What to do When the Weight Test Fails:
Methods to Distinguish between Full and Empty Achenes in *Echinacea angustifolia*

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Introduction

The goal of this project was to determine the most accurate method, or combination of methods, to distinguish between full and empty achenes. The most convenient method thus far has taken advantage of a clearly visible weight difference between full and empty achenes. When achene weights from an Echinacea flower head are sorted and displayed graphically using the statistical computing program R, there is almost always a large gap dividing full from empty achenes that tends to fall around 0.0020g (Figure 1 – click here for larger image). A small percentage of Echinacea heads, however, possess achenes that lack a discernible weight difference to mark the cutoff point between full and empty.

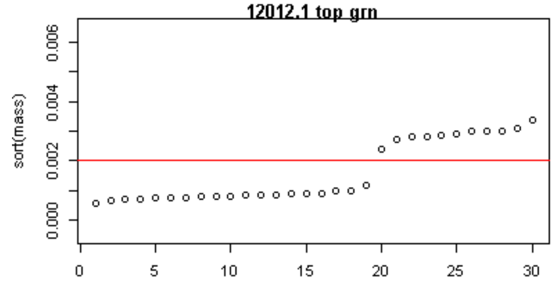


Figure 1. Graph depicting achene weights with a clear gap between full and empty. Generated in R.

20 samples of the bottom-most or top-most achenes from Echinacea heads that displayed a continuous pattern of variation in weight were selected for further study (Figure 2 - larger image). Achenes from these samples were reweighed and X-rayed to determine the cutoff value for each. Of the samples in the study, 60% had cutoff points that could be predicted by an intermediate transition weightbetween full and empty achenes. It was also found that there is no visual difference or size marker to distinguish full achenes from empty achenes.

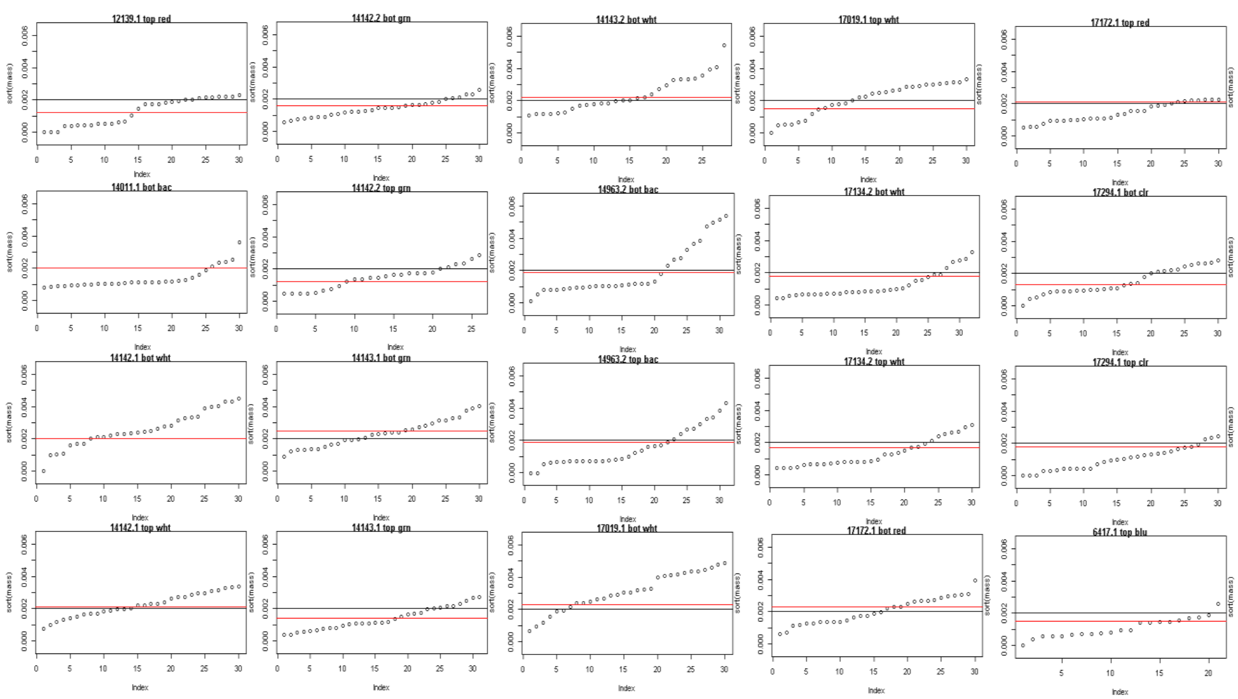


Figure 2. Achene weights from the samples selected for this study. All samples exhibit the absence of a weight gap between full and empty achenes. The red line marks the cutoff point for each after the conclusion of the project. The black line marks the standard 0.0020g cutoff point currently used for most heads. Graphs were generated using R.

