TERMINATION REPORT FOR

(TA) 2011-2R

Developing large scale seed production and roadside establishment protocols for four native Hawaiian ground covers.

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Developing Large Scale Seed Production and Roadside Establishment Protocols for Four Native Hawaiian Groundcovers

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Project performed in cooperation with the Hawaii Department of Transportation and the Federal Highways Administration.

Included in this termination report are detailed seed production and dormancy relief protocols for Pili grass (Heteropogon contortus) and Kakonakona (Panicum torridum). Since Emoloa (Eragrostis variabilis) seeds germinate immediately after harvest, a dormancy relief protocol for this grass was not necessary. Since the Kamanomano (Cenchrus agrimonioides) selection used in this project does not produce viable seed, our report will only describe vegetative propagation techniques for this species. Our report will focus on establishment of seed production plantings using transplants only. All native Hawaiian plant materials used in the research and demonstration projects described here were obtained through official transfers from the USDA/NRCS Plant Materials Center (PMC) on Molokai. The list of plant materials collected and used for the project are listed in this report. We determined that excellent weed control could be obtained in new plantings of all native species when transplants are used when treated with labeled rates of the granular formulation of Ronstar G. Safe and effective broadleaf weed control could be achieved in Emoloa plantings when Garlon 4 is applied to transplants 3 weeks after planting. Although some growth reduction is expected, full recovery is obtained 4-6 weeks after Garlon 4 application. Garlon 4 can also be safely used to control broadleaf weeds in Pili grass at 21 days after transplanting. We determined that transplants are the most effective way to establish plantings of Kakonakona in a seed production setting. Since Kakonakona is a very short-lived annual species, good weed control at planting is all that is required for the entire seed production cycle. We determined that Ronstar G can be used on newly transplants to provide excellent weed control until the mature seed recovery period is complete.
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Executive Summary of Project Accomplishments

The Hawaii Department of Transportation has provided funding in support of the research and development project titled: “Developing large scale seed production and roadside establishment protocols for four native Hawaiian ground covers”. The notice to proceed date was June 1, 2012 with final report due upon the termination date of May 31, 2015. The Task Agreement (TA) for this project is 2011-2R with Purchase Order No. 40054563. The Cooperative Agreement number is DOT-10-030.

Summary of “work required” performed during the project period

Collection of source identified seeds/planting materials for re-vegetation.

All native Hawaiian plant materials used in the research and demonstration projects described here were obtained through official transfers from the USDA/NRCS Plant Materials Center (PMC) on Molokai. The list of plant materials collected and used for the project are listed in Table 1 below.

Table 1. Source identified plant materials collected and established for this project.

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Historical accounts of the provenance of Emoloa (Eragrostis variabilis) and Kamanomano (Cenchrus agrimonioides) used in this project were conveyed during a recorded video interview with Glenn Sakamoto, station manager of NRCS/USDA Plant Material Center (PMC) on Molokai on April 23, 2015.

The Kamanomano (Cenchrus agrimonioides) stock plant used in this project was obtained by Glenn Sakamoto from Kahoolawe Island between 2007 and 2009. The Kahoolawe planting was originally made by Paul Higashino who was a member of the Kahoolawe Island Reserve Commission (KIRC) staff at that time. The material he planted was obtained from Anna Palomino owner/operator of Ho’olawa Farms located on Maui. This selection is considered sterile and has not been shown to produce viable seeds. Propagation of this selection can only be done through vegetative cuttings. Based on our interviews, the original provenance of the Kamanomano stock plant used in our project was derived from Maui via, the Molokai PMC and KIRC activity.
Glenn Sakamoto indicated that he had traveled to Kahoolawe in 1999 to survey and collect suitable plant materials for increase at the USDA/NRCS farm on Molokai. Glenn observed that the Emoloa form, used in our work, was growing very well at Landing Zone 3, a planting established by the Maui native plant collector and naturalist Rene Sylva. Mr. Sylva’s original collection of Emoloa seed came from the Olowalu area on Maui Island.

The Pili grass (*Heteropogon contortus*) provenance used in our project was collected by Glenn Sakamoto on Kahoolawe Island. It was later released by the USDA/NRCS farm on Molokai as the Kahoolawe Source Identified Natural Germplasm.

The Kakonakona (*Panicum torridum*) seeds used in our project were collected from naturally occurring stands in the Mo’omomi Preserve on the northeast corner of Molokai in 2013 and 2014.

Kamanomano is a long-lived perennial grass. We have a 4 ft. by 10 ft. planting established in a permanent bed at the UH Magoon Research and Teaching Facility in Manoa Valley (see Photo 1). We also have Kamanomano growing in compost filled raised beds at the H1/University Ave. demonstration site (see Photo 2). These beds are irrigated and can cuttings for expanded plantings. We wanted to expand plantings of Kamanomano to in-ground areas of the H1/University Ave. site but the soil lead issue prevented us from initiating this effort.

Photo 1. Kamanomano growing in a raised bed at the Magoon Research and Teaching Facility on UH campus in Manoa Valley.
Emoloa is not a long-lived perennial. This growth pattern was confirmed with the original planting at the H1/University Ave. demonstration site that showed decline in vigor after 2 years of growth and the withdrawal of irrigation. Our ability to plant a new stand of Emoloa at the H1/University Ave. site in January 2015 was halted due to concerns of elevated lead levels in the soil. The only exiting Oahu planting of Emoloa resulting from this project is currently located along the exit lane of the DuPont/Pioneer farm on Kunia Road, (photo 3). This planting was installed with the cooperation of Dr. John McHugh of DuPont/Pioneer to evaluate the response of Emoloa (and Pili grass) to spray application of preemergence herbicides applied over newly planted transplants. We have approximately 1 lb of Emoloa seed in storage at the termination of this project.
Pili grass is another long-lived perennial. A large well-maintained planting is located at the H1/University Ave. at the termination of this project. Two smaller plantings are located at the UH Magoon Research and Teaching Facility in Manoa Valley and Dupont Pioneer farm (see Photo 3). The size of the H1 planting is approximately 15 ft. x 200 ft. (Photo 4) and the Magoon Facility planting is approximately 15 ft. x 30 ft. (Photo 5).
Kakonakona is a short-lived annual that progresses from seed to seed in approximately 3 months. We currently maintain about 5 oz. of seed in storage at the termination of this project at the UH Manoa campus.

