Philippine Journal of Science
149 (3-a): 935-946, October 2020
ISSN 0031 - 7683
Date Received: 20 Apr 2020

Growth Response of *Thunbergia grandiflora* (Roxb. ex Rottler) Roxb. (Skyflower) on Shading and Anatomical Characteristics Under Various Soil Series

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Thunbergia grandiflora **Roxb. (Acanthaceae) or "skyflower" is an exotic species that was reported to be aggressively spreading out of control in the tropical and subtropical ecosystems. This study assessed the invasive characteristics of the species by analyzing its growth characteristics on varying shading intensity and soil series, as well as anatomical characteristics in varying soil series. Growth characteristics include the height and root collar diameter (RCD), while anatomical parameters include the thickness of all dermal, ground, and vascular tissues. Based on the results of the shading experiment,** ***T. grandiflora*** **grew significantly in terms of height and RCD under full light conditions (1784.75 lx). The species can survive intense light condition but is suppressed under partial to fully shaded treatments. In the soil series experiment, the highest growth and RCD were observed in the Luisiana soil series with strongly acidic soil (4.1 pH). The leaf and stem anatomical structures of** ***T. grandiflora*** **have the characteristics typical of invasive species that can adapt to certain harsh conditions. This includes the presence of dense non-glandular trichomes, cystolith, multi-layered collenchyma cells in the hypodermis, sclerenchymatous tissues, wider xylem pores, and closely pack parenchyma cells in the stem. Therefore, anatomical structures of this species suggest its ability as an invasive species. Consequently, the invasive characteristics can be suppressed by controlling the shade and soil conditions during the wildling stage of the species. The species can be bioinvasive and the extent may depend on the prevailing conditions in the site. However, further researches on the species' root physiological and anatomical responses to other biotic and abiotic factors in the environment are recommended. Also, knowing the invasiveness of** ***T. grandiflora*** **will help us identify possible strategies to limit the spread of the species, including early detection of the occurrence of species, avoiding disturbance in the area, and identifying species as biocontrol.**

Keywords: anatomical characteristics, bioinvasive, exotic species, growth response, shade intensity, soil series

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INTRODUCTION

Forests are considered an asset and natural wealth of every country in the world. The diverse mix of plants and tree species provides stability and dynamism for an ecosystem to function efficiently on a sustainable basis. However, the continuous entry and invasion of non-native or exotic species are tumultuously growing worldwide. Various studies reported that non-native species damage natural landscapes and suppress native plant communities through biological invasion.

Bioinvasion is defined as the proliferation of introduced species to distant ranges where their descendants proliferate, spread, and persist (Mack *et al.* 2000). Many bioinvasion cases have long been reported to alter many natural ecosystems and the species involved represent a vast array of taxonomic categories and geographic distribution (Thompson *et al.* 1995; Facon *et al*. 2006; Barbier 2007; Wardle and Peltzer 2017). The dramatic spread of non-native species worldwide has been facilitated by association with domestication and trade (*e.g.* introduction, seed contamination, horticultural trade), ability to disperse along with regional transport networks (*e.g*. roadsides, forest, canals, railways), and capacity for local colonization and population increase (Pysek and Hulme 2005). *Thunbergia grandiflora* or skyflower of the family Acanthaceae is considered an invasive species and it has been supported by many works of literature (Sarmiento *et al.* 2016; IUCN 2020; CABI 2020). It is native to the Indian subcontinent, southern China, and Myanmar and is mainly used as an ornamental and medicinal plant, green manure, poles, hedges, and fuelwood (Lusweti *et al.* 2011). It has been reported engulfing the natural habitats of Africa, Central America, the West Indies, and the numerous islands in the Pacific (PIER 2012). It has also been classified as a noxious weed in Australia (QDPIF 2007). In the Philippines, it has found to occur in Luzon, Visayas, and Mindanao (GBIF 2020). If all these events occur and management strategies will not be applied, chaos and trouble among native species' diversity will level down to extinction. Pysek and Hulme (2005) reported that bioinvasion is an emerging problem in both artificial and natural ecosystems because only a few of these ecosystems bear the immunity of having invaded. Anthropogenic causes include greater trade, transport, travel, and tourism brought by globalization that facilitate the introduction and spread of species that are not native to an area (CBD 2009), while natural causes include different environmental catastrophes (*e.g.* tsunami, flooding, *etc.*) brought by the changing climate (Hoffmann and Courchamp 2016).

