出國報告（出國類別：進修）

農委會農業菁英培訓計畫

-以熱處理

提昇熱帶水果低溫貯運性之研究

服務機關：行政院農業委員會臺南區農業改良場

姓名職稱：張嵐雁 助理研究員

派赴國家：美國

出國期間：106年8月14日至110年8月13日

報告日期：112年4月5日

摘要

筆者赴美國佛羅里達大學攻讀博士學位，原擬「以熱處理提昇熱帶水果低溫貯運性之研究」為題，後納入乙烯之交互反應為另一主題，但受疫情及出國期限完成部份研究。香蕉為重要熱帶更年性水果，對乙烯敏感且受寒害及果實後熟限制貯架壽命，研究以安喜培（1-MCP）阻斷綠熟香蕉之乙烯受體，釐清乙烯在寒害之角色。試驗以 25 $\mu g \cdot L^{-1}$ 以上之安喜培溶液浸泡綠熟香蕉可延後後熟反應，且果實未來仍可後熟。進一步研究初期寒害過程變化，比較浸泡 0 或 50 $\mu g \cdot L^{-1}$ 安喜培溶液的綠熟香蕉在植物生理、香氣及蛋白質體圖譜差異，結果顯示乙烯影響部份寒害徵狀速度，非決定性主因。熱處理雖可延緩寒害徵狀，但無法抑制其發生，然兩者機制與自由基消除之酵素系統有關，機制尚待後續研究釐清。

目次

目的

我國主要出口水果中香蕉、鳳梨、芒果、柑橘類、荔枝、番石榴、楊桃、鳳梨釋迦、蓮霧等，多屬原生於熱帶或亞熱帶地區之水果，這類水果多為低溫敏感型水果，果實置於 10-15℃以下、冰點以上的溫度一段時間後，果實代謝運作即受影響，將有細胞膜通透性改變、酵素活性改變，進一步衍生寒害次級反應如乙烯生成量累積、呼吸率上升、果實無法正常產能 （ATP 生成減少）、原生質流動（Protoplasmic streaming）變慢或停止、光合作用受阻（PSII 系統）等情形，當低溫逆境時間延長，上述改變將成為肉眼可見的低溫障礙徵狀，如果實表面損傷（如凹陷）、果肉變色、組織出現水浸狀或壞疽、風味改變、果實無法正常後熟、果實加速老化、增加腐敗菌類之感染機率等，特別是待果實由低溫貯藏環境移至常溫，徵狀會更加明顯。我國具利基性出口的果實，如番石榴、印度棗、楊桃等，可連皮食用，但長期低溫運銷易使果實表皮出現凹陷、果實表皮變色等，販售時外觀品質不良，而木瓜、香蕉、鳳梨則在低溫貯運後無法正常後熟，或果實低溫長期貯藏期間出現腐爛或果肉黑心等情形，也直接影響販售。

園產品種類多，形態、構造及生理上差異大，作物的原產地、遺傳物質組成、採收成熟度、組織代謝狀態、採收前管理方式、採收前後之環境因子如溫度、光照、相對濕度及大氣組成等因子皆造成作物對低溫敏感性不同。對果實本質而言，作物原產地與其自身基因中對於低溫環境之耐受能力相關，因此原生於熱帶之酪梨、芒果、木瓜等水果較原產溫帶之蘋果、梨等，對低溫更為敏感。其組織生理代謝方面，對寒害耐性較佳的植物組織中，細胞膜脂質中含較高比例之不飽和脂肪酸，或果實中含有較高比例的還原醣或脯氨酸（Proline），此外，不同採收成熟度之果實中，未完熟果實對低溫更敏感，其他採收前之栽培管理因子對採收後的低溫耐抗性之研究闕如。就外在環境因子而言，低溫溫度及處理時間會造成不同的低溫障礙徵狀程度，環境中相對濕度較高，低溫障礙發生程度會比相對濕度較低的環境下輕微。

目前造成低溫障礙，或稱寒害的徵狀假說有二，一為低溫造成細胞膜流動性下降，使細胞膜呈固態凝膠狀，抑制鑲嵌於膜上的酵素系統活性，而細胞膜流動性降低的程度與膜上的脂肪酸組成有關，但與低溫敏感性之關係尚不清楚。

另一假說則與活性氧化物（Reactive oxygen species, ROS）之新陳代謝有關，ROS 包含過氧化氫（H_2O_2）、超氧化自由基（Superoxide radical, $O_2^{-}\cdot$）或氫氧自由基（Hydroxyl radical,·OH）等，這些活性氧化物為生物體代謝之產物，活性極強，特別是氫氧自由基。若 ROS 未及時消除，會快速與其它分子如 DNA、蛋白質、不飽和脂肪酸（造成細胞膜脂質的過氧化反應）反應，使細胞受損，甚至造成細胞死亡。植物對活性氧化物應對模式推論有兩階段，第一為自身之抗氧化物質，如穀胱苷肽（Glutathione）、抗壞血酸（Ascorbate, or 維生素 C）、維生素 A、維生素 E、酚類化合物、花青素、類胡蘿蔔素等，上述抗氧化物可與 ROS 反應，以降低傷害。第二為抗氧化酵素，如將過氧自由基轉換為過氧化氫的過氧歧化酶（Superoxidase, SOD）、將過氧化氫轉為水的過氫化氫酶（Catalase, CAT）、抗壞血酸過氧化酶（Ascorbate peroxidase, APX）、穀胱苷肽過氧化酶（Glutathione peroxidase, GPX）等。一般情況下，這些酵素可控制 ROS 的濃度，以維持細胞內代謝的平衡，但逆境發生時，大量產生的 ROS 無法由原有的酵素完全代謝，造成細胞受損，目前果實遭逢低溫逆境的反應尚待釐清，在細胞未至死亡前，果實對低溫的耐逆境特性及因應逆境後誘發的代謝產物、酵素及其所扮演的角色仍不明，需要深入研究了解實際遭遇低溫障礙後果實的生理反應，對於採後處理技術端才能開發更為精確有效的預防方式。

本計畫原本規劃係以了解水果對低溫及高溫之敏感性切入，確定造成低溫及熱傷害之臨界溫度操作值，並以果實整體的蛋白質體或代謝質體分析切入，了解在臨界溫度內，植物的整體反應模式，及其誘發出之對逆境的耐抗性及其品質上改善的情形，設計開發低成本、對環境友善，有效減少果實貯運損耗之熱處理技術。

由於佛州所處緯度與臺灣中南部相仿，氣候條件類似，面臨環境限制因子近似，故以佛州為博士班申請目標。筆者申請農業菁英計畫獲准，同期間亦獲美國佛羅里達大學園藝系入學許可通知，於 Dr. Jeffrey K. Brecht 門下進行博士班訓練。農委會農業菁英培訓之經費含學費及生活支出，研究經費部份略有不足，經指導教授引薦美國 It’s Fresh! Ltd.公司，該公司同意贊助筆者四年論文研究經費，惟需執行該公司產品相關測試作為交換。因此原論文規劃外，亦擴充出乙烯與寒害關連為論文另一主軸。筆者博士班進修期間，除上課及論文實驗，

空檔需協助該公司測試原型商品，模擬不同作物慣行貯運過程中，配合使用該產品之品質變化及效益。於筆者而言，亦藉此機會了解美國內外銷通路的概略情形。

研究作物主題的選擇，與指導教授討論後，最後以材料特性及是否容易取得決定，雖佛州座落熱帶及亞熱帶，但熱帶水果生產僅集中於南佛，栽培方式粗放且產期集中，若為以此作為試驗材料將受限於短暫的產期，又美國本土初春易受早春霜害影響，多數熱帶作物生產所限，產量不大。但研究議題與寒害、乙烯及熱處理等相關，在文獻探討後，選擇以對低溫及乙烯皆敏感的綠熟香蕉為材料。美國香蕉多為由中南美洲進口，而取用的香蕉樣品來自 Chiquita 公司。該公司在生產及運銷管理十分嚴格，凡植株在生產過程中遭逢乾旱、淹水等逆境，即直接放棄受逆境而可能影響生長的植株批次，也不予採收。該公司在中南美洲有多點生產區，試驗中僅使用瓜地馬拉生產的果實，其為香蕉主要生產國家，週年氣溫穩定且較少受颶風影響，可降低樣本來自不同批次香蕉時所造成的影響。採後經船運抵達佛州的時間約 4-5 天，嚴格依循其公司採後標準作業程序以 14℃冷鏈運送，至抵港後才會催熟，而試驗則委託區域物流中心訂購未經催熟的綠熟香蕉，於隔天送達指定店面。

香蕉為世界重要的經濟作物，種植地擴及熱帶和亞熱帶地區，110 年產量達 1.16 億公噸，總種植面積超過 500 萬公頃。全球進口量約 22.9 億公斤，以美國進口量 460 萬公噸最多，佔總量 20.3%蔬果運輸，特別是國際運送管理，係以溫度為主要的控制因子，以低溫減緩植物代謝速率以維持品質及儘可能延長貯藏架壽命，延緩老化。然原生熱帶的香蕉對低溫十分敏感，在果實放置在低於特定臨界溫度會形成生理障礙，此類將果實存放在不會造成凍傷的低溫所造成的生理障礙即為寒害（Chilling injury），在香蕉上可見的寒害徵狀包含果皮褐變及果皮維管束褐變等，影響果實外觀，甚至可能導致顧客在驗貨即被退貨的情形。為此，若能更清楚了解寒害發生機制有助於減少寒害發生及避免食物浪費。因臺灣多數具利基的出口旗鑑作物為熱帶及亞熱帶蔬果，因此以寒害為研究主軸，試圖能找到有效的處理方案。

低溫敏感的作物被置於寒害溫度範圍內，會誘發不同的生理反應以維持細胞間代謝機能之穩定，為一自我調節的過程。受逆境的植物組織常有乙烯生成

量增加的情形，如酪梨及檸檬則曾有觀察到在貯藏於寒害溫度下產生大量的乙烯，此外，亦有作物自寒害溫度回溫後才出現明顯乙烯生成量增加，如小黃瓜。而香蕉除對低溫敏感外，亦對乙烯敏感，在 0.015~0.5 $\mu L \cdot L^{-1}$ 的乙烯濃度即可使綠熟果啟動後熟反應。前人研究以乙烯處理綠熟香蕉後，觀察到乙烯可誘導香蕉更具耐寒性，在番茄中則見寒害敏感性隨著果實越成熟而越低，紅熟番茄較綠熟番茄更耐低溫。由於乙烯處理同時會催動香蕉啟動後熟，越成熟的果實對低溫耐受性越好，推測在香蕉可能亦因乙烯事先啟動果實後熟作用，使寒害敏感性降低。為釐清寒害徵狀形成的過程，乙烯所扮演的角色，在研究中將部份果實事先處理乙烯作用抑制劑—安喜培（1-methylcyclopropene, 1-MCP），利用其能與乙烯受體緊密結合後並中斷乙烯作用的特性，將其在寒害溫度下之變化與未處理安喜培的果實進行比較，可了解在未有乙烯作用下的反應，能更如實了解乙烯在寒害中扮演的角色。

試驗假說為**綠熟香蕉經寒害誘導而出的內生乙烯在寒害及其徵狀發展中扮演重要角色。**

實驗目標如下：

一、 **決定可有效處理綠熟香蕉以延後其後熟作用的安喜培濃度，並比較在施用飽和乙烯促進催熟前或後的時間點施用安喜培對其恢復後熟能力之影響，並了解經處理的果實在後續恢復後熟的過程，如否能夠完全恢復，或呈現不規則、不同步的後熟反應。**

二、 **利用安喜培抑制乙烯作用，以釐清乙烯在香蕉寒害及寒害徵狀發生的過程所扮演的角色、在寒害逆境下受乙烯影響的生理參數。同時驗證作物之貯藏限制因子，以香蕉為材料，係以採收後熟作主要限制因子，倘若以安喜培處理後，延後後熟作用，並貯藏於潛在的寒害臨界溫度 14℃下，觀察在更長貯藏期階段，是否會有寒害徵狀發生。**

三、 **決定初期寒害階段，被低溫誘發的生理改變，並釐清其中與乙烯相關的徵狀、及不同寒害逆境在 5℃及 10℃處理不同時間下及回溫後造成的影響程度。**

四、 比較初期寒害發展階段綠熟香蕉之香氣圖譜，了解在早期寒害發展及其後回溫至完全成熟的階段，以安喜培中斷乙烯作業後，對綠熟香蕉在寒害期間及其後對香氣生合成過程中的影響情形。

五、 比較初期寒害發展階段綠熟香蕉之蛋白質圖譜，了解在早期寒害發展及其後回溫至完全成熟的階段，以安喜培中斷乙烯作業後，對綠熟香蕉在寒害期間及其後對蛋白質生合成及代謝之過程的影響情形，並且可釐清在寒害初期相關酵素之數量增減及活性變化。

六、 熱處理應用於香蕉以減緩其寒害發生之文獻探討。

利用 1-MCP 阻斷乙烯作用，以了解對寒害十分敏感的綠熟香蕉，在重度及輕度寒害溫度或最低建議儲溫的貯藏期間，以 1-MCP 處理綠熟香蕉，延遲其後熟反應，觀察在無乙烯作用下，與對照組比對，是否仍會發展出寒害徵狀（Chilling injury symptoms），以釐清乙烯在其中扮演的角色。在酪梨、鳳梨等作物中觀察到乙烯為加重果實寒害之因子，若香蕉也有類似反應，可望做為寒害調節應用。

試驗進度規劃原為五年，然實際允許在國外進修期限為四年，途中受疫情限制延後實驗進度，故和指導教授討論，決定先就四年期間已完成的試驗部份，提交論文以口試。原研究規劃中的熱處理對應於寒害逆境的研究在綠熟香蕉已有文獻討論過處理條件，因此規劃執行計畫在順序上較為壓後，並以寒害的臨界條件及乙烯在其中的關係率先執行，但因疫情影響，與熱處理相關研究未能如期執行完畢。故此，本次進修報告以博士論文所提交的內容，以綠熟香蕉上寒害與乙烯之關連為主，並附上當前香蕉經熱處理因應寒害之相關研究進展。

過程

職於 106 年 8 月 14 日起赴美，至 110 年 8 月 13 日返國服務，博士班前兩年以修課為主，並同步進行試驗研究。四年期間修習課程如下：

- Psychophysical Aspects of Foods
- Principles of postharvest horticulture
- Seminar preparation
- Plant biochemistry
- An introduction of computer programming with R
- Postharvest technologies for horticultural crops
- Laboratory methods in plant molecular biology
- Flavor chemistry
- Plant molecular biology and genomics
- Special topic- Plant hormonal Molecular biology
- Postharvest biology
- Horticultural Science Seminar
- Doctoral Research
- Advanced research

研究過程之內容摘要如下：

一、 乙烯燻蒸時機對安喜培處理過之綠熟香蕉的後熟反應

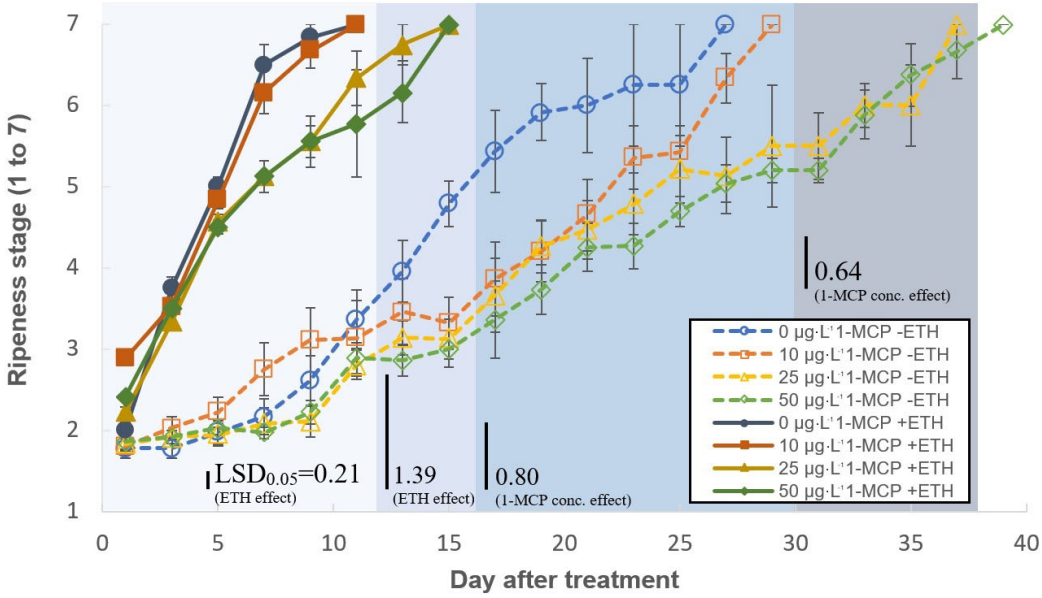
乙烯作為重要的植物荷爾蒙之一，參與許多植物生育過程及逆境反應，如更年性果實的後熟反應，乙烯即為重要的啟動因子，而其他逆境，如寒害（Chilling injury）與乙烯亦有關連，但其作用尚未完全釐清。安喜培（1-methlcyclopropene, 1-MCP）為可永久與乙烯受體結合之乙烯作用抑制劑（Ethylene action inhibitor），實際應用上可有效延續更年性果實後熟，亦可釐清乙烯在果實後熟階段所扮演的角色。傳統上安喜培係以長時間低劑量燻蒸使果實完全吸收，但以包裝場的流程，若能以短時間達成同樣效果，可望增加單日運轉量，但實際運用仍需針對作物實際狀況調整。試驗一為測試綠熟香蕉浸泡於不同濃度的 1-MCP 溶液 1 分鐘，以找尋最適當之處理。

試驗以綠熟香蕉處以 0 或 100 $\mu L \cdot L^{-1}$ 乙烯氣體於 20℃下 24 小時，隨後浸泡於 0、10、25 或 50 $\mu g \cdot L^{-1}$ 安喜培溶液 （23 ℃）中 1 分鐘，觀察 20℃貯藏期間，其果實受不同濃度的乙烯及安喜培溶液浸泡後熟變化及自乙烯抑制效應復原之情形。結果顯示，浸泡 10、25 或 50 $\mu g \cdot L^{-1}$ 安喜培處理後未薰蒸乙烯之果

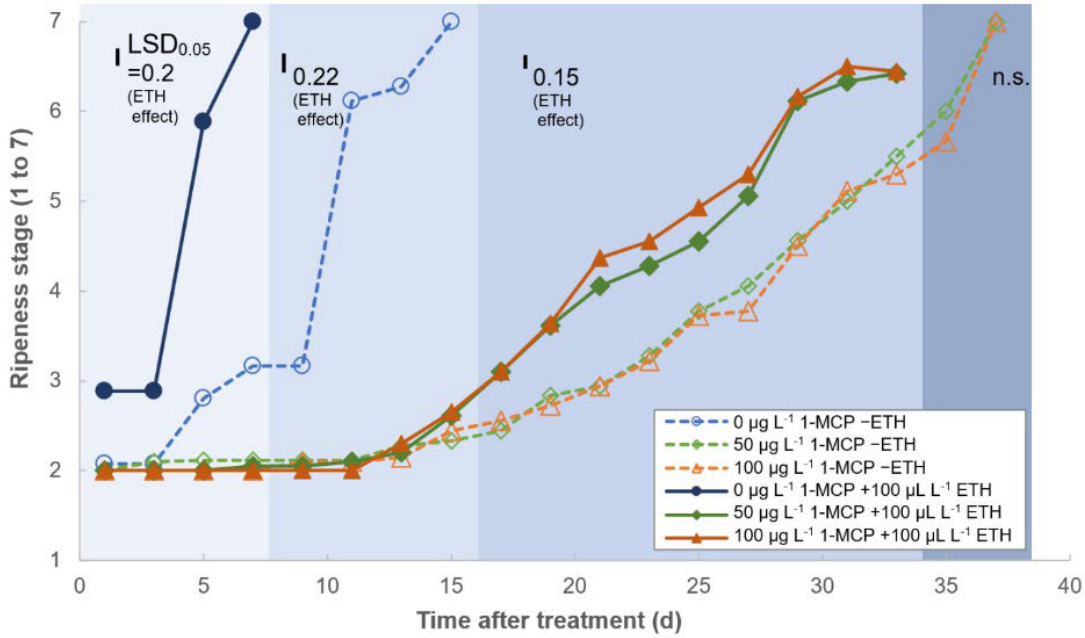
實與未處理乙烯及安喜培之果實各延遲 2、10 及 12 天，果實才達完熟（圖一、全果轉黃及出現褐斑）；經乙烯前處理之果實則幾乎不受安喜培之影響，僅後熟時間略緩 1-2 日。而進一步針對安喜培浸泡後對乙烯處理的效果，綠熟香蕉先行浸泡於浸泡於 0、50 或 100 $\mu g \cdot L^{-1}$ 安喜培溶液（23 ℃）中 1 分鐘，以 100 $\mu g \cdot L^{-1}$ 乙烯於 20℃下持續處理，並以新鮮流通空氣處理作為對照組。結果顯示安喜培+乙烯後處理的果實較浸泡安喜培後未經乙烯後處理之果實提前 4-6 天啟動後熟，而果實達完熟（全果轉為黃色並帶有褐斑）的時間，安喜培＋乙烯後處理較浸泡安喜培後未經乙烯後處理縮短 2 日（圖二）。處理安喜培的果實在轉色過程（果實黃色部份面積大於綠色，且僅果實兩端仍未轉色）中觀察到轉色不均勻，有部份色塊仍維持綠色無法褪去，此外，果肉亦有中心已軟化並呈水浸狀，但近果皮處質地偏硬的情形，但若以安喜培＋乙烯的處理，果肉軟化的過程較為一致（圖三）。

綜觀試驗結果，以綠熟香蕉而言，25$\mu g \cdot L^{-1}$ 安喜培溶液浸以 1 分鐘，為最低有效濃度，可延緩果實在 20 ℃後熟速度，與未處理安喜培及乙烯的果實相比，約可延後 10 日。而事前將綠熟香蕉處以乙烯後，再浸泡安喜培溶液，果實幾乎不受安喜培的延遲作用影響，仍正常進行其後熟反應。果實先浸泡安喜培溶液後再處以乙烯，果實貯藏初期後熟作用明顯被抑制，持續處以乙烯的果實恢復其後熟作用的時間較未處理者為短，乙烯可能促進果實生成新的乙烯受體，並加速啟動後熟。

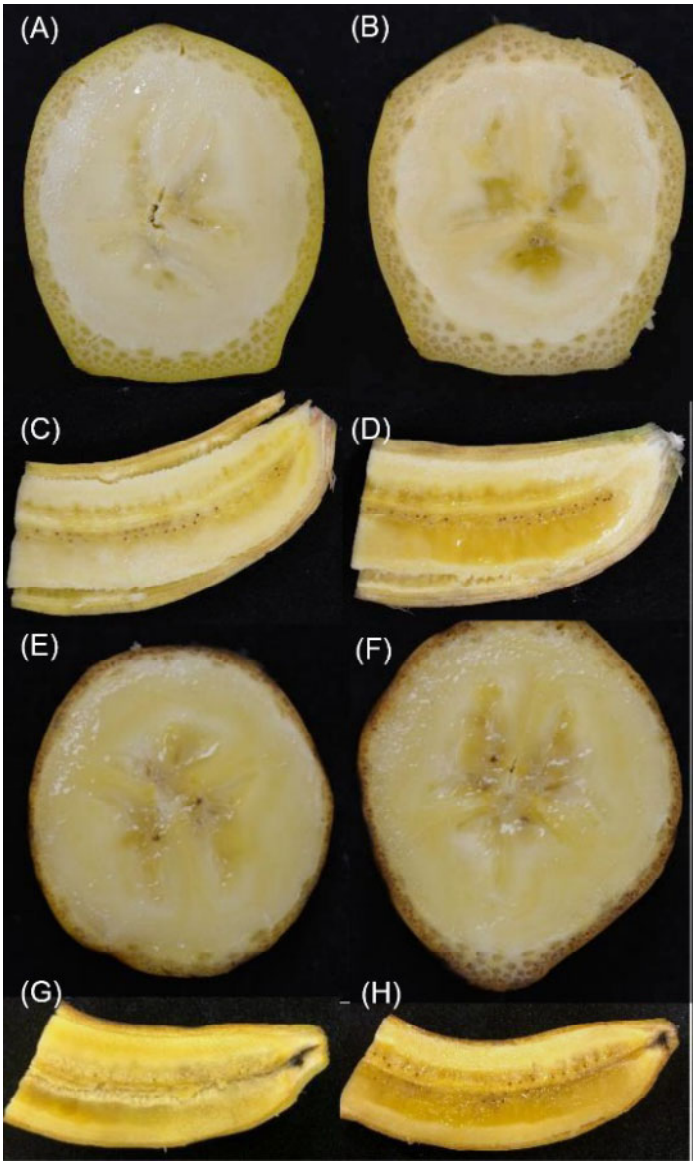
此一節試驗經修改投稿並刊於 Scientia Horticulturea （附件一）。



圖一、施用不同濃度乙烯於 1-MCP 處理之前，不同處理綠熟香蕉後熟情形。綠熟香蕉於 20°C 處以 0（-ETH, 以空心符號及虛線表示）或 100 µL·L^{-1}（+ETH, 以實心符號及實線表示） 乙烯 24 小時後，再浸泡於 0, 10, 25, or 50 µg·L^{-1} 安喜培溶液一分鐘並風乾後，貯藏於 20 °C, 95 % RH。數據之平均標準差以各點數值之垂直線條表示（n = 6）。LSD0.05 值在不同貯藏時期（Day 0-11; Day 13-15; Day 17-31）的值不同，相同者以同樣的底色呈現。



圖二、施用不同濃度乙烯於 1-MCP 處理之後，各處理綠熟香蕉後熟情形。綠熟香蕉先浸泡於 0、10、25 或 50 µg·L^{-1} 安喜培溶液一分鐘並風乾後，於 20°C, 95 % RH 下貯藏，並各連續處以 0 （-ETH, 以空心符號及虛線表示）或 100 µL·L^{-1} （+ETH, 以實心符號及實線表示） 乙烯。數據之平均標準差以各點數值之垂直線條表示（n = 6）。$LSD_{0.05}$ 值在不同貯藏時期（Day 0-7; Day 9-15; Day 17-33; Day 35-37）的值不同，相同者以同樣的底色呈現。



圖三、香蕉果實後熟期間果肉水浸狀及軟化的狀況。（A）、（B）、（E）、（F）為橫切面；（C）、（D）、（G）、（H）為縱切面。（A）、（C） 為處理第 13 天，自然後熟的香蕉（-ETH）的果肉狀況；（B）、（D） 為處理第 25 日，綠熟香蕉已先浸泡 100 μg·L^{-1} 安喜培溶液，連續處以 100 μL L^{-1} 乙烯於 20°C, 95 % RH 的果肉狀況（果實不均勻後熟）；（E）、（G） 為處理第 31 日，綠熟香蕉已先浸泡 100 μg·L^{-1} 安喜培溶液，連續處以 100 μL L^{-1} 乙烯的的果肉狀況（果實過熟）；（F）、（H） 為處理第 19 天，自然後熟的香蕉（-ETH）的果肉狀況（果實過熟）。

二、 延遲綠熟香蕉果實之後熟作用後，貯藏於公認的寒害臨界溫度中出現了寒害徵狀

熱帶水果如香蕉常會將果實置於公認為「寒害臨界溫度（Chilling threshold temperature, CTT）」中以避免果實寒害，但果實的貯藏壽命將由後熟中的老化情形決定，但所謂「寒害臨界溫度」是否完全不會對果實造成損害呢？為釐清此疑問，試驗以綠熟香蕉切分為單指後，浸泡於純水或 50 $\mu g \cdot L^{-1}$ 安喜培（1-MCP）溶液（有效當量, 23 ℃），使安喜培處理組可阻斷乙烯作用以延遲後熟並與純水進行對照，果實風乾後分為三組，貯藏於 5（重度寒害溫度）、13（輕度寒害溫度）或 14℃（綠熟香蕉之最低建議儲溫）觀察後續生理變化。結果顯示，經 1-MCP 處理之果實與經純水浸泡的對照組，若以果皮開始轉黃為判斷依據，約晚 10 日啟動轉色（表一）。就乙烯生成量而言，儲於 5℃的果實不論是否處理安喜培僅生成約 0.04 $ng \cdot kg^{-1} \cdot s^{-1}$ 乙烯量，且 31 日內未顯著增加（表二）；儲於 14℃的果實（處以 1-MCP 或純水）皆於第 33 天開始增加乙烯生成量；儲於 13℃中，處以純水的果實處理約第 38 天明顯增加、處以安喜培者則於 39 天開始上升。而乙烯的生成高峰，未處以 1-MCP 的果實儲於 13℃或 14℃下，果實各在第 44 天及第 39 天到達生成最大值；而處理 1-MCP 之果實儲於 13℃或 14℃者，則各於第 59 及 51 天達到高峰。

一般認為，果實貯藏在公認的寒害臨界溫度不會造成寒害，而是由其他儲存限制因子造成果實品質下降，直到不具食用價值而中止貯藏壽命。試驗假說為先以 1-MCP 處理綠熟香蕉以阻斷其乙烯作用，並貯藏於臨界溫度 14℃，更長低溫貯藏時間可能會出現香蕉寒害的徵狀。香蕉的典型寒害徵狀之一為果皮維管束褐變（Vascular browning, VB），其為其中最敏感也最早可由人肉眼辨別，試驗中儲於 5℃的果實皆於第 4 日即出現褐變，儲於 13℃的果實則皆於第 10 天出現褐變，但經處理安喜培之果實褐變程度比純水組更輕微。有趣的是儲於 14℃且未經安喜培處理（-1-MCP）的果實在第 19 天、若經安喜培處理的果實（+1-MCP）則是在第 28 天出現果皮維管束褐變，本試驗中即見綠熟香蕉在寒害臨界溫度貯藏更長的時間，形成較輕微的寒害徵狀，而維管束褐變為其中較敏感且不可逆的寒害指標，而果實於光適應下的葉綠素螢光讀值（Light-adapted quantum yield of photosystem II [Y(II)]）亦以對照組受寒害影響程度明顯較高（圖四），可作為非破壞性的初期寒害指標。

簡言之，當作物的主要貯藏限制因子，因施用其他技術而延長貯架壽命時，其他的次要貯藏限制因子，如一般認為是安全的寒害臨界溫度即可能轉變成寒害溫度，反而使作物受損明顯並出現寒害徵狀，因此延長貯架壽命的同時，需評估是否有其他次要影子會影響果實品質。本試驗中以果實先經安喜培浸泡後置於 13 或 14℃，其維管束褐變程度皆較對照組輕微，且儲於 14℃的果實中，安喜培處理明顯較對照組更晚出現寒害徵狀，顯示乙烯在初期寒害發生的過程中可能扮演部份角色。

此一節試驗經修改投稿並刊於 Frontier in Plant Science （附件二）。

表一、不同處理綠熟香蕉之後熟情形。綠熟香蕉先浸泡於 0 或 50 μg·L^{-1} 安喜培溶液一分鐘並風乾後，轉貯藏於 5℃、13℃或 14℃（相對溼度皆為 95%）中並定期調查（n=3）。

貯藏溫度（℃）	處理	實驗起始果實成熟度	果實啟動後熟（果色明顯轉黃）之平均日數	試驗第 61 天之果實平均成熟度
5. 0	純水	2.17a*	-**	-
	50 μL·L^{-1} 安喜培	2.17a	-*	-
13.0	純水	2.23a	45b	5.6b（頭尾綠到完全黃熟）
	50 μL·L^{-1} 安喜培	2.23a	57.7a	4c（果色中綠多於黃）
14.0	純水	2.17a	36c	7a（完全黃熟且出現褐點）
	50 μL·L^{-1} 安喜培	2.17a	38.7c	3.5c（果色中綠多過黃到黃多過綠）

*Mean separation within columns （lower case） were by Least Significant Different test （LSD） at 5% level.

