CHARACTERIZATION OF DORMANCY, ESTABLISHMENT AND SEED PRODUCTION OF WALThERIA INDICA AND PANICUM TORRIDUM

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Chapter 1

Dissertation Introduction

Characterization of dormancy, establishment and seed production of *Waltheria indica* and *Panicum torridum*.

Introduction

Over the years awareness has been continually developing that the world’s resources are not infinite. This consciousness has led to increased environmental awareness and stewardship. One of the most basic and fundamental philosophies of environmental stewardship is care of the land (Bradshaw and Chadwick 1980), which revolves around the concepts of preservation and restoration.

A major goal of restoration practitioners is to return a habitat to a more desirable condition involving a particular species composition, community structure, and/or set of ecosystem functions (Noss 1990). Non-native organisms have come to be recognized as one of the most serious ongoing causes of species declines and native habitat degradation (Vitousek et al. 1997). Currently in Hawaii, more than 50 invasive plant species have reached dominance in the natural environment. The invading plants impose a significant economic burden for control and eradication and threaten native biodiversity.
Roadside environments provide a distinctive habitat often supporting weedy and invasive plant species which are absent from natural communities (Gelbard and Belnap 2003). It is well accepted that roadsides function as movement corridors, acting as primary dispersion networks for invasive plants (Brothers 1992). The term road corridor refers to the road surface plus the maintained roadsides and any parallel vegetated strips, such as a median strip between lanes in a highway. Road corridors cover approximately 1% of the United States, equal to the area of Austria or South Carolina (Forman and Alexander 1998). However, the area directly affected ecologically is much greater. The ecological effects of roads impact about 15% of the land area of the US, an area equivalent in size to all the protected areas of the country combined, approximately 130 million hectares (Wilkinson et al. 2008). Despite the movement of invasive plant material spread over transportation corridors, roadside re-vegetation has traditionally been accomplished by utilizing exotic plant species because they are cost effective, readily available and quick to establish on disturbed sites (Landis et al. 2005). Controlling invasive species and re-vegetating road corridors with locally adapted species may mitigate some of the negative ecological effects of roads.

In 2006, President George W. Bush signed into law the Safe, Accountable, Flexible and Efficient Transportation Equity Act (SAFETEA-LU). Section 6006 of SAFETEA-LU includes a provision that makes activities for the control of noxious weeds and the establishment of native species eligible for Federal-aid funds (Anonymous 2011). Previous to the 2006 law, President Reagan had enacted the Surface Transportation and Uniform Relocation Assistance Act of 1987 (STURAA) which required that 0.25% of the landscape budget on a federally-funded project be reserved for establishment of native wildflowers (see 23 CFR §§ 752.4 and 752.11). SAFETEA-LU expands eligibility of STURAA to the establishment of a greater range of native
grasses, shrubs, trees, and vines (Anonymous 2011). To comply with the federal law, the State of Hawaii initiated the Statewide Noxious Invasive Pest Program (SNIPP). One aspect of SNIPP plan focuses explicitly on the re-vegetation of native species along roadway corridors of Hawaii (Anonymous 2011).

Achieving the re-vegetation goals of native plant species highlighted in the SNIPP plan is complicated by the lack of native plant materials for such large scale applications. Very limited seed stock is available from wild collection locations or seed producers. In response to the lack of native plant material the Hawaii Department of Transportation (HDOT) provided research funding for the development of large scale seed production and roadside establishment protocols for Native Hawaiian groundcovers. Two HDOT grants support the expansion of seed production sites for nine native plant species identified as candidates for roadside ground covers. This dissertation will focus on two of these species, *Panicum torridum*, an annual grass and *Waltheria indica*, a perennial shrub.

*Panicum* is a large genus of about 450 species of grasses native throughout various regions of the world. Very little research has been conducted on the native Hawaiian Panicum species. The research within the Panicum genus has focused on species that are of economic value or are considered weeds to be controlled in various agronomic settings. The main study species, reported in the literature are *Panicum dichotomiflorum* (Fall Panicum), *Panicum miliceum* (Proso millet), *Panicum virgatum* (Switchgrass) and *Panicum maximum* (Guineagrass).

*Panicum torridum* is an endemic grass ranging from 10 to 60 cm in height with velvety puberulant leaves. Distribution studies conducted in the year 1942 state that *P. torridum* is found on all Hawaiian Islands with sporadic distribution (Ripperton and Hosaka 1942). In 1992, *P.
*P. torridum* was found it to be a dominant species along the summit ridge of the Lehua islet on the remote island NiiHau (Evenhuis and Eldredge 2006). In Hawaii, annual grasses such as *P. torridum* normally emerge in months of April to May following the rainy season that lasts from November to January (Sakamoto, Personal communication 2013). *P. torridum* is found below 90 meters elevation in zones that receive 50-100 cm of rainfall per year (Ripperton and Hosaka 1942).

*Waltheria* is a genus of plants belonging to the order Malvales and family Sterculiaceae. *Waltheria indica* L. is a shrub sometimes reaching 2 m in height and 2 cm in stem diameter (Howard 1988). 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. Axillary inflorescences are usually dense glomerules that contain fragrant, yellow to orange flowers. Each 2-mm capsule holds one small, black, obovoid seed (Howard 1974).

*Waltheria indica* is a pan-tropical shrub species native to the New World which occurs in diverse populations in the Americas, Mexico and Brazil. It has been postulated that the small seeds may have attached to birds which transported *W. indica* to Hawaii (Wester 1992). During Captain Cook’s voyage in 1779, botanist David Nelson noted *W. indica* in regions with very dry, xerophytic scrub vegetation and an annual rainfall of about 50 cm (St John 1979). *W. indica* is found in well drained soils of all types, with elevation distributions from sea level to 1220 meters. Current populations tend to occur in altered sites, where soil disturbance has occurred (Long and Lakela 1971).
Traditionally *W. indica* was widely used to treat a variety of infections in humans (Mongalo et al. 2012). In traditional Hawaiian medicine, it is said that flower buds were chewed by infants, stems and leaves by older children, and roots by adults, although the whole plant was commonly utilized for adults. The bark of the taproots was chewed for sore throats. Whole plants and/or roots were boiled and juiced into a restorative, bitter tonic for fatigue or general debility. *W. indica* also served as a component in treating asthma, arthritis, neuralgia and pulmonary complications (Duane 2011).

In order to facilitate large scale seed production of the reported species, unknown factors of the seed production cycle need to be elucidated. Seed technology data gaps include; understanding methods to relieve various types of seed dormancy, seed crop establishment methods and seed harvest parameters for maximum mature seed recovery. Specifically this dissertation will focus on filling the knowledge gaps of seed dormancy relief, seed storage parameters, field establishment weed control methods, seed harvest timing for both *P. torridum* and *W. indica* and mechanized seed extraction from dried plant tissues.

This chapter (chapter 1) represents the justifications and introduction to the research encompassed within this dissertation. The second chapter will focus on the characterization of dormancy present in *W. indica* seeds. The determination of methods of seed dormancy relief and seed storage represent essential knowledge for large scale usage of *W. indica*. Specifically this chapter will evaluate physical dormancy relief methods of hand scarification, dry heat temperature exposure, hot water exposure and mechanical abrasion in an electric drum scarifier. As a compliment to dormancy relief experiments, long term storage parameters will be evaluated for scarified and non-scarified seeds. With the understanding of dormancy relief protocols and storage parameters discussed in chapter 2, the next logical step in the production cycle for *W.*
*indica* are field establishment procedures, which will be discussed in chapter 3. For seed production purposes, it is imperative that weeds are controlled in the production site to ensure *W. indica* seed lot purity from weed seed contamination. Weed interference is the primary constraint to successful establishment of native plant communities (Masters et al. 1996). The development of pre-emergent chemical establishment parameters for *W. indica* is necessary for increased usage, especially for weed free seed production. The intent of chapter 3 will be to determine the response of *W. indica* transplants and weeds to recommended labeled rates of the pre-emergence herbicides oxadiazon and indaziflam. With the ability to successfully germinate and grow *W. indica* and maintain a weed free production site the next step is to determine when to harvest flower clusters to maximize seed yield. When producing *W. indica* seed, correct timing of seed harvesting is essential due to the balance of maximizing mature seed yield before seed loss due to shattering occurs. Chapter 4 discusses an approach to determine optimum seed yield timing through visual descriptions of flower head characteristics and moisture content evaluation. The utilization of the techniques presented in chapter 4 can aid *W. indica* seed harvest decision making by restoration practitioners for individual plants in the wild or for in-field assessments. Similar to the topics discussed in chapters 2, 3 and 4 regarding the development of seed production protocols for *W. indica*, the following chapters will examine the same fundamental steps of the production cycle focusing on *P. torridum*. Chapter 5 will explore the physiologically imposed dormancy present in *P. torridum* seeds. This chapter will evaluate different methods to relieve dormancy than were discussed in chapter 2, because the previous techniques were employed to relief a specific type of dormancy (i.e. physical). Endogenous chemicals reported in the literature will be evaluated in an attempt to relieve *P. torridum* dormancy. Treatments tested will include an evaluation of potentially stimulatory exogenous
hormonal, reactive oxygen intermediates, and simulated combustion products. In certain occurrences, physiological dormancy is not relieved by exogenous chemical treatments (Bewley 1997), considering this, an experiment evaluating seed storage and after-ripening is also discussed in chapter 5. Seeds were exposed to storage conditions equilibrated to three levels of relative humidity, stored at three temperatures over 10 months to determine seed dormancy relief and storage optimization. The importance of field establishment, as discussed above in chapter 3, is fundamental to develop a species specific protocol to maintain weed control while minimizing phytotoxic effects on the production crop. Chapter 6 will evaluate *P. torridum* field establishment protocols for weed control. The specific intent of this chapter will be to determine the response of *P. torridum* transplants and weeds to recommended labeled rates of the pre-emergence herbicides oxadiazon and indaziflam. The final researched based chapter 7 will focus on optimization of harvest timing for *P. torridum*. The utilization of heat accumulation units as growing degree days (GDD’s) will be discussed as a way to characterize seed development based on thermal time, thus providing a specific quantifiable harvest time to maximize seed yields. The objective of this chapter is to optimize mature seed harvest timing of *P. torridum* by creating a quantifiable heat accumulative unit (GDD) indicator value. The last chapter, chapter 8 will summarize the overall conclusions represented in the previous chapters, along with the implications and recommendations of this research body for future restoration practitioners.
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Optimization of Waltheria indica seed dormancy relief methods and seed storage parameters.

Abstract
Seed dormancy is an ecological adaptation to temporally distribute germination to enhance seedling establishment success. From a crop production viewpoint, seeds that exhibit dormancy present a challenge due to low and unreliable germination rates. Characterization of the type of seed dormancy and the methods of dormancy relief represent essential knowledge for large scale field establishment and improved production and preservation of viable seeds. In Hawaii, Waltheria indica has been identified for expanded usage as a roadside ground cover in lowland dry ecosystems prevalent across many roadways in the Hawaiian archipelago. With the determination of physical dormancy present in W. indica seeds, relief methods evaluated included: hand scarification, dry heat temperature exposure, hot water exposure and mechanical abrasion in an electric drum scarifier. As a compliment to dormancy relief experiments, long term storage parameters were evaluated for scarified and non-scarified seeds. Results indicate that the greatest dormancy relief (most germination) was achieved with the mechanical electric drum scarifier lined with 80 grit sandpaper for a duration of 30 seconds. Dormancy relief was achieved through the other methods tested, but were less conducive to large scale seed handling than the drum scarifier. Seed viability was not affected with long term storage (5°C) when seed moisture levels were equilibrated to 12% RH and 50% RH. Non-scarified seeds exhibited
minimal loss in viability over ten months of storage compared to the significant decline in viability of drum scarified seeds.

**Introduction**

Plants produce seeds to ensure the greatest establishment and survival success for future generations (Stoehr and El-Kassaby, 2011). One innate mechanism to increase seedling success is seed dormancy. The critical function of dormancy is to prevent germination when conditions are suitable but when the probability of survival and growth of the seedling is low (Fenner and Thompson, 2005). Classification systems define five classes of seed dormancy: physiological dormancy (PD), morphological dormancy (MD), morphophysiological dormancy (MPD), physical dormancy (PY) and combinational dormancy (P + PD) (Baskin and Baskin, 2004). A single report on seed germination in of *Waltheria indica* L. indicated a 13% germination rate on freshly harvested seed after 16 weeks in a moist germination setting. This low level of germination in freshly harvested seed is an indication of some form of seed dormancy (Sánchez and Uranga, 1993). According to Baskin, water impermeable seeds (physical dormancy) are common in occur in the Sterculiaceae family, *W. indica* is a member of this plant family (Baskin, 2003). In a study conducted by Boyd, it was determined that in *Fremontodendron decumbens* (also a member of the Sterculiaceae) 97.8% of seeds were dormant due to an impermeable seed coat. Breaking of the seed coat, mechanically or by heat allowed for germination rates 18-26 times greater than with the untreated seeds (Boyd and Serafini, 1992; Vinod et al., 2014). Physical dormancy has been well documented in *Dodonaea viscosa*, having similarities to the “hard” seed testa of *W. indica*. In Baskin’s research, physical treatments were tested to break the dormancy imposed by *D. viscosa*’s hard seed testa. One treatment involved mechanically
scarifying seeds (Vinod et al., 2014), after 2 weeks of incubation, seeds that were mechanically scarified had germinated to 96–100% in light (Baskin et al., 2004). Non-scarified seeds, on the other hand, had germinated to only 0 and 1% in light and darkness, respectively (Baskin et al., 2004). Other methods use to scarified seeds involve exposure to dry heat at a range of 80–160 °C, emersion in boiling water and exposing seeds to low relative humidity conditions (Baskin et al., 2004).

Baskin suggests that storage can be utilized as a dormancy breaking treatment, but can also play a fundamental role in optimizing seed longevity. It is necessary to understand the optimum storage and dormancy relief conditions of seeds to maximize their usefulness. Ultimately, survival is directly related to the time the seed has been exposed to unfavorable conditions of temperature or humidity (Barton, 1961).

The impact of storage conditions on Cassia angustifolia seed was determined for four different levels of relative humidity (5.5%, 11%, 33%, and 75%), established and maintained using saturated salt solutions in airtight desiccators, and three different storage temperatures (5°C, 20°C and ambient). Storage temperatures of 5°C and 20°C at 5.5% and 11% relative humidity were found to be optimal for extending seed viability and maintain high levels of germination during storage (Santhoshkumar and Veena, 2012). Similar work was conducted in 2014 by Baldos et al. and characterized storage and after ripening parameters of the native Hawaiian grass Heteropogon contortus. It was determined that the optimum dormancy relieving conditions for H. contortus required a 28 to 30 day equilibration period in a 12% relative humidity desiccation chamber followed by a 30°C storage temperature for 9-12 months (Baldos et al., 2014). This research demonstrated how the factors of temperature and humidity work together to provide optimal storage conditions that maintain seed viability and dormancy relief.
The assessment of water imbibition can be used to elucidate physical dormancy in seeds (Rolston, 1978). Utilizing this approach it was determined that *W. indica* seeds possessed a water impermeable seed testa (i.e. physical dormancy). Thus, the purposes of this study are to: (1) evaluate optimal methods to relieve the physical dormancy imposed on *W. indica* seeds by determining the seed germination response to mechanical hand scarification, exposure to dry heat, hot water dipping and mechanical machine scarification; (2) determine the impact of storage humidity and duration on viability and germination response of non-scarified and scarified *W. indica* seeds at 5°C.

**Materials and Methods**

*Plant Material*

*Waltheria* is a genus of plants belonging to the order Malvales and family Sterculiaceae. *Waltheria indica* L., (ACC# 9079945) also known as *Waltheria americana* and *Waltheria elliptica* (Duvachelle, 2014; Wagner et al., 1990). *W. indica* 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 (Howard, 1988). A variety of upright and prostrate growth forms have been observed from seedling populations. 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 to 3.3 cm long and the blades are 2 to 12 cm long and 1 to 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 (Howard, 1974).
*W. indica* is a pan-tropical shrub species which occurs in diverse populations in the Americas, Mexico and Brazil. One report states that in Hawaii, *W. indica* was apparently naturalized soon after the arrival of nonnative colonists (Haselwood and Motter, 1966). Despite Haselwood’s report, *W. indica* is more widely classified as a native plant, postulating that the small seeds may have attached to birds which distributed to plant to Hawaii (Wester, 1992). Reinforcing the native status, during Captain Cook’s voyage to Hawaii in 1779, on board botanist, David Nelson noted *W. indica* in regions with very dry, xerophytic scrub vegetation and an annual rainfall of about 50 cm (St John, 1979). In certain regions of the world, *W. indica* is considered an invasive weed (Sánchez and Uranga, 1993). It is found in well drained soils of all types. In Hawaii, the species elevation ranges from sea level to 1220 meters. Current populations tend to occur in altered sites, where soil disturbance has occurred (Long and Lakela, 1971)

*W. indica* seeds used in this study were composed of two separate harvests / seed batches which were utilized for two repeated runs of the experiments conducted. Seed batch one (SB1) was harvested on the Island of Molokai by the United States Department of Agriculture, Natural Resource Conservation Service, Hoolehua Plant materials Center in July 2012 (Sakamoto, 2013). Seed batch two (SB2) was harvested from the same USDA facility in March 2014. Postharvest handling of both seed batches consisted of air drying, packaging and 5°C refrigeration until use (Duvachelle, 2014). All experiments were initiated first with SB1 between the dates 9/2013 – 4/2014. SB2 experimentation was initiated between 4/2014 – 5/2014.

