Optimization of *Waltheria indica* Seed Yield Using a Visual Evaluation Scale of Seed Head Cluster Maturity

Scott B. Lukas*, Joseph DeFrank and Orville C. Baldos

ABSTRACT

The determination of seed harvest timing is important to maximize seed production yields. Indeterminate flowering patterns present a challenge to optimize the harvest of mature seed, before the point of seed shattering and subsequent losses. A visual assessment of *Waltheria indica* seed heads was used to separate clusters into six discrete flower cluster categories (FCCs), based on visual parameters of flowering and green and necrotic tissue. The six FCCs were analyzed for mature seed yield and moisture content. Results indicated that the significantly greatest seed yield was obtained from the third FCC, containing 16.40 g of mature seed per 100 g of dried flower heads. The flower cluster moisture content for the optimal harvest of FCC 3 was 52%, and represented the first drop in moisture content that peaked at 57%. The initial decline in moisture content after FCC 2 can be used as a tracking indicator for the initiation of mature seed formation. Tracking moisture content and applying discrete visual flower head attributes of FCCs could enable practitioners to optimize seed yield during harvesting efforts. This method is recommended for in-field decision making to optimize seed recovery for *W. indica*.

INTRODUCTION

Waltheria is a genus of plants belonging to the order Malvales and family Sterculiaceae. *Waltheria indica* L. (ACC# 9079945) is also known as *Waltheria americana* and *Waltheria elliptica* (18). *Waltheria indica* (uhaloa) is a short-lived shrub or subshrub, sometimes reaching 2 m in height and 2 cm in stem diameter, usually with a single strong stem but frequently branches near the ground (8). The young stems and leaves are covered with a gray, velvety pubescence. The leaves are narrowly ovate or oblong with a rounded to subcordate base, irregularly serrate edges, and a rounded to acute tip. The petioles are 0.5–3.3 cm long and the blades 2–12 cm long and 1–7 cm broad. Axillary inflorescences are usually dense glomerules that contain fragrant, yellow to orange flowers. Each 2-mm capsule holds one small, black, obovoid seed.

In Hawaii, *W. indica* has been identified for increased usage in urban re-vegetation, including roadway corridors (7). However, no plant material or seed stock is commercially available, complicating re-vegetation efforts. *Waltheria indica* plants begin flowering at about 6 mon and bloom more or less continuously

Scott B. Lukas, Department of Horticulture, Hermiston Agricultural Research and Extension Center, Oregon State University, 2121 South 1st street, Hermiston, OR 97838; Joseph DeFrank and Orville C. Baldos, Department of Tropical Plant and Soil Sciences, University of Hawaii at Manoa, 3190 Maile Way, St. John Plant Science, Honolulu, HI 96822. *Corresponding author (scott.lukas@oregonstate.edu).
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until the plant weakens with age or stress (6). Reproduction of *W. indica* is most commonly achieved through seeds. A study by Sánchez and Uranga (15) reported that a collection of seeds averaged 0.0013 g $seed^{-1}$ or 764,000 seeds kg^{-1}. Development of protocols associated with seed harvest timing of *W. indica* are necessary to enable restoration practitioners' access to mature seed stock from wild populations or crop-like production settings.

A critical aspect in production of agricultural crops is correct timing of harvest to maximize yield and quality (2, 3, 13). This fundamental concept applies to almost every agronomic crop in production today. When producing native seed, correct timing of seed harvesting is essential due to the balance of maximizing mature seed yield before seed is lost due to shattering. In the case of *W. indica*, flower clusters form a gradient from immature clusters at apical branch tips, with increasing maturity moving towards the main stem. Indeterminate flowering patterns, as seen with *W. indica*, complicate proper timing of harvesting efforts to maximize yields. Difficulties emerge when seeds mature on one part of the inflorescence, becoming susceptible to shattering, while other seeds on the same inflorescence are continuing to develop (12). In such cases, harvest timing is a compromise between recoverable mature seeds and seeds lost to shattering.