**貯藏於 5℃的香蕉果皮直接轉為深褐色，且並於第 31 天後中止調查。

表二、不同處理綠熟香蕉之乙烯生成量變化。綠熟香蕉先浸泡於 0 或 50 μg·L^{-1} 安喜培溶液一分鐘並風乾後，轉貯藏於 5℃、13℃或 14℃（相對溼度皆為 95%）中並定期調查（n=3）。

貯藏溫度（℃）	處理	貯藏處理溫度後前三天之平均乙烯生成量（ng·kg^{-1}·s^{-1}）	乙烯生成量因開始後熟啟動而增加所費的天數	到達乙烯生成量高峰的試驗天數	到達乙烯生成量高峰的乙烯生成量（ng·kg^{-1}·s^{-1}）	到試驗結束（第 61 天）當日處理平均之乙烯生成量（ng·kg^{-1}·s^{-1}）*
5. 0	純水	0.106b**	-			0.095*
	50 μL·L^{-1} 安喜培	0.117a	-			0.103*
13.0	純水	0.104b	38.7b	54b	0.502c	0.409c
	50 μL·L^{-1} 安喜培	0.104b	46a	58a	1.018a	0.907a
14.0	純水	0.098c	37b	53.7b	0.647bc	0.439bc
	50 μL·L^{-1} 安喜培	0.108b	42.2ab	55.3ab	0.702b	0.569b

*貯藏於 5℃的香蕉果皮直接轉為深褐色，且並於第 31 天後中止調查，而貯藏期間果實皆未啟動後熟（如軟化等）。

**Mean separation within columns （lower case） were by Least Significant Different test （LSD） at 5% level.

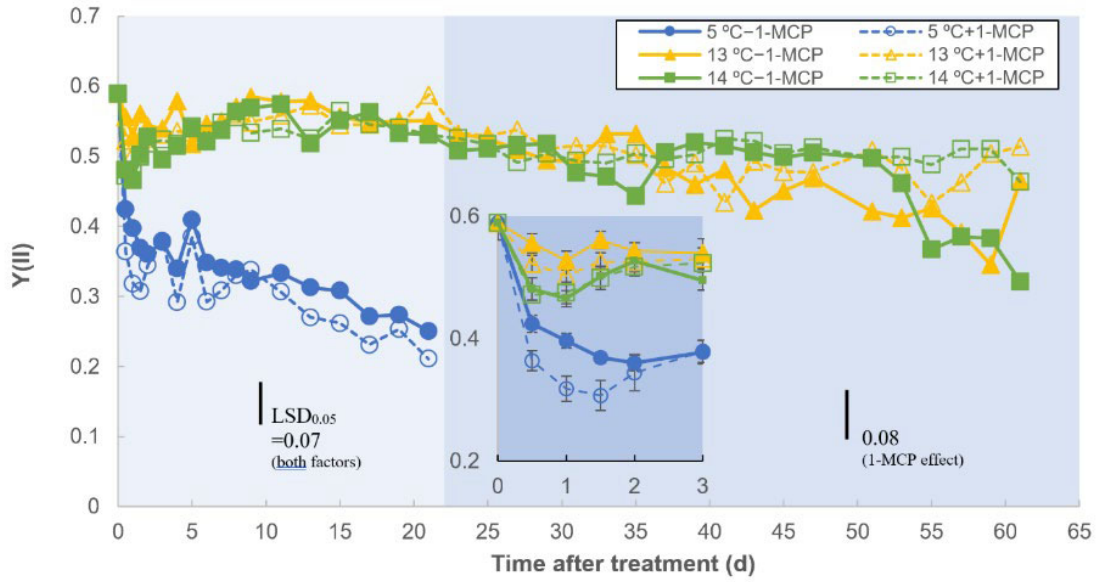
表三、不同處理綠熟香蕉之寒害徵狀發展情形。綠熟香蕉先浸泡於 0 或 50 μg·L^{-1} 安喜培溶液一分鐘並風乾後，轉貯藏於 5℃、13℃或 14℃（相對溼度皆為 95%）中並定期調查 （n=3）。

貯藏溫度（℃）	處理	果實表皮第一次出現**灰斑**的平均日數（括弧內為觀察到的面積百分比）	果皮第一次出現**維管束褐變**的平均日數（括弧內為觀察到的面積百分比）	試驗處理調查到最後一，果皮平均維管束褐變的面分百分比（括瓜內為該處理的調查結束天數）*
5.0	純水	4a**（40%）	4d（16.7%A）	95（31 日）
	50 μL·L^{-1} 安喜培	4a（40%）	4d（16.7%A）	95（31 日）
13.0	純水	-***	10c（8.3%B）	15（61 日）
	50 μL·L^{-1} 安喜培	-***	10c（2.3%C）	10（61 日）
14.0	純水	-***	19b（1%C）	8.3（61 日）
	50 μL·L^{-1} 安喜培	-***	28a（8.7%B）	10（61 日）

*貯藏於 5℃的香蕉果實遭逢嚴重寒害且直接轉為深褐色，且並於第 31 天後中止調查，而貯藏期間果實皆未啟動後熟（如軟化等）。

**Mean separation within columns （lower case and capital letter inside brackets） were by Least Significant Different test （LSD） at 5% level.

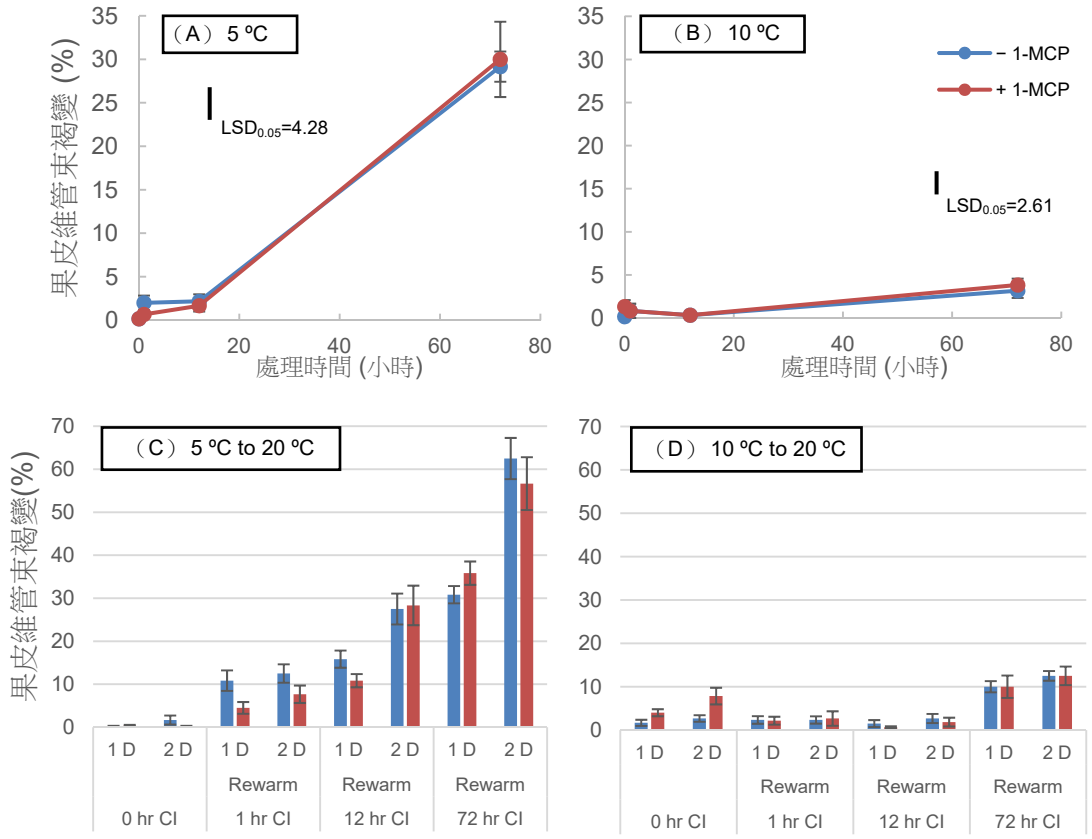
***貯藏於 13 或 14℃的香蕉果實未觀察到果皮灰斑出現。香蕉寒害特狀調查為實驗處理當天（第 0 天）、第 1 天、第 3 天及其後每 3 天調查一次。



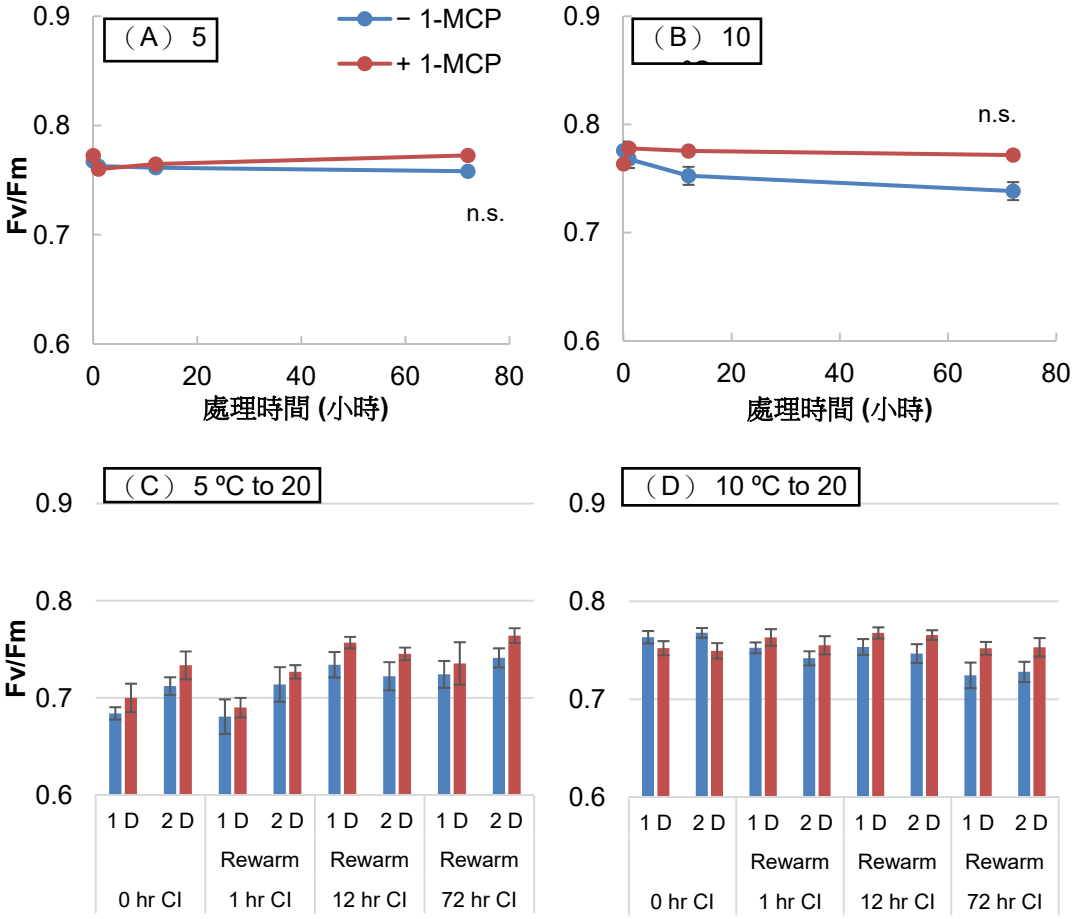
圖四、不同處理綠熟香蕉果皮之光化學效能。綠熟香蕉先浸泡於 0 或 50 μg·L^{-1} 安喜培溶液一分鐘並風乾後，轉貯藏於 5℃、13℃或 14℃（相對溼度皆為 95%）中並測量其光適應下光合作用系統二之葉綠素螢光之光量讀值（Quantum yield [Yield （II）], n=6）。數據之平均標準差以各點數值之垂直線條表示（n = 6）。$LSD_{0.05}$ 值在不同貯藏時期（Day 0-21; Day 23-61）的值不同，相同者以同樣的底色呈現。

三、綠熟香蕉在不同強度寒害逆境下，果實初期寒害發展及其後回溫之復原情形和與乙烯作用之影響

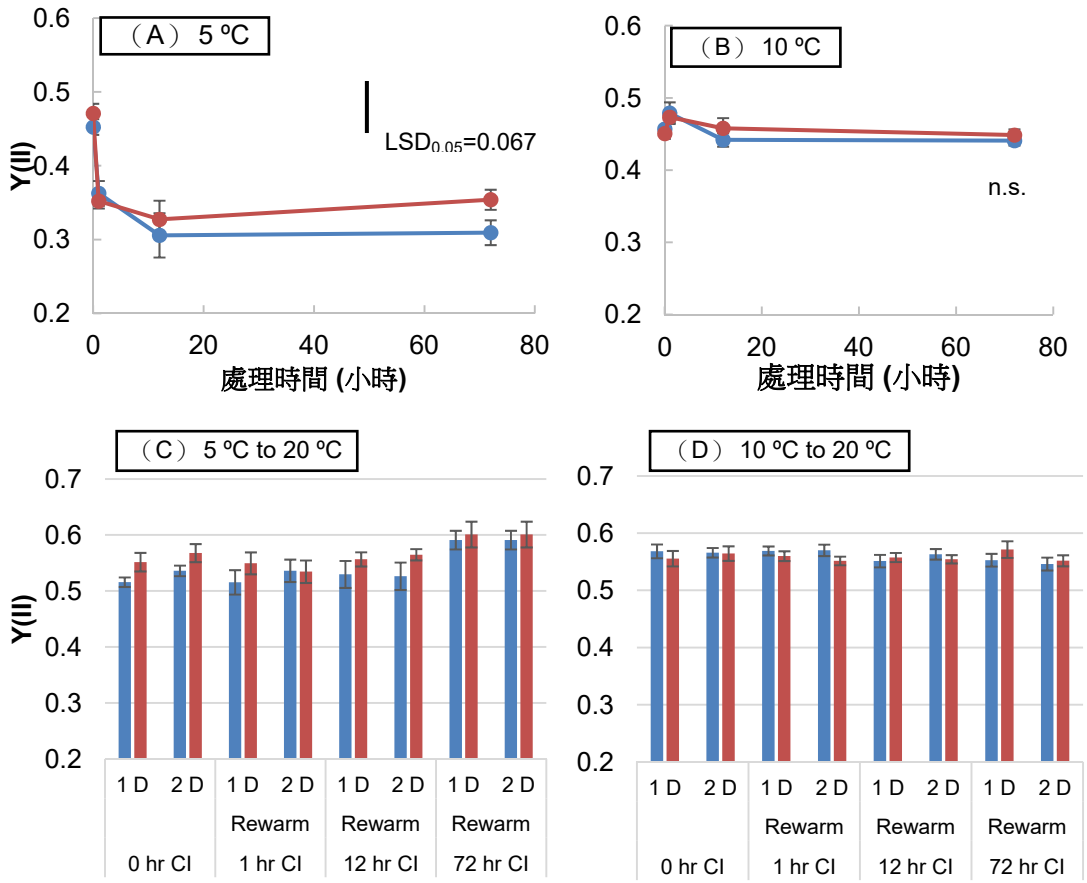
寒害的嚴重程度與「時間」×「低溫溫度」有關，以先前試驗中最敏感且不可逆的寒害徵狀「果皮維管束褐變」而言，綠熟香蕉在輕度寒害溫度（13/14℃）約 10-25 天即觀察到此特徵，而 5℃則在 1-12 小時內即見維管束褐變（Vascular browning, VB）發生，推測此二個不同溫度和時間的處理組合，寒害徵狀程度相仿，可能處於相近的逆境強度。因目前僅在果皮維管束褐變發展上有觀察到與乙烯相關的表現差異，為縮短試驗時間並同時了解寒害徵狀發展初期與乙烯之關連性，試驗調查香蕉綠熟果實低溫貯藏期間，寒害徵狀發展（Chilling injury symptoms development）過程，比較經 50 $\mu g \cdot L^{-1}$ 1-MCP 處理及純水處理之果實，貯藏 5℃或 10℃下 0、1、12 或 72 小時等不同寒害逆境（Chilling stress）下，貯藏期間及回溫（20℃）二日後的果實生理反應。結果顯示，寒害反應的強弱與逆境強度成正比，其中經 5℃或 10℃貯藏 72 小時的果實上，不可逆反應 VB 顯現，回溫後更為明顯（圖五）。其餘寒害反應如光適應或暗適應下光合作用系統二之葉綠素螢光反應（圖六及七）、組織離子滲漏率（圖八及九）、呼吸作用（圖十）及乙烯生成率（圖十一）等，皆隨逆境時間而降低，與逆境強度皆有負向的反應趨勢，回溫後恢復速率則依受逆境弱至強的程度恢復。各處理間以貯藏於 5℃下 72 小時的果實所受寒害逆境最為明顯，且回溫後逆境反應持續發展 1 至 2 天，甚至會惡化到影響生理表現，其餘處理組則是輕微甚至無明顯徵狀出現。有趣的是，經 1-MCP 預處理的果實貯藏於 5℃或 10℃，在葉綠素螢光、全果皮及果皮下維管束及周邊組織的離子滲漏率等與膜系統完整性相關的指標，皆顯現較輕微的寒害反應，可能具較佳耐寒害能力（Chilling injury tolerance）和回溫恢復反應（Recovery），顯示乙烯在寒害發展過程與細胞的膜系統，如細胞膜或葉綠體之膜系統結構之穩定性有關。



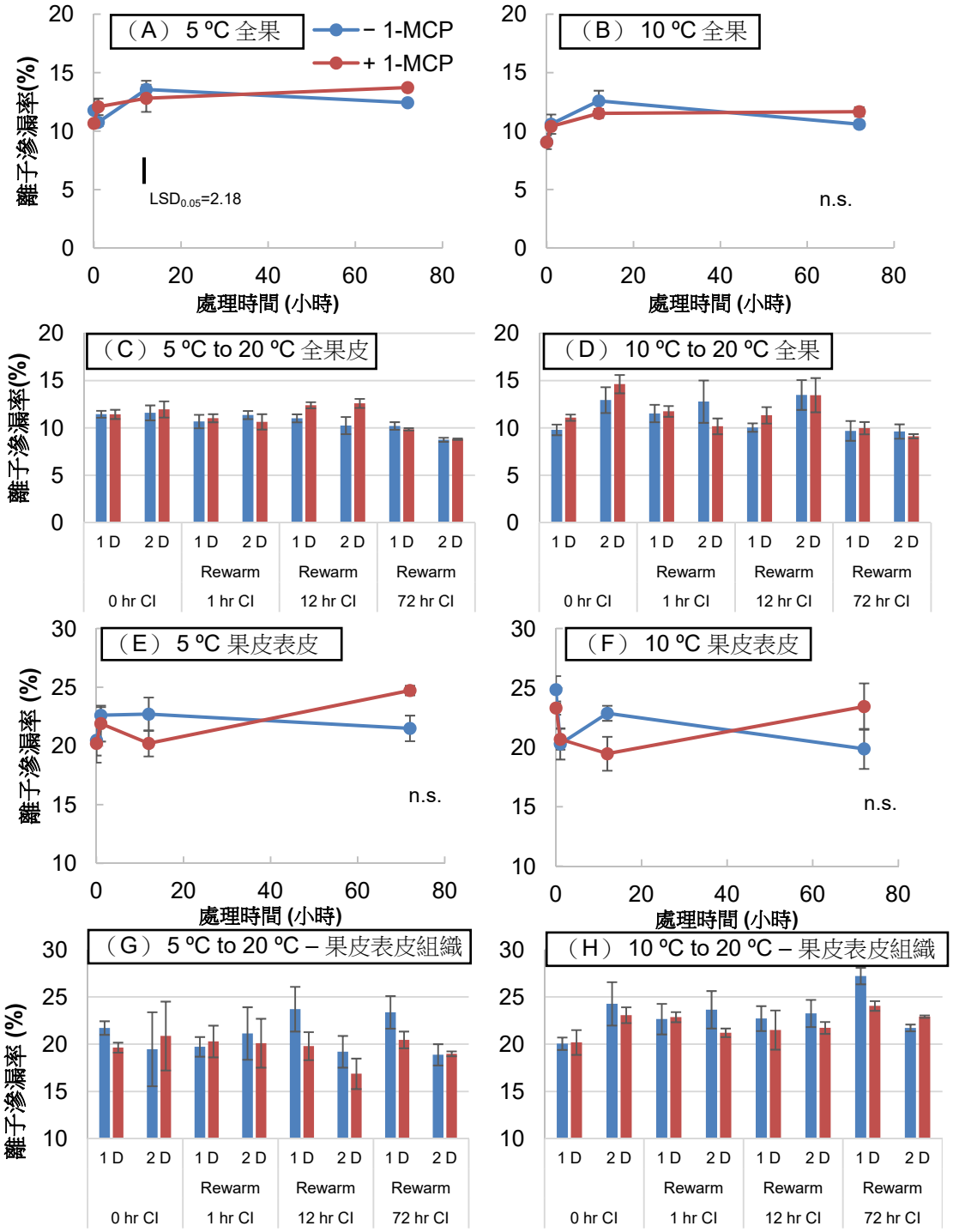
圖五、各處理之果皮維管束褐變情形。綠熟香蕉先浸泡於 0（-1-MCP）或 50 μg·L^{-1}（+1-MCP）安喜培溶液一分鐘並風乾後，經 14℃調節貯藏一夜後，轉貯藏於 5℃（A）或 10℃（B）下 0、1、12 及 72 小時（相對溼度皆為 95%）後移至 20℃環境回溫 1 及 2 天（5℃—C；10℃-D）。Vertical bars are standard errors of means （n = 6）.



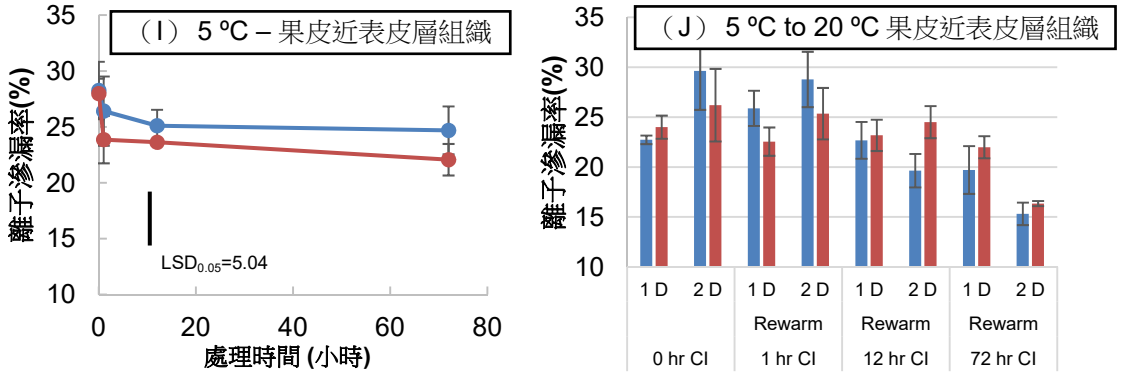
圖六、各處理之果皮暗適應下光合作用系統二葉綠素螢光值（Fv/Fm）變化情形。綠熟香蕉先浸泡於 0（-1-MCP）或 50 μg·L^{-1}（+1-MCP）安喜培溶液一分鐘並風乾後，經 14℃調節貯藏一夜後，轉貯藏於 5℃（A）或 10℃（B）下 0、1、12 及 72 小時（相對溼度皆為 95%）後移至 20℃環境回溫 1 及 2 天（5℃—C；10℃-D）。Vertical bars are standard errors of means（n = 6）.



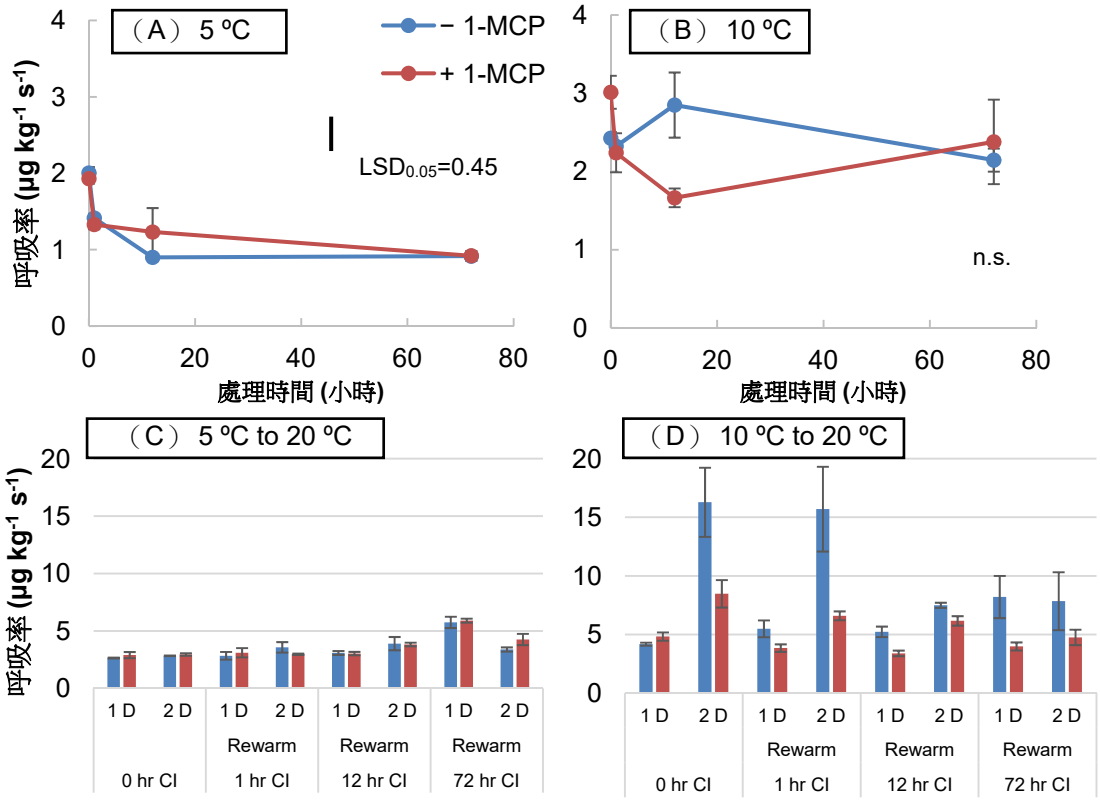
圖七、各處理之果皮光適應下光合作用系統二葉綠素螢光值[Y（II）]變化情形。綠熟香蕉先浸泡於 0（-1-MCP）或 50 μg·L^{-1}（+1-MCP）安喜培溶液一分鐘並風乾後，經 14℃調節貯藏一夜後，轉貯藏於 5℃（A）或 10℃（B）下 0、1、12 及 72 小時（相對溼度皆為 95%）後移至 20℃環境回溫 1 及 2 天（5℃—C；10℃-D）。Vertical bars are standard errors of means （n = 6）.



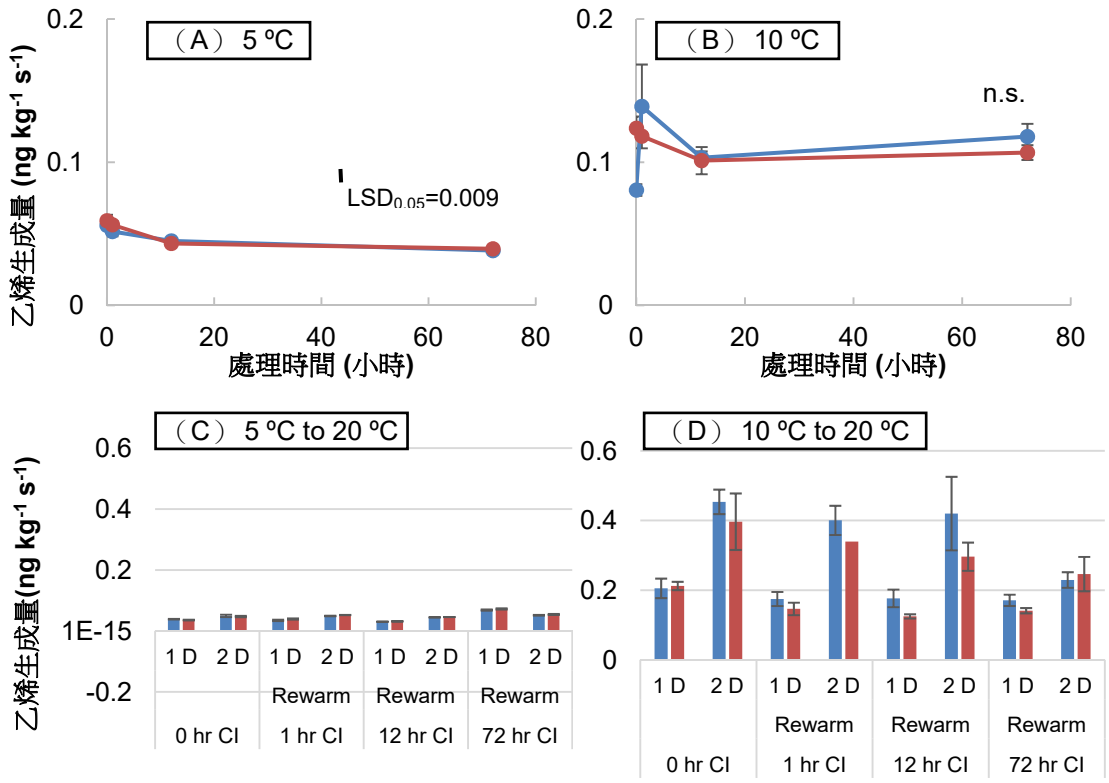
圖八、各處理之全果皮及果皮表皮組織之離子滲漏率變化。綠熟香蕉先浸泡於 0（-1-MCP）或 50 μg·L^{-1}（+1-MCP）安喜培溶液一分鐘並風乾後，經 14℃調節貯藏一夜後，轉貯藏於 5℃（全果皮-A; 果皮表皮組織-E）或 10℃（全果皮-B; 果皮表皮組織-F）下 0、1、12 及 72 小時（相對溼度皆為 95%）後移至 20℃環境回溫 1 及 2 天（5℃下全果皮-C 及 果皮表皮組織-G; 10℃下全果皮-D 及 果皮表皮組織-H）。Vertical bars are standard errors of means （n = 6）.



圖九、各處理之全果皮及果皮表皮組織之離子滲漏率變化。綠熟香蕉先浸泡於 0（-1-MCP）或 50 μg·L^{-1}（+1-MCP）安喜培溶液一分鐘並風乾後，經 14℃調節貯藏一夜後，轉貯藏於 5℃（全果皮-A; 果皮表皮組織-E）或 10℃（全果皮-B; 果皮表皮組織-F）下 0、1、12 及 72 小時（相對溼度皆為 95%）後移至 20℃環境回溫 1 及 2 天（5℃下全果皮-C 及 果皮表皮組織-G; 10℃下全果皮-D 及 果皮表皮組織-H）。Vertical bars are standard errors of means （n = 6）.



圖十、各處理之呼吸率變化。綠熟香蕉先浸泡於 0（-1-MCP）或 50 μg·L^{-1}（+1-MCP）安喜培溶液一分鐘並風乾後，經 14℃調節貯藏一夜後，轉貯藏於 5℃（A）或 10℃（B）下 0、1、12 及 72 小時（相對溼度皆為 95%）後移至 20℃環境回溫 1 及 2 天（5℃—C；10℃-D）。Vertical bars are standard errors of means （n = 3）.

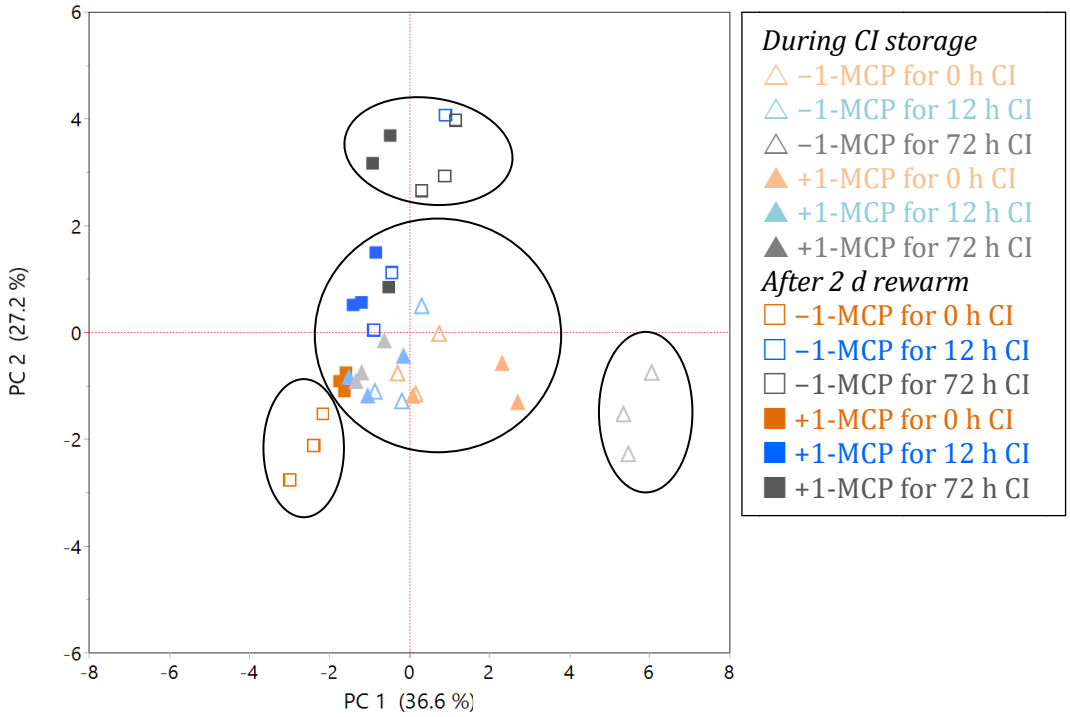


圖十一、各處理之乙烯生成量變化。綠熟香蕉先浸泡於 0（-1-MCP）或 50 μg·L^{-1}（+1-MCP）安喜培溶液一分鐘並風乾後，經 14℃調節貯藏一夜後，轉貯藏於 5℃（A）或 10℃（B）下 0、1、12 及 72 小時（相對溼度皆為 95%）後移至 20℃環境回溫 1 及 2 天（5℃—C；10℃-D）。Vertical bars are standard errors of means （n = 3）.