For all dormancy relief studies, experimental units consisted of 50 seeds exposed to the experimental treatment / treatment combinations with four replications, repeated for each seed batch. Each experimental unit was incubated in 90mm petri dishes pre-moistened with 3 ml distilled water and lined with filter paper (Whatman®#2, Little Chalfont, Buckinghamshire).
Petri dishes were placed in an alternating temperature germination chamber with four T5 high output 24W 6400K AgroBrite™ bulbs (Hydrofarm®, Petaluma, California) for 14 hours of light at 28°C and 10 hours of dark at 24°C. Experimental conditions during the seed germination period were monitored with a Hobo UX100 logger (Onset®, Cape Cod, Massachusetts). Distilled water was added to petri dishes as needed over the 10 day germination period. Germination was recorded when the seed radicle protruded 1 mm from the seed testa.

Seed batch viability testing
In both seed batches (SB1 and SB2) experimental units were sampled from the larger stock batches following standardized sampling procedures (Elias et al., 2012). Seeds were subjected to viability testing using standard 1% tetrazolium chloride (TZ) methods (Elias et al., 2012; ISTA, 1996). Data were analyzed as a random complete block design using the fit model function in the statistical program JMP® Pro 11 (SAS Institute Inc., Cary, NC.).

Dormancy relief using hand cut mechanical scarification
W. indica seeds were hand scarified using a scalpel with visual guidance provided with 20X magnification. A small piece of testa was removed to exposed unaffected endosperm. The experimental units for both seed batches were sampled following techniques outlined by Elias et al (Elias et al., 2012). Data were analyzed as a split plot design using the fit model function in the statistical program JMP® Pro 11. The main plot effect was seed batch, and the split plot effect was hand scarification.

Dormancy relief through exposure to dry heat
Seed germination response to dry heat temperatures of 50, 75, 100, and 125°C, in a Quincy™ lab model 40GC lab oven (Quincy Lab INC., Chicago, Illinois) and exposure times (0, 1, 5, 15, 30 and 60 minutes) was determined. During the seed exposure time, oven temperature was
manually recorded with a thermometer. After each exposure interval seeds were allowed to cool to room temperature for 60 seconds prior to the start of the seed germination phase of the experiment. Data were analyzed as a split split plot design using the fit model function in the statistical program JMP® Pro 11. The main plot effect was seed batch, the split plot effect was oven temperature and the split split plot effect was duration exposed to treatment temperatures.

**Dormancy relief effect through exposure to boiling water**

The relief of physical dormancy was evaluated by exposing *W. indica* seeds to a range of boiling water exposure times. Seed experimental units were dipped in boiling distilled water at intervals of 0, 1, 3, 5, 10, 15, 30 and 60 seconds. Immediately following the hot water dip, seeds were dipped in distilled ice water for five seconds then placed in a petri dish lined with filter paper with 3ml of distilled water. The hot water bath temperature was measured to quantify temperatures and a stir bar was utilized to ensure a homogenous heated water solution. The water temperature was held constant at 101°C throughout the entire experiment and the cold water bath ranged from 12.5°C to 14.0°C. Data were analyzed as a split plot design using the fit model in the statistical program JMP® Pro 11. The main plot effect was seed batch and the split plot effect was the duration of seed exposure to boiling water.

**Dormancy relief using mechanical sandpaper drum scarifier**

Seed scarification was evaluated using a commercially available mechanical sandpaper scarifier to relieve physical dormancy in *W. indica* seeds. Scarification was conducted in a Forsberg® seed scarifier with a 1/3 horsepower electric motor (Forsbergs, INC., Thief River Falls, Minnesota). With the Forsberg scarifier, seeds are placed in round cylinder with rotating metal impellers. The spinning action of the impellers rolls the seed against the cylinder walls covered with sandpaper. The movement of the seeds against the sandpaper provides a controlled and
repeatable method of seed scarification. Sandpaper coarseness is classified with a CAMI grit designation, the 80, 60 and 40 grits utilized have average particle diameters of 190 µm, 265 µm and 425 µm, respectively. This experiment will evaluate three grits of sandpaper (40, 60 and 80 grit) at three exposure times of 15, 30 and 45 seconds. Data were analyzed as a split split plot design using the fit model function in the statistical program JMP® Pro 11. The split split plot model was designed with seed batch as the main plot effect, sandpaper coarseness as the split plot effect and duration in the scarifier as the split split plot effect.

Impact of storage conditions on seed viability of scarified and non-scarified seed to evaluate seed preservation.

Both scarified and non-scarified seeds were partitioned into 0.25 gram (approximately 200 seeds) units and placed in open 1.5 ml micro-centrifuge tubes with O-ring gasket lid (Fisher Scientific®, Pittsburgh, Pennsylvania). Seeded tubes were equilibrated at two relative humidity (RH) levels inside sealed Bel-Art (Scienceware®, Wayne, New Jersey) non-vacuum desiccation chambers. Humidity levels were set and maintained using saturated salt solutions of lithium chloride (12%-RH) and calcium nitrate (50%-RH) (Baldos et al., 2014; Greenspan, 1977). After the 28 day humidity equilibrium period, seed sample tubes were sealed and transferred to a 5°C storage temperature. Seeds were withdrawn from the temperature chambers at intervals of 2 months with the last sample removed after 10 months. Seeds were evaluated for viability and germination percentages at each time interval. Seed moisture analysis was performed at the beginning and at each storage interval in a manner consistent with International Seed Testing Association (ISTA) protocols (ISTA, 2003). Data were analyzed as a split split split plot using the general analysis of variance fit model function in the statistical software program Statistix™ 10.0 (Analytical Software, Tallahassee, Florida). In the split split split plot model statement seed
batch was the main plot effect, scarification the split plot effect, storage humidity the split split plot effect and months of storage as the split split split plot effect. Response data included seed viability and percent germination. Non-scarified seeds were evaluated for germination at each storage interval to determine if dry storage alone could impact the physical dormancy imposed by the seed coat.

Results

Seed batch viability testing

Tetrazolium testing for seed viability indicated that 96.3% of seeds sampled from SB1 were viable with a significantly lower level of 62.5% recorded for SB2. Viability values for both seed batches will be used to standardize germination results in subsequent dormancy trials using the equation:

\[
\text{(Percent standardized germination} = \frac{\text{Percent observed germination}}{\text{Percent viable seeds}} \times 100\text{)}.
\]

Dormancy relief through hand cut mechanical scarification

The results of the analysis indicated that there was no interaction between the factors of seed batches and cutting treatment (P= 0.74), allowing for the pooling of treatment means across the factor of seed batch. Hand cut treatments resulted in an average germination of 96% compared to 8% of non-scarified treatments (P <0.001).

Dormancy relief effect through exposure to dry heat

The results of the analysis indicate that there is no significant interaction between the factors of seed batch × temperature × duration (P=0.0718), however a significant interaction between temperature × duration (P= <0.0001) was detected. Tukey’s HSD test at α= 0.05 was used for
means separation (Table 1). The treatment which resulted in the significantly highest germination was exposed to a temperature of 75°C for a 60 minute duration, with a mean germination of 39.4%. Treatments exposed to 75°C for 30 and 15 minutes were significantly lower than the 60 minute treatment but provided the second and third numerically highest germination. Minor stimulation in germination was recorded in treatments exposed to 50°C. The 75°C treatments exhibited a general positive linear trend until the maximum germination treatment. Temperature exposure of 100°C resulted in stimulation at 1 minute of exposure, then decreased steadily to zero germination at 30 minutes of exposure. A similar effect occurred at 125°C in which stimulation occurred at 1 minute of exposure then rapidly decreased to zero germination at 5 minutes (Figure 1). At high temperature treatments (100°C and 125°C), seeds with no germination were visually examined and determined to be non-viable due to complete endosperm desiccation.

**Dormancy relief effect through exposure to boiling water**

Results of the analysis indicated that there was no significant interaction detected between the factors of seed batch × treatment (P = 0.7719), therefore treatment means will be pooled for the factor of seed batch (Table 2). All boiling treatments resulted in significantly greater germination than the untreated seeds. Optimal germination was found at a boiling duration of 3 and 5 seconds, with 58.6% and 57.7% germination, respectively. Germination decreased as exposure times increased until the final 60 second duration, resulting in 38.8% germination (Figure 2).

**Dormancy relief using mechanical sandpaper drum scarifier**

Results of the analysis indicated that there was a significant interaction between the factors of seed batch × sandpaper × exposure time (P = 0.001), thus the results will be presented for each
In both seed batches, the highest level of germination occurred with 80 grit sandpaper exposed at 15 and 30 seconds, although the 30 second treatment in both seed batches was numerically optimal (Table 3). As exposure duration increased, especially as sandpaper grit decreased (more coarse) seeds were pulverized causing endosperm and embryo damage, lowering germination rates. The decrease in germination due to damage was more pronounced in SB2 (Figure 3).

**Impact of storage conditions on seed viability of scarified and non-scarified seed to evaluate seed preservation.**

The results of the analysis determined a significant interaction between the factors of seed batch × scarification × month, therefore results are presented for each seed batch (P=0.032), pooled over both humidity levels. Within SB1 the highest seed viability was found at month 0 (start of trial) in both the scarified and non-scarified treatments 95% and 98%, respectively). In non-scarified treatments, viability decreased to 92% at month 10 (Table 4). Scarification significantly reduced viability after month 2 of storage until month 10 (92% to 71%, respectively) (Figure 4). In SB2 there was no viability loss with storage of non-scarified seeds. Scarified seeds from SB2 followed a similar pattern of viability loss as seeds from SB1 (i.e. approximately 25% loss in viability with scarification and 10 months of storage).

In SB2 the highest seed viability was recorded at month 0 in the scarified treatment (65%). Seed viability remained stable throughout the 10 months of storage for the non-scarified seeds (Figure 4). Scarified seeds reduced viability significantly with time from month 2 until month 10 (58% to 40%, respectively) (Table 4). The decline recorded in viability in the scarified treatments over the durations in both seed batches indicate that scarification and storage is not the optimal method for seed preservation. Humidity levels evaluated during storage did not have a
significant effect on seed viability. Since viability in non-scarified seeds remained relatively stable, germination was analyzed to determine if dormancy relief was occurring over storage duration. The analysis indicated that there was no significant interaction between the factors of seed batch × months (P =0.199), therefore results will be pooled over seed batch. Germination analysis of non-scarified seeds indicated a slight reduction in germination from month 0 until month 10 (7.5% to 5%, respectively) (Table 5). Non-scarified seeds are not relieved of dormancy over storage durations up to 10 months (Figure 5).

**Discussion**

Seed viability was seen to have differed significantly between the two seed batches used throughout the range of experiments conducted. *W. indica* seed harvested by the USDA Hoolehua Plant Materials Center in 2012 (SB1) was harvested late (Sakamoto, 2013), as a result seeds were mostly mature and shedding from the mother plants. For the 2014 seed batch (SB2), harvest timing was earlier than the 2012 harvest time resulting in a larger percentage of immature seed. The 2012 harvest yielded 1.8 kg. of seed (from a 0.4 hectare area) compared to the 2014 harvest of 11.3 kg., from the same field. The harvester combine (Massey Ferguson MF-17/19; Kincade Equipment Manufacturing, Haven, Kansas) settings also differed from 2012 to 2014 to better clean the harvested seed. For the 2014 seed harvest, the alteration reduced the gap between the thresher wheel and the concave of the combine, causing more mechanical damage to the seeds harvested (Duvachelle, 2014). The combination of the greater percentage of immature seeds and more physical damage during harvesting helps to account for the reduced viability in the 2014 seed batch.
SB1 was stored in 5°C for the period between harvest and experimental use (14 months), seed batch specifications before use resulted in <10% *W. indica* germination, with greater than 90% viability. SB2 was stored in the same conditions for 1 month before experimental use, resulting in <5% germination but with less than 65% viability.

Dormancy was determined to be physically imposed based on the results of the hand cutting of *W. indica* seeds, however, the time to hand scarify 200 seeds is not practical for any large scale seed processing. Optimization of the dormancy relief methods proved to be most effective and efficient in both seed batches using an electric drum sandpaper scarifier with 80 grit sandpaper for a duration of 30 seconds. Utilizing sandpaper for seed scarification to relieve physical dormancy is well established (Egley, 1979; Hutchison and Ashton, 1979), but customizing the technique to a species specific regime is imperative (Olszewski et al., 2010). Storage parameters evaluating the effects of seeds scarified and non-scarified over a 10 months duration was designed to streamline the process of scarification and storage. The intent of this experiment was to enable to end user of the seeds a more streamline process, where seed could be immediately ready for use upon removal from storage. However, based on the decline in seed viability over ten months with scarified seeds, this procedure is not recommended. Based on the treatments evaluated, viability of *W. indica* seed is maintained at high levels when non-scarified seeds are stored at 5°C at both 12 and 50% RH for up to 10 months.

The research presented here supports the following protocols for the utilization of *W. indica* from seed stock. If seeds are not needed immediately after harvest, store in a 5°C refrigerator at either 12% or 50% RH. When seeds are desired for use, scarify using an electric drum scarifier with 80 grit sandpaper for a duration of 30 seconds to relieve physical dormancy. Once the seeds are scarified, viability will decline with storage conditions reported here.
Tables and Figures

Figure 2.1. Germination response of W. indica seeds to four dry heat temperatures over six exposure durations, with standard error bars.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>0</th>
<th>1</th>
<th>5</th>
<th>15</th>
<th>30</th>
<th>60</th>
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<tbody>
<tr>
<td>50</td>
<td>8.6</td>
<td>D</td>
<td>17.0</td>
<td>C</td>
<td>20.0</td>
<td>C</td>
</tr>
<tr>
<td>75</td>
<td>9.5</td>
<td>D</td>
<td>19.2</td>
<td>C</td>
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<td>C</td>
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<tr>
<td>100</td>
<td>8.4</td>
<td>D</td>
<td>22.0</td>
<td>BC</td>
<td>19.0</td>
<td>C</td>
</tr>
<tr>
<td>125</td>
<td>9.5</td>
<td>D</td>
<td>18.8</td>
<td>C</td>
<td>0.3</td>
<td>E</td>
</tr>
</tbody>
</table>

Table 2.1. Table of germination means for W. indica seeds exposed to dry heat treatments. Means are separated using Tukey’s HSD comparison at P=0.05. Means within columns and rows followed by the same letter are not significantly different.
Figure 2.2. Response of *W. indica* seed germination to hot water exposure durations with standard error bars.

<table>
<thead>
<tr>
<th>Duration (s)</th>
<th>Germination (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>8.8 E</td>
</tr>
<tr>
<td>1</td>
<td>37.3 D</td>
</tr>
<tr>
<td>3</td>
<td>58.6 A</td>
</tr>
<tr>
<td>5</td>
<td>57.7 AB</td>
</tr>
<tr>
<td>10</td>
<td>50.2 BC</td>
</tr>
<tr>
<td>15</td>
<td>46.5 C</td>
</tr>
<tr>
<td>30</td>
<td>43.7 CD</td>
</tr>
<tr>
<td>60</td>
<td>38.8 D</td>
</tr>
</tbody>
</table>

Table 2.2. Table of germination means for *W. indica* seeds exposed to boiling water treatments. Means are separated using Tukey’s HSD comparison at $P=0.05$. Means within columns and rows followed by the same letter are not significantly different.
Figure 2.3. Germination response of two experimental trials of W. indica seeds to mechanical abrasion in a sandpaper scarifier with three levels of sandpaper grit and four exposure durations, with standard error bars.

Table 2.3. Table of germination means for two seed batches of W. indica seeds exposed to mechanical abrasion in a sandpaper scarifier with three levels of sandpaper grit and four exposure durations. Means are separated using Tukey's HSD comparison at P=0.05. Means within columns and rows followed by the same letter are not significantly different.
Waltheria indica seed viability means (%)

<table>
<thead>
<tr>
<th>Seed treatment</th>
<th>Months of storage (seed batch 1)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-scarified</td>
<td></td>
<td>97.8</td>
<td>95.4</td>
<td>93.3</td>
<td>94.3</td>
<td>93.1</td>
<td>92.4</td>
</tr>
<tr>
<td>Scarified</td>
<td></td>
<td>95.4</td>
<td>AB</td>
<td>AB</td>
<td>AB</td>
<td>AB</td>
<td>AB</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Seed treatment</th>
<th>Months of storage (seed batch 2)</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>6</th>
<th>8</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-scarified</td>
<td></td>
<td>62.1</td>
<td>FG</td>
<td>FG</td>
<td>FG</td>
<td>FG</td>
<td>FG</td>
</tr>
<tr>
<td>Scarified</td>
<td></td>
<td>64.8</td>
<td>F</td>
<td>GH</td>
<td>HI</td>
<td>I</td>
<td>I</td>
</tr>
</tbody>
</table>

Table 2.4. Table of seed viability means of scarified and non-scarified W. indica seeds over 10 months, represented for both seed batches. Means are separated using Tukey’s HSD comparison at P=0.05. Means within columns and rows followed by the same letter are not significantly different.

