FINAL PROJECT REPORT
WTFRC Project Number:

Project Title: Ethylene ripening of pears by unconventional means

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Budget History:

Item	Year 1:	Year 2:	Year 3:
R&D Fees	26,240	32,000	30,200
Equipment	400	7,000	4,000
Supplies	500	1,500	8,000
Travel	2,860	7,200	10,000
Accommodation in US		1,600	2,000
Cool store fees		600	600
Taste panel and associated costs at NCSU			5,000
Miscellaneous			
Total	30,000	49,900	59,800

FINAL PROJECT REPORT

Project title:	**Ethylene ripening of pears by unconventional means**
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OVERALL PROJECT GOAL

This project aimed to test the potential of unconventional approaches to ethylene conditioning to expand the market window for winter pears, particularly 'Green Anjou'. This involved firstly confirming the reported need over the first month of storage for more prolonged and elevated exposures to ethylene than are practical using conventional conditioning methods. That knowledge has then been applied in testing the usefulness of a prototype Ethylene Release capsules (ERCs) as a viable alternative means of achieving optimal conditioning without requiring expensive conditioning facilities.

OBJECTIVES FOR 2005-06:

- Continue to determine the influence of ethylene concentration and length of conditioning period at 20°C (68°F) on subsequent softening and aroma production by 'Green Anjou' (in USA) and 'Comice' (in New Zealand) after one and 3 weeks of cold storage. (This included work carried out in March 2006, which is the main focus of this report).
- Test the use of ERCs for pre-conditioning 'Green Anjou' in boxes immediately prior to and during transport to the East Coast. Conditioned fruit to be compared in terms of eating quality and cosmetic attributes with fruit given the current industry standard conditioning, after all have been further ripened to a similar extent upon arrival. (This was completed in October 2005 and was the focus of our previous report).

Significant findings during the entire three year project:

In our experience, the following observations and conclusions apply to the conditioning of early storage (i.e. within the first month after harvest) Green Anjou in the US and Comice in New Zealand, provided they have been harvested at normal commercial maturity and are free of disorders.

Effects of ethylene concentration

- Warming alone was never sufficient to condition early season fruit adequately, unless they exhibited a high incidence of cork pit. Externally supplied ethylene was normally vital for success.
- Levels as low as 2 ppm ethylene produced definite stimulation of ripening (based on both firmness and aroma).
- Full softening was triggered by lower levels of ethylene than those required to trigger full aroma production. Effects on softening plateaued at about 10 ppm but to trigger full aroma potential required >100 ppm for Anjou, and >25 ppm for Comice.
- Higher levels of ethylene during conditioning resulted in a greater proportion of the fruit becoming autocatalytic (producing their own ethylene) after subsequent ripening.

Effects of temperature during conditioning

- Ethylene conditioning at 7°C had a significant positive effect on fruit capacity to subsequently soften and produce aroma (particularly the latter). However, conditioning at 20°C was markedly more effective than conditioning at 7°C in both respects.

Effects of length of conditioning period

- Longer periods of ethylene conditioning (e.g. 5 days) were more reliable than 3 days in triggering the capacity for aroma production. Shorter conditioning periods resulted in slower rates of aroma release during ripening. A single day of conditioning in ethylene often resulted in fruit that were capable of softening acceptably but produced little or no aroma.

Influence of period in cold storage

- Anjou and Comice became progressively less dependent on external ethylene with increased time in cold storage, and increasingly capable of producing their own ethylene.
- By around 3 weeks (Comice) and 5 weeks (Anjou) after harvest, ethylene conditioning no longer increased the capacity to soften, but still enhanced aroma production potential.

Usefulness of ERCs as a method of conditioning

- ERC prototypes were capable of producing and maintaining levels of ethylene sufficient to simply and effectively condition early season pears in a range of packaging, including clamshells, Euro-boxes and bushel boxes, using conventional perforated apple box liners.
- A half-pallet of cold 'Green Anjou' at two weeks after harvest, conventionally wrapped and packed in standard cartons and Euro-packs and sealed under a disposable pallet cover, was conditioned effectively and reasonably uniformly with ERCs in 5 days at ambient temperature.
- Conditioning of early season 'Green Anjou' using ERCs inside conventional cartons and Euro-packs for one day at ambient temperature, followed by gradual cooling before and during trucking across America with the ERCs still in place, resulted in a greater ripening potential and more aromatic and flavorsome fruit (according to a taste panel) than did standard one day forced air ethylene conditioning of pre-warmed fruit in a trailer or three days of warming without ethylene.

Results and discussion

The following section focuses on results from aspects of the first objective of the last year of this project that could not be included in our 2006 report. We then discuss these in relation to results and conclusions from earlier work in this project and by other research groups.