In the Mount Makiling Forest Reserve (MMFR), the vine skyflower (*Thunbergia grandiflora* R.) is now engulfing natural vegetation and some forest plantations,particularly in its lower slopes and vicinities, within the Molawin-Dampalit (1,626.10 ha) and Cambantoc (1,136.31 ha) subwatersheds. There was a study on the morpho-ecological characteristics of *T. grandiflora* in the Philippines but there is no detailed study yet on its leaf and stem anatomical characteristics, perhaps even in other areas in the world where the species occur. Further, its presence to both natural and plantation stands is now becoming a severe problem in the field of forest management in the country (Baguinon *et al*. 2003). Therefore, it is hypothesized in this study that *T. grandiflora* can grow even under the shade, as the species is said to be prolific and invasive species. Hence, there is a need to study the factors that have a possible significant influence on its growth and survival.

The objectives of the study were to examine the effects of shading and soil series on the growth response of *T. grandiflora*, and to characterize its leaf and stem anatomical structures in varying soil series.

Thunbergia grandiflora is introduced and is called as invasive species in the country (Baguinon 2011; Sarmiento *et al.* 2016). In this study, together with examining the leaf and stem anatomical characteristics, the growth responses of the species to different shading intensity and soil characteristics can serve as a timely and pioneering study in the field of bioinvasion in the country. Light suppression often serves as a key to slow down the growth of invasive plant species (Valladares *et al.* 2016). *Thunbergia grandiflora* can easily thrive in forest gaps and open areas in particular (DAF Queensland 2007). Another factor that contributes to its invasiveness is the alteration of soil pH and soil organic matter content (Gandal and Joshi 2014). Consequently, knowledge of these aspects may enable a deeper understanding of the species' adaptation mechanisms; hence, such a study may give insights on some possible control technologies for the species.

MATERIALS AND METHODS

Two separate experiments on shading and soil series were conducted with four treatments each in a similar location.

Study Area and Plant Material

There is difficulty in looking for seeds and seedlings of *T. grandiflora* throughout the country. However, wildlings of the species are conspicuously abundant in some portions of MMFR, one of the megadiverse tropical rainforests in the Philippines. The study was carried out within a portion of the BINHI Biodiversity Park (elevation: 91 masl; coordinates: N 14°9'16.20", E 121°14'13.20") at the back of the Department of Forest Biological Sciences of CFNR-UPLB from June to September 29, 2015 (wet season). The location experiences a Type I climate (tropical monsoon) with two pronounced seasons: a wet season from May to December and a short dry season from January to April. Heavy rainfall occurs between July and September, with a maximum of 24.3–39.6 cm per month and average annual rainfall and temperature of 210.3 cm and 31.9 °C, respectively (based on data for 2013–2015 collected at the UPLB National Agrometeorological Station). In this study, young wildlings of *T. grandiflora* measuring 11–12 cm high were collected. Wildlings were uprooted to obtain uniform RCD and height measurement. The selection of samples within the MMFR was based on the presence of tree host plants with abundant wildlings on the ground. Only straight wildlings without any deformations – such as leaf defoliation, leaf rolling, and the like – were considered on the nearest fully grown skyflower. After two weeks of replanting the wildlings in polyethylene bags to acclimatize in the pot environment, initial measurements on RCD and height were done and then daily during the experiment. Across treatments, wildlings were watered every 8 AM.

