PROOFS

A Comparison of Stereomicroscope and Image Analysis for Quantifying Fruit Traits

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ABSTRACT

Two methods, ocular measurements using a stereomicroscope and computerized image analysis, were compared to assess the usefulness of image analysis as an efficient and precise method to measure variation in fruit traits, such as fruit length. Individual fruits from nine plant species differing in size, shape, and the existence of dispersal-related structures were measured repeatedly with both methods. Results showed significant between-method differences in fruit length. Image analysis tended to overestimate fruit length, although differences between the two methods were very small. Both stereomicroscope and image analysis discriminated fruits of different sizes, whatever their shape, and no bias due to size was found in any case. However, low (though significant) correlations were found for measurements of elongated fruits with a tailed pappus obtained by both methods. Overall, we conclude that image analysis represents a good method to accurately estimate variation in fruit traits. The advantages of using image analysis are (1) the high amount of fruit parameters obtained with one single measurement, (2) the minimization of human errors, (3) the reduction of time needed to obtain large data sets concerning fruit trait variability, and (4) the possibility to estimate variability in traits of fruits with complicated shapes.

INTRODUCTION

There is substantial literature on several topics concerning fruit and seed morphology, dispersal capacity, and dormancy, as seeds represent an important life-cycle phase with influence on the ecology, demography, and life-history evolution of flowering plants (Fenner, 1985; Crawley, 1997; Baskin and Baskin, 1998). Thus, significant spatio-temporal variability in fruit traits (e.g., size, shape, dispersal-related structures, etc.) may have important implications for many fields in plant biology. The accuracy with which variation in fruit traits is measured is crucial to draw reliable conclusions. In addition, the quantity of fruits required is important (e.g., hundreds or even thousands may be necessary when fruits from different plants, treatments, and populations have to be measured) to have adequate sample sizes to achieve sufficient statistical robustness.

Traditionally, the method used to quantify fruit traits consisted in measuring fruits under a stereomicroscope, a time-consuming task often with human error that reduces accuracy. Other more sophisticated methods, such as computerized image analysis, represent an alternative to measuring different fruit traits at the same time, speeding up the process and retaining accuracy.

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However, the goodness of fit of image analysis to obtain accurate results on fruit traits has not yet been tested, even though image analysis has already been used to estimate fruit attributes (Illipronti et al., 1999; Keefe, 1999; Kruse, 2000; Sako et al., 2001), as well as to quantify weed biomass (Neeser et al., 2000), classify and count pollen grains (Aronne et al., 2001), and determine leaf area (Hunt and Hodson, 1999; Montero et al., 2000) and root length (Kimura et al., 1999).

The goal of this study was to compare different methods, i.e., stereomicroscope vs. image analysis, to assess the usefulness of image analysis as an efficient and precise method to measure fruit traits. To this end, fruit length of nine flowering plant species, which differed in size and shape, was measured twice: first, with a stereomicroscope, and second, with software for image analysis. The latter approach provides image-processing tools and measurement functions that permit the computer to recognize the features to be measured and produce repeatable results. The following questions were addressed: (1) do different methods, stereomicroscope and image analysis, differ in their respective measurements of fruit length? (2) To what extent can particular fruit traits bias measurements when using both methods?

MATERIALS AND METHODS

Nine plant species were selected that differed in fruit characteristics such as size, shape, and the existence of dispersal-related traits (Table 1). All species produce indehiscent fruits (i.e., fruits that usually originate from an ovary in which only one seed develops), except *Lobularia maritima* (L.) Desv. that produces fruits containing two seeds. Measurements for 15 filled fruits (seeds hereafter in the case of *L. maritima*) per species were carried out by using: (1) a ruled stereomicroscope (SMZ-10, Nikon), and (2) the image analysis software OPTIMAS 6.5 (Media Cybernetics, 1999). With the former, fruits were

TABLE 1. Fruit characteristics for the nine plant species selected in this study. Fruit size, shape, and the dispersal-related traits are qualitatively indicated. The lack of dispersal-related traits is indicated by dashes.