ERC-conditioning of early season US Anjou in clamshells; effects on fruit quality and aroma production

In 2006 we reported on a conditioning trial conducted in the fall of 2005 that tested the use of ERCs for pre-conditioning 'Green Anjou' in boxes immediately prior to and during transport to the East Coast. Conditioned fruit were compared in terms of eating quality and cosmetic attributes with fruit given the current industry standard conditioning, after ripening upon arrival. Early season Anjou were used in this trial, since such fruit, which have not had their chilling requirement satisfied, present the greatest challenge to condition effectively. It is particularly difficult to initiate full flavor and aroma development. These important aspects of fruit ripening are often neglected since they are more difficult to measure than softening.

In order to be able to easily monitor the effects of conditioning by ERCs on aroma development, one aspect of the 2005 shipping trial involved packing fruit into ripeSense® 4-piece clamshells containing sensor labels that change color in response to the accumulation of ripening-related aromas inside the clamshell. 'Green Anjou' pears used in this work were picked near Peshastin WA on 14 September

2005, graded into cherry bins and placed in storage at -1°C (30^{0}F). Cold fruit (90 ct) were then packed into ripeSense® 4-pack clamshells on 21, 23 and 25 September and conditioned at ambient temperatures (18-20°C) in a packing house until 26 September, when they were returned to the cold store and subsequently shipped to Raleigh, NC, where they arrived on 5 October. Controls comprised fruit that were packed in clams lacking ERCs, which were conditioned simply by warming to room temperature alone for three days, and others that were given no conditioning whatsoever. There were 50 clamshells per treatment, packed in commercial display boxes during conditioning and shipping.

Ethylene levels in clamshells containing ERCs were in the 50-100 ppm range during the conditioning period at room temperature in Wenatchee. Upon arrival in Raleigh ten days later, ethylene was still present, at levels around 5 ppm, in the clamshells containing ERCs. A trace (0.4 ppm) was found in the control set that had been warmed for three days without ERCs, while no ethylene was present around the control set of fruit in clamshells that had not been given any form of conditioning.

During ripening at 20°C (68°F), aroma and firmness were monitored for each of the clamshell treatments (Fig. 1). The fruit that had been conditioned with ERCs in their clamshells (Fig. 1 C & D) produced much more aroma and softened more rapidly than those of the control group that had just been warmed (B) or that had received no conditioning whatsoever (A). Fruit quality from a cosmetic perspective was perfectly acceptable in the clams that had been given 1 and 3 days conditioning with ERCs at room temperature (C & D), with less than 2% affected by bruising sufficient to render them unsaleable. This was despite arriving in Raleigh at 8 lb and 4.5 lb firmness respectively. The treatment that received 5 days conditioning with ERCs (not shown) exhibited 12% damaged rejects on arrival, presumably attributable to their soft state in transit (6 lb when shipped, 3 lb upon arrival).

Influence of ethylene concentration and length of conditioning period on subsequent ripening of early season New Zealand Comice

Harvest, storage and conditioning. Comice were picked near Wanganui, New Zealand on 15-16 February 2006 at normal commercial harvest maturity (average firmness of 6.02 kg, starch pattern index average of 0.65, average brix of 11.5%) and kept in cool storage -0.5°C (30°F) for two weeks. Fifteen samples of 50 fruit were then placed in perforated plastic bags and transferred to 20°C (68°F) on 27 February. After equilibrating for 24 h, ERCs in varying numbers (1, 2, 4 or 8) were added to the bags in triplicate. As controls, three bags did not have ERCs. Temperature tracking devices (iButton®) were placed inside fruit in a selection of the treatments. All the bags were then enclosed in cardboard boxes. These were not air tight but served to protect the bags from drafts. Ethylene concentrations inside the liners within the boxes were monitored daily using a Drager ethylene meter (calibrated against a gas chromatograph) throughout the conditioning period. After 24 hours of conditioning at 20°C, on 1 March one box of fruit from each triplicated treatment was returned to cold storage at -0.5°C, followed by the second and the third boxes after 72 and 120 hours (3 and 5 days) of conditioning respectively. All ERCs were removed from the boxes just prior to their return to cold storage.

