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Interspecific Crosses Yield Sterile Triploid Porterweed: A Sustainable Alternative to Invasive Nettleleaf Porterweed

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Abstract. **Porterweed plants (*Stachytarpheta* sp.), notably the nettleleaf porterweed (*Stachytarpheta cayennensis*), present both ecological benefits and challenges. While these plants attract diverse pollinators and are drought-resistant, nettleleaf porterweed is also an invasive species in the southeastern United States and Hawaii, threatening native ecosystems by outcompeting local flora and reducing biodiversity. Despite its invasive nature, it continues to be popular in nurseries because of its robust growth and flowering characteristics. Prior studies have demonstrated the prolific seed production of the species and potential for naturalization, emphasizing the need for sterile, noninvasive alternatives. This study explored the development of such alternatives through interploidy and interspecific hybridization by focusing on creating triploid hybrids that exhibit both male and female sterility. This was achieved by using interspecific and interploidy crosses between tetraploid nettleleaf porterweed and diploid dwarf red porterweed (*Stachytarpheta microphylla* 'Red Compact'). Growth, flowering, and sterility of the resulting hybrids were assessed in multiple controlled and field conditions. The results identified three triploid hybrids that exhibited desirable horticultural traits comparable to those of the invasive nettleleaf porterweed but, importantly, showed no viable seed or pollen production, confirming their sterility. These hybrids displayed robust growth, substantial flower production, and sterility across multiple environmental conditions, making them ideal candidates for replacing invasive species in landscape settings.**

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Porterweed plants (*Stachytarpheta* spp.) have become increasingly popular in pollinator gardens because of their ability to attract a diverse range of butterfly species and other pollinators (Gilman 2014). The plants are also valued for their drought tolerance and resistance to pests and diseases (Lamborn 2017). Although porterweeds offer important ecological benefits, one species, nettleleaf porterweed (*Stachytarpheta cayennensis*), has raised concerns because of its invasive traits. This heavy seed producer poses a threat to native ecosystems in the southeastern United States and Hawaii by outcompeting indigenous plants, disrupting ecological processes, and reducing biodiversity. Originally introduced to the United States from Central and South America, nettleleaf porterweed has escaped cultivation (US Department of Agriculture, Natural Resources Conservation Service 2023). The Florida Invasive Species Council has classified this species as a category II invasive plant, indicating its potential to impact ecosystems in the future (Florida Invasive Species Council 2023). Likewise, the University of Florida Institute of Food and Agricultural Sciences assessment of non-native plants in Florida's natural areas recommends that it should be used with caution throughout Florida (University of Florida Institute of Food and Agricultural Sciences 2024). Despite the concerns surrounding nettleleaf porterweed, it is sought after by consumers for its robust growth and abundant flowering (Parrish, unpublished data). The ongoing sale of this invasive species highlights the urgent need for sustainable alternatives that maintain the desirable traits of porterweed while minimizing the risk to native ecosystems. Previously, Wilson et al. (2009) assessed seed production and viability of eight porterweed selections in Florida and found that four of the selections exhibited high female sterility characterized by minimal or no seed production at various locations in Florida, suggesting a low potential for naturalization. In contrast, nettleleaf porterweed generated thousands of seeds and demonstrated seed viability ranging from 39% to 80% over a 28-week period. Their study also revealed that controlled crosses between nettleleaf porterweed and the Florida native jamaican porterweed (*Stachytarpheta jamaicensis*) could potentially result in hybridization. Porterweed cultivars Red Compact (*S. microphylla*) and J.P.'s Pink (*S. microphylla*) were identified as diploids, while the remaining selections were determined to be polyploids (Wilson et al. 2009). However, the research lacked critical information regarding pollen viability, which is essential for determining the potential of these porterweed species to hybridize with the native jamaican porterweed. In a more recent study by Qian et al. (2021), researchers examined plant morphology, ploidy levels, and pollen viability in five porterweed selections. Interestingly, the study identified nettleleaf porterweed as a tetraploid, positioning it as an ideal candidate for triploid production. Although coral (*Stachytarpheta mutabilis*) and 'Naples Lilac' (*S. cayennensis* × *S. mutabilis* 'Violacea') porterweed demonstrated female sterility in a previous study (Wilson et al. 2009), Qian et al. (2021) found evidence of potential pollen viability in these selections. This finding suggests that these accessions could potentially hybridize with jamaican porterweed, posing a risk to the native gene pool.

Commercial interest in breeding techniques designed to reduce the impact of popular ornamental invasive species is gaining momentum. For example, interploidy hybridization has demonstrated potential in creating sterile, noninvasive alternatives to aggressive plant species (Contreras and Hoskins 2020; Czarnecki et al. 2012; Rounsaville et al. 2011; Trueblood et al. 2010). Sterile triploid plants, characterized by three sets of chromosomes,

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provide environmentally friendly solutions to the challenges posed by invasive species. These plants retain desirable characteristics, such as ornamental value, without producing viable seeds, thereby reducing their spread and ecological impact (Wang et al. 2016).