Growth Response on Varying Shading Intensity and Soil Series

The experiment on shading intensity was established in a fully exposed to sunlight, no above tree canopy, and far from the walkway (undisturbed portion) of BINHI Biodiversity Park, CFNR-UPLB. Four shading levels served as the factor, which was no shading/full light (control, T1), partially exposed to light (T2), partially shaded (T3), and fully shaded (T4) conditions. The control treatment had 1,784.75 lx followed by the partially exposed to light with 588.23 lx, partially shaded with 113.50 lx, and the fully shaded with 38.25 lx. Treatments were located adjacent to each other. Except for control treatment, each treatment had a corresponding cubicle-like walling covered with plastic cover, which was holed (2 cm wide) to allow light penetration. The average light intensity for each treatment was obtained (10 AM and 3 PM) for two weeks using a light meter (Extech instruments – Light Meter 401025).

The growth response on the soil series experiment was also laid out in the same area where the shade experiment was conducted. Different levels of soil acidity were considered in selecting soil series. Soil samples were collected from different places – namely, Balujo, Cavinti, Laguna (Luisiana soil series, LUI); Bagumbayan, Tanauan, Batangas (Lipa soil series, LIP); Mt. Makiling Forest Reserve (Macolod soil series, MAC); and Bantog, San Miguel, Bulacan (Bantog soil series, BAN). According to Carating *et al.* (2014), the Luisiana soil series was formed from the physical and chemical weathered products of igneous rocks (of basalt and andesites) with thickness up to 200 cm, while the Lipa soil series was formed by the residual soil representing volcanic tuff material. It is dark brown, very friable, and easy to cultivate with thickness ranging from 25–35 cm. According to BSWM (2008), the Macolod soil series is tenacious, fine, and gravelly in texture at its surface level (sticky and compact when wet and granular when dry) with a depth of 30–60 cm. While the Bantog soil series has parent material from recent alluvium and coastal. The surface is brown sandy loam with a thickness of 25 cm. In each place, a 10 m x 10 m plot was established from which the soil samples (25 holes per treatment) were collected following a soil depth mark of 15–20 cm. Samples from each soil series were then prepared for soil pH and organic matter content analyses in the Soils Laboratory of the Institute of Renewable and Natural Resources, CFNR-UPLB (Table 1). The pot experiment was conducted using a completely randomized design (CRD). The size of the pot was 7.5 cm x 18 cm and was conducted for 3 mo. Wildlings were planted according to the soil series. A total of 25 wildlings were allotted to each of four soil series and arranged in five columns and five rows in full light condition.

Table 1. pH and organic matter (OM) content of soil series used in growing *Thunbergia grandiflora.*

Soil series	pH	Organic matter (%)
Luisiana (LUI)	4.1 (strongly acidic)	1.3782
Lipa (LIP)	5.4 (moderately acidic)	2.5833
Macolod (MAC)	6.0 (slightly acidic)	1.3782
Bantog (BAN)	5.8 (moderately acidic)	2.0977

Note: soil pH classification was based on DOST-PCARRD Philippine Recommends Series No. 36-D published in 2006.

In the shading intensity and soil series experiments, growth response parameters – namely, RCD and height – were measured using digital Vernier caliper (mm) and ruler (cm), respectively, every day for 3 mo. Signs and symptoms of fungal attack, diseases, abnormalities, and the like were also noted.

Anatomical Characteristics Measurement

In the soil series experiment, anatomical characteristics of *T. grandiflora* were observed. Transverse sections of the mid part of young stem and leaf (third fully expanded from the tip) were obtained, following the procedures of Johansen (1940). Specimens were randomly collected from each soil treatment. Samples were fixed in formalin: acetic acid: alcohol solution. Afterward, the samples were dehydrated in ethanol series. Embedding the samples into melted paraffin wax followed. Sections were cut at a thickness of 10–15 µm by a rotary microtome (HM 350 Microm, Heidelberg Germany). Sections were then stained with safranin and were counterstained with Fast Green dye solution. After staining, specimens were coated with the Entellan® solution. These slides were then air-dried to be ready for anatomical examination and analysis.

