. https://doi.org/10.3733/ucanr.7211

Li, H., Xia, Y., Liu, H. Y., Guo, H., He, X. Q., Liu, Y., Wu, D. T., Mai, Y. H., Li, H. B., & Zou, L. (2022). Nutritional values, beneficial effects, and food applications of broccoli (*Brassica oleracea* var. *italica* Plenck). *Trends in Food Science & Technology*, *119*, 288-308. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0924224421006725?via%3Dihub

Li, L., Lv, F. Y., Guo, Y. Y., & Wang, Z. Q. (2016). Respiratory pathway metabolism and energy metabolism associated with senescence in postharvest Broccoli (*Brassica*

oleracea L. var. *italica*) florets in response to O_2/CO_2 controlled atmospheres. *Postharvest Biology and Technology*, *111*, 330-336. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S092552141530137X?via%3Dihub

Li, Y., Wills, R., & Golding, J. (2017). Interaction of ethylene concentration and storage temperature on postharvest life of the green vegetables pak choi, broccoli, mint, and green bean. *The Journal of Horticultural Science and Biotechnology*, *92*(3), 288-293. https://www-tandfonline-com.ezproxy.massey.ac.nz/doi/full/10.1080/14620316.2016.1263545

Lin, Y. R., Lee, J. Y., Tseng, M. C., Lee, C. Y., Shen, C. H., Wang, C. S., Liou, C. C., Shuang, L. S., Paterson, A. H., & Hwu, K. K. (2018). Subtropical adaptation of a temperate plant (*Brassica oleracea* var. *italica*) utilizes non-vernalization-responsive QTLs. *Scientific Reports*, *8*(1), 13609. https://www.nature.com/articles/s41598-018-31987-1.pdf

Lindemann-Zutz, K. (2015). *Head size variation within broccoli (*Brassica oleracea *var.* italica*) plantings, causes and prediction for decision support* [Unpublished doctoral dissertation]. Leibniz Universität Hannover. https://edocs.tib.eu/files/e01dh15/835478459.pdf

Lindemann-Zutz, K., Fricke, A., & Stützel, H. (2016a). Prediction of time to harvest and its variability in broccoli (*Brassica oleracea* var. *italica*) Part I. Plant developmental variation and forecast of time to head induction. *Scientia Horticulturae*, *198*, 424-433. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0304423815303459?via%3Dihub

Lindemann-Zutz, K., Fricke, A., & Stützel, H. (2016b). Prediction of time to harvest and its variability of broccoli (*Brassica oleracea* var. *italica*) part II. Growth model description, parameterisation and field evaluation. *Scientia Horticulturae*, *200*, 151-160. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0304423816300103?via%3Dihub

Liu, D. Y., Chang, W. R., & Lin, J. Y. (2004). Performance comparison with effect of door opening on variable and fixed frequency refrigerators/freezers. *Applied Thermal Engineering*, *24*(14-15), 2281-2292. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S1359431104000390?via%3Dihub

Lohani, S., Trivedi, P. K., & Nath, P. (2004). Changes in activities of cell wall hydrolases during ethylene-induced ripening in banana: Effect of 1-MCP, ABA and IAA.

Postharvest Biology and Technology, *31*(2), 119-126. https://doi.org/10.1016/j.postharvbio.2003.08.001

Lu, X. (2020). *Ethylene as a contaminant in the industrial horticultural distribution chain* [Unpublished master's thesis]. Massey University. https://mro.massey.ac.nz/items/db76b8d2-03cb-4fde-9883-0b15c26ee3cf

Ludong, D. P. M. (2008). *Effects of irrigation rate on the growth, yield, nutritive value, and water use efficiency of Carrot* (Daucus carota) *and Broccoli* (Brasiola oleracea) [Unpublished master's thesis]. Curtin University. https://espace.curtin.edu.au/bitstream/handle/20.500.11937/1661/119758_Ludong2008.pdf?sequence=2&isAllowed=y

Luo, F., Cheng, S. C., Cai, J. H., Wei, B. D., Zhou, X., Zhou, Q., Zhao, Y. B., & Ji, S. J. (2019). Chlorophyll degradation and carotenoid biosynthetic pathways: Gene expression and pigment content in broccoli during yellowing. *Food Chemistry*, *297*, 124964. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0308814619310660?via%3Dihub