The University of Florida has successfully used interploidy crosses to develop sterile, noninvasive lantana (*Lantana camara*) cultivars as triploids (Czarnecki et al. 2012; Deng et al. 2017, 2020). These sterile cultivars maintain the esthetic appeal of their invasive counterparts while minimizing risks to native plant communities. This notable approach highlights the potential for applying similar strategies to other invasive species, such as nettleleaf porterweed, to develop sustainable alternatives for maintaining both ecological health and ornamental value.

Although alternatives to the invasive nettleleaf porterweed exist, no genotypes that are both female and male sterile have been discovered. Moreover, current alternatives do not share the same preferred growth habit as nettleleaf porterweed, limiting their effectiveness in replacing the invasive species in landscapes. As a result, this study aimed to explore the potential of interploidy and interspecific crosses to develop a porterweed genotype that is both female and male sterile. A secondary objective was to identify a sterile porterweed selection that exhibits growth and flowering habits comparable or even superior to those of nettleleaf porterweed, making it a suitable replacement in landscape settings.

Materials and Methods

Interspecific crosses. Interspecific crosses were conducted in Jul and Aug 2021 using nettleleaf ($2n = 4x$) and 'Red Compact' ($2n = 2x$) within the controlled environment of a greenhouse at the University of Florida Institute of Food and Agricultural Sciences Gulf Coast Research and Education Center in Wimauma, FL, USA. Stock plants of each parent were maintained in 3.8-L plastic containers filled with Jolly Gardener Proline HFC/B growing mix (BWI Companies Inc., Apopka, FL, USA). Flowers of nettleleaf stock plants were emasculated using a pair of forceps before anthesis. Pollen from 'Red Compact' flowers were transferred to the stigmas of emasculated nettleleaf flowers using a small paintbrush soon after anthesis. Seeds were collected and sown on soilless potting mix in 128-cell polystyrene trays using Jolly Gardener Proline HFC/B growing mix. To aid germination, a misting system was set to provide a 10-s spray every 15 min. As soon as the seedlings developed true leaves, they were transplanted to deeper 50-cell trays measuring 4.93 cm at the top and 11.43 cm in depth. The seedlings were then grown until they reached the flowering stage and interspecific hybrids were identified by flower color.

Greenhouse study. A greenhouse study was initiated in Jan 2022, with cuttings taken from the three identified hybrids (UF-NCR-1, UF-NRC-2, and UF-NRC-3), nettleleaf, and 'Red Compact'. These cuttings were rooted in 128-cell trays under the same conditions used for seed germination as detailed in the previous paragraph. For each genotype, seven rooted cuttings were cultivated in 3.8-L plastic containers filled with Jolly Gardener Proline HFC/B growing mix. They were arranged in a randomized complete block design within a temperature-controlled greenhouse in which temperatures were maintained at 29.4 °C during the day and 21.1 °C at night.

Each container-grown plant was hand-watered daily and supplemented with 1 tablespoon of controlled-release fertilizer (Osmocote, 14N–14P–14K, 3- to 4-month release at 21 °C; The Scotts Company, Marysville, OH, USA). Data collection took place 2 and 3 months post-transplant. Standard wooden meter sticks were used to measure plant height, width across two perpendicular axes, and inflorescence length. Leaf length, width, and internode length were measured using a stainless-steel ruler, while the internode diameter and flower width were determined using an electronic digital caliper (Max-Cal; Fowler & NSK, Tokyo, Japan).

Because of availability, inflorescence number, flower number, and flower size were only measured at the second data point. In addition, a visual rating was assigned to each plant based on its perceived likelihood of purchase, with 1 indicating a very low likelihood, 3 indicating a moderate likelihood, and 5 indicating a very high likelihood. Traits that were indicative of a very high visual rating score of 5 included a compact growth habit, abundant blooms, and overall plant fullness.

Pollen stainability. At the second data point, anthers were collected from each replicate of plants for pollen viability testing. A commercially available 2% acetocarmine solution (Carolina Biological Supply Co., Burlington, NC, USA) was heated on a hot plate (98 °C) to evaporate acetic acid and increase the concentration to a 4% acetocarmine solution. Anthers, harvested 1 d before anthesis, were submerged in the 4% acetocarmine staining solution and left to stain overnight at room temperature. On the subsequent day, the stained anthers were carefully placed on a glass slide with a drop of the staining solution and gently squashed beneath a coverslip. Pollen grains were observed at $\times 40$ and $\times 100$ magnification using a BX41 microscope equipped with an Olympus Q-color 5 camera (Olympus America Inc., Center Valley, PA, USA). Images of the pollen grains were captured and analyzed to assess pollen stainability. Pollen grains that were stained dark red were categorized as stainable.

Seed production. Upon completion of data collection, the plants were relocated to an open shade house outdoors. This allowed for insect-vectored pollination to take place over a span of 2 months. At the conclusion of this period, eight mature spikes from each genotype were randomly collected. Over a 10-cm section in the center of each spike, seed cavities were counted, and seeds were extracted when present.

Nuclear DNA content. The nuclear DNA content was assessed 3 months post-transplant using the one-step protocol for sample preparation as recommended by Doležel et al. (2007). This involved the use of LBO1 lysis buffer, RNase, and propidium iodide. 'Stupické polní rané' tomato (*Solanum lycopersicum* L.; 1.96 pg/2C) was used as the internal standard. Three technical replicates were analyzed for each of the three biological replicates. The holoploid DNA content (2C) was calculated by multiplying the DNA content of the standard by the mean fluorescence value of the sample and then dividing by the mean fluorescence of the internal standard.

