

## RESEARCH LETTER

# Quantification of palmer amaranth seed number using a computerized particle analyzer

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## Abstract

We evaluated the accuracy of a computerized particle analyzer (CPA) for high-throughput counting of Palmer amaranth (*Amaranthus palmeri* S. Watson) seeds and subsequently used the CPA to verify the accuracy of two subsampling methods for estimation of Palmer amaranth seed production. To determine accuracy of the CPA, 55 hand-counted samples, ranging from 500 to 5000 Palmer amaranth seeds, were drawn from field samples and counted with the CPA. The relationship between hand and CPA seed counts was described by a linear model ( $R^2 = 0.99$ ) with a slope of 0.987 and a y-intercept of 3.49. Thus, very little discrepancy exists between seed counts conducted by hand or the CPA. Additionally, two published methods for estimation of Palmer amaranth seed production were compared to the CPA and proven to be highly accurate. We conclude from these findings that the CPA offers a high-throughput alternative for weed scientists who frequently count large quantities of seed.

## 1 | INTRODUCTION

Renewed interest exists in the quantification of weed seed production as a measure of an effective weed management strategy. Monitoring weed seed production and contributions to the soil seed bank has been discussed and reviewed several times (Benoit, Derksen, & Panneton, 1992; Buhler, Forcella, & Hartzler, 1997); however, it remains an uncommon measurement in studies evaluating herbicides. A meta-analysis of literature from *Weed Science* and *Weed Technology* found that of 3,496 articles evaluating herbicide efficacy, only 81 articles included key words associated with weed seed production (Norsworthy, Korres, & Bagavathiannan, 2018). The promotion of zero-threshold strategies and investigation

of harvest weed seed control strategies are indicative of a paradigm shift to prioritize management strategies that reduce or eliminate weed seed (Bagavathiannan & Norsworthy, 2012; Norsworthy, Griffith, Griffin, Bagavathiannan, & Gbur, 2014; Somerville, Powles, Walsh, & Renton, 2018; Walsh, Newman, & Powles, 2013, Walsh et al., 2018).

As weed seed production has captured greater attention among researchers, we were inspired to evaluate the accuracy of previously reported methods for estimating Palmer amaranth (*Amaranthus palmeri* S. Watson) seed production and to evaluate the computerized particle analyzer (CPA) as a high-throughput alternative. Since hand-counting all seeds from harvested Palmer amaranth is prohibitively time consuming, subsamples are hand-counted, weighed, and extrapolated to calculate total seed number. Image analysis has been reported as an alternative to hand-counting (Buhler & Maxwell, 1993), but researchers have continued to hand-count Palmer

**Abbreviation:** CPA, computerized particle analyzer.

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amaranth seeds (Bertucci et al., 2018; Giacomini, Westra, & Ward, 2014; Schwartz et al., 2016). Preliminary research indicates that the CPA is capable of accurately counting tall fescue [*Schedonorus arundinaceus* (Schreb.) Dumort.] seeds (Bartley, Jackson, & Fonteno, 2016); however, no peer-reviewed research has evaluated the CPA for quantification of weed seed production. Further, the relative error of extrapolation associated with Palmer amaranth seed production estimates has yet to be explored.

Accurate modeling and forecasting of Palmer amaranth populations is contingent on the assumption that Palmer amaranth seed production estimates accurately reflect the number of seeds produced in the field. Thus, we designed the present study with two objectives: (i) verify accuracy of Palmer amaranth seed counts generated from a CPA and (ii) use CPA seed counts to determine accuracy of seed production estimates from two sampling methods.

## 2 | MATERIALS AND METHODS

Seeds were collected from female Palmer amaranth plants at the Horticultural Crops Research Station near Clinton, NC, in 2017. Terminal and axillary flowers were stripped in the field and then dried in ovens. Material was threshed by hand, sieved to remove large particles, and then separated from chaff in a vacuum-powered seed cleaning machine (The Real Seed Collection Ltd.). Finally, seeds were separated from remaining vegetative material using a vertical air column seed cleaner.