Ma, G., Wang, R., Wang, C. R., Kato, M., Yamawaki, K., Qin, F. F., & Xu, H. L. (2009). Effect of 1-methylcyclopropene on expression of genes for ethylene biosynthesis enzymes and ethylene receptors in post-harvest broccoli. *Plant Growth Regulation*, *57*, 223-232. https://link-springer-com.ezproxy.massey.ac.nz/article/10.1007/s10725-008-9339-7

Makhlouf, J., Willemot, C., Arul, J., Castaigne, F., & Emond, J. P. (1989). Regulation of ethylene biosynthesis in broccoli flower buds in controlled atmospheres. *Journal of the American Society for Horticultural Science*, *114*(6), 955-958. https://pdfs.semanticscholar.org/3855/3904b02a533df21df4284fcd07d70849166f.pdf

Martínez-Romero, D., Bailén, G., Serrano, M., Guillén, F., Valverde, J. M., Zapata, P., Castillo, S., & Valero, D. (2007). Tools to maintain postharvest fruit and vegetable quality through the inhibition of ethylene action: A review. *Critical Reviews in Food Science and Nutrition*, *47*(6), 543-560. https://www-tandfonline-com.ezproxy.massey.ac.nz/doi/full/10.1080/10408390600846390

Mattoo, A. K., & Handa, A. K. (2004). Ethylene signaling in plant cell death. In L. D. Noodén (Ed.), *Plant cell death processes* (pp. 125–142). Academic Press.

McMurchie, E. J., McGlasson, W. B., & Eaks, I. L. (1972). Treatment of fruit with propylene gives information about the biogenesis of ethylene. *Nature*, *237*(5352), 235-236. https://doi.org/10.1038/237235a0

Mlinarić, A., Horvat, M., & Šupak Smolčić, V. (2017). Dealing with the positive publication bias: Why you should really publish your negative results. *Biochemia Medica*, *27*(3), 447-452. https://hrcak.srce.hr/file/276521

Nagai, H., Miyake, N., Kato, S., Maekawa, D., Inoue, Y., & Takikawa, Y. (2017). Improved control of black rot of broccoli caused by *Xanthomonas campestris* pv. *campestris* using a bacteriophage and a nonpathogenic *Xanthomonas* sp. strain. *Journal of General Plant Pathology*, *83*, 373-381. https://link-springer-com.ezproxy.massey.ac.nz/article/10.1007/s10327-017-0745-4

Nath, A., Bagchi, B., Misra, L., & Deka, B. C. (2011). Changes in post-harvest phytochemical qualities of broccoli florets during ambient and refrigerated storage. *Food Chemistry*, *127*(4), 1510-1514. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0308814611002500?via%3Dihub

Nock, J., Watkins, C., James, H., Reed, N., & Oakes, R. (2009). Preharvest application of 1-methylcyclopropene (1-MCP) to control fruit drop of apples, and its effects on postharvest quality. *Acta Horticulturae, 877*, 365–374. https://www.actahort.org/books/877/877_46.htm?utm_source=chatgpt.com

Ogawara, T., Higashi, K., Kamada, H., & Ezura, H. (2003). Ethylene advances the transition from vegetative growth to flowering in *Arabidopsis thaliana*. *Journal of Plant Physiology*, *160*(11), 1335-1340. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0176161704705258?via%3Dihub

Ouda, B. A., & Mahadeen, A. Y. (2008). Effect of fertilizers on growth, yield, yield components, quality and certain nutrient contents in broccoli (*Brassica oleracea*). *International Journal of Agriculture and Biology*, *10*(6), 627-632. https://www.researchgate.net/profile/Atif-Mahadeen/publication/228641305_Effect_of_fertilizers_on_growth_yield_yield_components_quality_and_certain_nutrient_contents_in_broccoli_Brassica_oleracea/links/5e19c961299bf10bc3a466c4/Effect-of-fertilizers-on-growth-yield-yield-components-quality-and-certain-nutrient-contents-in-broccoli-Brassica-oleracea.pdf

Page, T., Griffiths, G., & Buchanan-Wollaston, V. (2001). Molecular and biochemical characterization of postharvest senescence in broccoli. *Plant Physiology*, *125*(2), 718-727. https://www-jstor-org.ezproxy.massey.ac.nz/stable/4279697