Field study 2022. In Jul 2022, five replicates of container-grown plants from the previous study were transplanted to raised beds in an open field to evaluate plant performance and seed production. Because of limited plant availability, only two replicates of 'Red Compact' were included. Measurements of plant height, width across two perpendicular axes, and plant ratings were conducted in a similar manner as that used during the greenhouse study. Additionally, flower density was rated using a visual scale of 1 to 5, with 1 indicating no blooms or spikes, 2 indicating the presence of visible spikes but no open flowers, 3 indicating one to several spikes with open flowers, 4 indicating the presence of many spikes with open flowers, and 5 indicating peak bloom with full plant coverage. A single spike was selected from each replicate during each data collection period, from which cavities were counted and seeds were extracted over a 10-cm section in the center of the spike according to the previously used methodology. Data collection began 3 months post-transplant and was conducted monthly for a total of 3 months.

Seeds collected from all data points were soaked in water overnight at 27 °C and then sown on water agar plates (10 $g \cdot L^{-1}$ agar). Any seeds that were floating after soaking were discarded. For each selection, 200 seeds were sown across four plates, with 50 seeds per plate. Germination was defined as occurring when the exposed radicle was at least 2.0 mm. The number of germinated seeds was counted weekly for 3 weeks. To avoid duplicate counts, germinated seeds were removed after each count. At the conclusion of data collection, nongerminated seeds were dissected and examined under a stereoscope to study seed morphology.

Field studies 2023. Cuttings from stock plants of nettleleaf, 'Red Compact', UF-NRC-1, UF-NRC-2, and UF-NRC-3 were taken during Jan 2023 for plant performance trials. In Mar 2023, three separate trials were initiated, each with four replicates arranged in a randomized complete block design. The trials took place in Gainesville, FL, USA (container trial), Balm, FL, USA (container trial), and Balm, FL, USA (raised bed trial).

In Gainesville (location 1), rooted cuttings were transplanted into 7.6-L plastic containers filled with Jolly Gardener Proline HFC/B growing mix (BWI Companies Inc.). Plants were maintained in an open greenhouse for

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the first month of growth and then transferred to an open plot on black landscape fabric. In the Balm container trial (location 2), rooted cuttings were transplanted into 7.6-L plastic containers filled with Jolly Gardener Proline HFC/B growing mix (BWI Companies Inc., Apopka, FL, USA) and immediately placed in an open plot on black landscape fabric. During the Balm raised bed trial (location 3), rooted cuttings were transplanted directly into raised white plastic-covered beds in an open field. All container plants were fertilized with 1 tablespoon of controlled-release fertilizer (Osmocote, 14N–6.1P–11.6K, 3- to 4-month release at 21 °C; The Scotts Company). All trials included automatic irrigation, and plants were watered as needed based on the plant growth stage.

Data were collected monthly for 4 months at each trial location. Metrics included plant height, width across two perpendicular axes, and the number of spikes. Plant ratings were determined using the following scale of 1 to 5: 1 = poor quality, severe leaf chlorosis, significant leaf spot/damage, not marketable; 2 = below-average quality, some leaf chlorosis, some leaf spot/damage, leggy and unattractive growth habit; 3 = average quality, moderate health, somewhat desirable growth habit; 4 = above-average quality, good health, vigorous growth, desirable and symmetrical growth habit, full appearance; and 5 = excellent quality, premium health, most desirable and symmetrical growth habit, peak plant fullness.

Bloom ratings were also determined using the following scale of 1 to 5: 1 = no flowers or spikes present; 2 = flower spikes visible, but no open flowers; 3 = one to several spikes with open flowers; 4 = many spikes with open flowers and multiple flowers open per spike; and 5 = abundant flowering, full plant coverage, numerous spikes, and multiple flowers open per spike.

Over the third and fourth months, three mature spikes, characterized by a yellowing to brown coloration, were harvested from each plant, with seeds subsequently extracted and cleaned. These cleaned seeds were then quantified by weight, where 100 seeds weighed 10 mg. Five hundred seeds per genotype and location were then sowed atop potting mix within seedling trays, which were subsequently covered with clear plastic domes to ensure high humidity. Weekly over the subsequent 3 weeks, the number of germinated seeds was tallied, and germinated seeds were removed following each count.

Native cross compatibility studies 2023. In Jan 2023, cuttings were taken from the stock plants of jamaican, UF-NRC-1, UF-NRC-2, and UF-NRC-3 porterweed for cross-compatibility evaluations within the controlled environment of a greenhouse at the University of Florida Institute of Food and Agricultural Sciences Gulf Coast Research and Education Center in Wimauma, FL, USA. Three rooted cuttings from each triploid and six cuttings from jamaican porterweed were transplanted into 3.8-L plastic containers filled with potting mix. The plants were maintained until each had at least two spikes with open flowers.