The freehand technique was also used. Leaf or stem sample was sandwiched to the middle of cassava pith and then sliced using a sharp razor blade. Thin cross-sections were then soaked in water in a petri dish.

Microscopic examination was done using a compound microscope (EUROMEX 1.3.0) at the Metallophytes Laboratory of the Department of Forest Biological Sciences, CFNR-UPLB. Photomicrographs were obtained using the compound microscope under 1000x and 400x magnification. Besides the identification of typologies of the structures, the thickness of all dermal, ground, and vascular tissues were measured.

Statistical Analysis

Descriptive statistics were used for the study. The mean growth per day of the wildlings was calculated. Analysis of variance (ANOVA) and Tukey's test were used to test the significance of the mean difference in height and RCD of wildlings of *T. grandiflora* across different shade intensity and soil series treatments. The Spearman's rank correlation test was used to explore the relationships among variables. All analyses were performed in R studio version 4.0.

RESULTS

Growth Response of *Thunbergia grandiflora* on Varying Shading Intensity

During the observation period, different responses across treatments were observed. ANOVA revealed significant differences in mean height and RCD on varying shading intensity treatments at α = 5% (Appendices I and II). Appendix III shows that the fully exposed to sunlight treatment (control, T1) obtained the highest mean height (86.95 cm) followed by partially exposed to sunlight treatment (T2) with 81.78 cm. The lowest was observed in partially shaded treatment (T3) with 75.02 cm and fully shaded treatment (T4) with 74.70 cm, as shown in Appendix IIIa. In terms of RCD, the control incurred the highest mean (1.42 mm) while the lowest was in the fully shaded (T4) with 1.26 mm (Appendix IIIb). Further, the mean RCD of partially shaded treatment (T3) is significantly lower compared to control treatment (Table 2)

Table 2. Tukey's test showing the mean difference in RCD of the four treatments of light exposure.

Treatment	diff	lwr	upr	p adj
T1–T2	0.716	0.06566508	1.3663349	0.0249760
T1–T3	1.040	0.38966508	1.690334	0.0003684*
T1–T4	0.684	0.03366508	1.3343349	0.0552986
T2–T3	0.324	–0.32633492	0.9743349	0.5635422
T2–T4	0.032	–0.68233492	0.6183349	0.9992341
T3–T4	–0.356	–1.00633492	0.2943349	0.4831520

*Denotes significance at ά = 5%

It can be deduced from these results that the amount of light may be one of the major factors for the growth of the species in terms of height and RCD. But correlations between shade intensity, height, and RCD of wildlings were not significant ($P = 0.291$ and $P = 0.384$, respectively).

In addition, the result in Appendix IV suggests that wildlings in T2 and T3 grow well in terms of height and RCD. In T4, wildlings grow well but discoloration, especially in leaves, was present. Some samples appeared to be even stunted. Chlorotic leaves were also present but were relatively small in size compared to the other treatments. The control (T1) showed no abnormalities (*e.g.* leaf discoloration), as observed in wildlings after the experiment. The plants grew well and produced relatively large leaves without any traces of curling and wilting.

Growth Response of *Thunbergia grandiflora* on Varying Soil Series

There were no significant differences in mean height on varying soil series at α = 5%. Appendix Va shows the mean height of all the treatments under different soil series. The Lipa soil series has the highest mean height, followed closely by the Macolod and Luisiana soil series. The Bantog soil series has the lowest mean height. In terms of RCD, results show that there is at least one mean RCD that is different among other treatments at α = 5% (Table 3; Appendix VI). This difference was observed between the Luisiana (strongly acidic) and Bantog soil series (moderately acidic) (Appendix Vb).