There are no plantings of native plants used in this project located at the UH Waimanalo Experiment station. We decided to concentrate our efforts for archival plantings at the H1/University Ave. demonstration site so that at the conclusion of our work the Department of Transportation would have a native plant resource under their direct control. An unforeseen complication to our efforts at this location was the discovery of elevated lead levels in the soil. At the termination of this project, it is unclear whether site access to maintain and obtain established perennial plant materials will be continued.

Development of seed production techniques for each grass species.

Included in this termination report are detailed seed production and dormancy relief protocols for Pili grass and Kakonakona. Since Emoloa seeds germinate immediately after harvest, a dormancy relief protocol for this grass was not necessary. Since the Kamanomano selection used in this project does not produce viable seed, our report will only describe vegetative propagation techniques for this species. Our report will focus on establishment of seed production plantings using transplants only.

Grain crops such as corn and sorghum require deeper soil planting than the very small-seeded species used in this project. With deeper soil plantings, preemergence herbicides can be used due to the separation of herbicide layer at the soil surface and
newly emerged grass roots. All the native species used in this project have very small seeds that can only be planted with surface or very shallow soil incorporation. Preemergence herbicides cannot be used in such crops due to close proximity of the herbicide layer and new roots emerging from seeds, both at or very near the soil surface. Acceptable weed control with very small seeded crops requires a relatively low weed seed soil bank (produced with repeated preplant weed flushing and kills) and high seeding rates to better compete with weeds for growing space. We did not conduct direct seeding studies with Kakonakona due to a chronic lack of adequate quantities of seed. It is our recommendation that transplants, of all species used in this project, be used for establishment of seed production blocks as well and landscape plantings.

_Evaluation of hydromulch planting rates and methods._

Our initial studies to conduct hydro seeding with Pili grass were not successful. When Pili grass seeds were added to the hydro seeding mix of organic mulch, water, tackifier (slurry lubricating and soil adherence agent) and nutrients and pumped onto experimental plots there was a complete failure in seed germination. We concluded that, the metal impeller blades of the centrifugal pump used to mix and deliver the hydro mulch/seed slurry destroyed these fragile seeds. We have concluded that hydro seeding of Pili grass will not be a practical way to establish this species. The recommended approach for direct seeding Pili grass is to hand drop seeds along drip irrigation lines and then cover the seed with a hydro mulch cap.

Prior to our hydro seeding work with Emoloa, we consulted the staff of the PMC on Molokai to leverage any experience they had with direct seeded plantings. They indicated that Emoloa field plantings that made use of overhead irrigation were heavily damaged by fungal attack on germinating seeds and seedlings. We decided to evaluate hydro seeding Emoloa and incorporate a fungicide into the seed/slurry mix. The fungicide recommended by the staff of the PMC was Apron XL (mefenoxam). Apron XL can be used on grasses and turf used in “erosion control projects”. With this label description of approved sites for use of Apron XL it was clear that plantings on the highway rights-of-way represented a legal application. We obtained enough seeds for the hydro seeding experiment from the Molokai PMC but our access to the H1/University Ave. demonstration site was suspended prior to our initiation of this aspect of the project. We were not able to conduct any hydro seeding experiments with Emoloa and no recommendations for hydro seeding this species were developed.

The Kamanomano selection used in this project can only be propagated using stem cuttings. We determined that a 24-hour soak in a hormone-based root inducing dip was an effective way to improve root initiation during establishment with cut stems. In a field establishment experiment, we placed hormone treated cut stems of Kamanomano on the surface of compost beds and capped them with hydro mulch slurry containing a
granular formulation of Ronstar preemergence herbicide. The stems did not develop roots in any of the treatments including plots treated with the hydro mulch cap that did not contain preemergence herbicide. We concluded that Kamanomano stems needed to be covered within the growth medium to reliably produce roots and establish successfully. The recommended way to establish Kamanomano is to produce rooted plugs in a nursery setting and then transplant these to field sites.

Kakonakona seed were obtained from wild stands on the windy plains of the Mo'omomi Preserve on Molokai. Seeds were collected by hand and used for determining dormancy relief conditions or for generating transplants used for seed harvest timing experiments. The lack of seed and time required to complete complex studies to determine dormancy relief resulted in insufficient quantities of non-dormant seed needed for hydro seeding studies.

**Post planting and establishment practices.**

We determined that Kamanomano can be best established by producing transplants in a nursery setting using stems soaked for 24 hours in a root inducing hormone solution. Weed control in newly planted stands can be obtained with the application of labeled rates of Ronstar G preemergence herbicide. Due to the withdrawal of access to the H1/University Ave. demonstration site, we were unable to establish experimental plots where post emergence herbicide studies could be conducted on this specie. We were not able to generate data to support the use of any post emergence herbicides in established stands of Kamanomano.

We determined that excellent weed control could be obtained in new plantings of Emoloa when transplants were treated with labeled rates of the granular formulation of Ronstar G. Safe and effective broadleaf weed control could be achieved in Emoloa plantings when Garlon 4 is applied to transplants 3 weeks after planting. Although some growth reduction is expected, full recovery is obtained 4-6 weeks after Garlon 4 application.

Pili grass transplants respond in a similar way with regards to response to Ronstar G applied during the establishment and maintenance phase of growth. Ronstar G applied at labeled rates provided excellent weed control in new plantings when transplants are used. Garlon 4 can also be safely used to control broadleaf weeds in Pili grass at 21 days after transplanting.

The successful use of preemergence herbicides in direct seeded Kakonakona plantings is unlikely since surface plantings are required for its very small seeds. We determined that transplants are the most effective way to establish plantings of Kakonakona in a seed production setting. Since Kakonakona is a very short-lived annual species, good weed control at planting is all that is required for the entire seed production cycle. We
determined that Ronstar G can be used on newly transplants to provide excellent weed control until the mature seed recovery period is complete.

