Design Improvements for Applied Postharvest Studies

PEF White Paper 22-01

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1. Introduction

Many postharvest researchers, extension specialists and trainers need to compare different postharvest treatments to demonstrate their efficacy and their suitability for adoption. Designing and conducting sound experiments is part of good decision making in postharvest handling, and relatively fast and simple to do. Postharvest experiments can be done quickly (over a few days or weeks) compared to production experiments which often require planting, cultivating and harvesting the crop before data collection and analyses. It is not recommended to change postharvest handling practices based on an opinion or on poorly designed applied research.

While evaluating the published information on reducing postharvest losses for the scoping review (Stathers et al., 2020) many interesting studies were excluded because the experiments used very small sample sizes, no replications or no controls. We referred to this as 'research loss and waste'. In fact, we screened 12907 abstracts, read 1906 research papers or reports and only include 334 studies in the review. Research for initial assessment of new technologies, or for understanding the physiology can be done with smaller sample sizes. However, when adopting new treatments or technologies in commercial or real-world situations one needs to be confident about success. In these cases, well designed experiments with larger sample sizes, replications and controls are necessary.

One of the best examples included in the scoping review (Stathers et al., 2020) was a study by Gilfillan & Saunt (1989) which used 160 cartons of fruit in the initial experiment, 50 x 8 kg cartons/treatment in the second experiment, and 5,000 cartons in the final commercial test. This series of experiments provided sound data to change commercial protocols with confidence.

This white paper aims to provide practical assistance on designing and conducting research experiments that can be used to make decisions about adopting new practices under real-world conditions.

2. Research question or hypothesis

Postharvest experiments are conducted to resolve many practical questions, including:

- What type and/or dose of a sanitizer or postharvest fungicide is most effective at controlling decay without adversely affecting produce quality?
- Which storage environment or temperature regime results in lower postharvest losses and better-quality product?
- Which harvest maturity results in good shelf life and high-quality produce?
- What type of packaging provides good protection at a reasonable cost?
- Is a new treatment or practice superior to the current or traditional treatment or practice?

Research scientists use the 'null hypothesis', which assumes that there will be no effect of the treatments, therefore, the data must be overwhelmingly convincing to prove otherwise. This is another way of stating that the treatments must be clearly superior and must convince you that they are worth adopting.

3. Variation

The biggest difficulty conducting experiments with biological products, like fruits and vegetables (or even humans) is the variability. Sometimes the variation is greater than the effect of the treatment. Even fruits or vegetables of the same variety harvested from the same tree, or from the same row of plants can vary considerably. A reasonably large sample of produce of the same variety, from the same field, and harvested on the same day, is recommended to reduce this variation. In some experiments, the produce is sorted into different sizes and the experiments are conducted on produce of the same size. It is not always possible to do this but making sure that each treatment has a similar range of size and quality, randomly assigned to each treatment sample and control sample(s), helps reduce the variation in the response.

4. Controls

Many poorly designed experiments lack a control (or check) treatment against which the new treatment should be compared. Depending on the type of experiment there may be more than

one control. For example, an experiment evaluating a sanitizer for use in the wash water in the packhouse may need to include a control that is not washed at all, as well as a control that is washed in water. However, when comparing a new sanitizer with the current standard sanitizer, e.g., chlorinated water, the experiment does not need an untreated control as the research question is does this new sanitizer (treatment) work as well as, or better, than the standard sanitizer (control)? When comparing fruits or vegetables that are normally stored in a shaded room versus those stored in a ZECC (zero energy cold chamber), the ZECC would be considered the improved practice (treatment) and the shaded room the current practice (control).

5. Measurements

The effect of a treatment is measured by certain produce maturity and quality factors, also known as dependent variables. These include decay, water loss, maturity, overall acceptability, as well as both external (colour, skin browning, wilting.) and internal quality (Brix, acid, flavour, aroma). These must be measured as accurately as possible to determine the effect of the treatment(s) which are the independent variables.

For example, when comparing a new water sanitizer with the standard sanitizer, the produce should be stored under typical conditions and the resulting decay and contamination should be measured. Quality should be assessed to make sure that the sanitizer did not have any unexpected negative effects.