Evaluation of a method of seed harvest timing for practitioners while in the field can be a valuable tool to facilitate optimal yields. Tracking moisture content of seeds or seed heads is one way of characterizing the optimum harvest time (10). Another method to determine optimum harvest timing is by correlating the accumulation of heat units (i.e., growing degree days) to specific stages of a crop production cycle (1, 11). These methods can provide a well-defined quantitative measure for harvest activities. However, they lack utility for determining the onset of mature seed formation and seed retention attributes for natural stands of wild plants, or for in-field decision making when plants are produced in a conventional crop setting. Utilizing discrete visual cues to characterize seed head or fruit maturity can be a reliable indicator of the optimal time to harvest seed (4, 17).

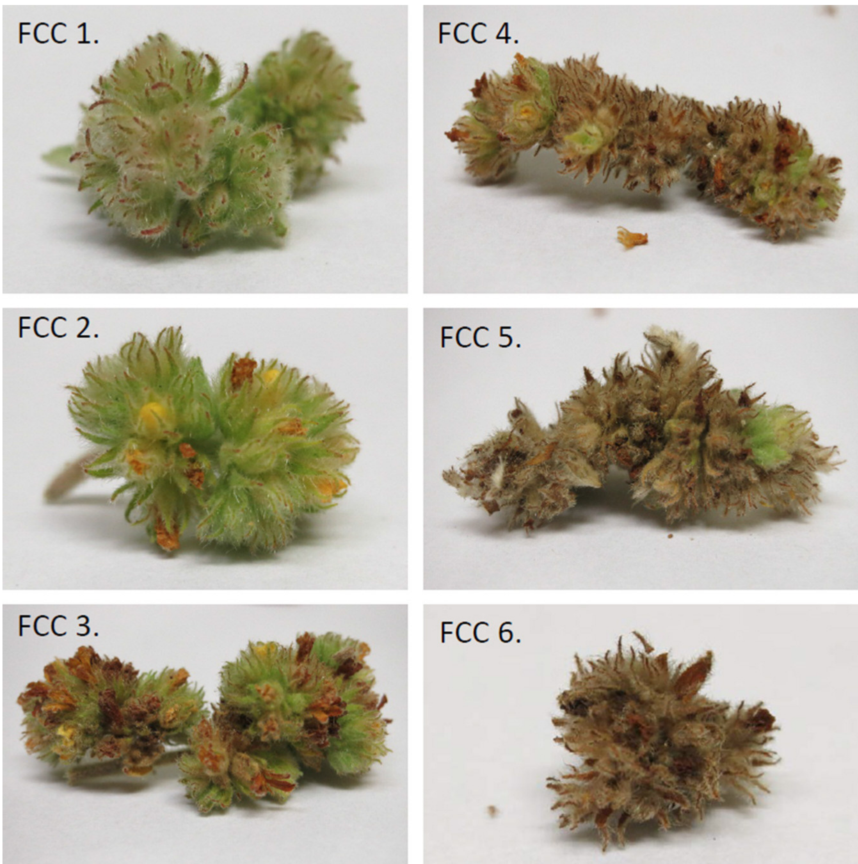
The intent of this research was to characterize visual cues that represent the discrete flower cluster categories (FCCs) of development, and correlate those FCCs to the amount of mature seed for *W. indica*. Data was collected to (1) characterize seed cluster development based on visual observations of flowering and floral tissue senescence, and (2) correlate discreet FCCs to flower cluster moisture and mature seed content. This trial was intended to provide preliminary recommendations for *W. indica* seed harvest indexing.

MATERIALS AND METHODS

Plant material. *Waltheria indica* seeds used to grow transplants for this study were harvested on the Island of Molokai from wild populations, by the United States Department of Agriculture (USDA), Natural Resource Conservation Service (NRCS), Hoolehua Plant Materials Center, in July 2012. *Waltheria indica* transplants were planted in field plots in December 2013 at a roadside demonstration site located in Honolulu, Hawaii. Transplants were 4 mon of age at time of planting. Characterization of visual floral cluster development and sample collection was conducted in January 2015 when *W. indica* plants were physically mature in comparison to wild plant populations. The experimental field was covered in Beltech™ 1859 woven polypropylene fabric (Belton Industries®, Belton, South Carolina) prior to planting to prevent weed competition and provide a clean surface to collect shattered seeds. Openings were made in the ground cover fabric to accommodate transplants. Drip irrigation and fertilization were supplied as needed to maintain active crop growth and flower development.