In the following sections, detailed protocols are provided to successfully establish the 4 species used in this project. Establishment protocols were designed primarily for plantings used to produce seed or obtain vegetative propagation materials. Although the protocols were designed with seed production as the primary goal, much of the technology here can also apply to landscape plantings.
Seed Production Protocols for Pili grass
(Heteropogon contortus)

Orville Baldos
Department of Tropical Plant and Soil Sciences
University of Hawaii at Manoa
Seed Production Protocol for Piligrass (Heteropogon contortus)

Common name: Piligrass  
Scientific name: Heteropogon contortus  
Seed source: Kahoolawe Island source identified natural germplasm, USDA/NRCS accession number #: 9079683, HA-5748

Piligrass (Heteropogon contortus) is a fire tolerant and clump growing, native grass commonly found on the dry leeward sides of the Hawaiian Islands, from 0 to 2300 ft above sea level. Piligrass is a culturally important grass in Hawaii since the ancient Hawaiians have used it for house construction, floor coverings and torches. Because of its drought tolerance and ability to grow in low fertility soils, piligrass has been extensively used as a restoration and erosion control species for severely degraded sites. Piligrass produces viable seed, allowing for direct seeding protocols for re-vegetation efforts. However, fresh seeds are dormant and require a postharvest storage treatment period of 12 months at 86°F and 6% seed moisture content (dry weight basis-dwb).

Crop establishment protocol

Site preparation

Clear the seed production site of trash and debris and any unwanted tall shrubs or grasses that might interfere with planting. Once cleared, install a temporary overhead irrigation to encourage active weed growth prior to systemic herbicide application. Conduct sequential flushing and killing of weeds for at least 3 to 6 months prior to planting to exhaust the weed seed bank and stored reserves of perennial species. To kill existing vegetation, conduct a spray to wet application of Round ProMAX (48.7% glyphosate) at a rate of 1.3 to 2.0 oz/gallon for spot treatment. For annual weeds apply 1.0 to 2.7 quarts per acre and or 1.5 to 3.3 quarts per acre for perennial weeds. When broadleaf weed are present at tank mix with Garlon Ultra can be used to expand the weed control spectrum over Roundup ProMax alone. Use 3 to 4 quarts per acre of Garlon 4 for foliage treatment of broadleaf weeds.
**Seed treatments prior to planting**

Freshly harvested seeds of piligrass exhibit very poor germination because of dormancy. To relieve dormancy, seeds can be either dry stored or treated with smoke water. The most effective means of removing piligrass dormancy is to store the seeds for 12 months at 86°F and 6% seed moisture content (dwb). Seeds stored at this condition exhibit >90% germination. Another alternative seed treatment to enhance germination is through presoaking dormant seeds for 15 minutes in either 1% Colgin Natural Mesquite food grade liquid smoke solution or undiluted smoke water generated by burning piligrass. Piligrass smoke water can be produced by burning 4.2 oz of dry piligrass and bubbling this smoke into 1 gallon of water. Smoke treatments allow up to 50% germination in freshly harvested seeds.

**Establishment by plugs**

Sow pre-stored or presoaked (in smoke water) seeds in plastic trays filled with a commercially prepared mixture of potting media (Pro-mix ‘BX’). After one week, transplant seedlings into dibble tubes (Ray-Leach Cone-tainers) filled with a mixture of potting media (Pro-mix ‘BX’) and fertilizer (10-4-16, Best Microgreen 10, applied at 1 lb per 2 cubic ft of potting mix). Allow the plugs to grow under overhead-irrigated conditions for 2 months. Cut the grass to 4 inches height before transplanting to prevent transplant shock and to remove flower heads that may have formed. Row planting is recommended to facilitate weed control during piligrass growth and establishment. The recommended minimum spacing for planting is 3.5 ft between rows and 1.5 to 3 ft in rows (Figure 1). Install drip tape on the surface (Figure 2) and fertilize at a rate of 100 lbs N/acre to stimulate rapid growth, generate weed suppressing mulch and canopy fill in. Apply an over the top spray of Ronstar® 50 WP (oxadiazon 50%, 4 lbs/acre) or a granular application of Ronstar® G (oxadiazon 2%, 100 lbs/acre) over the seedlings to control germinating weed seeds (Figure 3).

![Figure 1. Plant piligrass seedlings at an in-row spacing of 1.5 to 3 ft.](image-url)
Figure 2. After clearing and flushing the weeds, install drip tape on rows spaced at 3.5 ft apart.

Figure 3. Right after planting, fertilize the field with 100 lbs N/acre. Spray over the top with Ronstar® 50 WP (oxadiazon 50%, 4 lbs/acre) or apply granular Ronstar® G (oxadiazon 2%, 100 lbs/acre) to control germinating weed seeds.

At 2 months after transplanting, control emerging weeds with directed sprays of Garlon 4 (triclopyr 44.3%, 1.12 gallons/acre) for broadleaf weed control or Assure II (quizalofop p-ethyl, 10.3%) at 8 fl oz/acre for grassy weed control (Figure 4). At 3 to 4 months after planting, mow the pili grass plants to 4 inches height in order to promote upright growth.
and increased number of inflorescence bearing tillers (Figure 5). Use a hedge trimmer or sickle bar mower to make a clean cut, reduce plant shock and encourage stem proliferation.

Figure 4. After transplanting, allow plants to grow for the next 2 months. To control emerging weeds in the plot, conduct directed sprays of Garlon 4 (triclopyr 44.3%, 1.12 gallons /acre) for broadleaf weed control or use Assure II (quizalofop p-ethyl, 10.3%) at 8 fl. oz/acre for grassy weed control.

Figure 5. Mow newly established piligrass to about 4 inches above the ground to promote tillering. Begin counting the days from cutting to determine the date of seed harvest.
**Pre- and postemergence weed control**

For pre-emergence weed control of newly transplanted plugs, apply an over the top spray of Ronstar® 50 WP (oxadiazon 50%, 4 lbs/acre) or granular application of Ronstar® G (oxadiazon 2%, 100 lbs/acre). For postemergence weed control of broadleaf weeds, an over the top spray of Garlon 4 (triclopyr 44.3%, 1.12 gallons /acre) is recommended starting at 21 days after planting. To control emerged grassy weeds, use Assure II (quizalofop p-ethyl, 10.3%) at 8 fl. oz/acre. Directly spray the weeds to minimize injury to pili grass.