6. Replications

Field experiments comparing different rates of fertilization require several plots of land randomly selected for each treatment to overcome the variations in results caused by different soil types and microclimates. However, in postharvest experiments comparing different storage environments e.g., shaded rooms, ZECC and mechanically controlled cold stores it is not practically possible to replicate the rooms. Therefore, replication of treatments is used to increase the ability to detect differences in the treatments and reduce the effects of natural variation in plants or produce. Replicates could include boxes, bags, or trays containing many individual items, or simply consist of individual produce depending on the type of experiment. For example, a replicate (rep) could be four 5 kg cartons of tomatoes (e.g., approximately 33 x 150 g tomatoes per carton),

20 individual pumpkins, 10 hands of bananas, 10 bunches of spinach, or 4 crates of cabbage (5-6 per crate) per treatment.

Four replicates are a good minimum size for most postharvest experiments, although some experiments use as few as 2 reps or as many as 10 reps. For initial experiments a smaller sample size and many treatments may be evaluated. This first step can help eliminate some of the treatments. But to make a commercial decision it is better to repeat the experiment using less treatments and more replications. These replicates should each contain at least one commercial unit, for example if tomatoes are sold in 10 kg cartons, then 10 kg would be a good size for a replicate and 4 x 10 kg boxes should be used for each treatment.

Some data can be measured non-destructively e.g., weight loss over time, but typically postharvest experiments of fruits and vegetables need to be assessed destructively at different times. The need for a representative sample at each evaluation stage increases the sample size of the treatments, and ultimately the experiment.

7. Repetition

Since there are so many factors that affect the responses of fruits and vegetables, it is important to repeat experiments. While experiments can be repeated in the same way several times, it is often more useful to design preliminary experiments to eliminate non significant or low performing treatments, and then repeat with less treatments and more replication.

Initial experiments to determine rates of fungicides or sanitizers often start with higher numbers of treatments and smaller replicates. For example, if there is little known information on the dosage the best option would be to evaluate a log scale concentration (e.g., 0, x, 10x, 100x), whereas, if some idea of the concentration range is known then a dosage rate of 0.5x, x, 2x is recommended. Once initial data is gathered, the experiment would be repeated with a smaller range of concentrations, or simply, one concentration versus a control.

One of the limitations of practical postharvest research is the failure to repeat the experiments at a larger scale to make informed decisions, and failure to include a cost: benefit or return on investment (ROI) analysis. It would be difficult to implement changes with confidence if the experiment had measured 10 cabbages or 3 bunches of spinach. The recent scoping review

(Stathers et al., 2020) rejected many postharvest studies because they were conducted at with small sample sizes. Conversely, one of the best examples of evaluating postharvest treatments was initially conducted using 50 cartons per treatment, repeated with 50 cartons per treatment and finally confirmed with 5,000 cartons per treatment (Gilfillan & Saunt, 1991).

8. Examples of experimental designs

8.1 Sanitizers for bell peppers

Often bell peppers need to be washed to remove the dust from the field but washing increases the risk of spreading disease. In this case the new product (at three rates) would be compared to an unwashed (dry) control and a water washed control. The research question asks if the sanitizer is effective at reducing cross contamination of bell peppers during washing and at what dose it is most effective, while the null hypothesis would state that the sanitizer is no better than water, and there is no effect of dose. In this case I have selected 3 reps of 10 units with 4 evaluation dates as a starting point (Table 1). However, this uses 600 bell peppers. It would be possible to reduce the number of bell peppers required in the experiment to 150 by assessing their decay non-destructively during storage. Other quality factors should also be measured e.g., water loss, overall acceptability and even microbial counts in the water after use.

The bell peppers must be stored in the same environment during the study and ideally this should mimic the typical conditions from production to consumptions e.g., a shaded room, a ZECC, room controlled by a CoolBot, or a cold room at 10°C.

In this experiment one would expect the control washed in water to have the highest decay. If there were no differences between treatments and the controls, it may mean that the bell peppers were free of most spores. The experiment would need to be repeated at a time of year when the bell peppers have a higher risk of final and bacterial contamination in the field.

If the current practice was washing bell peppers in chlorinated water (150 ppm) then a typical experiment would be comparing this with a new sanitizer. The research question asks if the new sanitizer is more effective than the current sanitizer, while the null hypothesis states that there is no difference between the two sanitizers.

The smaller number of treatments allows an increase in replication e.g., 4 reps of 10 peppers (Table 2). Again, if the response to treatments can be assessed non-destructively, it is possible to further increase the number of replications and/or the sample size without designing an experiment that is too large to handle or too expensive to conduct e.g., 5 reps of 20 peppers (Table 3).

