**Report on performance of Bluapple technology for storage of fruits and vegetables.**

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**Summary**

*Bluapples stored with fruits and vegetables were found to significantly reduce ripening and softening. The benefits were greatest for less ripe fruit and lowest for ripe and overripe fruit. While the response varied between fruit improvements in storage life from 2 to 4 days can be found for fruit stored in fruit bowls, with the biggest effect being for limes. For vegetables stored in refrigerator crispers, again differences between vegetables were observed, with improvements in storage live ranging from 1 to 6 days. The biggest benefits were seen for delaying yellowing of broccolini and for reducing rots of mini-cucumbers.*

*It was found that Bluapples can reduce typical ethylene levels found around stored fruit and vegetables to close to zero within several hours. The ethylene absorbance capacity of a bluapple was calculated as being about 600 mls of ethylene gas. This is equivalent to the level of ethylene produced by 10kg of fully ripe apples at room temperatures after 100 days.*

This report was requested by Biofarm Artel who provided packages of Bluapples for use in the testing. The benefit of Bluapples for fruit and vegetables is based on the absorbtion by Bluapples of the fruit ripening gas ethylene, and consequently a slow down in ripening or overripening of fruit and vegetables. The testing was designed to assess the performance of Bluapples in a fruit bowl at room temperatures and in a fruit/ vegetable crisper in a refrigerator. Testing was done using six 10L plastic boxes with a clip-on lids stored in a large refrigerator at 4 $\pm$1°C and with six 11L glass bowls stored in a controlled temperature room at 22 $\pm$2°C. Three containers contained fruit and vegetables alone, with the other three containers containing fruit and vegetables with a Bluapple.

Testing was done frequently during storage and fruit and vegetables were assessed for firmness and quality using objective scoring scales. Photos were also taken for each examination were also taken under carefully controlled conditions for each examination. These photos were analysed and converted into scientifically used colour values for each fruit or vegetables to allow precise measurements of colour changes over time (see Appendix for details).

Testing was also carried out of the effectiveness of Bluapples at absorbing ethylene and in order to assess the ethylene absorbing capacity of each Blueapple.

Fig 1.  The containers used for bluapple testing.




**Fruit Bowl Results**

The fruits used in these tests were bananas, mangoes, avocadoes and limes.  They were obtained at the Flemington Markets shortly after harvest in as green a condition as possible. The results shown in the figures are in different colours for each fruit and the control fruit is represented by a solid line and symbols and the fruit stored with a Bluapple by a broken line and open symbols.

Bluapples had a highly significant effect on slowing the ripening of fruit as shown by colour changes (high colour values are greener and lower colour values are more yellow/orange) slower) and also by slowing the softness of fruit.  The overall biggest effect of Bluapples was seen in slowing the yellowing limes.  The benefit of Bluapples was greatest for less ripe fruit and it was least for fruit that were already or almost fully ripe when placed in the fruit bowl. While the effects in the fruit bowl varied depending on the type of fruit and its ripeness, on average Bluapples in a bowl resulted in an extra 1 - 3 days of storage, with an extra 3 - 5 days storage for limes (see Table 1)

Figure 2  Quality changes of fruit stored in a fruit bowl at 22°C.
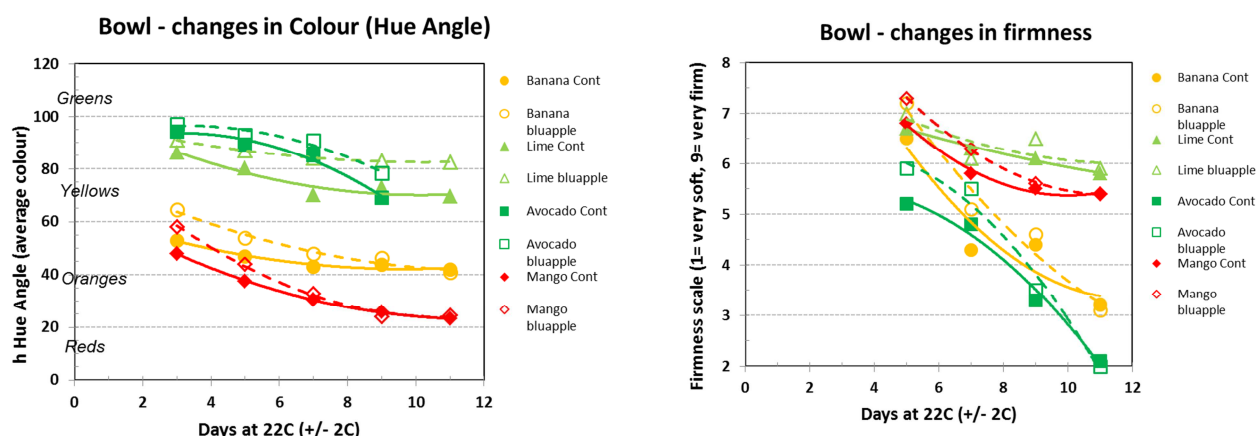