			Fruit trait		
Species	**Code**	**Family**	**Size**	**Shape**	**Dispersal**
Lobularia maritima (L.) Desv.†	1	Brassicaceae	small	rounded	—
Anemone hepatica L.	2	Ranunculaceae	medium	hooked	elaiosome
Daucus carota L.	3	Umbelliferae	small	ellipsoidal	bristles
Hypochaeris radicata L.	4	Compositae	medium	elongated	tailed pappus
Knautia arvensis (L.) Coult.	5	Dipsacaceae	medium	cylindrical	—
Ranunculus bulbosus L.	6	Ranunculaceae	medium	rounded	—
Pimpinella saxifraga L.	7	Umbelliferae	small	hooked	—
Tragopogon pratensis L.	8	Compositae	large	elongated	tailed pappus
Succisa pratensis Moench	9	Dipsacaceae	medium	cylindrical	teeth crown

† Seed traits for *L. maritima*, since this plant produces fruits containing two seeds.

measured individually to the nearest 1 mm. With the latter, fruits were placed individually in a light box of a stereomicroscope (SMT-4, Askania) where the image is captured electronically with a camera (CV-M70, DVS) attached to the stereomicroscope. The system identifies fruits by electronically separating the fruits, seen as dark objects, from the white background in the image. To accomplish this, a macro (ALI programming language) was developed, which automatically calculates several traits (e.g., maximum length, maximum width, perimeter, area, etc.) of fruits placed in the light box. It must be noted that fruits have to be placed in the light box totally individualized. If two fruits touch, the software may recognize them as one single unit. Because image resolution differed depending on fruit size, a resolution of 237 pixels cm^{-1} was used for medium and large fruits of *Hypochaeris radicata* L. and *Tragopogon pratensis* L., and of 821 pixels cm^{-1} for the other fruit types of study. Fruit measurements were saved as ASCII data, which in turn were imported to different statistical packages for later analyses.

Fruit length was measured as the distance from the base of the fruit to the apex. Dispersal-related structures of the pericarp, such as the elaiosome in *Anemone hepatica*, the bristles in *Daucus carota* L. and the teeth crown in *Succisa pratensis* Moench were also included in the measurements, but not the pappus of *H. radicata* and *T. pratensis* (the pappus was removed prior to measurements). Each fruit of each species was first measured with the stereomicroscope, and then, the same individualized fruits were measured with image analysis. From all possible traits that the image analysis can provide, only fruit length was used, which included the dispersal-related structures mentioned above. In fact, image analysis cannot differentiate dispersal-related structures, unless such structures show a marked contrast (dark/light) with the bulk of the fruit. In the case of *H. radicata*, the extension of the pericarp to which the pappus is attached was too thin to be considered by the image analysis as part of the fruit. Thus, only for *H. radicata*, the fruit length measurement included only the bulk of the fruit but not the extension of the pericarp supporting the pappus.

For each species studied, differences between methods in the measurement of fruit length were tested with paired samples test, while the correlation between fruit length using both methods was tested with Pearson's correlation test. To test whether fruit shape and size produced a methodological bias, the standardized regression coefficient (β) for each study species was computed, and tested whether it differed significantly from 1 with a t-test. Variables were log-transformed for improving normality, homoscedasticity and linearity (Zar 1996).

RESULTS

In general, fruit length obtained with image analysis was significantly greater than that recorded with a stereomicroscope (Figure 1 and Table 2), and only fruit length estimates of *Pimpinella saxifraga* L. and *T. pratensis* did not differ between the two methods (Table 2). Nevertheless, there was a highly significant correlation between fruit length estimates obtained from both methods for all species of study (Figure 2 and Table 2), indicating that both stereomicroscope and image analysis accurately discriminated fruits of different sizes. Albeit significant, measurements for both *H. radicata* and *T. pratensis* fruits showed the lowest correlation coefficients (Table 2) and therefore the greatest inaccuracy (Figure 2). However, standardized regression coefficients

Figure 1. Means (±SE) of the fruit length (seed length in the case of *L. maritima*) (N = 15) of nine plant species calculated using a stereomicroscope (filled bars) and image analysis (clear bars). Species codes as in Table 1.