Paradis, C., Castaigne, F., Desrosiers, T., Fortin, J., Rodrigue, N., & Willemot, C. (1996). Sensory, nutrient and chlorophyll changes in broccoli florets during controlled

atmosphere storage. *Journal of Food Quality*, *19*(4), 303-316. https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.1745-4557.1996.tb00425.x

Patra, S. K., Poddar, R., Pramanik, S., Gaber, A., & Hossain, A. (2022). Crop and water productivity and profitability of broccoli (*Brassica oleracea* L. var. *italica*) under gravity drip irrigation with mulching condition in a humid sub-tropical climate. *PLoS ONE*, *17*(3), e0265439. https://doi.org/10.1371/journal.pone.0265439

Paul, V., Pandey, R., & Srivastava, G. C. (2012). The fading distinctions between classical patterns of ripening in climacteric and non-climacteric fruit and the ubiquity of ethylene – An overview. *Journal of Food Science and Technology*, *49*(1), 1-21. https://doi.org/10.1007/s13197-011-0293-4

Paulsen, E., Lema, P., Martínez-Romero, D., & García-Viguera, C. (2022). Use of PLA/PBAT stretch-cling film as an ecofriendly alternative for individual wrapping of broccoli heads. *Scientia Horticulturae*, *304*, 111260. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0304423822003818?via%3Dihub

Pellegrino, R., Wheeler, J., Sams, C. E., & Luckett, C. R. (2019). Storage time and temperature on the sensory properties broccoli. *Foods*, *8*(5), 162. https://www.mdpi.com/2304-8158/8/5/162

Phuong, N. T. H., Uchino, T., Tanaka, F., & Tanaka, F. (2022). Effect of 1-methylcyclopropene (1-MCP) and temperature on the quality of broccoli during storage. *Environmental Control in Biology*, *60*(1), 43-51. https://www.jstage.jst.go.jp/article/ecb/60/1/60_43/_pdf

Phuong, N. T. H., Uchino, T., Tanaka, F., & Tanaka, F. (2023). Effect of 1-MCP concentration on the quality of broccoli (*Brassica oleracea* L., var. *italica*) during storage. *Journal of the Faculty of Agriculture, Kyushu University, 68*(1), 45–53. https://doi.org/10.5109/6770287

Phan, C.-T. (1987). Biochemical and physiological changes during the harvest period. In J. Weichmann (Ed.), *Postharvest physiology of vegetables* (pp. 9–22). Marcel Dekker, Inc.

Pierce, R. (2023). *Volume of horizontal cylinder*. https://www.mathsisfun.com/geometry/cylinder-horizontal-volume.html

Pinera, D. R. (1995). *The effect of plant density, cultivar and season on the growth and development of broccoli* [Unpublished master's thesis]. Massey University. https://mro.massey.ac.nz/items/39aad6ce-f8ae-4367-ab55-acf9302b061a

Pogson, B. J., & Morris, S. C. (1997). Consequences of cool storage of broccoli on physiological and biochemical changes and subsequent senescence at 20 °C. *Journal of the American Society for Horticultural Science, 122*(4), 553–558. https://doi.org/10.21273/JASHS.122.4.553

Porter, K. L., Collins, G., & Klieber, A. (2005). 1-MCP does not improve the shelf-life of Chinese cabbage. *Journal of the Science of Food and Agriculture*, *85*(2), 293-296. https://scijournals.onlinelibrary.wiley.com/doi/pdf/10.1002/jsfa.1955?casa_token=qnn1Kanm6U8AAAAA:7Y6wQCUkJCqZGyyJ7pQUEM8uuZpwioEhi_xDEdK1Jw2AhbVxEOrwTJZ57Z9E-S5itVAohLbwrxEUaJ8

Rangkadilok, N., Tomkins, B., Nicolas, M. E., Premier, R. R., Bennett, R. N., Eagling, D. R., & Taylor, P. W. (2002). The effect of post-harvest and packaging treatments on glucoraphanin concentration in broccoli (*Brassica oleracea* var. *italica*). *Journal of Agricultural and Food Chemistry*, *50*(25), 7386-7391. https://pubs.acs.org/doi/pdf/10.1021/jf0203592

Reyes Jara, A. M., Gómez-Lobato, M. E., Civello, P. M., & Martínez, G. A. (2021). Expression of BoNOL and BoHCAR genes during postharvest senescence of broccoli heads. *Journal of the Science of Food and Agriculture*, *101*(4), 1629-1635. https://doi.org/10.1002/jsfa.10783