Pollen from jamaican porterweed flowers was transferred to the stamens of the triploid lines using a small paintbrush. Over a span of 10 d, all open flowers on two spikes per plant were pollinated. Reciprocal crosses were also made by using anthers of UF-NRC-2 crossed onto emasculated jamaican flowers. For quality control, six spikes of jamaican porterweed plants were self-pollinated. Flowers on a total of six spikes from three clonal jamaican porterweed plants were pollinated daily for 10 d.

At 2, 3, and 4 weeks after pollination, eight developing seeds (two per plant) were collected for analysis. The collected seeds were cut longitudinally, stained in a 1.0% tetrazolium (2, 3, 5-triphenyl chloride) solution at 35 °C for 18 h, and observed under a stereoscope, with dark red staining indicating viability. Six weeks after pollination, all remaining seeds were collected, cleaned, sowed, and evaluated in the same manner as that used during the previous experiment.

Statistical analysis. To identify significant differences ($P \leq 0.05$) in pollen viability, seed production, and seed germination among porterweed selections, we conducted an analysis of variance using R version 4.2.2 (R Core Team 2022). When significant differences were detected, Tukey's honestly significant difference test was used to differentiate the means.

Results

Interspecific crosses. Approximately 80 interspecific crosses were conducted, yielding 90 seeds, which resulted in 50 seedlings suitable for visual evaluation. The seedlings commenced flowering 3 months post-germination. Despite their highly similar plant morphologies, 20 seedlings were identified as successful hybrids. These were recognized by their distinctive vivid purple (RHS86 80A, RGB 102, 15, 108) flower color and smooth leaves, which presented a stark contrast to the vivid violet (RHS86 87A, RGB 74, 18, 130) hue of the flowers of the maternal parent and textured leaf surface (Fig. 1). Little variation was seen in the F1 hybrids as expected; therefore, the best three were selected for further trials based on bloom coverage, plant compactness, and fullness.

Greenhouse study. No significant differences in plant height, leaf length, and leaf width were detected between nettleleaf porterweed and the three hybrids at either of the data points (Supplemental Table 1). All were notably taller than 'Red Compact'. At both times of observation, UF-NRC-3 presented the greatest plant width. By the second data collection, all three hybrids were significantly wider than both nettleleaf and 'Red Compact' porterweed. While there were no significant differences in internode length between all three hybrids and nettleleaf at the first data point, UF-NRC-2 displayed significantly shorter internodes than those of nettleleaf porterweed at the second data point. Internode diameter was similar among the three hybrids and nettleleaf, all of which were larger than that of 'Red Compact'. Across both data points, the three hybrids had significantly longer inflorescences than those of both nettleleaf and 'Red Compact'. UF-NRC-2 had a significantly higher count of inflorescences at the second

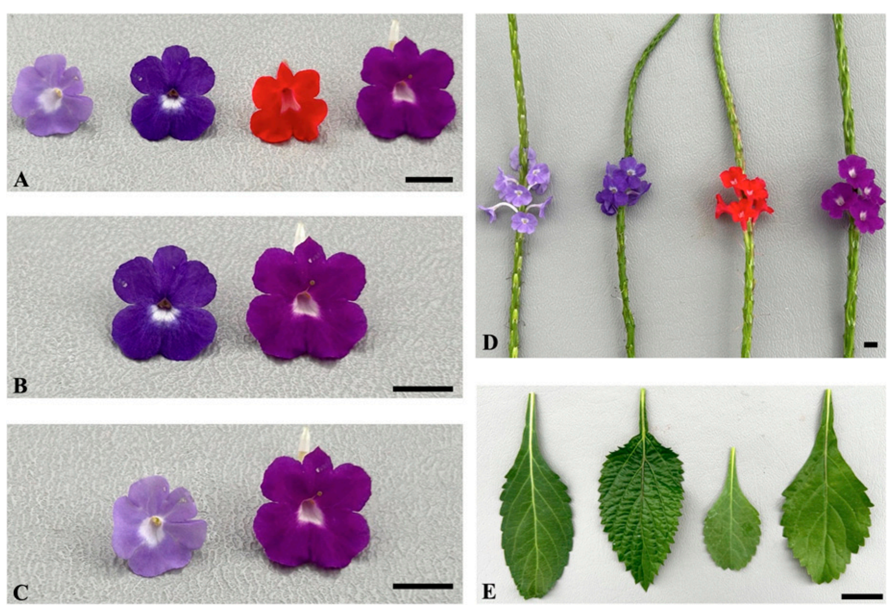

Fig. 1. Flowers and inflorescences of *Stachytarpheta jamaicencis*, *Stachytarpheta cayennensis*, *Stachytarpheta microphylla* 'Red Compact', and *Stachytarpheta* × 'UF-NRC-2'. Genotypes are pictured from left to right: (**A**) *Stachytarpheta jamaicencis*, *Stachytarpheta cayennensis*, *Stachytarpheta microphylla* 'Red Compact', and 'UF-NRC-2'; (**B**) *Stachytarpheta cayennensis* and *Stachytarpheta* × 'UF-NRC-2'; (**C**) *Stachytarpheta jamaicencis* and *Stachytarpheta* × 'UF-NRC-2'; (**D**) *Stachytarpheta jamaicencis*, *Stachytarpheta cayennensis*, *Stachytarpheta microphylla* 'Red Compact', and *Stachytarpheta* × 'UF-NRC-2'; and (**E**) *Starchytarpheta jamaicencis*, *Stachytarpheta cayennensis*, *Stachytarpheta microphylla* 'Red Compact', and *Stachytarpheta* × 'UF-NRC-2'. (**A–D**) Scale bars = 5 mm. (**E**) Scale bar = 2 cm.