Ripening after a short intervening period (4-8 days) of cold storage. On 9 March (4-8 days after being returned to cold storage, depending on the conditioning period), four ripeSense® clamshells containing aroma sensing labels were filled with fruit from each of the 15 boxes in the cold store, and a further four with fruit that had received no conditioning treatment. The filled clamshells were transferred to 20°C where the fruit were allowed to ripen. Daily measurements were made of ethylene, carbon dioxide and oxygen concentrations in the headspace of each clamshell, and colors of the aroma sensing labels were recorded relative to the standard ripeSense® eight point color scale. In order to monitor changes in firmness non-destructively, a bench top Sinclair IQ tester was used. The fruit were thus briefly removed from the clamshells each day to permit this measurement,

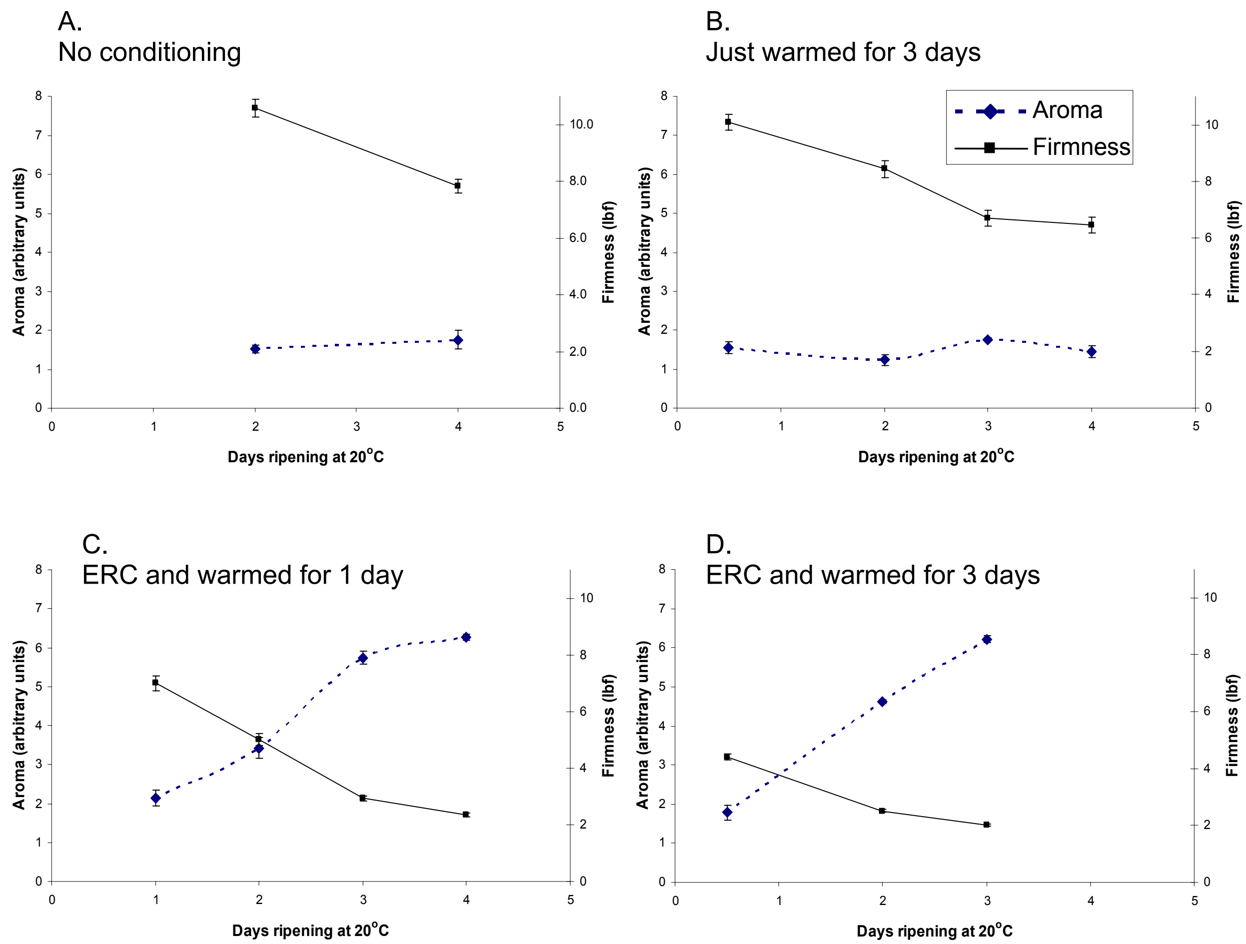

Figure 1 Effects of ethylene from an ERC inside a 4-pack ripeSense® clamshell on the rate of softening and aroma production by early season US Green Anjou pears. One ERC was added to each clamshell when pears, harvested one week earlier, were packed. Resulting ethylene levels inside the clamshells were 50 – 100 ppm. The packed clamshells were left to warm at 20°C for 1 day (C) and 3 days (D) before re-cooling and shipment by truck to North Carolina under normal commercial conditions, followed by ripening at 20°C. Control groups of fruit packed in clamshells without ERCs were either given no conditioning whatsoever (A), or were simply warmed for 3 days without any source of artificial ethylene (B).

after the head space gases had been sampled. Firmness was finally assessed destructively, using a GUSS Fruit Texture Analyser, at staggered times for each conditioning treatment, so that fruit in each treatment had been exposed to 20°C a total of 250 hours (just over 10 days) including both the conditioning and the post storage ripening periods.