Figure 2.4. Waltheria indica storage viability over storage duration of 10 months, represented over two seed batches, with standard error bars.
Germination response means of non-scarified W. indica in storage

<table>
<thead>
<tr>
<th>Months</th>
<th>Germination (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>7.5 A</td>
</tr>
<tr>
<td>2</td>
<td>7.0 AB</td>
</tr>
<tr>
<td>4</td>
<td>5.5 AB</td>
</tr>
<tr>
<td>6</td>
<td>5.8 AB</td>
</tr>
<tr>
<td>8</td>
<td>5.6 AB</td>
</tr>
<tr>
<td>10</td>
<td>5.0 B</td>
</tr>
</tbody>
</table>

Table 2.5. Table of seed germination means for storage potential of non-scarified W. indica seeds over 10 months, pooled over two seed batches. Means are separated using Tukey’s HSD comparison at P=0.05. Means within columns and rows followed by the same letter are not significantly different.

Figure 2.5. Waltheria indica germination over storage duration of 10 months, pooled over two seed batches, with standard error bars
Literature Cited


St John, H. (1979) The Vegetation of Hawaii as Seen on Captain Cook’s Voyage in 1779.


Chapter 3

Weed control and plant response of transplanted *Waltheria indica* in the presence of two pre-emergence herbicides applied at the time of planting.

Abstract

The Hawaii Statewide Noxious Invasive Pest Program (SNIPP) and associated storm water management plans provide statutory justification for increased use of native plants along State of Hawaii transportation corridors. The demand for native plants outweighs the availability of plant materials or seed. In order to produce seed stock and ensure seed lot purity, establishment protocols for weed control must first be defined. *Waltheria indica*, a native Hawaiian broadleaf shrub has been identified for increased roadside usage, thus will be the focus of this research. Weed control during the establishment phase of *W. indica* is essential for optimizing establishment success. In this study, the efficacy and phytotoxicity of the pre-emergence herbicides oxadiazon and indaziflam applied over *W. indica* transplants were evaluated. Crop and weed response to granular oxadiazon at 2.24 kg ai ha\(^{-1}\) and 4.48 kg ai ha\(^{-1}\) and flowable indaziflam at 24 g ai ha\(^{-1}\) and 49 g ai ha\(^{-1}\) were determined. Unacceptable *W. indica* injury with both rates of indaziflam was recorded. Oxadiazon provided excellent broad spectrum weed control with acceptable injury to *W. indica*. 
Introduction

Utilizing pre-emergent herbicides to reduce weed competition is well established in crop production. Production of native plant species for seed or restoration purposes does not differ in the requirements of weed control (Grilz and Romo 1995; Hitchmough et al. 1994; Tjelmeland et al. 2008). However, there is a limited number commercial herbicides available in North America for application to native plant species (Smith and Whalley 2002). In the State of Hawaii, initiatives are calling for planting of native species along Department of Transportation roadway corridors (Tamimi 1999). To comply with federal presidential acts, the State of Hawaii initiated the Statewide Noxious Invasive Pest Program (SNIPP) (Anonymous 2011). One aspect of the SNIPP plan specifically calls for the re-vegetation of native species along roadway corridors. Aside from the SNIPP plan, statutory regulations of the Clean Water Act, National Pollutant Discharge Elimination System (NPDES) permits and associated storm water pollution prevention plans for roadways on Oahu, Hawaii integrate the planting of native species as a best management practice (BMP) (Anonymous 2007). Weed interference is the primary constraint to successful establishment of native plant communities (Masters et al. 1996). Herbicides are an essential component of management strategies that are being developed to establish native grasses in restoration projects (Masters et al. 1996). Using roadways as sites for growing native plants allows for legal usage of herbicides labeled for rights-of-way sites. Most modern herbicides have product labels that list “rights-of-way” or “non-crop area” as legal sites of application. When native Hawaiian plants are used in roadside landscapes or grown on roadsides for seed production, herbicides labeled for use on highway rights-of-way can be applied during establishment and for maintaining weed free status without violating label instructions. Roadside rights-of-way represent a unique area for growing native plants due to the wide range
of chemical tools available for weed control during establishment, growth and seed production cycle.

*Waltheria indica* L. is one of the target species identified for development of seed production protocols in Hawaii and will be the focus of this research. The identification of preemergence herbicides effective for use in the establishment of *W. indica* is necessary for increased usage as a ground cover and for weed free seed production. The identification of *W. indica* response to pre-emergent chemicals has not been reported in scientific literature based on keyword search parameters (*W. indica* + herbicides + response + phytotoxicity + chemical) in the databases, CAB abstracts, Biological abstracts, Google scholar and Agricola as of April 2015. Recent work conducted on weed control in native Hawaiian plant species indicates that the best establishment success is achieved by applying pre-emergence herbicides over the top of transplanted plants. In the native Hawaiian grass *Sporobolus virginicus*, transplants treated with granular oxadiazon at 4.48 kg ai ha$^{-1}$ exhibited the highest aboveground dry biomass followed by the plants treated with oxadiazon at 2.24 kg ai ha$^{-1}$, while maintaining weed control throughout the duration of the experiment (Baldos et al. 2010). Similarly, newly transplanted *Fimbristylis cymosa* (a native Hawaiian sedge) was determined to have a tolerance to the pre-emergence herbicide oxadiazon, while maintaining acceptable weed control (Baldos et al. 2012).

Conclusions from the research on other native Hawaiian species reinforce oxadiazon as effective at controlling weeds and at specific rates do not exhibit high levels of phytotoxicity. Another pre-emergent herbicide effective at low rates of application for 3-5 months with common name indaziflam, was recently introduced in 2010 to control grasses and broadleaf weeds in turf (Shaner 2014). There have been no published reports on the phytotoxic effects of indaziflam on newly established native Hawaiian plants. The herbicides and application rate selection reported
here is based on related literature and instructions on the product label oxadiazon (Anonymous 2012) and indaziflam (Anonymous 2010).

The intent of this experiment will be to determine the response of W. indica transplants and weeds to recommended labeled rates of oxadiazon and indaziflam.

Materials and Methods

Plant material

Waltheria is a genus of plants belonging to the order Malvales and family Sterculiaceae. W. indica L., (ACC# 9079945) is a short-lived shrub or subshrub sometimes reaching 2 m in height and 2 cm in stem diameter (Duvachelle, personal communication, 2014). This shrub usually has a single strong stem, but frequently branches near the ground. A variety of upright and prostrate growth forms have been observed from seedling populations. Axillary inflorescences are usually dense glomerules that contain fragrant, yellow to orange flowers. Each 2-mm capsule holds one small, black, obovoid seed (Howard 1974).

W. indica is a pan-tropical shrub species which occurs in diverse populations in the Americas, Mexico and Brazil. One report states that in Hawaii, W. indica was apparently naturalized soon after the arrival of nonnative colonists (Haselwood and Motter 1966). Despite Haselwood’s report, W. indica is more widely classified as a native plant, postulating that the small seeds may have attached to birds which distributed the plant to Hawaii (Wester 1992). Reinforcing the native status, during Captain Cook’s voyage to Hawaii in 1779, on board botanist, David Nelson noted W. indica in regions with very dry, xerophytic scrub vegetation and an annual rainfall of about 50 cm (St John 1979). In certain regions of the world, W. indica is considered an invasive
weed (Sánchez and Uranga 1993). It is found in well drained soils of all types. In Hawaii, the species elevation ranges from sea level to 1220 meters. Current populations tend to occur in altered sites, where soil disturbance has occurred (Long and Lakela 1971). *W. indica* is a good candidate for re-vegetation and seed production purposes due to high drought tolerance and continual production of flowers and seed year round.

In this research, *W. indica* seeds were sown 70 days prior to the start of experiment (2/15/2014 for the first experiment and 4/11/2014 for the second experimental repeat). Seeds were sown in Sunshine mix #4 with mycorrhizae (Sun Gro Horticulture®, Agawam, Massachusetts) in SC-10 dibble tubes (Stuewe & Sons Inc., Tangent, Oregon). Fertilization and irrigation were provided as needed throughout the pre-transplant production period. The herbicide tolerance experiment was conducted twice with starting dates of 04/02/2014 and 06/20/2014.

**Experimental plan**

Planting of transplants and treatment applications were conducted on the first experiment in April 2014 and the replicate experiment in June 2014 in a field adjacent to the first. Before planting, the soil was rototilled to incorporate 112 kg nitrogen acre⁻¹ (formulation 21-4-7) to a depth of 4 inches in both experimental fields. Soil type in both of the experimental fields was Makiki stony clay loam (isohyperthermic typic haplustepts). Soil nutrient analysis was conducted at the completion of both experiments by the University of Hawaii diagnostic service center. The first experimental field contained 221 ppm phosphorus (P), 1203 ppm potassium (K), 5056 ppm calcium (Ca), 1468 magnesium (Mg), 0.30% nitrogen (N) with a 7.2 pH. The second experimental field contained 201 ppm P, 803 ppm K, 8199 ppm Ca, 986 ppm Mg, 0.14% N with a 7.4 pH.
The experiments were designed as a random complete block with four chemical treatments and one untreated plot with four replications. Four *W. indica* transplants were used in each 0.65 m × 1.5 m (1 m²) experimental plot. Chemicals treatments consisted of granular oxadiazon (Ronstar® G, Bayer CropScience, Research Triangle Park, NC) and flowable indaziflam (Specticle® FLO, Bayer CropScience, Research Triangle Park, NC) applied over the top of transplanted *W. indica* at 2.24 & 4.48 kg ai ha⁻¹ and 24 & 49 g ai ha⁻¹, respectively. Granular oxadiazon was applied in pre-weighed aliquots by hand to individual plots to ensure uniform distribution. Spray treatments of indaziflam were applied using a compressed carbon dioxide gas sprayer operating at 241 kPa, outfitted with a single Teejet® (TeeJet Technologies, Wheaton, Illinois) 9095 EVS nozzle (even spray pattern) calibrated to deliver 375 l ha⁻¹.

After application of chemical treatments overhead irrigation was applied for 10 minutes to activate the chemicals. Trial one and two irrigation volumes measured were 1612 L ha⁻¹ min⁻¹ and 1520 L ha⁻¹ min⁻¹, respectively (these rates were maintained for all irrigation applications). Starting on day 2, overhead sprinkler irrigation was delivered two times per day at 6:30am and 12:00pm for 15 minutes each start time for the first trial. Moisture levels of the first trial were determined to be too high based on visual assessment so the second trial irrigation level was reduced to 10 minutes each start time for the first 6 days then reduced to one start time at 6:00am for ten minutes on Monday, Wednesday and Friday.

*Data collection*

During the first trial, four predominate weeds were present within the experimental plots, which were assessed individually for percent control at 45 DAP. Trial one weed species present were *Commelina diffusa* (spreading dayflower, honohono), *Portulaca oleracea* (common purslane), *Euphorbia hypericifolia* (graceful spurge) and *Eleusine indica* (goosegrass). During the second
trial only two predominate weeds were present, *Ipomoea triloba* (morning glory) and *Eleusine indica* (goosegrass).

Data were collected at three time points at 15 day intervals starting at 15 days after planting (DAP), 30 DAP and 45 DAP. The first (15 DAP) and second (30 DAP) data collection points consisted of visual *W. indica* percent of maximum growth vigor ratings. Percent of maximum vigor ratings of *W. indica* were based on the author’s familiarity of uninhibited plants grown as nursery stock and personal observations of wild plants. The third (45 DAP) and final data collection point consisted of *W. indica* percent of maximum vigor, *W. indica* dried plant biomass (g/4 plants) accumulation, timed removal of weeds from experimental plots (s/m²), dried weed biomass (g/m²), and percent control of individual weed species present. The second replicate trial was conducted with all data collection times and ratings consistent with the first trial.

### Data analysis

Data were analyzed in the statistical analysis program Statistix™ 10.0 (Analytical Software, Tallahassee, Florida). Separate analyses were conducted for *W. indica* percent of maximum vigor for each data collection time and were analyzed over both trials as a split plot with experimental trial as the main effect and treatment as the split plot effect. *W. indica* dry aboveground biomass accumulation in the third rating period (45 DAP) was analyzed over both trials as a split plot with experimental trial as the main effect and treatment as the split plot effect. If significant trial × treatment interactions were detected, data from trials were combined (Brosnan et al. 2011).

Due to different weed species present in trials one and two, all weed response data will be analyzed individually based on trial. Weed response data were analyzed using a random complete block design analysis of variance in the statistical analysis program Statistix™ 10.0.
Individual species percent control data in both trials were square root transformed to conform to the assumptions of the ANOVA (Ahrens et al. 1990). When significant effects were detected, means were separated using Tukey’s all pairwise HSD test at α= 0.05.

Results and Discussion

Data collection 1 (15 DAP)

Results of the analysis for percent of maximum growth vigor did not detect a significant trial × treatment interaction (P=0.118), thus results will be pooled over trial, however a significant treatment effect was detected (P< 0.001). No significant differences were detected in plant vigor of W. indica between the untreated and the low rate of oxadiazon plots (99% and 98%, respectively). A significant reduction in percent of maximum vigor was recorded for the high rate of oxadiazon (88%) and both rates of indaziflam (low rate = 57% and high rate = 26%) (Table 1).

Data collection 2 (30 DAP)

The results of the analysis for W. indica percent of maximum vigor did not reveal a significant interaction between the factors of trial × treatment (P= 0.078), thus results will be pooled over trials. A significant treatment effect was detected (P< 0.001). The untreated and low rate of oxadiazon treatments resulted in the significantly highest percent of maximum vigor (97% and 94%, respectively), followed by the high rate of oxadiazon and the low rate of indaziflam (69% and 46%, respectively), the high rate of indaziflam imposed the highest level of percent of maximum vigor suppression (Figure 1) (Table 1).
Data collection 3 (45 DAP)

Results of the analysis for *W. indica* percent of maximum vigor did not indicate an interaction between the factors of trial × treatment (P= 0.056), thus results will be pooled over trials. A significant treatment effect was detected (P <0.001). The significantly highest percent of maximum vigor was found with the low rate of oxadiazon (82%), the untreated and high rate of oxadiazon significantly lowered the percent of maximum vigor (69% and 60%, respectively). The lowest level of percent of maximum vigor was recorded in plots treated with indaziflam (Table 1). The decreased *W. indica* percent of maximum vigor in the untreated plots was attributed to increased weed competition.

Results of the analysis for the aboveground *W. indica* dry plant biomass indicated a significant interaction between the factors of trial × treatment (P= 0.003), thus treatment means will be presented separately over trials. Significantly highest *W. indica* biomass was found in the second trial with both low and high rates of oxadiazon (137 g and 141 g, respectively). In both trials the numerically lowest biomass was detected with the high rate of indaziflam (trial 1 = 26 g and trial 2 = 7 g) (Table 3). Based on the treatment means by trial, the second trial exhibited increased biomass of *W. indica*, this can be attributed to seasonal variation as the second trial was in June compared to the first in April. Another reason for the increased biomass in the second trial may be due to the reduction of irrigation, as mentioned previously *W. indica* is extremely drought tolerant, and appears to be inhibited by excessive watering.

During the first trial, the analysis indicated a significant effect of treatment on weeding times (P< 0.001). Weeding times were significantly lower in all herbicide treated plots compared to the untreated control (Table 3). Numerically lowest weeding times were found in the high rate of the oxadiazon treatment (5 seconds). During the second trial, the analysis indicated a significant
effect of treatment on weeding times (P < 0.001). Significantly lower weeding times were found in all herbicide treated plots compared to the untreated control (Table 3).

During the first trial, the results of the analysis indicated a significant effect of treatment on weed biomass within experimental plots (P< 0.001). Weed biomass was significantly lower in all herbicide treated plots compared to the untreated control (Table 3). During the second trial, the results of the analysis indicated a significant effect of treatment on weed biomass within experimental plots (P< 0.001). Weed biomass was significantly lower in all herbicide treated plots compared to the untreated control (Table 3). Morning glory was present during this trial and was minimally controlled with the low rate of indaziflam.

During the first trial, spreading dayflower control in herbicide treated plots did not differ from the untreated control (Table 2) indicating a lack of commercially acceptable control. Common purslane and Graceful spurge were well controlled by all herbicide treatments with oxadiazon at the high rate providing 100% control. The low rate of indaziflam did not provide commercially acceptable control of Common purslane and Graceful spurge. Total (100%) control of Goosegrass was recorded for both herbicides at both high and low rates of application during both runs of the experiment. During the second trial, morning glory emergence was variable throughout the experimental area with only weak suppression imposed by the low rate of indaziflam.