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Table 1. Nuclear DNA content, ploidy level, and pollen stainability of porterweed (*Stachytarpheta* sp.) accessions, grown in Balm, FL, USA, in 2022.

Porterweed	Nuclear DNA content ± *SD* (pg/2C)	Ploidy level	Pollen grains examined (no.)	Pollen stainability (%)[i]
UF-NRC-2	2.17 ± 0.04 b	3*x*	436	1.5 a
UF-NRC-1	2.18 ± 0.04 b	3*x*	443	3.2 a
UF-NRC-3	2.17 ± 0.03 b	3*x*	347	0.2 a
Nettleleaf	2.76 ± 0.04 a	4*x*	332	95.8 b
Red Compact	1.42 ± 0.04 c	2*x*	379	94.3 b

[i] Means with the same letter within the column are not significantly different according to the Tukey honestly significant difference procedure at $P \leq 0.05$.
SD = standard deviation.

data point compared with those of both nettleleaf and 'Red Compact', with UF-NRC-1 and UF-NRC-3 closely trailing, averaging approximately 41 spikes per plant. Interestingly, UF-NRC-1 and UF-NRC-3 also exhibited significantly larger flowers than those of both nettleleaf and 'Red Compact', with UF-NRC-2 lagging behind by just 0.02 mm, but still 1 mm larger than the nettleleaf flowers.

A flow cytometry analysis confirmed the triploid nature of the three interspecific hybrids, UF-NRC-1, UF-NRC-2, and UF-NRC-3 (Table 1). Their nuclear DNA content was approximately 1.5-times greater than the 2C value of 'Red Compact' and 21% smaller than that of nettleleaf porterweed. Furthermore, the nuclear DNA content among the three hybrids demonstrated negligible variation, with all observed changes falling within 1 standard deviation of one another.

Observations from the aceto-carmine stainability analysis spanned a total of 1242 pollen grains across the five porterweed genotypes (Fig. 2; Table 1). The maximum number of grains (n = 332) was observed in the nettleleaf genotype. The highest pollen stainability was exhibited by 'Red Compact', with 94% of its pollen grains becoming fully stained by the dye. Nettleleaf followed closely with a stainability of nearly 96%. In stark contrast, all three triploid hybrids, UF-NRC-1, UF-NRC-2, and UF-NRC-3, demonstrated significantly lower stainability, with less than 3.2% of their pollen grains staining fully.

Significant differences were noted in the percent seed set per 10 cm of flower spike after a 2-month period in a shade house setting across the two parental genotypes and the three hybrids (Table 2). 'Red Compact' stood out, with the highest recorded seed set percentage, with almost 84% of its flowers producing seeds. This was significantly higher than that of the nettleleaf genotype, which produced seeds from approximately 63% of counted flowers. In contrast, the triploid hybrids had a nearly nonexistent seed set; only a single seed was found, and it belonged to UF-NRC-3.

Field study 2022. Observations from three time points indicated that the three hybrid genotypes (UF-NRC-1, UF-NRC-2, and UF-NRC-3) significantly outperformed nettleleaf and 'Red Compact' in terms of plant and bloom ratings (Fig. 3; Supplemental Table 2). Similarly, the hybrids also surpassed both parents in terms of plant height and width, with UF-NRC-3 showing significantly higher measurements in both aspects. The number of flowers per 10 cm of spike was comparable across all accessions; however, a slight but significant decrease was noted for UF-NRC-1 and UF-NRC-3. Interestingly, despite their overall performance, UF-NRC-2 and UF-NRC-3 exhibited the lowest seed production, with only 50% and 47% of flowers producing seed, respectively.

From the 200 seeds sown, a stark contrast in germination rates was observed: 89% of nettleleaf seeds germinated within 3 weeks, whereas no seeds from any of the three hybrids showed any signs of germination (Fig. 4; Table 3). Upon dissecting the ungerminated hybrid seeds, they were found to contain empty cavities, with embryos shriveled and confined to one side of the seed.