Sabir, F. K. (2012). Postharvest quality response of broccoli florets to combined application of 1-methylcyclopropene and modified atmosphere packaging. *Agricultural and Food Science*, *21*(4), 421-429. https://doi.org/10.23986/afsci.6387

Sakaldas, M., & Gundogdu, M. (2015). The effects of preharvest 1-methylcyclopropene (Harvista) treatments on harvest maturity of 'Golden Delicious' apple cultivar. *Acta Horticulturae, 1139*, 601–606. https://doi.org/10.17660/ActaHortic.2016.1139.104

Saltveit, M. E. (2005). Aminoethoxyvinylglycine (AVG) reduces ethylene and protein biosynthesis in excised discs of mature-green tomato pericarp tissue. *Postharvest Biology and Technology*, *35*(2), 183-190. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0925521404001875?via%3Dihub

Scariot, V., Paradiso, R., Rogers, H., & De Pascale, S. (2014). Ethylene control in cut flowers: Classical and innovative approaches. *Postharvest Biology and Technology*, *97*, 83-92. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0925521414001653?via%3Dihub

Serek, M., Sisler, E. C., Tirosh, T., & Mayak, S. (1995). 1-Methylcyclopropene prevents bud, flower, and leaf abscission of Geraldton waxflower. *HortScience, 30*(6), 1310. https://doi.org/10.21273/HORTSCI.30.6.1310

Serek, M., Sisler, E. C., & Reid, M. S. (1995a). Effects of 1-MCP on the vase life and ethylene response of cut flowers. *Plant Growth Regulation*, *16*(1), 93-97.

Serek, M., Woltering, E., Sisler, E., Frello, S., & Sriskandarajah, S. (2006). Controlling ethylene responses in flowers at the receptor level. *Biotechnology Advances*, *24*(4), 368-381. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0734975006000243?via%3Dihub

Serrano, M., Martinez-Romero, D., Guillen, F., Castillo, S., & Valero, D. (2006). Maintenance of broccoli quality and functional properties during cold storage as affected by modified atmosphere packaging. *Postharvest Biology and Technology*, *39*(1), 61-68. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0925521405001912?via%3Dihub

Singh, S., Rai, A. K., Alam, T., & Singh, B. (2018). Influence of modified atmosphere packaging (MAP) on the shelf life and quality of broccoli during storage. *Journal of Packaging Technology and Research*, *2*, 105-113. https://link-springer-com.ezproxy.massey.ac.nz/article/10.1007/s41783-018-0030-9

Siomos, A. S., Koularmanis, K., & Tsouvaltzis, P. (2022). The impacts of the emerging climate change on broccoli (*Brassica oleracea* L. var. *italica* Plenck.) crop. *Horticulturae*, *8*(11), 1032. https://www.mdpi.com/2311-7524/8/11/1032

Sisler, E., & Serek, M. (2003). Compounds interacting with the ethylene receptor in plants. *Plant Biology*, *5*(05), 473-480. https://onlinelibrary-wiley-com.ezproxy.massey.ac.nz/doi/full/10.1055/s-2003-44782

Sisler, E. C., & Serek, M. (1997). Inhibitors of ethylene responses in plants at the receptor level: Recent developments. *Physiologia Plantarum*, *100*(3), 577-582. https://onlinelibrary-wiley-com.ezproxy.massey.ac.nz/doi/full/10.1111/j.1399-3054.1997.tb03063.x

Song, H. J., Kim, M. H., & Ku, K.-M. (2021). A double-edged sword of surfactant effect on hydrophobic surface broccoli leaf as a model plant: Promotion of pathogenic microbial contamination and improvement to disinfection efficiency of ozonated water. *Processes*, *9*(4), 679. https://www.mdpi.com/2227-9717/9/4/679/pdf

Suzuki, Y., Uji, T., & Terai, H. (2004). Inhibition of senescence in broccoli florets with ethanol vapor from alcohol powder. *Postharvest Biology and Technology*, *31*(2), 177-

182. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0925521403001571?via%3Dihub

Tallei, T. E., Kepel, B. J., Wungouw, H. I. S., Nurkolis, F., Adam, A. A., & Fatimawali. (2024). A comprehensive review on the antioxidant activities and health benefits of microgreens: Current insights and future perspectives. *International Journal of Food Science and Technology*, *59*(1), 58-71. https://ifst-onlinelibrary-wiley-com.ezproxy.massey.ac.nz/doi/full/10.1111/ijfs.16805

