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Brief Report

Landscape and Fruit Evaluation of Three Privet (*Ligustrum* sp.) Cultivars in Florida

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Abstract: Chinese privet (*Ligustrum sinense*) has escaped cultivation in 20 states in the USA and is classified as a noxious weed in Florida, where its use is prohibited. There is confusion among producers and consumers surrounding the invasive status of its cultivars. In Florida, cultivars of an invasive wildtype species are also invasive unless proven otherwise and exempted from an invasive ruling. This study evaluated the fruiting and landscape performance of two cultivars of Chinese privet, 'Variegatum' (variegated privet) and 'Sunshine' (sunshine privet), and an interspecific hybrid of another form of privet, 'NCLX1' (*L.* × *vicaryi*, Golden Ticket®), planted in southwest, northcentral, and north Florida. 'Sunshine' and 'Variegatum' privet both performed well throughout the study with average quality ratings ranging from 3.2 to 4.9 (scale of 1–5) and a 100% survival rate during the nearly two-year study. 'NCLX1' privet also had a 100% survival rate at the southwest and northcentral sites, but 80% survival at the north Florida site. 'Variegatum' privet grew the largest in both height and width compared to 'Sunshine' and 'NCLX1' privet, that had similar heights. 'Variegatum' and 'NCLX1' privet flowered during the study, but 'Sunshine' privet did not. 'Variegatum' privet was the only cultivar evaluated to produce mature fruit and show clear signs of reversion back to its green wildtype form. The DNA content of all three cultivars suggests they are diploids, as is the wildtype form of Chinese privet. These results provide potential alternatives to Chinese privet for use in landscapes but recommend avoiding 'Variegatum' privet, due to its frequent reversion to the wildtype and its abundant fruit production.

Keywords: privet; invasive plants; ploidy; woody shrubs

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1. Introduction

Although most introduced ornamental plants do not escape cultivation, some plants spread into natural areas, develop self-sustaining populations, and subsequently disrupt the function and form of natural ecosystems [1]. In the past decade, significant progress has been made by the ornamental plant industry to minimize the risk of invasive plant introductions [2]. Voluntary codes of conduct have been adopted nationally by botanic gardens and the horticulture trade to help reduce the pathway of invasive plants [3]. Simultaneously, plant breeders worldwide have been looking for and developing new cultivars with significantly reduced or eliminated invasive potential that can replace invasive ones [4]. For example, sterile cultivars have been developed for popular landscape plants such as heavenly bamboo (*Nandina domestica*) [5], Mexican petunia (*Ruellia simplex*) [6], and lantana (*Lantana camara*) [7], providing safe alternatives for landscapes and gardens, with large commercial companies such as Ball Horticulture and Proven Winners now incorporating these non-invasive traits into their marketing campaigns [2].

Another popular ornamental plant in need of sterile cultivar replacements is privet (*Ligustrum* sp). These evergreen shrubs have dark green foliage and white, slightly fragrant flowers in the summer. With a USDA cold hardiness zone between 7 and 11, privets are versatile in a range of landscapes, and are known for having high drought tolerance, disease resistance, fast growth, responsiveness to pruning, and tolerance to a variety of growing conditions. Despite their ornamental appeal, several types of privet are now considered invasive, as determined by the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) Status Assessment of Non-native Plants in Florida's Natural Areas, including Chinese privet (*Ligustrum sinense*), glossy privet (*Ligustrum lucidum*), and Japanese privet (*Ligustrum japonicum*) [8]. Among these, Chinese privet has caused the greatest concern due to its widespread impacts on natural areas of the southeast and the threat of its expansion globally. First introduced to the USA from China in 1852 and naturalized as early as 1900, Chinese privet has escaped in 20 states in the USA (predominately in the southeast), including 31 counties in Florida, and its growth/sale/transport is now forbidden in Florida [8]. Spreading through both root suckers from its extensive root system and through fruit dispersal, a single plant can produce an average of 2800 fruit per stem [9] creating a relatively transient seed bank with germination with little dependence on the liberation of seeds from their fruit [10]. More than two-thirds of the seeds can germinate within 60 days under a range of controlled seasonal temperatures, independent of light [11].

