Floriculture crops had a wholesale value of $4.4 billion in the United States in 2015 (U.S. Department of Agriculture, 2016). The floriculture industry produces many crops using specialized substrates, and many of these crops are produced from seeds. An ideal seed propagation substrate is firm enough to support a seed, allows for gas exchange to ensure oxygen is present for developing roots, holds water to facilitate hydration of the seed without promoting anoxia, is free of pathogens, and is economical (Hartmann et al., 2011). Sphagnum peat, perlite, and vermiculite are commonly used in propagation substrates. Growing concern for turfgrass (Morley, 1929), commercial horticultural production (Dumroese et al., 2011; Graber et al., 2010; Headlee et al., 2014; Vaughn et al., 2013). Most research testing biochar or other forms of PCM in seed propagation or plant establishment substrates has focused on ecological or field agricultural applications, as opposed to soilless substrate culture in container production settings. Seed germination studies with potato (Solanum tuberosum L.; Bamberg et al., 1986) and sunflower (Helianthus annuus L.; Alburquerque et al., 2014) have shown increased germination rates when activated charcoal was added to the respective substrates. Keeley and Pizzorno (1986) reported increased germination of herbaceous California chaparral plant seeds [Emmenanthe pendaliflora Bentham. and Eriphyllum confer- tiforum (DC.) A. Gray] when exposed to char or heat-treated wood products in screened potting soil. They concluded that the presence of heated xylan and glucuronic acid were involved in promoting germination. Two studies have reported an increase in wheat (Triticum aestivum L.) germination in soils amended with biochar (Solaiman et al., 2012; Van Zwieten et al., 2010). Nair and Carpenter (2016) reported that germination increases in peppers (Capsicum annuum L.) produced in cell trays with soilless mixes amended with hardwood biochar, whereas Liopa-Tsakalidi and Barouchas (2017) reported that pepperoncini (C. annuum L. ‘Stavros’) germination increases in an acidic soil amended with wood chip biochar and no germination change in alkaline soil amended with the same biochar. Also, biochar addition has resulted in no significant changes in maize (Zea mays L.), soybean [Glycine max (L.) Merr.], radish (Raphanus sativus L.), mung bean [Vigna radiata (L.) R. Wileczek], sunflower, or wheat germination (Free et al., 2010; Paneque et al., 2016; Solaiman et al., 2012; Van Zwieten et al., 2010). A reduction in germination percentage after biochar amendment has been reported for mung bean, subterranean clover [Trifolium subterraneum L.], and wheat (Solaiman et al., 2012). Plant establishment and growth in substrates amended with biochar or other forms of PCM have been variable. Increases in fresh and dry weight, root length, and plant height have been reported, as well as decreases. The rate of biochar inclusion, base substrate or soil, biochar volatile matter, biochar-free radicals, synergistic effect with fertilizers, and crop selection have all been identified as factors (Deenik et al., 2010; Liao et al., 2014; Solaiman et al., 2012; Van Zwieten et al., 2010). The effect of differences in biochar feedstock, processing, and physical properties between experiments is not known. Plant establishment and growth study data are often collected as plant height (Grabber et al., 2010; Sun et al., 2014; Vaughn et al., 2013), dry

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Table 1. End-of-study physical and chemical properties of cornell seed germination mix amended with coconut shell biochar used in germination and seedling growth experiments.

| Substrate biochar (%) | pH | Conductivity (mS cm⁻¹) | Total exchangeable cations (meq kg⁻¹) | N | P | K (mg kg⁻¹) | Ca | Mg | C/N ratio | Dry bulk density (kg m⁻³) |
|-----------------------|----|------------------------|---------------------------------------|---|---|------------|----|----|-----------|--------------------------|
| 0                     | 6.0 ± 0.1 | 0.27 ± 0.03 | 369 | 489 | 135 | 1,029 | 4,606 | 979 | 28.4 | 189.8 |
| 5                     | 6.1 ± 0.1 | 0.24 ± 0.02 | 260 | 279 | 124 | 1,310 | 3,121 | 703 | 49.0 | 227.2 |
| 10                    | 6.0 ± 0.1 | 0.26 ± 0.01 | 240 | 289 | 150 | 1,433 | 2,729 | 617 | 60.9 | 255.1 |
| 20                    | 6.1 ± 0.1 | 0.27 ± 0.02 | 210 | 256 | 107 | 1,675 | 2,170 | 481 | 87.9 | 310.3 |
| 40                    | 6.0 ± 0.1 | 0.25 ± 0.02 | 146 | 55 | 112 | 1,815 | 1,201 | 327 | 80.1 | 417.1 |