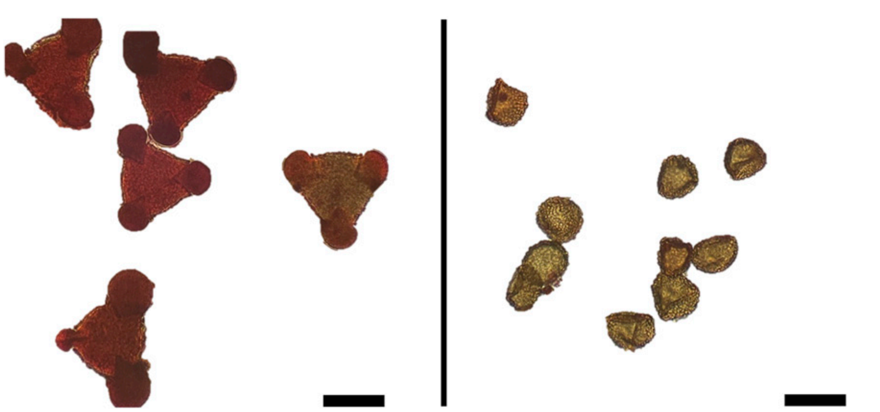

Fig. 2. Acetocarmine-stained pollen grains of the resident porterweed species (*Stachytarpheta cayennensis*) (left) and *Stachytarpheta* × 'UF-NRC-2' (right). Pollen was collected from field-grown plants from the 2022 field trials in Balm, FL, USA. Dark red color indicates pollen viability. Scale bar = 100 μm.

Field studies 2023. In this study, no significant differences were observed in plant ratings across all time points for locations 1 and 3 (Supplemental table 3). However, location 2 presented a different scenario (Fig. 5). Specifically, UF-NRC-2 had the highest average plant rating at 3.88, which was significantly greater than 3.25 observed for nettleleaf and 2.63 observed for 'Red Compact'.

In terms of bloom ratings, each location demonstrated unique patterns. At location 1, all three hybrids, UF-NRC-1, UF-NRC-2, and UF-NRC-3, had significantly higher recorded bloom ratings than that of 'Red Compact' (Supplemental Table 3). Although not statistically significant, there was a notable increase in the bloom rating from 3.75 for nettleleaf to 4.06 for the three hybrids. At location 2, UF-NRC-2 outperformed both parent plants, with a significantly higher average bloom rating of 3.44. Furthermore, UF-NRC-1 and UF-NRC-3 also had significantly higher bloom ratings than that of 'Red Compact'. At location 3, all three hybrids had significantly higher bloom ratings than those of both parent plants, with an average rating of approximately 3.81.

When evaluating the number of spikes across the three different locations, distinct patterns emerged for each hybrid as compared with those of their parent plants. Specifically, UF-NRC-2 consistently outperformed both parents, with significantly more spikes recorded at all three locations (Fig. 6; Supplemental Table 3). At location 1, UF-NRC-1 also exhibited superior performance by registering significantly more spikes than those of both of its parents. However, UF-NRC-3 was only significantly better than 'Red Compact' in this location. At location 2, both UF-NRC-1 and UF-NRC-3 had significantly more average spikes than those of 'Red Compact', although neither hybrid significantly outperformed nettleleaf. Moving to location 3, UF-NRC-1 continued to show strong results, with significantly more average spikes than those of both parent plants. However, UF-NRC-3 was only significantly better than 'Red Compact'. It is worth noting that at locations 2 and 3, nettleleaf did not exhibit a significant advantage in spike count compared with that of 'Red Compact'.

When assessing the height of the plant hybrids across the three locations, clear differences in performance were noted among the parent plants and hybrids (Supplemental Table 3). At location 1, all three hybrids demonstrated significantly greater heights compared with that of 'Red Compact'. However, these heights were not statistically significant when compared with that of nettleleaf. Moving to location 2, all three hybrids were significantly shorter than nettleleaf, but significantly taller than 'Red Compact'. At location 3, UF-NRC-1 stood out by being significantly taller than both parent plants, while UF-NRC-2 and UF-NRC-3 were only significantly taller than 'Red Compact'.

When it comes to plant width, the three hybrids generally showed no significant increase over nettleleaf, but they were significantly

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Table 2. Seed production of porterweed (*Stachytarpheta* sp.) accessions grown in the greenhouse and field in Balm, FL, USA, in 2022.

	Flower cavities counted (no.)		Seeds produced (no.)[i]		Seed set (%)	
Porterweed	Greenhouse	Field	Greenhouse	Field	Greenhouse	Field
UF-NRC-2	316	636	0 b	319 b	0 c	50.1 b
UF-NRC-1	314	528	0 b	328 b	0 c	61.6 b
UF-NRC-3	309	598	1 b	280 b	0 c	47.1 c
Nettleleaf	336	673	211 a	659 a	62.6 b	98.0 a
Red Compact	331	242	278 a	238 b	83.8 a	98.4 a

[i] Means with the same letter within the column are not significantly different according to the Tukey honestly significant difference procedure at $P \leq 0.05$.

Fig. 3. Plants of the resident porterweed species (*Stachytarpheta cayennensis*) in the 2022 replicated field trials in Balm, FL, USA, on 26 Sep 2022. *Stachytarpheta cayennensis* was very vigorous, with an upright growth habit and low flower coverage and foliage near the base of the plant.

wider than 'Red Compact' (Supplemental Table 3). At location 2, both UF-NRC-2 and UF-NRC-3 were significantly wider than both parent plants, while UF-NRC-1 was only significantly wider than 'Red Compact'. A similar trend was observed at location 3. Here, UF-NRC-1 and UF-NRC-3 were significantly wider than both nettleleaf and 'Red Compact', while UF-NRC-2 was only significantly wider than 'Red Compact'.