Table 1. A theoretical experimental design to determine the optimum concentration of a new sanitizer for bell peppers

Experimental design	**Replicates per treatment**	**Number per rep**	**Number per treatment**
Treatments (5)			
Control - dry	3	10	30
Control - washed in water	3	10	30
2.5 mg/L sanitizer (0.5x)	3	10	30
5 mg/L sanitizer (x)	3	10	30
10 mg/L sanitizer (2x)	3	10	30
Total/evaluation date			***150***
Evaluation dates (4)			
0 days			150
5 days			150
10 days			150
15 days			150
Total/experiment			***600***

Table 2 A theoretical experimental design to determine if a new water sanitizer can replace the current sanitizer (e.g., 100 ppm chlorine) and using destructive assessments at two dates

Experimental design	**Replicates per treatment**	**Number per rep**	**Number per treatment**
Treatment (2)			
Chlorine 150 ppm	4	10	40
Sanitizer Y 2.5 ppm	4	10	40
Total/evaluation date			80
Evaluation dates (2)			
10 days			80
20 days			80
Total/experiment			***160***

Table 3 A theoretical experimental design to determine if a new water sanitizer can replace the current sanitizer (e.g., 100 ppm chlorine) and using non-destructive evaluation with greater replication

Experimental design	Replicates per treatment	Number per rep	Number per treatment
Treatment (2)			
Chlorine 150 ppm	5	20	100
Sanitizer Y 2.5 ppm	5	20	100
Total/evaluation date			**200**

8.2 Waxes or coating on tomatoes

An initial experiment evaluating four coating agents or waxes on tomatoes against an untreated control, (i.e., 5 treatments) could use 30 single tomatoes per treatment. However, it is more realistic to pack tomatoes in a container and consider that a replicate. Each treatment would have 3 replicates (container) of 10 tomatoes (tray) that would be measured on each evaluation date (Table 4). A simple experiment would use 300 tomatoes! If there were not enough tomatoes the experiment could be modified so that there was only one batch that was weighed every day and evaluated for acceptability after 14 days. This would reduce the experiment to 150 tomatoes.

The tomatoes must be stored in the same environment during the study and ideally this should mimic the typical postharvest handling conditions. The containers would be weighed at the start of the experiment since weight loss, which is an important variable to consider when using a wax or coating agent. After 7 and 14 days of storage the container of tomatoes would be weighed again. The tomatoes would be evaluated for overall appearance, decay, colour (using a 6-point scale), any unexpected response from the coating, shelf life and quality on both day 7 and day 14. On the final day of data collection (day 14), the tomatoes should be assessed for typical aromas and taste as waxes can make tomatoes anaerobic. The fruit must be handled carefully to avoid any impact bruises or mechanical damages while taking observations.

In this theoretical experiment Wax B made the tomatoes anaerobic and completely unacceptable in terms of aroma and taste and Coating D was not different to the control. Both were excluded and the experiment was repeated with three treatments and more replication (5 trays of 10 fruits, i.e., 50 tomatoes per treatment per time) and more evaluation times to be sure that the

result are consistent. The initial quality of the tomatoes is measured on a single batch of fruit as the quality is the same for all treatments. When water loss is the focus then a set of samples that is measured non-destructively over the storage period can yield the best results. In this example the wax B and coating C extended shelf life in the first experiment so the storage time was extended to 21 days. This experiment would use 650 tomatoes. Again, it would be possible to reduce the size to 150 by measuring tomatoes non-destructively. However, effects on the internal quality and flavor changes may be missed.

If the results showed that both products were better than the control but not different from each other, the choice could be made on costs and ease of application. A final test would be to pack waxed and control tomatoes in the typical boxes used for the market (e.g., 10 kg fiberboard cartons or RPC Reusable Plastic Crates) send them to the market as well as keeping four cartons of each to evaluate (retain samples). Ideally, the tomatoes should be evaluated further down the handling chain markets and the feedback assessed.

Table 4 A theoretical experimental design to evaluate different waxes and coatings on tomatoes during storage.

Experimental design	**Replicates per treatment**	**Number per rep**	**Number per treatment**
Treatments (5)			
Control (no wax)	3	10	30
Wax A	3	10	30
Wax B	3	10	30
Coating C	3	10	30
Coating D	3	10	30
Total/evaluation date	***15***		***150***
Evaluation dates (2)			
Day 7			150
Day 14			150
Total/experiment	***45***		***300***

Table 5 A theoretical experimental design to evaluate different waxes and coatings on tomatoes with fewer treatments and more replications.