GR = coconut shell biochar <0.001 <0.001 <0.001 <0.001 <0.001 <0.001
Block 0.438 0.901 0.559 0.170 0.038 0.227
GR 0.140 <0.001 0.546 <0.001 <0.001 0.131

*1:5 dilution testing (substrate to deionized water), n = 5.
*2:1 dilution testing (substrate to deionized water), n = 5.

| Biochar (%) | Final germination (%) Coreopsis Eschscholzia Leucanthemum² | Timson’s index³ | Coreopsis Eschscholzia Leucanthemum² |
|-------------|-------------------------------------------------------------|----------------|-------------------------------------|
| GR1 0       | 82.0 ± 14.0                                                 | 53.0 ± 14.9    | 59.0 ± 16.8                         |
| 5           | 80.0 ± 16.3                                                 | 40.0 ± 22.1    | 47.0 ± 14.2                         |
| 10          | 80.0 ± 8.2                                                  | 67.0 ± 18.9    | 65.0 ± 16.5                         |
| 20          | 73.0 ± 12.5                                                 | 52.0 ± 14.0    | 70.0 ± 14.1                         |
| 40          | 79.0 ± 11.0                                                 | 52.0 ± 22.5    | 54.0 ± 20.7                         |
| GR1 mean    | 78.8 ± 12.6                                                 | 52.8 ± 20.0 a   | 59.0 ± 17.9                         |
| GR2 0       | 81.0 ± 9.9                                                  | 77.0 ± 13.4    | 58.0 ± 15.5                         |
| 5           | 84.0 ± 8.4                                                  | 80.0 ± 13.3    | 62.0 ± 19.3                         |
| 10          | 82.0 ± 14.0                                                 | 81.0 ± 13.7    | 60.0 ± 18.9                         |
| 20          | 81.0 ± 16.0                                                 | 84.0 ± 10.7    | 57.0 ± 25.4                         |
| 40          | 75.0 ± 15.8                                                 | 80.0 ± 11.5    | 49.0 ± 27.3                         |
| GR2 mean    | 80.0 ± 13.0                                                 | 80.4 ± 12.3 b   | 57.2 ± 21.3                         |
| General linear model analysis of variance (P values) | | | |
| GR          | 0.499                                                      | <0.001         | 0.485                              |
| Block       | 0.838                                                      | 0.194          | 0.051                              |
| Biochar     | 0.590                                                      | 0.089          | 0.194                              |
| GR × biochar | 0.661                                                      | 0.121          | 0.199                              |

Table 2. Final germination (%) and Timson’s index means for herbaceous seedlings after 3 weeks of growth in cornell seed germination mix amended with coconut shell biochar (v/v) repeated in two germination rooms (GRs), with so (n = 10).

| Biochar (%) | Shoot length (mm) Coreopsis Eschscholzia Leucanthemum | Root length (mm) Coreopsis Eschscholzia Leucanthemum |
|-------------|--------------------------------------------------------|-------------------------------------------------------|
| GR1 0       | 16.6 ± 3.1 a                                           | 42.5 ± 13.1 a                                         |
| 5           | 15.9 ± 3.8 a                                           | 32.0 ± 11.0 a                                         |
| 10          | 25.5 ± 1.9 c                                           | 75.2 ± 5.0 b                                         |
| 20          | 22.4 ± 4.9 bc                                          | 75.0 ± 8.8 b                                         |
| 40          | 25.9 ± 1.7 c                                           | 72.3 ± 9.1 b                                         |
| GR2 0       | 22.8 ± 2.0 bc                                          | 63.7 ± 8.1 a                                         |
| 5           | 21.9 ± 1.8 b                                           | 69.9 ± 6.4 a                                         |
| 10          | 21.0 ± 1.8 b                                           | 72.1 ± 4.8 a                                         |
| 20          | 21.3 ± 2.7 b                                           | 70.9 ± 7.0 a                                         |
| 40          | 23.5 ± 2.2 b                                           | 70.5 ± 9.9 a                                         |
| General linear model analysis of variance (P values) | | |
| GR          | 0.140                                                   | <0.001                                               |
| Block       | 0.438                                                   | 0.901                                                |
| Biochar     | <0.001                                                  | 0.059                                                |
| GR × biochar | <0.001                                                  | <0.001                                               |

