The effects of pre-harvest application of hexanal formulations on time to ripening and senescence and fruit retention time in limequat (*Citrofortunella floridana* J.W. Ingram & H.E. Moore)

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Limequat (*Citrofortunella floridana* J.W.Ingram & H.E.Moore), unlike many tropical fruits, is produced year-round in Trinidad and Tobago and there are periods where other seasonally available varieties of limes, for example, the West Indian variety, are in greater demand than the limequat, resulting in glut supplies and high losses in the field. Limequat has been known to produce in excess of 250,000 fruits per hectare and during periods of low demand, much of these fruits go to waste. Retention time of the fruit on the tree is usually around 35 days, after full colour change from green to yellow. Due to its soft texture, the fruit bruises very easily leading to high post-harvest losses and loss of earnings to farmers. This study was conducted to determine the effects of pre-harvest treatments with enhanced freshness formulation (EFF), applied at different concentrations and different application intervals, on retention time of the fruit on the tree and time to colour change of the fruit as an indicator of onset of senescence. Trials conducted showed that pre-harvest biweekly applications of EFF at four percent was more effective in delaying the onset of colour change of the limequat fruits than EFF application at two per cent. It was observed that application of two per cent EFF bi-weekly for four weeks gave a greater reduction in the average number of fruits showing full colour change after treatment when compared to weekly application. Results of the study indicated that application of four percent EFF at bi-weekly spray intervals for one month significantly reduced the rate of colour change of limequats on the trees. Weekly applications of two percent EFF gave the best retention times on the tree, in excess of 99 days, after treatment. Thus, treatment with EFF reduced pre-harvest losses and increased the time for which marketable quality of fruits was maintained.

Keywords: Enhanced freshness formulation, hexanal, limequat, pre-harvest, applications, fruit retention

There are several cultivars of limes grown in the twin island nation of Trinidad and Tobago but the West Indian variety remains predominant while the limequat variety (*Citrofortunella floridana*) is least propagated since not many farmers have access to the planting material. Nevertheless the limequat, once properly managed, is available year-round, while other preferred varieties are seasonally available. The limequat has the capacity to produce a minimum of 250,000 units of limes per hectare per six month bearing cycle. These values were captured based on a three year study (NAMDEVCO records) on a farm located in Central Trinidad. Traditional uses include domestic utilization for the preparation of beverages, fish and meat meals and condiments.

The limequat is a smooth-skinned, soft-textured fruit with an average diameter of 48mm, average juice content of 19 ml, which is comparable to the juice content of a medium-sized lime of the West Indian variety, and an average weight of 35g. The fruit is soft, thus excessive force is not require to express its juice. However, the softness of the skin increases the susceptibility of the fruit to high post-harvest losses if poor handling practices are employed. Brown discolouration is the major indicator of end of shelf life and normally develops only after full colour change from green to yellow takes place, signalling loss of marketability of the fruit.

The limequat is harvested at the full, green, mature stage for optimum juice content and less post-harvest losses rather than at its full

yellow colour stage, while limes of the West Indian variety are best utilized when at the full yellow colour stage, since the juice content is high and less force is required to express it. Limequat fruits harvested at physiological maturity have better post-harvest shelf life properties (Baldwin 1993). Along with its other quality parameters that provide good marketability, the retention of the green colour increases its demand; as such, retention of green colour of the fruit in the field is significant importance to the farmer. Chlorophyll degradation is the main reason for yellowing of lime fruit (Drazkiewice 1994; Sriloang et al. 2011). During ripening and senescence, membrane degradation occurs as a result of the presence of phospholipase D (PLD). Degradation is also enhanced as a result of stress and the presence of reactive oxygen species (ROS) (Paliyath and Droillard 1992). Research has shown that hexanal, a naturally occurring volatile aldehyde, is a significant inhibitor of PLD activity and can enhance the shelf life and marketability of flowers, fruits and vegetables significantly (Paliyath et al. 1999, 2003; Paliyath and Murr 2007).