Experimental design	Replicates per treatment	Fruit number per rep	Fruit number per treatment
Treatments (3)			
Control	5	10	50
Wax B	5	10	50
Coating C	5	10	50
Total/evaluation date			***150***
Evaluation dates (5)			
1 set for weight loss			150
Initial			50
7 days			150
14 days			150
21 days			150
Total/experiment			***650***

8.3 Storage environments for leafy amaranth

In an experiment conducted by Ambuko et al. (2017), crates of leafy amaranth in bundles of 300 g were stored in three environments (treatments); zero energy brick cooler (ZEBC), evaporative charcoal cooler (ECC), or ambient room conditions. Each treatment had three bunches per evaluation date (900 g) and the amaranth was evaluated four times over eight days (Table 9). In this study they measured water loss, colour, wilting using a hedonic scale (1 = extreme wilting, 2 = very severe wilting, 3 = severe wilting, 4 = moderate wilting, 5 = slight wilting, 6 = very slight wilting, and 7 = no wilting) and vitamin C concentration.

Table 6. An actual experimental design used by Ambuko et al. (2017) to evaluate different storage environments for leafy amaranth where ZEBC is zero energy brick cooler and ECC is evaporative charcoal cooler

Experimental design	**Bunches per treatment**	**g per bunch**	**g per treatment**
Treatment (3)			
Ambient room (control)	3	300	900
ZEBC	3	300	900
ECC	3	300	900
Total/evaluation date			2700
Evaluation dates (4)	**Bunches per eval date**		**kg per eval date**
0 days	9	300	2.7
2 days	9	300	2.7
5 days	9	300	2.7
8 days	9	300	2.7
Total/experiment	***36***		***10.8***

8.4 Maturity and packaging of tomato

Gautam et al. (2017) harvested tomato at two maturity stages and packed them in plastic crates with either no liner, paper or plastic liners, or perforated plastic bags. Each treatment had three 20 kg crates (Table 7). The crates of tomatoes were transported about 200 km, in a truck. The damage to the fruits was assessed on arrival. About 2 kg fruits were allowed to ripen and weight loss and fruit colour (using a hedonic scale where 1 = breaker, 2 = turning, 3 = pink, 4 = orange, 5 = red, 6 = deep red) were recorded every 3 days. Firmness, total soluble solids (TSS), titratable acidity (TA), and vitamin C were measured when the tomatoes were ripe. The experiment was terminated when the overall acceptability decreased below 50%.

Table 7. An actual experimental design used by Gautam et al. (2017) to evaluate the effect of maturity and type of packaging on the quality of tomatoes after transportation.

Experimental design		**Crates (reps)**	**kg per crate**	**kg per treatment**
Treatments (8)				
Maturity (2)	**Packaging (4)**			
Breaker stage	PC (plastic crates) (control)	3	20	60
Breaker stage	PC lined with newspaper	3	20	60
Breaker stage	PC lined with polyethylene	3	20	60
Breaker stage	PC with perforated plastic bags	3	20	60
Orange-yellow stage	PC (plastic crates) (control)	3	20	60
Orange-yellow stage	PC lined with newspaper	3	20	60
Orange-yellow stage	PC lined with polyethylene	3	20	60
Orange-yellow stage	PC with perforated plastic bags	3	20	60
Total/evaluation date		24		480
Evaluation dates (1)				

9. Using spreadsheets for data collection and analysis

Managing data collection with an Excel spreadsheet is very simple. The spreadsheet can be set up prior to the start of the experiment and include each evaluation date, treatments and variables (Figure 1). Setting up a table, with no blank rows at the top or blank columns on the left, allows one to use the pivot table function in Excel, which is essentially a summary table of the data that can be rapidly generated. Pivot tables save time and reduce the errors that can occur when making calculations on a spreadsheet, and they can be set to automatically refresh (see Resources section for training on pivot tables)

	A	B	C	D	E	F	G	H
1	**Treatment**	**Carton**	**Day**	**Date**	**Color**	**Initial wt (kg)**	**Final wt (kg)**	**Wt loss (%)**
2	Control	1	0	10/10/2022				
3	Control	2	0	10/10/2022				
4	Control	3	0	10/10/2022				
5	Control	4	0	10/10/2022				
6	Ethylene absorber	1	0	10/10/2022				
7	Ethylene absorber	2	0	10/10/2022				
8	Ethylene absorber	3	0	10/10/2022				
9	Ethylene absorber	4	0	10/10/2022				
10	Control	1	2	12/10/2022				#DIV/0!
11	Control	2	2	12/10/2022				#DIV/0!
12	Control	3	2	12/10/2022				#DIV/0!
13	Control	4	2	12/10/2022				#DIV/0!
14	Ethylene absorber	1	2	12/10/2022				#DIV/0!
15	Ethylene absorber	2	2	12/10/2022				#DIV/0!