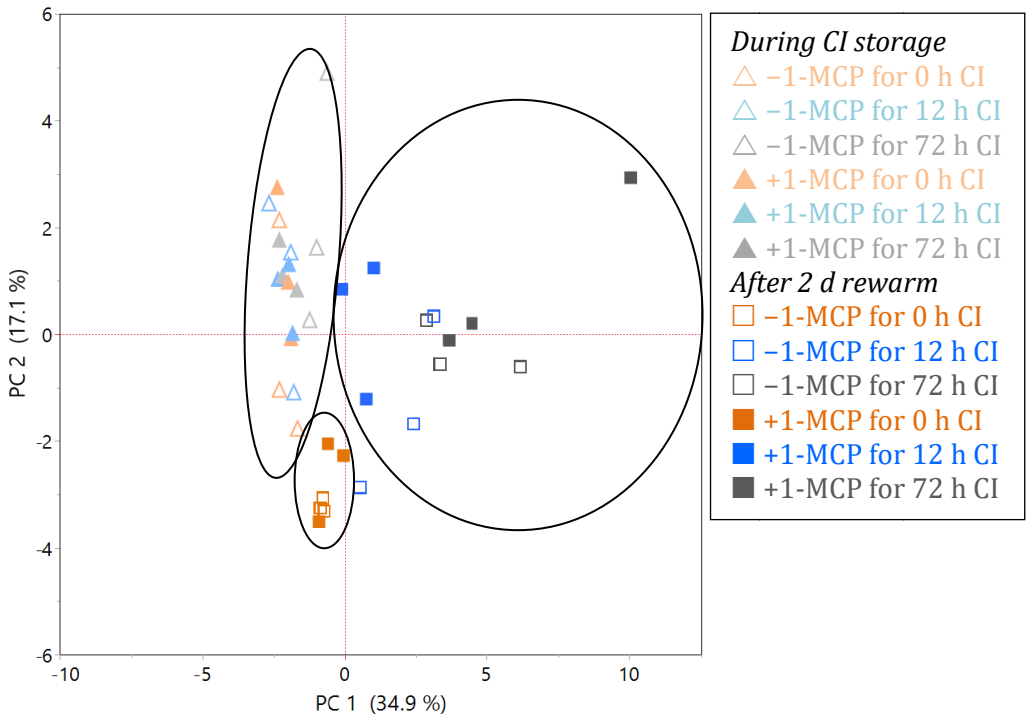
四、初期寒害逆境下，綠熟香蕉乙烯感知能力對香氣圖譜之影響

香蕉主要特徵香氣在果實後熟期，特別是呼吸率及乙烯生成量的生成高峰後大量形成，但其他因子如不適當的儲藏溫度會影響香氣分子生合成，在番茄即有觀察到因過低的貯溫中，果實香氣生成量大幅減少，在香蕉中亦發現儲藏於過低的溫度會減少香氣生成。香氣成分係由細胞組織之脂類及蛋白質經酵素轉化生成，然細胞膜系統異常為主要寒害反應之一，未知寒害後膜的異常是否會影響香氣的生成來源，因此為了解寒害對綠熟果實之香氣揮發物生合成之影響，及安喜培阻斷乙烯感知並阻止乙烯作用後，是否會改變香氣圖譜，試驗四利用綠熟香蕉果實處以寒害溫度，針對初期寒害階段所生成之香氣揮發物進行差異性分析，將綠熟香蕉處以 1-MCP 處理或純水後，貯藏 5℃下 0、12 或 72 小時、及於 20℃回溫 2 天後或至完熟期（果皮轉黃且具褐斑）等階段，比較其果皮和果肉的香氣圖譜之異同，以釐清輕微寒害處理下，經安喜培阻斷的乙烯感知（Ethylene perception）是否會影響低溫貯藏時，及其後熟階段之香氣生合成。結果顯示經歷低溫、回溫 2 日後甚至是回溫後到果實完熟階段，果皮及果肉組織都受到寒害逆境及 1-MCP 處理不同程度的影響，寒害逆境越大，影響亦越大。經主成分分析可知，果皮組織 72 小時低溫後香氣圖譜即與其他處理會有些微不同（圖十二及十三），回溫之後則更明顯區分，但果肉組織在處理時未有明顯差異，在回溫後才會明顯與未經寒害的果實不同。果皮的香氣含量經低溫 12 小時處理即受到影響，而果肉在低溫期間皆未有明顯差異，推測揮發物生成部份，果皮比果肉對低溫更敏感。在眾多香氣物質中，5℃的寒害溫度可誘導對照組果實在低溫貯藏期間及回溫階段生成更多綠葉揮發物（己醛、反式己烯醛、己醇和反式己烯醇等），且隨著處理時間越久且回溫後，生成量更多（表四及五）。綠葉揮發物係昆蟲或動物在嚙食植物葉片時會大量生成，並會啟動抗逆境或系統性的防禦機制，亦有觀察到其他作物上會在低溫逆境下被大量誘發，推測對照組可能有明顯啟動防禦或抗逆境相關的反應，而經安喜培浸泡過的果實在面對低溫時所生成之綠葉揮發物僅 50-75%，配合果皮維管束褐變之結果，推測經安喜培處理之果實可能在初期寒害階段具有較佳的耐寒性。而果實經低溫貯藏 0、12 或 72 小時後，或回溫 2 天，或至完熟階段，以主成份分析各時間點果肉及果皮之揮發物圖譜（圖十四及十五），結果顯示受到寒害逆境壓力越大，其受

影響的揮發物在比例上會越不同，但整體而言，果皮及果肉組織皆具有相似的果肉及果皮的揮發物組成，僅各物質含量比例不同，表示本試驗寒害處理的果實恢復其揮發物生合成途徑，而雖經 1-MCP 處理過的果實亦可在貯藏過程中恢復其乙烯作用（Ethylene action），並啟動後熟，包含生合成香氣揮發物的製造能力。



圖十二、以主成份分析綠熟香蕉處理純水或安喜培後，於 5℃貯藏不同時間及其後續 2 日回溫後，各處理果皮生成香氣成分的各處理群集分佈。綠熟香蕉先浸泡於 0（-1-MCP）或 50 μg·L^{-1}（+1-MCP）安喜培溶液一分鐘並風乾，經 14℃調節貯藏一夜後，轉貯藏於 5℃下 0、12 及 72 小時（相對溼度皆為 95%）後移至 20℃環境回溫 2 天。



圖十三、以主成份分析綠熟香蕉處理純水或安喜培後，於 5℃貯藏不同時間及其後續 2 日回溫後，各處理果肉生成香氣成分的各處理群集分佈。綠熟香蕉先浸泡於 0（-1-MCP）或 50 μg·L^{-1}（+1-MCP）安喜培溶液一分鐘並風乾，經 14℃調節貯藏一夜後，轉貯藏於 5℃下 0、12 及 72 小時（相對溼度皆為 95%）後移至 20℃環境回溫 2 天。

表三、綠熟香蕉之果肉及果皮經低溫貯藏後，受影響之香氣成份生成量（μg kg^{-1} FW benzyl butanoate equivalents）。綠熟香蕉先浸泡於 0（-1-MCP）或 50 μg·L^{-1}（+1-MCP）安喜培溶液一分鐘並風乾，經 14℃調節貯藏一夜後，轉貯藏於 5℃下 0、12 及 72 小時（相對溼度皆為 95%）。

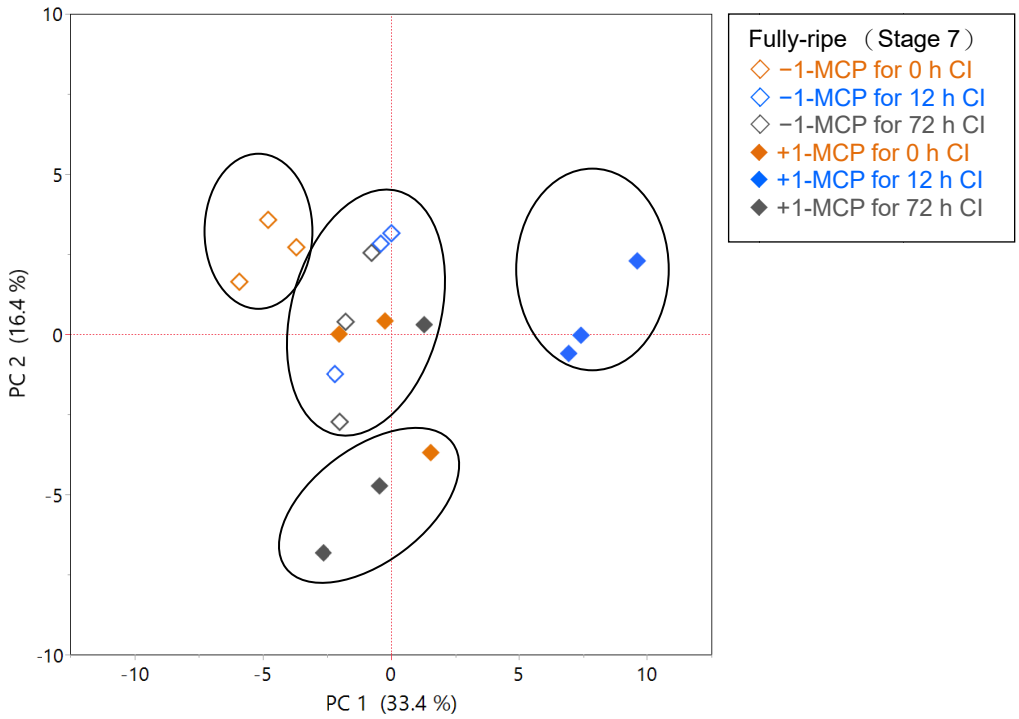
香氣成分	*低溫貯藏（5 ℃）*					
	0 h（14 °C）		12 h CI		72 h CI	
	−	+	−	+	−	+
	果皮					
醛類						
反式己烯醛(E)-2-Hexenal	106.6b*	129.7b	188.5b	132.5b	140.5b	103.2b
反式己烯醇(E,Z)-2,6-Nonadienal	116.1ab	108.0b	80.5bc	82.1bc	150.7a	78.4bc
己醛 Hexanal	87.7b	167.0b	147.9b	129.8b	251.2b	130.4b
總醛類	395.8	508.0	486.3	388.9	692.6	379.8
總酯類	-	-	-	-	-	-
總香氣成分	399.5	514.9	491.8	402.1	709.4	379.8
	果肉					
醛類						
反式己烯醛(E)-2-Hexenal	936.6	1287.4	1355.6	1085.4	2317.2	2439.7
反式己烯醇(E,Z)-2,6-Nonadienal	100.1	143.9	82.4	124.6	169.0	101.1
己醛 Hexanal	979.7	1758.3	2281.8	1423.0	5349.0	4494.9
總醛類	2212.4	3477.9	4085.0	2934.1	8333.0	7337.2
總酯類	49.0	38.5	29.7	57.5	55.1	97.8
總香氣成分	2365.3	3741.2	4453.6	3183.0	8479.3	8007.9

*Mean separation within the same row with different letters are significantly different（$p>0.05$）.

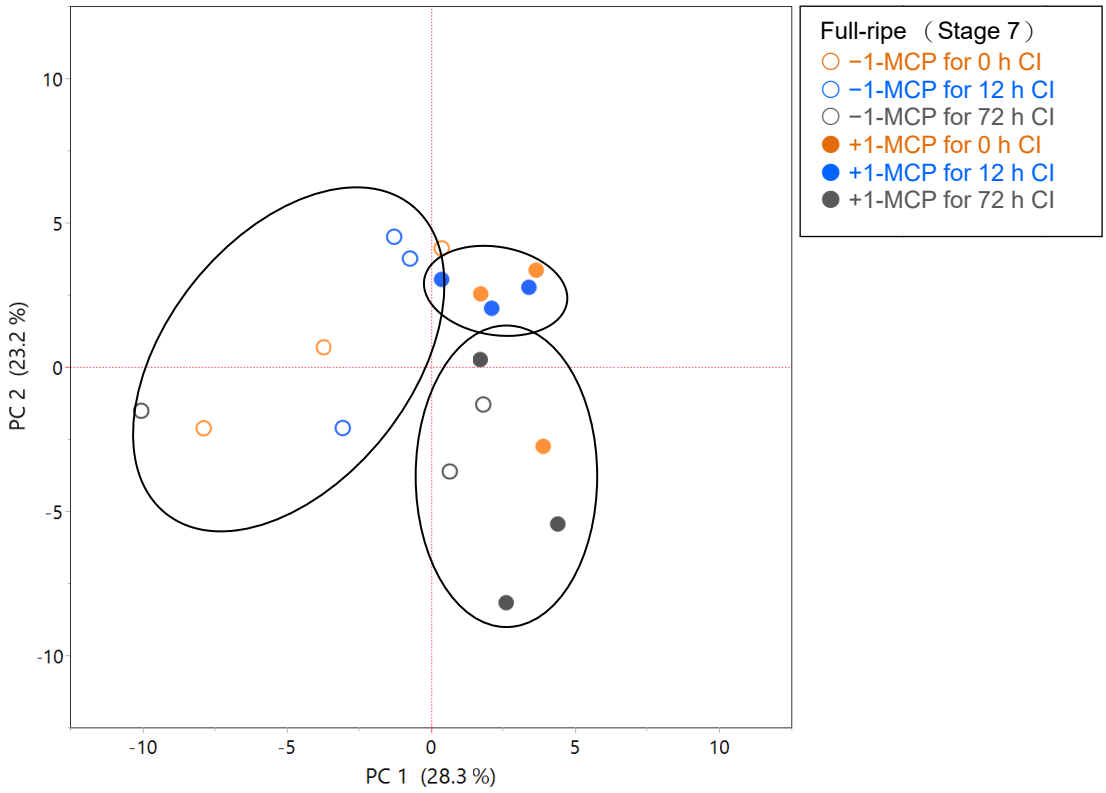
表四、綠熟香蕉之果肉及果皮經低溫貯藏並回溫（**20 °C**）**2 日後**，受影響之香氣成份生成量（μg kg^{-1} FW benzyl butanoate equivalents）。綠熟香蕉先浸泡於 0（-1-MCP）或 50 μg·L^{-1}（+1-MCP）安喜培溶液一分鐘並風乾，經 14℃調節貯藏一夜後，轉貯藏於 5℃下 0、12 及 72 小時（相對溼度皆為 95%）後移至 20℃環境回溫 2 天。

香氣成分	**低溫貯藏後經 2 日回溫處理（20 °C）**					
	0 h		12 h CI		72 h CI	
	−	+	−	+	−	+
	果皮					
醛類						
反式己烯醛 (E)-2-Hexenal	73.4b*	129.6b	252.7ab	171.2b	432.9a	389.9a
反式己烯醇 (E,Z)-2,6-Nonadienal	68.8c	75.0bc	80.7bc	77.6bc	150.9a	75.8bc
己醛 Hexanal	53.7b	146.1b	572.4a	284.4b	535.8a	525.3a
總醛類	235.7	399.4	991.7	595.5	1245.2	1079.2
總酯類	0.0	2.8	13.2	2.1	36.9	35.4
總香氣成分	303.0	443.7	1021.4	653.0	1300.3	1177.0
	果肉					
醛類						
反式己烯醛 (E)-2-Hexenal	2041.4	2975.8	8053.7	7288.6	9699.1	13799.8
反式己烯醇 (E,Z)-2,6-Nonadienal	-	-	78.0	137.0	90.4	106.3
己醛 Hexanal	3167.0	4993.7	29960.7	24324.9	19268.8	28899.6
總醛類	5406.0	8247.3	38547.5	32200.2	29701.6	43681.8
總酯類	124.9	97.6	133.5	83.5	275.2	328.9
總香氣成分	5545.7	8387.5	38738.0	32427.3	30051.3	44089.6

*Mean separation within the same row with different letters are significantly different（$p>0.05$）.



圖十四、以主成份分析綠熟香蕉處理純水或安喜培後，於 5℃貯藏不同時間後果實達到完熟階段，各果皮生成香氣成分的處理群集分佈。綠熟香蕉先浸泡於 0（-1-MCP）或 50 μg·L^{-1}（+1-MCP）安喜培溶液一分鐘並風乾，經 14℃調節貯藏一夜後，轉貯藏於 5℃下 0、12 及 72 小時（相對溼度皆為 95%）後移至 20℃環境貯藏至完熟。

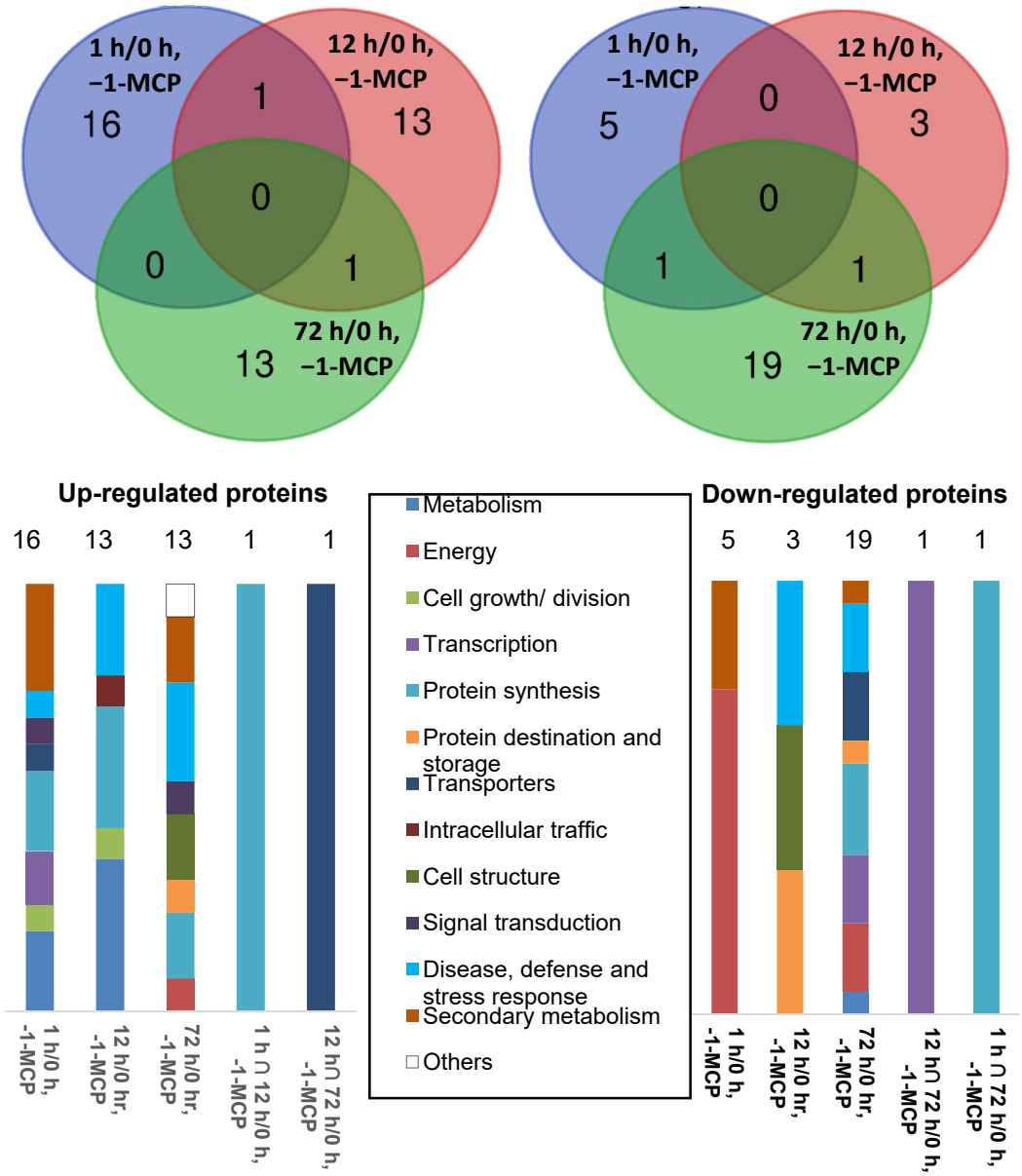


圖十五、以主成份分析綠熟香蕉處理純水或安喜培後，於 5℃貯藏不同時間後果實達到完熟階段，各果肉生成香氣成分的處理群集分佈。綠熟香蕉先浸泡於 0（-1-MCP）或 50 μg·L^{-1}（+1-MCP）安喜培溶液一分鐘並風乾，經 14℃調節貯藏一夜後，轉貯藏於 5℃下 0、12 及 72 小時（相對溼度皆為 95%）後移至 20℃環境貯藏至完熟。

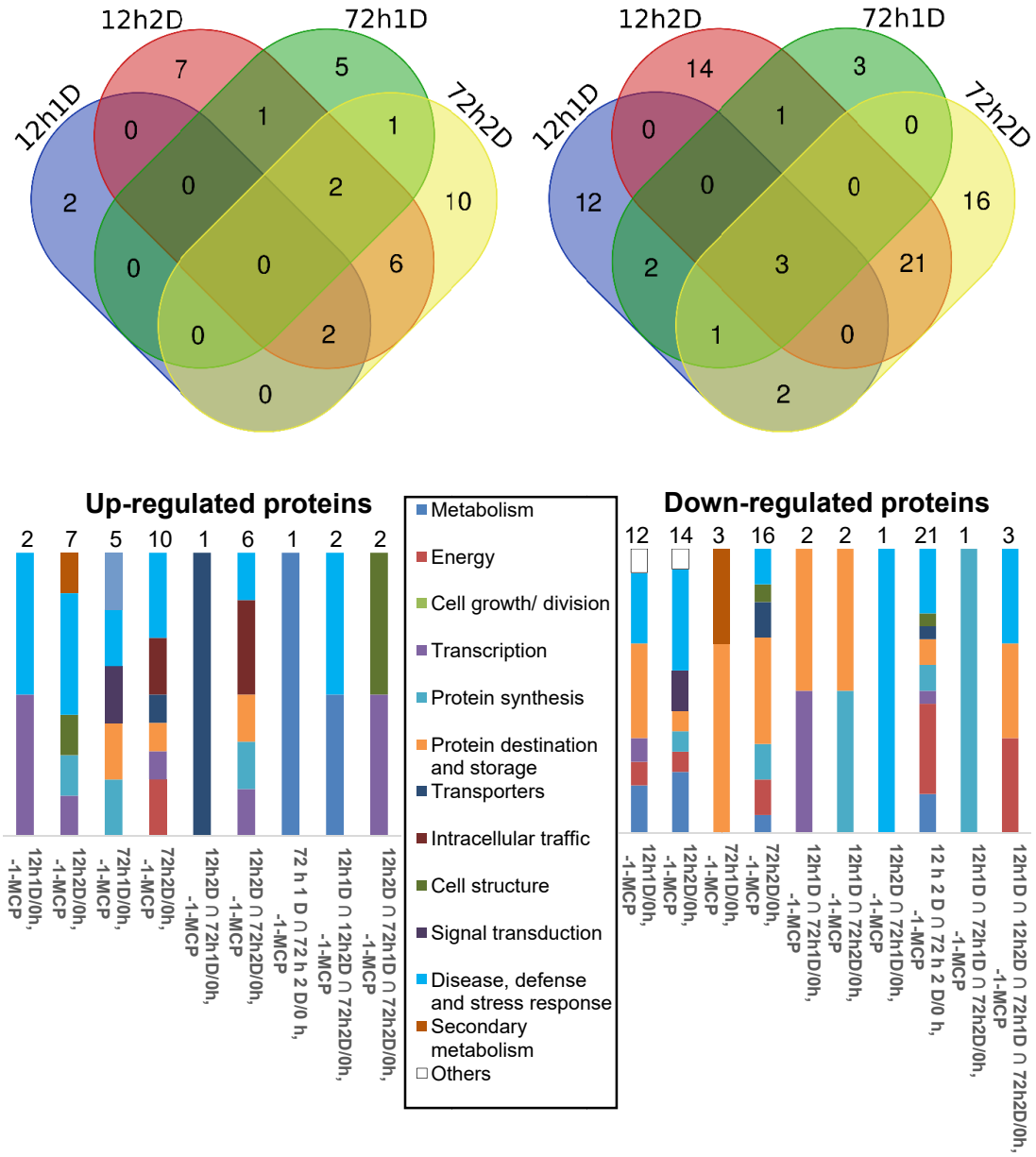
五、初期寒害逆境下，綠熟香蕉乙烯感知與否對蛋白質圖譜之影響

寒害溫度下，植物細胞膜系統的流動性降低為理論初期寒害現象之一。目前研究進展亦發現，植物細胞因低溫活化如鈣離子或 Reactive oxygen species（ROS）等訊號，經膜上的蛋白質傳遞訊息至細胞內部，以對逆境產生「反應」，此外，低溫下部份酶或蛋白質亦出現活性改變或數量增減的情形，例如脂肪酸去飽和酶（Fatty acid desaturase, FAD）即常因低溫而增加數量及活性，且相對應之細胞膜不飽和脂肪酸隨之含量增加，增加低溫下細胞膜的流動性；與細胞膜分解相關的磷酸脂肪酶 D（Phospholipase D）亦有觀察到受低溫而提升活性，加速改變細胞膜通透性，且膜系統受損。故此，逆境後整體蛋白質變化，可了解低溫下植物細胞的反應，並有助做逆境診斷及逆境減緩策略的制定。於前敘試驗中，已知寒害溫度下，果皮維管束褐變初期發展與乙烯相關，為使用蛋白質體圖譜更具體了解乙烯感知在寒害過程上可能參與的狀況，試驗五以經 1-MCP 處理後之綠熟香蕉在 5 度 C 下 0、1、12 或 72 小時的貯藏期間、於 20℃回溫 1、2 天後與僅浸泡純水之對照組處理，針對果實中對寒害最敏感的果皮維管束組織取樣，進行蛋白質體圖譜的比對分析。結果顯示受寒害低溫刺激，整體蛋白質體圖譜與逆境反應（包含對低溫、氧化逆境及乾旱等）、蛋白質生合成（Glutamate, methionine 或 cysteine）、轉譯、轉錄、運輸及訊息傳導相關的蛋白質皆有明顯增加，而醣解作用（Glycosis）相關蛋白質則有明顯減少（圖十六）。回溫後，參與碳水化合物及部份脂類代謝途徑、運輸、胞間傳遞、訊息傳導相關的蛋白質明顯增加，但與白胺酸（Leucine）、丙酮酸鹽（Pyruvate）、部份脂類及能量等代謝、蛋白質折疊、組合及分解等相關蛋白質則生成量顯著減少（圖十七）。經 1-MCP 處理者在醣解作用、碳水化合物代謝、蛋白質水解作用、胞吞作用（Endocytosis）相關之蛋白質明顯增加；而胺基酸及六碳醣代謝、粒腺體電子傳遞鏈、DNA 複製、蛋白質合成、標記、修飾及胞間傳遞相關者則顯著減少（圖十八），特別是回溫貯藏後（圖十九）。其中比較特別的是與細胞膜分解相關的蛋白質 9S-脂質氧化酶（9S-Lipoxygenase, 9S-LOX）在對照組之寒害溫度貯藏初期（1 小時 5 度 C）較 1-MCP 處理組有明顯偏高，顯示含較少量 9-LOX 的 1-MCP 處理可能與膜系統較少部份被氧化有關，而試驗三曾調查 1-MCP 處理的細胞膜的脂肪氧化程度（MDA assay），亦吻合此結果，推測乙烯可

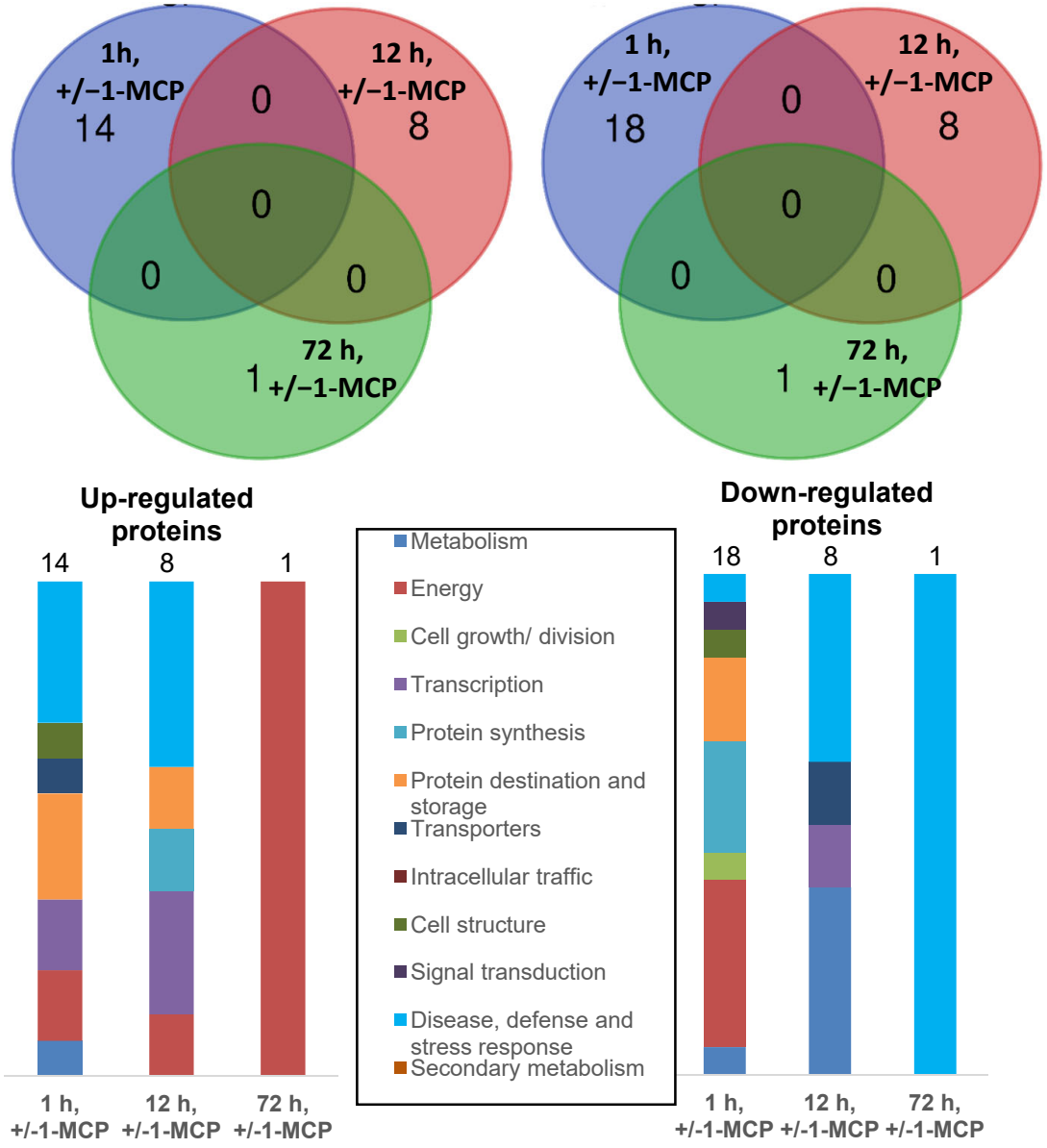
能以間接的方式，如正向調節 9S-LOX，以影響寒害下細胞膜的分解過程。



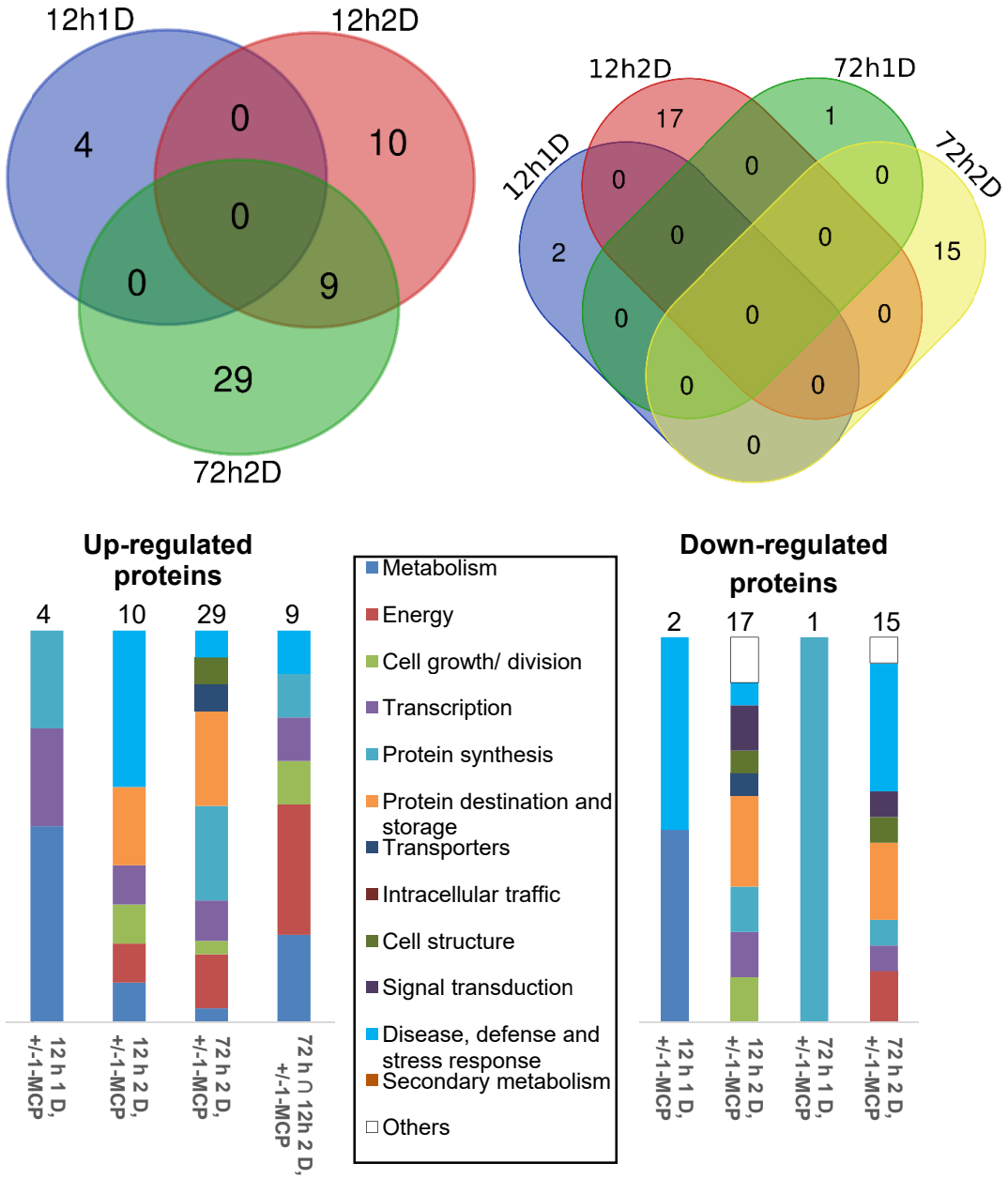
圖十六、綠熟香蕉處理純水的對照組（-1-MCP），於 5℃貯藏 1（1h）、12（12h）及 72 小時（72h）後，果皮維管束組織內累積已知蛋白質之功能性特徵分類情形。文氏圖內為經 1、12 及 72 小時低溫處理的果實，與處理前之果實（0hr）相比，蛋白質含量具顯著數量倍數表現差異（$p < 0.05$, fold change > 1.2 or < 0.8）之蛋白質種類數量。蛋白質之功能性分類係依 Bevan et al.（1998）所示。柱狀圖上所示之數字為各處理中具有明顯表現量變化之蛋白質數量。