Cultivars of invasive plants are also considered invasive in Florida unless exempted from the noxious weed ruling or proven otherwise through an Infraspecific Taxon Protocol (ITP) assessment [2]. Wilson et al. [11] evaluated 12 privet selections in northern and southern Florida and found low or no fruit production among cultivars (with novel traits and/or foliage color) compared to their respective wildtype or resident taxa. Among these selections, 'Swift Creek' Chinese privet (*Ligustrum sinense* 'Swift Creek') showed promise, with 99.8% fruit reduction in north Florida and 100% in south Florida when compared to the green wildtype form without evidence of chimeral breakdown (reversion). Thus, sterile cultivar development and evaluation can play a prominent role in identifying safe alternatives to ornamental invasives. Large-scale commercial companies are now incorporating these non-invasive traits into their marketing campaigns. The objectives of this study were to: (1) determine the effects of location and genetics on the landscape performance, growth, and fruiting of three privet cultivars, and (2) determine the ploidy of these cultivars in order to assess invasion risks.

2. Materials and Methods

2.1. Plant Material and Site Locations

Three privet cultivars, 'Sunshine', 'Variegatum', and 'NCLX1', finished in 11.4 L pots, were evaluated in this study, as described in Table 1. The experiments were conducted in southwest Florida [Gulf Coast Research and Education Center (GCREC), Balm], northern Florida [North Florida Research and Education Center (NFREC), Quincy], and northcentral Florida [Plant Science Research and Education Unit (PSREU), Citra], as described by Wilson and Deng [2]. Before planting, the beds were slightly disked and covered with black semipermeable landscape fabric (Lumite Inc., Baldwin, GA, USA). The plants were spaced 2.33 m from the center and grown in full-sun conditions at each location. Plants were drip irrigated at least once or twice each day for 35 to 60 min as needed for each site and fertilized with approximately 84 g of 15N–3.9P–10K 8–9 month controlled-release fertilizer (Osmocote Plus; Scotts, Marysville, OH, USA). The maximum and minimum daily temperature at two meters, total rainfall, and relative humidity were recorded on site by the Florida Automated Weather Network (FAWN https://fawn.ifas.ufl.edu accessed on 22 March 2021). Prior to planting, soil samples were collected from each row at each site, mixed for uniformity, and air dried for standard analysis (UF Extension Soil Testing Laboratory, Gainesville, FL, USA). The initial potassium (K), phosphorous (P), magnesium (Mg), and calcium (Ca) of soils based on Mehlich-3 extraction indicated sufficient nutrient ranges at all three field sites. The field conditions in southwest Florida were as follows: 2.14% organic matter, pH 6.35, electrical conductivity (EC) 0.05 dS/m, average monthly rainfall 11.43 cm, average monthly relative humidity 79.4%, average monthly temperature 25.8 °C, average monthly minimum temperature 21.5 °C, and average monthly maximum temperature 28.8 °C. The field conditions in northcentral Florida were as follows: 1.01% organic matter, pH 5.65, EC 0.10 dS/m, average monthly rainfall 9.7 cm, average monthly relative humidity 81.1%, average monthly temperature 25.4 °C, average monthly minimum temperature 18.6 °C, and average monthly maximum temperature 33.1 °C. The field conditions in northern Florida were as follows: 2.1% organic matter, pH 5.35, EC 0.07 dS/m, average monthly rainfall 13.97 cm, average monthly relative humidity 80.6%, average monthly temperature 20.8 °C, average monthly minimum temperature 13.7 °C, and average maximum temperature 28.5 °C.

Table 1. Cultivar and trade name, description, and nursery source of three privet taxa evaluated for landscape performance, fruiting, and ploidy level.

Taxa	Cultivar Name	Common Name	Description and Plant Patent (PP)
Ligustrum × vicaryi	'NCLX1'	Golden Ticket® privet	Glossy foliage emerges as bright yellow and changes to chartreuse as it matures. Product of the North Carolina State University breeding program. Interspecific hybrid of *L. × vicaryi* and *L. tschonoskii* 'Little Thomas'. Patented in 2014 (PP27301P3) and trademarked as Golden Ticket).
Ligustrum sinense	'Sunshine'	Sunshine privet	Discovered by Thomas McCracken in 2002, Zebulon, NC. Naturally occurring sport of unnamed *Ligustrum sinense*. Bright yellow foliage that turns a medium green color in the shade. Patented in 2007 (PP20379P2) and marketed as part of the Southern Living® plant collection.
Ligustrum sinense	'Variegatum'	Variegated privet	Leaves have creamy white margins surrounding a slate-green center.