Thompson, J. F., & Cantwell, M. I. (2001). *How to measure produce firmness and toughness*. University of California, Postharvest Technology Center. https://my.ucanr.edu/sites/Postharvest_Technology_Center_/files/228177.pdf

Tian, M., Downs, C., Lill, R., & King, G. (1994). A role for ethylene in the yellowing of broccoli after harvest. *Journal of the American Society for Horticultural Science*, *119*(2), 276-281. https://journals.ashs.org/downloadpdf/view/journals/jashs/119/2/article-p276.pdf

Tian, M. S., Prakash, S., Elgar, H. J., Young, H., Burmeister, D. M., & Ross, G. S. (2000). Responses of strawberry fruit to 1-methylcyclopropene (1-MCP) and ethylene. *Plant Growth Regulation*, *32*(1), 83-90. https://doi.org/10.1023/A:1006409719333

Toivonen, P. M., & DeEll, J. R. (1998). Differences in chlorophyll fluorescence and chlorophyll content of broccoli associated with maturity and sampling section. *Postharvest Biology and Technology*, *14*(1), 61-64. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0925521498000222?via%3Dihub

UC Statewide IPM Program. (2020). *UC IPM pest management guidelines: Cole crops.* https://ipm.ucanr.edu/legacy_assets/PDF/PMG/pmgcolecrops.pdf

University of Massachusetts Amherst. (2024). *UMass extension vegetable program: Brassicas, downy mildew.* https://ag.umass.edu/vegetable/fact-sheets/brassicas-downy-mildew

Uthaichay, N., Ketsa, S., & van Doorn, W. G. (2007). 1-MCP pretreatment prevents bud and flower abscission in *Dendrobium* orchids. *Postharvest Biology and Technology*, *43*(3), 374-380. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0925521406002560?via%3Dihub

Vasconcelos, I. S. A., & Almeida, D. P. F. (2003). Treatment with 1-methylcyclopropene complements temperature management in maintaining postharvest quality of broccoli. *Acta Horticulturae, 628,* 227–232. https://doi.org/10.17660/ActaHortic.2003.628.27

Verschoor, J. A. (2017). *Evaluation of MACView® Portable Ethylene Postharvest Gas Analyser: Independent test of suitabilityand performance for use in horticultural settings*. (Report No. 1753). Wageningen Food & Biobased Research. https://edepot.wur.nl/511838

Vilhena, N. Q., Cervera-Chiner, L., Moreno, A., & Salvador, A. (2023). Recent development in the preharvest 1-MCP application to improve postharvest fruit quality. *New Advances in Postharvest Technology*, 1-18. https://redivia.gva.es/bitstream/handle/20.500.11939/8867/2023_Vilhena_Recent.pdf?sequence=1

Walker, G., Cameron, P., & Berry, N. (2001). Implementing an IPM programme for vegetable brassicas in New Zealand. In N. M. Endersby & P. M. Ridland (Eds.), *The management of diamondback moth and other crucifer pests: Proceedings of the Fourth International Workshop* (pp. 365–370). International Society for Horticultural Science. https://www.conservationevidencejournal.com/individual-study/5216

Warton, M., Wills, R., & Ku, V. (2000). Ethylene levels associated with fruit and vegetables during marketing. *Australian Journal of Experimental Agriculture*, *40*(3), 465-470. https://www.researchgate.net/profile/Ron-Wills/publication/248891535_Ethylene_levels_associated_with_fruit_and_vegetables_during_marketing/links/5c6b290f299bf1e3a5b259ee/Ethylene-levels-associated-with-fruit-and-vegetables-during-marketing

Watkins, C. B. (2006). The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. *Biotechnology Advances*, *24*(4), 389-409. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S073497500600022X?via%3Dihub

Watkins, C. B. (2016). *Advances in the use of 1-MCP* (pp. 117–146). In Wills & Golding (Eds.), *Advances in postharvest fruit and vegetable technology*. CRC Press. https://dlib.scu.ac.ir/bitstream/Hannan/370836/2/9781482216967.pdf#page=139

Wichrowska, D., Kozera, W., Knapowski, T., Prus, P., & Ligocka, A. (2021). Assessment of the interactive effect of the use of 1-methylcyclopropene and cultivars on the nutritional value of broccoli during storage. *Agronomy*, *11*(12), 2575. https://www.mdpi.com/2073-4395/11/12/2575

Wills, R., & Golding, J. (2016). *Postharvest: An introduction to the physiology and handling of fruit and vegetables* (6 th ed.). CABI.