圖十七、綠熟香蕉處理純水的對照組，於 5℃貯藏 12（12h）及 72 小時（72h）後回溫 1（1D）或 2 天（2D），果皮維管束組織內累積已知蛋白質之功能性特徵分類。文氏圖為經 1、12 及 72 小時低溫處理後回溫 1 或 2 天的果實，與處理前（0hr, -1-MCP）之果實相比，蛋白質含量具顯著數量倍數表現差異（$p < 0.05$, fold change > 1.2 or < 0.8）之蛋白質種類數量。蛋白質之功能性分類係依 Bevan et al.（1998）所示。柱狀圖上所示之數字為各處理中具有明顯表現量變化之蛋白質數量。



圖十八、綠熟香蕉處理純水的對照組（-1-MCP）與經安喜培浸泡（+1-MCP）處理組進行比較，於 5℃貯藏 1（1h）、12（12h）及 72 小時（72h）後，果皮維管束組織內具明顯表現差異的蛋白質功能性特徵分類情形。文氏圖內為對照組（-1-MCP）與經安喜培浸泡（+1-MCP）處理組進行比較其經 1、12 及 72 小時低溫處理的蛋白質體圖譜中，具顯著數量倍數表現差異（$p < 0.05$, fold change > 1.2 or < 0.8）之蛋白質種類數量。蛋白質之功能性分類係依 Bevan et al.（1998）所示。柱狀圖上所示之數字為各處理中具有明顯表現量變化之蛋白質數量。



圖十九、綠熟香蕉處理純水的對照組（-1-MCP）與經安喜培浸泡（+1-MCP）處理組進行比較，於 5℃貯藏 12（12h）及 72 小時（72h）後回溫 1（1D）或 2 天（2D），果皮維管束組織內具明顯表現差異的蛋白質功能性特徵分類情形。文氏圖內為對照組（-1-MCP）與經安喜培浸泡（+1-MCP）處理組進行比較其經 1、12 及 72 小時低溫處理的蛋白質體圖譜中，具顯著數量倍數表現差異（$p < 0.05$, fold change > 1.2 or < 0.8）之蛋白質種類數量。蛋白質之功能性分類係依 Bevan et al.（1998）所示。柱狀圖上所示之數字為各處理中具有明顯表現量變化之蛋白質數量。

六、熱處理對延緩香蕉寒害徵狀之研究進展

香蕉寒害徵狀初期先為果皮維管束褐變，接著果皮表皮細胞也開始出現褐斑，甚至整條香蕉呈現褐色，預試驗中即發現約維管束褐變程度達 20%以上，果表皮的褐斑才陸續出現。在前人研究中即發現果皮表面褐變與苯丙胺酸脫氨裂解酶(Phenylalanine ammonia lyase, PAL)及苯鄰二酚氧化酶(catechol oxidase, CO)在褐變過程中活性增加。在前述寒害初期過程，蛋白質體研究未見果皮維管束組織中，此二酵素的數量出現明顯變化，可能是較偏後才啟動之反應機制。為減緩寒害徵狀的發生，熱處理可激發作物的防禦反應，如以短時間（2 小時以下）中高溫（40-60℃）水溫進行溫湯處理，對於後續可預期的逆境具較加的反應，Promyou 等人（2008）即以 AAA 型的‘Gros Michel’及較耐寒的 ABB 型‘Namwa’香蕉各浸泡於 42℃溫湯 5、10 及 15 分鐘，並以浸泡 25℃常溫水作為對照，並轉貯至 4℃下觀察。結果顯示 AAA 型的‘Gros Michel’的對照組在 4℃下貯藏 2 天即出現褐斑，在第 10 天即幾乎全黑，較耐寒的 ABB 型‘Namwa’香蕉的對照組則在 4℃下貯藏 4 天後出現褐斑，但至多約 80%面積褐變。經 42℃溫湯 5 及 10 分鐘的兩品系，其寒害發生情形與對照組相仿，而溫湯處理 15 分鐘下的‘Gros Michel’則至第 6 天才出現褐斑、而‘Namwa’香蕉亦至第 6 天出現褐斑，且同時果皮組織不飽和脂肪酸對飽和脂肪酸的比例皆有上昇，顯示熱處理可減緩寒害發生。而經溫湯處理 15 分鐘之‘Gros Michel’與對照組相比具較低的脂肪氧化酶（Lipoxygenase, LOX）活性及較低的脂肪氧化情形，經基因表達分析，溫湯過的‘Gros Michel’香蕉其延後褐變的趨勢與苯鄰二酚氧化酶表達量呈正相關，而熱休克蛋白 70 (*Hsp 70*)的基因轉譯量亦在溫湯處理中有明顯增加，有趣的是同樣經溫湯處理 15 分鐘的‘Namwa’香蕉則未類似的反應，而兩品系皆共同的反應為不飽和脂肪酸對飽和脂肪酸的比例經溫湯處理後皆有增加，顯示較具耐寒害的香蕉品系可能是以不同機制提高其對低溫之耐受性，而 AAA 型香蕉則可能是由溫湯處理後提昇其脂肪氧化酶及熱休克蛋白等，亦有其餘作物文獻中發現溫湯會提昇其 ROS 消除的酵素系統，皆與降低細胞膜在寒害時受到破壞的不同機制有關。原計畫係針對試驗所作的 AAA 型‘Cavendish’香蕉進行測試，以 42℃溫湯處理 15、30 至 60 分鐘，以確認其對熱處理的極限及適用之條件，再轉至 4℃下貯藏，並調查其生理指標變化以釐清其經熱處理後對寒害之

耐性。由溫湯後轉低溫貯藏的果實處理中，選出適合處理進行深入調查；再納入經 1-MCP 處理後香蕉經熱處理後，對其低溫耐寒性之影響及果實後熟之變化（亦含香氣及蛋白質體圖譜等），以釐清熱處理過程是否與乙烯有關。惟因受疫情及時程不及影響，未完成此部份之研究。

七、結論

綜前述研究利用 1-MCP 釐清乙烯於綠熟香蕉寒害徵狀初期發展之作用，寒害所誘發的逆境乙烯確實參與綠熟香蕉初期可見但不可逆轉的寒害徵狀及一些不可見但可恢復的寒害反應。但不可逆轉的嚴重寒害徵狀，如細胞膜破損、果實生理反應受損（如無法正常後熟等）則無法確認其與乙烯的直接關連。綜上，在綠熟香蕉的寒害徵狀發展過程，乙烯推測僅影響部份之生理變化，但乙烯不是決定其發展方向的主要因子。熱處理經前人研究發現對香蕉確實有延緩寒害的作用，但亦無法完全抑制寒害徵狀發生，係與激發其自由基消除系統相關酵素有關，且外觀果皮表面之褐變與脂肪氧化酶（Lipoxygenase, LOX）及苯鄰二酚氧化酶有正向關連，未來可朝此方向探入討論延緩寒害徵狀發生之策略。

心得及建議

1. 在美期間，側面觀察實驗室運作經費主要從（1）官方來源，如州政府或跨州與各相關作物的研究人員合作的大型研究計畫，或是向美國聯邦政府轄下之農業部或國際組織（如 FAO 等）提出緊急產業缺口研究，或（2）產業界之私人公司（農企業，或生產、包裝資材等產業鏈的某一個單位）、或是生產者組織（如藍莓生產協會等），而各合作單位聯絡密切，會有定期簡單的口頭報告反饋，或（3）系上經費支應（但這部份不多）。校方定位除了研究外，亦有要求不同佔比的教學、推廣的業務，筆者的指導教授每年皆有達成產業推廣的目標任務，在校期間常看到業者來訪請教包裝或作物處理等問題，有時重要的部份可形成研究計畫，並由校方法務部門協助擬定合作契約。筆者研究經費係由業者贊助，合作過程業者特別重視實驗主要目的及產品「經濟」效益是否符合公司需求，實驗規劃除實驗室內部測試，配合包裝廠實際出貨的貯運試驗，會一再確認其有效程度，於不同品種、作物間的反應差異及是否符合成本效益進行調整，無效處理也會檢討可能的原因，未來試驗再依據試驗修正或調整方向。與業者合作，通常是由業者方提出需求，由老師建議方向或直接排實驗驗證，可有效解決實務方面的問題。產業與學界的合作十分順暢，研究也貼合業界實際需求。而不同的主要作物皆有一個由農民組成的協會，每年都有針對生產的作物進行討論，也會邀集栽培、採後的老師進行年度的生產檢討，而協會實際運作的經費也是由農民從生產利潤中撥出，並每年投入經費給學校針對目前育種需求、栽培或採後的問題研究開發，形成一個正向的循環進行產業發展。在臺灣也有類似的農民團體，但果樹類作物相較為多元且面積較不集中，除少數作物可形成品項團隊（文旦、鳳梨及蓮霧等），但仍以政府單位及學校為主要策劃者，農友規模較小或零散，若能形成農企業，可針對田間管理及實銷售端有效統籌並計畫生產，或許可以農友立場發揮更大的效能。

2. 農業人力的問題也在美國發生，由於美國農業生產者多由大農組成，所

屬面積多於數十公頃，並且常是跨州生產以分散產季壓力，為了能大量完成田間操作，大型農機及相對應的栽培模式也會配合研發而出，但園藝作物仍有大多工作無法以機械取代，如採收等。以佛州的柑橘生產為例，果園的作業規劃，如噴藥（包括無人機等）、灌溉系統由管理人員配合天氣資料調整施用時間，修剪也有大型機具進入田間修成特定形狀，但果實生長分佈在樹冠各區，採收仍需以人力協助，但美國本地勞工不足，美國政府開放短期外國農業移工以 H-2A 簽證，由公司依需求申請名額，並提供具體食宿規劃。據業者表示，移工管制及抽檢十分嚴格，需提供移工適合的生活環境，若為合住式移工宿舍，臥室需要至少以每人 4.6 平方公尺計，並且具備床或雙人床，及個人專用儲藏櫃，家具之間間隔需至少 91 公分；若每人單間房，睡眠、烹飪及居家空間需每人 9.2 平方公尺。房間需有加溫及冷氣設備，並有合適方便的供水（至少每天每人 35 加崙）及合適的洗浴空間（馬桶、洗衣間、洗手臺、淋浴設備等）並提供衛生紙等等（詳 https://www.dol.gov/agencies/whd/fact-sheets/26g-housing-standards-for-rental-and-public-accommodations-H-2A ），需依實呈報工作及住宿紀錄，若不合格可能會影響下一年度的申請資格。美國政府端對移工的福祉也逐年重視，包含在美工作期間各式保險費用也逐年要求，業者面也反應人工成本也逐年增加，可運作的利潤有限，可能會需要調整營運策略。與業者洽談時也提到「中南美洲黑工」，聽說幾乎是以不合理條約對待黑工的人員，近幾年美國政府查核的力度有越來越加大，可能數量有減少，但很多訊息也更不流通。整體產業對目前 AI 配合判斷的農機具的需求更加迫切，也希望出現更有效率執行智能判斷的農作補助機具。

圖二十、佛州柑橘採收仍以人工為主

3. 原僅在亞洲流通的蔬果在美國陸續增加種植面積。美國人口組成多元，亞裔（含東北亞、東南亞等）及印度裔人口數日漸增加，就 UF 而言，

研究生以中國人及印度人為最主要的外國學生組成，接下來即為中南美洲及北美等地學生。筆者求學期間，亞洲風味的食物及食材越來越常見，如豆腐、亞洲嗜好性蔬果的種類也越來越多，如苦瓜、絲瓜、長豇豆及竹筍等，或柿子、荔枝、龍眼等水果。佛州及加州皆有亞裔經營的農場專門生產亞洲蔬菜及水果，但目前仍主要僅鎖定亞裔客群，未有大規模生產，但近年來亦有大型通路商開始在各分店舖貨，如青江菜及大白菜等，且幾乎全年供應，可見市場接受度及供銷皆十分穩定。由於佛州地區除 1 月易有霜害風險外，其餘月份之氣候條件與臺灣十分相似，作物環境適應性可能也類似，但病蟲害相可能會較不同，未來在蔬果品種上也可望與美方合作更密切交流。

圖二十一、佛州生產的苦瓜（左圖，Chinese type）及水耕綠豆芽產品(右圖)

圖二十二、在大型量販店見到的富有柿、楊桃及番石榴(左)和包心白菜(右，圖內左上)

4. 佛州近年來以小型農場或有機農場為主開始發展簡易溫室栽培，或是以 high-tunnel 式種植蔬果，生產項目以高經濟作物如草莓、沙拉菜或少數精緻的食用花或擺盤用的 microgreens 為主。但由於量較少，因此在銷售端多與鄰近的高端餐廳業者直接合作並計畫生產，配合後端冷鏈作業，採收後降溫後出貨，才有良好的週轉率及生產品質。亦有業者說明部份種植的產品規格會直接由餐廳訂出，而對方甚至會針對食用風味及烹飪需求提出相關建議，業者也著手進行更符合餐廳需求的育種。臺灣目前育種方向以解決生產問題及大眾嗜好性趨向為主，但較小眾的特殊風味品系可能也是未來值得發展的一環。

圖二十一、High-tunnel 式溫室 (https://centralfloridaagnews.com/qa-high-tunnels/)

圖二十二、塑膠布溫室(側向僅有捲揚)
(https://centralfloridaagnews.com/qa-high-tunnels/)

圖二十三、Microgreen 生產溫室及已包裝好準備出貨的成品

附錄

Scientia Horticulturae 309 (2023) 111636

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Responses of 1-methylcyclopropene (1-MCP)−treated banana fruit to pre− and post−treatment ethylene exposure

Lan-Yen Chang [a,b,*], Jeffrey K. Brecht [a]

[a] Horticultural Sciences Department, University of Florida, 1219 Fifield Hall, Gainesville, FL 32611-0690, USA
[b] Tainan District Agricultural Research and Extension Station, Council of Agriculture, 70 Muchang, Tainan 71246, Taiwan

ARTICLE INFO

Keywords:
Ripeness stage
Climacteric respiration
Degreening
Asynchronized ripening

ABSTRACT

1-Methylcyclopropene (1-MCP), an ethylene receptor blocker, is mostly applied in gas form to inhibit ethylene action. An alternative immersion application method was used to investigate how pre- or post-1-MCP exposure to ethylene influences banana fruit response to 1-MCP. Mature-green (MG) banana fruit were immersed in 0, 10, 25 or 50 μg L^{-1} 1-MCP (a.i.) solution at 20 °C to determine the most effective concentration. The bananas were exposed to 0 or 100 μL L^{-1} ethylene for 24 h prior to (+/−preETH) or continuously following (+/−postETH) 1-MCP treatment to determine its effect on 1-MCP inhibition of ripening. Ripening of 1-MCP-treated fruit was delayed by up to 12 d at 20 °C, but that effect was reduced by circa 60% with ethylene pre-treatment. Recovery from 1-MCP in terms of ripening initiation occurred 4 to 6 d earlier in the presence of continuous post-treatment ethylene compared with air and the time to the fully ripe stage for 1-MCP plus ethylene fruit was 2 d shorter than for fruit without ethylene. Uneven recovery of fruit from 1-MCP was observed at stage 5, when the fruit had softer central pericarp and firmer external pericarp that adhered to the peel, but 'normal', uniformly soft pulp developed at stage 7 and later. Since post-treatment ethylene exposure accelerated the recovery of ripening after 1-MCP application in terms of earlier onset and accelerated rate of ripening, it could be suggested that ethylene may promote generation of new ethylene receptors.

1. Introduction

Ethylene is associated with many plant developmental processes including fruit ripening and tissue senescence (Able et al., 2002). Overcoming ethylene effects is an important strategy to extend post-harvest life by delaying the ripening of climacteric crops such as apple, avocado, banana, tomato, and others. The efficacy of the ethylene action inhibitor, 1-methylcyclopropene (1-MCP), which binds permanently to plant ethylene receptors (Blankenship and Dole, 2003; Golding et al., 1998; Sisler et al., 1996a), depends on the active concentration, treatment duration, temperature, and application method, along with the size and developmental stage of the crop (Blankenship and Dole, 2003; Choi and Huber, 2008; Golding et al., 1998; Harris et al., 2000; Satekge and Magwaza, 2021). The commercial use of 1-MCP to control ethylene action and thereby extend storage life is primarily being used for apples in conjunction with controlled atmosphere storage using 1-MCP in its gaseous form. Moreover, 1-MCP is registered for use on at least 24 different horticultural commodities in almost 30 countries (Sozzi and Beaudry, 2007).

Some 1-MCP-treated crops have been found to generally begin to regain ethylene sensitivity after 10 to 15 d (Sisler et al., 1996a; Sisler and Serek, 1997), which is in line with the previously determined half-life of ethylene receptors in plants (In et al., 2013; Tatsuki et al., 2009), leading to the suggestion that recovery of ethylene sensitivity and ripening following 1-MCP treatment may be related to synthesis of new, unblocked ethylene receptors (Blankenship and Dole, 2003; Watkins et al., 2000). Ethylene has been found to promote synthesis of ethylene receptors in Arabidopsis (Chen et al., 2007) and tomato (Kevany et al., 2007). However, whether the recovery from 1-MCP treatment when that treatment is followed by ethylene exposure can be ascribed to an earlier onset of ripening or an increased rate of ripening after recovery is not clear.

Banana (*Musa acuminata*, AAA group), one of the most important items in fruit trading worldwide, is an ethylene-sensitive climacteric fruit. Ripening of mature-green (MG) 'Dwarf Cavendish' banana fruit can be triggered by 0.015 − 0.5 μL L^{-1} ethylene (Marriott and Palmer, 1980) and the ripening rate induced by a 20 h ethylene incubation increased as the ethylene concentration was increased from 0 to 100 μL

* Corresponding author at: Tainan District Agricultural Research and Extension Station, Council of Agriculture, 70 Muchang, Tainan 71246, Taiwan.
E-mail address: shingsu@gmail.com (L.-Y. Chang).

https://doi.org/10.1016/j.scienta.2022.111636
Received 31 July 2022; Received in revised form 20 October 2022; Accepted 21 October 2022

L^{-1} (Kubo et al., 1993). Ripening initiation of MG bananas prior to ethylene application in special ripening rooms is unacceptable commercially.

Effective 1-MCP application procedures in terms of ethylene action inhibition for shelf-life extension as reported in previous studies varied from 10 nL L^{-1} 1-MCP fumigation at 24 °C for 24 h (Sisler et al., 1996a) to 450 μL L^{-1} 1-MCP fumigation at 20 °C for 6 h ('Cavendish' subgroup, cultivar 'Williams'; Golding et al., 1998). These treatments were shown to delay fruit color (Golding et al., 1998) and texture (Harris et al., 2000; Jiang et al., 1999a) changes and to suppress the onset of climacteric respiration, ethylene, and aroma volatile production (Golding et al., 1998). Use of much higher 1-MCP concentrations with banana has been reported to result in development of uneven color development during the resumption of ripening (Jiang et al., 2004) suggesting the need to evaluate recovery in order to properly determine the appropriate 1-MCP application procedure.

In contrast to the typical 12 to 24−h gaseous 1-MCP treatment in sealed chambers, a 1−min immersion in a higher concentration of aqueous 1-MCP solution has shown similar efficacy in tomato (Choi and Huber, 2008). It was suggested that immersion of 'Cavendish' banana fruit in 400 μg L^{-1} aqueous 1-MCP for 10 min was effective in prolonging the shelf-life by up to 30 d at 20 °C without noting any compromising effects (Rahman et al., 2013). Hagan et al. (2017) treated plantains with 100 μg L^{-1} aqueous 1-MCP for 1 min at ripeness stage 2−3 (i.e., ripening initiated). They found that the fruit shelf life was extended by 6 to 21 d without significantly affecting sensory quality.

In this study, the first objective was to determine an effective 1-MCP concentration for a 1−min aqueous application on MG banana. To test the hypothesis that recovery from 1-MCP may be accelerated by ethylene, the second objective was to examine the effect of exposure to a saturating dose of exogenous ethylene before or after 1-MCP treatment on the timing of the onset and the rate of recovery of ripening. The third objective was to evaluate the effect of 1-MCP immersion on the banana ripening process and compare it with the natural ripe fruit for evidence of irregular or asynchronous ripening, which has been reported to be a problem with gaseous 1-MCP treatment.

2. Materials and methods

Mature green banana fruit (*Musa sp.*, AAA group, 'Cavendish' subgroup, harvested as the angularity of the sides of the banana fruit became rounded) exported from Guatemala to Florida, USA by Chiquita Brands International and not treated with ethylene were obtained through a retailer in Florida immediately upon receipt at the retailer's local distribution center, about 4–5 days after harvest in Guatemala, with the bananas maintained continuously at 13.5 to 14.0 °C. Once received by the local store in Gainesville, the fruit were transferred to the Postharvest Laboratory, University of Florida, Gainesville. The development of banana hands proceeds from the proximal to the distal end and the flower primordia in each bunch develop from right to left in a 'zigzag' pattern (Fahn, 1953). Therefore, based on preliminary testing, the peel a* and hue angle values were measured on the most distal one third of the rightmost fingers of each hand (distal end pointing upward) and only bunches with a* lower than −14 and hue higher than 105° were used for the experiment. Single fruit (fingers) at stage 1.5 [between dark and light green based on a 1 (dark green) to 7 (yellow with brown spots) scale were used for all experiments, Supplementary Fig. 1; (von Loesecke, 1950)]. The fruit were selected for uniformity of size, color, and shape, and freedom from defects. The fingers were randomized and then washed and sanitized using 100 μL L^{-1} peroxyacetic acid for 3 min and allowed to air dry before further treatment.

2.1. Exp. 1 Determination of optimum aqueous 1-MCP concentration for delaying ripening and the effect of ethylene pre-treatment on response to 1-MCP

In a preliminary test, 0, 50, 100, 250, 500, and 1000 μg L^{-1} aqueous 1-MCP (a.i.; AF × RD-380, AgroFresh, Inc., Rohm and Haas, Philadelphia, PA) solutions were applied to 24 fingers of bananas per treatment by immersion for 60 s at 23 °C. In the first experiment that we conducted, fruit receiving different 1-MCP concentrations that had been air dried in the same area were all unable to ripen properly after 30 d at 20 °C (Supplementary Fig. 2). These results suggested the possibility of 1-MCP cross contamination during air drying and that the minimum effective 1-MCP concentration to delay ripening might be lower than 50 μg L^{-1}. Subsequently, +1-MCP and −1-MCP fruit were prepared and treated at different times and maintained in separate controlled temperature and humidity chambers to avoid the possibility of 1-MCP cross-contamination.

To determine the optimum 1-MCP dose along with effect of ethylene pre-treatment on banana fruit response to 1-MCP, a batch of graded fruit was divided into two groups (Exp. 1). Prior to 1-MCP treatment, half of the fruit were treated with 100 μL L^{-1} ethylene mixed in a continuous flow of air (20% oxygen, 0.05% carbon dioxide, and balance nitrogen) in a flow−through system for 24 h at 20 °C (+preETH) and the other half held in air at 20 °C (−preETH). Exposure of MG banana fruit to 100 μL L^{-1} ethylene can be expected to saturate the fruit response to ethylene (Inaba and Nakamura, 1986). The fruit from the +preETH and −preETH treatments were then each separated into four subgroups of 24 banana fingers each. The subgroups were then treated separately with 1-MCP to avoid cross−contamination, being immersed in 0, 10, 25, or 50 μg L^{-1} 1-MCP (a.i.) solution at 23 °C for 60 s (Choi and Huber, 2008; Hagan et al., 2017) followed by air drying for at least 3 h (until the time when 1-MCP was not detected in the storage chamber by GC measurement). The day that all the fruit had been treated with 1-MCP was designated as day 0 and the fruit were then all stored in controlled temperature and humidity chambers at 20 °C and 95% RH. Ripening features were evaluated every 1 or 2 d based on the rate of ripening in terms of measured respiration and ethylene production rates and objective color measurements; ripeness stage was also subjectively determined using the von Loesecke 1 to 7 rating system (von Loesecke, 1950).

2.2. Exp. 2. Recovery of 1-MCP-treated banana ripening in continuous air versus ethylene

Another experiment (Exp. 2) was conducted to determine the effect of ethylene post−treatment on banana fruit recovery from 1-MCP treatment. Bananas for Exp. 2 were handled according to the same preparation procedures described for Exp. 1. The fruit were divided into three groups of 30 fingers that were treated separately by being immersed in 0, 50, or 100 μg L^{-1} 1-MCP (a.i.) solution at 23 °C for 60 s followed by air drying. The day that all the fruit had been treated with 1-MCP was designated as day 0 and the fruit were then transferred to controlled temperature chambers at 20 °C and 95% RH. Half of the fruit were continuously exposed to 100 μL L^{-1} ethylene (+postETH) at 20 °C in a flowing system while the other half were held in ethylene−free air (−postETH) at 20 °C. The ethylene− and air−treated fruit were isolated from each other in separate flowing systems. The rates of respiration and ethylene production, and color changes were measured every other day until the fruit in a treatment reached ripeness stage 4 (approximately one-half ripe; more yellow than green); the fruit were then subjected to daily measurements and ripeness stage evaluations. Each sample that was held in 100 μL L^{-1} ethylene was transferred to an air−circulating environment for 4 h before measurement of respiration and ethylene to reduce the effect of exogenous ethylene residue on the measurements.

2.3. Respiration (CO_2 production) and ethylene production

Three replicates of three banana fingers from each treatment were placed in 1.75−L airtight glass containers with screw−on metal lids with holes that were fitted with rubber serum stoppers and the containers were sealed for 1 h at 20 °C. A 3.0 mL head space sample was withdrawn for CO_2 and ethylene measurements using a Varian CP-3800 gas chromatograph (Varian Inc., Walnut Creek, CA) equipped with a thermal conductivity detector (TCD) and a pulse discharge helium ionization detector (PDHID) as described in detail by Chang and Brecht (2020).

2.4. Ripeness stage assessment

Six randomly selected banana fingers within the same treatment were examined and their ripeness stage from 1 to 7 judged visually by the external skin color using the von Loesecke rating system (1950). The same six banana fingers were evaluated at each subsequent sampling time. Peel color was measured on two opposite sides at the equatorial point along the longitudinal axis of three fingers per treatment using a Minolta Chroma Meter (CR-400, Minolta Camera Co. Ltd., Japan) with an 8−mm aperture and D65 light source on the CIE L*, a*, b* color space in which L* represents the lightness from 0 (black) to 100 (white), a* represents green when negative and red when positive, and b* represents blue when negative and yellow when positive. The CIE color space parameters a* and b* were used to calculate the hue angle [h = arc tan (b*/a*)] and chroma [C = sqrt(a^{*2}+b^{*2})].

In the preliminary experiment that was previously described, treating MG bananas with the three highest concentrations of aqueous 1-MCP (i.e., 250, 500 and 1000 μg L^{-1}) resulted in mottled green and yellow bananas that remained hard in the external portion of the pericarp, but with translucent, apparently overripe tissue in the internal pericarp portion. Therefore, examination of the pericarp status of the 100 μg L^{-1} 1-MCP-treated fruit followed by 100 μL L^{-1} ethylene or not at stages 5−7 was included in Exp. 2 to determine if the ripening of the pericarp tissue proceeded normally or not.

2.5. Statistical analysis

Data were subjected to repeated measures analysis of variance (RM-ANOVA) using JMP statistical software (Version 8, SAS Institute, Cary, NC, USA). Fisher's least significant differences (LSD, $p \leq 0.05$) were determined and used to compare differences between treatment means following identification of a significant ANOVA F−value. The experiments reported were each conducted twice with similar results, but the data sets were not combined due to differences in absolute values.

3. Results

3.1. Exp. 1 Determination of the optimum aqueous 1-MCP concentration for delaying ripening and the effect of ethylene pre-treatment on response to 1-MCP

3.1.1. Ripeness stages

Control (−preETH) fruit immersed in 0, 10, 25, or 50 μg L^{-1} 1-MCP reached ripeness stage 7 (yellow with first appearance of brown flecks) after 27, 29, 37, and 39 d at 20 °C and 95% RH (Fig. 1). In contrast, the ripening of ethylene pre-treated green bananas (+preETH) was significantly less affected by 1-MCP, with those fruit reaching stage 7 on days 11 to 15, a circa 60% reduction of ripening inhibition (Fig. 1). 1-MCP concentrations of 25 and 50 μg L^{-1} delayed attainment of stage 7 in +preETH fruit by 4 d. The +preETH fruit treated with 50 μg L^{-1} 1-MCP remained longer in stages 5−6 (yellow with green tips to all yellow) compared with +preETH fruit treated with 25 μg L^{-1} 1-MCP. The two higher concentrations of 1-MCP for both −preETH and +preETH treatments showed delayed ripening.

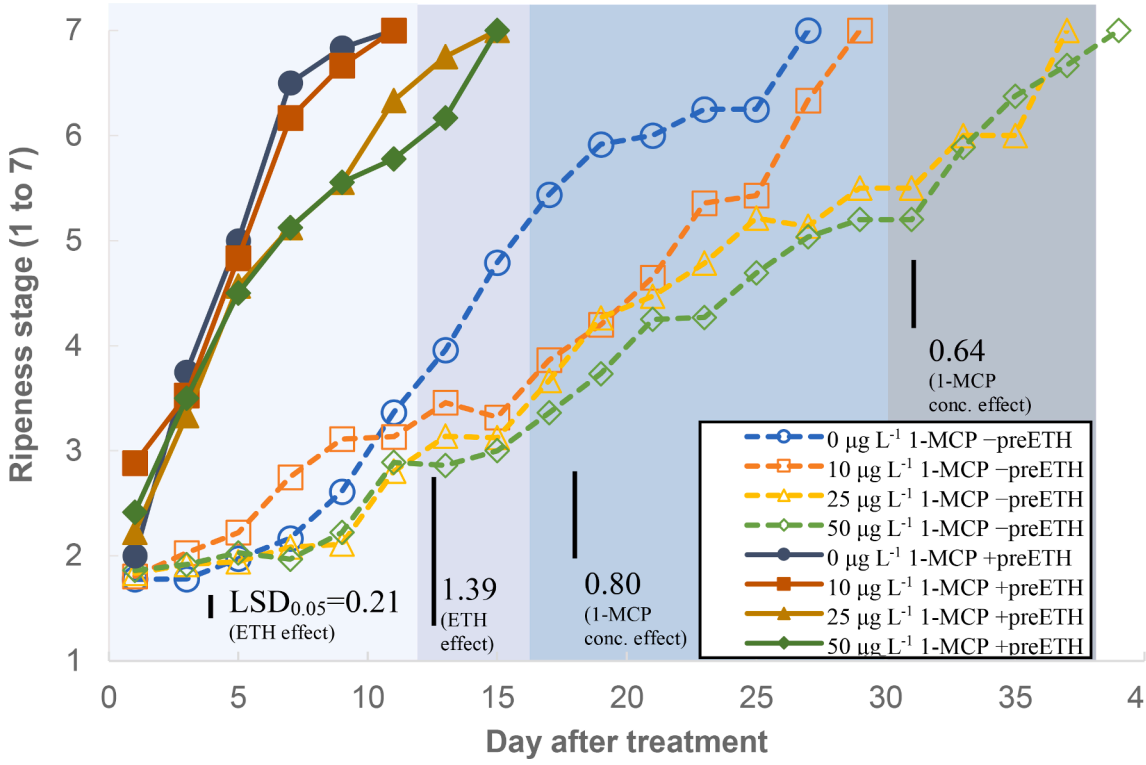


Fig. 1. Ripeness stage during storage at 20 °C and 95% RH for banana fruit initially treated with 0 (−preETH, open symbols with broken lines) or 100 μL L^{-1} (+preETH, solid symbols with solid lines) ethylene for 24 h (Day −1) at 20 °C followed by 0, 10, 25, or 50 μg L^{-1} aqueous 1-MCP (Day 0) (n = 6). Vertical bars are $LSD_{0.05}$ values which varied within different time frames marked as different shades (Days 0 to 11; Days 13 to 15; Days 17 to 29; Days 31 to 37) due to the various shelf-life of treatments.