Table 3. Primary shoot and primary root length (mm) means for herbaceous seedlings after 3 weeks of growth in cornell seed germination mix amended with coconut shell biochar (v/v) repeated in two germination rooms (GRs), with so (n = 10).

| Biochar (%) | Shoot length (mm) Coreopsis Eschscholzia Leucanthemum | Root length (mm) Coreopsis Eschscholzia Leucanthemum |
|-------------|--------------------------------------------------------|-------------------------------------------------------|
| GR1 0       | 16.6 ± 3.1 a                                           | 42.5 ± 13.1 a                                         |
| 5           | 15.9 ± 3.8 a                                           | 32.0 ± 11.0 a                                         |
| 10          | 25.5 ± 1.9 c                                           | 75.2 ± 5.0 b                                         |
| 20          | 22.4 ± 4.9 bc                                          | 75.0 ± 8.8 b                                         |
| 40          | 25.9 ± 1.7 c                                           | 72.3 ± 9.1 b                                         |
| GR2 0       | 22.8 ± 2.0 bc                                          | 63.7 ± 8.1 a                                         |
| 5           | 21.9 ± 1.8 b                                           | 69.9 ± 6.4 a                                         |
| 10          | 21.0 ± 1.8 b                                           | 72.1 ± 4.8 a                                         |
| 20          | 21.3 ± 2.7 b                                           | 70.9 ± 7.0 a                                         |
| 40          | 23.5 ± 2.2 b                                           | 70.5 ± 9.9 a                                         |

Digital imaging and analysis have been used to quantify plant growth in micropropagation (Smith et al., 1989), aquatic plant establishment (Sher-Kaul et al., 1995), and in plant phenotyping studies (Goizarian et al., 2011; Leister et al., 1999). The equipment and software required ranged widely and in some cases, the software may be free (Tajima and Kato, 2011). Digital imaging allows seedling length and two-dimensional area data to be easily calculated with software. To date, most biochar studies with plant growth rely on weight or length measurement data as dependent variables, so it is important to understand how two-dimensional area data correlate with these metrics.

Digital imaging analysis can be promising, but more research is needed in the context of ornamental plant propagation. The species in this study were selected from the ornamental perennial plant industry (Carpentia grandiflora Hogg ex Sweet ‘Early Sunrise’ and Leucanthemum ×superbum Bergman ex J. Ingram ‘Silver Princess’) and the restoration and revegetation industry (Eschscholzia californica Cham.; Montalvo...
et al., 2002) because their germination and seedling establishment responses to a PCM have not been documented. The primary objective was to assess the effects of coconut shell biochar in propagation substrate on seed germination and seedling growth for these three species. A secondary objective was to examine the relationship between digital imaging data and traditional, manually collected data.

Materials and Methods

A germination study comprising three experiments was conducted in two germination rooms (GRs) in California. GR1 (July 2015) was in San Luis Obispo and GR2 (Nov. to Dec. 2015) was in Arroyo Grande. The GRs were climate controlled, contained benches with bottom-heat mats, and had cool white fluorescent lighting (Toole, 1963). The GR1 mean air temperature was 24.5 °C (1.1 so), mean substrate temperature was 24.6 °C (1.1 so), mean relative humidity (RH) was 72.9% (9.4 so), and the 24-h mean photosynthetically active radiation (PAR) level was 14.0 μmol·m⁻²·s⁻¹. The GR2 mean air temperature was 21.0 °C (1.2 so), mean substrate temperature was 21.2 °C (1.1 so), mean RH was 65.3% (4.3 so), and the 24-h mean PAR level was 15.9 μmol·m⁻²·s⁻¹.