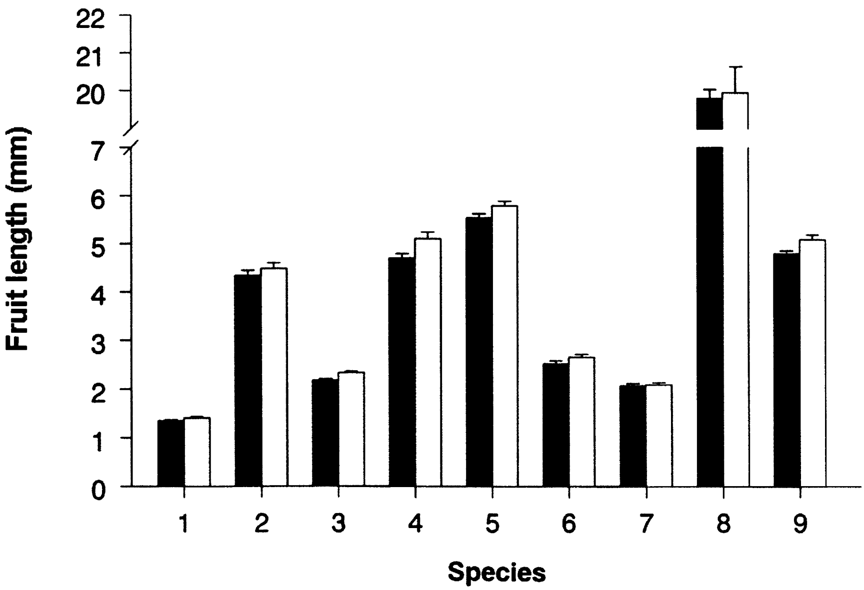
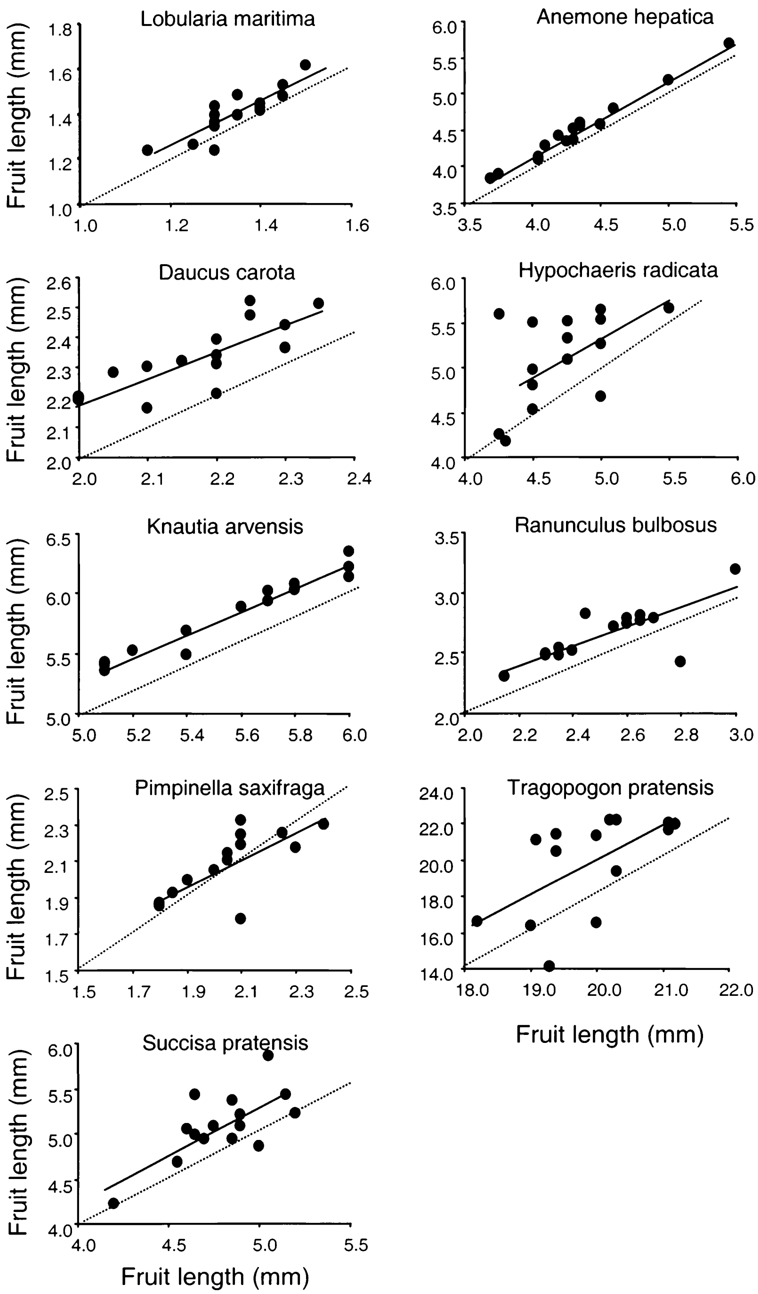