Among all the seed collected and sown from the experimental plots, nettleleaf demonstrated the highest germination percentages (Table 3), with 66%, 56%, and 82% of seeds germinated from locations 1, 2, and 3, respectively. This was followed by 'Red Compact', which had much lower germination percentages of 13%, 10%, and 23% across the same three locations, respectively. Strikingly, none of the seeds sown from the three hybrids germinated.

Over a span of 2 full weeks, 311 flowers from UF-NRC-1, 291 from UF-NRC-2, and 308 from UF-NRC-3 were hand-pollinated using pollen from the native species, jamaican porterweed (Table 4). Additionally, 304 flowers of jamaican porterweed were pollinated using anthers from NRC-2. The yield from these pollinated flowers resulted in the harvest of 92, 57, 78, and 0 seeds from UF-NRC-1, UF-NRC-2, UF-NRC-3, and jamaican porterweed, respectively. Despite these efforts, none of the sown seeds germinated. Further investigation using tetrazolium seed staining revealed that embryos were aborting their development approximately 2 weeks after pollination. Immature seeds examined at 3 and 4 weeks post-pollination displayed shriveled embryos that did not take-up the stain (Fig. 7).

Fig. 4. Seed of the resident porterweed species (*Stachytarpheta cayennensis*) (left) and *Stachytarpheta* × 'UF-NRC-2' (right) collected from 2022 field trials in Balm, FL, USA. Seed of *Stachytarpheta cayennensis* was dark black and slightly larger than the brown seed of 'UF-NRC-2'. Scale bar = 5 mm.

Discussion

The primary objective of this research was to develop interspecific hybrids that were morphologically and functionally equal to or better than the invasive nettleleaf porterweed yet sterile to prevent environmental risks associated with invasiveness or genetic contamination of native species. The results largely support the success of these objectives, especially in terms of morphological attributes and sterility, and underscore the potential to replace nettleleaf porterweed in the market.

The successful cultivation of three interspecific hybrids (UF-NRC-1, UF-NRC-2, and UF-NRC-3) with distinct lavender flower colors demonstrated that the hybrids could be differentiated from nettleleaf porterweed in the wild and could potentially offer esthetic advantages. This feature could be particularly beneficial for horticultural applications for which visual appeal is a priority.

The three hybrid breeding lines showed performance comparable to the invasive nettleleaf in the 2022 greenhouse trial. With leaf size and shape similar to those of nettleleaf, the hybrids maintained the overall desirable foliage. Surprisingly, the hybrids showed shorter internodes than those of nettleleaf, which gave the plants a fuller compact appearance, which is a common advantageous trait of ornamental plants. The increased length of inflorescences further enhanced the flowering capability of these hybrids, allowing them to produce more flowers per spike while already producing more inflorescences than those of their parents.

The genome size estimated using flow cytometry for nettleleaf porterweed was in agreement with that previously reported (2.81 pg/2C) by Qian et al. (2021). While a genome size was not reported, Wilson et al. (2009) identified 'Red Compact' as a possible diploid. This claim was confirmed in this study with a nuclear DNA content of 1.42, which is approximately half that of the tetraploid nettleleaf. Qian et al. (2021) reported a strong correlation ($R^2 = 0.9831$) between chromosome number and DNA content, allowing the assumption of diploidy to be reasonable. The three hybrids had nuclear DNA content directly between those of the two parents, indicating triploidy.

Acetocarmine pollen staining indicated surprisingly high pollen stainability of the two parents in this study, which is likely indicative of their ability to set seed in the wild. All three hybrids, however, had extremely low pollen stainability, with pollen grains being deformed and misshaped, which further confirmed low male fertility.

After 2 months in an open shade house, the low male fertility was observed firsthand because the three hybrids were unable to produce seed. Nettleleaf and 'Red Compact' were still able to produce a significant amount of seed, likely because of self-pollinations with their fertile pollen because pollinator abundance was not significantly high based on the observations.

Moving the plants to an open field setting in Fall 2022 allowed the plants to exponentially increase in size and attract a suite of pollinators. The field study provided valuable insights regarding the morphological advantages of these hybrids. They were similar to or surpassed nettleleaf porterweed in several parameters such as plant width, inflorescence length, and plant ratings. These traits point to their potential utility in horticultural contexts, where these hybrids could serve as desirable alternatives to nettleleaf porterweed.

Both nettleleaf and 'Red Compact' managed to produce a seed from nearly every

Table 3. Seed germination of porterweed (*Stachytarpheta* sp.) accessions grown in a field setting in Balm, FL, USA, in 2022 and in an outside pot trial in Balm, FL, USA, and Gainesville, FL, USA, 2023.

	Seeds sowed (no.)		Seeds germinated (no.)		Seed germination (%)	
Porterweed	2022	2023	2022	2023	2022	2023
UF-NRC-2	200	1500	0	0	0	0
UF-NRC-1	200	1500	0	0	0	0
UF-NRC-3	200	1500	0	0	0	0
Nettleleaf	200	1500	177	1021	88.5	68.1
Red Compact	N/A	1500	N/A	230	N/A	15.3

N/A = not available.