Seeds and substrates. Seeds of C. grandiflora ‘Early Sunrise’, Leucanthemum ×superbum ‘Silver Princess’ (W. Atlee Burpee & Co., Warminster, PA), and E. californica (Anderson’s Seed Co., Escondido, CA) were obtained from commercial suppliers. The base mix was amended with biochar from a commercial supplier (Bay Area Biochar, Concord, CA) at rates of 0%, 5%, 10%, 20%, or 40% (v/v). The biochar was made from coconut shell feedstock treated to fast pyrolysis at ~600 °C, then ground to small particles and acidified. The chemical and physical properties of the biochar were analyzed by a commercial laboratory (Waypoint Analytical, Anaheim, CA): pH: 6.4; dry bulk density: 362.1 kg·m⁻³; C/N ratio: 107.6; total exchangeable cations: 95 meq·kg⁻¹; and dry weight percentage passing screen sizes: 6.4 mm (99.8%), 4.8 mm (99.4%), 2.4 mm (97.7%), 1.0 mm (37.5%), and 0.5 mm (15.4%). The biochar was a coarse powder according to the classification system proposed by Camps-Arbestain et al. (2015).

Sphagnum peat and biochar were screened to a maximum particle size of 6 mm before use. At the conclusion of the study, substrate samples were sent to a commercial laboratory for chemical and physical analysis (Waypoint Analytical). Additional pH (IQ150; Spectrum Technologies, Inc., Aurora, IL) and electrical conductivity (2265FS; Spectrum Technologies, Inc.) substrate testing was conducted using 1:5 (substrate:deionized water, v/v) diluted samples (Table 1).

Experimental design and setup. Each species was treated as a separate experiment with 10 replicates. The experiments were randomized complete block designs, with blocks and biochar substrate amendment as independent factors. Seeds were sown in 7.9-cm-deep square pots with a volume of 538 cm³ (SVT-400; T.O. Plastics, Inc., Clearwater, MN). Each pot was an experimental unit with 10 seeds, and the seeds were observational units. Seeds were lightly covered (2 mm) with coarse vermiculite after sowing (Vermiculite 3 Medium; Therm-O-Rock Industries, Inc., Chandler, AZ). Misting was provided on a weekly basis in GR1 and twice weekly in GR2. Each experimental unit received ~5 mL of water per misting.

Data collection. Data were collected daily as the number of seeds visibly germinated in each pot. The emergence of any plant structure from the seedcoat was considered visible germination (Bewley et al., 2013). The seedlings were removed from the substrate after 21 d, and any substrate particles clinging to the root system were gently removed with a soft brush and a spray bottle. Mean seedling primary root length and primary shoot length for each experimental unit were measured manually to the closest millimeter using a ruler.

After the experiments in GR2, seedling roots and shoots were separated with a razor after they were manually measured. The roots and shoots from each experimental unit were then placed on a transparent cellulose acetate
shoot taking care to avoid overlapping the plant tissues. They were subsequently scanned at 600 dpi against a white background (Epson Perfection V19; Epson America, Inc., Long Beach, CA). Seedling tissue was oven dried at 50 °C for 48 h, then weighed. Scans were analyzed with ImageJ, ver. 1.50a (National Institutes of Health, Bethesda, MD) to determine mean seedling two-dimensional area (cm²) for each experimental unit. The enhance contrast function was used at 0.25% saturated pixels, followed by conversion to an 8-bit binary image, then the analyze particles function (size: 0.01–infinity cm², circularity: 0.00–1.00) to measure the area and generate masks. The masks were visually compared with the original scans to ensure that the software had identified the plant tissue and removed the background. Digital tracing of shoots and roots to the closest millimeter was conducted in ImageJ with the Measure and Label plugin (Rasband, 2006).