**Crop harvest index**

To prepare the plantings for seed production, cut the grass stands to a height of no less than 4 inches above the ground. Use a hedge trimmer or sickle bar mower to make a clean cut to the stand. After cutting, fertilize the plants at a rate of 100 lbs N/acre to stimulate rapid growth and seed production. Regularly monitor spike and seed development as plants approach the 79th day after cutting. Seeds are ready for harvesting between 79 to 82 days after cutting or when extensive tangling of spikes is observed (Figure 6) and the spike moisture content (measured without the awn) reaches 68% to 72% moisture content (dwb).

Figure 6. Extensive appearance of tangled seed heads between 79 to 82 days after cutting indicates that piligrass seeds are ready for harvest.

To calculate percent moisture content (dwb) of spikes, obtain the fresh weight of a 10-spike sample (awns removed). Dry the sample for 1 day in an oven set at 217°F. Obtain the dry weight of the sample and calculate percent moisture content (dwb) using this equation:
Harvest the seed mass by hand using a steel rake to comb the grass (Figure 7 and 8). Transport seed mass indoors (e.g. an open air garage) and place on a plastic tarp. Spread to an even layer (3 inches or less) and allow to air dry for one week (Figure 9).

Figure 7. Harvesting of the piligrass seed mass can be accomplished by combing the tangled portion of the grass using a steel rake.

Figure 8. Pile seed mass on a plastic tarp.
Figure 9. Transfer the seed mass to a drying area and spread making a thin layer. This makes it easier for the seed mass to dry.

**Seed cleaning protocols**

Free up the seed from the dried seed mass by combing with a rubber pet brush (Figure 10) or by passing it through a thresher (Almaco Small Bundle Thresher). Place the seed mass again on a tarp and allow to air dry for another week. After a week of drying, most of the seeds will settle at the bottom of the tarp (Figure 11). Collect the seeds at the bottom by gently removing the tangled mass of awns on the top layer of the tarp. Slightly shake the mass of awns to remove any remaining seed. Clean (i.e. remove awns) the seeds further by sieving through a ½ x ½ inch wire mesh and then pass through ¼ x ¼ inch wire mesh (Figure 12). Remove dust and chaff using an Almaco Air Blast seed cleaner calibrated so that the seeds fall at the bottom chute (Figure 13). To calibrate the seed cleaner, adjust the airflow to a point at which a sample of clean seed falls down the bottom chute and is not blown on the upper chute. Place seeds in clean canvas or paper bags and label them. Write the date of harvest, date of air-drying and date of cleaning (Figure 14).
Figure 10. After drying, extract the seeds by rubbing the dried seedmass with a rubber pet brush. Remove the awns by hand.

Figure 11. During the air drying process, the awns will expand and contract causing loose seeds to fall at the bottom of the tray. Collect loose seeds and clean.
Figure 12. Pass the collected seeds thru a series of sieves (1/2 x 1/2 square inch and ¼ x 1/4 square inch wire mesh) to further remove the awns.

Figure 13. Remove chaff and lighter material by passing the extracted seed through a calibrated seed cleaner (Almaco Air Blast Seed Cleaner). Collect clean seed at the bottom of the machine.
Figure 14. Store clean seeds in plastic zip bags, airtight glass or plastic wide-rimmed bottles. Label the container with the species name, accession, date of harvest, date of drying and date of cleaning.

Post-harvest seed treatment to produce germinable seed

The after ripening bucket system used for drying down and seed dormancy removal is composed of a 5 gallon bucket lined with bubble wrap for insulation. The wall of the bucket contains an opening affixed with an electrical bulk head fitting that accommodates an electric cord that powers a thermostat temperature probe (Hydrofarm MTPRTC Digital Thermostat for Heat Mats) connected to a greenhouse heating pad (Hydrofarm MT10006 9-by-19-1/2-Inch Seedling Heat Mat). Prior to use, the electrical bulk head fitting must be sealed with silicon caulking to make it airtight (Figure 15).

To dry down piligrass seeds to 6% moisture (dwb), place bags/containers (opened) of cleaned seed inside the after-ripening bucket system. Place a 750 gram canister of silica gel (Dry-Packs Portable Dehumidifier, 750 grams) beside the seeds and seal the plastic bucket with an airtight cover (Gamma Seal Lids). You will need to monitor seed moisture loss for approximately 28 days or until seed moisture content (dwb) reaches 6%. To calculate percent moisture content (dwb) of seeds, during the silica gel enhanced drying process, periodically collect a 1-gram sample of seeds and place it into an oven set at 217°F. You will need to re-weigh the samples several times during the oven drying process until the seed weight no longer changes, this way you can be sure
that all moisture in the seed has been removed. Once you have a stable weight calculate percent moisture content (dwb) using this equation:

\[
\frac{\text{(Fresh weight} - \text{container weight}) - \text{(Dry weight} - \text{container weight})}{\text{Dry weight} - \text{container weight}} \times 100
\]

In this example, the 1-gram sample of seeds will need to be dried to a finalized non-changing weight of 0.943 grams. Using the equation above: \([1.0 - 0.943)/(0.943)] X 100 = 6\%.

When the seeds reach 6\% moisture (dwb), remove the silica gel canister and tightly seal the bucket.

Start the dormancy loss process by turning on the heating pad to 86°F. Store the seeds under heated conditions for 12 months. Figure 16 illustrates the entire dry down and dormancy loss procedure in the fully assembled heated after ripening bucket system.