The benefits of Bluapple in a fruit bowl can maximized by using a more enclosed shape bowl and especially by regular removal of ripe fruit and any with damage or decay.  This allows the less ripe fruit to get the maximum benefit of the ethylene removal by the Bluapples.  Such removal could be ideally done by regularly eating or using the riper fruit.

Table 1  Summary of the overall effects of Bluapples on storage life of fruit and vegetables.

| Fruit /vegetables | Significant* extra days | Reduced | | |
|---|---|---|---|---|
| | | colour change | softening | rots |
| **Bowl (22°C)** | | | | |
| Avocado | 1 – 2** | ▓ | ▓ | |
| Banana | 1 – 3 | ▓ | ▓ | |
| Lime | 3 – 5 | ▓ | ▓ | |
| Mango | 1 | ▓ | ▓ | |
| **Crisper (4°C)** | | | | |
| Broccolini | 4 – 6 | ▓ | | |
| Dill | 1 – 2 | ▓ | | |
| Mini-cucumber | 2 – 4 | | | ▓ |
| Plum | 0 | | | |
| Truss tomato | 1 – 2 | ▓ | | |
| Tuscan cabbage | 0 | | | |

\*   Significant at the p<0.1 level

\*\* Lower limit for riper fruit/ vegetables, higher limit for less ripe fruit/ vegetables

## Crisper Results

The products tested used crisper conditions were mainly vegetables rather than fruit.  This was because at the time of the year the testing was done (mid-summer) fruit usually stored in the crisper such as apples and pears have been in storage almost a year before sale and reliable results are difficult to obtain with them.  Vegetables have much lower rates of ethylene production, therefore in order to have some ethylene present for any benefit of Bluapples to be seen, two ripe bananas were added, with one being replaced every 2-3 days.

The fruits and vegetables used in these tests were plums, truss tomatoes, broccolini, mini-cucumbers, dill and Tuscan cabbage. The wide range of colours from dark purple plums to dark green Tuscan cabbage meant that colour change results needed to split into two figures each. A major difference between these results and those from the fruit bowl is that changes under these cooler conditions are much slower.  Again there was a highly significant effect of Bluapples amongst the fruit and vegetables tested on colour changes.  The largest effect was on broccolini, with a delay in yellowing by 4- 6 days, smaller but significant effects on reducing colour changes by several days (1-3 days) were observed with dill and with truss tomatoes (measuring the quality of the truss stems), (see Table 1).