Flower stage determination. Flower clusters of *W. indica* were separated into six FCCs based on visually distinct attributes of development (Fig. 1). FCC 1 was visually identified as pre-flowering, where no flower buds were open, with

Figure 1. Six *Waltheria indica* flower cluster categories (FCCs) used for the determination of seed harvest timing visual indicators. FCCs were classified from immature to mature (FCC 1 to FCC 6, respectively) by presence of flowers, senesced flowers, green tissue and necrotic tissue.



100% green tissue. FCC 2 consisted of open flower buds with approximately 10% senescing flowers with 100% green flower cluster tissue. FCC 3 was characterized by 40–50% senesced flowers with 10% necrotic flower cluster tissue. FCC 4, 5 and 6 were identified by 50%, 75% and 100% necrotic flower cluster tissue, respectively. On a representative 17-mon old (4 mon for transplant production and 13 mon in the ground) *W. indica* plant with a 1.5 m long side branch, a visual approximation of percentage composition of each flowering FCC would be: 1% FCC 1, 5% FCC 2, 30% FCC 3, 30% FCC 4, 30% FCC 5, and 4% FCC 6.

Experimental design. The field planting was partitioned into four blocks established on separate drip irrigation Netafim™ TLCV9-1210 lines (Netafim USA, Fresno, California). Drip irrigation specifications indicated a flow rate of 3.4 L h^{-1} delivered for each emitter spaced by 30 cm. Individual blocks contained eight *W. indica* plants, and were flanked by buffer plants not included in the sampling pool. Plants were numbered from 1–8 in each block, and a random number generator was used to select four plants for flower cluster sampling to characterize the six FCCs.

From each randomly selected plant, 15 g of each flower FCC were collected. The 15 g color FCC samples were then composited to one sample for each block (60 g total fresh weight for each FCC per block). Sub-samples from each composite sample from each flower cluster were evaluated independently for moisture content and total mature seed yield. Flower cluster moisture analysis (FW basis) was performed following ISTA's constant temperature oven drying method (9). After drying, the composite samples were reduced to 20 grams. Each 20 g sample of individual FCCs was processed to extract mature seed. Mature seeds were classified based on seed size and presence of brown coloration with a hardened seed testa. In this study, seed viability and germination were not evaluated. Seeds were extracted from flower clusters by passing the samples through two types of mechanized seed cleaning equipment. In the first cleaning device, seeds were removed from flower clusters using a Westrup® LA-H brush machine fitted with a #14 mantle (1.0 × 1.0 mm square mesh) and medium nylon 0.5 mm brushes (Westrup® Inc., Plano, Texas). As the name implies, the brush machine presses the seed heads against a perforated metal cylinder, and seeds either push through the cylinder or remain within the machine, while debris are removed by gravity and dust recovered by a power vacuum. The brush machine was run at full power for 1 min with the front discharge door closed and 1 min with discharge door fully open. In the second device, seeds were separated from pulverized flower head components with a Clipper™ Office Tester fitted with a 1.651 × 1.651 mm wire mesh top screen and a 0.927 × 0.927 mm wire mesh bottom screen (A.T. Ferrell Company Inc., Bluffton, Indiana). The seed separator blower air ducts were open at 75% capacity and run until all material traveled past the screen sifters. Cleaned seeds were weighed to determine the yield (g) from 20 g of dry flower head clusters, and yield data reported per 100 g of dry cluster weight.

Data analyses. Seed yield was used to characterize the discrete FCC that contained the maximum amount of seed. Moisture data was also used to characterize each FCC. Data for seed yield (g) and moisture content (%) from 20 g

TABLE 1. Average seed yield and percentage moisture content of *Waltheria indica* flower heads separated into six flower cluster categories (FCCs) based on seed cluster maturity.

FCCs	Seed yield	Moisture content
	(g per 100 g of dry cluster weight)	(%)
1	0.00 e†	53 b
2	2.85 d	57 a
3	16.40 a	52 b
4	13.65 b	44 c
5	14.05 b	30 d
6	10.85 c	13 e

†Means with the same letter, in the same column, do not significantly differ ($p \leq 0.05$) according to Tukey's HSD test.