### 2.1 | Computerized particle analyzer

In general, image analysis is a three-step process composed of staging a material, capturing the image, and using algorithms to process images and collect data. However, these labor-intensive processes are performed simultaneously with instruments like the CPA (Tyler CPA II Conveyor, W.S. Tyler). The instrument vibrates a sample, channeling it to a conveyor system, which propels sample particles between a line-scan camera and light source. As particles pass across the light source, a two-dimensional image of each individual particle is captured. To identify and count particles of interest (e.g., Palmer amaranth seed), digital filters can be applied to the CPA output, based on particle dimensions. In the case of seed counts, criteria were entered to match the circularity, size, and length/width ratio characteristics of Palmer amaranth seeds. Inclusion criteria for this experiment were determined using hand-counted samples and visually reviewing excluded particles using the CPA software. Based on visual output, for a particle to be counted as a seed of Palmer amaranth, criteria were set to a minimum size of 0.4 mm, minimum circularity of 0.8, and a maximum length-to-width ratio of 3:1. Thus,

#### Core ideas

- The computerized particle analyzer counts large quantities of weed seeds accurately.
- Both published methods for estimation of weed seed production are equally accurate.
- Automated seed counting offers a high-throughput alternative to counting seed by hand.

the instrument was able to exclude nonseed particles from reported counts: small particles (dust), noncircular particles (stem fragments), and oblong particles (chaff and bracts).

### 2.2 | Accuracy of computerized particle analyzer seed counts

CPA seed counting accuracy was determined using hand-counted Palmer amaranth seed samples as a standard. Samples were hand-counted to quantities of 500 (7), 1,000 (2), 1,500 (2), 2,000 (2), 2,500 (2), 3,000 (3), 3,500 (2), 4,000 (2), and 5,000 (3) seeds, with numbers in parenthesis indicating the number of samples per quantity. Each hand-counted sample was counted by the CPA three times, generating 75 observations from 25 hand-counted samples. Additional hand-counted samples from subsequent analyses were included to enrich the data pool, resulting in a total of 117 CPA observations from 55 hand-counted samples ranging from 500 to 5,000 seeds. The full data set was used to estimate parameters for a linear model describing the relationship between CPA count and hand-count, described by Equation 1:

$$\text{CPA}_{\text{count}} = (a \times \text{Hand}_{\text{count}}) + b \quad (1)$$

where  $\text{CPA}_{\text{count}}$  is the seed count produced by the CPA,  $\text{Hand}_{\text{count}}$  is the seed number from hand-counted samples,  $a$  is the slope, and  $b$  is the y-intercept.

### 2.3 | Accuracy of methods for seed production estimates

Cleaned Palmer amaranth seeds from eight field-collected samples were homogenized, and 25-g aliquots were weighed from each sample. Each 25-g aliquot then represented the collection of seeds to be tested using two methods to estimate seed number: the count-based method and biomass-based method. Each method was replicated three times, for a total of 24 observations for each method. The count-based method was reported by Sellers, Smeda, Johnson, Kendig, and Ellersieck (2003). The first step is hand-counting subsamples

(in this case it was 100 seed, but it can vary to any number), and the second step is weighing then extrapolating total seed number using the following equation:

$$S_{\text{total}} = (M_{\text{total}}/M_{\text{sample}}) \times 100 \quad (2)$$

where  $S_{\text{total}}$  and  $M_{\text{total}}$  are seed number and mass of clean seed from harvested Palmer amaranth, respectively, and  $M_{\text{sample}}$  is the average mass of the five 100-seed samples. In the present study,  $M_{\text{total}}$  was 25 g, based on aliquots from field-collected samples.

For the biomass-based method, the first step is weighing subsamples to a predetermined mass (0.5 g here, but it can be any number), and the second step is to hand-count then extrapolate total seed number using the formula proposed by Sosnoskie, Webster, Grey, and Culpepper (2014):

$$S_{\text{total}} = \frac{(M_{\text{total}})(S_{\text{sample}})}{M_{\text{sample}}} \quad (3)$$

where  $S_{\text{total}}$  and  $M_{\text{total}}$  are seed number and mass of clean seed from harvested Palmer amaranth, respectively, and  $S_{\text{sample}}$  and  $M_{\text{sample}}$  are seed number and mass of clean seed from 0.5-g subsamples, respectively. In the present study,  $M_{\text{total}}$  was 25 g, based on aliquots from field-collected samples.