Atmospheric water uptake quantification. Based on the observations in GR1, a follow-up experiment was conducted to quantify differences in water uptake from the atmosphere between the substrates. The substrates were oven dried for 72 h at 80 °C, then 200 cm³ were placed in a 7.3-cm-deep, 7-cm-wide square plastic pot (#1642; Anderson Die & Mfg., Co., Portland, OR). There were five replications. The substrates were weighed, and then placed in a chamber with 72% RH in cool storage for 4 months between the GR1 experiments and the GR2 experiments. Timson’s index data showed no biochar effect (Table 2). Eschscholzia had a higher index in GR2 (1368.8) than GR1 (895.0; P < 0.001; Table 2), which was the result of the high germination rate in GR2. Leucanthemum had a higher Timson’s index in GR1 (929.8) than GR2 (674.8; P < 0.001; Table 2), which was the result of a faster rate of germination in GR1 than GR2.

Shoot and root length. An interaction occurred between GR and biochar with regard to shoot and primary root length for all three species (P ≤ 0.002; Table 3). Coreopsis, Eschscholzia, and Leucanthemum

Shoot and root length data were correlated with biochar amendment rate. Two-dimensional area was linearly regressed with seedling dry weight and seedling length in Minitab. A multiple regression model was constructed with seedling length and seedling weight as predictors of two-dimensional area. Digital tracing and traditionally collected data were correlated in Minitab.

Water uptake after 7 d was analyzed as VMC using the general linear model function in Minitab, with block as a random factor. Post hoc mean separation was performed using Tukey’s studentized range test. The significance level (α) used to reject null hypotheses in all statistical tests was 0.05.

Results and Discussion

Germination. The biochar used in this study did not have a significant effect on final seed germination percentage for any of the three species (Table 2). These results are similar to the findings with other biochars and their effect on maize, radish, soybean, mung bean, and wheat germination (Free et al., 2010; Solaiman et al., 2012; Van Zwieten et al., 2010) and suggest that coconut shell biochar may be acceptable in seed propagation substrates used for Coreopsis, Eschscholzia, and Leucanthemum.

Eschscholzia germination was higher in GR2 (80.4%) than in GR1 (52.8%; P < 0.001; Table 2). A final germination percentage as low as 50% is consistent with published Eschscholzia germination rates (Montalvo et al., 2002). Eschscholzia is thought to have morphological or morphophysiological dormancies that reduce germination percentages (Baskin and Baskin, 2014; Cook, 1962). Fire and temperature may break Eschscholzia dormancy, with higher germination percentages reported for seeds exposed to liquid and dry smoke or chilling (Montalvo et al., 2002). The higher Eschscholzia germination percentage in GR2 compared with GR1 may have been a result of the seeds being held in cool storage for 4 months between the GR1 experiments and the GR2 experiments. Timson’s index data showed no biochar effect (Table 2). Eschscholzia had a higher index in GR2 (1368.8) than GR1 (895.0; P < 0.001; Table 2), which was the result of the high germination rate in GR2. Leucanthemum had a higher Timson’s index in GR1 (929.8) than GR2 (674.8; P < 0.001; Table 2), which was the result of a faster rate of germination in GR1 than GR2.

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shoot length increased at the three highest rates of biochar amendment compared with the lowest two rates in GR1 (P ≤ 0.05), though that response was not repeated in GR2 (Table 3). Primary root length increased with 40% biochar amendment compared with the control for all three species, and that response was repeated in GR2 (P ≤ 0.05; Table 3).

Plant growth differences between GRs may have been caused by environmental conditions. The substrate temperatures in both GRs remained in a favorable range for Coreopsis and Leucanthemum germination; however, the GR1 substrate temperatures 24.6 °C were higher than optimal (21 °C) for subsequent growth (Nau, 2011). This could possibly explain differences in root growth, but it is not probable because the 0% and 5% biochar substrates were disproportionately affected. A more likely reason for differences between GRs was water availability.