2.2. Visual Quality, Plant Size, and Reversion

Assessments of visual quality (plant performance) were performed by the same individual at 3-month intervals at each site on a scale from 1 to 5, where 1 = very poor quality, severe leaf necrosis; 2 = poor quality; 3 = fair quality, adequate color and form; 4 = good quality; and 5 = excellent quality and premium color and form. Averaged among the 21 months of the experiment, means are presented in Table 2. Final plant heights and widths were recorded at the end of the experiment (21 months), and growth indices were calculated as an average of the measured height (measured from crown to natural break in foliage) and two perpendicular widths [height + (width 1 + width 2)/2]. Notation of any phenotypic reversion (chimeral breakdown to green tissue) of cultivars was recorded every month.

Table 2. Mean quality, final width, height, growth index, total fruit number (n = 5), reversion percentage, and nuclear DNA content of three privet cultivars grown in southwestern (Gulf Coast Research and Education Center, Balm, FL, USA), northcentral (Plant Science Research and Education Unit, Citra, FL, USA), and northern (North Florida Research and Education Center, Quincy, FL, USA) locations for 21 months.

Cultivar Name	Mean Quality ^{w} ± SE (1–5 Scale)	Width (cm)	Height (cm)	Growth Index (cm) ^{x}	Total Fruit No.	Reversion (%) ^{y}	Nuclear DNA Content ± SD (pg/2C) ^{z}	Proportion of Plants That Flowered (%)
				Southwest				
'NCLX1'	3.23 ± 0.22 b	63.67 c	114.00 b	88.84 b	0	0	2.85 ± 0.06	100
'Sunshine'	4.80 ± 0.08 a	112.20 b	68.60 c	90.40 b	0	0	2.66 ± 0.05	0
'Variegatum'	4.80 ± 0.08 a	265.50 a	183.40 a	224.45 a	0	20	2.72 ± 0.07	20
				Northcentral				
'NCLX1'	3.60 ± 0.22 b	71.86 c	101.20 b	86.53 b	0	0	--	100
'Sunshine'	4.94 ± 0.04 a	129.10 b	112.40 b	120.75 b	0	0	--	0
'Variegatum'	4.71 ± 0.11 a	230.10 a	152.00 a	191.05 a	0	60	--	100
				North				
'NCLX1'	3.05 ± 0.23 b	56.19 c	92.28 ab	74.23 b	0	0	--	100
'Sunshine'	4.40 ± 0.15 a	97.82 b	70.52 b	84.17 b	0	60	--	0
'Variegatum'	4.03 ± 0.20 a	133.71 a	129.06 a	131.39 a	2904	80	--	80

^{w} Mean quality over the course of 21 months on a qualitative scale (one to five) where one = very poor quality, two = poor quality, three = adequate quality, four = good quality, and five = excellent quality. ^{x} Growth index determined by (average of two perpendicular widths + height)/2. ^{y} A plant was considered reverted if the phenotypic green color of the wildtype was visible down to the base of a branch. ^{z} Nuclear DNA content was only tested at the southwest Florida location (GREC, Balm, FL, USA). The nuclear DNA content of the wildtype was 2.67 ± 0.04 pg/2C, and this was used as a reference. Different letters within columns for each site are significantly different according to Tukey–Kramer's honestly significant difference range test at $p \leq 0.05$.

2.3. Seed Viability

Each month, observations of flowering and fruiting were recorded for all plants at all sites. Before fruit ripening, mesh netting was placed over panicles to prevent predation. Mature fruit was manually collected and counted at each location. The mature fruit, each containing a single seed, was harvested and depulped by hand using a dehulling trough (Hoffman Manufacturing, Inc., Albany, OR, USA). Seeds were surface sterilized with a 20% sodium hypochlorite solution (6% active ingredient) for 20 min and then triple rinsed with distilled water. Viability tests were performed by an independent seed testing facility (US Forest Service National Seed Laboratory, Dry Branch, GA, USA) using a tetrazolium (TZ) staining test adapted from the Association of Official Seeds Analysts (AOSA) rules for Tetrazolium testing. Two replications of 50 seeds ('Variegatum' privet only) were cut laterally and stained overnight (12–18 h) at 37 °C in a 1.0% TZ solution. Seeds were considered viable when firm embryos were stained evenly red. X-ray analysis (Faxitron Ultrafocus, Tucson, AZ, USA) was used to non-destructively determine the presence of an embryo.