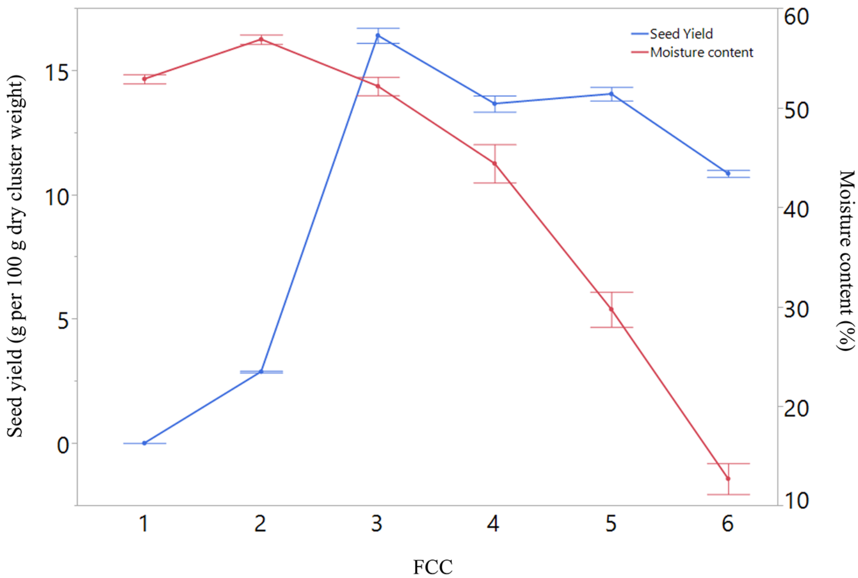
of composite sample were analyzed separately using a randomized complete block design analysis of variance (Statistix™ 10.0; Analytical Software, Tallahassee, Florida). When significant effects were detected, means were separated using Tukey's HSD test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Analysis of variance of seed yield results for each color FCC indicated a significant effect of flower cluster FCC on seed yield ($p < 0.001$). The significantly highest mature seed content was extracted from the 3rd FCC, with a mean seed yield of 16.40 g per 100 g of dry cluster weight (Table 1). The 4th and 5th FCCs resulted in the next highest yields of 13.65 and 14.05 g of seed, respectively. No mature seed was found in the first FCC and only 2.85 g in FCC 2. The fully senesced FCC 6 contained 10.85 g of seed (Table 1). Moisture analysis indicated that the 3rd FCC contained 52% moisture (Table 1). Flower cluster moisture content increased slightly from FCC 1 to FCC 2 as a result of the floral developmental processes. FCC 3 represented the first drop in moisture content following peak levels in FCC 2 (Fig. 2), as the flower cluster began to senesce. The highest seed yield found in FCC 3 was attributed to the peak time point where seeds had matured in the flower cluster, but had yet to be shattered. In FCC 1 and 2, seeds were predominately undeveloped or immature, resulting in lower mature seed yield. In FCC 4-6, seeds were mature, but due to moisture decline as a result of flower senescence the seeds were shattered from the cluster.

Tracking the initial decline in moisture at FCC 3 could be utilized to determine where optimized mature seed recovery can be obtained. The determination of visual indicators for optimization of seed harvest timing can be a helpful tool that seed managers can employ (14). Native plants in the wild or in production settings can be visually assessed for optimized harvest time (19). The utilization of this method can aid decision making for seed harvesting from wild populations, allowing for an immediate assessment of harvest timing while in the field. Agronomists utilize similar visual techniques to

Figure 2. Average (± SE) seed yield and seed head moisture content of *Waltheria indica* represented over six seed head flower cluster categories (FCCs). Maximum seed yield was obtained at the third visual FCC. Moisture content decline after FCC 2 indicated the presence of mature seed formation.



judge crop readiness for food crops (4, 5, 16). However, limited literature is available for the adoption of these visual techniques in native seed harvesting and production settings. Harvest practitioners assessing *W. indica* seed maturity can track flower cluster moisture content and/or visual indicators to compare FCC development and subsequent seed maturity. Understanding that optimum seed harvest timing for *W. indica* can be achieved by assessing the visual attributes associated with FCC 3 and by the initial decline in flower cluster moisture can aid in harvest and management decisions. It should be noted that results and conclusions are based on one year of field experimentation to gain initial insight into *W. indica* seed harvest indexing. Future studies should be expanded to assess the relationship between seed maturity and viability, and conducted over multiple years to increase confidence and applicability of conclusions and recommendations.

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