The need for prolonged ethylene treatment during conditioning of Comice at two weeks from harvest, if the fruit are to be marketed shortly afterwards, is clearly evident from data summarized in Figures 2, 3 and 4. Fruit exposed to ethylene for 3 days during conditioning produced significantly more aroma (Fig 2B-D), ethylene (Fig 2F-H) and CO_2 (Fig 3B-D) than those given just 1 day of ethylene, even when all were given the same total time at 20°C, including conditioning and subsequent ripening. Prolonged ethylene treatment beyond 3 days had little additional impact on fruit capacity to eventually produce ethylene (Fig 2F-H) and CO_2 (Fig 3B-D), or to soften (Fig 4), but brought about significant further increases in aroma production capacity when ethylene was supplied at concentrations of 13-50 ppm (Fig 2B-C).

A single day of conditioning produced significant changes in ethylene production (Fig 2 F-H) and firmness (Fig 4), but had little or no significant effects on aroma (Fig 2B-D) or CO_2 (Fig 3B-D) production relative to controls (Figs 2A and 3A) during ripening.

Control fruit (simply warmed without external ethylene for one, three or five days during conditioning), produced only trace amounts of aroma (Fig 2A), ethylene (Fig 2E), maintained flat baseline levels of respiratory CO_2 (Fig. 3A) and remained markedly firmer than any of the conditioned fruit (Fig. 4), even after a total of 10 days at 20°C (which includes the conditioning period). The low level of ethylene (1 ppm) that was detected within the control clamshells during conditioning was evidently insufficient to trigger subsequent autocatalytic ethylene production, which is likely to be involved in activating and accelerating other ripening related changes.

Autocatalytic ethylene production was evident as soon as fruit that had been conditioned with ethylene for 5 days were returned to 20°C, and commenced 5 and 8 days later in fruit conditioned for 3 and 1 days respectively (Fig 2F-H).

Increasing the concentration of ethylene from around 13 ppm to around 25 ppm (achieved with two ERCs, data not shown) enhanced aroma production, particularly for the 3 and 5 day exposures, but had no effect on firmness and ethylene production after a total of 10 days at 20°C. Further increases in ethylene concentration to around 50 and 85 ppm (4 and 8 ERCs respectively) produced no greater effects on any of the Comice ripening responses monitored after a total of 10 days at 20°C than those produced by 25 ppm ethylene (data not shown).

Ripening after a longer intervening period (11 weeks) of cold storage On 16 May, after 11 weeks of storage at -0.5°C following conditioning at two weeks after harvest, ethylene levels inside the boxes containing the conditioned fruit in the cold room were measured and rot incidence assessed. Most of the rots (14 out of the total of 19 rotten fruit across all treatments) occurred amongst fruit that had been conditioned for 5 days in ethylene. The remaining sound fruit from each box were put into 15 sets of eight clamshells and permitted to ripen at 20°C. A further set was filled with fruit that had received no conditioning. During this second ripening test, firmness was monitored destructively almost every day during ripening. Aroma was monitored daily in each clamshell but ethylene, CO_2 and O_2 measurements were restricted to early in the ripening period.

Ethylene was detected in all the boxes after 11 weeks while still in cold storage. The lowest levels (5-10 ppm) were found in the boxes of fruit that had simply been warmed for 1-5 days, without ethylene from ERCs. The boxes of ethylene conditioned fruit contained ethylene in the 10-40 ppm range, with the highest levels generally occurring in those boxes that had been conditioned longest (data not shown). Ethylene concentrations in individual boxes did not closely reflect the incidence of rots.

Ethylene was produced in considerable quantities during the first day of ripening by fruit of all treatments, including controls. Levels in the 60-90 ppm range were detected inside all clamshells containing conditioned fruit, in no apparent relationship with period of conditioning nor concentration of ethylene in the range 13-85 ppm during conditioning (data not shown). Those containing control fruit that had received no conditioning, or had been conditioned by warming alone, were significantly slower to produce ethylene (20-40 ppm inside the clamshells after one day of ripening), but all treatments were clearly autocatalytic at this stage.