Table 2. Paired samples test for the effects of method (stereomicroscope vs. image analysis) on fruit length (seed length in the case of *L. maritima*) for nine plant species. The t-values and paired samples correlations are given. Degrees of freedom of all analyses, df = 14. Significance: ***, P < 0.0001; **, P < 0.01; *, P < 0.05; ns, non-significant.

Species	t-value	Correlation
Lobularia maritima	-3.71 **	0.90 ***
Anemone hepatica	-8.76 ***	0.99 ***
Daucus carota	-8.52 ***	0.73 **
Hypochaeris radicata	-3.41 **	0.54 *
Knautia arvensis	-12.81 ***	0.97 ***
Ranunculus bulbosus	-3.34 **	0.88 ***
Pimpinella saxifraga	-0.74 ns	0.84 ***
Tragopogon pratensis	-0.02 ns	0.57 *
Succisa pratensis	-4.34 **	0.75 **

FIGURE 2. Scatter plots of fruit length estimates (seed length in the case of *L. maritima*) obtained by stereomicroscope (X–axis) and image analysis (Y–axis) for nine plant species. Solid lines indicate the least-square linear regressions, whereas dotted lines represent the perfect regression with slope of 1.



(β) obtained by regressing measurements from both methods did not differ significantly from 1 ($\beta > 0.54$, $t_s < 1.50$, $P > 0.05$ in all cases), indicating that there was no bias due to fruit shape and size (Figure 2).

We could not compare differences between methods in fruit length excluding dispersal-related structures because image analysis could not differentiate the bulk of the fruit from elaiosomes or bristles. We estimated the length of such dispersal-related structures by subtracting the length of the bulk of the fruit without dispersal-related structures (measured with stereomicroscope) from the fruit length obtained with the stereomicroscope. In all cases, the length of the bulk of the fruit and fruit length were not statistically correlated ($P > 0.07$ for all Pearson's correlation tests), indicating that dispersal-related structures did not vary as a function of fruit size.

DISCUSSION

In this study we examined whether stereomicroscope and image analysis yielded comparable fruit trait measurements. Therefore, we evaluated the usefulness of image analysis as an accurate and fast method to obtain reliable data. The results show that fruit length records given by image analysis were systematically greater than stereomicroscope measurements. Although these differences were significant in seven of the nine species studied (Table 2), the magnitude of the difference was low in all cases (see Figure 1). Apparently, fruit characteristics did not account for between-method differences. In fact, measurements of round-shaped seeds with no secondary structures (e.g., *L. maritima*) differed between methods, whereas hooked fruits (*P. saxifraga*) or with tailed pappus (*T. pratensis*) did not show significant differences between methods.

Three explanations can be given to account for between-method differences in fruit length found in this study. First, differences in the criteria used between the stereomicroscope user and the macro developed to define the fruit might explain the systematic overestimation given by image analysis. Second, image analysis estimated fruit length as the longest axis of the fruit. For uniaxial-type fruits, this does not represent a problem because the criterion applied for both methods is the same. However, for multiaxial-type fruits, the stereomicroscope user will measure all fruits with the same criterion (e.g., from the base of the fruit to the apex), whereas image analysis will seek the longest axis whatever the position of the fruit. Finally, the stereomicroscope measured fruits to the nearest 1 mm whereas image analysis gave records to the nearest 10 μm, which might also account for the small differences between methods found in this study (Figure 1). In all cases, fruit length estimates obtained by both methods were significantly correlated, meaning that fruit size differences were concordant between stereomicroscope and image analysis. In addition, in all species, regression coefficients did not deviate from 1, indicating that the small bias was independent of fruit size. Overall, our results indicate that image analysis can be considered a good method to accurately estimate fruit length and that all fruit types tested produced no methodological bias (Figure 2). However, elongated fruits with tailed pappus, (i.e., *H. radicata* and *T. pratensis*), showed the lowest correlation coefficients (Table 2), perhaps because the extension of the pericarp that supports the pappus cannot be clearly identified by image analysis (e.g. *H. radicata*).