Methods

I obtained the Echinacea heads used in this study from the ongoing Staffanson project. The achenes from the tops and bottoms of these heads were cleaned and weighed, generating a total of ninety-six samples. I plotted the achene weights for each sample using R, and determined new cutoff points where necessary if the weight gap did not fall at 0.0020g. Of the original ninety-six samples, twenty did not have a discernible cutoff point and were selected for use in this project (Figure 2).

After each sample was assigned a best-guess cutoff value, I reweighed the achenesfrom these samples individually using a Metler Toledo balance. I placed the achenes in the range of ±0.0005g of their sample's cutoff on a post-it in ascending order of their recorded weights. The selected achenes were then X-rayed at 18kv with a 20 second exposure time to determine at what weight achenes from each sample transitioned from full to empty. New cutoff values for each sample were then assigned and plotted in R.

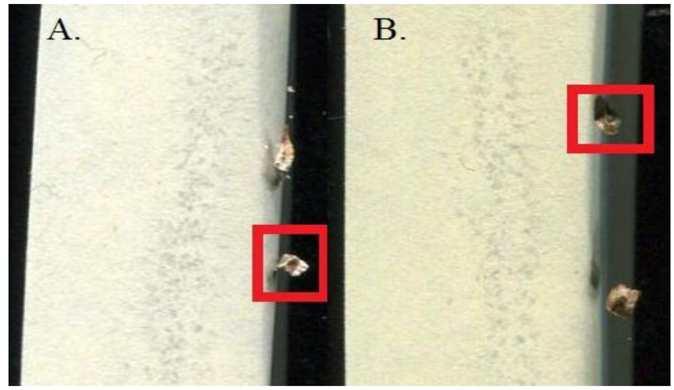
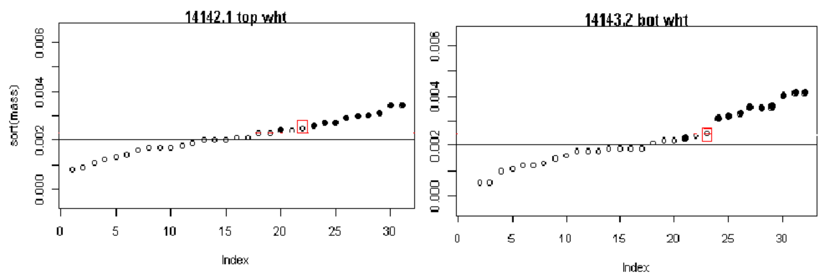


Figure 3. Side-by-side comparison of a full and empty achene with the same weight. The full achene is highlighted in red. (A). 12139.1 bot red: two achenes weighing 0.0012g. (B). 14142.1 top wht: two achenes weighing 0.0023g.



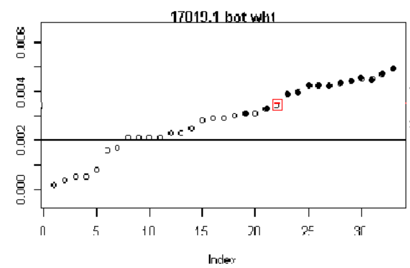


Figure 4. Clockwise from top left: samples 28, 33, and 55. Full achenes are shown in black. An empty achene with a greater weight than the transition weight is boxed in red.

Results

There is no visual, and sometimes no weight guide, to distinguish between full and empty achenes. Over half of the samples had two or more achenes that had an identical weight, but differed in whether or not they were full. In some cases, the full achene was actually smaller in size than the empty achene (Figure 3 - larger image).The push test works for achenes with the same weight. The achenes shown in red in Figure 3 both passed the push test, while their empty counterparts did not. Subsequent X-ray images confirmed these results. Empty achenes may thus be larger and identical in weight or other physical characteristics to full achenes.

A large number of achenes in one sample still retained their florets. Removing these florets and reweighing the achenes resolved the weight gap issue in this sample.

Of the other samples, twelve samples had one or more groups of achenes with the same weight. These weights fell under the category of Predicted Transition weight (Table 1). A transition weight is a weight that two or more achenes in the same sample possess. This weight divides the full and empty achene populations in each sample. Achenes with a transition weight differ in whether they are full or empty, which can easily be determined by the push test (Figure 5 - larger image). The slope of achene weight distribution tends to level off after the transition weight, although this is not always a useful guide (Figure 2). Sample 31 did not exhibit a transition weight, and the cutoff was determined solely by X-ray and push-test (Table 1). Seven samples had one or more likely transition weights. X-ray analyses of these samples revealed that all achenes with likely transition weights were empty (Table 1). In addition, samples 28, 33, and 55 had one empty achene that was 0.0001g or 0.0002g heavier than the transition weight (Figure 4 – larger image). This suggests that the transition between full and empty achenes is more likely a narrow range of weights rather than a single weight.