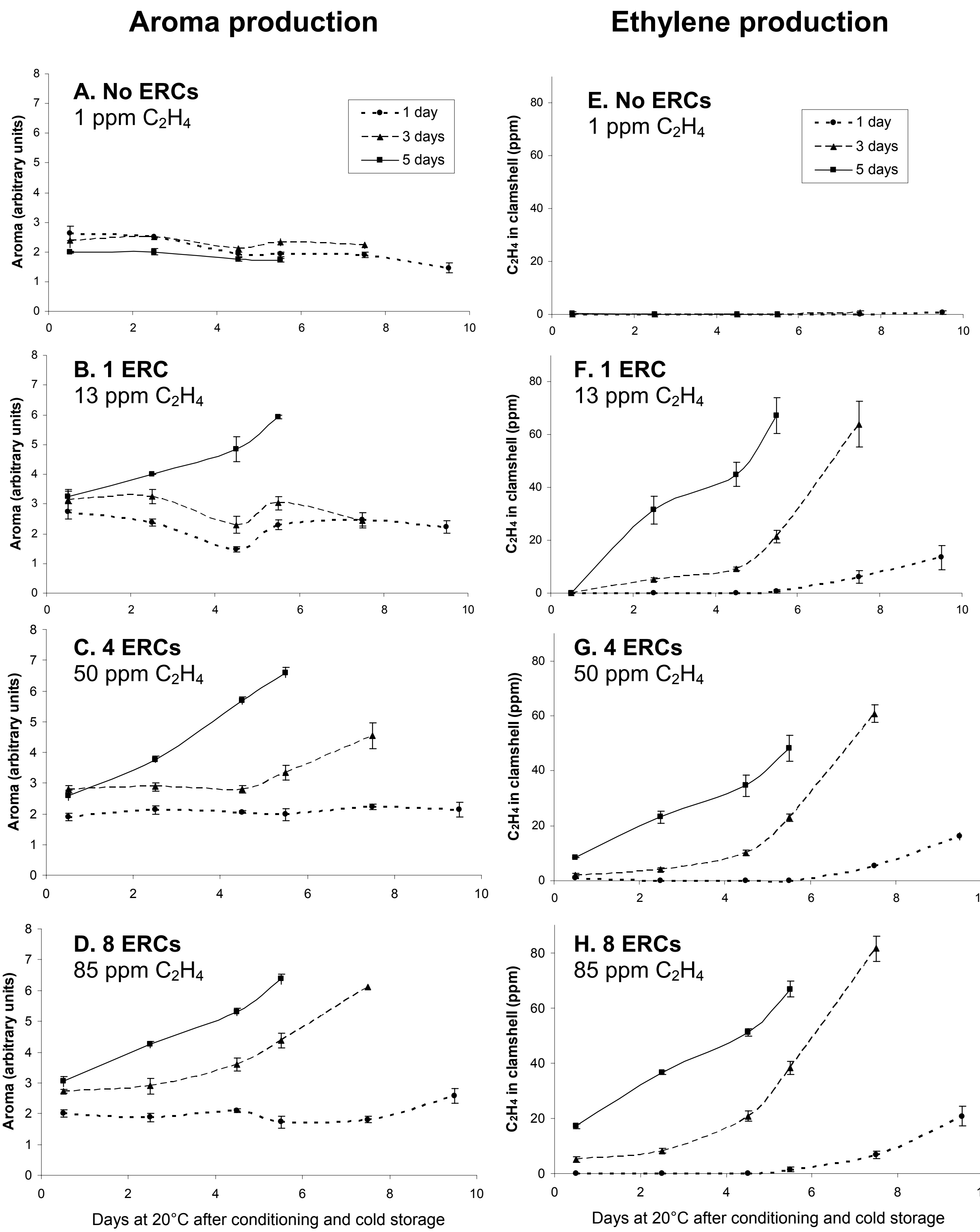

Figure 2. Influence of ethylene concentration and period of exposure during conditioning of NZ Comice pears two weeks after harvest at 20°C on the production of aroma and ethylene during subsequent ripening following a short intervening cold storage period of 4-8 days. To supply ethylene, various numbers of ERCs, as shown, were included with each batch of 50 fruit inside a perforated bag within a cardboard box.