3.1.2. Respiration (CO_2 production) and ethylene production rates

Respiration (Fig. 2) and ethylene (Fig. 3) production rates were suppressed by 1-MCP, especially for the fruit without ethylene pre-treatment (−preETH/+1-MCP). Initially, +preETH fruit had higher respiration (CO_2 production) rates, over 14 μg kg^{-1} s^{-1}, compared with −preETH fruit, which remained below 5 μg kg^{-1} s^{-1}. The climacteric peaks of respiration reached up to 22, 19, and 18 μg kg^{-1} s^{-1} on the 5th day for the 0, 10, and 25 μg L^{-1} +preETH/+1-MCP treatments, and 15 μg kg^{-1} s^{-1} on the 10th day for 50 μg L^{-1} +preETH/+1-MCP. The climacteric peaks for the −preETH treatments occurred after 15, 21, 30, and 31 d for 0, 10, 25, and 50 μg L^{-1} 1-MCP, respectively. The respiration rate reached up to 15 μg kg^{-1} s^{-1} for the 0 and 10 μg L^{-1} −preETH/+1-MCP fruit while it reached close to 12 μg kg^{-1} s^{-1} for the 25 and 50 μg L^{-1} −preETH/+1-MCP fruit.

Ethylene production rates of +preETH fruit were initially over 0.2 ng kg^{-1} s^{-1} while the rates for the −preETH fruit remained below 0.2 ng kg^{-1} s^{-1}. The peak of ethylene production was the same for all +preETH treatments, around 0.5 ng kg^{-1} s^{-1}, and occurred at around 13 to 15 d, while the −preETH fruit reached the ethylene climacteric peak on days 15, 17, 24, and 24 for 0, 10, 25, and 50 μg L^{-1} 1-MCP, respectively. However, the peak of ethylene production for all +preETH treatments might not be the actual climacteric peak, which should occur close to the climacteric peak of respiration. Besides, the respiration rate after 13−15 d showed another rise which was accompanied by the appearance of decay. The climacteric peak of ethylene production likely occurred during the early stages of the experiment and the following higher peak might be due to decay.

3.1.3. Peel color development

The +preETH bananas showed similar patterns of color development regardless of the 1-MCP concentration applied (Fig. 4). Lightness of the peel (Fig. 4a) increased from 60 to 70 on day 5 (at stage 5) then dropped back to 60. The −preETH fruit had relatively lower peel lightness and chroma (Fig. 4b) than the +preETH fruit. The peel lightness and chroma of −preETH/+1-MCP fruit rose slightly after day 9, was maintained until day 19, and then decreased until the end of the investigation, corresponding to the change from green to lighter yellow color and the later appearance of darker brown spots. The same characteristics of the −preETH/−1-MCP fruit remained the same during the first 23 d and declined afterward. The peel color change in terms of hue angle (Fig. 4c) in the −preETH treatments indicated a possible effect of 1-MCP dosage, showing the inhibition of green color loss. Other than in the +preETH treatments, in which the fruit turned from green to yellow faster than in −preETH, the relative rate of color change of fruit in the rest of the treatments corresponded to the 1-MCP concentration.

3.2. Exp. 2. Recovery of 1-MCP-treated banana ripening in continuous air versus ethylene

3.2.1. Ripeness stages

When the effects of 1-MCP treatment followed or not by continuous ethylene exposure were investigated, the −1-MCP/+postETH fruit began to ripen earliest, followed by the −1-MCP/−postETH fruit; the +1-MCP/+postETH fruit were next with the fruit from both 1-MCP concentration treatments ripening together, and lastly the +1-MCP/−postETH fruit ripened (both 1-MCP concentrations; Fig. 5). The −1-MCP/+postETH fruit reached stage 7 fastest, on day 7, and the −1-MCP/−postETH fruit did so on day 15. Fruit from the 50 and 100 μg L^{-1} +1-MCP/+postETH treatments also turned yellow starting from day 15, which was 4 to 6 d earlier than the +1-MCP/−postETH fruit. There was no obvious difference in ripening inhibition and recovery between fruit treated with 50 or 100 μg L^{-1} 1-MCP. However, fruit in the +1-MCP/+postETH treatment developed blotchy color during stages 4 to 5, remained at stage 5–6 (with green tips) past day 25 (Fig. 5), and failed to reach stage 7.

3.2.2. Rates of respiration (CO_2 production) and ethylene production

The onset of the climacteric rises in respiration and ethylene

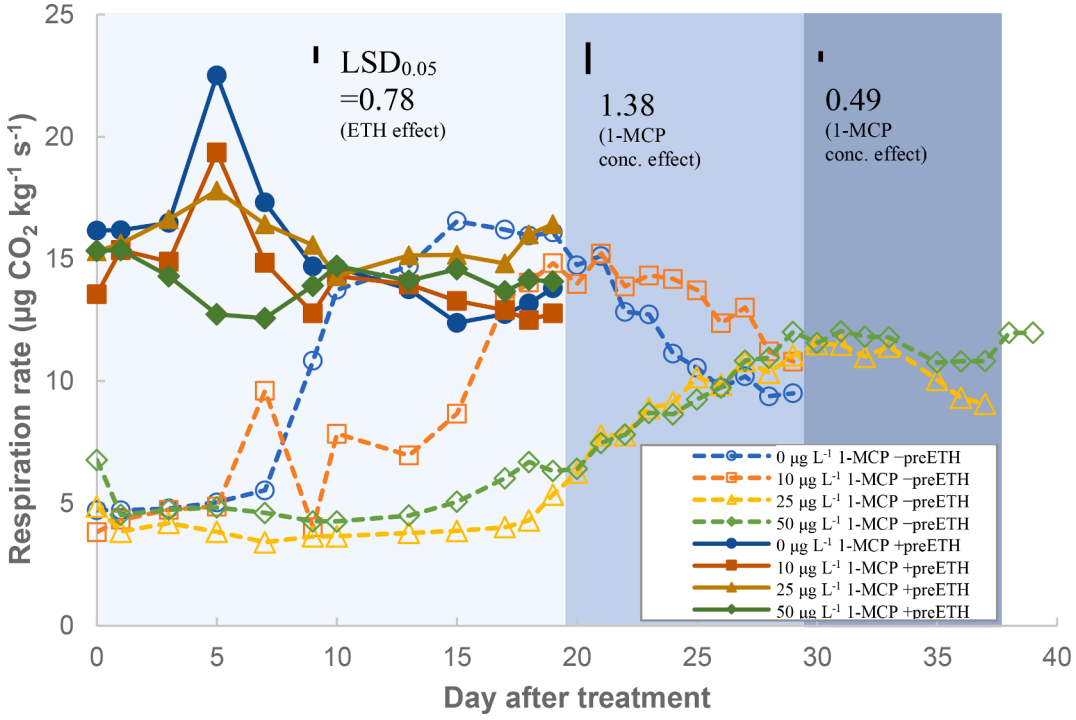


Fig. 2. Respiration (CO_2 production) during storage at 20 °C and 95% RH for banana fruit initially treated with 0 (−preETH, open symbols with broken lines) or 100 μL L^{-1} (+preETH, solid symbols with solid lines) ethylene pre-treatment for 24 h at 20 °C followed by 0, 10, 25, or 50 μg L^{-1} aqueous 1-MCP (Day 0) (n = 3, each replicate is a set of 3 fingers). Vertical bars are $LSD_{0.05}$ values which varied within different time frames marked as different shades (Days 0 to 19; Days 20 to 29; Days 30 to 37) due to the various shelf-life of treatments.

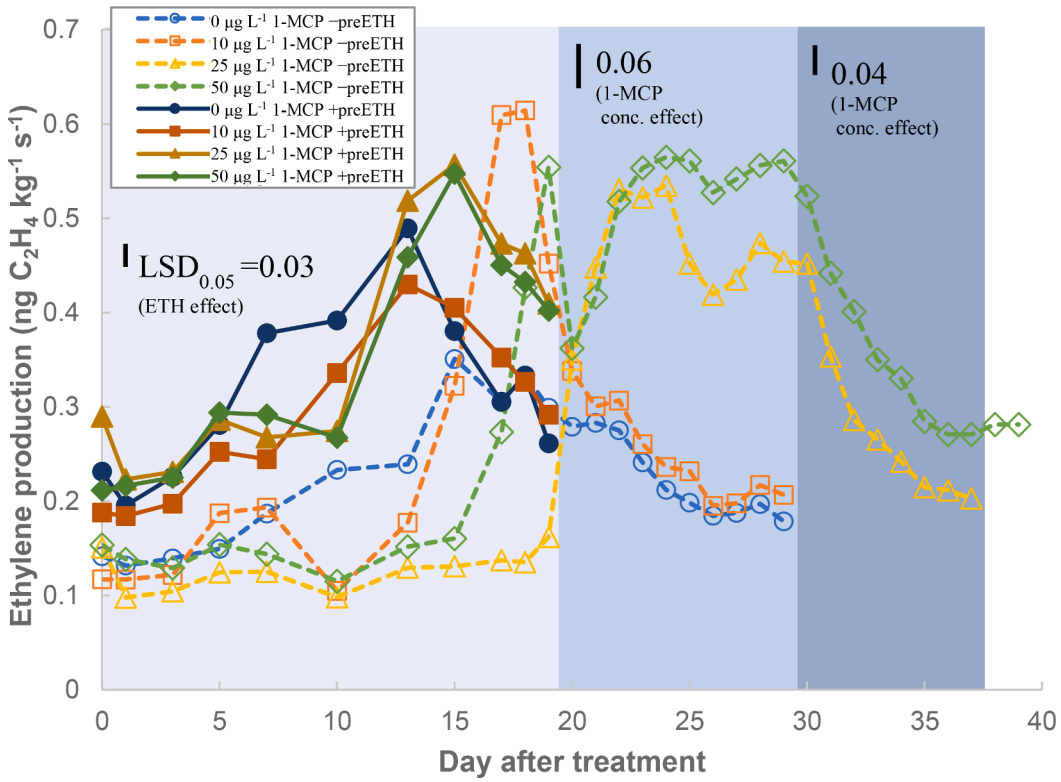


Fig. 3. Ethylene production during storage at 20 °C and 95% RH for banana fruit initially treated with 0 (−preETH, open symbols with broken lines) or 100 μL L^{-1} (+preETH, solid symbols with solid lines) ethylene for 24 h at 20 °C followed by 0, 10, 25, or 50 μg L^{-1} aqueous 1-MCP ($n = 3$, each replicate is a set of 3 fingers). Vertical bars are $LSD_{0.05}$ values which varied within different time frames marked as different shades (Days 0 to 19; Days 20 to 29; Days 30 to 37) due to the various shelf-life of treatments.

production were delayed by 1-MCP, whether the 1-MCP treatment was followed by continuous ethylene application or not (Figs. 6 and 7). Fruit without 1-MCP treatment that were exposed to 0 or 100 μL L^{-1} ethylene produced CO_2 at rates on day 1 of 4 and 9 μg kg^{-1} s^{-1}, respectively, which correspondingly increased to respiration peaks on day 11 without ethylene and day 3 with ethylene, both with peak rates of 17–18 μg kg^{-1} s^{-1}. For 1-MCP-treated fruit, the respiration rate remained low at around 2–4 μg kg^{-1} s^{-1} until day 15. The respiration rates of the +1-MCP/+postETH treatments increased faster than for the +1-MCP/−postETH treatments until reaching around 10–12 μg kg^{-1} s^{-1}, staying at that relatively constant level until the end of the investigation.

Fruit without 1-MCP but with ETH produced ethylene at 0.1 ng kg^{-1} s^{-1} on day 1, slightly increasing to 0.22 ng kg^{-1} s^{-1} on day 7, when the fruit were at stage 7. The ethylene production by the −1-MCP/−postETH treatment started from 0.03 ng kg^{-1} s^{-1} and rose to 0.5 ng kg^{-1} s^{-1} on day 15. 1-MCP-treated fruit produced around 0.03 ng kg^{-1} s^{-1} ethylene until day 15. Ethylene production for +1-MCP/+postETH treatments rose earlier and reached a peak of around 0.8 ng kg^{-1} s^{-1} on day 23–25. For the −postETH treatments, ethylene production started to increase from day 19 and reached around 0.65 ng kg^{-1} s^{-1} on day 25, which was slightly lower than that of +postETH fruit. The +postETH treatment accelerated the climacteric respiration and ethylene production without a significant 1-MCP concentration effect.

3.2.3. Peel color components

Treatment of bananas with 1-MCP regardless of post-treatment ethylene exposure delayed peel color development (Fig. 8). The peel lightness and chroma of −1-MCP/+postETH fruit showed sharp increases and reached their highest values on day 5 before declining. Color development of −1-MCP/−postETH fruit proceeded slowly and reached a peak around days 13 to 15. Color development of fruit from the +1-MCP/+postETH treatments started to sharply increase on days 17 and 15 for lightness and chroma, respectively. The peaks for lightness and chroma were reached on day 25 and days 21 to 25 for fruit from the 50 and 100 μg L^{-1} +1-MCP/+postETH treatments, respectively, and dropped over the last few days as the appearance of senescence spots increased on the peel. Fruit treated solely with 1-MCP (+1-MCP/−postETH) were the last to turn yellow. The lightness in peel color of fruit from the 50 μg L^{-1} +1-MCP/−postETH treatment began to turn on day 21 while the lightness of fruit receiving 100 μg L^{-1} 1-MCP/−postETH started to change on day 23. The chroma rose sharply on day 19 and day 23 for fruit that received 50 or 100 μg L^{-1} 1-MCP/−postETH, respectively, and peaked on day 27 and day 29, respectively. The hue angle (turning from green to yellow) in all treatments decreased throughout the investigation (Fig. 8c) and changed sharply on different days. Fruit from the −1-MCP/+postETH treatment turned on day 3, fruit from −1-MCP/−postETH turned on day 5, fruit receiving 50 or 100 μg L^{-1} +1-MCP/+postETH turned on day 17, fruit from 50 μg L^{-1} +1-MCP/−postETH turned on day 21, and fruit from 100 μg L^{-1} +1-MCP/−postETH turned on day 23. The results suggest that 1-MCP delayed the ripening-related peel color development of banana fruit and that ethylene application after 1-MCP accelerated the ripening process.

3.2.4. Ripening of the pericarp

As shown in Fig. 9a, −1-MCP fruit exhibited uniform ripening of the pericarp at stage 6. In contrast, fruit from the 100 μg L^{-1} +1-MCP/+postETH treatment developed translucency at the center of the fruit pericarp by stage 5 (Fig. 9b). When peeling the fruit without 1-MCP application, peel tissues were easily separated from the pericarp (Fig. 9c); but for +1-MCP/+postETH fruit, a layer of relatively firm pericarp tissue of about 2 mm thickness remained attached to the peel (Fig. 9d). The pericarp of fruit treated with the same 1-MCP concentration but −postETH had a similar translucent appearance. Although the +1-MCP fruit remained at stage 5 peel color longer than the −1-MCP

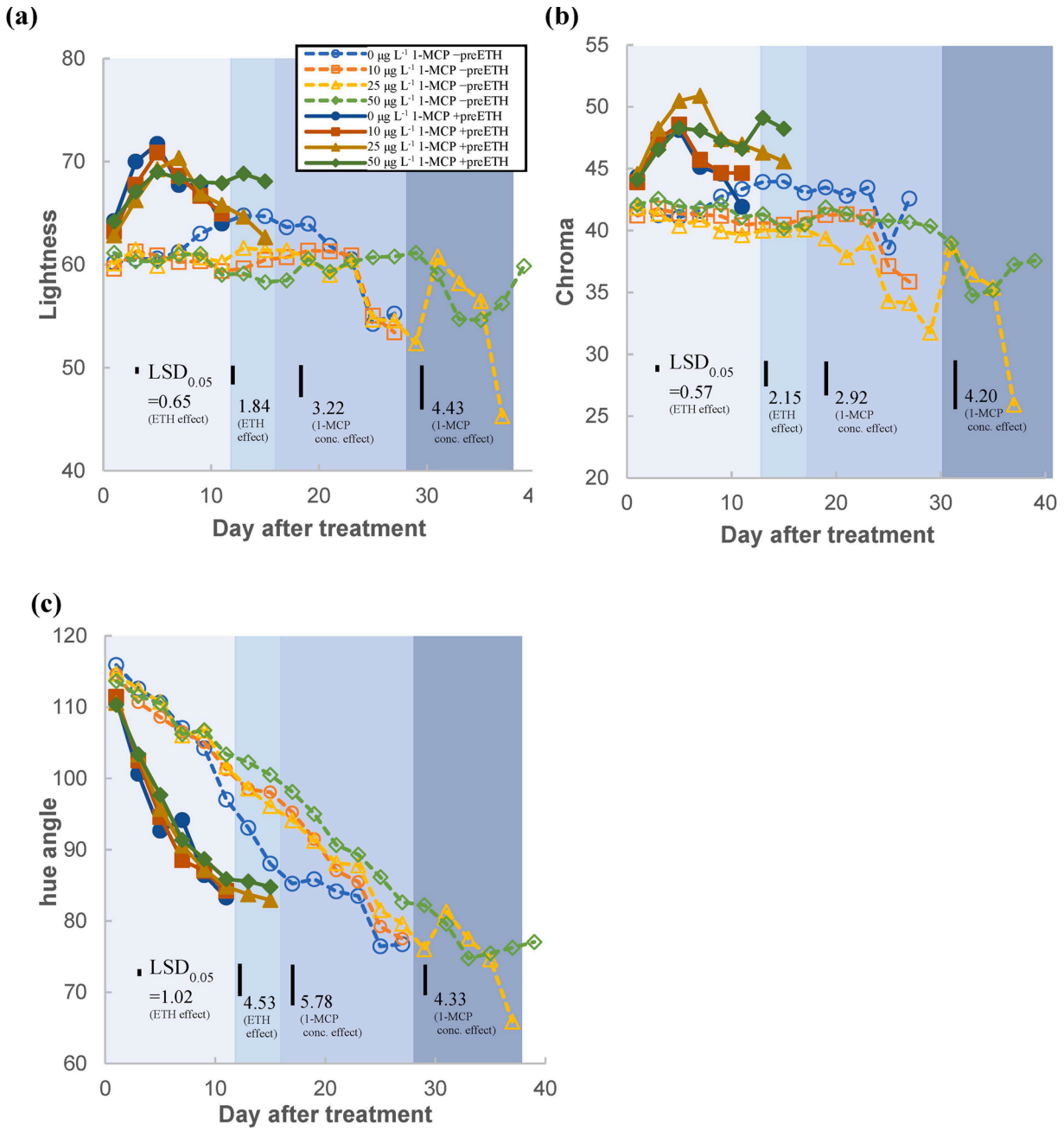


Fig. 4. Peel color response during storage at 20 °C and 95% RH in terms of lightness (a), chroma (b), and hue angle (c) for banana fruit initially treated with 0 (−preETH, open symbols with broken lines) or 100 μL L^{-1} (+preETH, solid symbols with solid lines) ethylene for 24 h at 20 °C followed by 0, 10, 25, or 50 μg L^{-1} aqueous 1-MCP (Day 0). ($n = 6$). Vertical bars are $LSD_{0.05}$ values which varied within different time frames marked as different shades (Days 0 to 11; Days 13 to 15; Days 17 to 27; Days 29 to 37) due to the various shelf-life of treatments.

fruit, the whole pericarp of the +1-MCP fruit was translucent at peel color stages 6 to 7 (Fig. 9f and h) like the over-ripe fruit without 1-MCP (Fig. 9e and g) at stage 7. This observation suggests that although the higher concentration of 1-MCP might have led to uneven ripening of the pericarp tissue during the early part of ripening (stage 5), the entire pericarp eventually still became translucent.

4. Discussion

In this study, aqueous 1-MCP immersion for 60 s was shown to be a time-efficient method that is comparable to gaseous application for 12–24 h in delaying the ripening of banana fruit. This is in agreement with previous reports for tomato (Choi and Huber, 2008) and banana (Rahman et al., 2014), respectively. With selection of an appropriate concentration, 1-MCP immersion is a promising add-on process in packinghouses to increase the green-life of bananas for export markets and to increase the through-put of the packing-line procedure.

The response of banana fruit to 1-MCP is known to be "concentration × exposure time"-dependent (Bagnato et al., 2003; Harris et al., 2000; Jiang et al., 1999a). In our study, MG banana fruit treated with 10 μg L^{-1} 1-MCP solution had similar responses as for fruit without 1-MCP treatment while the fruit immersed in 25 μg L^{-1} 1-MCP solution or higher showed obvious delays in the development of ripening parameters. The most effective concentration suggested in our study was lower than previously reported for banana fruit, such as 100 μg L^{-1} 1-MCP for 10 min immersion for 'Cavendish' (Rahman et al., 2013) or 100 μg L^{-1}

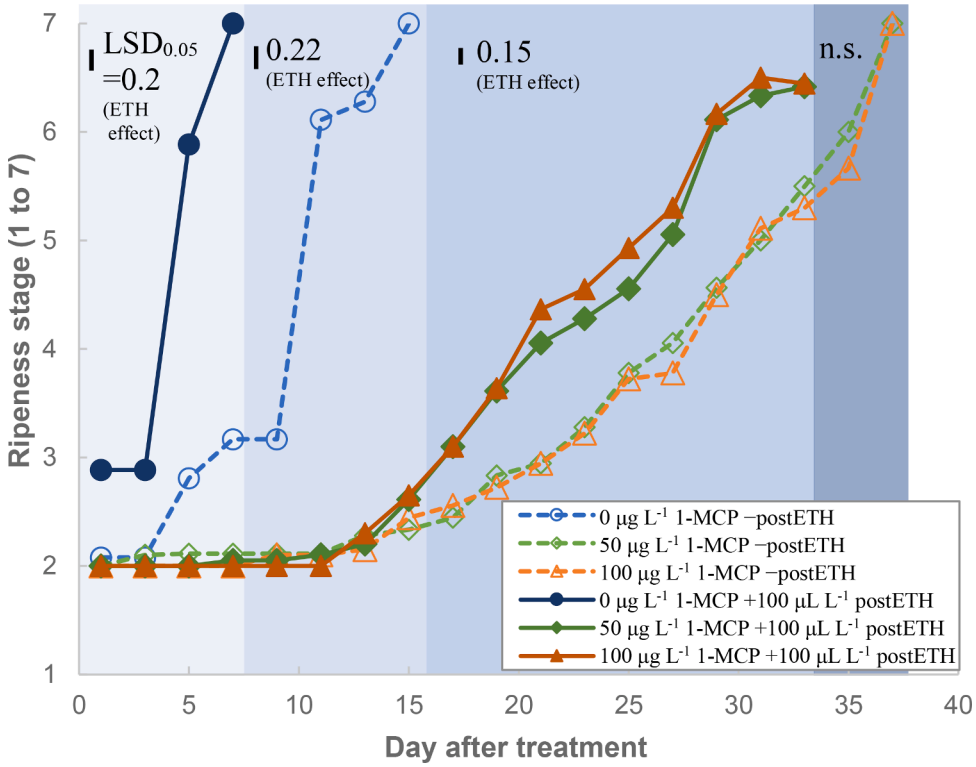


Fig. 5. Ripeness stages of banana fruit during storage at 20 °C and 95% RH after treatment with 0, 50, or 100 µg L^{-1} aqueous 1-MCP followed by 0 (−postETH, open symbols with broken lines) or 100 µL L^{-1} (+postETH, solid symbols with solid lines) ethylene ($n = 6$). Vertical bars are $LSD_{0.05}$ values which varied within different time frames marked as different shades (Days 0 to 7; Days 9 to 15; Days 17 to 33; Days 35 to 37) due to the various shelf-life of treatments. (n.s.= not significant).

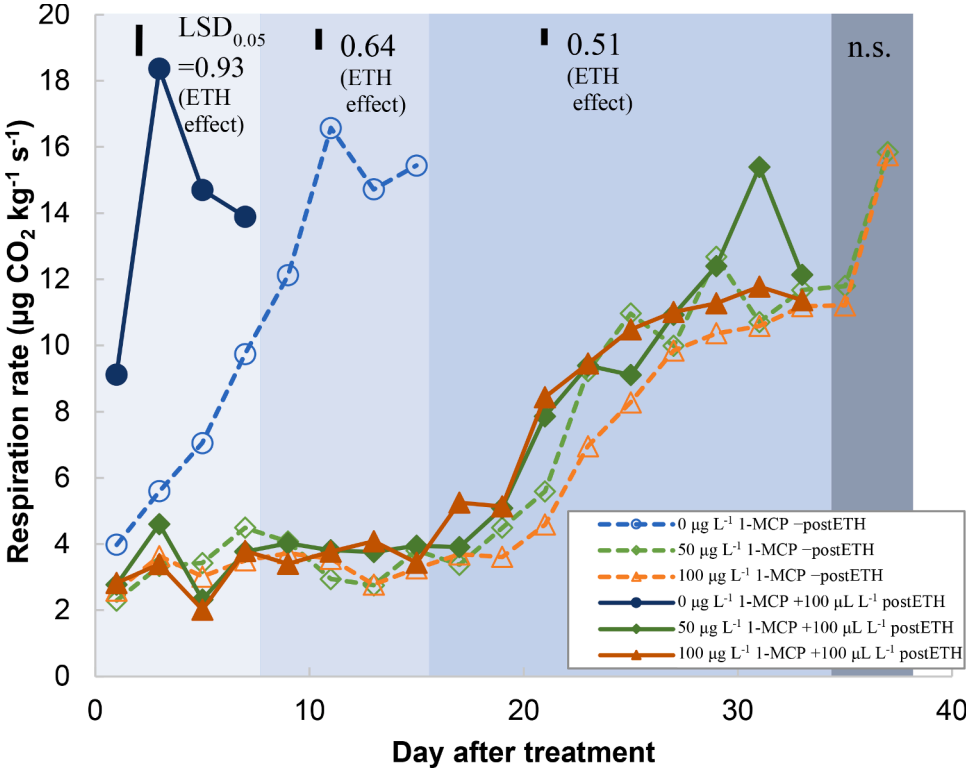


Fig. 6. Respiration (CO_2 production) rates of banana fruit during storage at 20 °C and 95% RH after treatment with 0, 50, or 100 µg L^{-1} aqueous 1-MCP followed by 0 (−postETH, open symbols with broken lines) or 100 µL L^{-1} (+postETH, solid symbols with solid lines) ethylene($n = 3$, each replicate is a set of 3 fingers). Vertical bars are $LSD_{0.05}$ values which varied within different time frames marked as different shades (Days 0 to 7; Days 9 to 15; Days 17 to 33; Days 35 to 37) due to the various shelf-life of treatments. (n.s.= not significant).

1-MCP for 5 min immersion for 'Gros Michel' (Rahman et al., 2014). The difference may be due to lack of testing of lower 1-MCP concentrations in the previous research as well as variation in experimental designs, including preharvest factors, banana variety, and packaging and storage methods. Higher aqueous 1-MCP concentrations and longer immersion times than the 25 µg L^{-1} for 60 s treatment reported here may not only

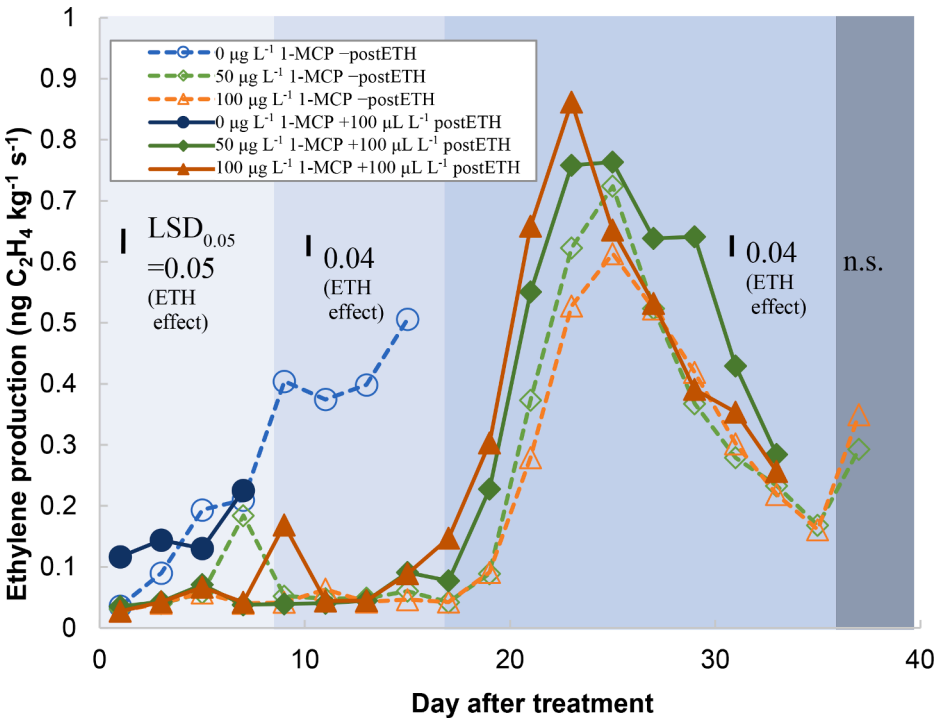


Fig. 7. Ethylene production rates of banana fruit during storage 20 °C and 95% RH after treatment with 0, 50, or 100 μg L^{-1} aqueous 1-MCP followed by 0 (−postETH, open symbols with broken lines) or 100 μL L^{-1} (+postETH, solid symbols with solid lines) ethylene (n = 3, each replicate is a set of 3 fingers). Vertical bars are $LSD_{0.05}$ values which varied within different time frames marked as different shades (Days 0 to 7; Days 9 to 15; Days 17 to 33; Days 35 to 37) due to the various shelf-life of treatments. (n.s.= not significant).

have stronger effects in terms of delaying onset of ripening but may also cause abnormal ripening recovery as observed in banana (Rahman et al., 2013, 2014) and in tomato (Choi and Huber, 2008).

In Exp. 2., The ripening inhibition responses to 50 and 100 μg L^{-1} 1-MCP application for 60 s without post-treatment ethylene were not significantly different, which suggests a possible saturation point of around 50 μg L^{-1} 1-MCP on banana fruit. A concentration-dependent effect of aqueous 1-MCP for the maximum ripening inhibition in MG tomato occurred at 400 μg L^{-1} for a 1 min immersion (Choi and Huber, 2008). In our study, 25–50 μg L^{-1} 1-MCP was shown to be an appropriate concentration to delay banana ripening with no problematic developmental defects such as blotchy peel color or translucent central pericarp tissue being observed during ripening recovery. For 100 μg L^{-1} 1-MCP plus post-treatment ethylene, abnormal ripening was observed in that the pulp softened and turned translucent from the center while the peel remained at stage 5 with no senescent spots forming.

Ethylene exposure before or after 1-MCP application showed different patterns of ripening and ripening recovery. To maximize ethylene action and ensure uniform ripening of a climacteric fruit like banana, the continuous presence of a saturating dose of ethylene is essential. In a previous study it was found that application of gaseous 1-MCP for 24 h after initiation of the climacteric using 500 μL L^{-1} propylene (equivalent to 5 μL L^{-1} ethylene) (Burg and Burg, 1967) had little effect on banana ripening (Golding et al., 1998). It was suggested that the ripening process could be considered irreversible after a 24 h ethylene or propylene treatment at 15–20 °C (Golding et al., 1998). The ethylene application in Exp. 1 involved exposure to 100 μL L^{-1} ethylene for 24 h, which initiated the climacteric. The ethylene treatment was then followed by 1-MCP treatment, and our results are in agreement with previous reports showing the effects of exogenous ethylene treatments in simultaneously promoting ripening and mitigating 1-MCP effects. In contrast, continuous exposure to 100 μL L^{-1} ethylene after applying 1-MCP modulated the inhibition of ethylene action by 1-MCP. In Exp. 2, the fruit treated with either 50 or 100 μg L^{-1} 1-MCP showed similar ripening suppression periods and post-treatment ethylene exposure reduced the time for ripening to recommence by 2–4 d.

The recovery of ethylene sensitivity and ethylene action following 1-MCP treatment has been proposed to be due to the regeneration of ethylene receptors or the renewed availability of ethylene receptors. However, Sisler et al. (1996b) determined using radiolabelled 1-MCP that its binding to ethylene receptors is irreversible. Ethylene induces a subset of receptor genes in Arabidopsis (Chen et al., 2007) and turnover of ethylene receptors triggered by ethylene binding was observed in Arabidopsis (Chen et al., 2007) and tomato (Kevany et al., 2007). However, when 1-MCP bound to ethylene receptors, the regeneration of receptor genes was reduced (Tatsuki and Endo, 2006), suggesting a role for ethylene action in ethylene receptor production. It was shown in broccoli (Able et al., 2002) and tomato (Sisler et al., 1996a) that, for maximum effectiveness, 1-MCP should be periodically re-applied. Commercial practice for 1-MCP treatment of apples in storage also includes periodic re-applications. If 1-MCP binding is irreversible, the need for periodic reapplication of 1-MCP implies that production of new, unbound receptors is involved in the recovery from 1-MCP treatment. Jiang et al. (1999b) showed that 1-MCP-treated bananas in polyethylene bags eventually ripened, and they suggested that regeneration of ethylene receptors was involved. In our study, continuous exposure to ethylene following 1-MCP treatment speeded up the recovery of the ripening process, which was also observed by others (Jiang et al., 1999a; Zhu et al., 2015). This suggests the potential use of ethylene to accelerate the regeneration of ethylene receptors to optimize full ripening recovery of 1-MCP-treated fruit.