Figure 3  Quality changes  of fruit and vegetables stored in a crisper at 4°C



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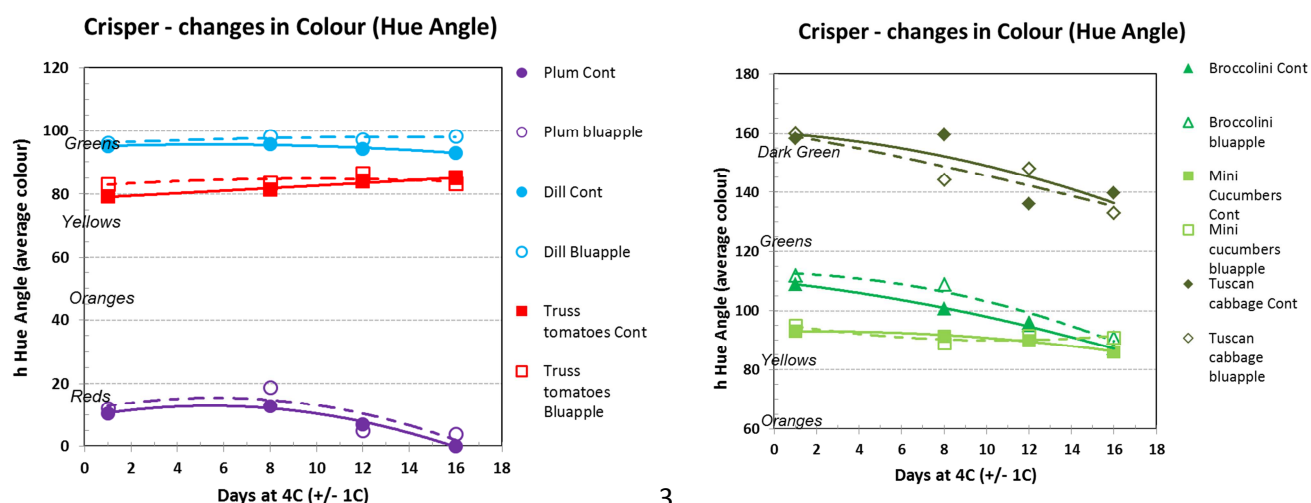
Significant effects of Bluapple on colour changes of plums, mini-cucumbers and Tuscan cabbage were not observed. However, if there had been higher ethylene levels in the crisper and if stone fruit for which green colour can be readily observed had been used, then a significant effect may well have been observed. Significant changes in colour lightness, rather than the type of colour due to Bluapples were observed for dill, tomatoes,and mini-cucumbers. The effect was especially noticeable for mini-cucumbers, with this change being due to a significant delay in decay by as much as 7 days.
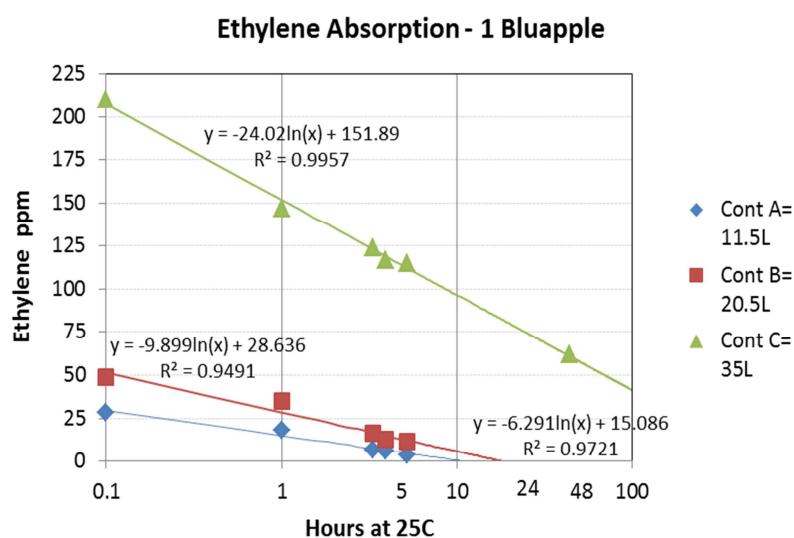
The benefits of Bluapples in the crisper would be likely to be considerably greater than those found here, if considerable quantities of fruit were also stored in the crisper, as fruit produce much higher concentrating of ethylene than vegetables. This situation is very likely to occur in the normal domestic refrigerator with fruits such as apples and pears.

**Ethylene Absorbtion Results**

Normal levels of ethylene in food storage areas would typically be well below 10ppm, while increases to ripening and decay can occur for ethylene levels as low as 0.3 ppm of ethylene. To test the Bluapples under very difficult conditions, levels of 25, 50 and 200 ppm of ethylene were used in large containers with capacities of 11.5, 20.5 and 35 litres respectively. The reduction of ethylene levels was seen to occur in a logarithmic manner, namely it started of rapidly and then became slower as levels of ethylene became lower. It can be seen that levels in the 25 and 50 ppm container rapidly reduced to unmeasurably low levels after about 8 and 15 hours respectively. For the extremely high level of 200ppm, a time period in excess of four days would be required for ethylene to approach zero. However, for levels below 10ppm typically found around fruit and vegetables, the reduction to almost zero would occur in a matter of hours.

Figure 4  Absorption of ethylene by a Bluapple.

The absorption capacity of each blue apple containing 10g of absorbent, based on these results and other tests done over much longer periods was calculated as being about 700 mls of ethylene. In order to give a relative idea of scale, it would take 10kg of fully ripe apples held at room temperatures about 100 days to produce this much ethylene. Since, this quantity of fully ripe fruit would never be stored for this long under room temperatures, replacement of Bluapples every three months would seem to be a sensible precaution.