Figure 15. Bucket setup for drying and after-ripening piligrass seeds: 1) Airtight screw type lid (Gamma Seal Lids); 2) seedling heat mat (Hydrofarm MT10006 9-by-19-1/2-Inch Seedling Heat Mat) connected to a 3) heat mat thermostat with a temperature probe (Hydrofarm MTPRTC Digital Thermostat for Heat Mats); 4) Bubble wrap.
Figure 16. Drying and dormancy loss procedure using the after ripening bucket system. Dry the seeds to 6% moisture (dwb) by placing the opened seed bag/container in the after-ripening bucket containing 2) silica gel dessicant (Dry-Packs Portable Dehumidifier, 750 grams) and 3) humidity indicator papers (Uline 10-60% and 5,10,15% Humidity Indicators). During the drying phase, the bucket is not heated. Once the seed moisture is reduced to 6% (dwb), the silica gel cylinder is removed. Seal the bucket tightly and plug in the thermostat which is connected to the heating pad. Set the inside temperature of the bucket to 86°F and store the seeds at these conditions for 12 months.
Optimized seed storage conditions

After 12 months of storage in the after-ripening bucket system, remove the bags/containers of seeds, seal them tight and store in a reefer set at 41°F until use (Figure 17). Make sure to write the date of removal from the after-ripening bucket. Monitor the seed moisture content by inserting humidity indicator papers in each bag/container (Uline 10-60% and 5,10,15% Humidity Indicators). The ideal relative humidity for long-term storage is 12%.

Figure 17. After incubating the seeds for 12 months at 86°F and 6% seed moisture content (dwb), remove the bags/containers of seeds from the bucket, seal them (make sure they are airtight) and store at 41°F until use.
Seed Production Protocols for Emoloa *Eragrostis variabilis*

Presented by
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University of Hawaii at Manoa
Seed production protocol for Emoloa (Eragrostis variabilis).

Eragrostis variabilis (Gaud.) Steud. was selected for use as native Hawaiian plant suitable as a ground cover on roadside areas. This species has common names of emoloa, kawelu, kalamalo and variable lovegrass (Joy, 2009). Emoloa is a short lived perennial grass, that has a bunch grass growth form. Emoloa is an endemic that occurs in the Hawaiian Islands on sand dunes, grasslands, open sites in dryland forests and exposed slopes and ridges or cliffs from sea level to about 3,700 feet. It grows in areas that receive rainfall in the range of 40 to 100 inches annually.

Crop Establishment protocol

Site preparation

The recommended site preparation for Emoloa is similar to the other native species discussed in this report. Clear the seed production site of trash and debris and any unwanted tall shrubs or grasses that might interfere with planting. Once cleared, install a temporary overhead irrigation to encourage active weed growth prior to systemic herbicide application. Conduct sequential flushing and killing of weeds for at least 3 to 6 months prior to planting to exhaust the weed seed bank and stored reserves of perennial species. To kill existing vegetation, conduct a spray to wet application of Round ProMAX (48.7% glyphosate) at a rate of 1.3 to 2.0 oz/gallon for spot treatment. For annual weeds apply 1.0 to 2.7 quarts per acre and or 1.5 to 3.3 quarts per acre for perennial weeds. When broadleaf weed are present at tank mix with Garlon Ultra can be used to expand the weed control spectrum over Roundup ProMax alone. Use 3 to 4 quarts per acre of Garlon 4 for foliage treatment of broadleaf weeds.

Establishment by plugs

Research has documented the susceptibility of Emoloa seedlings to fungal disease problems at 14 to 21 days after planting in a nursery setting to produce transplants (Duvauchell, 2009). For Pythium damping off protection apply the systemic fungicide Apron XL (mefenoxam) as a preplant seed treatment of 0.64 – 1.28 fl oz/100 lb (0.1 to 0.2 ml/200 grams) of seed. Sow seeds treated ¼ inch deep in plastic trays filled with a 1:1 mixture of perlite and commercially prepared mixture of potting media (Pro-mix 'BX'). After one week, transplant seedlings into dibble tubes (Ray-Leach Cone-tainers) filled with a mixture of 1:1 perlite and potting media (Pro-mix 'BX') and fertilizer (10-4-16, Best Microgreen 10, applied at 1 lb per 2 cubic ft of potting mix). Allow the plugs to grow under overhead-irrigated conditions for 2 months. Automated irrigation should be timed to occur at mid-morning to insure drying of the foliage and growth media surface prior to sunset. Remove any flower heads that emerge prior to transplanting to allow plants to establish and larger mass of foliage prior to seed production. Row planting is recommended to facilitate weed control during Emoloa growth and establishment. The
recommended minimum spacing for planting is 3.5 ft between rows and 1.5 to 3 ft in rows. Install drip tape on the surface and fertilize at a rate of 100 lbs N/acre to stimulate rapid growth.

Pre- and postemergence weed control

Apply an over the top spray of Ronstar® 50 WP (oxadiazon 50%, 4 lbs/acre) or a granular application of Ronstar® G (oxadiazon 2%, 100 lbs/acre) over the seedlings to control germinating weed seeds. Susceptible broadleaf weeds can be controlled with spray applications of Garlon Ultra (triclopyr) at 2 qt./acre. Since Garlon Ultra applications caused a noticeable reduction in Emoloa growth when applied twice to 21 day old transplants (DeFrank, 2003), it is recommended that Garlon Ultra sprays be directed away from Emoloa foliage as much as possible to minimize foliar injury. To control emerged grassy weeds, spot treatments with the selective herbicides Poast (sethoxydim), Arrow 2EC (and other clethodim containing herbicides) and Plateau (imazipic) can be used when sprays are directed away from Emoloa foliage (DeFrank, 2007) to minimize foliar damage.

Crop harvest index

The harvest index for Emoloa was developed by establishing distinct stages of seed head development and then extracting mature seeds to identify which stage contained the highest level of seeds. In this study Emoloa transplants were planted to the field on March 20, 2014. Overhead irrigation and fertilization was applied to maximize growth and seed production. By January 14, 2015 6 distinct levels of seed head development were identified and are depicted in Photo 1.