Year-round availability, high yields and numerous marketing avenues make the limequat non-competitive when compared to limes of the West Indian variety, which can create gluts on the market whenever the latter is in production. Further, fruit of the West Indian variety normally have a lower selling price than limequat because of the lower cost of production, contributing to the lower demands for limequat when both are in season and glut conditions exist. With very few agro-processing options, the advantage obtained from year-round production in the limequat, is eroded by high post-harvest losses resulting from glut supplies and reduced demand. The problem can be solved if the retention time of limequats on the tree can be increased in order to extend fruit availability beyond the glut period. Previous studies conducted on mango fruits using hexanal formulations showed that pre-harvest applications of hexanal formulations increased the retention time of fruits on the trees (Anusuya et al. 2016).

In this study, pre-harvest application of hexanal was used to observe the effects on the rate of senescence as indicated by fruit yellowing, and retention time of fruit on the trees. Treatments were applied at different concentrations and at different time intervals in an attempt to determine the efficacy of combination of treatment time and concentration to increase fruit retention and reduce the rate of senescence.

Materials and methods

Experiment procedure

Research investigations were conducted on a limequat orchard located in Central Trinidad. The trees were approximately 15 years old and are pruned periodically to encourage increased flower production and to support ease of harvesting. Treatment plots were selected to prevent drift affecting selected plots. Four pre-harvest spray treatments of EFF (Paliyath and Murr 2007) were applied as follows:

1. Hexanal at 2% v/v - enhanced freshness formulation **(EFF1)** applied weekly for 3 weeks (total of 4 applications).
2. Hexanal at 2% v/v - enhanced freshness formulation **(EFF1)** applied bi-weekly for 4 weeks (total of 3 applications).
3. Hexanal at 4% v/v - enhanced freshness formulation **(EFF2)** applied weekly for 3 weeks (total of 4 applications).
4. Hexanal at 4% v/v - enhanced freshness formulation **(EFF2)** applied bi-weekly for 4 weeks (total of 3 applications).

Corresponding control plots were treated with control solutions, **EFF0**, comprising EFF1 or EFF2 minus hexanal. Treatment solutions were applied to trees using a pressurized nozzle sprayer to give good coverage. A 2 x 3 factorial in a completely

randomized experimental design was used to collect data.

Data Analysis

Limequats are estimated to reach harvest maturity within 10 weeks of flower drop. Maturity indices used for limequats were loss in glossy appearance of the fruit and full green skin colour with no signs of yellowing. All fruit that were at harvest maturity as well as all fruit showing signs of yellowing were removed from the trees prior to application of spray solutions. The variables measured (response variables) were:

(1) Number of fruits showing 50% colour change by 49 days after spraying,

(2) Number of fruits showing full colour change (green to yellow) by specified number of days after spraying and

(3) Number of fallen fruit observed after treatment.

Fruits were counted to have 50% colour change when visual observation noted 50% skin colour had changed from green to yellow. Fruits were counted to have full colour change when visual observation noted that 100% skin colour had changed to full yellow. Fruit fall was recorded as the total number of fruits that fell from the trees during the recording period. Fallen fruits were removed from the plot each week. Observations were done on a total of 8600 mature fruit from the six groups of ten trees (replicates) selected from each treatment x spraying frequency combination (Table 1).

Table 1: Number of fruit observed per treatment (EFF0- Control spray application; EFF1- 1% EFF spray; EFF2-2% EFF spray)

	Spraying frequency	
Treatment	Biweekly Interval	Weekly Intervals
EFF0	1500	1500
EFF1	1800	1800
EFF2	1650	1650

The data collected were analysed using two-way ANOVA modelling. The estimated marginal mean effects were then derived for number of fruits observed with 50% and 100% colour change and for the number of fruits fallen observed across the trees that were sprayed with EFF0, EFF1 and EFF2 weekly and bi-weekly. Two-way ANOVA modelling was adopted for this study because of the significant interaction effect between treatment type and spray frequency for which comparisons could have been made. All models were assessed using at least a 5% significance level. If the interaction effect analysed in the ANOVA model was not significant, then the individual variables (treatment, and spray frequency) were then analysed separately for statistical differences.