Image analysis was not able to discriminate between the body of the fruit and their dispersal-related parts. Nevertheless, fruit length records can be considered as good estimates of fruit size because, for the species studied, fruit length was primarily accounted for by the body of the fruit, which is positively related to seed quality in terms of embryo size and nutrients available for embryo development, a relationship with high relevance for plant biologists (Fenner, 1985). It must be noted that for ecological and evolutionary studies focusing on spatio-temporal variability in dispersal-related traits, the image analysis technique described cannot provide precise information on variation in generative and dispersal-related structures at the same time. Nevertheless, highly significant relationships between generative and dispersal-related structures can be fitted from a sub-sample of fruits, and then applied to estimate such parameters for the entire dataset of fruit length obtained by using image analysis.

The advantages of using image analysis are many. First, fruit length was used as a trait to compare both methods, but image analysis can provide several parameters related to fruit shape and make them readily available in a database. Second, image analysis minimizes human errors, eliminating undesired sources of variation not accounted for by ecological factors of study. Third, fruit size data collection becomes a fast and easy task once the image analysis process has been defined. Finally, image analysis can provide size estimates of fruits with very particular shapes or complicated secondary structures whose variability would be also difficult and inaccurate to record with stereomicroscope.

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REFERENCES

Aronne, G., Cavuoto, D. and Eduardo, P. 2001. Classification and counting of fluorescent pollen using an image analysis system. *Biotechnic and Histochemistry* 76:35–40.

Baskin, C. C. and Baskin, J. M. 1998. *Seeds. Ecology, Biogeography, and Evolution of Dormancy and Germination*. Academic Press, San Diego.

Crawley, M.J. 1997. *Plant Ecology*. Blackwell Science Ltd., Oxford.

Fenner, M. 1985. *Seed Ecology*. Chapman and Hall, London.

Hunt, M. A. and Hodson, S. L. 1999. Accurate area determination of complex leaves using digital image analysis. *Australian Journal of Plant Physiology* 26:767–772.

Illipronti, R. A., Langerak, C. J. and Lommen, W. J. M. 1999. Variation in physical seed attributes relates to variation in growth of soybean seedlings within a seed lot. *Seed Science and Technology* 27:339–357.

Keefe, P. D. 1999. Measurement of linseed (*Linum usitatissimum* L.) seed characters for distinctness, uniformity and stability testing using image analysis. *Plant Varieties and Seeds* 12:79–90.

Kimura, K., Kikuchi, S. and Yamasaki, S. 1999. Accurate root length measurement by image analysis. *Plant and Soil* 216:117–127.

Kruse, M. 2000. The effect of moisture content on linear dimensions in cereal seeds measured by image analysis. *Seed Science and Technology* 28:779-791.

Media Cybernetics. 1999. OPTIMAS release 6.5. Media Cybernetics, Silver Spring, Maryland.

Montero, F. J., de Juan, J. A., Cuesta, A. and Brasa, A. 2000. Nondestructive methods to estimate leaf area in *Vitis vinifera* L. *Hortscience* 35:696–698.

Neeser, C., Martin, A. R., Juroszek, P. and Mortensen, D. A. 2000. A comparison of visual and photographic estimates of weed biomass and weed control. *Weed Technology* 14:586–590.

Sako, Y., Regnier, E. E., Daoust, T., Fujimura, K., Harrison, S.K. and McDonald, M.B. 2001. Computer image analysis and classification of giant ragweed seeds. *Weed Science* 49:738–745.

Zar, J.H. 1996. *Biostatistical Analysis*. Prentice Hall, Upper Saddle River.