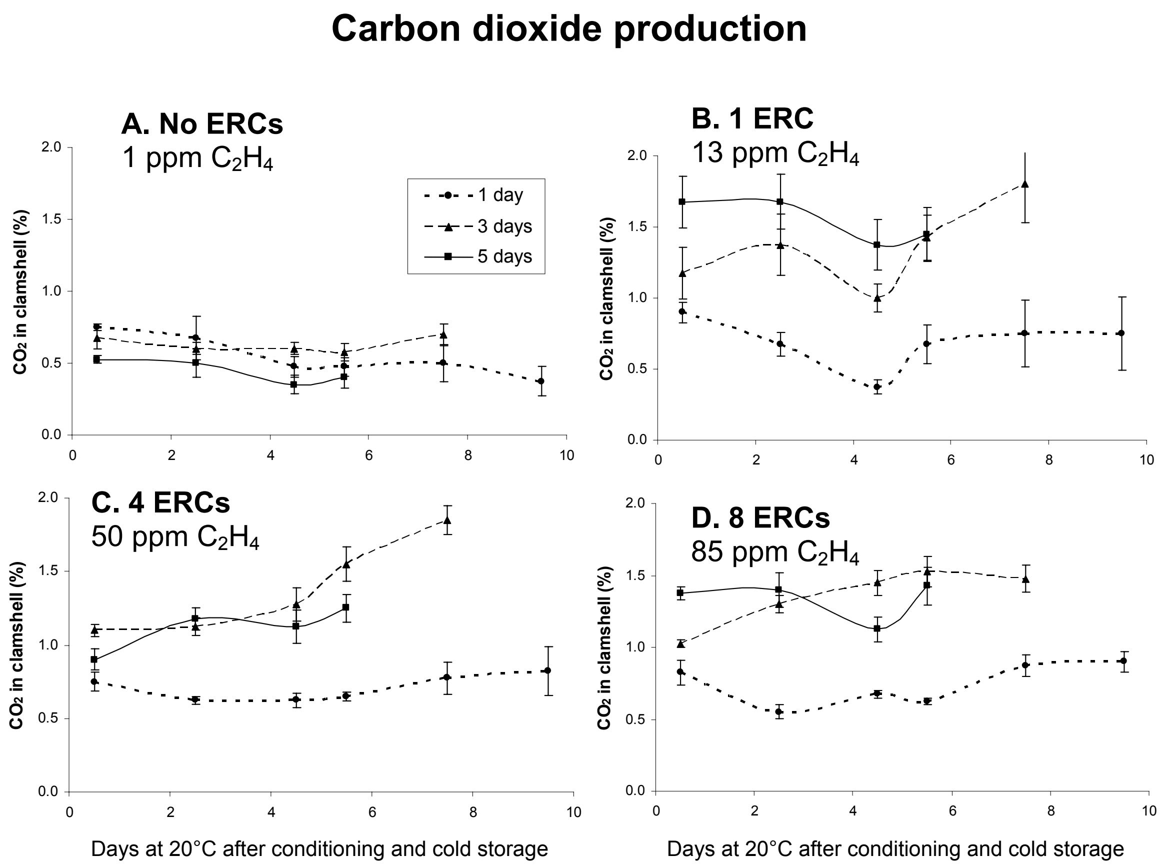

Figure 3. Influence of ethylene concentration and period of exposure during conditioning of NZ Comice pears two weeks after harvest at 20°C on the production of carbon dioxide during subsequent ripening following a short intervening cold storage period of 4-8 days. To supply ethylene, various numbers of ERCs, as shown, were included with each batch of 50 fruit inside a perforated bag within a cardboard box.

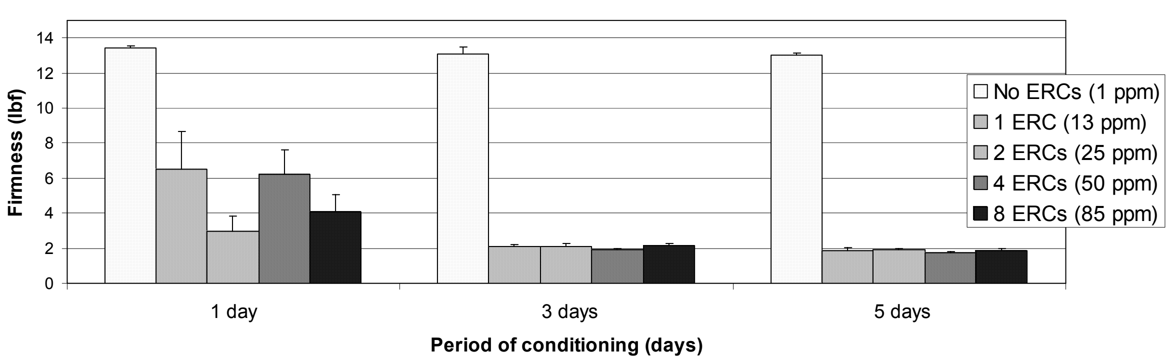

Figure 4. Influence of ethylene concentration and period of exposure during conditioning of Comice pears two weeks after harvest at 20°C on fruit firmness after a total of 10 days at 20°C (including conditioning) following a short intervening cold storage period of 4-8 days. To supply ethylene at different concentrations, various numbers of ERCs, as shown, were included with each batch of 50 fruit inside a perforated bag within a cardboard box.