Because the CPA can rapidly count a fixed mass of seed, a CPA biomass-based treatment was included to test the accuracy of the CPA-counts extrapolated from 0.5-g samples, relative to the CPA-counts from the 25-g aliquots. The calculated  $S_{\text{total}}$  for each method, Equations 2 and 3, was compared to the CPA-counts of the 25-g aliquots, and the relative error of each method was calculated using the following equation:

$$\text{Relative error} = \frac{S_{\text{total}} - CPA_{\text{count}}}{CPA_{\text{count}}} \times 100 \quad (4)$$

where  $S_{\text{total}}$  is the Palmer amaranth seed number from calculated from Equation 2 or 3 and  $CPA_{\text{count}}$  is the seed number reported by CPA.

## 2.4 | Statistical analysis

Accuracy of CPA-counts was evaluated using PROC GLM in SAS version 9.4 (SAS Institute, 2016), treating hand-counted samples as the standard. The model statement defined  $CPA_{\text{count}}$  as the dependent variable and  $S_{\text{total}}$  as the independent variable and then tested for a linear relationship between the terms. A solution statement generated the slope and y-intercept, describing a linear relationship between the two variables (Equation 1).

Analysis of variance (ANOVA) was conducted on relative error for seed production estimates of the biomass-based and count-based methods (Equation 2). Relative error for seed

production estimates was subjected to ANOVA using PROC MIXED in SAS, with seed production estimation method (i.e., biomass-based or count-based) treated as a fixed effect and seed source and seed source  $\times$  method treated as random effects.

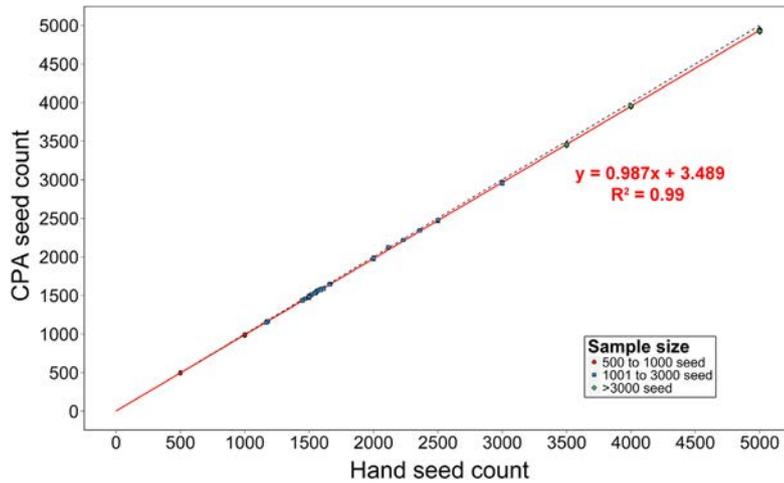
## 3 | RESULTS AND DISCUSSION

### 3.1 | Accuracy of computerized particle analyzer seed counts

The ANOVA provided strong evidence that Palmer amaranth seed counts generated by the CPA differed from hand-counted seeds ( $P < .0001$ ). However, the relationship between counting methods was best described by a linear model with a slope of 0.987 and an intercept of 3.49 (Figure 1). The coefficient of determination was close to 1 ( $R^2 = 0.99$ ), indicating that little variation between the two methods of counting is unexplained by the model. Most important, the model predicts a slope of 0.987, which is close to perfect accuracy (i.e., slope = 1), indicating high accuracy of CPA counts. Still, a slope less than 1 shows that the CPA underreports seed numbers relative to hand-counted samples. Indeed, 110 of 111 samples reported fewer seeds from CPA counts, while only one sample recorded a higher seed number than the hand-counted number. The trend for the CPA to underreport seed was likely due to the exclusion of particles by digital filters. Based on these results, we are confident that the CPA can be used as a standard for seed counting as reliably as hand-counting, which has previously served as the scientific standard (Chahal, Irmak, Jugulam, & Jhala, 2018; Vila-Aiub, Gundel, & Preston, 2015).