In both experiments broadleaf weed control was consistently higher with oxadiazon than indaziflam. The single grass species (goosegrass) present during both experiments was well controlled by both herbicides at rates of application recommended by the product label. Reported literature confirms indaziflam is very effective in controlling grass species such as
Digitatia ischaemum (smooth crabgrass) and Poa annua (annual bluegrass) (Brosnan et al. 2011; Hunter Perry et al. 2011).

W. indica growth in indaziflam treated plots was significantly reduced indicating that this herbicide will not be useful for weed control during early establishment using transplants. Oxadiazon, applied in a granular formulation to W. indica transplants was shown to provide a useful level of grass and broadleaf weed control with an acceptable level of growth inhibition.
Tables and Figures

Figure 3.1. Experimental plots (1 m²) with four W. indica plants. W. indica percent of maximum vigor response at 30 DAP to oxadiazon at 2.24 kg ai ha⁻¹ (left) compared to indaziflam at 49 g ai ha⁻¹ (right).

Figure 3.2. Experimental plots (1 m²) with close up image of W. indica plants. W. indica percent of maximum vigor response at 30 DAP to oxadiazon at 2.24 kg ai ha⁻¹ (left) compared to indaziflam at 49 g ai ha⁻¹ (right). Unacceptable injury is visible in the W. indica plants treated with the high rate on indaziflam.
### Table 3.1. *W. indica* percent of maximum vigor response to the pre-emergence herbicides oxadiazon and indaziflam applied at low and high label rates. Means are separated using Tukey's HSD comparison at $P=0.05$. Means within columns followed by the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>15 DAP</th>
<th>30 DAP</th>
<th>45 DAP</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxadiazon</td>
<td>98 A</td>
<td>94 A</td>
<td>82 A</td>
</tr>
<tr>
<td>2.24</td>
<td>88 B</td>
<td>69 B</td>
<td>60 B</td>
</tr>
<tr>
<td>4.48</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indaziflam</td>
<td>57 C</td>
<td>46 B</td>
<td>37 B</td>
</tr>
<tr>
<td>0.024</td>
<td>26 D</td>
<td>18 C</td>
<td>24 B</td>
</tr>
<tr>
<td>0.049</td>
<td>99 A</td>
<td>97 A</td>
<td>69 B</td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### Table 3.2. Percent control of individual weed species present in trials one and two at 45 DAP. Means are separated using Tukey's HSD comparison at $P=0.05$. Means within columns followed by the same letter are not significantly different.

#### Trial one

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Spreading dayflower</th>
<th>Common purslane</th>
<th>Graceful spurge</th>
<th>Goosegrass</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxadiazon</td>
<td>72 A</td>
<td>93 A</td>
<td>95 A</td>
<td>98 A</td>
</tr>
<tr>
<td>2.24</td>
<td>82 A</td>
<td>100 A</td>
<td>100 A</td>
<td>100 A</td>
</tr>
<tr>
<td>4.48</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indaziflam</td>
<td>72 A</td>
<td>71 A</td>
<td>72 A</td>
<td>94 A</td>
</tr>
<tr>
<td>0.024</td>
<td>72 A</td>
<td>97 A</td>
<td>94 A</td>
<td>98 A</td>
</tr>
<tr>
<td>0.049</td>
<td>72 A</td>
<td>3 B</td>
<td>0 B</td>
<td>0 B</td>
</tr>
<tr>
<td>Untreated</td>
<td>72 A</td>
<td>3 B</td>
<td>0 B</td>
<td>0 B</td>
</tr>
</tbody>
</table>

#### Trial two

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Morning glory</th>
<th>Goosegrass</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chemical</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxadiazon</td>
<td>66 AB</td>
<td>100 A</td>
</tr>
<tr>
<td>2.24</td>
<td>92 A</td>
<td>100 A</td>
</tr>
<tr>
<td>4.48</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indaziflam</td>
<td>28 AB</td>
<td>100 A</td>
</tr>
<tr>
<td>0.024</td>
<td>65 AB</td>
<td>100 A</td>
</tr>
<tr>
<td>0.049</td>
<td>2 B</td>
<td>5 B</td>
</tr>
<tr>
<td>Untreated</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chemical</td>
<td>Rate (kg ai ha⁻¹)</td>
<td>Weed response</td>
</tr>
<tr>
<td>--------------</td>
<td>------------------</td>
<td>---------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxadiazon</td>
<td>2.24</td>
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</tr>
<tr>
<td></td>
<td>4.48</td>
<td></td>
</tr>
<tr>
<td>Indaziflam</td>
<td>0.024</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.049</td>
<td></td>
</tr>
<tr>
<td>Untreated</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.3. Weed and W. indica response to the pre-emergence herbicides oxadiazon and indaziflam applied at low and high label rates at 45 DAP. Means are separated using Tukey’s HSD comparison at P=0.05. Means for weed response within columns followed by the same letter are not significantly different. Means for W. indica response within columns and rows followed by the same letter are not significantly different.
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Optimization of Waltheria indica seed yield using a visual evaluation scale of six seed head cluster maturity categories.

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 were used to separate clusters in to six discrete color categories (CATs) based on parameters of flowering, green tissue and necrosis. The six cluster CATs were analyzed for mature seed yield and percent moisture content. Results indicated that the significantly greatest seed yield was obtained from the third cluster color CAT, containing 3.28 g of mature seed in 20 grams of dried flower heads. The flower cluster moisture content for the optimal harvest CAT was 52 percent and represented the first drop in moisture content that peaked at 56%. The initial decline in moisture content after CAT 2 can be used as a tracking indicator for the initiation of mature seed formation. Tracking discrete visual flower head color CATs in which practitioners can utilize to optimize seed yield.

Introduction
In Hawaii, the native shrub Waltheria indica L. has been identified for increased usage in urban re-vegetation, including roadway corridors (ANONYMOUS, 2011). However, no plant material or
seed stock is commercially available, complicating re-vegetation efforts. *W. indica* plants begin flowering at about 6 months of age and bloom more or less continuously until the plant weakens with age or stress (FRANCIS, 1989). Reproduction of *W. indica* is most commonly achieved through seeds. A study conducted by Sanchez determined that a collection of seeds averaged 0.0013 grams/seed or 764,000 seeds/kg (SÁNCHEZ and URANGA, 1993). Development of protocols associated with seed harvest timing of *W. indica* is necessary to enable restoration practitioner’s access to mature seed stock from wild populations or crop-like production settings.

A critical aspect in production of agricultural crops is the correct timing of harvest to maximize yield and quality (BEDNARZ et al., 2002; COPELAND, 1995; RUSSO, 1996). 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 as you move to 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 (MCDONALD and COPELAND, 1997). 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 (KLEIN and HARMOND, 1971). 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 (BALL et al., 2009; LINDGREN
and Walker, 2012). These methods can provide a well-defined quantitative measure for harvest activities. However, they lack utility for determining the onset 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 (Crookston and Hill, 1978; Srimathi et al., 2013).

The intent of this research is to characterize visual cues that represent the discrete flower cluster categories (CATs) of development and correlate those CATs to the amount of mature seed for W. indica. Data will be collected to: (1) characterize seed cluster development based on visual observations of flowering and floral tissue senescence; (2) correlate discreet CATs to flower cluster moisture and mature seed content.

Materials and methods

Plant material

Waltheria indica transplants were installed to field plots in December 2013 at a roadside demonstration site located in Honolulu Hawaii. Characterization of visual floral cluster development and sample collection was conducted in January 2015. 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. Transplants were 4 months of age at time of planting in the experimental field. 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 CATs based on visually distinct attributes of development (figure 1). CAT 1 was visually identified as pre-flowering, where no flower buds were open, with 100% green tissue. CAT 2 consisted of open flower buds with approximately 10% senescing flowers with 100% green flower cluster tissue. CAT 3 was identified by 40-50% senesced flowers with 10% necrotic flower cluster tissue. CAT 4, 5 and 6 were identified by 50%, 75% and 100% necrotic flower cluster tissue, respectively.

On a representative 17-month old (4 months for transplant production and 13 months in the ground) *W. indica* plant with a 1.5 meters long side branch, a visual approximation of the percentage composition of each flowering CAT is: 1% CAT 1, 5% CAT 2, 30% CAT 3, 30% CAT 4, 30% CAT 5, and 4% CAT 6.

**Experimental design**

The field planting was partitioned into four blocks established on separate drip irrigation lines. Drip irrigation was provided by Netafim™ TLCV9-1210 lines (Netafim USA, Fresno, California). Drip irrigation specifications indicate a flow rate of 3.4 liters 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, a random number generator was used to select four plants for flower cluster sampling to characterize the six CATs.

From each randomly selected plant, 15 grams of each flower CAT was collected. Each 15 gram color CAT sample was composited to one sample for each block (60 gram total fresh weight for each CAT / block). Flower cluster moisture content in each composite sample and total mature seed yield data were gathered. Flower cluster moisture analysis was performed following a
constant temperature oven drying fresh weight method based on International Seed Testing
Association (ISTA) protocols (ISTA, 2003). After drying, composite samples were reduced to
20 grams. Each 20 g sample for individual CATs were processed to extract mature seed. 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 x 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, seeds are allowed to either
push through the cylinder or remain within the machine while debris is removed (figure 2) by
gravity and dust recovered with a power vacuum. The brush machine was run at full power for
1 minute with the front discharge door closed and 1 minute with discharge door fully open
(figure 3). In the second device, seeds were separated from pulverized flower head components
with a Clipper™ Office Tester fitted with a 1.651 x 1.651 mm wire mesh top screen and a 0.927
x 0.927 mm wire mesh bottom screen (A.T. Ferrell Company Inc., Bluffton, Indiana) (figure 4).
The seed separator blower air ducts were open at 75% capacity and was run until all material
traveled past the screen sifters. Cleaned seeds were weighed to determine the yield from 20
grams of dry flower head clusters.

Data analysis
Seed yield in grams was used to characterize the discrete flower cluster color CAT which
contained the maximum amount of seed. Moisture data was also used to characterize each
flower cluster CAT. Data for seed yield and moisture content were analyzed separately using a
random complete block design in the statistical software program Statistix™ 10.0 (Analytical
When significant effects were detected, means were separated using Tukey’s HSD test at $\alpha = 0.05$.

**Results and Discussion**

The results of the analysis for grams of seed yield for each color CAT indicated a significant effect of flower cluster CAT on seed yield ($P < 0.001$). The significantly highest mature seed content was extracted from the 3rd cluster CAT with a mean seed yield of 3.28 grams (table 1). The 4th and 5th CATs resulted in the next highest yield of 2.73 and 2.81 grams of seed, respectively. No mature seed was found in the first CAT and only 0.57 grams in CAT 2. The fully senesced CAT 6 contained 2.17 grams of seed (figure 5). Moisture analysis indicated that that at the 3rd CAT flower cluster contained 52.2% moisture (table 1). Flower cluster moisture content increased slightly from CAT 1 to CAT 2. Cat 3 represents the first drop in moisture content following peak levels in CAT 2 (figure 5).

Tracking the initial decline in moisture at CAT 3 can be utilized to determine flower cluster CAT where mature seed begin to form. The determination of visual indicators for optimization of seed harvest timing can be a helpful tool that seed managers can employ (SAMARAH et al., 2004). Native plants in the wild, or in production settings can be visually assessed for optimized harvest time (WANG et al., 2006). The utilization of this method can aid decision making for seed harvesting from wild population allowing for an immediate assessment of harvest timing while in the field. Agronomists utilize similar visual techniques to judge crop readiness for food crops (CROOKSTON and HILL, 1978; ELIAS and COPELAND, 2001; SINNECKER et al., 2002), however limited literature is available for the adoption in native seed harvesting and production settings. Harvest practitioners assessing *W. indica* seed maturity can follow a color indicator swatch to
compare CAT development and subsequent seed maturity. Understanding that optimum seed harvest timing for *W. indica* can be achieved at color CAT 3 can aid in harvest and management decisions.
Figure 4.1. Six Waltheria indica flower cluster CATs used for determination of seed harvest timing visual indicators. CATs were classified from immature to mature (CAT 1 to 6, respectively) by presence of flowers, senesced flowers, green tissue and necrotic tissue.
Figure 4.2. Westrup LA-H brush machine inner rotating brush mechanism with #14 (1.0 x 1.0 mm) perforated mantle and medium nylon 0.5 mm brushes.

Figure 4.3. Westrup LA-H brush machine front with discharge door fully open. The discharge door is notated in this image by the letter A.
Seed yield and moisture content of *W. indica* based on seed cluster maturity category

<table>
<thead>
<tr>
<th>Category</th>
<th>Seed yield (g)</th>
<th>Moisture content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0 E</td>
<td>53 B</td>
</tr>
<tr>
<td>2</td>
<td>0.57 D</td>
<td>57 A</td>
</tr>
<tr>
<td>3</td>
<td>3.28 A</td>
<td>52 B</td>
</tr>
<tr>
<td>4</td>
<td>2.73 B</td>
<td>44 C</td>
</tr>
<tr>
<td>5</td>
<td>2.81 B</td>
<td>30 D</td>
</tr>
<tr>
<td>6</td>
<td>2.17 C</td>
<td>13 E</td>
</tr>
</tbody>
</table>

Table 4.1. Table of seed yield means and percent moisture content for *W. indica* classified over six visual color CATs of flower head clusters. Means are separated using Tukey’s HSD comparison at *P*=0.05. Means within columns followed by the same letter are not significantly different.

Figure 4.4. Clipper Office Tester seed separator cleaning *W. indica* plant material. Plant material visible is seen passing over the top screen (notated by the letter A) due to excessive size, seeds which are smaller in diameter will pass through the screen (A) to a secondary screen followed by further separation by an aspiration air column.
Figure 4.5. Seed yield means and seed head moisture content of *W. indica* represented over six discrete color CATs of seed head clusters, presented with standard error bars. Maximum seed yield was obtained at the third visual cluster CAT. Moisture content decline after CAT 2 indicated the presence of mature seed formation.
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Chapman & Hall.


Chapter 5

Evaluation of *Panicum torridum* dormancy relief through exogenous chemical treatments and seed storage optimization of temperature and relative humidity parameters

Abstract

Seed dormancy is an evolutionary adaptation for increasing seedling survival by delaying germination and is found in many taxonomic families. Although dormancy is an important ecological survival technique, it imposes hardships for agronomic production using seeds. *Panicum torridum* is a native Hawaiian annual grass that has been identified as a re-vegetation candidate for seed production. *P. torridum* appears to possess an intermediate to deep physiological dormancy (PD). This research aims to characterize relief parameters by: 1) evaluating exogenous hormonal, reactive oxygen intermediates, and simulated combustion product treatments; 2) determining optimized storage conditions of relative humidity (RH) and temperature over a 10 month duration. Results indicate that all exogenous chemical treatments tested were not effective at relieving the dormancy present in *P. torridum*. Optimal storage conditions to relieve dormancy were found with seeds equilibrated to 12% RH, stored at 30°C for a period of 8 months resulting in 55% germination. Maintenance of viability for long term storage up to ten months is best achieved with seeds stored at 12% RH. The results support the specified storage parameters as the recommended procedure to relieve dormancy and maintain viability in *P. torridum* seeds.
Introduction

Seed dormancy is an evolutionary adaptation for increasing seedling survival by delaying germination (Fenner and Thompson, 2005). Dormancy of mature seeds have been identified in numerous studies (Baskin and Baskin, 2004), spanning multiple taxonomic families (Baskin et al., 2000). Although dormancy is an important ecological survival technique, it imposes hardships for agronomic production. Classification systems define five classes of seed dormancy: physiological dormancy (PD), morphological dormancy (MD), morphophysiological dormancy (MPD) physical dormancy (PY) and combinational dormancy (PY + PD) (Baskin and Baskin, 2004). Within the PD class, three levels can be defined as non-deep, intermediate and deep, which can be partially elucidated by germination response to stimulatory chemicals (Baskin and Baskin, 2004).

_Panicum torridum_ Gaudich. is a native Hawaiian annual grass that has been identified as a re-vegetation candidate for roadsides and conservation plantings. Preliminary germination evaluations have indicated that dormancy is present, and there are general reports of dormancy existing throughout the Panicum genus (Simpson, 2007). Postharvest seed studies of _Panicum dichotomiflorum_ have shown that deep seed dormancy exists and is difficult to overcome by pretreatment of the dry seeds. A further potential requirement for germination in the Panicum genus is an alternating temperature cycle during the germination period, suggesting that multiple forms of dormancy could exist (Taylorson, 1979). In many species, the depth of seed dormancy is controlled by the relationship between levels of gibberellic acid (GA) and abscisic acid (ABA) and is generally referred to as the hormone-balance theory (Wareing and Saunders, 1971). This theory proposes that as dormant seeds reach the optimum ratio of endogenous GA:ABA, germination can proceed. This is the foundation for the widely accepted practice of introducing...
GA to dormant seeds, which is a well-established dormancy breaking chemical. In 2010, Kirmizi reported that germination in *Panicum olympia* seeds commenced when exposed to a 24 hour GA\(^3\) imbibition period. Seed germination increased from 0% in the untreated seed to 49% and 52% with 100 and 250 ppm GA\(^3\), respectively (Kirmizi et al., 2010). An article by Hilhorst states that although GA\(^3\) is a well-recognized and accepted dormancy breaking chemical, GA\(^4+7\) is almost 1000 times more effective (Hilhorst, 2011). Germination stimulation by GA is limited to the non-deep and intermediate depths of dormancy (Baskin and Baskin, 2004). Another method to shift the GA:ABA balance to induce germination involves the use of fluridone which decreases the levels of endogenous ABA during early stages of imbibition (Grappin et al., 2000).