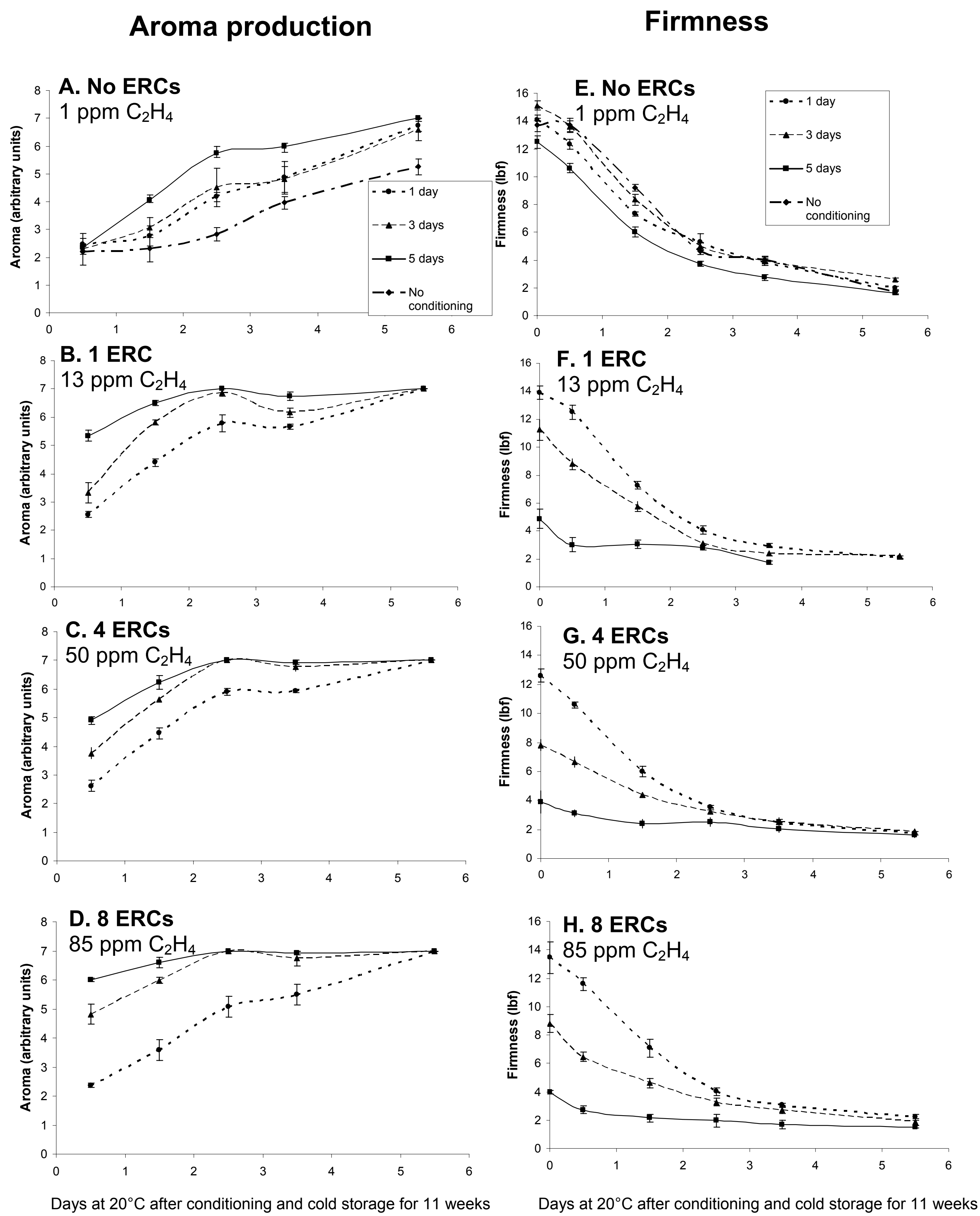

Figure 5. Influence of ethylene concentration and period of exposure during conditioning of Comice pears two weeks after harvest at 20°C on the production of aroma and firmness during subsequent ripening following an intervening cold storage period of 11 weeks. To supply ethylene, various numbers of ERCs, as shown, were included with each batch of 50 fruit inside a perforated bag within a cardboard box.

Aroma production was initially much more rapid in fruit that had been conditioned for 3 or 5 days than in those that had received only one day of ethylene conditioning (Fig 5B-D), or that had simply been warmed (Fig 5 A). This initial rate of aroma production was inversely related to the initial firmness of the fruit of the various treatments (Fig 5A-D vs. E-H). Initially large differences in aroma and firmness between fruit of the various conditioning treatments had all disappeared by the end of the 5 day ripening period (Fig 5). The fruit that had received no conditioning whatsoever remained significantly less aromatic than the rest after 5 days of ripening, but were indistinguishable in terms of firmness at that point (Fig 5 A and E).

In summary, the effects of conditioning fruit at two weeks and then returning the fruit to storage were very dependent on when the fruit were subsequently ripened. In the first part of the experiment, in which ripening potential was assessed just a week after returning fruit to cold storage, differences caused by the various conditioning treatments persisted, and in some cases became more accentuated, during the ripening period (Figs 2 and 3). In contrast, during the second evaluation of the ripening potential of the same batches of conditioned fruit that had been stored for a further 11 weeks, differences in firmness and aroma production attributable to the various conditioning treatments were immediately apparent upon returning to 20°C, but rapidly diminished during the course of ripening. Presumably this was due to the over-riding effects of autocatalytic ethylene production that was occurring in all fruit in the latter ripening experiment only.