Winkler, S., Faragher, J., Franz, P., Imsic, M., & Jones, R. (2007). Glucoraphanin and flavonoid levels remain stable during simulated transport and marketing of broccoli

(*Brassica oleracea* var. *italica*) heads. *Postharvest Biology and Technology*, *43*(1), 89-94. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0925521406002031?via%3Dihub

Wu, W., Gao, H., Chen, H., Fang, X., Han, Q., & Zhong, Q. (2019). Combined effects of aqueous chlorine dioxide and ultrasonic treatments on shelf-life and nutritional quality of bok choy (*Brassica chinensis*). *Lwt*, *101*, 757-763. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S002364381831034X?via%3Dihub

Xu, F., Chen, X., Yang, Z., Jin, P., Wang, K., Shang, H., Wang, X., & Zheng, Y. (2013). Maintaining quality and bioactive compounds of broccoli by combined treatment with 1-methylcyclopropene and 6-benzylaminopurine. *Journal of the Science of Food and Agriculture*, *93*(5), 1156-1161. https://doi.org/10.1002/jsfa.5867

Xu, F., Wang, H., Tang, Y., Dong, S., Qiao, X., Chen, X., & Zheng, Y. (2016). Effect of 1-methylcyclopropene on senescence and sugar metabolism in harvested broccoli florets. *Postharvest Biology and Technology*, *116*, 45-49. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0925521416300035?via%3Dihub

Yang, Y. W., Tsai, C. C., & Wang, T. T. (1998). A heat-tolerant broccoli F1 hybrid, 'Ching-Long 45'. *HortScience*, *33*(6), 1090-1091. https://journals.ashs.org/view/journals/hortsci/33/6/article-p1090.xml

Yuan, G., Sun, B., Yuan, J., & Wang, Q. (2010). Effect of 1-methylcyclopropene on shelf life, visual quality, antioxidant enzymes and health-promoting compounds in broccoli florets. *Food Chemistry*, *118*(3), 774-781. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0308814609007432?via%3Dihub

Zhao, Y., Tang, Z., & Chen, S. (2022). Loading model and mechanical properties of mature broccoli (*Brassica oleracea* L. Var. *italica* Plenck) stems at harvest. *Agriculture*, *12*(10), 1519. https://www.mdpi.com/2077-0472/12/10/1519

Zhu, Y., Ji, S., Li, P., Kang, Y., Wei, B., Luo, M., & Zhou, Q. (2025). Integrating proteomics and phosphoproteomics to reveal the regulatory mechanism of yellowing in postharvest cold storage of broccoli. *Postharvest Biology and Technology*, *225*, 113531. https://doi.org/https://doi.org/10.1016/j.postharvbio.2025.113531

Zhu, Y., Liu, C., Zhao, M., Duan, Y., Xie, J., & Wang, C. (2024). Transcriptome profiling reveals key regulatory factors and metabolic pathways associated with curd formation and development in broccoli. *Frontiers in Plant Science*, *15*, 1418319. https://www.frontiersin.org/journals/plant-science/articles/10.3389/fpls.2024.1418319/pdf

Zhuang, H., Hildebrand, D., & Barth, M. (1997). Temperature influenced lipid peroxidation and deterioration in broccoli buds during postharvest storage. *Postharvest Biology and Technology*, *10*(1), 49-58. https://www-sciencedirect-com.ezproxy.massey.ac.nz/science/article/pii/S0925521496000543?via%3Dihub

Zhuang, H., Hildebrand, D. F., & Barth, M. M. (1995). Senescence of broccoli buds is related to changes in lipid peroxidation. *Journal of Agricultural and Food Chemistry*, *43*(10), 2585-2591. https://pubs-acs-org.ezproxy.massey.ac.nz/doi/pdf/10.1021/jf00058a006

Zsom, T., Polgari, P., Nguyen, L. P. L., Hitka, G., & Zsom-Muha, V. (2020). Quality maintenance of broccoli by the use of 1-MCP treatments. *Progress in Agricultural Engineering Sciences, 16*(S1), 95–103. https://doi.org/10.1556/446.2020.10010