Sample Number	Head ID/ Position	Assigned Cutoff	Actual Cutoff	Predicted Transition Weight	Transition Weight
10	12139.1 top red	0.001g	0.00162	0.0012	.0012
23	14011.1 bot bac	0.003g	0.002	0.0012 (0.0011, 0.001)	None
27	14142.1 bot wht	0.002	0.0021	0.002 (0.0023)	0.002
28	14142.1 top wht	0.002	0.0024	0.0017 (0.0021, 0.0023, 0.0024)	0.0021 *0.0023
29	14142.2 bot grn	0.002	0.0017	0.0016 (0.0017)	0.0016
30	14142.2 top grn	0.001	0.0012	0.0012 (0.0013)	0.0012
31	14143.1 bot grn	0.002	0.0025	0.0019 (0.0023)	None
32	14143.1 top grn	0.0018	0.0014	None	None
33	14143.2 bot wht	0.0025	0.0024	0.0022	0.0022 *0.0023
42	14963.2 top bac	0.0018	>0.0018	0.0014 (0.0012, 0.001)	None
41	14963.2 bot bac	0.0015	0.0019	0.0017	None
55	17019.1 bot wht	0.0035	0.0028	0.0023 (0.0029, 0.0031)	0.0023 *0.0025
56	17019.1 top wht	0.001	0.0015	0.0017	None
60	17134.2 top wht	0.002	0.0018	0.0017 (0.0025)	0.0017
59	17134.2 bot wht	0.002	0.0022	0.0018 (0.0022, 0.0025)	0.0018
63	17172.1 bot red	0.0033	0.0023	0.0026 (0.0028, 0.003)	None
64	17172.1 top red	0.0016	>0.002	0.0016 (0.0013)	None observed
79	17294.1 bot clr	0.0015	0.0017	0.002 (0.0013, 0.001)	0.0013
80	17294.1 top clr	0.002	0.0019	0.0018	0.0018
92	6417.1 top blu	0.002	0.0016	0.0015	0.0015

Table 1. Assigned cutoff refers to original cutoff prediction obtained by visual examination of achene weight distribution for each sample using R-generated graphs. Sample number was automatically assigned by R. Actual cutoffs are the weights at which all subsequent achenes were full. Predicted transition weight lists weight(s) that two or more achenes possessed in each sample. Weights listed in parentheses were deemed less likely to be the actual transition weight due to their lower weight value or fewer achenes with that weight.

*Weight of an empty achene that does not have a transition weight

Figure 5. Side-by-side comparison of X-ray and photograph images of achenes within 0.0005g of the original cutoff weight for each sample with a transition weight (TW). Achenes that are part of the transition weight are shown in red, as well as empty achenes that are heavier than the transition weight. (A). 12139.1 top red; TW – 0.0012g. (B). 14142.1 bot wht; TW – 0.002g. (C). 14142.1 top wht; TW – 0.0023. (D). 14142.2 bot grn; TW – 0.0016. (E). 14142.2 top grn; TW – 0.0012g. (F). 14143.2 bot wht; TW – 0.0023g. (G). 17019.1 bot wht; TW – 0.0023g. (H). 17134.2 top wht; TW – 0.0017g. (I). 17134.2 bot wht; TW – 0.0018g. (J). 17294.1 bot clr; TW – 0.0013. (K). 17294.1 top clr; TW – 0.0018. (L). 6417.1 top blu; TW – 0.0015g.

Conclusion

Practically, the weight test is still the most convenient method for distinguishing between full and empty achenes. When there is no clear cutoff point, however, the next step should be to identify possible transition weights that one or more achenes possess. Identify the transition weight after which the slope of achene weight distribution levels off, and use that weight as the cutoff. If there is no point after which achene weights level off, use the transition weight closest to 0.002g as the cutoff. To verify the chosen cutoff, use the push test to assess whether the achenes in the transition weight are full. For more accuracy in ambiguous cases, X-raying achenes within 0.0002g of the likely transition weight can provide a conclusive determination.

This study was significantly limited by the small sample size used. Of the ninety-six samples in the Staffanson project, only twenty lacked a clear cutoff weight. As such, future studies on this topic should draw samples from several hundred, or even thousands, of Echinacea heads. In addition, it would be useful to assess the accuracy of using predicted transition weights as cutoff points in a study with a larger sample size. The results of such a study would indicate how often verification by push test or X-ray is needed. Another interesting avenue of investigation would be to study whether cutoff points for the top and bottom achenes on a single head are correlated in any way. The identification of a trend in the variance of cutoff weight of achenes from the same Echinacea head has the potential to increase accuracy inidentifying the correct transition weight for achenes lacking a distinct cutoff point.