Ethylene which is also a hormonal treatment has been recognized to relieve dormancy in seeds. Ethylene is produced in at least trace amounts by almost all higher plants and is involved in the control of growth and development processes that range from germination to senescence (Abeles et al., 2012). In most species studied, exogenous ethylene or ethephon stimulates the germination of dormant and non-dormant seeds although in some cases seed germination is unaffected by this hormone (KeÇpczyński and KeÇpczyńska, 1997). Ethephon, an ethylene releasing compound, was found to break secondary dormancy in seeds allowing for more efficient treatment applications compared to ethylene (a gas at room temperatures). Tischler and Young found that 0.3M ethanol added to the growing substrate significantly increased germination of *Panicum coloratum*, compared to the control (Tischler and Young, 1983).

Aside from hormonal treatments, reactive oxygen donors such as sodium nitroprusside (SNP) or hydrogen peroxide can enhance germination or relieve dormancy in seeds (Beligni and Lamattina, 2000). A 2008 study conducted by Sarath determined that 20mM hydrogen peroxide added to petri dishes with *Panicum virgatum* exhibited the highest germination across seed lots.
after 2 and 4 days of treatment, compared to treatments of SNP and potassium ferrocyanide (Sarath and Mitchell, 2008). Although Sarath determined that hydrogen peroxide was superior to SNP for *P. virgatum*, SNP is still shown to relieve seed dormancy. Many compounds are nitric oxide (NO) donors but SNP is the one of the most commonly used substance for seed dormancy relief (Bethke et al., 2011).

In the native Hawaiian grass species, *Heteropogon contortus*, the simulated combustion products liquid smoke (a commercial food grade flavoring) diluted to a 1% solution and 0.5mM cyanide solutions were found to be effective germination promoters. Optimum treatment rates for liquid smoke and several other germination enhancers were determined by Baldos (Baldos, 2013).

Since seed exhibiting intermediate or deep PD are not generally affected by stimulatory dormancy relief chemicals, it is necessary to understand optimum after-ripening storage conditions to maximize seed germination (Baskin and Baskin, 2004). Ultimate seed survival is directly related to the time the seed has been exposed to unfavorable conditions of temperature or humidity (Barton, 1961). In the storage condition study of *Cassia angustifolia*, seed was stored at four different levels of relative humidity (RH) (5.5%, 11%, 33%, and 75%) established and maintained using saturated salt solutions in airtight desiccators and three different storage temperatures (5°C, 20°C and ambient). Storage temperatures of 5°C and 20°C at 5.5% and 11% RH were found to be optimal for extending seed viability and maintain high levels of germination when removed from storage (Santhoshkumar and Veena, 2012). Similar work has been conducted to characterize after-ripening parameters of *H. contortus*. It was determined that the optimum dormancy relieving conditions for *H. contortus* required 28 days of seed equilibration in a 12% RH desiccation chamber followed by a 30°C incubation temperature for 9-12 months depending on seed batch (Baldos et al., 2014). Although saturated salts are well
established in the maintenance of specific target RH levels (Winston and Bates, 1960; Young, 1967), proportions of water added to the salt (forming the saturated salt solution) to desiccate a given volume of water (contained in fresh seeds) are unclear.

The intent of this study is to evaluate seed dormancy relief in *P. torridum* through: 1) exposure to the exogenous chemicals cyanide, ethanol, GA\(^3\), GA\(^4+7\), liquid smoke, hydrogen peroxide, fluridone, ethephon and sodium nitroprusside; 2) storage conditions equilibrated to three levels of relative humidity, stored at three temperatures over 10 months. Prior to the dormancy relief storage study, a calibration procedure was utilized to determine the components of a saturated salt (LiCl) solution that could absorb moisture released by seeds in a sealed vessel while lowering and maintaining the target RH level.

**Materials and Methods**

*Plant seed material*

Two separate batches of *Panicum torridum* seeds were used throughout these experiments to determine the consistency of the treatment response in replicated trials. Both seed batches were harvested from wild populations found on the Hawaiian island of Molokai and stored in sealed bags in a 10°C refrigerator until use. Seed batch 1 (SB1) was harvested in May 2013. Seed batch 2 (SB2) was harvested in May 2014. Both seed batches showed higher than 90% seed viability before experimental use using a 1% tetrazolium chloride (TZ) solution following standardized methods outlined by the ISTA (ISTA, 1996).
Germination parameters

For all germination studies, experimental units consisted of 50 seeds exposed to the experimental treatment/treatment combinations with four replications, repeated for each seed batch. Each experimental unit was incubated in 90mm petri dishes pre-moistened with 3 ml distilled water and lined with filter paper (Whatman® #2, Little Chalfont, Buckinghamshire). Petri dishes were placed in an alternating temperature germination chamber with four T5 high output 24W 6400K AgroBrite™ bulbs (Hydrofarm®, Petaluma, California) for 14 hours of light at 28°C and 10 hours of dark at 24°C. Experimental conditions during the seed germination period were monitored with a Hobo UX100 logger (Onset®, Cape Cod, Massachusetts). Distilled water was added to petri dishes as needed over the 14 day germination period. Germination was recorded when the seed radicle protruded 1 mm from the seed testa.

Water imbibition of seed

To help classify dormancy type, it is necessary to determine if the seed testa is permitting water to pass through to the seed endosperm. This study evaluated the ability of after-ripened germinable P. torridum seeds (provided by G. Sakamoto, USDA Hoolehua Plant Materials Center) to imbibe water compared to freshly harvested non-germinable seeds. Post-harvest handling parameters of the germinable seeds used in this trial were not determined by USDA/NRCS staff. If the fresh seeds are able to imbibe water and not germinate, it is unlikely that a physical dormancy mechanism is prohibiting germination. Weight increases were measured in 100 seed units over a 24 hour period exposed to distilled water on filter paper, with three replications. Weights were taken prior to exposure to water, and after exposure at 1 h, 2 h, 4 h, 12 h, and 24 h. At each interval, seeds were removed from the saturated filter paper, blotted dry, weighed, and returned to the filter paper (Baskin et al., 2004). Weights were measured
using an analytical balance with a weight sensitivity range of 0.1 mg. The amount of water taken up was determined by actual weight increases and converted to percentage increase in weight:

\[ Wi = \frac{(Wi - Wd)}{Wd} \times 100 \]

where \( Wi \) and \( Wd \) are weights of imbibed and dry seeds, respectively.

A random sample of the germinable seed batch utilized for the imbibition control had a 55% germination rate at the start of the experiment, with high viability (90%). The freshly harvested seed was of high viability (96%) but fully dormant with no germination. Data expressed as percent seed moisture increase will be compared between germinable \( P. torridum \) seed and freshly harvested seed over imbibition exposure times. Data were analyzed for weight increases as a split plot with seed batch (i.e. germinable and fresh) as the main effect and imbibition time as the split plot effect, in the statistical software program Statistix™ 10.0 (Analytical Software, Tallahassee, Florida).

**Dormancy relief through exogenous chemicals**

After determining that freshly harvested seed could not germinate, seed response to known germination stimulators was determined to characterize the physiological nature of \( P. torridum \) dormancy. Table 1 contains a list of chemicals and rates reported to stimulate germination in seeds with physiological dormancy. \( P. torridum \) seeds were exposed to treatments of cyanide, ethanol, \( GA^3 \), \( GA^{4+7} \), liquid smoke, hydrogen peroxide, fluridone, ethephon and sodium nitroprusside individually to determine their effect on dormancy relief.

Germination responses of zero to less than 4% will be excluded from the analysis. Treatments that have zero or low variance will reduce the magnitude of mean square error, which is the denominator for F tests and also a component of multiple comparison procedures used to
compare treatments (Reeve and Strom, 2004). Thus, zero germination and other low variance treatments could potentially affect the statistical analysis of dormancy relief by increasing apparent treatment effects (Reeve and Strom, 2004). Data were analyzed as a random complete block design using standard least squares fit model in the statistical program JMP® Pro 11 (SAS Institute Inc., Cary, NC).

*Desiccation chamber calibration*

Published reports were not clear on either the optimal composition of saturated salt solutions (the proportions of salt and water) or the desiccation chamber’s moisture levels that could be absorbed to reduce and maintain the reported level of RH. The ability of lithium chloride (LiCl) to reduce the RH and then maintain the reduced level, in a sealed chamber, in the presence of a known quantity of water was determined. Baldos determined that 28 days of exposure to 12% RH, reduced *H. contortus* seed from 11.5% to 6% seed moisture content (Baldos et al., 2014). It is reasonable to consider that *P. torridum* seeds would respond in a similar way in a desiccating atmosphere as *H. contortus* seeds (a grass that occupies a similar environmental niche in Hawaii), and that moisture content of fresh seeds (11.5%) could be reduced by 5.5% after 12% RH equilibration with a water and LiCl salt mixture. With 11.0 grams of total seed placed in a sealed desiccation chamber, the water removed from the total amount of *P. torridum* seeds by the saturated salt needed to be 0.61 grams (equivalent to 0.61 ml water) to arrive at a seed moisture content of 6.0%.

Twenty grams of LiCl salt was mixed with 0 ml, 3.0 ml, 6.0 ml, 12.0 ml and 24.0 ml of distilled water. These water/salt mixtures were placed in a sealed desiccation chamber that contained 5 ml of water in a separate container. The drop in RH was recorded inside the chamber for 18 days with a Hobo UX100 data logger.
Dormancy relief and viability maintenance through storage conditions

Seed after ripening conditions were evaluated for the parameters of temperature and relative humidity. Three RH levels were imposed on seeds placed into unsealed 1.5 ml micro-centrifuge tubes with O-ring gasket lids (Fisher Scientific®, Pittsburgh, Pennsylvania) and placed in sealed Bel-Art (Scienceware®, Wayne, New Jersey) non-vacuum desiccation chambers. Humidity levels were maintained using saturated salt solutions of LiCl (12% RH), calcium nitrate (50% RH) and sodium chloride (75% RH) (Winston and Bates, 1960; Young, 1967). Each desiccation chamber contained a petri dish with, 20 grams of salt mixed with 6 ml of distilled water to reduce and maintain specific levels of RH based on the type of salt used. After 30 days in the sealed desiccator, seed filled tubes were immediately sealed and transferred to three incubation temperature (10°C, 20°C and 30°C) chambers. Seeds were withdrawn from the temperature chambers at 0, 2, 4, 6, 8 and 10 months to test for germination, seed viability (TZ) and seed moisture content. Throughout the experiment, temperature and humidity levels within the chambers were monitored in real time and logged electronically with Onset© UX-100 data loggers.

Germination data were analyzed as a split split split split plot, classifying seed batch at the main plot effect, RH as split plot effect, temperature as spilt split plot effect and duration in storage as split split split split plot effect in the statistical software program Statistix™ 10.0. Due to inconsistent germination found in month 0 in SB1, and no germination in SB2, data for month 0 was removed from the analysis. The remaining analysis for germination data was conducted over the three factors at durations of 2, 4, 6, 8 and 10 months. The analysis for seed viability was conducted with the same data analysis format as germination, but all months of storage were included in the analysis.
Results

Water imbibition of seeds

Results of the analysis indicate that no interactions were present between the factors of seed batch × imbibition time on seed weight increases, thus treatment means will be pooled over seed batch (P= 0.057). The results indicated a significant weight increase over the 24 hour imbibition period (P< 0.001). Both germinable seeds and freshly harvested seeds were similar in their ability to imbibe water, with both seed batches showing a 15.3% final seed weight increase. Both 1 and 2 hour of imbibition weights were significantly greater than the starting point, but significantly less than the maximum increases of 15.6%, 14.6% and 15.3% at 4 h, 12 h and 24 h, respectively (table 2). This result indicates that the P. torridum seed testa is not limiting the permeation of water to the endosperm, an indication that dormancy is not physically based (PY) (Baskin et al., 2004).

Dormancy relief through exogenous chemicals

The results indicated that P. torridum seeds are not stimulated by any of the exogenous chemicals tested. All treatments and rates presented in table 1 resulted in zero germination. The lack of response indicates that the dormancy present is an intermediate or deep form of PD (Baldos et al., 2015; Baskin and Baskin, 2004).

Desiccation chamber calibration

Calibration of saturated salt solutions indicated that 20 grams of LiCl combined with 6 ml distilled water reduced ambient humidity levels (50%) to target 12.5% RH within 6 days in a 1700 cm³ chamber in the presence of 5 ml of distilled water. The 12.5% target RH was not achieved with the other volumes of water added to the 20 grams of LiCl (table 3). These results
supported the use of the salt and water mixture that could remove the required amount of seed moisture in *P. torridum* seeds during dry seed storage to provide dormancy relief and viability maintenance. The other salts used in this research (calcium nitrate and sodium chloride) are required to absorb less moisture (due to higher RH levels of 50% and 75%) from the chambers, therefore the water volumes to produce their saturated salt solutions were based on the LiCl calibration.

**Dormancy relief through storage conditions**

The results of the analysis indicated the highest level significant interaction was found between the three factors of temperature $\times$ RH $\times$ months in storage ($P < 0.001$), allowing for means to be pooled across seed batches. The highest level of germination was recorded when storage conditions of 12% humidity at 30°C were imposed on dormant seeds (figure 1). Under these conditions, percent germination increased steadily up to 55% at 8 months in storage and then dropped slightly to 49% at 10 months (table 4). Storage at 20°C also showed similar but consistently lower levels of germination enhancement that peaked at 6 months at 20% and did not significantly change with an additional 4 months of storage. Storage at 10°C resulted in minimal germination, peaking at less than 10% germination with 8 months of storage. At 75% humidity, there was minor stimulation in germination with storage. The germination response at 50% RH was similar to that at 75% with only slight enhancement during the storage duration when the 30°C temperature was imposed on dormant seeds.

**Viability maintenance through storage conditions**

The analysis of seed viability indicated that a significant four way interaction was present between the factors of seed batch $\times$ temperature $\times$ RH $\times$ months in storage ($P < 0.001$), thus factors of temperature, RH and storage duration will be presented separately for both seed
batches. The general trend between both seed batches was that at the lowest RH level (12%) viability loss was minimized over the ten month duration. As RH level was raised to 50% and 75% increasing losses of viability were detected over the storage duration, with lowest seed viability at 75% RH. Within the 12% RH level, temperature increases produced a slight decline in viability. The 50% RH level exhibited a more defined loss of viability as temperatures increased over time. Within the 75% RH treatments, increased temperatures produced the most defined and reduced losses of viability, with the greatest viability losses found with the highest temperature of 30°C (figure 2). In SB2, the magnitude of viability loss was greater than SB1 and most obvious at the highest RH (75%) and the warmest temperature (30°C) over ten months. *P. torridum* seed viability was maintained at the highest levels at 12% RH during the ten months of storage within the temperature range of 10°C to 30°C. Relative humidity levels above 12% reduced seed viability and are not recommended during storage.

**Discussion**

A visual examination of the thin seed testa and the water imbibition experiment indicated that there is no restriction of water passing through the through seed testa to the endosperm. This reinforces the hypothesis that the dormancy present is not physically based. The very small size (<1mm) of *P. torridum* seed did not allow for the excision of the embryo to test for MD or to further the understanding of depth of PD (Baskin and Baskin, 1998). Following the logical progression of treatments to relieve dormancy led to the evaluation of PD exogenous chemical treatments. As noted in the results, no germination stimulation was detected in response to any of the chemicals evaluated. It is well established that different plant species frequently exhibit
significant variations in their germination responses to exogenously imposed chemical stimulants (Bewley, 1997).

Differences detected between the storage response of SB 1 and SB 2 at the initiation (month 0) are ascribed to the duration that SB 1 was stored before use in the experiment. Seed batch 1 was stored in a 10°C refrigerator for 4 months before evaluation of storage variables on dormancy relief, compared to SB 2 which was used 2 weeks after harvest. The storage differences before experimentation between seed batches can account for an elevated level of dormancy relief in SB 1. Ideally, studies on factors impacting seed dormancy relief should be conducted on seeds collected from the field as soon after harvest as possible. The wild stands of *P. torridum* that were the source of seeds for these studies, were located in remote areas of Molokai, making timings of seed collection and the initiation of experiments very challenging. It has also been reported that depth of dormancy can be affected by time of year harvesting of *H. contortus* seeds (Baldos et al., 2014). Both *P. torridum* seed batches used in this research were harvested in May, but were separated by one year, thus the unrecorded environmental conditions at the collection sites could account for differences in the observed depth of dormancy (Andersson and Milberg, 1998).