At the conclusion of the GR1 experiment, leftover substrate stored in containers in the GR exhibited differences in water adsorption as affected by biochar amendment level. This observation prompted an experiment to quantify the amount of water adsorbed from the atmosphere by each substrate. After 7 d, at 72% atmospheric RH, substrates amended with biochar at a level of 20% or 40% had the highest WVC followed by substrates amended with 5% or 10% biochar; the control had the lowest WVC (P ≤ 0.05; Fig. 1). These data suggest that the 40% biochar substrate experimental unit absorbed ≈5.4 mL water per week more than the control in GR1. When misting was increased to twice weekly in GR2, biochar level did not affect root and shoot growth.

Biochars may be hydrophilic or hydrophobic. The temperature of pyrolysis may affect biochar water adsorption capacity. Pyrolysis at ≈600 °C has produced biochars with high water adsorption capacity (Gray et al., 2014; Kinney et al., 2012), which aligns with the biochar used in this study. Seedling shoot and root lengths had low to moderate positive correlations with biochar amendment rate for each plant species (r = 0.33–0.54). These increases are consistent with reported positive plant growth responses to other biochars (Solaíman et al., 2012; Vaughan et al., 2013). Previous studies have attributed increased plant growth to synergism between biochar and fertilizers (Deenik et al., 2010), biochar-induced plant responses (Graber et al., 2010), microorganism balance (Graber et al., 2010), nutrient availability (Headlee et al., 2014), and improved substrate properties (Alburquerque et al., 2014). This study suggests that water absorption may be another potential benefit, particularly when atmospheric humidity is high and irrigation rate is low.

**Dry weight and scans.** Propagation substrate amended with 40% coconut shell biochar increased Coreopsis seedling dry weight (0.9 mg) compared with the control (0.7 mg; P ≤ 0.05). Biochar percentage did not affect Leucanthemum seedling dry weight. These mixed results are consistent with similar studies of plant growth responses to biochar incorporation in soils and substrates (Solaíman et al., 2012; Van Zwieten et al., 2010).

Biochar level had no effect on the two-dimensional area (cm²) of Coreopsis and Leucanthemum; however, seedling dry weight and seedling length (shoot length + primary root length) predicted a significant percentage of the variation in seedling two-dimensional area (Fig. 2) for Coreopsis and Leucanthemum. When seedling dry weight and seedling length where combined as a two-factor regression to predict root and shoot length, 73% of the variation in Coreopsis area (R² = 0.73; P < 0.001) and 87% of Leucanthemum area (R² = 0.87; P < 0.001) could be explained by these factors. The equation for Coreopsis was S-AREA = 0.0125 + 0.2936 SDW + 0.00784 SL [where S-AREA = seedling two-dimensional area (cm²), SDW = seedling dry weight (mg), and SL = seedling length (mm)]. The equation for Leucanthemum was S-AREA = 0.309 + 0.2533 SDW + 0.00603 SL. These results suggest two-dimensional area provides meaningful data closely allied with more traditional weight and length measurements. Digitally traced shoot and root lengths were strongly positively correlated (r = 0.99–0.97) with shoot and root length measurements collected manually with a ruler (Fig. 3). This strong association between manually collected data and digitally collected data suggests both methods are acceptable. The practicality of digital imaging will be influenced by the plant and substrate. In this study, seedlings were easy to scan because substrate was easily removed from roots and the seedling shoots and roots had minimal branching. It was easy to avoid overlapping the plant tissue. Plants with highly developed root or shoot systems would increase the difficulty of collecting digital images free of overlap.

Benefits of digitally collected data include avoidance of handling small, dried samples and the presence of easy-to-store sets for verification or reanalysis of data. Using digital methods for area and length measurements may increase research efficiency by allowing one scan to provide multiple data points. In this study, manual root length data were collected faster than digitally traced data; however, manual root weight data were much slower to collect than digital root area data. When plants appropriate for scanning are paired with multiple metrics per scan, the advantages of digital data collection are substantial.

Not all biochar will be suitable for propagation substrates; however, this study indicates that coconut shell biochar may be used successfully when germinating Coreopsis, Eschscholzia, and Leucanthemum seed. In this study, biochar either had no effect or a minimal effect on seed germination, and it had either no effect or a slightly positive effect on seedling growth. Therefore, coconut shell biochar can maintain the favorable properties of a substrate while displacing ingredients with high economic or ecological cost.

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