Emoloa produces a single crop of seed per growing season with mature seed production starting in late January and extends to the end of March. Mature Emoloa seeds do not possess any form of seed dormancy present in many native species. If overhead irrigation is used to establish and grow a seed crop it should be turned off once the seed heads are fully formed. Once mature seed start to form, overhead irrigation or a rainy period will cause seed to germinate within the seed head and greatly reduce the amount of viable mature seeds that can be harvested. Once Emoloa seed heads start to mature (tissue turns from green to brown) harvesters must be on alert to harvest seeds if warm rainy conditions are expected.
Photo 1. Seven distinct stages of Emoloa seed head development at 300 days after planting transplants. Stages are: 1) 100% green tissue, 2) 85% green tissue, 3) 75% green tissue, 4) 50% green tissue, 5) 25% green tissue, 6) 5% green tissue and 7) 0% green tissue. Image to the right provides a comparison of mature seed to and an adult index finger.

Mature seed can be extracted from all levels of seed head development except stage 1, 100% green tissue. Once dry senescing seed head tissue starts to form (i.e. seed head tissues changes from green to brown) mature seed can be extracted from Emoloa seed heads. Seed head that are less than 50% to 5% green tissue should be harvested for mature seed extraction. An additional field indicator of mature seed is the expansion of the seed head and the release small black seeds with gentle shaking (see Photo 1, right side image)
Seed cleaning methods

The process of seed cleaning uses two commercially available machines to clean Emoloa seeds. The first step in the seed cleaning process of Emoloa utilizes 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 1). The brush machine should be run at full power for 1 minute with the front discharge door closed. Seed collected in the bottom catch pan indicated in Figure 1 will be further cleaned using a secondary machine. In the second device, seeds can be separated from the 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 2). The seed separator blower air ducts should be open at 25% capacity and run until all material traveled past the screen sifters.

Figure 1. Westrup LA-H brush machine used for the first seed cleaning stage of Emoloa to separate seeds from seed heads. A) Discharge door adjustment knob to be set fully closed. B) Rotating inner brushes push seed through perforated cylinder. C) Catch pan at bottom of machine, this location will collect the material that will be processed by the Clipper Office Tester.
Figure 2. Second stage seed cleaning of Emoloa using a Clipper Office Tester machine. The right diagram indicates the seed path within the machine. A) The hopper into which bulk material produced from the Westrup brush machine can be placed. B) The upper 0.927 x 0.927 mm screen which allows Emoloa seed to pass through and removes larger plant material. C) The solid sheet bottom screen allows no material to penetrate. D) The air blower separates dust and other fine material from the seeds when set at 25% blower flow capacity. E) The bottom collection receptacle which gathers final cleaned seeds.

Citations


Production Protocols for Native Hawaiian Kakonakona Grass

Presented by Scott Lukas

Tropical Plant & Soil Sciences Department
University of Hawaii at Manoa

U.S. Department of Agriculture
Natural Resources Conservation Service

Department of Transportation
State of Hawaii
Seed Production protocol for Kakonakona (*Panicum torridum*)

Common name: Kakonakona, torrid panicgrass  
Scientific name: *Panicum torridum*  
Seed source: Wild collected from Moomomi Preserve on Molokai, Hawaii.  
USDA accession number #: 9107411

Introduction

Kakonakona (*Panicum torridum*) is a Hawaiian endemic grass ranging from 4 to 25 inches in height with velvety leaves. In Hawaii, annual grasses such as Kakonakona normally emerge in months of April to May following the rainy season that lasts from November to January. Kakonakona is found below 300 feet elevation in zones that receive 20-40 inches of rainfall per year. Kakonakona is well adapted for re-vegetation sites with low rainfall conditions. Kakonakona seed was successfully collected in 2014 from remote wild populations in the Moomomi Preserve on the northeast corner on Molokai. Seeds can be collected from available wild populations if the appropriate collection permits have been obtained. Sales of Kakonakona seed or plant material are not currently available as of 2015.

Post-harvest seed treatment to produce germinable seed

*Germination test*

Kakonakona seeds have been found to possess dormancy which prevents freshly harvested seed from germinating. After seeds are obtained, a germination test is recommended to determine if the seed batch is still dormant. This can be achieved by placing a test sample of seeds on a damp paper towel folded to cover seeds as seen in figure 1. Place paper towel with seeds in a warm location exposed to natural light (such as a windowsill).
Figure 1. Paper towel seed germination test to evaluate dormancy.

Care should be taken to ensure that the paper towel containing the seeds is not allowed to dry and that it is not exposed to extreme temperatures. Non-dormant seeds should germinate within 10 days. If seeds do not germinate, it can be assumed that seeds are dormant. If the viability of the seed batch is unknown, seed can be evaluated for viability through the Hawaii Dept. of Agriculture seed laboratory (1428 South King Street, Honolulu Hi 96822) using a tetrazolium chloride viability test.

**Dormancy relief**

Place bags/containers (opened) of cleaned Kakonakona seed inside a 5 gallon plastic bucket containing a 750 gram can of silica gel (Dry-Packs Portable Dehumidifier, 750 grams). Seal the plastic bucket with an airtight cover (Gamma Seal Lids). You will need to monitor seed moisture loss for approximately 28 days or until seed moisture content (dry weight basis - dwb) reaches 6%. To calculate percent moisture content (dwb) of seeds during the silica gel enhanced drying process, periodically collect a 1 gram sample of seeds and place into an oven set at 217º F. You will need to re-weigh the sample several times until the seed weight remains stable, this way you can be sure that all moisture in the seed has been removed. Once your have a stable weight calculate percent moisture content (dry weight basis) using this equation:

\[
\frac{(\text{Fresh weight} - \text{container weight}) - (\text{Dry weight} - \text{container weight})}{\text{[Dry weight} - \text{container weight}] \times 100
\]