When utilizing saturated salts for humidity regulation, it is recommended that salt and water proportions are calibrated before desiccation of seeds. This research supports the addition of 6 ml distilled water to 20 grams of LiCl (0.3 ml of distilled water added to each gram of LiCl) to provide the reduction to and maintenance of the target 12% RH for 11 grams of *P. torridum* seed in a 1700 cm³ desiccator. Calibration methods discussed in this paper can be up-scaled for seed desiccation in a larger capacity chamber. A practitioner can calculate the amount of saturated
salts needed to desiccate greater amounts of seed within an airtight commercially available vessel such a sealable plastic bucket or insulated beverage cooler.

Seed viability of *P. torridum* remained stable over the duration of the experiment when equilibrated to 12% RH, however as RH increased (50% and 75%) seed viability decreased. Ultimate seed survival is directly related to the time the seed has been exposed to unfavorable conditions of temperature or humidity (Barton, 1961; Harrington, 1972; Roberts, 1972). Storage parameters can greatly impact seeds both in terms of relief of dormancy and persistence over time (Priestley, 1986).

In summary, the research indicated that exogenous chemical treatments did not relieve *P. torridum* dormancy; however, seed germination was promoted to a maximum of 55% germination by storage maintained at 12% RH at 30°C for a duration of 8 months. *P. torridum* seeds can maintain viability for up to 10 months when a storage RH of 12% is imposed and consistently maintained.
Tables and Figures

**P. torridum** germination response to exogenous chemicals

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Rate</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>0.3 M</td>
<td>Tischler and Young, 1983</td>
</tr>
<tr>
<td>Hydrogen Peroxide</td>
<td>20 mM</td>
<td>Sarath and Mitchell, 2008</td>
</tr>
<tr>
<td>GA³</td>
<td>100 ppm</td>
<td>Kirmizi et al., 2010</td>
</tr>
<tr>
<td></td>
<td>200 ppm</td>
<td></td>
</tr>
<tr>
<td>GA⁴⁺⁷</td>
<td>50 uM</td>
<td>Hilhorst, 2011</td>
</tr>
<tr>
<td></td>
<td>100 uM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 uM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>400 uM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>800 uM</td>
<td></td>
</tr>
<tr>
<td>Liquid smoke</td>
<td>1%</td>
<td>Baldos, personal communication, 2013</td>
</tr>
<tr>
<td>Potassium cyanide</td>
<td>0.5 mM</td>
<td>Bethke et al., 2011</td>
</tr>
<tr>
<td>Fluridone</td>
<td>10 uM</td>
<td>Grappin et al., 2000</td>
</tr>
<tr>
<td></td>
<td>20 uM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40 uM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>80 uM</td>
<td></td>
</tr>
<tr>
<td>Ethephon</td>
<td>0.5 mM</td>
<td>KeÇpczyński and KeÇpczyńska, 1997</td>
</tr>
<tr>
<td></td>
<td>1.0 mM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0 mM</td>
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</tr>
<tr>
<td></td>
<td>4.0 mM</td>
<td></td>
</tr>
<tr>
<td>SNP</td>
<td>50 uM</td>
<td>Bethke et al., 2011</td>
</tr>
<tr>
<td></td>
<td>100 uM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>150 uM</td>
<td></td>
</tr>
<tr>
<td></td>
<td>200 uM</td>
<td></td>
</tr>
<tr>
<td>Distilled water</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.1. List of exogenous chemicals, rates and respective citations tested to relieve dormancy in *P. torridum* seeds

**P. torridum** seed weight increase over 24 hours of water imbibition

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Weight increase (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.00 C</td>
</tr>
<tr>
<td>1</td>
<td>5.21 B</td>
</tr>
<tr>
<td>2</td>
<td>8.14 B</td>
</tr>
<tr>
<td>4</td>
<td>15.64 A</td>
</tr>
<tr>
<td>12</td>
<td>14.66 A</td>
</tr>
<tr>
<td>24</td>
<td>15.31 A</td>
</tr>
</tbody>
</table>

Table 5.2. *Panicum torridum* seed percent mass increase over a 24 hour distilled water imbibition period. Means are separated using Tukey's HSD comparison at *P*=0.05. Means within columns followed by the same letter are not significantly different.
Table 5.3. Desiccation chamber relative humidity calibration using saturated lithium chloride. The optimal RH level of 12.5% was obtained by adding 6 ml distilled water to 20 grams lithium chloride in a 1700cm³ desiccation chamber.

<table>
<thead>
<tr>
<th>Water added (ml)</th>
<th>RH level achieved (%)</th>
<th>Days to RH equilibration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>13.0</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>13.0</td>
<td>6</td>
</tr>
<tr>
<td>6</td>
<td>12.5</td>
<td>6</td>
</tr>
<tr>
<td>12</td>
<td>13.5</td>
<td>5</td>
</tr>
<tr>
<td>24</td>
<td>26.0</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 5.4. Panicum torridum germination means representing dormancy relief based on varying levels of RH and temperature over a ten month storage duration. Optimum germination was found in seeds exposed to 12% RH at 30°C for 8 months. Means are separated using Tukey’s HSD comparison at P=0.05. Means within columns and rows followed by the same letter are not significantly different.
Figure 5.1. Seed dormancy relief through germination of *P. torridum* exposed to varying levels of RH and temperature over a ten month storage duration. Optimum germination was found in seeds exposed to 12% RH at 30°C for 8 months. Data are pooled over two seed batches and presented with standard error bars.
Figure 5.2. Seed viability maintenance of *P. torridum* seeds exposed to varying levels of RH and temperature over a ten month storage duration. Optimum viability was maintained within the 12% RH, greatest losses were discovered at 75% RH at 30°C over the ten month experiment. Data is represented over two seed batches and plotted with standard error bars.
References


Baldos, O.C. (2013) Seed dormancy, smoke-stimulated germination and harvest timing of pili grass (Heteropogon contortus), a native Hawaiian grass with potential for expanded re-vegetation use. UNIVERSITY OF HAWAI‘I AT MANOA.


Chapter 6

Weed control efficacy and plant response of transplanted *Panicum torridum* in the presence of two pre-emergence herbicides applied at the time of planting.

Abstract

State of Hawaii initiatives are calling for increased usage of native plants, especially along roadway corridors. *Panicum torridum*, a native Hawaiian annual grass has recently been identified as a candidate species for development of establishment protocols related to the production of seed along roadway rights-of-way. In order to produce a native seed crop and ensure seed lot purity, establishment protocols for weed control must first be defined. To accomplish this, the utilization of pre-emergence chemicals may be necessary. However, the determination of the type of herbicide and the rate used need to provide a useful level of weed control while minimizing phytotoxic effects on the crop species. This study reports the response of weeds and *P. torridum* phytotoxicity to granular oxadiazon applied at 2.24 and 4.48 kg ai ha\(^{-1}\) and spray applications of indaziflam applied at 24 and 49 g ai ha\(^{-1}\) at the time of transplanting. Oxadiazon applied at 4.48 kg ai ha\(^{-1}\) provided the highest level of weed control with little to know impact on the *P. torridum* growth response. Indaziflam provided less weed suppression than oxadiazon and imposed phytotoxic effects that proved to be excessive to *P. torridum* transplants.
Introduction

In the State of Hawaii, initiatives are calling for planting of native species along Department of Transportation roadway corridors (Tamimi 1999). To comply with federal presidential acts, the State of Hawaii initiated the Statewide Noxious Invasive Pest Program (SNIPP) (Anonymous 2011). One aspect of the SNIPP plan specifically calls for the re-vegetation of native species along roadway corridors. Aside from the SNIPP plan, statutory regulations of the Clean Water Act, National Pollutant Discharge Elimination System (NPDES) permits and associated storm water pollution prevention plans for roadways on Oahu integrate the planting of native species as a best management practice (BMP) to realize reduced inputs for roadside vegetation management (Anonymous 2007). In Hawaii, the current demand for native plants outweighs the availability of plant materials or seed (Ricordi et al. 2014). In order to produce seed stock and ensure seed lot purity from weed seeds, establishment protocols for weed control must first be defined.

Weed interference is the primary constraint to successful establishment of native plants (Masters et al. 1996). Herbicides are an important tool for suppressing weed competition during initial seedling growth and removing exotic species in native plant communities (Bahm and Barnes 2011). The heavy and rapid establishment of weeds when irrigation and supplemental nutrients are applied to production sites highlights the need for pre-emergence herbicides as a fundamental crop establishment protocol. Utilizing pre-emergent herbicides to reduce weed competition is a well-established method in agronomy. The aim of weed management is shifting from complete weed control, to reducing weeds to an acceptable threshold level within the production site (Boström and Fogelfors 2002). However, the cultivation of native plant species for seed requires more complete weed suppression to enhance harvest efficiency and minimize weed seed contamination (Grilz and Romo 1995; Hitchmough et al. 1994; Tjelmeland et al. 2008).
Panicum torridum Gaudich., a native Hawaiian annual grass has recently been identified as a candidate species for development of establishment protocols that seek to make use of roadway rights-of-way for seed production. However, in Hawaii, limited research has been conducted to facilitate the expanded usage of native species. The determination of seedling establishment protocols needs to be created to improve the efficiency of practitioners conducting planting and maintenance procedures. A fundamental aspect of crop production is the control of aggressive weedy species during early establishment. Pre-emergent herbicides have proven effective in suppressing weeds in plantings of native Hawaiian plants and a multitude of other crops (Baldos et al. 2010; Brecke et al. 2010; Pimentel et al. 1978).

Violations of state and federal pesticide laws are a concern when utilizing crop protection chemicals on native plants produced on sites not listed on the commercial product label. A very limited number of herbicide labels contain a use pattern that allows application to specific native plants (Smith and Whalley 2002). Utilizing roadway rights-of-ways as a site for growing native plants allows for legal use of any pre or post-emergence herbicides that lists this site on the product label. Many modern herbicides have product labels that list “rights-of-way” or “non-crop area” as legal sites of application. When native Hawaiian plants are used in roadside landscapes or grown on roadsides for seed production, herbicides labeled for use on rights-of-way can be applied during establishment and for maintaining weed free status without violating label instructions. Roadside rights-of-way represent a special area for growing native plants due to the wide range of chemical tools available for weed control during establishment, growth and the seed production cycle. Additionally, native plants grown on roadside rights-of-way cannot be considered a crop because they have no retail value either as landscape stock, seed crop or
animal feed. Roadside rights-of-way are truly a non-crop area due to the lack of economic motive for producing plants and seeds in these areas.

Important ecological benefits accompany roadside use of native plants. Roadside environments provide a distinctive habitat often supporting weedy and invasive plant species and pose a threat to the integrity of natural communities (Gelbard and Belnap 2003). It is well accepted that roadsides function as primary dispersion corridors for exotic plant dispersal (Brothers 1992; Mortensen et al. 2009). The displacement of weedy species with native plants along distribution corridors offers the opportunity to spread native species using the same biotic and abiotic agents employed by invasive weeds.

Previous work conducted on weed control in native Hawaiian plant species indicates that establishment success is greatly enhanced when pre-emergence herbicides are applied over the top of transplanted plants. In the native Hawaiian grass *Sporobolus virginicus*, transplants treated with granular oxadiazon at 4.48 kg ai ha⁻¹ produced the highest aboveground dry biomass accumulation, while maintaining weed control throughout the duration of the 38 day experiment (Baldos et al. 2010). Similarly, newly transplanted *Fimbristylis cymosa* (a native Hawaiian sedge) was tolerant to applications of oxadiazon, while maintaining acceptable levels of weed control (Baldos et al. 2012). Oxadiazon has also been shown to be safe and effective in a variety of container grown ornamental grasses when applied at labeled rates (Neal and Senesac 1991). Research on a variety of native Hawaiian species supports the evaluation of oxadiazon in the development of establishment protocols that require safe and effective weed control agents when using transplants.

Another pre-emergent herbicide, indaziflam, was recently introduced in 2010 to control grasses and broadleaf weeds in turf (Shaner 2014). Indaziflam is an alkylazine herbicide that inhibits
cellulose biosynthesis in susceptible species (Anonymous 2010; Myers et al. 2009), and has approximately 10 to 15 times lower use rates than most pre-emergence herbicides (Gómez de Barreda et al. 2013). Safe use of indaziflam has been reported for bermuda grass, centipede grass, St. Augustine grass and zoysia grass (Brosnan et al. 2011; Gómez de Barreda et al. 2013). Indaziflam has been shown to provide longer residual weed control at lower application rates than many industry standards (Perry et al. 2011).

The intent of this research is to determine the response *P. torridum* transplants and weeds to granular oxadiazon applied at 2.24 and 4.48 kg ai ha$^{-1}$ and spray applications of indaziflam applied at 24 and 49 g ai ha$^{-1}$.

**Materials and Methods**

*Plant material*

*Panicum torridum* is an endemic grass in Hawaii, ranging from 10 to 60 cm in height with velvety puberulant leaves. Distribution studies conducted in 1942 indicated that *P. torridum* is found on all Hawaiian Islands with sporadic distribution (Ripperton and Hosaka 1942). In 1992, *P. torridum* was found to be a dominant species along the summit ridge of the Lehua islet on the remote island NiiHau (Evenhuis and Eldredge 2006). In Hawaii, annual grasses such as *P. torridum* normally emerge in months of April to May following the rainy season that lasts from November to January (Sakamoto, personal communication). *P. torridum* is found below 90 meters elevation in zones that receive 50-100 cm of rainfall per year (Ripperton and Hosaka 1942).

In this research, *P. torridum* seeds were sown 40 days before the time of planting and experimental treatment application (2/21/2014 for the first experiment and 5/23/2014 for the
second experimental repeat). Seeds were sown in Sunshine mix #4 with mycorrhizae (Sun Gro Horticulture®, Agawam, Massachusetts) in 38 cell seedling trays (Landmark Plastic®, Akron, Ohio). Fertilization and irrigation were provided as needed throughout the transplant production period. The herbicide tolerance experiment was conducted twice with starting dates of 04/02/2014 and 07/02/2014, maintaining similar experimental parameters for seedling preparation and planting procedures.

Experimental plan

Planting of transplants and treatment applications were conducted on the first experiment in April 2014 and the replicate experiment in July 2014 in a field adjacent to the first at the University of Hawaii at Manoa, Magoon Research facility on Oahu.

Before planting, the soil was rototilled to incorporate 112 kg nitrogen acre⁻¹ (formulation 21-4-7) to a depth of 4 inches in both experimental fields. Soil type on both of the experimental fields was Makiki stony clay loam (isohyperthermic typic haplustepts). Soil nutrient analysis was conducted at the completion of both experiments by the University of Hawaii agricultural diagnostic service center. The first experimental field contained 221 ppm phosphorus (P), 1203 ppm potassium (K), 5056 ppm calcium (Ca), 1468 magnesium (Mg), 0.30% nitrogen (N) with a 7.2 pH. The second experimental field contained 201 ppm P, 803 ppm K, 8199 ppm Ca, 986 ppm Mg, 0.14% N with a 7.4 pH.

The experimental design was a randomized complete block with four chemical treatments and one untreated plot with four replications. Four P. torridum transplants were used in each 0.65 m × 1.5 m (1 m²) experimental plot. Chemical treatments consisted of granular oxadiazon (Ronstar® G, Bayer CropScience, Research Triangle Park, NC) and flowable indaziflam (Specticle® FLO, Bayer CropScience, Research Triangle Park, NC) applied over the top of
transplanted *P. torridum* at 2.24 & 4.48 kg ai ha\(^{-1}\) and 24.0 & 49.0 g ai ha\(^{-1}\), respectively.

Granular oxadiazon was applied in pre-weighed aliquots by hand to individual plots to ensure uniform application. Spray treatments of indaziflam were applied using a compressed carbon dioxide gas sprayer operating at 241 kPa, outfitted with a single Teejet\(^{®}\) (TeeJet Technologies, Wheaton, Illinois) 9095 EVS (even spray pattern) nozzle calibrated to deliver 375 l ha\(^{-1}\).

After herbicide application, overhead irrigation was applied for 10 minutes to activate the chemicals. Trial one and two irrigation volumes were measured to be 1612 L ha\(^{-1}\) min\(^{-1}\) and 1520 L ha\(^{-1}\) min\(^{-1}\), respectively (these rates were maintained for all irrigation applications). Starting on day 2, overhead sprinkler irrigation was delivered two times per day at 6:30am and 12:00pm for 15 minutes at each start time for the first trial. Moisture levels of the first trial were determined to be too high based on visual assessment so the second trial irrigation was reduced to 10 minutes each start time for the first 6 days then reduced to one start time at 6:00am for ten minutes on Monday, Wednesday and Friday.