Figure 1. Example of a datasheet for a simple banana experiment comparing banana stored in cartons at room temperature with or without an ethylene absorber. This datasheet would be extended to the end of shelf life. #DIV/0! is the formula for weight loss entered in the spreadsheet ([[initial wt.-final wt.]/initial wt.] *100) and used to create a pivot table

In the example in Table 8, banana fruit were stored with or without an ethylene absorber and the overall changes in colour (using a hedonic colour scale from 1 to 7) for the fruit in the carton, and weight loss, assessed daily. The cumulative weight loss formula was built into the spreadsheet). Pivot tables of average data of one of more variables with other statistical functions, such as standard deviation, can be rapidly calculated using the pivot tables (Table 9)

Table 8 Example of a datasheet for a banana experiment with data.

Treatments	Carton	Day	Date	Color	Initial weight (kg)	Final weight (kg)	Weight loss (%)
Control	1	0	10-10-2022	3	16.00		
Control	2	0	10-10-2022	2	16.30		
Control	3	0	10-10-2022	3	16.10		
Control	4	0	10-10-2022	3	16.00		
Ethylene absorber	1	0	10-10-2022	3	16.10		
Ethylene absorber	2	0	10-10-2022	3	16.20		
Ethylene absorber	3	0	10-10-2022	3	16.10		
Ethylene absorber	4	0	10-10-2022	2	16.00		
Control	1	2	12-10-2022	4	16.00	16.00	0.0
Control	2	2	12-10-2022	3.5	16.30	16.20	0.6
Control	3	2	12-10-2022	4	16.10	16.00	0.6
Control	4	2	12-10-2022	4.5	16.00	15.90	0.6
Ethylene absorber	1	2	12-10-2022	3.5	16.10	16.00	0.6
Ethylene absorber	2	2	12-10-2022	3.5	16.20	16.10	0.6
Ethylene absorber	3	2	12-10-2022	4	16.10	16.05	0.3
Ethylene absorber	4	4	12-10-2022	3	16.00	15.95	0.3
Control	1	4	14-10-2022	5	16.00	15.90	0.6
Control	2	4	14-10-2022	5	16.30	16.25	0.3
Control	3	4	14-10-2022	5.5	16.10	16.00	0.6
Control	4	4	14-10-2022	4.5	16.00	15.80	1.3
Ethylene absorber	1	4	14-10-2022	4	16.10	16.00	0.6
Ethylene absorber	2	4	14-10-2022	4	16.20	16.10	0.6
Ethylene absorber	3	4	14-10-2022	4.5	16.10	16.00	0.6
Ethylene absorber	4	4	14-10-2022	3.5	16.00	15.90	0.6
Control	1	6	16-10-2022	6	16.00	15.30	4.4
Control	2	6	16-10-2022	6	16.30	15.75	3.4
Control	3	6	16-10-2022	6.5	16.10	15.55	3.4
Control	4	6	16-10-2022	6	16.00	15.40	3.8
Ethylene absorber	1	6	16-10-2022	5	16.10	15.40	4.3
Ethylene absorber	2	6	16-10-2022	5	16.20	15.80	2.5
Ethylene absorber	3	6	16-10-2022	5	16.10	15.50	3.7
Ethylene absorber	4	6	16-10-2022	4.5	16.00	15.30	4.4
Control	1	8	18-10-2022	7	16.00	15.10	5.6
Control	2	8	18-10-2022	7	16.30	15.20	6.7
Control	3	8	18-10-2022	7	16.10	15.35	4.7
Control	4	8	18-10-2022	6.5	16.00	15.15	5.3
Ethylene absorber	1	8	18-10-2022	5.5	16.10	15.35	4.7
Ethylene absorber	2	8	18-10-2022	5.5	16.20	15.20	6.2

Treatments	Carton	Day	Date	Color	Initial weight (kg)	Final weight (kg)	Weight loss (%)
Ethylene absorber	3	8	18-10-2022	5.5	16.10	15.25	5.3
Ethylene absorber	4	8	18-10-2022	5	16.00	15.15	5.3
Control	1	10	20-10-2022	7	16.00	14.80	7.5
Control	2	10	20-10-2022	7	16.30	14.95	8.3
Control	3	10	20-10-2022	7	16.10	14.85	7.8
Control	4	10	20-10-2022	7	16.00	14.70	8.1
Ethylene absorber	1	10	20-10-2022	6	16.10	14.80	8.1
Ethylene absorber	2	10	20-10-2022	6	16.20	14.85	8.3
Ethylene absorber	3	10	20-10-2022	6	16.10	14.75	8.4
Ethylene absorber	4	10	20-10-2022	6	16.00	14.85	7.2

Table 9 Examples of pivot table created from the spreadsheet of data in Table 8.