General discussion of early season conditioning of US Anjou and NZ Comice

Prolonged ethylene treatments lasting 3-4 days were found to be required by Anjou during the first month after harvest in order to induce the capacity to ripen fully (Chen *et al.*, 1996, Facteau and Mielke, 1998). Assessment of the capacity to ripen fully has previously normally been based primarily on firmness measurements, sometimes accompanied by measurement of free juice. Aroma development, which impacts strongly on flavor, can now be easily monitored continuously through the use of ripeSense® sensors (www.ripeSense.com). Using this approach we have shown on several occasions that treatments that are sufficient to induce full softening of Anjou pears may not necessarily be sufficient to trigger release of the full aroma potential, particularly early in the season for pears that have a chilling requirement (e.g. Figs 3B and 3D in our 2005 report to the NW Pear Research Review, comparing effects of 3 days vs. 6 days exposure of early season Anjou to various levels of ethylene at 20°C).

Similarly, for NZ Comice during the first month after harvest, we have sound that short conditioning treatments (e.g. one day) may trigger the capacity to soften and produce ethylene without necessarily triggering aroma production. Longer ethylene treatments of 3 to 5 days were more likely to coordinate acceptable aroma production with softening. Since such long periods of ethylene treatment are logistically and economically challenging for normal commercial practice, it is not surprising that Comice has been claimed to not normally achieve its optimum flavor intensity until after more than two months of cold storage (Elgar *et al*., 1997). A recent report by Sugar and Basile (2006), aimed at opening up the early season market to Comice, reported that 3 days of 100 ppm ethylene at 20°C was sufficient to reduce the postharvest chilling requirement to just three days. Aroma was not monitored, but the eating quality was reported to range narrowly from fair to good, without a clear association between treatment and eating quality. Juiciness and texture of these early season Comice that had been conditioned and ripened were reported as generally acceptable, but flavor "was equivalent to that of early season Comice" (i.e. weaker than optimal). Perhaps an even longer ethylene treatment than three days is required to induce optimal flavor in these very early fruit?

We have also attempted to investigate the threshold limits of concentration for ethylene to be effective. In the 2004 NW Pear Research Review we reported that exposure to levels as low as 2 ppm for 7 days at 20°C produced definite stimulation of ripening of US Anjou. Interestingly, the effects on softening of increasing ethylene concentrations plateaued at about 10 ppm, whereas the effects on aroma production were still continuing to increase at 100 ppm. Our results obtained this year with Comice in New Zealand suggest a similar pattern of response to increasing ethylene, but for this fruit little appears to be gained by exceeding 25 ppm ethylene during conditioning.

Wang *et al* (1972) conducted an extensive investigation of the influence of a wide range of ethylene concentrations on ripening, assessed in terms of firmness, ethylene production and respiration rate. They reported threshold ethylene levels to induce ripening were between 0.5 and 2 ppm, depending on maturity. They also found that the concentrations of ethylene and the times required to initiate a response were different for respiration, ethylene production and softening. Their data (and of others cited by them) indicate that softening of pears occurs prior to, and independently of, development of the climacteric rise in respiration. They did not monitor aroma volatile production, which may be another distinct physiological process that can be initiated independently by ethylene at various concentrations, provided other conditions are met. For example, for Anjou at 1 week after harvest, we found that exposure to >10 ppm ethylene for 7 days (but not 3 days) at 7°C had a marked impact on subsequent aroma production, but little effect on potential to soften (our 2004 report, p 97, Fig. 1 parts D, G and H). This interesting observation probably merits repetition and further investigation, since it is the reverse of the normal order of effects of increasing ethylene concentration and exposure period at higher temperatures, in which softening is more easily triggered than aroma production.

The first year of our investigations involved individual fruit in sealed jars, into which ethylene could be precisely injected to achieve the desired range of concentrations. Our studies of the ethylene concentration requirements in the second and third years of this project involved less precise control of the concentration, since we employed ERCs to deliver the ethylene into a ventilated clamshell or polyliner box environment. The advantages of this approach were (i) more replication became possible, since the method was less labor intensive; and (ii) it served to pave the way for commercial application of the ERC technology in the future. As described in most detail in our 2006 report, the ERC technology has been shown to provide a potential alternative method to condition fruit without the need for a controlled conditioning room. This should allow (1) longer-term conditioning at no extra cost (2) greater flexibility in coping with bottle-necks in the conditioning chain and (3) the possibility of conditioning individual pallet loads of packaged fruit taken directly from cold-storage. Our 2006 FPC project is to evaluate the potential of the ERC technology for conditioning full pallet lots of Green Anjou, since all our previous research has been on a smaller scale.

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