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Fig. 5. The 2023 replicated porterweed pot trials in Balm, FL, USA, on 26 Apr 2023. ‘UF-NRC-2’ (*Stachytarpheta* ×) seen on the left, ‘Red Compact’ (*Stachytarpheta microphylla*) in the center, and nettleleaf porterweed (*Stachytarpheta cayennensis*) on the right. These plants were evaluated monthly (March–June) for plant and flower performance and seed production.

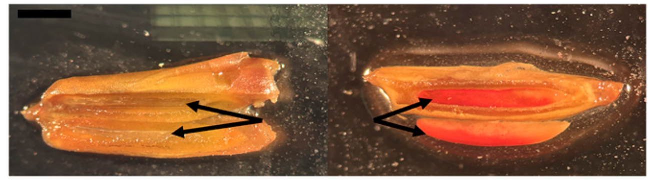

Fig. 7. *Stachytarpheta* × ‘UF-NRC-2’ (left) and the resident porterweed species (*Stachytarpheta cayennensis*) (right) developing porterweed seeds 4 weeks after pollination with *Stachytarpheta jamaicencis* pollen and stained with 1.0% tetrazolium at 35 °C for 18 h. Red indicates embryo viability in seed. No viable embryos are observed in NRC-2 seeds. Arrows indicate the seed embryo. Scale bar = 1 mm.

flower observed, which was attributable to the invasive potential of these non-native species. Surprisingly, while at a significantly lower frequency than that of nettleleaf and ‘Red Compact’, the three hybrids managed to produce a seed (without viable embryos) on approximately 50% of the flowers observed. This finding was unexpected because, in other crops, seed development often fails soon after pollination or does not occur at all on triploid plants (Varoquaux et al. 2000).

The 2022 field germination study provided encouraging results regarding the sterility of the three triploid hybrids. Of 600 seeds collected across these hybrids, not a single one germinated, strongly suggesting a fundamental issue in seed viability. The results of a further analysis, which revealed shriveled (or aborted) embryos within these seeds, corroborated this hypothesis and called for additional investigations. In stark contrast, the nettleleaf genotype exhibited an impressive 89% seed germination rate. Because this species not only produces seeds from nearly all its flowers but also boasts such a high germination rate, the mechanisms underlying its invasive potential in various ecosystems have become glaringly apparent.

Fig. 6. A plant of *Stachytarpheta* × ‘UF-NRC-2’ porterweed in the 2023 raised bed field trials in Balm, FL, USA, on 30 May 2023.

The 2023 pot and field trials demonstrated that the hybrids consistently performed well across multiple locations, often surpassing both nettleleaf and ‘Red Compact’ in various metrics. Notably, the hybrids were more compact than nettleleaf in all tested locations, and they also exhibited an increased number of spikes and greater flower density. These characteristics enhance their appeal for broad horticultural adoption, thus raising the possibility that they could become the preferred choice over the invasive nettleleaf. Importantly, while the triploid hybrids did produce seeds in all 2023 trials, the embryos were aborted and shriveled, and none of these seeds germinated, reaffirming their female sterility.

In addition to evaluating the self-reproductive capabilities of the triploid hybrids, it was crucial to examine their ability to cross-pollinate with the native jamaican species. As was the case in the field trials, the triploids produced seeds, but those seeds were not viable. Evidence of successful pollination was seen because the embryos were present in early development, but they degenerated over time, as is seen in sugar apple (*Annona squamosa*) (Dos Santos et al. 2014). The inability of the triploids to successfully cross-pollinate with native species underscores their environmental safety and aligns with the primary objective of minimizing ecological risks while developing functionally superior hybrids.

In summary, this study successfully achieved its primary objective of developing triploid interspecific hybrids that are both morphologically and functionally comparable to or even superior to the invasive nettleleaf porterweed while displaying reproductive sterility. The hybrids exhibited consistent performance across diverse environments and conditions, ranging from controlled greenhouse settings to open field and pot trials. Most notably, the lack of viable seed production in both self-pollination and cross-pollination experiments substantiates their sterile nature, thus addressing ecological concerns related to invasiveness or potential genetic contamination of native species. The breeding line ‘UF-NRC-2’ has been released as a cultivar and is available for licensing through the Florida Foundation Seed Producers Inc. (http://ffsp.net/). This hybrid was selected based on its consistent plant performance across multiple years and environments. ‘UF-NRC-2’ was also highly favored because of its superior spike production, which surpassed that of all other lines and parent plants (Supplemental Table 3). The findings from this study offer promising avenues for the future development of environmentally responsible yet esthetically appealing ornamental plants.

Table 4. Hybridization potential of porterweed (*Stachytarpheta* sp.) accessions with *S. jamaicensis.* Hand pollinations were made in a greenhouse in Balm, FL, USA, in Apr 2023.

	S. jamaicensis as the maternal parent			*S. jamaicensis* as the paternal parent		
Porterweed	Flowers pollinated (no.)	Seed set (%)	Seedling emergence (%)	Flowers pollinated (no.)	Seed set (%)	Seedling emergence (%)
UF-NRC-2	304	0	0	291	19.6	0
UF-NRC-1	299	0	0	311	29.6	0
UF-NRC-3	287	0	0	308	25.3	0
Nettleleaf	280	52	67.8	285	64.1	51.2

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