2.4. Nuclear DNA Content and Ploidy Level

Three leaf samples (biological replicates) were analyzed per cultivar, and the analysis was repeated three times. Flow cytometry was performed to analyze the DNA content and infer the ploidy. The methodology followed that reported by Wilson et al. [5], who reported inferred ploidy in heavenly bamboo (*Nandina domestica*). Briefly, leaf tissue (≈20 mg) was chopped in a nuclei isolation buffer, and the released nuclei were stained with 50 $\mu g \cdot mL^{-1}$ propidium iodide and 50 $\mu g \cdot mL^{-1}$ RNase (New England BioLabs, Ipswich, MA, USA) before analysis. The LB01 buffer contained 15 mM Tris, 2 mM Na2EDTA, 0.5 mM spermine tetrahydrochloride, 80 mM KCl, 20 mM NaCl, and 0.1% (v/v) Triton X-100 and was adjusted to pH 7.5. The isolated nuclei were analyzed using a Cyflow® Ploidy Analyser (Sysmex Europe GmbH, Norderstedt, Germany) cytometer for fluorescence intensity using tomato (*Lycopersicum* 'Stupické polní rané') (2C nuclear DNA content = 1.96 pg/2C) as the internal reference.

2.5. Experimental Design and Data Analysis

The field experiments utilized a randomized complete block experimental design that was randomized separately for each site (n = 45). There were five blocks and three treatments (three cultivars of privet) at each of the three locations (southwest, northcentral, and north, FL). Data were analyzed using R (R.3.5.2, The R Foundation, Vienna, Austria) and RStudio (R 1.1.463, Boston, MA, USA) linear mixed effects-models assuming normally distributed data. The assumptions for linear models were confirmed via QQ plots and plotting model residuals. No model selection was used, as this was a planned experiment. Quality ratings measured across the entire experiment were modeled in response to cultivar, location, sampling interval, and all possible interactions with the plot nested within the block was treated as a random effect. The height, width, and growth index at the end of the experiment were modeled in response to the cultivar, location, and cultivar*location interaction, with the experimental block being treated as a random effect. For all response variables, Tukey's HSD was then used to detect differences among treatment levels ($p \leq 0.05$) of statistically significant model terms.

3. Results and Discussion

3.1. Landscape Performance

All three types of privet were tolerant to the three different site conditions included in this study. The main effects of visual quality ratings were statistically significant for location (F2,32 = 15.38; $p < 0.001$) and cultivar (F2,32 = 71.62; $p < 0.001$), but the interaction between these two terms was statistically non-significant (F12,211 = 15.38; $p < 0.497$). On average, the visual quality ratings of plants evaluated in this study ranged from 3.23 to 4.80 (southwest), 3.60 to 4.94 (northcentral), and 3.05 to 4.03 (north) (Table 2). Regardless of location, visual quality ratings were similarly high for 'Sunshine' and 'Variegatum' privet, and 37–43% higher than that of 'NCLX1' privet. The peak performance of 'NCLX1' privet was seasonally influenced, with the highest visual quality ratings occurring from late fall to the early winter months. This may suggest that Florida is the southern extreme of its preferred growing range.

Two factors may have contributed to the lower visual quality ratings of 'NCLX1' privet. First, the containers were filled with a single plant per pot rather than three plants per pot, which is often done in the industry for a fuller appearance. This influenced the plants' overall form, as the plants grew more vertical than wide, with some irregularity. Second, during the winter months, the plants lost 75% or more of their foliage, regardless of location. The 'NCLX1' privet is regarded as deciduous, and even in Florida, we found it to be semideciduous.