**Data collection**

During the first trial, four predominate weeds were present within the experimental plots, which were assessed individually for percent control at 45 DAP. Trial one weed species present were *Commelina diffusa* (spreading dayflower, honohono), *Portulaca oleracea* (common purslane), *Euphorbia hypericifolia* (graceful spurge) and *Eleusine indica* (goosegrass). During the second trial only three predominate weeds were present, *Ipomoea triloba* (morning glory), *Digitaria sanguinalis* (hairy crabgrass) and *Eleusine indica* (goosegrass).

Data were collected at three time points at 15 day intervals starting at 15 days after planting (DAP), 30 DAP and 45 DAP. The first (15 DAP) and second (30 DAP) data collection points consisted of visual ratings of *P. torridum* percent of maximum growth vigor ratings. Percent of
maximum vigor ratings of *P. torridum* were based on the author’s familiarity of uninhibited plants grown as nursery stock and personal observations of wild plants. The third (45 DAP) and final data collection point consisted of *P. torridum* percent of maximum vigor ratings, *P. torridum* dried plant biomass (g/4 plants) accumulation, timed removal of weeds from experimental plots (s/m²), dried weed biomass (g/m²), and percent control of individual weed species present. The second replicate trial was conducted with all data collection times and ratings consistent with the first trial.

*Data analysis*

Data were analyzed in the statistical analysis program Statistix™ 10.0 (Analytical Software, Tallahassee, Florida). Separate analyses were conducted for *P. torridum* percent of maximum vigor for each data collection time and were analyzed over both trials as a split plot with experimental trial as the main effect and herbicide treatment as the split plot effect. *P. torridum* dry aboveground biomass accumulation in the third data collection period (45 DAP) was analyzed over both trials as a split plot with experimental trial as the main effect and treatment as the split plot effect. If significant trial × treatment interactions were detected, data from the two trials were combined (Brosnan et al. 2011).

Due to different weed species present in trials one and two, all weed response data were analyzed individually based on trial. Weed response data were analyzed using a randomized complete block design analysis of variance in the statistical analysis program Statistix™ 10.0. Individual species percent control data in both trials were square root transformed to conform to the assumptions of the ANOVA (Ahrens et al. 1990). When significant effects were detected, means were separated using Tukey’s all pairwise HSD test at α= 0.05.
Results and Discussion

Data collection 1 (15 DAP)

The analysis for percent of maximum growth vigor of *P. torridum* did not indicate a significant interaction between the factors of experimental trial × treatment (P=0.100), thus results will be pooled over trial. Significant treatment effects were detected (P=0.007), with the highest vigor ratings found in the untreated plots (97%), followed by both low and high indaziflam rates and the high rate of oxadiazon (96%, 96% and 94%, respectively). The lowest *P. torridum* vigor rating was detected in the low rate of oxadiazon (Table 1).

Data collection 2 (30 DAP)

The analysis for percent of maximum growth vigor of *P. torridum* did not indicate a significant interaction between the factors of experimental trial × treatment (P=0.367), thus results will be pooled over trial. A significant treatment effect on vigor ratings was detected (P=.010) (Table 1). The highest vigor was found with granular oxadiazon at the low rate (99%), followed by the high rate and the untreated control (96% and 95% respectively). A significant reduction in percent of maximum vigor was recorded for the both levels of indaziflam.

Data collection 3 (45 DAP)

Results of the analysis for *P. torridum* percent of maximum vigor did not indicate an interaction between the factors of experimental trial × treatment (P= 0.074), thus results will be pooled over trials. A significant treatment effect on plant vigor was detected (P<0.001). The significantly highest percent of maximum vigor was found with the low rate of oxadiazon (91%) followed by the high rate of oxadiazon (90%) (Table 1) (Figure 1). The lowest level of percent of maximum vigor was recorded with the high rate of indaziflam and the untreated control (71% and 77%
respectively). The decreased *P. torridum* percent of maximum vigor in the untreated plots was attributed to weed competition.

The results of the analysis for *P. torridum* plant biomass did not indicate a significant interaction between the factors of experimental trial × treatment (*P* = 0.578), thus treatment means will be pooled over trials. A significant treatment effect on *P. torridum* biomass was detected (*P* < 0.001), with the significantly greatest biomass found with the high rate of oxadiazon (Table 3). The lowest level of *P. torridum* biomass was recorded with the high rate of indaziflam and the untreated control.

During the first trial, the analysis indicated a significant effect of treatment on weeding times (*P* < 0.001). Weeding times were significantly lower in all herbicide treated plots compared to the untreated control (Table 3). In herbicide treated plots, numerically lowest weeding times were found in the high rate of the oxadiazon treatment (6 seconds) compared to the highest weeding times found with the low rate of indaziflam (50 seconds). During the second trial, the analysis indicated a significant effect of treatment on weeding times (*P* < 0.001). Significantly lower weeding times were found in all herbicide treated plots compared to the untreated control (Table 3). In herbicide treated plots, numerically lowest weeding times were found in the high rate of the oxadiazon treatment (3 seconds) compared to the highest weeding times found with the low rate of indaziflam (21 seconds).

During the first trial, the results of the analysis indicated a significant effect of treatment on weed biomass (*P* < 0.001). Weed biomass between the untreated control and the low rate of indaziflam were not significantly different, but were significantly greater than the high rate of indaziflam and both rates of oxadiazon (Table 3). In herbicide treated plots, numerically lowest weed biomass was found in the high rate of the oxadiazon treatment (4 g/m²) compared to the highest
weed biomass found with the low rate of indaziflam (65 g/m²). During the second trial, the results of the analysis indicated a significant effect of treatment on weed biomass (P= 0.002). Weed biomass between the untreated control and the low rate of indaziflam were not significantly different, but were significantly greater than the high rate of indaziflam and both rates of oxadiazon (Table 3). In herbicide treated plots, numerically lowest weed biomass was found in the high rate of the oxadiazon treatment (6 g/m²) compared to the highest weed biomass found with the low rate of indaziflam (114 g/m²). Morning glory was present during this trial and was not adequately controlled with the low rate of indaziflam (Figure 1).

During the first trial, spreading dayflower control was considered acceptable with both herbicides applied at the high rate (Table 2). The low rate of indaziflam did not differ from the untreated plots, indicating a lack of commercially acceptable control. Common purslane was well controlled by all herbicide treatments, except in the low rate of indaziflam which did not provide commercially acceptable control. Graceful spurge was well controlled by all herbicide treatments, with oxadiazon at the high rate providing 100% control. Goosegrass was well controlled by all herbicide treatments. During the second trial, morning glory emergence was variable throughout the experimental area with unacceptable control provided by the low rate of indaziflam (Table 2). Oxadiazon at both rates provided the highest level of morning glory control. Total (100%) control of goosegrass was recorded for all herbicide treatments. Hairy crabgrass was very well controlled by all herbicide treatments.

In both experiments broadleaf weed control was consistently higher with oxadiazon than indaziflam. Both grass species (goosegrass and large crabgrass) present during both experiments were well controlled by both herbicides at rates of application recommended by the product label. Reported literature confirms indaziflam is very effective in controlling grass species such
as *Digitatia ischaemum* (smooth crabgrass) and *Poa annua* (annual bluegrass) (Brosnan et al. 2011; Hunter Perry et al. 2011).

The results of the three rating periods on weed and *P. torridum* response indicate that granular oxadiazon applied at 4.48 kg ai ha\(^{-1}\) provides the greatest weed control while minimizing phytotoxic effects. Although the *P. torridum* percent of maximum vigor ratings appeared to be greater during the last rating period with the low rate of oxadiazon, biomass accumulation was greatest with the high level of oxadiazon. The low *P. torridum* biomass and vigor found in the untreated controls was attributed to high weed competition, highlighting the need for pre-emergent weed control.
### Tables and Figures

#### Table 6.1: *P. torridum* percent of maximum vigor response to the pre-emergence herbicides oxadiazon and indaziflam applied at low and high label rates. Means are separated using Tukey’s HSD comparison at P=0.05. Means within columns followed by the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Rate (kg ai ha(^{-1}))</th>
<th>15 DAP</th>
<th>30 DAP</th>
<th>45 DAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxadiazon</td>
<td>2.24</td>
<td>93 B</td>
<td>99 A</td>
<td>91 A</td>
</tr>
<tr>
<td></td>
<td>4.48</td>
<td>94 AB</td>
<td>96 AB</td>
<td>90 AB</td>
</tr>
<tr>
<td>Indaziflam</td>
<td>0.024</td>
<td>96 AB</td>
<td>93 B</td>
<td>79 BC</td>
</tr>
<tr>
<td></td>
<td>0.049</td>
<td>96 AB</td>
<td>92 B</td>
<td>71 C</td>
</tr>
<tr>
<td>Untreated</td>
<td>-</td>
<td>97 A</td>
<td>95 AB</td>
<td>77 C</td>
</tr>
</tbody>
</table>

#### Table 6.2: Percent control of individual weed species present in trials one and two at 45 DAP. Means are separated using Tukey’s HSD comparison at P=0.05. Means within columns followed by the same letter are not significantly different.

#### Trial one

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percent of weed species controlled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical</td>
<td>Rate (kg ai ha(^{-1}))</td>
</tr>
<tr>
<td>Oxadiazon</td>
<td>2.24</td>
</tr>
<tr>
<td></td>
<td>4.48</td>
</tr>
<tr>
<td>Indaziflam</td>
<td>0.024</td>
</tr>
<tr>
<td></td>
<td>0.049</td>
</tr>
<tr>
<td>Untreated</td>
<td>-</td>
</tr>
</tbody>
</table>

#### Trial two

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Rate (kg ai ha(^{-1}))</th>
<th>Morning glory</th>
<th>Goosegrass</th>
<th>Hairy crabgrass</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxadiazon</td>
<td>2.24</td>
<td>80 AB</td>
<td>100 A</td>
<td>97 A</td>
</tr>
<tr>
<td></td>
<td>4.48</td>
<td>87 A</td>
<td>100 A</td>
<td>97 A</td>
</tr>
<tr>
<td>Indaziflam</td>
<td>0.024</td>
<td>33 BC</td>
<td>100 A</td>
<td>100 A</td>
</tr>
<tr>
<td></td>
<td>0.049</td>
<td>71 AB</td>
<td>100 A</td>
<td>100 A</td>
</tr>
<tr>
<td>Untreated</td>
<td>-</td>
<td>0 C</td>
<td>0 B</td>
<td>0 B</td>
</tr>
</tbody>
</table>
Treatment | Weed response | P. torridum response
---|---|---
**Chemical** | **Rate (kg ai ha⁻¹)** | **Weed Free Time (s/m²)** | **Weed biomass (g/m²)** | **Dry biomass (g)**
| | **Trial 1** | **Trial 2** | **Trial 1** | **Trial 2** | |
Oxadiazon | 2.24 | 26 A | 12 A | 16 A | 13 A | 97 AB |
| | 4.48 | 6 A | 3 A | 4 A | 6 A | 99 A |
Indaziflam | 0.024 | 50 A | 21 A | 65 AB | 114 AB | 92 AB |
| | 0.049 | 15 A | 6 A | 9 A | 26 A | 77 BC |
Untreated | - | 147 B | 136 B | 116 B | 250 B | 63 C |

Table 6.3. Weed and P. torridum response to the pre-emergence herbicides oxadiazon and indaziflam applied at low and high label rates at 45 DAP. Means are separated using Tukey’s HSD comparison at \( P=0.05 \). Means within columns followed by the same letter are not significantly different.

Figure 6.1. Response of weeds and P. torridum to low and high label application rates of oxadiazon and indaziflam at 45 DAP. The low rate of indaziflam allowed for plots to be heavily invaded with Morning glory.
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Chapter 7

Determination of optimal seed harvest timing for *Panicum torridum* based on growing degree day heat unit accumulation.

Abstract

The native Hawaiian annual grass *Panicum torridum* has been selected for development of seed production protocols to aid in re-vegetation efforts throughout Hawaii. The determination of harvest timing is an important factor needed to optimize mature seed yield. Numerous studies have detailed the difficulties associated with rapid seed shattering in grass seed production systems, as seen in *P. torridum*. The utilization of heat accumulation units as growing degree days (GDD’s) can be used to characterize mature seed development based on thermal time, thus providing a specific quantifiable harvest time to maximize seed yields. The objective of this study is to optimize mature seed harvest timing of *P. torridum* by creating a quantifiable heat accumulative unit (GDD) indicator value. The results indicate that maximize seed yield occurs when accumulated GDD values after anthesis initiation are at 249 and 259 for August and January planting periods in Hawaii, respectively. If the calculation of GDD’s are not practical, the recommended harvest time for maximize seed yield in *P. torridum* is 9 days after flower anthesis with August plantings and 12 days with a January planting in Hawaii.
Introduction

A critical stage in the production of agricultural crops is the correct timing of harvest to maximize yield and quality (Bednarz et al., 2002; Copeland, 1995; Russo, 1996). For seed crop production, ideally all seeds would mature uniformly at the same location on the plant and be ready for harvest at the same time, however this seldom happens (McDonald and Copeland, 1997). Panicum torridum Gaudich., is a native Hawaiian annual grass that has been selected for development of seed production protocols to aid in early-stage re-vegetation efforts throughout Hawaii. However, parameters for seed production are unknown. P. torridum seed appears to mature rapidly in an indeterminate pattern. Indeterminate maturation causes shedding of mature seeds on one portion of the inflorescence while other regions remain immature (McDonald and Copeland, 1997). In such cases, the timing of harvest is a compromise to enable the greatest yields of high quality mature seed while minimizing seed loss to shattering (Garcia-Diaz and Steiner, 2000). Numerous studies have detailed the difficulties associated with rapid seed shattering in grass seed production systems (Hides, 1987; Wang et al., 2006). In many grass species, seed harvesting is initiated after initial shedding begins, but delays in harvesting results in substantial seed losses (Pegler, 1976). There are many factors that are known to influence flowering time in angiosperms, foremost of these are length of day (photoperiod) and temperature (Blázquez et al., 2003; Ellis et al., 1997; Karsai et al., 2008). The influences of flowering based on temperature and photoperiod relate to the accumulation of heat units. The degree day heat accumulation unit was developed by Réaumur in 1735 to describe the relationship between plant morphological development rate and temperature (Bonhomme, 2000). Plant growth and development have a closer correlation to thermal time.
than chronological time (SEVERINO and AULD, 2014). The degree day unit or growing degree day (GDD) approach is a method widely used for quantification of thermal time (SHRESTHA et al., 2010). It is based on the assumption that growth ceases below a given temperature (base temperature), and that growth increases linearly in response to incremental temperature increases (YANG et al., 1995). Relationships between GDD and rates of morphological development have been used to schedule management of warm season grasses (MADAKADZE et al., 2003; MULLAHEY et al., 1990; SANDERSON and WOLF, 1995). GDD’s have been successfully used to characterize seed harvest production timing in the native Hawaiian grass Heteropogon contortus (BALDOS, 2013).

It has been found that the linearity between development rate and temperature is only valuable for a relatively limited range of temperatures (BONHOMME, 2000), thus highlighting the importance of the base temperature (YANG et al., 1995). For tropical Panicum grasses little or no growth is expected when temperatures are below 15°C, this threshold value is generally accepted as the base temperature for other tropical species (BALDOS, 2013; MCWILLIAM, 1978; MORENO et al., 2014).

Understanding more precise timing methods for the optimization of seed harvesting for P. torridum could enhance production capacity and minimize seed losses, which are especially important due to the rarity of this endemic species. The objective of this study is to optimize mature seed harvest timing of P. torridum by creating a quantifiable heat accumulative unit (GDD) indicator.
Materials and Methods

Plant material

*Panicum torridum* is an endemic grass in Hawaii, ranging from 10 to 60 cm in height with velvety puberulant leaves. Distribution studies conducted in the year 1942 indicated that *P. torridum* is found on all Hawaiian Islands with sporadic distribution (RIPPERTON and HOSAKA, 1942). In 1992, *P. torridum* was found it to be a dominant species along the summit ridge of the Lehua islet on the remote island Niihau (EVENHUIS and ELDREDGE, 2006). In Hawaii, annual grasses such as *P. torridum* normally emerge in months of April to May following the rainy season that lasts from November to January (Sakamoto, personal communication). *P. torridum* is found below 90 meters elevation in zones that receive 50-100 cm of rainfall per year (RIPPERTON and HOSAKA, 1942).

In this research, *P. torridum* seeds were sown in Sunshine mix #4 with mycorrhizae (Sun Gro Horticulture®, Agawam, Massachusetts) in 38 cell seedling trays (Landmark Plastic®, Akron, Ohio). Seedlings were identified as suitable for transplantation when root balls remained intact when extracted from seedling trays. This growth stage corresponded to 55 days (trial 1-August planting) and 75 days (trial 2-January planting) after direct seeding into tray cells. Fertilization and irrigation were provided as needed throughout the pre-transplantation period to maximize growth.