Altered climacteric bursts of respiration and ethylene production have been observed in most 1-MCP-treated crops (Watkins, 2006). In this study, the climacteric respiration of 1-MCP-treated banana fruit was delayed and reduced in response to increasing 1-MCP concentrations, whether the fruit received post-treatment ethylene or not, which was previously reported for banana (Golding et al., 1998; Rahman et al., 2014; Zhu et al., 2015) and tomato (Choi and Huber, 2008). However, while the ethylene production of the 1-MCP-treated fruit was delayed, the peak was higher than for fruit without 1-MCP treatment, which is supported by other researchers (Golding et al., 1998; Pelayo et al., 2003; Zhu et al., 2015). Preclimacteric bananas have been shown to exhibit a kind of autoinhibition of ethylene production, producing less ethylene when treated with higher concentrations of ethylene or its analogues (e.g., propylene) to initiate climacteric ripening (Golding et al., 1998; Inaba et al., 1986; Vendrell and McGlasson, 1971). Atta-Aly et al. (2000)

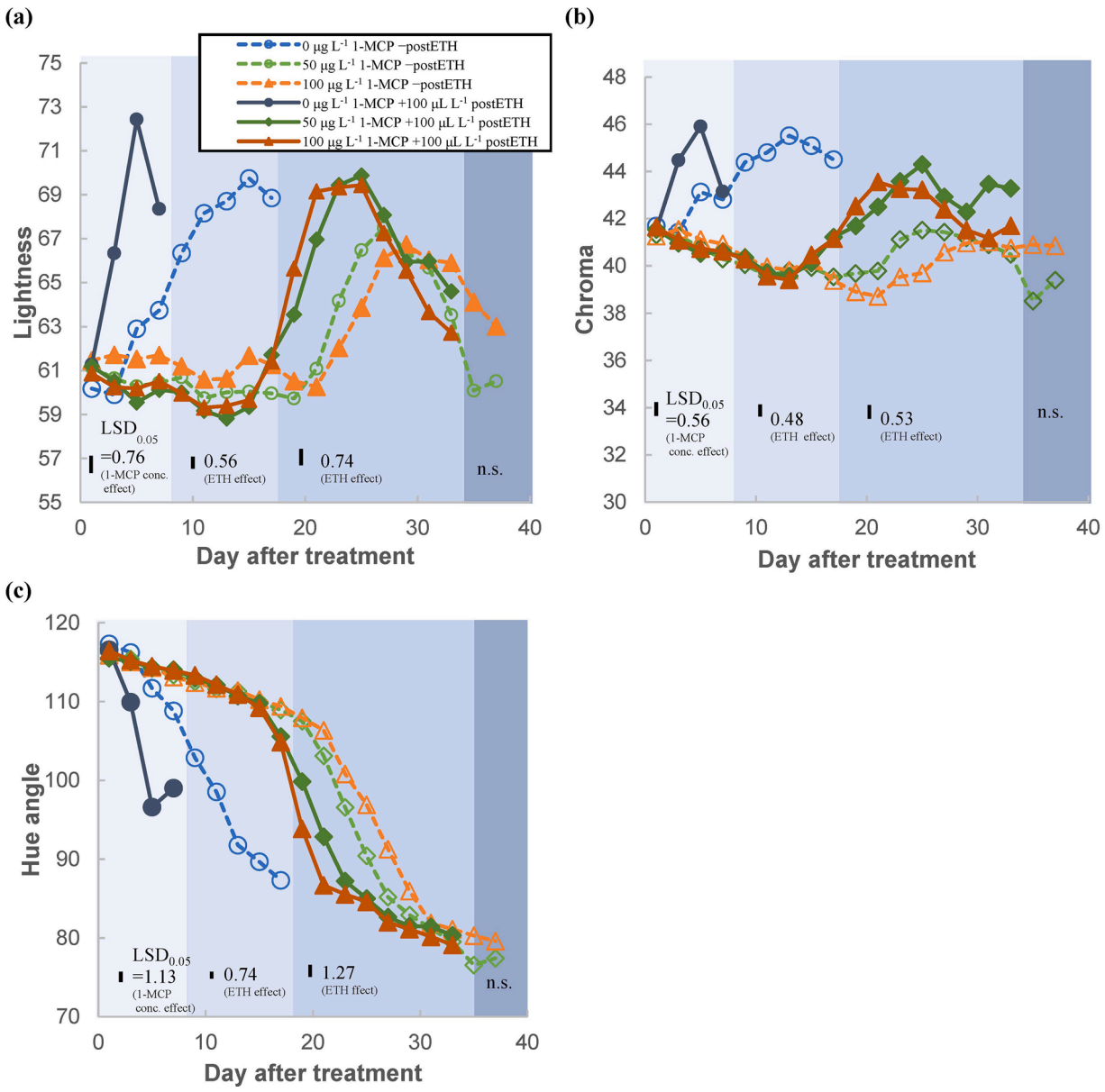


Fig. 8. Peel color changes of banana fruit during storage at 20 °C and 95% RH after treatment with 0, 50, or 100 μg L^{-1} aqueous 1-MCP followed by 0 (−postETH, open symbol with solid lines) or 100 μL L^{-1} (+postETH, solid symbol with solid lines) ethylene in terms of lightness (a), chroma (b), and hue angle (c)($n = 6$). Vertical bars are $LSD_{0.05}$ values which varied within different time frames marked as different shades (Days 0 to 7; Days 9 to 15; Days 17 to 33; Days 35 to 37) due to the various shelf-life of treatments. (n.s., not significant).

found that autoinhibition of ethylene production is the normal state in nonclimacteric strawberry fruit, but that there was a transition from autoinhibition to autocatalysis in climacteric tomato fruit during the transition from the immature green to mature green developmental stages. It was suggested that the normal autocatalytic feedback mechanism of ethylene biosynthesis upon exposure to exogenous ethylene was blocked in MG banana by exposure to 1-MCP (Golding et al., 1998). Inaba et al. (2007) found differential feedback regulation of ethylene biosynthesis in banana pulp and peel. 1-MCP-treatment of preclimacteric bananas followed by 500 μL L^{-1} propylene, or treatment of bananas with 1-MCP after the onset of ripening, resulted in higher ethylene production, higher content of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC), and ACC synthase activity, but lower ACC oxidase activity in the pericarp, while the opposite effect was observed in peel tissues (Inaba et al., 1986).

The banana peel comprises about 35% of the whole fruit weight (Vu et al., 2016) and the changes in the peel during ripening may be overshadowed by the biochemical changes in the greater amount of pericarp tissue. Therefore, the ethylene production rate could be increasing in 1-MCP-treated fruit without corresponding appearance of ripening-related changes in the peel. The gas composition of the internal tissues (data not shown) showed similar results with static measurement of whole fruit except for the exogenous ethylene application on 1-MCP-treated fruit. 1-MCP responsiveness has been shown to be strongly decreased or increased by treatments that increase or decrease internal ethylene concentration in apple (Watkins et al., 2000), avocado (Zhang et al., 2009), and tomato (Zhang et al., 2011). The results from Exp. 2 in our study showed that, during ripening recovery of 1-MCP-treated fruit, post-treatment ethylene application resulted in more ethylene production than for fruit receiving only 1-MCP treatment. This suggests that the ethylene treatment may have accelerated the production of new ethylene receptors and thus offset the inhibition of endogenous ethylene production associated with 1-MCP.

As ripening proceeds, chlorophyll breakdown and carotenoid

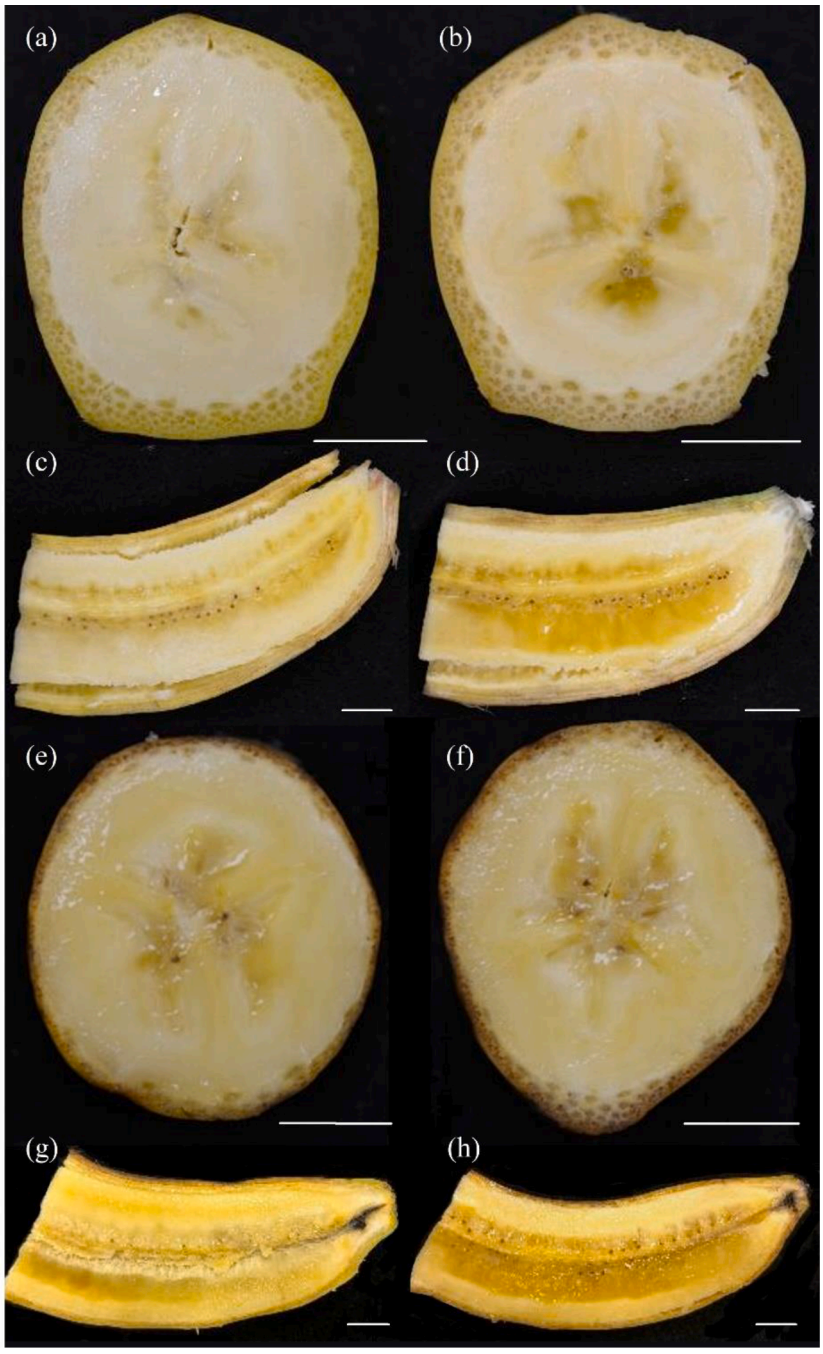


Fig. 9. Pericarp translucency during ripening and softening shown in cross section (a, b, e, f) and longitudinal section (c, d, g, h). (a, c) normally ripe banana (−postETH) on Day 13; (b, d) banana treated with 100 μg L^{-1} 1-MCP followed by 100 μL L^{-1} ethylene (+postETH) on Day 25 (unevenly ripe); (e, g) banana treated with 100 μg L^{-1} 1-MCP followed by 100 μL L^{-1} ethylene (+postETH) on Day 31 (overripe); (f, h) normally ripening banana (−postETH) on Day 19 (overripe). Horizontal bars equal to 1 cm.

biosynthesis occur in the banana peel, which turns from green to yellow (Kulkarni et al., 2011; Vu et al., 2019). Besides carotenoids, flavonoids can also contribute to yellow color of plants (Nishihara and Nakatsuka, 2011) and may also be involved in banana peel color development. 1-MCP treatment delays the ripening-associated color development of banana fruit as shown in previous reports (Golding et al., 1998; Zhu et al., 2015). Peel degreening is influenced by ethylene, which promotes chlorophyll degradation, unmasking pre-existing, stable carotenoids present in the peel (Gross et al., 1976). The values for lightness, b* and chroma of 1-MCP-treated fruit were lower than for fruit without 1-MCP treatment, indicative of darker, less yellow and less pure color for fruit treated with 1-MCP, and lighter, brighter and more pure yellow color for fruit without 1-MCP. While partial retention of green chlorophyll could be involved in the observed color difference between 1-MCP treated and untreated bananas, the process of turning yellow could also be affected by inhibition of ethylene perception. Phytoene synthase (PSY) is an important upstream enzyme for carotenoid biosynthesis that has been found to be involved in ethylene-induced ripening in tomato, for which 1-MCP application was shown to alter the mRNA abundance of PSY, which suppressed color development (Hoeberichts et al., 2002). It is possible that the carotenoid biosynthesis pathway in the banana peel was similarly altered by the inhibition of ethylene action via 1-MCP. Moreover, the apparent overdosing of 1-MCP in the 100 μg L^{-1} 1-MCP treatment, with ethylene or not, resulted in asynchronous color development within the same tissues such that green tips were retained on the fruit even while senescent spots were observed to develop (Supplementary Fig. 3).

Uneven coloration during ripening recovery has been addressed as a critical issue for 1-MCP protocol development (Golding et al., 1998; Harris et al., 2000). In our study, fruit with ethylene pre-treatment

followed by 1-MCP application didn't develop the blotchy appearance on the peel that was previously described as being a consequence of 1-MCP overdosing. The rest of the 1-MCP-treated fruit, whether followed by ethylene exposure or not, exhibited mottled yellow and green coloration during stages 4 - 5. There were also green tips retained on the fruit even when senescent spots appeared on fruit treated with 100 μg L^{-1} 1-MCP.

Abnormal softening may be induced by 1-MCP application, as has been observed in tomato (Hurr et al., 2005) and papaya (Manenoi et al., 2007) in which less mature fruit receiving excess 1-MCP ripened abnormally upon recovery. This abnormal ripening included incomplete softening. However, fruit treated at appropriate maturity with optimal 1-MCP concentration could recover from 1-MCP and soften normally (Dong et al., 2002; Hofman et al., 2001). Few observations have been reported of detailed anatomical aspects of this incomplete softening. In our study, fruit treated with 100 μg L^{-1} 1-MCP followed by ethylene treatment developed abnormal translucency in the core of the pericarp at stage 5 that was similar to the translucent appearance of over-ripe pericarp tissue; however, the pulp tissues recovered a normal soft texture at stage 7, similar to what was observed in control fruit.

It seems possible that the inner, translucent pericarp tissue may be the fruit endocarp and the outer, hard tissue that adhered to the peel (exocarp) may be the fruit mesocarp. Since there was no clear demarcation between endocarp and mesocarp tissue in normally softening fruit, this observation should be further investigated to determine if the symptoms of hard and soft, translucent tissues are limited to the mesocarp and endocarp, respectively.

Uneven banana peel color development and abnormal softening of the pericarp may result from possible excess 1-MCP sorption and desorption in the exocarp, which has been observed in fruit of the closely related plantain (Choi and Huber, 2009). 1-MCP sorption by asparagus spears and plantain fruit has been shown to be associated with high lignin concentrations (Choi and Huber, 2009). Starch, which is the major storage component in the pericarp of MG bananas, also exhibited low 1-MCP sorption (Choi and Huber, 2009). Thus, there is potentially a large amount of 1-MCP available for non-specific binding since it has been estimated that 1-MCP treatments typically involve a huge excess of 1-MCP molecules compared with calculated ethylene binding sites – on the order of 0.5 to 4.3 $\times$ 10^6 molecules of 1-MCP available per physiological binding site (Nanthachia et al., 2007). This could result in uneven distribution and potential reservoirs of 1-MCP within banana fruit. Once the tissues recover from 1-MCP treatment and generate new ethylene receptors, desorption of 1-MCP from non-specific binding sites in the banana exocarp might result in availability of free 1-MCP to bind to the new receptors. However, the simultaneous presence of exogenous ethylene during binding site regeneration could increase the chances of functional binding sites being present, favoring recovery of ripening as observed in the present study.

Climacteric ethylene production and presumably respiration as well starts in the pericarp tissues of banana (Vendrell and McGlasson, 1971) where there has been shown to be significant resistance to diffusion of CO_2 and O_2 (Perez and Beaudry, 1998). This might indicate that ethylene accumulates to some extent in the inner portion of the banana fruit pulp while the peel tissues may continue releasing 1-MCP from non-specific binding sites to the external portion of the fruit. This may explain why the pericarp develops an over-ripe-like core during ripening recovery following 1-MCP treatment, but with unripe or uneven coloration of the peel.

5. Conclusions

Aqueous 1-MCP application is comparable to the traditional gaseous application method in its effect on MG banana. After 1-MCP immersion, inhibition of ethylene action in MG banana results in delayed ripening. This includes delayed and attenuated climacteric respiration and ethylene production, and inhibition of color development and softening. For a 60-s exposure time, the minimum concentration of aqueous 1-MCP necessary to inhibit ripening while still allowing full recovery of normal ripening was 25 μg L^{-1}. Ethylene exposure for 24 h prior to treatment with 1-MCP largely overcame the ripening inhibition, while continuous ethylene exposure following 1-MCP treatment promoted earlier onset of climacteric changes and accelerated the recovery of ripening, which supports the suggestion that ethylene may promote generation of new ethylene receptors. From a practical standpoint, immersion treatment of bananas with 25 μg L^{-1} 1-MCP without ETH resulted in shelf life of 37 d at 20 °C versus about 15 days without 1-MCP or ETH, extending the time at the full yellow color (stage 6) while also inhibiting senescent spotting.

Funding

This research was supported by research funding from It's Fresh! Ltd.

Data availability statement

In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study.

CRediT authorship contribution statement

Lan-Yen Chang: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing – original draft. **Jeffrey K. Brecht:** Conceptualization, Methodology, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgments

We thank Dr. Donald J. Huber, University of Florida (Emeritus) for helpful discussions during the conceptualization and planning of this research and assistance with the aqueous 1-MCP application technique. We also thank Dr. Patrick E. Brecht, President, P.E.B. Commodities (formerly Corporate Director Quality Control, United Brands Co.), for useful descriptions of commercial banana postharvest handling practices. This work was supported by research funding from It's Fresh! Ltd.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scienta.2022.111636.

Reference

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 Biol. Technol. 26, 147–155. https://doi.org/10.1016/S0925-5214(02)00011-X.

Atta-Aly, M.A., Brecht, J.K., Huber, D.J., 2000. Ethylene feedback mechanisms in tomato and strawberry fruit tissues in relation to fruit ripening and climacteric patterns. Postharvest Biol. Technol. 20, 151–162. https://doi.org/10.1016/S0925-5214(00)00124-1.

Bagnato, N., Barret, R., Sedgley, M., Klieber, A., 2003. The effects on the quality of Cavendish bananas, which have been treated with ethylene, of exposure to 1-methylcyclopropene. Intl. J. Food Sci. Technol. 38, 745–750. https://doi.org/10.1046/j.1365-2621.2003.00726.x.

Blankenship, S.M., Dole, J.M., 2003. 1-Methylcyclopropene: a review. Postharvest Biol. Technol. 28, 1–25. https://doi.org/10.1016/S0925-5214(02)00246-6.

Burg, S.P., Burg, E.A., 1967. Molecular requirements for the biological activity of ethylene. Plant Physiol. 42, 144–152.

Chang, L.Y., Brecht, J.K., 2020. Variation among strawberry cultivars in bruising susceptibility related to wound ethylene production and sensitivity. HortScience 55, 444–448. https://doi.org/10.21273/HORTSCI14733-19.

Chen, Y.F., Shakeel, S.N., Bowers, J., Zhao, X.C., Etheridge, N., Schaller, G.E., 2007. Ligand-induced degradation of the ethylene receptor etr2 through a proteasome-dependent pathway in Arabidopsis. J. Biol. Chem. 282, 24752–24758. https://doi.org/10.1074/jbc.M704419200.

Choi, S.T., Huber, D.J., 2008. Influence of aqueous 1-methylcyclopropene concentration, immersion duration, and solution longevity on the postharvest ripening of breaker-turning tomato (*Solanum lycopersicum* L.) fruit. Postharvest Biol. Technol. 49, 147–154. https://doi.org/10.1016/j.postharvbio.2008.01.003.

Choi, S.T., Huber, D.J., 2009. Differential sorption of 1-methylcyclopropene to fruit and vegetable tissues, storage and cell wall polysaccharides, oils, and lignins. Postharvest Biol. Technol. 52, 62–70. https://doi.org/10.1016/j.postharvbio.2008.11.002.

Dong, L., Lurie, S., Zhou, H.W., 2002. Effect of 1-methylcyclopropene on ripening of 'Canino' apricots and 'Royal Zee' plums. Postharvest Biol. Technol. 24, 135–145. https://doi.org/10.1016/S0925-5214(01)00130-2.

Fahn, A., 1953. The origin of the banana inflorescence. Kew Bull. 8 (3), 299–306.

Golding, J.B., Shearer, D., Wyllie, S.G., McGlasson, W.B., 1998. Application of 1-MCP and propylene to identify ethylene-dependent ripening processes in mature banana fruit. Postharvest Biol. Technol. 14, 87–98. https://doi.org/10.1016/S0925-5214(98)00032-5.

Gross, J., Carmon, M., Lifshitz, A., Costes, C., 1976. Carotenoids of banana pulp, peel and leaves. Food Sci. Technol. 9, 211–214.

Hagan, L.L., Johnson, P.N.T., Sargent, S.A., Huber, D.J., Berry, A., 2017. 1-Methylcyclopropene treatment and storage condition delay the ripening of plantain fruit while maintaining sensory characteristics of ampesi, the boiled food product. Intl. Food Res. J. 24, 630–636.

Harris, D.R., Seberry, J.A., Wills, R.B.H., Spohr, L.J., 2000. Effect of fruit maturity on efficiency of 1-methylcyclopropene to delay the ripening of banana. Postharvest Biol. Technol. 20, 303–308. https://doi.org/10.1016/S0925-5214(00)00150-2.

Hoeberichts, F.A., Van Der Plas, L.H.W., Woltering, E.J., 2002. Ethylene perception is required for the expression of tomato ripening-related genes and associated physiological changes even at advanced stages of ripening. Postharvest Biol. Technol. 26, 125–133. https://doi.org/10.1016/S0925-5214(02)00012-1.

Hofman, P.J., Jobin-Decor, M., Meiburg, G.F., Macnish, A.J., Joyce, D.C., 2001. Ripening and quality responses of avocado, custard apple, mango and papaya fruit to 1-methylcyclopropene. Aust. J. Exp. Agric. 41, 567–572. https://doi.org/10.1071/EA00152.

Hurr, B.M., Huber, D.J., Lee, J.H., 2005. Differential responses in color changes and softening of 'Florida 47' tomato fruit treated at green and advanced ripening stages with the ethylene antagonist 1-methylcyclopropene. HortTechnology 15, 617–622.

In, B.C., Strable, J., Binder, B.M., Falbel, T.G., Patterson, S.E., 2013. Morphological and molecular characterization of ethylene binding inhibition in carnations. Postharvest Biol. Technol. 86, 272–279. https://doi.org/10.1016/j.postharvbio.2013.07.007.

Inaba, A., Nakamura, R., 1986. Effect of exogenous ethylene concentration and fruit temperature on the minimum treatment time necessary to induce ripening in banana fruit. J. Jpn. Soc. Hortic. Sci. 55, 348–354.

Inaba, A., Liu, X., Yokotani, N., Yamane, M., Lu, W., Nakano, R., Kubo, Y., 2007. Differential feedback regulation of ethylene biosynthesis in pulp and peel tissues of banana fruit. J. Exp. Bot. 58, 1047–1057. https://doi.org/10.1093/jxb/erl265.

Jiang, W., Zhang, M., He, J.H., Zhou, L., 2004. Regulation of 1-MCP-treated banana fruit quality by exogenous ethylene and temperature. Food Sci. Technol. Int. 10, 15–20. https://doi.org/10.1177/1082013204042189.

Jiang, Y., Joyce, D.C., Macnish, A.J., 1999a. Responses of banana fruit to treatment with 1-methylcyclopropene. Plant Growth Regul 28, 77–82. https://doi.org/10.1023/A:1006222631666.

Jiang, Y., Joyce, D.C., Macnish, A.J., 1999b. Extension of the shelf life of banana fruit by 1-methylcyclopropene in combination with polyethylene bags. Postharvest Biol. Technol. 6, 187–193. https://doi.org/10.1016/S0925-5214(99)00009-5.

Kevany, B.M., Tieman, D.M., Taylor, M.G., Dal Cin, V., Klee, H.J., 2007. Ethylene receptor degradation controls the timing of ripening in tomato fruit. Plant J. 51, 458–467. https://doi.org/10.1111/j.1365-313X.2007.03170.x.

Kubo, Y., Tsuji, H., Inaba, A., Nakamura, R., 1993. Effects of elevated CO_2 concentrations on the ripening in banana fruit by exogenous C_2H_4. J. Jpn. Soc. Hortic. Sci. 62, 451–455.

Kulkarni, S.G., Kudachikar, V.B., Keshava Prakash, M.N, 2011. Studies on physico-chemical changes during artificial ripening of banana (*Musa sp.*) variety 'Robusta. J. Food Sci. Technol. 48, 730–734. https://doi.org/10.1007/s13197-010-0133-y.

Manenoi, A., Bayogan, E.R.V., Thumdee, S., Paull, R.E., 2007. Utility of 1-methylcyclopropene as a papaya postharvest treatment. Postharvest Biol. Technol. 44, 55–62. https://doi.org/10.1016/j.postharvbio.2006.11.005.

Marriott, J., Palmer, J.K., 1980. Bananas - Physiology and biochemistry of storage and ripening for optimum quality. Crit. Rev. Food Sci. Nutr. 13, 41–88.

Nanthachai, N., Ratanachinakorn, B., Kosittrakun, M., Beaudry, R.M., 2007. Absorption of 1-MCP by fresh produce. Postharvest Biol. Technol. 43, 291–297. https://doi.org/10.1016/j.postharvbio.2006.10.003.

Nishihara, M., Nakatsuka, T., 2011. Genetic engineering of flavonoid pigments to modify flower color in floricultural plants. Biotechnol. Lett. 33, 433–441. https://doi.org/10.1007/s10529-010-0461-z.

Pelayo, C., Vilas-Boas, E.V., de, B., Benichou, M., Kader, A.A., 2003. Variability in responses of partially ripe bananas to 1-methylcyclopropene. Postharvest Biol. Technol. 28, 75–85. https://doi.org/10.1016/S0925-5214(02)00124-2.

Perez, R., Beaudry, R.M., 1998. Fractional surface coating modifies gas diffusion and ripening in bananas. J. Am. Soc. Hortic. Sci. 123, 115–118. https://doi.org/10.21273/JASHS.123.1.115.

Rahman, M.A., Hossain, M.A., Begum, M.M., Banu, S.P., Arfin, M.S., 2014. Evaluating the effects of 1-methylcyclopropene concentration and immersion duration on ripening and quality of banana fruit. J. Postharvest Technol. 2, 54–67.

Rahman, M.A., Miaruddin, M., Chowdhury, M.G.F., Begum, M.M., Islam, M.N., 2013. Influence of aqueous 1-methylcyclopropene on postharvest ripening and quality of banana. Int. J. Postharvest Technol. Innov. 3, 304–316.

Satekge, T.K., Magwaza, L.S., 2021. Investigating the effect of fruit size on ripening recovery of banana treated with 1-methylcyclopropene. Horticulturae 7, 357. https://doi.org/10.3390/horticulturae7100357.

Sisler, E.C., Serek, M., Dupille, E., 1996a. Comparison of cyclopropane, 1-methylcyclopropene, and 3,3-dimethylcyclopropene as ethylene antagonists in plants. Plant Growth Regul. 18, 169–174. https://doi.org/10.1007/BF00024378.

Sisler, E.C., Serek, M., 1997. Inhibitors of ethylene responses in plants at the receptor level: recent developments. Physiol. Plant 100, 577–582. https://doi.org/10.1111/j.1399-3054.1997.tb03063.x.

Sisler, E.C., Dupille, E., Serek, M., Smith, A.R., et al., 1996b. Effect of 1-methylcyclopropene and methylenecyclopropane on ethylene binding and ethylene action on cut carnations. Plant Hormone Signal Perception and Transduction. Springer, Dordrecht. https://doi.org/10.1007/978-94-009-0131-5_17.

Sozzi, G.O., Beaudry, R.M.Beaudry, 2007. Current perspectives on the use of 1-methylcyclopropene in tree fruit crops: an international survey. Stewart Postharvest Rev. 3 (8), 16. https://doi.org/10.2212/spr.2007.2.8.

Tatsuki, M., Endo, A., 2006. Analyses of expression patterns of ethylene receptor genes in apple (*Malus domestica* Borkh.) fruits treated with or without 1-methylcyclopropene (1-MCP). J. Jpn. Soc. Hortic. Sci. 75, 481–487. https://doi.org/10.2503/jjshs.75.481.

Tatsuki, M., Hayama, H., Nakamura, Y., 2009. Apple ethylene receptor protein concentrations are affected by ethylene, and differ in cultivars that have different storage life. Planta 230, 407–417. https://doi.org/10.1007/s00425-009-0953-z.

Vendrell, M., McGlasson, W.B., 1971. Inhibition of ethylene production in banana fruit tissue by ethylene treatment. Aust. J. Biol. Sci. 24, 885–895.

von Loesecke, H.W., 1950. Bananas, 2nd ed. Interscience, New York, pp. 52–66.

Vu, H.T., Scarlett, C.J., Vuong, Q.V., 2016. Optimization of ultrasound-assisted extraction conditions for recovery of phenolic compounds and antioxidant capacity from banana (*Musa cavendish*) peel. J. Food Process. Preserv. 41, e13148. https://doi.org/10.1111/jfpp.13148.

Vu, H.T., Scarlett, C.J., Vuong, Q.V., 2019. Changes of phytochemicals and antioxidant capacity of banana peel during the ripening process with and without ethylene treatment. Sci. Hortic. 253, 255–262. https://doi.org/10.1016/j.scienta.2019.04.043.

Watkins, C.B., 2006. The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. Biotechnol. Adv. 24, 389–409. https://doi.org/10.1016/j.biotechadv.2006.01.005.

Watkins, C.B., Nock, J.F., Whitaker, B.D., 2000. Responses of early, mid and late season apple cultivars to postharvest application of 1-methylcyclopropene (1-MCP) under air and controlled atmosphere storage conditions. Postharvest Biol. Technol. 19, 17–32. https://doi.org/10.1016/S0925-5214(00)00070-3.

Zhang, Z., Huber, D.J., Rao, J., 2011. Ripening delay of mid-climacteric avocado fruit in response to elevated doses of 1-methylcyclopropene and hypoxia-mediated reduction in internal ethylene concentration. Postharvest Biol. Technol. 60, 83–91. https://doi.org/10.1016/j.postharvbio.2011.01.001.

Zhang, Z., Huber, D.J., Hurr, B.M., Rao, J., 2009. Delay of tomato fruit ripening in response to 1-methylcyclopropene is influenced by internal ethylene levels. Postharvest Biol. Technol. 54, 1–8. https://doi.org/10.1016/j.postharvbio.2009.06.003.

Zhu, X., Lin, S., Fu, D., Si, Z., Wu, B., Chen, W., Li, X., 2015. Effect of the combination treatment of 1-MCP and ethylene on the ripening of harvested banana fruit. Postharvest Biol. Technol. 107, 23–32. https://doi.org/10.1016/j.postharvbio.2015.04.010.

frontiers | Frontiers in Plant Science

TYPE Original Research
PUBLISHED 15 September 2022
DOI 10.3389/fpls.2022.966789

OPEN ACCESS

EDITED BY
Isabel Lara,
Universitat de Lleida,
Spain

REVIEWED BY
Si Ma,
China Agricultural University,
China
Noureddine Benkeblia,
University of the West Indies, Mona,
Jamaica

*CORRESPONDENCE
Lan-Yen Chang
shingsu@gmail.com

SPECIALTY SECTION
This article was submitted to
Crop and Product Physiology,
a section of the journal
Frontiers in Plant Science

RECEIVED 11 June 2022
ACCEPTED 24 August 2022
PUBLISHED 15 September 2022

CITATION
Chang L-Y, Sargent SA, Kim J and
Brecht JK (2022) Delaying ripening using
1-MCP reveals chilling injury symptom
development at the putative chilling
threshold temperature for mature green
banana.
Front. Plant Sci. 13:966789.
doi: 10.3389/fpls.2022.966789

Delaying ripening using 1-MCP reveals chilling injury symptom development at the putative chilling threshold temperature for mature green banana

Lan-Yen Chang[1,2]*, Steven A. Sargent[2], Jeongim Kim[2] and Jeffrey K. Brecht[2]

[1]Division of Crop Improvement, Tainan District Agricultural Research and Extension Station, Tainan, Taiwan, [2]Horticultural Sciences Department, University of Florida, Gainesville, FL, United States

Storage at the putative chilling threshold temperature (CTT) to avoid chilling injury still limits postharvest handling of tropical fruit like banana in that ripening may occur at the CTT. To determine whether chilling injury (CI) symptoms would develop in mature green (MG) banana fruit if the CTT exposure was extended by inhibiting ethylene action and thus ripening, 1-methylcyclopropene (1-MCP) was applied. Individual 'fingers' from multiple 'clusters' of MG bananas were either immersed in water or 50 μg L^{-1} 1-MCP (a.i.) solution and each treatment was divided into three subgroups for storage at 5.0°C (severe CI), 13.0°C (mild CI), or 14.0°C (CTT) ± 0.1°C. 1-MCP delayed ripening in terms of color change for 10 days for fruit stored at the CTT. Ethylene production by fruit at 5.0°C remained around 0.04 ng kg^{-1} s^{-1} with no obvious increase during 31-day storage. Ethylene production at 14.0°C (–1-MCP/+1-MCP) increased on Day 33 while increasing on Day 38 for 13.0°C fruit without 1-MCP and on Day 39 for fruit with 1-MCP. Peak climacteric ethylene occurred on Days 44 and 39 for 13.0 and 14.0°C fruit without 1-MCP, respectively, and on Days 59 and 51 for 13.0°C and 14.0°C 1-MCP-treated fruit, respectively. As hypothesized, longer exposure of MG banana fruit to the CTT of 14.0°C without onset of ripening as was allowed by prior 1-MCP treatment allowed CI to develop at that normally non-chilling temperature. Vascular browning was the first visual and most sensitive CI symptom in the experiment and was observed on Day 4 at 5.0°C, Day 10 at 13.0°C, Day 19 at 14.0°C without 1-MCP, and on Day 28 at 14.0°C with 1-MCP. Using a 1-MCP pre-treatment to remove the influence of ethylene from bananas stored at 13°C or 14°C also resulted in slight reduction in vascular browning severity. In conclusion, a putative safe temperature may become a CI temperature if the shelf-life-limiting factor is removed, allowing longer exposure. Chilling at the CTT caused relatively mild injury on fruit, and vascular browning is a sensitive indicator of CI status, while the light-adapted quantum yield of photosystem II [Y(II)] could be a non-destructive indicator of early CI stress in MG banana. Fruit at 13.0/14.0°C developed CI symptoms slightly later with 1-MCP than without 1-MCP. This suggests that ethylene might be involved in early CI symptom development.