Results and discussion

Colour change in limequats fruits

There was a general reduction in the rate of colour change from full green to full yellow for fruit from treated trees when compared to fruit from control trees at both the biweekly and weekly treatment intervals for both EFF1 and EFF2 pre-harvest treatments. Fruits sprayed at one week intervals per month with EFF2 showed the least mean percentage change in colour when compared to fruit that had been sprayed twice per month with the same EFF concentration.

Colour change from green to 50 percent yellow was observed within the first seven days from spraying in fruits from trees treated with EFF 1 as well as in those from control trees. However, the mean number of fruits showing colour change was less than 2% in the latter when compared to greater than 40% for those from control trees. Generally, fruits treated with EFF2 had the least mean number of fruits showing initial change in colour compared to the other treatments.

The association between treatment concentration and spray interval on the rate of colour change for limequat fruits is represented

in Figure 4. It should be noted that the mean percentage of fruits showing 50 per cent colour change remained the same for biweekly applications of both EFF1 and EFF2 treatments when compared to the control.

Therefore, treatments with EFF1 and EFF2 gave significant reductions in the mean percentage of fruits showing 50 per cent colour change by day 49 when compared to fruit on control trees. Differences were found in mean number of fruits with 50% and full colour change at 49 days after spray application between the EFF1 and the EFF2 at the same spraying frequencies (biweekly or weekly) (Table 2). Treatment with EFF1 at biweekly intervals showed a higher mean number of fruits with 50% colour change when compared to treatment with EFF1 at weekly intervals. Treatment with EFF2 at weekly intervals however; showed a higher mean number of fruits with 50% colour change and full colour change when compared to EFF2 at biweekly treatment intervals. Generally, application of EFF2 biweekly showed a lower mean number of fruits with 50% colour change by day 49 when compared to treatment with EFF1 at weekly and biweekly intervals. Biweekly applications of EFF1 showed a lower mean percentage of fruits with full colour change by day 49 when compared to applications of EFF2 at the same rate of application.

Fifty percent colour change

The Analysis of Variance (ANOVA) table for number of fruit per 100 with 50% colour change (Table 3) indicates significant treatment x spraying frequency interaction; for which the interaction plot is shown in Figure 1. The plot shows that spraying EFF2 solution at biweekly intervals results in the smallest mean number of fruit with 50% colour change. There was no significant difference in the efficacy of spraying EFF1 and EFF2 at weekly intervals, with respect to number of fruit with 50% colour change, compared to spraying EFF2 at biweekly intervals (Figure 1).

Full colour change

Trees treated with EFF1 at the biweekly spray interval showed the least average percentage of fruits with full colour development after 49 days when compared trees treated with EFF1 at weekly spray intervals.

Table 2: Mean number of fruit (and standard deviations) showing colour change at 49 days after spray application

	Spraying frequency/month	
Treatment	Biweekly intervals	Weekly Intervals
Number of fruit with 50% colour change per 100 fruit		
EFF0 (Control)	82.7 (8.07)	68.3 (13.79)
EFF1	82.9 (14.16)	18.1 (8.46)
EFF2	9.3 (5.58)	16.5 (6.73)
Number of fruit with full colour change per 100 fruit		
EFF0 (Control)	19.4 (5.03)	9.0 (10.62)
EFF1	9.8 (4.14)	23.2 (27.24)
EFF2	13.9 (14.59)	15.0 (7.57)

Table 3: Analysis of Variance: Number of fruit per 100 with 50% colour change

Source	Sum of Squares	df	Mean Square	F	P
Treatment	39724.542	2	19862.271	197.369	≤ 0.001
Spraying	8611.383	1	8611.383	85.570	≤ 0.001
Treatment *Spraying	13666.041	2	6833.020	67.899	≤ 0.001
Error	5434.299	54	100.635		
Total	67436.265	59			