Ethylene Absorption - 1 Bluapple

$y = -24.02\ln(x) + 151.89$
$R^2 = 0.9957$

$y = -9.899\ln(x) + 28.636$
$R^2 = 0.9491$

$y = -6.291\ln(x) + 15.086$
$R^2 = 0.9721$

Ethylene  ppm

Hours at 25C

Cont A= 11.5L
Cont B= 20.5L
Cont C= 35L

# Appendix

**Methods Used**

For each assessment digital photos were taken with Canon IXUS700 camera with flash and backlit with xenon floodlighting. All photos were analysed with Digimizer software (Digimizer Ver.4.2.2.0, MedCalc Software, Belgium) and then converted to RGB values for each fruit measured, with corrections for values of white and black from a colour card in each photo (see Figure Appendix 1). The RGB values from the photos were then converted to the scientifically defined CIE (Commission Internationale de l'Eclairage) colour values of L, a, b and then finally to the CIE colour values of L, C, h (Lightness (0-100), Chroma (0-100), hue angle (0-360°)). The L,C,h colour model is frequently used to scientifically describe colour changes in fruits, vegetables and plants. The conversion of values from RGB to L,C,h was done using the large and very comprehensive Excel Colour Conversion Spreadsheet model, produced by Gabor Boronkay, Colour Conversion Centre (4.0a) 2010 Budapest (http:// http://ccc.orgfree.com/ accessed January 2013), see Appendix Figure 2. The illuminance values used in the model to produce the colour conversions were the D50 illuminant and an observation angle of 10°. Fruit firmness was assessed on a 1-9 scale with 1 being totally soft, 4 soft eating ripe, 5 eating ripe, 6 firm eating ripe and 9 being very firm.

The results were analysed using the statistical package Minitab Version 16 (Minitab Inc., Pennslyvania, USA).

Figure Appendix 1  Typical photo taken for detailed colour evaluation.



| Mea... | Avg.... | Red ... | Gree... | Blue ... |
|--------|---------|---------|---------|----------|
| Rect... | 0.4523 | 0.6200 | 0.5420 | 0.1948 |
| Rect... | 0.4610 | 0.5769 | 0.5457 | 0.2604 |
| Rect... | 0.5473 | 0.7535 | 0.6346 | 0.2537 |
| Rect... | 0.1035 | 0.1035 | 0.0982 | 0.1087 |
| Rect... | 0.8966 | 0.9041 | 0.8975 | 0.8882 |

Figure Appendix 2.  Software used to convert image RGB values to CIE L, C, h values (Gabor Boronkay 2010)

## Conditions for Analysis of Ethylene

Analysis was done on a Gowmac Model 580 Gas Chromatograph, with a 2m Activated Alumina column and a Flame Ionisation Detector.  The nitrogen carrier gas flow was 40mls/min (300mls/min air and 30mls/min hydrogen).  Temperature conditions used were Injector 70°C, Column 100°C and Detector 120°C.

## Results of Statistical Analysis for Fruit in Bowls

Analysis of Variance for L, using Adjusted SS for Tests

| Source | DF | SeqSS | AdjSS | MS | F | P |
|---|---|---|---|---|---|---|
| Day | 3 | 1289.4 | 250.3 | 83.4 | 1.39 | 0.246 |
| Trt | 1 | 63 | 717.3 | 717.3 | 11.97 | 0.001 |
| Frt | 3 | 141742.9 | 126190 | 42063.3 | 701.72 | 0 |
| Day*Trt | 3 | 271.6 | 198.1 | 66 | 1.1 | 0.349 |
| Day*Frt | 9 | 3286.4 | 3334.5 | 370.5 | 6.18 | 0 |
| Trt*Frt | 3 | 2757.2 | 2700.3 | 900.1 | 15.02 | 0 |
| Day*Trt*Frt | 9 | 461.7 | 461.7 | 51.3 | 0.86 | 0.566 |
| Error | 226 | 13547.1 | 13547.1 | 59.9 | | |
| Total | 257 | 163419.2 | | | | |

S = 7.74229   R-Sq = 91.71%   R-Sq(adj) = 90.57%

Analysis Of Variance for C, using Adjusted SS for Tests
C

| Source | DF | SeqSS | AdjSS | MS | F | P |
|---|---|---|---|---|---|---|
| Day | 3 | 1805.7 | 1188.4 | 396.1 | 10.78 | 0 |
| Trt | 1 | 0.7 | 267.2 | 267.2 | 7.27 | 0.008 |
| Frt | 3 | 75844 | 66874 | 22291.3 | 606.85 | 0 |
| Day*Trt | 3 | 255.1 | 166.8 | 55.6 | 1.51 | 0.212 |
| Day*Frt | 9 | 5429.6 | 5468.8 | 607.6 | 16.54 | 0 |
| Trt*Frt | 3 | 839.3 | 797.2 | 265.7 | 7.23 | 0 |
| Day*Trt*Frt | 9 | 190.7 | 190.7 | 21.2 | 0.58 | 0.816 |
| Error | 226 | 8301.6 | 8301.6 | 36.7 | | |
| Total | 257 | 92666.6 | | | | |