KEYWORDS

texture analysis, vascular browning, chlorophyll fluorescence, electrolyte efflux, shelf-life limiting factor

Introduction

Temperature management is essential to control crop metabolic activity and therefore maintain the quality of a commodity for the longest shelf-life before deterioration from senescence or decay occurs. However, most tropical crops, which are chilling sensitive, can be dramatically and negatively affected by low-temperature storage. Below some temperature limit, physiological damage, known as chilling injury (CI), occurs in plant tissues (Lyons, 1973). The temperature limit, or chilling threshold temperature (CTT) is a practical term within the produce industry for the lowest safe storage or transport temperature at which CI is never encountered under usual postharvest handling conditions. Therefore, for climacteric tropical fruit species, the major shelf-life limitation is CI at chilling temperatures and ripening at non-chilling temperatures. Banana (*Musa* spp., AAA group, Cavendish type), one of the most economically important horticultural crops worldwide, is a chilling-sensitive, climacteric tropical fruit requiring strict temperature control [recommended storage temperature range from 13.3 to 14.4°C (Thompson, 2011)] to maintain its commercial value. Classical CI symptoms of banana fruit include peel surface discoloration, subepidermal vascular browning, delayed or abnormal ripening, and sometimes failure to ripen, which are related to both temperature and exposure time. Underpeel discoloration, the industry term for vascular browning, is the earliest visible symptom of banana CI (Harvey, 2005), and reputedly can result from exposing mature-green (MG) banana to 1 h at 10°C, 5 h at 11.7°C, 24 h at 12.2°C, or 72 h at 12.8°C (P.E. Brecht, PEB Commodities, formerly Corp. Dir. Qual. Control, United Brands/Chiquita, personal communication). However, the alteration of membrane lipids is generally regarded as the first step of CI response in plant cells and precedes the appearance of visible CI symptoms (Marangoni et al., 1996).

Low temperature is perceived by plants as a kind of stress. When plant tissues are exposed to an injurious low temperature, the membrane conformation and structure are firstly affected, and parameters related to membrane permeability, such as electrolyte efflux (EE) and lipid oxidation, composition adjustment, or phase transition of membrane lipids, proceed as the severity of chilling increases (Lyons, 1973; Saltveit, 1991). However, the longer the duration of chilling exposure or the lower the temperature during exposure, the more severe the CI symptoms that were observed in lemon (*Citrus limon* L. cv. Eureka; Eaks, 1980), avocado (*Persea americana* cv. 'Hass'; Eaks, 1983), and banana (Trejo-Márquez and Vendrell, 2010). As CI and severe CI symptoms are irreversible, cells probably die in the extreme condition.

Ripening-related ethylene production by climacteric fruits like banana is triggered by maturation stage, controlled by specific silent genes (Yang and Hoffman, 1984; Manning et al., 2006; Lü et al., 2018). Ethylene production is also often regarded as being involved in the response of plants to various stresses, including chilling, freezing, high temperature, and mechanical injury. The ethylene produced by fruit tissues in response to chilling stress could play a role in the development of CI or CI symptoms. Some fruit such as zucchini (*Cucurbita pepo* L.) have been reported to produce increased amounts of ethylene as storage duration below the CTT is extended (Megías et al., 2016); some fruit produce a great amount of ethylene upon rewarming from the chilling temperature (e.g., cucumber, *Cucumis sativus* L.; Eaks and Morris, 1956; Andersen and Kent, 1982). For banana, Li et al. (2015) applied ethylene to mature-green (MG) banana fruit before exposure to chilling conditions and suggested that the ethylene alleviated CI symptom development. However, the ethylene application also accelerated the ripening process, which would have likely reduced CI sensitivity as shown for tomatoes (Chomchalow et al., 2002; Biswas et al., 2014), since banana fruit are extremely ethylene-sensitive. It has been reported that the ripening process in physiologically MG banana fruit can be triggered by 0.015 to 0.5 μL L^{-1} ethylene (Marriott and Palmer, 1980; Chang and Hwang, 1990).

1-Methylcyclopropene (1-MCP) is an ethylene action inhibitor that effectively binds irreversibly to ethylene receptors, preventing the signal transduction regulating ethylene action (Sisler et al., 1996a). It has been found that 1-MCP treatment inhibited the development of CI in avocado (Pesis et al., 2002), pineapple (*Anana comosus* L. Merr; Selvarajah et al., 2001), and plum (*Prunus salicina* L.; Candan et al., 2006); however, 1-MCP was reported to exacerbate CI symptoms in "Empire" apple (*Malus domestica* Borkh; Watkins, 2008). These results suggest that the response of different species of fruit to ethylene under chilling stress varies and that the mechanisms of ethylene action on CI and its development in various harvested fruit are relatively complex.

Since banana shelf life is limited by ripening at the CTT, application of ethylene antagonists such as 1-MCP could be a promising practice to delay the onset of the ripening process and thus extend the shelf life. However, the resulting extension of banana shelf-life at the CTT by 1-MCP application raises the possibility that CI could replace ripening as the shelf-life limiting factor. That is because the longer-than-normal exposure to the slightly higher temperature that would be made possible by 1-MCP treatment might allow CI to occur that is usually obviated by ripening.

The basis for conducting the research being reported here was related to the following two suppositions: first, that tropical climacteric fruit shelf life is limited by CI at chilling temperatures

and by ripening at non-chilling temperatures; second, that the ethylene produced by fruit tissues in response to chilling stress could play a role in the development of CI. We used 1-MCP treatment prior to storage of MG bananas at their CTT (i.e., their lowest non-chilling temperature of 14.0°C) or at a chilling temperature just below the CTT (i.e., 13.0°C) to explore those two suppositions. For comparison, we also stored bananas at the extreme chilling temperature of 5.0°C.

The objectives of this study were to determine the possible role of ethylene in the development of banana CI and/or CI symptom development by using 1-MCP to remove ethylene involvement, and to examine the potential chilling stress parameters that might be affected by ethylene.

Materials and methods

Sample preparation and treatments

MG banana fruit (*Musa* spp., AAA group Cavendish subgroup) without exogenous exposure to ethylene were obtained through a local retailer in Florida directly after receipt at the retailer's local distribution center, and about 4–5 days after harvest in Guatemala, with the bananas having been maintained at 13.5–14.0°C, depending on the season. Once received at a local store in Gainesville, the fruit were transferred to the Postharvest Laboratory, University of Florida, FL, United States. To reduce variation in maturity, clusters with $a^* < -14$ and hue angle (h^*) < 105 ° were selected from a group representing twice the number required for each experiment. Selected clusters (i.e., "hands") were divided into individual fruit (i.e., "fingers"), selecting only those at stage 2 (1–7 scale; von Loesecke, 1950; Supplementary Figure 1) and discarding fruit with nonuniform size and color, or with defects. The selected fingers were randomized and then washed and sanitized using 100 μL L^{-1} peroxyacetic acid for 3 min and allowed to air dry before further treatment. The experiment described here was conducted twice using fruit obtained and handled in this way with similar results and representative results are presented.

Preparation of aqueous 1-MCP solution was based on Choi and Huber (2008) and Hagan et al. (2017). Solutions were prepared with 1-MCP powder (AF × RD-380, AgroFresh, Inc., Rohm and Haas, Philadelphia, PA, United States) at 0 or 50 μg L^{-1} (active ingredient; a.i.), with the powder suspended in 10 L of distilled water in a 20 L plastic bucket by stirring for 1 min. Based on our preliminary measurements of 1-MCP release kinetics, we determined that loss of 1-MCP due to volatilization was minimal if the 1-MCP solution was used less than 45 min after preparation. Therefore, within 10 to 45 min following preparation, banana fingers for the two treatments were immersed in the solutions for 60 s at 23.0°C followed by air drying. The immersion and drying of the bananas for the two treatments were done separately to avoid 1-MCP cross-contamination. After drying, the control and 1-MCP bananas were stored overnight in separate rooms at 14.0°C and 95% relative humidity (RH).

On the following day, bananas treated with 0 or 50 μg L^{-1} aqueous 1-MCP (a.i.) solutions were then divided into three subgroups (51 fingers per subgroup plus an additional approximately 10% fruit for observations) and transferred to 5°C (severe CI), 13°C (mild CI), or 14°C (CTT; ± 0.1°C) and 95% RH (± 1%) in the darkness. The customized Conviron controlled temperature rooms (Controlled Environments Ltd., Winnipeg, Manitoba, Canada) that were used for these experiments maintained the setpoint temperature ± 0.1°C using a hot gas bypass system and continuous compressor and fan operation; the RH was maintained at ±1% by a dry fog system (ES100, Smart Fog Inc., Reno, NV, United States).

Ripeness stage

Three randomly selected banana fingers within the same treatment were selected and their ripeness stage, from 2 (light green) to 7 (yellow with brown spots), was judged visually by the external skin color using a standard banana color chart (von Loesecke, 1950; Supplementary Figure 1). The ripeness stages of the same three banana fingers per treatment were reevaluated at each subsequent sampling time.

Respiration rate and ethylene production measurement

Respiration and ethylene production were measured every 4 h during the first 3 d, then every day until Day 9, then every 2 d during Days 11–37, and back to daily until the last measurement (up to 61 days depending on the treatment). Three replicates of three banana fingers from each treatment were maintained in 1.75 L glass containers equipped with lids with rubber septa and were sealed for 1 h at 13°C or 14°C and 1.5 h for 5°C. A 3.0-mL headspace sample was withdrawn with a syringe for CO_2 and ethylene measurements using a Varian CP-3800 gas chromatograph (Varian Inc., Walnut Creek, CA, United States) equipped with a thermal conductivity detector (TCD) and a pulse discharge helium ionization detector (PDHID) as described in detail by Chang and Brecht (2020b). The carrier gas (helium) flow rate was 0.33 mL s^{-1}. The injector and columns were operated at 220°C and 50°C, respectively. The PDHID was operated at 120°C and the TCD was operated at 130°C.

Texture analysis

The texture of the peel and pulp of three banana fingers per treatment was determined by using a texture analyzer (TA.XT plus, Texture Technologies Corp. and Stable Micro Systems, Ltd., Hamilton, MA, United States) every 3 d beginning on Day 1, except for the last three evaluations, which were conducted on Days 43, 51, and 61. Samples were prepared after equilibrating to

room temperature (23°C). For peel texture analysis, banana peel from near the equatorial region was cut into two, 2.5 × 2.5 cm squares per finger. Banana peel shear resistance was evaluated by putting the epidermal side of the peel tissue downward and using a 50-kg load cell fitted with a 3-mm thick stainless steel flat-edge blade positioned parallel to the fiber direction. The peel was then sheared to a depth of 5 mm at a rate of 10 mm min^{-1} and the maximum load force was recorded and expressed in Newton (N). Pulp texture analysis was performed on two 5-cm-thick cross sections of pulp tissue sliced from whole fruit near the equatorial region. The measurement was performed using a 50-kg load cell fitted with an 8-mm stainless steel Magness–Taylor probe (convex tip), which was positioned at zero-force contact on the equatorial region of the sample, positioned horizontally on its side on a solid platform. Pulp slices were compressed to a depth of 10 mm at a rate of 10 mm min^{-1}. The maximum load force over the distance of the probe travel was recorded and expressed in Newton (N).

Evaluation of peel graying, vascular browning, and chlorophyll fluorescence

Chilling injury severity in banana fruit was scored visually on three banana fingers per treatment every 3 day beginning on Day 1, except for the last three evaluations, which were conducted on Days 43, 51, and 61. Peel graying was determined by estimating the percentage area of banana peel turning gray or grayish-brown. Vascular browning in the peel aerenchyma tissue was expressed as percentage area of browning after removing the peel epidermal layer from the middle portion (*ca.* 3 × 5 cm^2) of a finger using a potato peeler (Supplementary Figure 2).

Chlorophyll fluorescence was measured every half-day during the first 3 d, then every day until Day 9, then every 2 d until the last day at each treatment temperature (5 ± 0.1, 13 ± 0.1, or 14 ± 0.1°C). Photosystem II (PSII) function of chloroplasts was determined through the modulated chlorophyll fluorometer OS5p + (Opti-Science Ltd., Hudson, United States). For the dark-adapted test of maximum quantum yield (photochemical efficiency of PSII), the fruit were placed in continuous dark for at least 30 min and the equatorial region of samples was evaluated in the dark without light disruption while collecting data of Fv/Fm. For the light-adapted test on the quantum yield of PSII, fruit samples were exposed to illumination from a full spectrum LED light fixture (40 W, 5000 K, 4100 lumens, Sunco Lighting, United States) for at least 30 min and the equatorial region of the sample exposed to light evaluated using Y(II) mode.

Peel electrolyte efflux and malondialdehyde content

Fruit samples were evaluated every 6 days, except for the last three intervals at Days 43, 51, and 61. Electrolyte efflux (EE) of banana peel was evaluated using peel tissue disks ($n = 3$) excised from the equatorial region using a 9-mm diameter (No. 5) cork borer. Disks of each fruit peel sample were rinsed with distilled water followed by drying with paper towels and immersion in 10 mL of 0.7 M mannitol solution at 23.0°C for 4 h (Villalta and Sargent, 2004) with gentle shaking. The conductivity of the mannitol bathing solution after 4 h incubation was measured using a YSI-31A conductivity meter (Model 3,403, Yellow Springs, OH, USA). Total electrolyte content was determined after freezing the tissue samples at −20°C for at least 24 h. Frozen samples were thawed and boiled for 30 min and the conductivity of the bathing solution was re-measured. Electrolyte efflux was expressed as a percentage of total tissue electrolyte content.

Malondialdehyde (MDA) measurement was done as described by Huang et al. (2013). Frozen peel tissue (2.0 g) was homogenized in 10 mL of 100 g L^{-1} trichloroacetic acid and centrifuged at 10,000 × g_n for 20 min. A 1-mL aliquot of the resulting supernatant was collected and mixed with 3 mL of 5 g L^{-1} thiobarbituric acid. The mixture was boiled for 15 min, cooled in an ice bath, and centrifuged at 12,000 × g_n for 15 min. The clear supernatant was collected and used to measure absorbance at 450, 532, and 600 nm. The MDA concentration was calculated according to the formula: 6.453 × (A532 − A600) − 0.563 × A450. The concentration of MDA on a fresh weight basis was calculated as mmol kg^{-1}.

Statistical analysis

The experimental design was a two-factor repeated measurement completely randomized design. Data were subjected to repeated measures analysis of variance (RM-ANOVA) using JMP statistical software (Version 8, SAS Institute, Cary, NC, USA). Fisher's least significant differences (LSD, $p \leq 0.05$) were determined to compare differences between treatment means following identification of a significant ANOVA effect. The experiment reported was conducted two times and representative results presented.

Results

Ripening

The repeated experiments gave statistically similar results; therefore, only the data of the second experiment are presented here. Fruit stored at 5.0°C did not ripen during the 31-day storage period and the peel turned to dark brown after 16 days whether treated with 50 μg L^{-1} 1-MCP or not (data not shown). The fruit in the remaining treatments were maintained at stage 2–2.5 until at least Day 34 and only the greenness of the peel faded slowly and the h* decreased slightly from 115° to 110° during the first 30 days for the fruit stored at 13.0/14.0°C (color data not shown). Fruit without 1-MCP treatment (−1-MCP) stored at 13.0 or 14.0°C "turned" (appearance of yellow color; initiation of ripening) after 45 or 36 days, respectively (Table 1). The drastic decline of the h^*

corresponded to the ripeness stage and occurred first in the −1-MCP fruit at 14.0°C and then in the −1-MCP fruit at 13.0°C. Fruit treated with 1-MCP (+1-MCP) and stored at either 13.0 or 14.0°C started to ripen after 57.7 and 53 days, showing little difference in appearance. On the last day of the experiment (Day 61), fruit from the −1-MCP treatments stored at 13.0/14.0°C had reached color stage 6 or 7 while fruit from the +1-MCP treatments were still at color stage 3.5 or 4. Thus, the 1-MCP treatment delayed the ripening process significantly.

Respiration and ethylene production

Respiration (CO_2 production) and ethylene production exhibited similar patterns during storage. The initial respiration rate of the fruit at 14.0°C was 1.4 $\mu g\,kg^{-1}\,s^{-1}$. After transfer to the severe chilling treatment (5.0°C), respiration rate declined to 0.8–0.9 $\mu g\,kg^{-1}\,s^{-1}$ and remained constant (Table 2). Fruit stored at 13.0 or 14.0°C maintained a similar respiration rate about 1.28 or 1.35–1.41 $\mu g\,kg^{-1}\,s^{-1}$ for first 29 days. The first 3 days were intensively examined in order to detect irreversible CI development, but no significant stress-related respiratory burst was observed. The critical climacteric respiration characters are shown in Table 2. Fruit in the 14.0°C/−1-MCP treatment began firstly to increase respiration rate after 29 days, followed by fruit in the 13.0°C/−1-MCP treatment after 35 days. The increase in respiration rate for 1-MCP-treated fruit was delayed. Fruit at 14.0°C/+1-MCP were observed to commence the respiratory climacteric after 48 days while at 13.0°C/+1-MCP the respiration rate began to rise on Day 54. The peak climacteric respiration rate was also affected, reaching 8.85 $\mu g\,kg^{-1}\,s^{-1}$ on Day 43 for the 14.0°C/−1-MCP fruit, 7.0 $\mu g\,kg^{-1}\,s^{-1}$ around Day 51 for the 13.0°C/−1-MCP fruit, 6.29 $\mu g\,kg^{-1}\,s^{-1}$ after 58 days for the 14.0°C/+1-MCP fruit, and 6.0 $\mu g\,kg^{-1}\,s^{-1}$ on Day 56 for the 13.0°C/+1-MCP fruit.

In terms of ethylene production, all fruit produced around 0.04 $ng\,kg^{-1}\,s^{-1}$ before being initially transferred from 14.0°C to the different treatment temperatures (Table 3). The ethylene production rate of fruit stored at 13.0°C/14.0°C − 1-MCP began to increase after 37 days, following by 14.0°C/+1-MCP (42 days) and 13.0°C/+1-MCP (46 days). The climacteric peak of ethylene production was 0.16–0.23 $ng\,kg^{-1}\,s^{-1}$ on around Day 53–55 for fruit in the 13.0°C, 14. 0°C/−1-MCP, and 14.0°C/+1-MCP treatments, and on Day 58 for the 13.0°C/+1-MCP treatment.

Textural analysis of banana peel and pulp

The textural changes in the banana peel and pulp were asynchronous. Resistance of banana peel to shear force for the

TABLE 1 Changes in ripeness stage of banana fruit that were initially treated with 0 (−1-MCP) or 50 $\mu L\,L^{-1}$ (+1-MCP) aqueous 1-MCP for 60s at 23.0°C and then transferred to 5.0°C, 13.0°C or 14.0°C storage with 95% RH (n=3).

Storage temperature (°C)	Treatment	Initial ripeness stage	Average days to initiate the ripening process (obvious color changes)	Average color stage at the end of the experiment (Day 61)*
5. 0	−1-MCP	2.17a**	–*	–
	+1-MCP	2.17a	–*	–
13.0	−1-MCP	2.23a	45c	5.6b (Green tip to fully yellow)
	+1-MCP	2.23a	57.7a	4c (Yellow more than green)
14.0	−1-MCP	2.17a	36d	7a (Fully yellow with brown spots)
	+1-MCP	2.17a	53b	3.5c (Green more than yellow to yellow more than green)

*Banana fruit stored at 5.0°C turned brown and were discarded after 31 days. Before then, there were no ripening characteristics observed.
**Different letters in the same column indicate significant differences by treatments.

TABLE 2 Changes in respiration rate (CO_2 production) of banana fruit that were initially treated with 0 (−1-MCP) or 50 $\mu L\,L^{-1}$ (+1-MCP) aqueous 1-MCP for 60s at 23.0°C and then transferred to 5.0°C, 13.0°C or 14.0°C storage with 95% RH (n=3).

Storage temperature (°C)	Treatment	Average respiration rate ($\mu g\,kg^{-1}\,s^{-1}$) during the first 3 days	Days to the climacteric rise of respiration	Days to the climacteric peak of respiration	Average respiration rate ($\mu g\,kg^{-1}\,s^{-1}$) at the climacteric peak	Average respiration rate ($\mu g\,kg^{-1}\,s^{-1}$) at the end of the experiment (day 31 at 5°C; day 61 at 13/14°C)*
5. 0	−1-MCP	0.85d**	–*	–*	–*	1.55
	+1-MCP	0.91d	–*	–*	–*	1.89
13.0	−1-MCP	1.28c	35.7c	51.3c	7.01b	6.49a
	+1-MCP	1.28c	54a	60.8a	7.49ab	7.22a
14.0	−1-MCP	1.35b	29.7d	43.7d	8.85a	6.99a
	+1-MCP	1.41a	48b	58.5b	6.29b	4.64b

*Banana fruit stored at 5.0°C turned brown and were discarded after 31 days. Before then, there were no ripening characteristics observed.
**Different letters in the same column indicate significant differences by treatments.

fruit stored at 5.0°C remained within the range of 100 to 120 N during the entire 31-day storage period while the peel shear resistance of the fruit at 13.0/14.0°C increased from 100 to 140 N over the same period (Table 4). After 34 days, the peel shear resistance for the −1-MCP treatments stored at 13.0 or 14.0°C began declining, falling below 100 N by Day 51 and Day 37, respectively, while the 13.0 or 14.0°C/+1-MCP fruit retained a peel shear resistance of approximately 100 N throughout storage. Peel shear resistance was affected mostly by temperature before 31 days, but 1-MCP was the major factor determining peel shear resistance after 34 days.

Firmness of banana pulp was initially 31–34N (Table 4). Pulp firmness of fruit stored at 5.0°C (+1-MCP/−1-MCP) remained constant throughout storage while the pulp firmness in the other treatments declined beginning approximately on Day 34 (data not shown). Pulp firmness in the −1-MCP treatments at 13.0/14.0°C and + 1-MCP treatment at 14.0°C declined drastically to below 20N after 37–42 days while the +1-MCP/13.0°C fruit softened more gradually to the same firmness over 51 days.

Gray peel and vascular browning

Results for visual CI symptoms are presented in Table 5. Only fruit stored at 5.0°C (+1-MCP/−1-MCP) exhibited gray skin development, a symptom of CI, which turned to dark brown as storage proceeded. The other treatments did not show significant external discoloration. Storage temperature was the major factor controlling gray peel discoloration, and only during the 31 days of storage duration for the 5.0°C treatments.

Fruit stored at 5.0°C (+1-MCP/−1-MCP) developed brown streaks from discoloration of vascular strands in the aerenchyma tissue of the peel after 4 days and this browning area in vascular tissues kept increasing to 100% on Day 20 (data not shown). Treatments at 13.0°C (+1-MCP/−1-MCP) showed some brown spots and streaks after 10 days, but the severity was reduced by 1-MCP treatment so that around 3% area was affected for the +1-MCP fruit compared with around 8% for the −1-MCP fruit. The −1-MCP fruit at 14.0°C developed vascular browning after 19 days while the +1-MCP fruit required 28 days to develop

TABLE 3 Changes in ethylene production rate of banana fruit that were initially treated with 0 (−1-MCP) or 50 μL L^{-1} (+1-MCP) aqueous 1-MCP for 60s at 23.0°C and then transferred to 5.0°C, 13.0°C or 14.0°C storage with 95% RH (n=3).

Storage temperature (°C)	Treatment	Average ethylene production (ng kg^{-1} s^{-1}) during the first 3 days	Days to the climacteric rise of ethylene production	Days to the climacteric peak of ethylene production	Average ethylene production (ng kg^{-1} s^{-1}) at the climacteric peak	Average ethylene production (ng kg^{-1} s^{-1}) at the end of the experiment (day 31 at 5°C; day 61 at 13/14°C)*
5. 0	−1-MCP	0.036b**	–			0.032
	+1-MCP	0.040a	–			0.037
13.0	−1-MCP	0.035b	38.7b	54b	0.166c	0.136c
	+1-MCP	0.034b	46a	58a	0.338a	0.301a
14.0	−1-MCP	0.032c	37b	53.7b	0.214bc	0.145bc
	+1-MCP	0.036b	42.2ab	55.3ab	0.232b	0.188b

*Banana fruit stored at 5.0°C turned brown and were discarded after 31 days. Before then, there were no ripening characteristics observed.
**Different letters in the same column indicate significant differences by treatments.

TABLE 4 Changes in peel resistance to shear force (N) and pulp firmness (N) of banana fruit that were initially treated with 0 (−1-MCP) or 50 μL L^{-1} (+1-MCP) aqueous 1-MCP for 60s at 23.0°C and then transferred to 5.0°C, 13.0°C or 14.0°C storage with 95% RH (n=3).

Storage temperature (°C)	Treatment	Initial banana peel resistance to shear force (N) on the first day	Days to peel resistance to shear force below 100 N	Banana peel resistance to shear force (N) at the end of the experiment	Initial banana pulp firmness (N) on the first day	Days to the pulp firmness below 20 N	Banana pulp firmness (N) at the end of the experiment (day 31 at 5°C; day 61 at 13/14°C)*
5.0	−1-MCP	100.68d**	–	107.21*	34.11a	–	34.11
	+1-MCP	100.74d	–	119.13*	31.73a	–	31.73
13.0	−1-MCP	107.34bc	51	43.17b	34.11a	42ab	1.66a
	+1-MCP	119.34a	-	119.79a	31.73a	51a	1.92a
14.0	−1-MCP	111.78b	37	47.29b	34.11a	37b	1.82a
	+1-MCP	111.69b	-	106.72a	31.73a	42ab	1.65a

Banana peel resistance to sheer force (N) with the epidermis oriented downward and the fiber direction oriented parallel to a 3-mm diameter flat blade and banana pulp firmness (N) measured using a 8-mm diameter Magness-Taylor probe.
*Banana fruit stored at 5.0°C turned brown and were discarded after 31 days. Before then, there were no ripening characteristics observed.
**Different letters in the same column indicate significant differences by treatments.

TABLE 5 Chilling injury symptom development on banana fruit that were initially treated with 0 (−1-MCP) or 50 μL L^{-1} (+1-MCP) aqueous 1-MCP for 60s at 23.0°C and then transferred to 5.0°C, 13.0°C or 14.0°C storage with 95% RH (n=3).

Storage temperature (°C)	Treatment	Average days to show the first sign of gray peel (percentage area observed)	Average days to show the first sign of vascular browning (percentage area observed)	Average percentage vascular browning area observed at the end of the experiment (day 31 at 5°C; day 61 at 13/14°C)*
5.0	−1-MCP	4a***** (40%)	4d (16.7%A)	95
	+1-MCP	4a (40%)	4d (16.7%A)	95
13.0	−1-MCP	–***	10c (8.3%B)	15
	+1-MCP	–***	10c (2.3%C)	10
14.0	−1-MCP	–***	19b (1%C)	8.3
	+1-MCP	–***	28a (8.7%B)	10

*Banana fruit stored at 5.0°C suffered severe chilling injury turning brown and were discarded after 31 days. Before then, there were no ripening characteristics observed.
**Different letters in the same column indicate significant differences by treatments.
***There was no gray peel observed on banana fruit stored at 13 or 14°C.

vascular browning. Vascular browning area did not exceed 10% throughout the experiment for fruit stored at 13.0/14.0°C.

Chlorophyll fluorescence

Yield (II) [Y(II)], known as the quantum yield of photosystem II (PSII) at light-adapted status, indicates operating efficiency of PSII under real-time environmental conditions. The initial Y(II) value on Day 0 was 0.6 (Figure 1). The Y(II) value of fruit stored at 5.0°C (+1-MCP/−1-MCP) rapidly decreased within the first 0.5 d and thereafter continued declining more slowly, reaching 0.2 to 0.3 at the end of observation (Day 21; see insert in Figure 1). The Y(II) values of fruit at 13.0/14.0°C and + 1-MCP/−1-MCP changed little during the first 5 weeks of storage. Beginning around Day 37, the Y(II) of the 13.0°C/−1-MCP fruit declined faster than the 14.0°C fruit and the 13.0°C/+1-MCP fruit. However, beginning around Day 53, the Y(II) of the 14.0°C/−1-MCP decreased more rapidly, equaling the 13.0°C/−1-MCP treatment over the last week of the experiment.

The other chlorophyll fluorescence indicator, Fv/Fm, represents the maximum photochemical efficiency of PSII in dark-adapted green tissues (Baker, 2008). Similar, to the Y(II) results, the Fv/Fm of fruit stored at 5.0°C (+1-MCP/−1-MCP) decreased from 0.8 to around 0.7 after 1 day, thereafter declining further over the next 10 days, to 0.64 for −1-MCP fruit and 0.58 for +1-MCP fruit (Figure 2). The treatments at 13.0/14.0°C (+1-MCP/−1-MCP) slowly decreased from 0.8 to 0.75 over 45 days. By Day 47, the fruit at 13.0°C briefly declined compared with the 14.0°C fruit, but then rose again to the baseline (0.7–0.75), like the rest of the treatments.

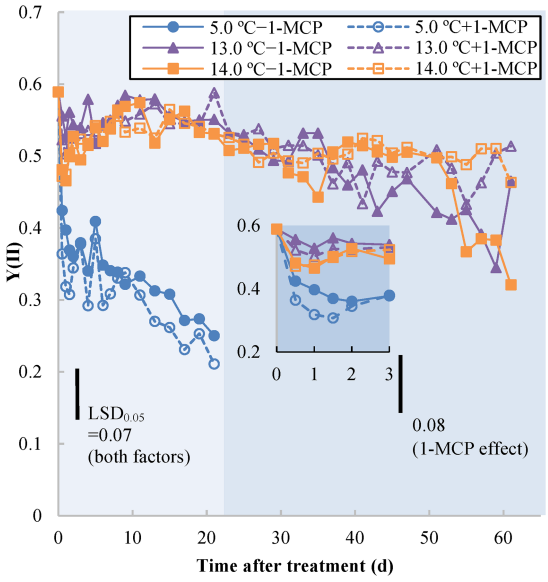


FIGURE 1
Photochemical efficiency as measured by light-adapted status photosystem II chlorophyll fluorescence quantum yield [Yield (II)] of banana peel initially treated with 0 (−1-MCP) or 50 μL L^{-1} (+1-MCP) aqueous 1-MCP for 60s at 23.0°C and transferred to 5.0, 13.0 or 14.0°C storage with 95% RH (n=3). $LSD_{0.05}$ values varied within different time frames shown by different background shading (days 0–21; days 23–61) due to the various shelf-life durations of treatments.

Electrolyte efflux and malondialdehyde content

Electrolyte efflux indicates the status of membrane function of plant cells in terms of their ability to regulate transport of solutes. Electrolyte efflux of fruit at 5.0°C (+1-MCP/−1-MCP) increased gradually from 15 to 30% during the 31 days of storage while that of fruit at 13.0/14.0°C (+1-MCP/−1-MCP) remained around 13% but increased gradually later during the storage (Table 6). The EE for the −1-MCP treatments at 13.0/14.0°C slightly increased after Day 42; thereafter further increase in EE was more dramatic in the 14.0°C/−1-MCP treatment than in the 13.0°C/−1-MCP treatment. In contrast, the EE of the + 1-MCP treatments at 13.0/14.0°C stayed constant until Day 51, rising only on the last day of storage (Day 61).

Membrane lipid peroxidation resulting from environmental stress can be evaluated by measuring MDA content. The MDA content of banana peel from fruit stored at 5°C was higher than that of the 13/14°C treatments (Supplementary Figure 3) but there was no difference among 1-MCP treatments at 13/14°C. Pulp MDA content is presented in Supplementary Figure 4, showing that similar patterns were shared by all treatments.