*** indicates a p-value of less than 1% significant

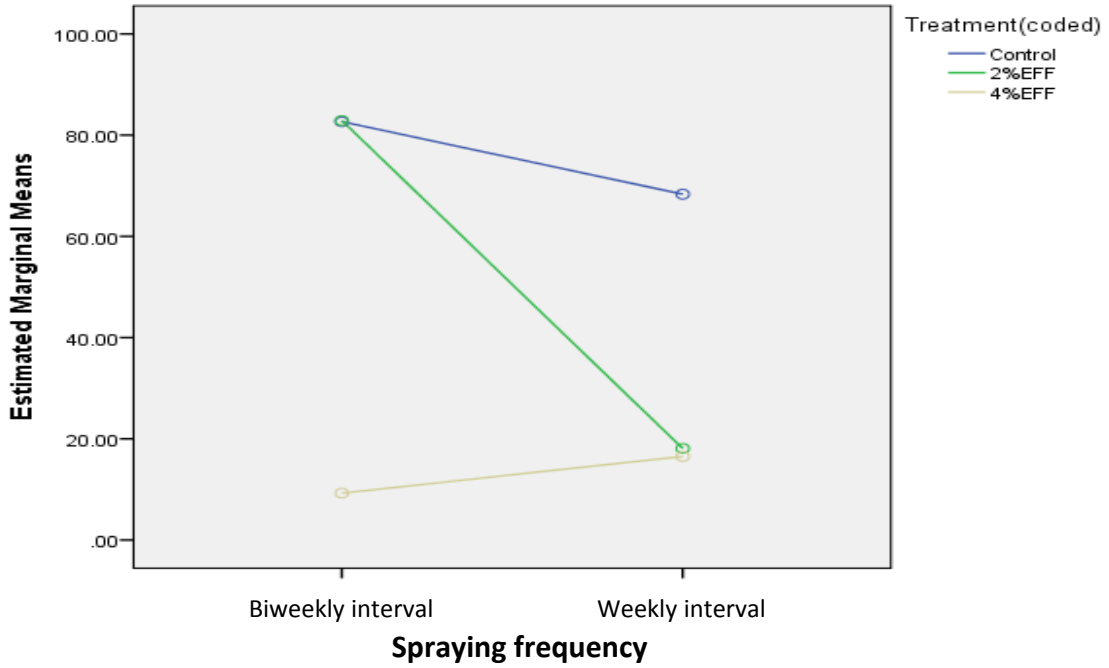


Figure 1: Estimated marginal means of number of fruit with 50% colour change per 100 fruit. Treatment x spraying frequency interaction plot.

There was no significant difference in the mean percentage of fruits showing full colour change at different spray intervals for treatments with EFF2 when compared to EFF1. The results indicate that biweekly applications of EFF2 had the same effect as weekly applications of EFF2 on full colour change in limequats fruits.

It can be deduced from the results of the trials that pre-harvest spray applications of EFF1 applied biweekly for approximately thirty days before harvest, significantly reduces the rate of full colour change from green to yellow in limequats fruits.

The general delay in the development of full colour was noticeable for up to 99 days after application of EFF1 and EFF2 as pre-harvest treatments when compared to control. Fruit on control trees showed full colour change by day 42.

The interaction plot shows that biweekly applications of EFF1 solution is the preferred treatment for retarding full colour change.

Table 4 shows the Analysis of Variance ANOVA table for number of fruit per 100 with full colour change. The table shows significant treatment x spraying frequency interaction ($p = 0.034$); for which the interaction plot is shown in Figure 2.