In this example, the 1 gram sample of seeds will need to be dried to a finalized non-changing weight of .943 grams. Using the equation above: 1.0 - 0.943/0.943 X 100 = 6%. When the seeds reach 6% moisture, remove the silica gel from the bucket and assemble the heated seed after ripening systems as shown in figure 2. The after ripening system is composed of a 5 gallon bucket lined with bubble wrap for insulation. The wall of the bucket contains an opening fitted with an electrical bulk head fitting that accommodates an electric cord that powers a greenhouse heating pad (Hydrofarm MT10006 9-by-19-1/2-Inch Seedling Heat Mat) and matched thermostat temperature probe the (Hydrofarm MTPRTC Digital Thermostat for Heat Mats). The seeds, reduced to 6% moisture by the silica gel, will be sealed in the heated (86°F) after ripening bucket for 8 months. Figure 3 illustrates the entire dry down and fully assembled heated after ripening system. At the start of the process, the silica gel cylinder is placed in close proximity to an open bag of seeds to facilitate a reduction in seed moisture content. The bucket is not heated during this phase. Once the seed moisture is reduced to 6% (dwb) the silica gel cylinder is removed, the bucket is then sealed and the heating pad connected to the thermostat is plugged in. The inside temperature of the bucket is to be set at 86°F with the lid tightly sealed to maintain seed at the required moisture level.
Figure 2. Bucket setup for drying and after-ripening Kakonakona seeds: 1) Airtight screw type lid (Gamma Seal Lids); 2) seedling heat mat (Hydrofarm MT10006 9-by-19-1/2-Inch Seedling Heat Mat) connected to a 3) heat mat thermostat with a temperature probe (Hydrofarm MTPRTC Digital Thermostat for Heat Mats); 4) Bubble wrap.
Figure 3. Dry the seeds to 6% moisture (dry weight basis) by placing the 1) opened seed bag/container in the after-ripening bucket containing 2) silica gel desiccant (Dry-Packs Portable Dehumidifier, 750 grams) and 3) humidity indicator papers (Uline 10-60% and 5,10,15% Humidity Indicators). Seal the container and dry the seeds for approximately 4 weeks. After 6% seed moisture content has been achieved, remove the silica gel pack, seal the bucket and incubate for 8 months at 86°F.
Optimized seed storage conditions

After 8 months of storage in the after-ripening bucket, remove the bags/containers of seeds, seal and store in a refrigerator set at 41°F until use (Figure 4). Make sure to write the date of removal from the after-ripening bucket. Monitor the seed moisture content by inserting humidity indicator papers in each bag/container. The ideal humidity, within the container used for long-term storage is 12%.

Figure 4. After incubating the seeds for 8 months at 86°F and 6% seed moisture content, remove the bags/containers of seeds from the bucket, seal them (make sure they are airtight) and store at 41°F until use.

Crop establishment protocol

Site preparation

Before transplanting Kakonakona, the planting site should be prepared to reduce weed pressure by stimulating weeds to grow then chemically removing them using herbicides. This can be accomplished by providing overhead irrigation and chemical fertilizers so that the soil profile is moist to a depth of 3 to 4 inches to stimulate weed species to germinate and grow. After 3-4 weeks, when weeds are actively growing, turn off irrigation and apply commercially available broad spectrum herbicides to kill the establishing weeds. When herbicide treated weeds are
visibly dead (1-3 weeks depending on weed species present), resume irrigation and repeat the process of flushing and killing the weeds. Complete the irrigation weed flush and herbicide spray process at least 3 times to reduce the weed seed bank present in the planting site. Time spent executing this process will greatly enhance Kakonakona establishment success by reducing future weed competition.

**Seedling and transplant preparation**

Kakonakona establishment is best achieved by planting transplants. Seeds should be sown in Sunshine mix #4 with mycorrhizae planting media in 38 cell seedling trays (or similar size). Seeds cannot be buried or covered with soil, place 2-3 seeds on soil surface of each individual planter cell. During this period, irrigation must be minimized, allowing for soil to be damp during the day and partially dry at night. Excess irrigation resulting in soggy soil will support fungal infections leading to seedling death. If all 2-3 seeds emerge in the planting cell, after 3 weeks thin to one seedling by clipping the smallest plants away at the soil surface with scissors. When seedlings are 3 weeks old, or are visibly yellowing, light foliar fertilizer (Miracle Gro™ or comparable product) can be applied (figure 5). Seedlings should be visible within two weeks, but should not be transplanted until 40-50 days after seeding (figure 5).

![Figure 5. Kakonakona ready for transplanting 40 days after seeding in a 38 cell tray. Yellowing on the blades indicates the need for foliar fertilization.](image)

**Transplanting and weed control methods**

When transplants are ready (40-50 days after seeding), they can be planted by creating a small hole in the soil to insert one plant (from the seedling tray). Irrigation can be applied by overhead sprinklers or drip lines. Drip lines are optimal to minimize water use and to direct water to the root zone of the plant. Kakonakona is a drought tolerant species, so watering levels should be maintained to provide a dry appearing soil surface but visibly damp at 1 inch under the surface. Excess irrigation will produce weak plants and foster unwanted weed growth. Directly after
planting, pre-emergence herbicides must be applied to suppress weeds from germinating. The pre-emergent herbicide Ronstar® 2G (Bayer CropScience, Research Triangle Park, NC) can be safely applied over the top of Kakonakona transplants at 200 pounds per acre. Overhead irrigation needs to be briefly applied to the point of saturation but before runoff or puddles occur to activate (dissolve) the Ronstar granular chemical. If necessary, a second application of Ronstar® 2G at the same rate can be applied 45 days after the previous application following weeding to remove visible weeds.

Seed harvest timing to optimize mature seed recovery

In many grass species, seed harvesting is initiated after initial shedding begins, but with Kakonakona, delays in harvesting results in substantial seed losses. 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. Kakonakona seeds can be optimally harvested by counting days after seed heads initiate the visual flowering cue of anthesis. Anthesis is the stage in flowering when the flowers are open and fully functioning. In Kakonakona, the presence of anthesis appears as bright yellow to orange flowers which appear at the top of the seed head (figure 6).

Figure 6. Illustration of Kakonakona at initiation of flowering anthesis. This visual cue, with bright orange anthers, is to be used as the starting point for harvest timing.
After anthesis is observed at the top of the seed heads, seen in figure 6, the recommended harvest time to maximize seed yield is 9 days post anthesis for August plantings and 12 days for January plantings. Figure 7 displays the appearance of seed heads at the optimum time of harvest in both August and January plantings, respectively.

Figure 7. Representative Kakonakona seed heads at optimal harvest time 9 days after anthesis for August (left image) and 12 days after anthesis in January (right image).