Experimental design

The experiment was designed as a randomized complete block with four replications. Two replicate experimental trials were conducted in the same experimental plots separated temporally by six months. Trials one and two were initiated in August 2014 and January 2015, respectively. Each trial replicate consisted of a separate raised 3 × 3 meter planting bed, filled with Tropical...
Blend compost (Hawaiian Earth Products™, Kapolei, Hawaii) with bed borders provided by 5 cm diameter white PVC pipe. Tropical Blend compost is composed of 33% compost, 33% 5/8” black cinder, 33% screened soil and 11-52-0 fertilizer, with a pH of 7.76 (HAWAIIAN EARTH PRODUCTS, 2013). *P. torridum* plants were planted in rows with a between row and in row spacing of 0.30 m with border plants excluded from data sampling. Overhead irrigation was provided two times daily at 5:00 am and 12:00 pm for a 15 minute duration at each start time. Trial one and two irrigation volumes were measured to be 2100 L ha\(^{-1}\) min\(^{-1}\) and 2000 L ha\(^{-1}\) min\(^{-1}\), respectively (these rates were maintained for all irrigation applications). During the second trial, cooler ambient temperatures imposed less moisture demand resulting in reduced irrigation times from 15 to 12 minutes. Experimental plots were amended with 56 kg nitrogen ha\(^{-1}\) (formulation 21-4-7) 7 days before planting in both experimental trials. Previous research indicates the pre-emergence herbicide oxadiazon in a granular form applied at 4.48 kg ai ha\(^{-1}\) can be safely applied to *P. torridum* transplants without a reduction in biomass. In order to suppress weed growth during both experimental trials, oxadiazon at 4.48 kg ai ha\(^{-1}\) was applied to the soil 3 days after planting. Before the initiation of the trial two, a seed germination bioassay using Bermuda grass seeds confirmed that no residual herbicide had persisted from the previous experimental run.

**Data collection**

In both trials, data collection did not commence until 50% of the *P. torridum* population had initiated anthesis (Figure 1). Data collection consisted of sampling *P. torridum* seed heads at 3 day intervals beginning at the anthesis point. At each sample time, three dominate seed heads were removed from one randomly selected *P. torridum* plant in each of the four replications. Dominate seed heads consisted of the largest and most developed seed heads on each plant at the
given sample interval. Sampling continued until P. torridum seeds were fully shed from the inflorescence, at which point data collection ceased. During both trials, 11 samples were taken in each replication, spanning a sampling period of 33 days. At each sampling time, seed heads were individually bagged for each plant and assessed for moisture content following constant oven moisture analysis techniques outlined by the International Seed Testing Association (ISTA) (ISTA, 2003).

Seed cleaning

At the end of the data collection period, seed heads (groups of 3 sampled) were processed to remove seeds from the inflorescence. Seeds were extracted and separated from dried pulverized flower head tissues using two devices specifically designed for this purpose. The first step made use of a Westrup® LA-H brush machine fitted with a #14 mantle (1.0 x 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, seeds are allowed to push through the cylinder and larger plant parts are retained within the cylinder (figure 2). The brush machine was run at full power for 1 minute with the front discharge door closed. In the second device, seeds were separated from pulverized seed head components with a Clipper™ Office Tester fitted with a 0.927 x 0.927 mm wire mesh top screen and a solid sheet bottom screen (A.T. Ferrell Company Inc., Bluffton, Indiana) (Figure 3). The seed separator blower air ducts were open at 25% capacity and was run until all material traveled past the screen sifters. Cleaned seeds were weighed to assess grams of seed recovered from each plant at each time interval.
Growing degree day equation

Cumulative GDD’s during each trial were calculated by using daily minimum and maximum temperature (°C). Temperature measurements were recorded from the center of the experimental plots using a Hobo® Pro V2 logger (Onset®, Cape Cod, Massachusetts). GDD values were calculated using the following equation:

\[ GDD \text{ daily} = \frac{T_{max} + T_{min}}{2} - T_{base} \]

where

\[ \sum GDD \text{ daily} = \text{cumulative GDD} \]

where \( T_{max} \) is the maximum daily temperature, \( T_{min} \) is the minimum daily temperature, and \( T_{base} \) is the base temperature where \( P. \) torridum growth and development is deemed not to occur (Lawson et al., 2009; McMaster and Wilhelm, 1997; Shrestha et al., 2010). In this study a \( T_{base} \) of 15°C was utilized based on similar reported developmental parameters of native Hawaiian \( H. \) contortus grass, tropical Panicum grass species and tropical pasture grasses. (Baldos, 2013; McWilliam, 1978; Moreno et al., 2014).

Data analysis

Seed yield data were analyzed over both experimental trials using a split plot analysis of variance, with experimental trial as the main effect and sample time as the split plot effect. If a significant interaction between the factors of experimental trial \( \times \) sample interval are detected, seed yield means will be presented separately over trials. Significant effects of seed yield over sample times will be separated using Tukey’s HSD test at \( \alpha = 0.05 \). The analysis of variance was conducted using the statistical software program Statistix™ 10.0 (Analytical Software, Tallahassee, Florida).
Seed yield data were plotted against the cumulative GDD for each experimental trial to produce parametric equations and to determine the local maximum inflection point corresponding to maximum seed yield and GDD accumulation. Predictive equations and parameter estimates of the polynomial regression curves were plotted using statistical program JMP® Pro 11 (SAS Institute Inc., Cary, NC).

**Results and Discussion**

The results of the analysis detected a significant interaction between the factors of experimental trial × sample interval on seed yield (P< 0.001), thus results will be presented separately by trial. A significant effect of sample time on seed yield was detected (P< 0.001). In the first experimental trial, maximum seed yield was found at the forth sample interval (9-days after anthesis) amounting to 0.312 grams of *P. torridum* seed (Table 1). In the second experimental trial, maximum seed yield was found at the fifth sample interval (12-days after anthesis) amounting to 0.319 grams of seed. The maximum seed yield was not significantly different between the forth sample interval in the first trial and the fifth sample interval in the second trial (Figure 4). Seed yield maximums were recorded at differing sampling time intervals for plantings initiated in August and January. The shorter time to reach maximum mature seed yield with the August planting is attributed to warmer temperatures than those associated with the January planting. This is expected as the intervals were based on chronological time of alternate seasons, August and January. Accumulated GDD units required for maximum seed yield followed as similar trend with fewer units required for the August planting (249) than the January planting (259). A total of 775 GDD units accumulated in the August planting cycle (i.e.
anthesis to complete seed shatter) and 573 GDD accumulated in the January planting cycle. (Figures 5 and 6).

The least squares predictive equations for both experimental trials indicated that seed yield required a fifth degree polynomial expression. In the first trial, the predictive equation resulted in a $R^2$ value of 0.82 (Figure 7):

$$
\text{Mature seed yield} = 0.5520302 - 0.0009395 \times GDD - 9.5105e^{-7} \times (GDD - 403.873)^2 + 1.9195e^{-8} \times (GDD - 403.873)^3 - 2.34e^{-12} \times (GDD - 403.873)^4 - 8.921e^{-14} \times (GDD - 403.873)^5.
$$

The second trial predictive equation resulted in a $R^2$ value of 0.83:

$$
\text{Mature seed yield} = 0.5626582 - 0.001232 \times GDD - 5.5622e^{-6} \times (GDD - 296.597)^2 + 3.8346e^{-8} \times (GDD - 296.597)^3 + 3.956e^{-11} \times (GDD - 296.597)^4 - 2.895e^{-13} \times (GDD - 296.597)^5.
$$

The results indicate that maximum mature seed yield for crops planted in August in Hawaii requires 249 GDD after anthesis and crops planted in January require 259 GDD. Seed head moisture content corresponding to maximum seed yield for the first trial was 18.6% compared to the second trial of 16.1%. The general trend of seed head moisture content for both trials indicates that the highest moisture content is found three days (one sample interval) before the peak harvest time followed by a steady decline to full desiccation (Figure 8 and 9). If the calculation of GDD’s is not available to predict maximum mature seed yield then the recommended harvest time to maximize seed yield in $P. \text{torridum}$ is 9 chronological days post anthesis for August plantings and 12 days for a January planting.
Tables and Figures

Figure 7.1. Illustration of P. torridum at initiation of flowering anthesis. This visual cue, with bright orange anthers, was used as the starting point for GGD accumulation to characterize the harvest index.

Figure 7.2. Westrup LA-II brush machine inner rotating brush mechanism with #14 (1.0 x 1.0 mm) perforated mantle and medium nylon 0.5 mm brushes.
Figure 7.3. Clipper Office Tester seed separator for cleaning P. torridum seeds. Seeds are separated by two sifting screens and aspirated through an air column during separation.

Figure 7.4. Representative P. torridum seed heads at optimal harvest time for trial one (left image) and trial two (right image). Seed heads from trial one yielded 0.312 grams of mature seed 9 days after anthesis and trial two heads yielded 0.319 g of mature seed 12 days after anthesis.
**Table 7.1.** Panicum torridum seed yield means represented over sample intervals for two experimental trials. Means are separated using Tukey’s HSD comparison at $P=0.05$. Means within columns and rows followed by the same letter are not significantly different.

<table>
<thead>
<tr>
<th>Sample interval (days after anthesis)</th>
<th>Seed yield (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
</tr>
<tr>
<td>0</td>
<td>0.000 F</td>
</tr>
<tr>
<td>3</td>
<td>0.040 DEF</td>
</tr>
<tr>
<td>6</td>
<td>0.144 C</td>
</tr>
<tr>
<td>9</td>
<td>0.312 A</td>
</tr>
<tr>
<td>12</td>
<td>0.164 BC</td>
</tr>
<tr>
<td>15</td>
<td>0.166 BC</td>
</tr>
<tr>
<td>18</td>
<td>0.127 C</td>
</tr>
<tr>
<td>21</td>
<td>0.065 DE</td>
</tr>
<tr>
<td>24</td>
<td>0.080 D</td>
</tr>
<tr>
<td>27</td>
<td>0.077 D</td>
</tr>
<tr>
<td>30</td>
<td>0.007 F</td>
</tr>
</tbody>
</table>

**Figure 7.5.** Panicum torridum seed yield expressed over accumulated growing degree day units. Maximum seed yield was obtained at 249 GDD’s.
Figure 7.6. Panicum torridum seed yield expressed over accumulated growing degree day units. Maximum seed yield was obtained at 259 GDD's.

Figure 7.7. Panicum torridum predictive fifth degree polynomial curves representing seed yield estimates over growing degree days. Predictive equations:

**Mature seed yield trial 1**

\[ \text{Mature seed yield trial 1} = 0.5520302 - 0.0009395 \cdot GDD - 9.5105e^{-7} \cdot (GDD - 403.873)^2 + 1.9195e^{-8} \cdot (GDD - 403.873)^3 - 2.34e^{-11} \cdot (GDD - 403.873)^4 - 8.921e^{-14} \cdot (GDD - 403.873)^5 \]

**Mature seed yield trial 2**

\[ \text{Mature seed yield trial 2} = 0.5626582 - 0.001232 \cdot GDD - 5.5622e^{-6} \cdot (GDD - 296.597)^2 + 3.8346e^{-10} \cdot (GDD - 296.597)^3 + 3.956e^{-11} \cdot (GDD - 296.597)^4 - 2.895e^{-13} \cdot (GDD - 296.597)^5 \]
Figure 7.8. Seed head moisture content and seed yield of Panicum torridum in August, represented over days after flower anthesis.

Figure 7.9. Seed head moisture content and seed yield of Panicum torridum in January, represented over days after flower anthesis.
Literature Cited

Baldos, O.C., 2013. Seed dormancy, smoke-stimulated germination and harvest timing of pili grass (Heteropogon contortus), a native Hawaiian grass with potential for expanded re-vegetation use. UNIVERSITY OF HAWAII AT MANOA.


Chapter 8

Dissertation Conclusions

Overall conclusions, implications and future research ideas

The research contained within this dissertation can provide useful knowledge regarding fundamental agronomic procedures for the native plant species *Waltheria indica* (Uhaloa) and *Panicum torridum* (Kakonakona). Although the research focuses on the two listed species, broader applications for other species not contained in this dissertation can apply. The adoption of methodologies proposed within this dissertation can be a valuable resource for research or practical applications. The depth or continuation of dissertation research must be limited, so aside from the conclusions drawn, it is intended to provoke and provide a foundation for future research endeavors.

Seed dormancy is defined as the critical function to prevent germination when conditions are suitable but when the probability of survival and growth of the seedling is low (Fenner and Thompson 2005). Although dormancy may be of ecological significance for survival and persistence, it is a hindrance for agronomic purposes. As discussed in chapters 2 and 4, the first step to relieving seed dormancy is accurate characterization of dormancy. In chapter 2, *W. indica*’s seed testa was found to impose physical dormancy. Nearly complete relief of dormancy was achieved through a mechanical scarification procedure using a commercially available drum scarifier lined with 80 grit sandpaper for a duration of 30 seconds. In chapter 5, *P. torridum*’s seed testa was determined to be permeable to water but seed germination did not occur.
Considering the permeability of the seed testa and the failure of *P. torridum* to respond to exogenous germination stimulators led to the conclusion that seeds possess an intermediate to deep physiological dormancy. Dormancy was relieved through long term storage in sealed containers with an atmosphere of 12% relative humidity (RH) at 30°C for a duration of 8 months (resulting in 55% germination). The conclusions presented in both chapters 2 and 5 regarding seed dormancy mechanisms and relief are supported by extensive research conducted by Baskin, a leading researcher in the field of seed dormancy (Baldos et al. 2015; Baskin and Baskin 1998; Baskin 2001; Baskin 2003; Baskin and Baskin 2004; Baskin et al. 2000; Baskin et al. 2004).

In order to achieve the state of Hawaii Department of Transportation’s roadside re-vegetation goals, large quantities of seeds must be acquired. The acquisition of sufficient seeds in a one-time harvest for any large scale planting is currently impossible, requiring multiple seed collection events coupled with seed storage that can maintain seed viability over period of time. Along with seed dormancy relief, chapters 2 and 5 also discuss storage parameters necessary for long term seed storage, allowing for the creation of a stored seed bank. It was determined that *P. torridum* seeds can maintain viability for up to 10 months when a storage RH of 12% is imposed and consistently maintained. *W. indica* seed viability was preserved with when non-scarified seeds were stored at 5°C at either 12 or 50% RH for up to 10 months. Although longer storage periods seem likely with proper conditions, research reported here can only certify appropriate conditions for no longer than 10 months for both species evaluated. Further research that quantifies conditions for longer terms of seed storage would be extremely useful to efforts to expand native plant usage along roadways and conservation plantings.

The initial steps necessary for seed production are the awareness of how to store seeds to maintain viability then understanding how to germinate seeds when they are required for use. As
discussed in chapters 3 and 6, the next step in the seed production cycle is the development of establishment procedures coupled with efficient weed control methods. Weed interference is the primary constraint to successful establishment of native plant communities (Masters et al. 1996). In order to manage weeds in a cost effective production system, herbicides must be considered for the establishment of native plants in large scale ecosystem restoration projects (Masters et al. 1996). Chemical weed control research must be conducted to characterize the response of weeds and native plants to use rates consistent with the product labels. The pre-emergence herbicide oxadiazon, applied in a granular formulation at 2.24 kg ai ha$^{-1}$ to $W. indica$ transplants was shown to provide a useful level of grass and broadleaf weed control with an acceptable level of growth inhibition. The $P. torridum$ response indicated that granular oxadiazon applied at 4.48 kg ai ha$^{-1}$ provides the greatest weed control while minimizing phytotoxic effects. The use of oxadiazon can provide a nearly weed free production site for the two native plants discussed while minimizing negative growth effects.

The final step presented in this dissertation discusses the determination of timing for maximum seed yield and species-specific operating settings for commercially available seed cleaning equipment. Chapters 4 and 7 discuss reliable methods to characterize stages of flower head development and their use as markers to initiate and maximize seed harvest. With $W. indica$, mature seed harvest timing can be optimized by utilizing a visual cue. In Chapter 7, seed harvest of $P. torridum$ is maximized when GDD values of 249 (August planting) and 259 (January planting) are obtained following the onset of anthesis. The harvest indexing procedures reported here can aid in maximizing the recovery of mature seed for two native Hawaiian plants. Species-specific seed cleaning methods were optimized for two types of seed processing equipment. Processing times and accuracy for seed cleaning was greatly enhanced using the
mechanized process compared to manual methods. In the first cleaning device, seeds are removed from intact seed heads with a Westrup® LA-H brush machine fitted with a #14 mantle (1.0 x 1.0 mm square mesh) and medium nylon 0.5 mm brushes (Westrup® Inc., Plano, Texas). In the second device, seeds were separated from pulverized seed head components with a Clipper™ Office Tester. Both of the machines used were chosen based on their flexibility and adaptability to process seed from a wide variety of species. For both species studied, further research could explore ways to mechanize in-field harvesting of seeds.

The information reported here can have a significant impact on public and private efforts to restore larger populations of native plants and help to address the expanded use of native plants in public landscapes and conservation plantings (Anonymous 2007; Anonymous 2011; Ricordi et al. 2014; Tamimi 1999).
Literature Cited


Anonymous (2011) Statewide noxious invasive pest program.