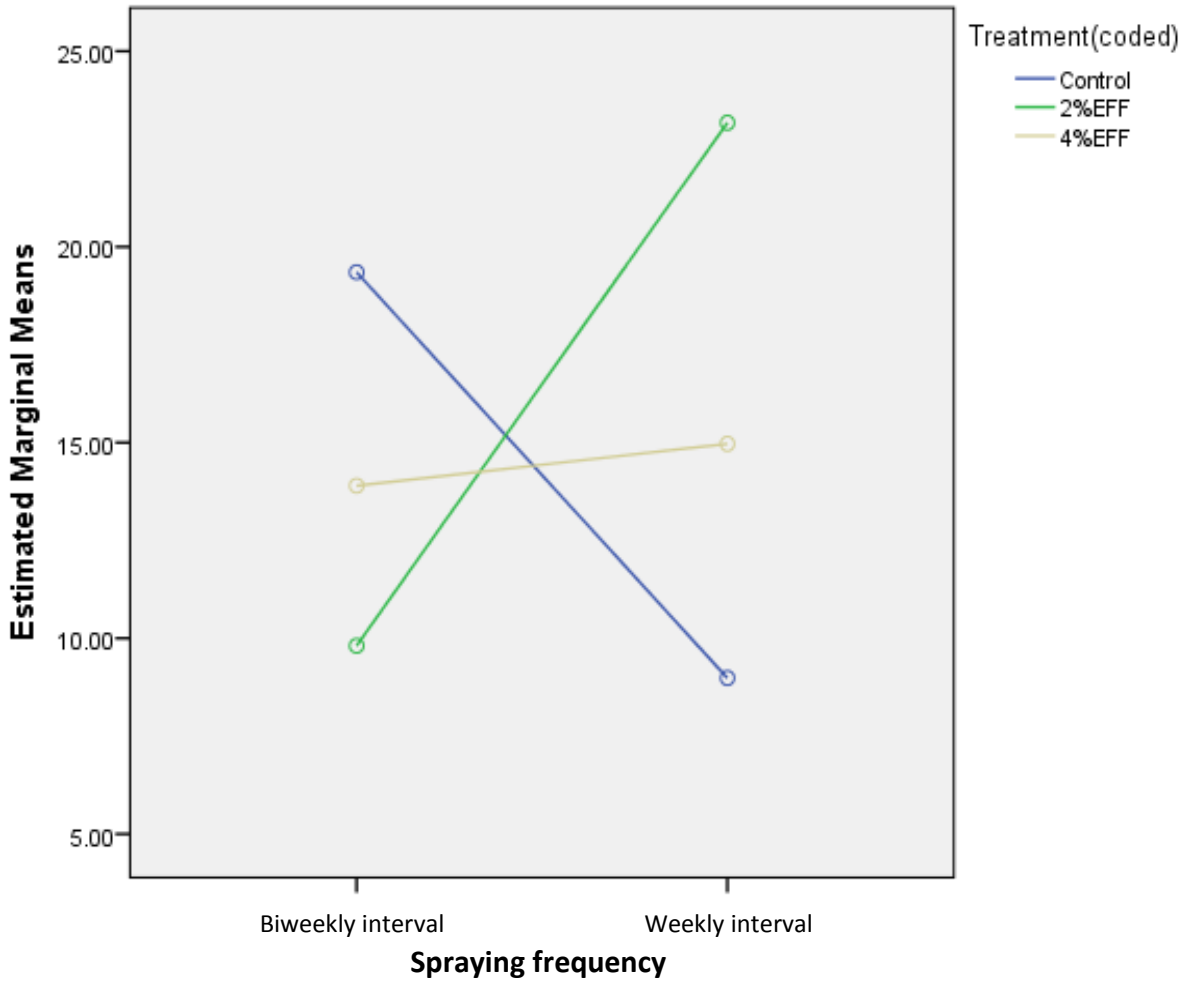


Figure 2: Estimated marginal means of number of fruit fall with full colour change per 100 fruit. Treatment x spraying frequency interaction plot.

Fruit fall

A total of 176 out of 8600, that is, 2.05% fruit fell before the last spray application and as such were not considered in this study. The mean and standard deviation of fruit fall per 100 fruit are given in Table 5: treatment and spraying frequency. The corresponding ANOVA table is Table 6 and the interaction plot is shown in Figure 3.

The ANOVA model (Table 6) developed for fruit fall as explained by the interaction between treatment type and spray frequency showed that a significant difference ($p \leq 0.001$) exists in the average number of fruits observed falling among the various treatment and spray frequency combinations.

Measuring the estimated marginal means effect (Figure 3) showed that bi-weekly spraying resulted in a comparatively smaller average fruit fall for EFF1 and EFF2 when compared to the control. Further, weekly spraying with EFF resulted in a decrease in the observed number of fruit fall. Thus, the results suggest that the number of fruit fall can be decreased by weekly application of EFF1.