3.2. Plant Size and Growth

The final plant heights, widths, and growth indices varied among cultivars and locations (Table 2) with significant main effects ($p \leq 0.001$) for each measured trait and significant interactions for perpendicular widths (F4,33 = 13.28; $p \leq 0.001$), height (F4,33 = 5.46; $p \leq 0.002$), and growth index (F4,33 = 9.73; $p \leq 0.001$) (Table 2). Across locations, plant widths of 'Variegatum' privet were 1.3 to 1.9 times greater than those of 'Sunshine' privet and 1.6 to 3.4 times greater than those of 'NCLX1' privet. Similarly, 'Variegatum' privet grew taller than 'Sunshine' privet at each of the three locations. 'NCLX1' and 'Sunshine' privet reached similar heights in northcentral and north FL. Throughout much of the study, the plant sizes (growth index) of cultivars grown in northcentral FL were slightly greater or similar to those of cultivars grown in southwest FL (Table 2). The 'Sunshine' privet had a domed shape with compact branching, making it a desirable foundation plant. The 'NCLX1' privet had more vertical than horizontal growth, and the foliage was not densely packed. In contrast, ‘Variegatum’ grew very wide, indicating that it may need more regular trimming to keep it from overtaking an area.

3.3. Flowering, Fruiting, and Seed Viability

At each of the three locations, flowering was observed for ‘Variegatum’ and ‘NCLX1’ privet, but not for ‘Sunshine’ privet. Flowering typically began in May and continued until August. However, fruiting was only observed for ‘Variegatum’ privet and only in the northern most location, where 2904 fruit were collected in the final year of the study (Table 2). Surprisingly, only 4.0% of the seeds collected from north FL were viable, with less than a quarter (23%) having embryos present. This contradicts a prior study, in which ‘Variegatum’ privet planted in north FL had 78.5% germination [11], suggesting that geographical and abiotic factors may play a role in the embryo development of this cultivar. Low viability could be associated with the presence of a seed-attacking ligustrum weevil (*Ochyromera ligustri*) that has been documented attacking Chinese privet in Florida [12]. Lack of fruiting of ‘Sunshine’ and ‘NCLX1’ privet indicate these cultivars are female-sterile and may serve as acceptable alternatives to both the variegated and wildtype privet.

3.4. Reversion, DNA Nuclear Content, and Ploidy

The chimeral integrity of ‘Variegatum’ cultivars is a particular concern when it comes to its status as an invasive species, as is the trueness to type [11]. ‘NCLX1’ maintained its chartreuse foliage color throughout the study. It was noted that towards the latter part of the study, several of the ‘Sunshine’ privet plants had solid green leaves rather than the typically yellow-green foliage. However, fruit was never observed on the green tissue, so it was not confirmed to have reverted to wildtype characteristics. In contrast, as early as one month after installation, ‘Variegatum’ privet showed clear signs of green reversion as a result of chimeral breakdown. In fact, by the end of the study, 80%, 60%, and 20% of ‘Variegatum’ privet plants reverted back to the green wildtype form, in northern, northcentral FL, and southwestern locations, respectively (Table 2). Regardless of fruiting ability, the likelihood of variegated privet reverting is high; therefore, this is not a cultivar we recommend for planting. A link between polyploidy and invasive predisposition has been suggested with invasiveness being 12% more likely as the chromosome number doubles, and 20% more likely for polyploids compared with diploids [13]. In this study, regardless of the absence or presence of fruit, nuclear DNA content ranged from 2.66 to 2.85 pg/2C among cultivars compared to the wildtype (2.67 pg/2C) (Table 2). This indicates that all cultivars evaluated in this study are diploids and that polyploidy does not seem to be the cause of female infertility.

4. Conclusions

Our study addressed the female fertility of three privet cultivars common in nursery production. The data presented herein suggest two cultivars have non-invasive traits that could serve as alternatives to the invasive wildtype form of privet, if approved by the Infraspecific Taxon Protocol developed for Florida [8]. This information, along with prior successful tetraploid induction of wildtype privet [14], offers effective approaches to combatting the invasiveness of ligustrum (and other plants with invasion potential) through the development, evaluation, and selection of non-invasive traits. In future work, marker analysis of the identified non-invasive cultivars and other available ligustrum cultivars may enable a better understanding of the genetic diversity within cultivated ligustrum and potential identification of molecular markers associated with non-invasive traits.

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