Discussion

Extension of produce shelf-life with retention of high quality is one of the ultimate goals in postharvest handling. Temperature management is a major strategy that is used to reduce metabolic reactions and thus extend shelf life. Another strategy used with climacteric fruits is to delay the onset of the climacteric by avoiding direct ethylene exposure and reducing ethylene production and action by atmosphere control or blockage of ethylene receptors (Watkins, 2006). Temperature management cannot be fully utilized to extend the shelf-life of chilling-sensitive climacteric fruit due to relatively high CTTs that allow ripening to occur while avoiding CI; in the case of banana, shelf life is limited by ripening at the CTT of 14.0°C. Therefore, blocking ethylene action and thus the onset of the climacteric using 1-MCP could be a viable strategy for extending banana shelf life. However, the possibility exists that longer than normal exposure to 14.0°C could result in CI.

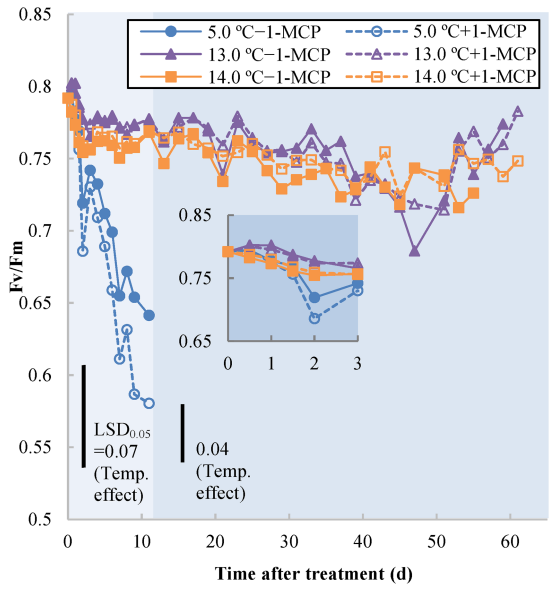


FIGURE 2
Photochemical efficiency as measured by dark-adapted status photosystem II chlorophyll fluorescence (Fv/Fm) of banana peel initially treated with 0 (−1-MCP) or 50 μL L^{-1} (+1-MCP) aqueous 1-MCP for 60s at 23.0°C and transferred to 5.0, 13.0 or 14.0°C storage with 95% RH (n=6). $LSD_{0.05}$ values varied within different time frames shown by different background shading (days 0–11; days 13–61) due to the various shelf-life durations of treatments.

Before beginning the experiment, fruit exhibiting any defects, including possible CI symptoms on the peel were discarded. However, CI is cumulative and there remains a possibility that chilling exposure could have occurred in the field or during transportation from Guatemala to Florida without visual symptom development that could be detected by us. There were slight differences in the darkness of the peel green color between lots of bananas, suggesting that some exposure to higher than optimal temperatures may have occurred, but any fruit not initially at stage 2 were discarded.

Banana fruit stored at 5.0°C, whether treated with 1-MCP or not, remained at the initial green, preclimacteric developmental stage during storage, in terms of peel color, texture of peel and pulp, and total pulp soluble solids, until the onset of CI symptoms occurred. The CI symptoms observed at 5.0°C were severe and included inhibition of peel color change, gray epidermal area, vascular browning, and increased stress indicators such as chlorophyll fluorescence [Y(II) and Fv/Fm], EE, and peel MDA content (data not shown) as previous research has indicated (Grierson et al., 1967; Abd El-Wahab and Nawwar, 1977; Chaiprasart et al., 2001; Promyou et al., 2008). Moreover, no obvious 1-MCP effect was observed on fruit at 5.0°C in this study, suggesting no role for ethylene action in CI development under severe chilling temperatures.

Results during storage at 13.0/14.0°C (+1-MCP/−1-MCP) indicated that the delay of the ripening process was affected by both low temperature and 1-MCP. Based on the ripeness stage ratings (Table 1), fruit in the 14.0°C/−1-MCP treatment began to ripen after 34 days, followed by 13.0°C/−1-MCP after 42 days; and 13.0/14.0°C + 1-MCP fruit on Day 51. The ripening rate was affected by both temperature and 1-MCP as the onset of ripening for +1-MCP fruit was delayed by a few more days compared to −1-MCP fruit. Ethylene receptor level has been reported to be the major determinant of initiation of ripening of tomato fruit, and it appears that degradation of ethylene receptors may be induced by ethylene binding, but not 1-MCP binding (Kevany

TABLE 6 Changes in electrolyte efflux (EE) of banana fruit initially treated with 0 (−1-MCP) or 50 μL L^{-1} (+1-MCP) aqueous 1-MCP for 60s at 23.0°C and transferred to 5.0, 13.0 or 14.0°C storage with 95% RH (n=6).

Storage temperature (°C)	Treatment	Banana peel electrolyte efflux after 1 day of storage (%)	Days to peel electrolyte efflux above 20%	Banana peel electrolyte efflux (%) at the end of the experiment (day 31 at 5°C; day 61 at 13/14°C)*
5.0	−1-MCP	15.07a**	13b	31.15
	+1-MCP	15.74a	10b	28.47
13.0	−1-MCP	14.43a	-	16.39b
	+1-MCP	13.78a	61a	22.55ab
14.0	−1-MCP	14.36a	61a	33.31a
	+1-MCP	15.03a	-	17.10b

*Banana fruit stored at 5.0°C suffered severe chilling injury turning brown and were discarded after 31 days. Before then, there were no ripening characteristics observed.
**Different letters in the same column indicate significant differences by treatments.

et al., 2007). However, the inhibition of ethylene action by 1-MCP may be alleviated after a certain period of time, and its recovery rate would then be affected by temperature.

Peel color changes, on which the ripeness stage ratings were based, showed minor differences between −1-MCP and + 1-MCP bananas for lightness (L*) and chroma or color purity (C*), but 1-MCP significantly inhibited changes in h* and also a* value corresponding to loss of green color (Supplementary Figure 5). This 1-MCP effect on color development of banana peel is in agreement with observations that 1-MCP inhibits the breakdown of chloroplasts in most crops (Ku and Wills, 1999; Harris et al., 2000; Mir et al., 2001). However, in the final investigation, there was no obvious difference in peel color at the mild chilling temperature of 13.0°C or the putative lowest safe storage temperature or CTT of 14.0°C, which was supported by Facundo et al. (2015) who reported that mild chilling temperatures (10–13°C) did not affect the color development when cv. Nanicão (AAA type) bananas were fully ripe.

Preclimacteric stage (MG) bananas stored at severe chilling temperature (5.0°C), mild chilling temperature (13.0°C), or CTT (14.0°C) all maintained the same respiration rate and ethylene production rate during storage, which comports with the reports of Gemma et al. (1994) and Sánchez (2016) for chilled bananas; however, Gemma et al. (1994) also observed a burst of respiration and ethylene production after rewarming severely chilled banana fruit (1–5°C) at room temperature. The temperature quotient (Q_{10}) is useful to estimate metabolic rates within a known temperature range. The Q_{10} is known to be higher at chilling temperatures for chilling-sensitive crops (Lyons and Breidenbach, 1990). Based on the ethylene production rate measured at 14.0°C in the current study, the expected ethylene production at 5.0°C was estimated using the Q_{10} to be 0.024 ng kg^{-1} s^{-1}, but the actual rate measured was 0.04 ng kg^{-1} s^{-1}, which was close to the ethylene production rate of the higher temperature treatment. This suggests that there was elevated stress-induced ethylene production at 5.0°C.

The onsets of the climacteric burst in respiration and ethylene production were influenced by the storage temperature and by 1-MCP treatment. Interestingly, there was a 10-day delay between the climacteric rises in respiration and ethylene production for 14.0°C/−1-MCP versus 14.0°C/+1-MCP and 13.0°C/−1-MCP, and another 10-day delay before the climacteric occurred in the 13.0°C/+1-MCP treatment. Peak respiration rates for all treatments at 13.0/14.0°C were similar (5–6 μg kg^{-1} s^{-1}) although respiration of +1-MCP fruit at 13.0°C was slightly higher than that of the 13.0°C/−1-MCP fruit. The peak ethylene production of +1-MCP fruit was higher (0.18 ng kg^{-1} s^{-1} at 14.0°C; 0.29 ng kg^{-1} s^{-1} at 13.0°C) than the −1-MCP controls at 13.0/14.0°C (0.12 ng kg^{-1} s^{-1}). Delays in the onset of the climacteric along with higher climacteric peaks of respiration and ethylene production in +1-MCP banana fruit were also observed by Golding et al. (1998) and in our previous research (Chang and Brecht, 2020a).

Neither the severe chilling temperature of 5.0°C nor 1-MCP treatment affected the texture of banana peel and pulp during the initial 31-days storage period. In contrast, there was a difference in the 1-MCP effect on texture at 13.0/14.0°C between the peel and pulp tissues. Peel sheer force of −1-MCP fruit at 13.0/14.0°C declined during storage while +1-MCP fruit retained constant shear force throughout the experiment. The softening of the banana fruit pulp at 13.0/14.0°C began after 34 d and proceeded at different rates. The −1-MCP fruit pulp tissue was completely soft (< 2 N) by Day 51 while the pulp of + 1-MCP fruit did not soften to the same firmness until Day 61. Treatment with 1-MCP also delayed softening in papaya fruit (*Carica papaya* L., Hofman et al., 2001; Ergun and Huber, 2004) and was associated with lower pectin methyl esterase (Façanha et al., 2019) and polygalacturonase activities (PG; Fabi et al., 2014) delaying the total pectin degradation (Asmar et al., 2010). Banana softening resulted from the cell wall changes, specifically the synchronized degradation of pectin, hemicellulosic polysaccharides, and starch (Kojima et al.,1994; John and Marchal, 1995). 1-MCP might have affected the banana cell wall degradation during ripening, resulting in the differences among treatments in the current study.

Storage at 13.0°C, just a single degree below the CTT, is a relatively mild chilling stress to banana fruit, and the development of CI symptoms would be expected to develop at a very slow rate. The severity of CI symptoms is determined by a "time × temperature" relationship in which more severe symptoms develop, and develop faster, at lower temperatures and vice versa (Paull, 1990). Therefore, we hypothesized that the CTT of 14.0°C could also be a chilling temperature when the exposure time is extended by inhibiting the shelf-life limiting factor of ripening. Alteration of a few CI symptoms were observed in this study, but more CI symptoms were found to occur at both 13.0°C and 14.0°C if given enough time (Table 6).

Vascular browning was the first CI symptom that appeared, developing earlier in −1-MCP fruit stored at the mild chilling temperature (13.0°C) than at the CTT (14.0°C); +1-MCP delayed but did not prevent the appearance of vascular browning at both of those temperatures. Regarding the external peel appearance related to CI, only fruit held at 5.0°C developed obvious gray skin, but no trace of that CI symptom was found on bananas held at 13.0/14.0°C. Interestingly, when the epidermal layer of the mildly chilled fruit without gray peel was removed, up to 20% of the vascular tissue was affected by vascular browning (data not shown). Therefore, the development of typical peel color in the epidermal layer may obscure mild vascular discoloration. Moreover, vascular browning of +1-MCP fruit at 13.0/14.0°C developed later and with less severity (i.e., lighter browning) than in the control −1-MCP fruit, suggesting involvement of ethylene in vascular browning.

Vascular browning of chilled banana peel has been observed to occur in the laticifer cells, also known as latex vessels, that are associated with vascular bundles (John and Marchal, 1995; Harvey, 2005). Latex in laticifer cells consists of various organelles in a colloidal fluid cytoplasm (Baker et al., 1990). Lutoid vesicles in banana latex may compartmentalize polyphenol oxidase (PPO) and phenols (Kallarackal et al., 1986; Harvey, 2005). Browning reaction of banana fruit results from the oxidation of mainly dopamine by PPO (Griffiths, 1959; Abd El-Wahab and Nawwar, 1977) to produce dopaminequinone-H$^+$, which is hypothesized to

cooxidize with salsolinol to form salsolinol-*o*-quinone (Sojo et al., 2000). This highly active *o*-quinone would then polymerize to produce melanins as the major browning substance (Castaner et al., 1996). The major enzymes controlling browning in chilling storage were proposed to be phenylalanine ammonia lyase (PAL) and PPO (Nguyen et al., 2003), which are also related to ethylene action in crops (Couture et al., 1993; Pesis et al., 2002; Jin et al., 2011). The interaction of ethylene and those two enzymes at chilling temperature needs to be further investigated.

Chlorophyll fluorescence parameters indirectly reflected the stress status of chlorophyll-containing tissues among different treatments. Quantum yield [Y(II)] is an estimation of PSII operating efficiency (i.e., the rate of electron transport of PSII); Fv/Fm represents the maximal quantum efficiency of PSII photochemistry to detect the loss of function of PSII reaction centers (Öquist et al., 1992; Baker, 2008). The decrease in chlorophyll fluorescence results from the inactivation of the PSII reaction center by stress (e.g., CI) and ripening (breakdown of chloroplasts to form chromoplasts; Chaiprasart et al., 2001) and the inhibition of degreening in 1-MCP-treated fruit may also delay the change of chlorophyll fluorescence (Mir et al., 2001). Of both chlorophyll parameters, Y(II) of 5.0°C fruit decreased rapidly, after only after 0.5 day of storage, followed soon after by a decline in Fv/Fm after 1 day. The chlorophyll fluorescence decline in fruit at 13.0°C after Day 37 might have been due to CI, but the subsequent drop of the 14.0°C/−1-MCP fruit may have been a result of ripening-induced chlorophyll breakdown. The early CI stress status of 13.0°C fruit was also detectable from Y(II) and occurred before the onset of ripening. Therefore, Y(II) could be an appropriate tool to examine early CI stress on the MG banana.

Chilling temperature may lead to alteration of the physical properties of lipids in plant membrane systems (Lyons, 1973), possibly resulting in unbalanced metabolism that leads to lipid peroxidation and accumulation of reactive oxygen species (ROS; Zhang et al., 2005; Nukuntornprakit et al., 2015). Therefore, EE and MDA content are used as essential markers to evaluate membrane permeability and peroxidation, respectively, as indicators of membrane integrity. Malondialdehyde content, one parameter of membrane integrity, showed the temperature effect but no significant 1-MCP effect (Supplementary Figures 3, 4). However, changes in EE and MDA can be affected by ripening and senescence as well as by CI (Gemma et al., 1994; Lim et al., 2007), all of which lead to breakdown of membrane systems. Increased EE in bananas at 5.0°C (+1-MCP/−1-MCP) coincided with the appearance and severity of CI symptoms, including vascular browning and the reduction in chlorophyll fluorescence [Y(II) and Fv/Fm]. In contrast, the changes of EE at 13.0/14.0°C (+1-MCP/−1-MCP) followed the progress of ripening as shown by changes in peel color, and fruit respiration rate, ethylene production, and texture.

Compared to the sensitive CI response of banana peel, the pulp appears to be less affected by chilling temperature based on texture. The changes were delayed by temperature and 1-MCP, but recovered as storage progressed. The difference in chilling sensitivity between banana peel and pulp was also observed by Gemma et al. (1994) in terms of the patterns of EE at different temperatures in the two tissues, which showed breakpoints at 8.9 and 3.0°C, respectively, indicating a similar conclusion that the peel of bananas is more sensitive to CI than the pulp.

Conclusion

Chilling threshold temperature is a limitation for postharvest handling of climacteric tropical fruit like banana in that CI is avoided but ripening may occur at the CTT. This research demonstrates that this putative safe temperature may become a *de facto* chilling temperature if the shelf-life-limiting factor is removed, allowing longer exposure. Longer exposure time at the reported banana fruit CTT of 14.0°C (19 days for −1-MCP treatment; 25 days for +1-MCP treatment) caused relatively mild CI on the fruit before recovery from 1-MCP-induced ripening inhibition occurred. Vascular browning was the most sensitive indicator of CI status while Y(II) was also determined to be a potential non-destructive tool to detect early CI stress in MG banana. 1-MCP-treated fruit at 13.0/14.0°C developed less vascular discoloration, less EE, and higher quantum yield [Y(II)] than fruit without 1-MCP, but 1-MCP did not reduce development of external peel discoloration or affect Fv/Fm at the same temperatures, which suggests that ethylene might be involved in early development of some, but not all CI symptoms.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

L-YC and JB conceived and designed the experiments, analyzed the data, and wrote the manuscript. L-YC performed the experiments. L-YC, SS, JK, and JB reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

L-YC was sponsored by the Elite program of the Council of Agriculture, Taiwan for her Ph.D. The authors declare that this study received funding from It's Fresh! Ltd. The funder was not involved in the study design, collection, analysis, interpretation of data, the writing of this article, or the decision to submit it for publication.

Acknowledgments

We thank Donald J. Huber for helpful discussions during the conceptualization and planning of this research and assistance with the aqueous 1-MCP application technique.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

Supplementary material

The Supplementary material for this article can be found online at: https: https://www.frontiersin.org/articles/10.3389/fpls.2022.966789/full#supplementary-material

References

Abd El-Wahab, F. K., and Nawwar, M. A. M. (1977). Physiological and biochemical studies on chilling-injury of banana. *Scientia Hortic.* 7, 373–376. doi: 10.1016/0304-4238(77)90010-3

Andersen, C. R., and Kent, M. W. (1982). Storage of cucumber fruit at chilling temperatures. *HortScience* 17:527.

Asmar, S. A., Abreu, C. M. P., Lima, R. A. Z., Correa, A. D. A., and Santos, C. D. (2010). Firmeza de mamão tratado com 1-MCP em diferentes tempos de exposição. *Clin. E Agrotecnol* 34, 440–444. doi: 10.1590/S1413-70542010000200024

Baker, D. A., Kallarackal, J., and Milburn, J. S. (1990). Water relations of the banana. II. Physicochemical aspects of the latex and other tissue fluids. *Func. Plant Biol.* 17, 69–77. doi: 10.1071/PP9900069

Baker, N. R. (2008). Chlorophyll fluorescence: a probe of photosynthesis *in vivo*. *Annu. Rev. Plant Biol.* 59, 89–113. doi: 10.1146/annurev.arplant.59.032607.092759

Biswas, P., East, A. R., Hewett, E. W., and Heyes, J. A. (2014). Ripening delay caused by 1-MCP may increase tomato chilling sensitivity. *New Zeal. J. Crop Hort.* 42, 145–150. doi: 10.1080/01140671.2013.870218

Candan, A. P., Graell, J., and Larrigaudiere, C. (2006). Postharvest quality and chilling injury of plums: benefits of 1-methylcyclopropene. *Span. J. Agric. Res.* 9, 554–564. doi: 10.5424/sjar/20110902-259-10

Castaner, M., Gil, M. I., Artes, F., and Tomas-Barberan, F. A. (1996). Inhibition of browning of harvested head lettuce. *J. Food Sci.* 61, 314–316. doi: 10.1111/j.1365-2621.1996.tb14184.x

Chaiprasart, P., Gemma, H., and Iwahori, S. (2001). Changes in chorophyll fluorescence and enzyme activity for scavenging of free radicals in banana fruits stored at low temperature. *Jpn. J. Trop. Agri.* 45, 181–191. doi: 10.11248/jsta1957.45.181

Chang, L. Y., and Brecht, J. K. (2020a). Recovery of ripening by mature green banana fruit following 1-methylcyclopropene application. *Proc. Fla. State Hort. Soc.* 133:148.

Chang, L. Y., and Brecht, J. K. (2020b). Variation among strawberry cultivars in bruising susceptibility related to wound ethylene production and sensitivity. *HortScience* 55, 444–448. doi: 10.21273/HORTSCI14733-19

Chang, W. H., and Hwang, Y. J. (1990). Effect of ethylene treatment on the ripening, polyphenoloxydase activity and water soluble tannin content of Taiwan northern banana at different maturity stages and the stability of banana polyphenoloxydase. *Acta Hortic.* 275, 603–610. doi: 10.17660/ActaHortic.1990.275.75

Choi, S. T., and Huber, D. J. (2008). Influence of aqueous 1-methylcyclopropene concentration, immersion duration, and solution longevity on the postharvest ripening of breaker-turning tomato (*Solanum lycopersicum* L.) fruit. *Postharvest Biol. Technol.* 49, 147–154. doi: 10.1016/j.postharvbio.2008.01.003

Chomchalow, S., El Assi, N. M., Sargent, S. A., and Brecht, J. K. (2002). Fruit maturity and timing of ethylene treatment affect storage performance of green tomatoes at chilling and nonchilling temperatures. *Hort Technology* 12, 104–114. doi: 10.21273/HORTTECH.12.1.104

Couture, R., Cantwell, M. I., Ke, D., and Saltveit, M. E. (1993). Physiological attributes related to quality attributes and storage life of minimally processed lettuce. *HortScience* 28, 723–725. doi: 10.21273/HORTSCI.28.7.723

Eaks, I. L. (1980). Effect of chilling on respiration and volatiles of California lemon fruit. *J. Amer. Soc. Hort. Sci.* 105, 865–869. doi: 10.21273/JASHS.105.6.865

Eaks, I. L. (1983). Effects of chilling on respiration and ethylene production of 'Hass' avocado fruit at 20°C. *HortScience* 18, 235–237.

Eaks, I. L., and Morris, L. L. (1956). Respiration of cucumber fruits associated with physiological injury at chilling temperatures. *Plant Physiol.* 31, 308–314. doi: 10.1104/pp.31.4.308

Ergun, M., and Huber, D. J. (2004). Suppression of ethylene perception extends shelf-life and quality of 'sunrise solo' papaya fruit at both pre-ripe and ripe stages of development. *Eur. J. Hortic. Sci.* 69, 184–192.

Fabi, J. P., Broetto, S. G., de Silva, S. L. G. L., Zhong, S., Lajolo, F. M., and do Nascimento, J. R. O. (2014). Analysis of papaya cell wall-related genes during fruit ripening indicates a central role of polygalacturonases during pulp softening. *PLoS One* 9:e105685. doi: 10.1371/journal.pone.0105685

Façanha, R. V., Spricigo, P. C., Purgatto, E., and Jacomino, A. P. (2019). Combined application of ethylene and 1-methylcyclopropene on ripening and volatile compound production of 'Golden' papaya. *Postharvest Biol. Technol.* 151, 160–169. doi: 10.1016/j.postharvbio.2019.02.005

Facundo, H. V. D. V., Gurak, P. D., Mercadante, A. Z., Lajolo, F. M., and Cordenunsi, B. R. (2015). Storage at low temperature differentially affects the colour and carotenoid composition of two cultivars of banana. *Food Chem.* 170, 102–109. doi: 10.1016/j.foodchem.2014.08.069

Gemma, H., Matuyama, Y., and Wang, H. G. (1994). Ripening characteristics and chilling injury of banana fruit. I. Effect of storage temperature on respiration, ethylene production and membrane permeability of peel and pulp tissues. *Jpn. J. Trop. Agr.* 38, 216–220. doi: 10.11248/jsta1957.38.216

Golding, J. B., Shearer, D., Wyllie, S. G., and McGlasson, W. B. (1998). Application of 1-MCP and propylene to identify ethylene-dependent ripening processes in mature banana fruit. *Postharvest Biol. Technol.* 14, 87–98. doi: 10.1016/S0925-5214(98)00032-5

Grierson, E. B., Grierson, W., and Soule, J. (1967). Chilling injury in tropical fruit. I. Bananas (*Musa paradisiaca* var. *sapientum* cv. Lacatan). *Proc. Trop. Reg. Am. Soc. Hort. Sci.* 11, 82–94.

Griffiths, L. A. (1959). Detection and identification of polyphenol oxidase substrate of banana. *Nature* 184, 58–59. doi: 10.1038/184058a0

Hagan, L. L., Johnson, P. N. T., Sargent, S. A., Huber, D. J., and Berry, A. (2017). 1-methylcyclopropene treatment and storage condition delay the ripening of plantain fruit while maintaining sensory characteristics of *ampesi*, the boiled food product. *Intl. Food Research J.* 24, 630–636.

Harris, D. R., Seberry, J. A., Wills, R. B. H., and Spohr, L. J. (2000). Effect of fruit maturity on efficiency of 1-methylcyclopropene to delay the ripening of banana. *Postharvest Biol. Technol.* 20, 303–308. doi: 10.1016/S0925-5214(00)00150-2

Harvey, B. V. (2005). Mild chilling injury of banana (Cavendish cv. Williams) and its control in the field. dissertation. Adelaide, Australia: University of Adelaide.

Hofman, P. J., Jobin-Decor, M., Meilburg, G. F., Macnish, A. J., and Joyce, D. C. (2001). Ripening and quality responses of avocado, custard apple, mango and papaya fruit to 1- methylcyclopropene. *Austral. J. Exp. Agric.* 41, 567–572. doi: 10.1071/Ea00152

Huang, H., Jing, G., Gua, L., Zhang, D., Yang, B., Duan, X., et al. (2013). Effect of oxalic acid on ripening attributes of banana fruit during storage. *Postharvest Biol. Technol.* 84, 22–27. doi: 10.1016/j.postharvbio.2013.04.002

Jin, P., Shang, H., Chen, J., Zhu, H., Zhao, Y., and Zheng, Y. (2011). Effect of 1-methylcyclo-propene on chilling injury and quality of peach fruit during cold storage. *J. Food Sci.* 76, S485–S491. doi: 10.1111/j.1750-3841.2011.02349.x

John, P., and Marchal, J. (1995). "Ripening and biochemistry of the fruit," in *Bananas and Plantains*. ed. S. Gowen (Netherlands: Springer), 434–467.

Kallarackal, J., Garlick, P. R., and Milburn, J. A. (1986). Characterization of the structural inclusion in the latex of banana (*Musa sp.*) can. *J. Bot.* 64, 2591–2601.

Kevany, B. M., Tieman, D. M., Taylor, M. G., Dal Cin, V., and Klee, H. J. (2007). Ethylene receptor degradation controls the timing of ripening in tomato fruit. *Plant J.* 51, 458–467. doi: 10.1111/j.1365-313X.2007.03170.x

Kojima, K., Sakurai, N., and Kuraishi, S. (1994). Fruit softening in banana: correlation among stress-relaxation parameters, cell wall components and starch during ripening. *Physiol. Planta.* 90, 772–778. doi: 10.1111/j.1399-3054.1994.tb02536.x

Ku, V. V. V., and Wills, R. B. H. (1999). Effect of 1-methylcyclopropene on the storage life of broccoli. *Postharvest Biol. Tech.* 17, 127–132. doi: 10.1016/S0925-5214(99)00042-3

Li, T., Yun, Z., Zhang, D., Yang, C., Zhu, H., Jiang, Y., et al. (2015). Proteomics analysis of differentially expressed proteins involved in ethylene-induced chilling tolerance in harvested banana fruit. *Front. Plant Sci.* 6:845. doi: 10.3389/fpls.2015.00845

Lim, C. S., Kang, S. M., Cho, J. L., Gross, K. C., and Woolf, A. B. (2007). Bell pepper (*Capsicum annuum* L.) fruits are susceptible to chilling injury at the breaker stage of ripeness. *HortScience* 42, 1659–1664. doi: 10.21273/HORTSCI.42.7.1659

Lü, P., Yu, S., Zhu, N., Chen, Y. R., Zhou, B., Pan, Y., et al. (2018). Genome encode analyses reveal the basis of convergent evolution of fleshy fruit ripening. *Nature Plants.* 4, 784–791. doi: 10.1038/s41477-018-0249-z

Lyons, J. M. (1973). Chilling injury in plants. *Annu. Rev. Plant Physiol.* 24, 445–466. doi: 10.1146/annurev.pp.24.060173.002305

Lyons, J. M., and Breidenbach, R. W. (1990). "Relation of chilling stress to respiration," in *Chilling Injury of Horticultural Crops*. ed. C. Y. Wang (Boca Raton, FL: CRC Press), 224–231.

Manning, K., Tör, M., Poole, M., Hong, Y., Thompson, A. J., King, G. J., et al. (2006). A naturally occurring epigenetic mutation in a gene encoding an SBP-box transcription factor inhibits tomato fruit ripening. *Nature Genet.* 38, 948–952. doi: 10.1038/ng1841

Marangoni, A. G., Palma, T., and Stanley, D. W. (1996). Membrane effects in postharvest physiology. *Postharvest Biol. Tech.* 7, 193–217. doi: 10.1016/0925-5214(95)00042-9

Marriott, J., and Palmer, J. K. (1980). Bananas – physiology and biochemistry of storage and ripening for optimum quality. *Critical Rev. Food Sci Nutri.* 13, 41–88. doi: 10.1080/10408398009527284

Megías, Z., Manzano, S., Martínez, C., Garcia, A., Aguado, E., Garrido, D., et al. (2016). Postharvest cold tolerance in summer squash and its association with reduced cold-induced ethylene production. *Euphytica* 213, 1–12. doi: 10.1007/s10681-016-1805-0

Mir, N. A., Curell, E., Khan, N., Whitaker, M., and Beaudry, R. M. (2001). Harvest maturity, storage, temperature, and 1-MCP application frequency alter firmness retention and chlorophyll fluorescence of 'Redchief Delicous' apples. *J. Amer. Soc. Hort. Sci.* 126, 618–624. doi: 10.21273/JASHS.126.5.618

Nguyen, T. B. T., Ketsa, S., and van Doorn, W. G. (2003). Relationship between browning and the activities of polyphenol oxidase and phenylalanine ammonia lyase in banana peel during low temperature storage. *Postharvest Biol. Technol.* 30, 187–193. doi: 10.1016/S0925-5214(03)00103-0

Nukuntornprakit, O., Chanjirakul, K., van Doorn, W. G., and Siriphanich, J. (2015). Chilling injury in pineapple fruit: fatty acid composition and antioxidant metabolism. *Postharvest Biol. Technol.* 99, 20–26. doi: 10.1016/j.postharvbio.2014.07.010

Öquist, G., Chow, W. S., and Anderson, J. M. (1992). Photoinhibition of photosynthesis represents a mechanism for the long-term regulation of photosystem II. *Planta* 186, 450–460. doi: 10.1007/BF00195327

Paull, R. E. (1990). "Chilling injury of crops of tropical and subtropical origin," in *Chilling Injury Of Horticultural Crops*. ed. C. Y. Wang (Boca Raton, FL: CRC Press), 17–36.

Pesis, E., Ackerman, M., Ben-Arie, R., Feygenberg, O., Feng, X., Apelbaum, A., et al. (2002). Ethylene involvement in chilling injury symptoms of avocado during cold storage. *Postharvest Biol. Technol.* 24, 171–181. doi: 10.1016/S0925-5214(01)00134-X

Promyou, S., Ketsa, S., and van Doorn, W. G. (2008). Hot water treatments delay cold-induced banana peel blackening. *Postharvest Biol. Technol.* 48, 132–138. doi: 10.1016/j.postharvbio.2007.09.006

Saltveit, M. E. (1991). Prior temperature exposure affects subsequent chilling sensitivity. *Physiol. Plantarum.* 82, 529–536. doi: 10.1111/j.1399-3054.1991.tb02943.x

Sánchez, M. R. (2016). Evidence of programmed cell death in degradative processes in banana fruit (Musa spp., AAA group, Cavendish subgroup) during ripening, over-ripening and after exposure to abiotic stress. PhD dissertation. Gainesville, FL: University of Florida.

Selvarajah, S., Bauchot, A. D., and John, P. (2001). Internal browning in cold-stored pineapple is suppressed by a postharvest application of 1-methylocyclopropene. *Postharvest Biol. Technol.* 23, 167–170. doi: 10.1016/S0925-5214(01)00099-0

Sisler, E. C., Dupille, E., and Serek, M. (1996a). Effect of 1-methylcyclopropene and methylenecyclopropene on ethylene binding and ethylene action on cut carnations. *Plant Growth Regul.* 18, 79–86. doi: 10.1007/BF00028491

Sojo, M. M., Nunez-Delicoda, E., Sanchez-Ferrer, A., and Garcia-Carmona, F. (2000). Oxidation of salsolinol by banana pulp polyphenol oxidase and its kinetic synergism with dopamine. *J. Agric. Food Chem.* 48, 5543–5547. doi: 10.1021/jf000293f

Thompson, A. K. (2011). "Banana (Musa spp.)," in *Postharvest Biology and Technology of Tropical and Subtropical Fruits. Vol. 2*. ed. E. M. Yahia (Cambridge, UK: Woodhead Publishing), 216–244e.

Trejo-Márquez, M. A., and Vendrell, M. (2010). Physiological response to chilling temperature of banana fruit (*Musa acuminata* cv. Dwarf cavendish). *Acta Hort.* 864, 387–393. doi: 10.17660/ActaHortic.2010.864.53

Villalta, A. M., and Sargent, S. A. (2004). Response of beit alpha-type cucumbers (*Cucumis sativus* L., 'Manar') to continuous ethylene exposure. *Proc. Fla. State Hort. Soc.* 117, 368–372.

von Loesecke, H. W. (1950). *Bananas. 2nd Edn*. New York, NY: Interscience, 52–66.

Watkins, C. B. (2006). The use of 1-methylcyclopropene (1-MCP) on fruits and vegetables. *Biotechnol. Advances* 24, 389–409. doi: 10.1016/j.biotechadv.2006.01.005

Watkins, C. B. (2008). Overview of 1-methylcyclopropene (1-MCP) trials and uses for edible horticultural crops. *HortScience* 43, 86–94. doi: 10.21273/HORTSCI.43.1.86

Yang, S. F., and Hoffman, N. E. (1984). Ethylene biosynthesis and its regulation in higher plants. *Ann. Rev. Plant physiol.* 35, 155–189. doi: 10.1146/annurev.pp.35.060184.001103

Zhang, J., Huang, W., Pan, Q., and Liu, Y. (2005). Improvement of chilling tolerance and accumulation of heat shock proteins in grape berries (*Vitis vinifera* cv. Jingxiu) by heat pretreatment. *Postharvest Biol. Technol.* 38, 80–90. doi: 10.1016/j.postharvbio.2005.05.008