Table 4: ANOVA table for number of fruit per 100 with full colour change

Source	Sum of Squares	Df	Mean Square	F	P
Treatment	64.641	2	32.321	.166	.847
Spraying	27.563	1	27.563	.142	.708
Treatment *S	1408.271	2	704.136	3.618	.034
Error	10509.337	54	194.617		
Total	12009.812	59			

Table 5: Summary Statistics for fruit fall for spray treatments

	Spraying frequency/month	
	Two times	Four times
Treatment	Number of fruit per 100	Number of fruit per 100
EFF0 (Control)	6.2 (3.89)	0.1 (0.35)
EFF1	1.59 (1.14)	0.0 (-)
EFF2	1.9 (2.62)	1.4(1.59)

Table 6: ANOVA: Number of fruit fall per 100 fruit

Source	Sum of Squares	df	Mean Square	F	P
Treatment	59.908	2	29.954	6.933	0.002
Spraying	109.350	1	109.350	25.309	≤ 0.001
treatment * Spraying	88.261	2	44.131	10.214	≤ 0.001
Error	233.310	54	4.321		
Total	490.829	59			

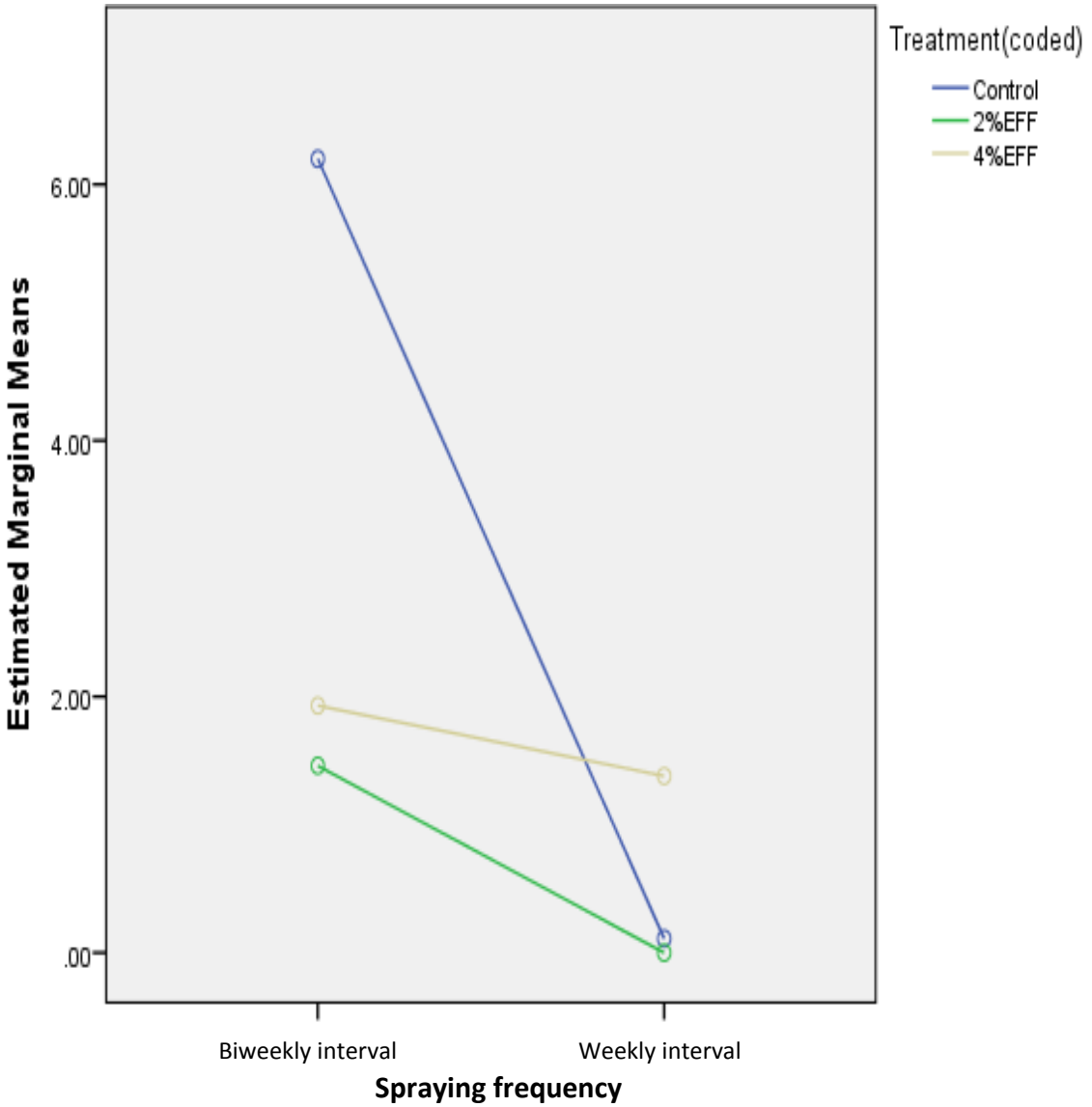


Figure 3: Estimated marginal means of number of fruit fall per 100 fruit for treatment concentrations EFF1 and EFF2 applied at biweekly and weekly spray intervals on fruit fall in Limequat fruits.

Retention time

Fruit fall from both control and treated trees occurred only after fruits showed full yellow colour change. Full yellowing and subsequent fruit drop commenced in control trees after 35 days. Development of full yellow colour and accompanying fruit fall was not observed in treated trees until day 99. The trials demonstrated that mean percentage fruit drop was predicted by treatment applications of EFF and treatment intervals. Fruit drop was lowest for treatments with EFF1 applied at weekly intervals when compared to EFF2 treatments (Table 5). Additionally, the application of EFF2 at weekly intervals and biweekly intervals showed no significant difference on the rate of fruit fall when compared to fruit fall from trees treated with EFF1.

Fruits treated with EFF1 and EFF2 showed fruit drop after a minimum of 99 days (when weekly and biweekly spray treatments were applied) and a maximum of 120 days fruit retention after an initial colour change (for fruits treated with EFF2). However, results indicated that fruits treated with EFF1 at weekly treatment intervals had the lowest mean percentage of fruit fall when compared to biweekly applications using the same concentration (Table 6).