The shorter time to reach maximum mature seed yield with the August planting is due to commonly warmer temperatures than those associated with the January planting time. Seed heads can be harvested by using shears to individually cut from the plant. Care should be taken to immediately place the seed heads in a bag because seeds may be falling from the heads.

Seed cleaning methods

The process of seed cleaning uses two commercially available machines to clean Kakonakona seeds. The first step in the Kakonakona seed cleaning process of Kakonakona utilizes 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 8). The brush machine should be run at full power for 1 minute with the front discharge door closed. Seed collected in the bottom catch pan indicated in figure 8 will be further cleaned using a secondary machine. In the second device, seeds can be separated from the 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 9). The seed separator blower air ducts should be open at 25% capacity and run until all material traveled past the screen sifters.
Figure 8. Westrup LA-H brush machine used for the first seed cleaning stage of Kakonakona to separate seeds from seed heads. A) Discharge door adjustment knob to be set fully closed. B) Rotating inner brushes push seed through perforated cylinder. C) Catch pan at bottom of machine, this location will collect the material that will be processed by the Clipper Office Tester.

Figure 9. Second stage seed cleaning of Kakonakona using a Clipper Office Tester machine. The right diagram indicates the seed path within the machine. A) The hopper into which bulk material produced from the Westrup brush machine can be placed. B) The upper 0.927 x 0.927 mm screen which allows Kakonakona seed to pass through and removes larger plant material.
C) The solid sheet bottom screen allows no material to penetrate. D) The air blower separates dust and other fine material from the seeds when set at 25% blower flow capacity. E) The bottom collection receptacle which gathers final cleaned seeds.
Stock Plant/Plug Production Protocols for Kamanomano
(Cechrus agrimonioides var. agrimonioides)

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University of Hawaii at Manoa
Stock Plant/Plug Production Protocol for Kamanomano
(*Cenchrus agrimonioides var. agrimonioides*)

Common name: Kamanomano  
Scientific name: *Cenchrus agrimonioides var. agrimonioides*  
Seed source: Maui Island, USDA/NRCS  
accession number #9107361

Kamanomano (*Cenchrus agrimonioides var. agrimonioides*) is a low growing, endemic, shortlived perennial grass that spreads by stolons and seeds. It is found on the islands of Oahu, Lanai and Maui usually on dry rocky slopes or ridges between 1900 to 2500 ft above sea level. Because of its low growth habit and drought tolerance, kamanomano has been successfully used for re-vegetation of severely degraded sites on the island of Kahoolawe. These characteristics also make it an ideal groundcover for roadsides. Kamanomano is a federally endangered species, with wild populations consisting only of several individuals on parts of Maui and Oahu.

Crop establishment protocol

*Site preparation*

Clear the production site of trash and debris and any unwanted tall shrubs or grasses that might interfere with planting. Once cleared, install temporary overhead irrigation to encourage active weed growth prior to systemic herbicide application. Conduct sequential flushing and killing of weeds for at least 3 to 6 months prior to planting to exhaust the weed seed bank and stored reserves of perennial species. To kill existing vegetation, conduct a spray to wet application of Roundup ProMax (48.71% glyphosate) at a rate of 1.3 to 2.0 fl. oz./gallon for spot treatment. For annual weeds apply 1.0 to 2.7 quarts per acre and or 1.5 to 3.3 quarts per acre for perennial weeds. When broadleaf weeds are present, a tank mix with Garlon 4 Ultra can be used to expand the weed control spectrum over Roundup ProMax alone. Use 3 to 4 quarts per acre of Garlon 4 Ultra for foliage treatment of broadleaf weeds.
Establishment by plugs

The Kamanomano Maui Island form used in studies for roadside use has not been shown to produce viable seed but can be propagated by making cuttings of runners or by division of clumps. To produce grass plugs, collect 8-inch long tip cuttings (flower spikes removed from healthy stock plants) (Figure 1). Keep cuttings under cool and moist conditions, away from direct sunlight to prevent drying. Prepare a 1:20 soaking solution of Dip ‘N Grow (500 ppm indole-butyric acid and 250 ppm 1-naphthaleneacetic acid) by mixing 1 part of Dip ‘N Grow to 19 parts of water. Soak tip cuttings in solution for 24 hours (Figure 2) and plant into dibble tubes (Ray-Leach Cone-tainers) filled with potting media (Pro-mix ‘BX’) (Figure 3). The top portions of the cuttings will dry out and appear to be dead. The stem cuttings will root if overhead-irrigation is applied to maintain good soil moisture in approximately 45 days. Transplant rooted plugs 8 to 10 ft between rows and 3 ft within in rows. Install drip tape on the surface and fertilize at a rate of 100 lbs N/acre to stimulate rapid growth, generating weed suppressing canopy cover. Apply an over the top spray of Ronstar® 50 WP (oxadiazon 50%, 4 lbs/acre) or a granular application of Ronstar® G (oxadiazon 2%, 100 lbs/acre) over the seedlings to control germinating weed seeds. At 45 days after planting, conduct light handweeding of the plots followed by a re-application of Ronstar® G (oxadiazon 2%, 100 lbs/acre). Ratoon/mow the grass once a year to promote vigorous growth. Topdressing the stem runners with clean compost will induce rooting and enhance the erosion control potential of this species.

Figure 1. Well watered, healthy stock plants provide good material for tip cuttings
Figure 2. Soak harvested tip cuttings in a 1:20 dilution of Dip ‘N Grow. To ensure maximum soaking, place a weight on top of the cuttings.

Figure 3. Plant soaked cuttings in dibble tubes filled with potting media. Allow to root under overhead irrigated conditions.
Crop harvest index (stolons)

Stolons or tip cuttings can be harvested anytime after the plantings have established. To produce more stems and encourage vigorous growth, a once a year ratooning/mowing is recommended followed by light hand weeding, applications of fertilizer (100 lbs N/acre) and Ronstar® 50 WP (oxadiazon 50%, 4 lbs/acre) or Ronstar® G (oxadiazon 2%, 100 lbs/acre).