### 3.2 | Relative error of seed production estimates

Because the CPA was determined to count seeds with high fidelity (Figure 1), relative error associated with the biomass-based methods and count-based method were assessed using the CPA as a standard (Equation 4). Output from ANOVA determined that no significant differences exist between extrapolated seed counts ( $P = 0.9456$ ) and relative error of seed counts ( $P = 0.4513$ ) among seed production estimation methods (Table 1). Thus, each method was determined to estimate seed counts with similar accuracy. As expected, the biomass-based method exhibited the highest accuracy (within 0.01%) when seed counts were conducted using the CPA (Table 1). This is unsurprising because the comparison was simply a measure of the CPA's ability to consistently count seeds among two seed quantities, the 0.5-g subsamples and the 25-g aliquots. More interesting is the comparison of relative error between the hand-counting methods, biomass-based and



**FIGURE 1** Linear relationship comparing hand-counted and computerized particle analyzer (CPA) counted samples of Palmer amaranth seeds. The slope of the fitted line (—, in red) was 0.987 and indicates a consistent under estimation by the CPA relative to hand-counted samples. A perfect linear correlation is portrayed by a dashed line (- -) with a slope of 1 and y-intercept of 0

**TABLE 1** Comparison of extrapolated Palmer amaranth seed counts and relative error associated with each method for estimation of seed production, using computerized particle analyzer (CPA) counts as a standard

Seed production estimation method	Extrapolated seed count <sup>a</sup>	Relative error <sup>b</sup>
	Seed 25-g <sup>-1</sup> sample	%
CPA biomass-based method	79,792	-0.01
Biomass-based method	78,404	0.80
Count-based method	79,620	0.90
<i>P</i> value	0.4555	0.9456

Note: Least square means generated using output from ANOVA, using the MIXED procedure in SAS 9.4.

<sup>a</sup>Extrapolated seed counts for each seed production estimation method were calculated to determine the predicted seed number in a 25-g aliquot of field-collected Palmer amaranth seed. Estimates were determined using Equations (2) and 3.

<sup>b</sup>Relative error for each seed production estimation method was calculated as percent, relative to the CPA-count of a 25-g aliquot. Relative error was determined using Equation 4.

count-based. Each of these methods produced very small relative errors and estimated seed counts within 1% of the CPA extrapolated standard.

### 3.3 | Research implications

The CPA produces accurate Palmer amaranth seed counts and can filter nonseed particles reliably. Dynamic image analysis with the CPA is more rapid than counting by hand and offers researchers a high-throughput option for estimating seed production. Given the success with Palmer amaranth, we expect that this technology would work well for other weeds in the genus *Amaranthus*, which share seed characteristics (Sauer, 1957).

The present findings affirm that researchers can confidently report seed production estimates using either count-based or biomass-based Palmer amaranth seed production estimation

methods. However, it must be noted that we were working with meticulously cleaned materials, with limited amounts of nonseed particles. Generally, extrapolations of biomass-based methods are preferable because they account for mass contributed by contaminant particles such as mineral substrate or vegetative materials. In contrast, extrapolations based on a fixed seed number are less desirable because they fail to account for contaminants and their contribution to the mass of a sample.

No previous work has evaluated relative error associated with biomass-based and count-based methods for Palmer amaranth seed estimation. The present study found no significant difference between the two methods and determined that both methods were reliable and accurate. Further, the CPA represents a new technology that may be used to efficiently and accurately count seeds as well as either previously published method. We conclude that researchers with large amounts of seeds to quantify may consider the CPA as a high-throughput alternative to hand-counting.

### CONFLICT OF INTEREST

The authors have no conflicts of interest to declare.

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