Fruit drop was observed in control trees from 35 days after full colour change. Fruits from trees treated with EFF1 and EFF2 showed fruit fall after ninety-nine (99) days. The mean percentage of fruit fall per tree for treated trees was <1% per week compared to >6% per week for control trees.

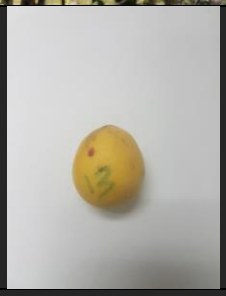
Days	Control	2% Treatment - 2 week spray interval	4% Treatment - 2 week spray interval
0			
35			
49	N/A		

Figure 4: The effect of EFF1 and EFF2 treatments at biweekly applications on colour change and fruit fall on limequat fruits.

Conclusion

Pre-harvest applications of EFF1 and EFF2 (either at biweekly or weekly spray intervals) were effective in extending time of fruit retention on treated trees than non-treated trees. Applications of EFF1 at biweekly spray intervals were most effective than weekly and biweekly spray applications of EFF2 for the same period, resulting in a reduction in the rate of full colour change for fruits on the tree. Applications of EFF2 (4%) bi-weekly gave greater delay in 50% colour change than weekly application of EFF2. Additionally, the rate of senescence, as indicated by the rate of colour change from full green to full yellow, was also decreased in fruits on treated trees, so that overall time to full colour change in the treated fruit was longer than for fruits on the control trees up to in excess of ninety-nine (99) days.

However, given the interaction between spray concentration and spray intervals, it appears that spraying weekly at the higher concentration, i.e., four percent, accelerated colour change enough to make application of EFF1 (two percent) at biweekly intervals more effective at delaying full colour change. This was probably as a result of a slight toxicity effect of the higher application concentration. Therefore, treatment with EFF1 at biweekly intervals is recommended for use by farmers to increase income generation, given the advantage on the reduced rate of colour change from full green to full yellow. Weekly applications of EFF1 however, gave the highest retention time for lime fruit on the trees.

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