S       =       6.06074   R-Sq   =       91.04%  R-Sq(adj)       =       89.81%

Analysis Of Variance for h, using Adjusted SS for Tests
h

| Source | DF | SeqSS | AdjSS | MS | F | P |
|---|---|---|---|---|---|---|
| Day | 3 | 11163.5 | 12718.3 | 4239.4 | 82.79 | 0 |
| Trt | 1 | 660.7 | 1667.7 | 1667.7 | 32.57 | 0 |
| Frt | 3 | 103824.7 | 96649.2 | 32216.4 | 629.14 | 0 |
| Day*Trt | 3 | 108.5 | 83.1 | 27.7 | 0.54 | 0.655 |
| Day*Frt | 9 | 3685.5 | 3461.5 | 384.6 | 7.51 | 0 |
| Trt*Frt | 3 | 575.3 | 528.6 | 176.2 | 3.44 | 0.018 |
| Day*Trt*Frt | 9 | 1252.6 | 1252.6 | 139.2 | 2.72 | 0.005 |
| Error | 226 | 11572.8 | 11572.8 | 51.2 | | |
| Total | 257 | 132843.7 | | | | |

S       =       7.15591   R-Sq   =       91.29%  R-Sq(adj)       =       90.09%

**Results of Statistical Analysis for Vegetables and Fruit in Refrigerator Crispers**

Analysis Of Variance for L, using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Day | 3 | 697.1 | 749.7 | 249.9 | 6.04 | 0.001 |
| Trt | 1 | 14.2 | 3.2 | 3.2 | 0.08 | 0.78 |
| Frt | 5 | 87958 | 87291.6 | 17458.3 | 421.98 | 0 |
| Day*Trt | 3 | 59.2 | 37.2 | 12.4 | 0.3 | 0.826 |
| Day*Frt | 15 | 1579.6 | 1667.4 | 111.2 | 2.69 | 0.001 |
| Trt*Frt | 5 | 635.5 | 693.1 | 138.6 | 3.35 | 0.006 |
| Day*Trt*Frt | 15 | 808.1 | 808.1 | 53.9 | 1.3 | 0.198 |

| | | | | | |
|---|---|---|---|---|---|
| Error | 372 | 15390.4 | 15390.4 | 41.4 | |
| Total | 419 | 107142.1 | | | |

S = 6.43212    R-Sq = 85.64%    R-Sq(adj) = 83.82%

Analysis Of Variance for C, using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Day | 3 | 931.6 | 768.6 | 256.2 | 11.29 | 0 |
| Trt | 1 | 1.7 | 39.1 | 39.1 | 1.72 | 0.19 |
| Frt | 5 | 55937.1 | 54252.8 | 10850.6 | 478.05 | 0 |
| Day*Trt | 3 | 127.2 | 94.4 | 31.5 | 1.39 | 0.247 |
| Day*Frt | 15 | 2780.7 | 2760 | 184 | 8.11 | 0 |
| Trt*Frt | 5 | 608.1 | 646.8 | 129.4 | 5.7 | 0 |
| Day*Trt*Frt | 15 | 333.5 | 333.5 | 22.2 | 0.98 | 0.476 |
| Error | 372 | 8443.4 | 8443.4 | 22.7 | | |
| Total | 419 | 69163.3 | | | | |

S = 4.76417    R-Sq = 87.79%    R-Sq(adj) = 86.25%

Analysis of Variance for h, using Adjusted SS for Tests

| Source | DF | Seq SS | Adj SS | Adj MS | F | P |
|---|---|---|---|---|---|---|
| Day | 3 | 3162 | 3243 | 1081 | 11.56 | 0 |
| Trt | 1 | 936 | 53 | 53 | 0.57 | 0.451 |
| Frt | 5 | 676177 | 664183 | 132837 | 1420.21 | 0 |
| Day*Trt | 3 | 386 | 367 | 122 | 1.31 | 0.271 |
| Day*Frt | 15 | 4961 | 4975 | 332 | 3.55 | 0 |
| Trt*Frt | 5 | 1323 | 1322 | 264 | 2.83 | 0.016 |
| Day*Trt*Frt | 15 | 1081 | 1081 | 72 | 0.77 | 0.711 |
| Error | 372 | 34794 | 34794 | 94 | | |
| Total | 419 | 722819 | | | | |

S = 9.67124    R-Sq = 95.19%    R-Sq(adj) = 94.58%