A) Average of banana skin colour (1-7) and weight loss (%) for each evaluation day

	Column Labels											
	Average of Colour						Average of Weight loss (%)					
Row Labels	0	2	4	6	8	10	0	2	4	6	8	10
Control	2.8	4.0	5.0	6.1	6.9	7.0	0.00	0.70	3.73	5.59	7.92	
Ethylene absorber	2.8	3.7	3.8	4.9	5.4	6.0	0.00	0.50	3.73	5.36	8.00	

B). Average and standard deviation of banana skin colour (1-7).

	Column Labels											
	Average of Colour						Std Dev of Colour					
Row Labels	0	2	4	6	8	10	0	2	4	6	8	10
Control	2.8	4.0	5.0	6.1	6.9	7.0	0.50	0.41	0.41	0.25	0.25	0.00
Ethylene absorber	2.8	3.7	3.8	4.9	5.4	6.0	0.50	0.29	0.57	0.25	0.25	0.00

10. Conclusions and recommendations

A well-designed experiment with sufficient sample sizes, replications and controls can assist in answering practical questions and making informed decisions to change postharvest handling practices. Conducting an initial experiment to screen a larger of treatments, followed by

experiments with lower treatment numbers and higher replications can be a practical way to answer postharvest questions.

11.References

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12. Resources

12.1 Pivot tables

Excel Pivot Table Tutorial for Beginners https://www.youtube.com/watch?v=igSovq_H24A

How to Use Pivot Tables in Excel 2013 for Dummies https://www.youtube.com/watch?v=vdxnO8O4Ke0

Pivot Table Tutorial – Learn PivotTables in 1 Hour – Excel Crash Course https://www.youtube.com/watch?v=2RKw-HGCLB0

And many more…

12.2 Visual or color charts for rating fresh produce

UC Davis Postharvest Technology Center: Produce Facts postharvest.ucdavis.edu/PF/

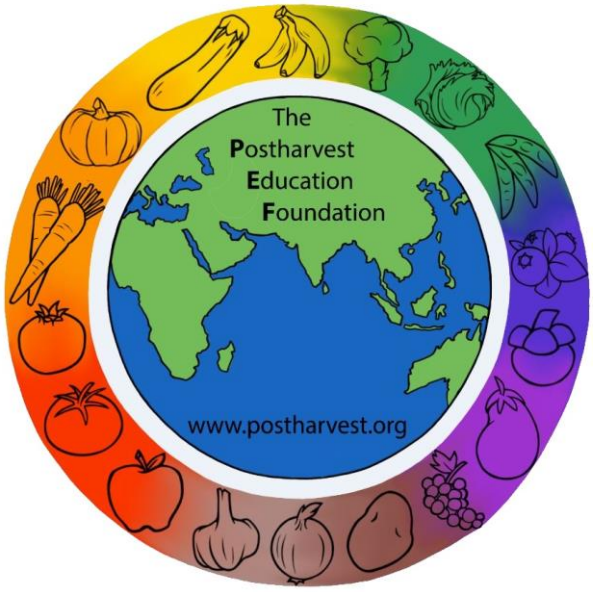
OECD International standards for fruits and vegetables (in French and English) https://www.oecd-ilibrary.org/agriculture-and-food/international-standards-for-fruit-and-vegetables_19935668

USDA Index of Official Visual Aids (for fruits and vegetables) https://www.ams.usda.gov/sites/default/files/media/Official%20Inventory%20of%20FV%20Inspection%20Aids.pdf

Barrett, D. How to use 1) a color chart 2) a refractometer 3) measure temperature and relative humidity. http://postharvest.org/videos1.aspx

12.3 Designing field trials

Davis, R.F., Harris, G.H., Roberts, P.M. & MacDonald, G.E. 2017. Extension Agronomist Designing Research and Demonstration Tests for Farmers' Fields. University of Georgia Extension Bulletin 1177. https://extension.uga.edu/publications/detail.html?number=B1177



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