Leaf and Stem Anatomical Characteristics Grown in Different Soil Series

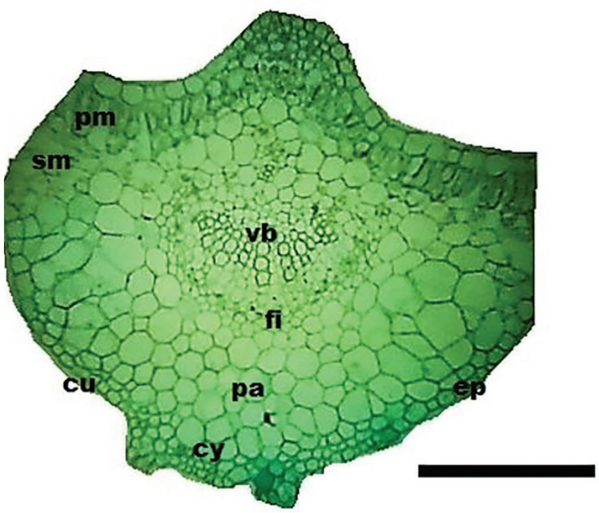
All leaves from all the treatments (Macolod, Lipa, Luisiana, and Bantog soil series) have single-layered upper and lower epidermis and are covered with a thick cuticle layer (11.52 µm) (Appendix VII). Leaf anatomical characteristics, *i.e.* in Macolod soil series, are shown in Figure 1.

Across treatments, the dorsiventral leaf, which is typical of most vascular plants, was observed. Palisade mesophyll is composed of single-layered, large, and elongated to

Table 3. Tukey's test showing the mean difference in RCD of four treatments of soil series.

Treatment	diff	lwr	upr	p adj
LUI-LIP	35.945806	–15.15659	87.04820	0.2558153
LUI-MAC	22.499570	–28.60283	73.60197	0.6506561
LUI-BAN	26.956774	–24.14562	78.05917	0.0046491*
LIP-MAC	–13.446237	–64.54863	37.65616	0.8979153
LIP-BAN	–8.989032	–60.09143	42.11336	0.9662130
MAC-BAN	4.457204	–46.64519	55.55960	0.9956098

*Denotes significance at ά = 5%



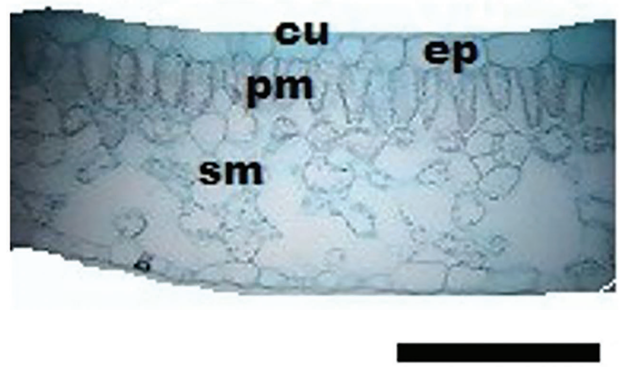


Figure 1. Transverse section of a leaf of *Thunbergia grandiflora* from Macolod soil series treatment showing (A) midrib and (B) leaf blade under 1000x. Abbr.: cu – cuticle, e – epidermis, cy – cystolith crystals, pa – parenchyma, sm – spongy mesophyll, pm – palisade mesophyll, vb – vascular bundle, and fi – sclerenchyma fiber. Bar represents 83.14 µm.

slightly oval-shaped compactly arranged parenchyma cells. Spongy mesophyll, on the other hand, is made up of four to five layers of large, round to slightly hexagonal parenchyma cells with large intercellular spaces. Such a structure of mesophyll in *T. grandiflora* can thrive under high light intensity conditions.

Further, two to three layers of collenchyma cells and sclerenchyma cells were conspicuously present in the hypodermis and in vascular tissues, respectively. The vascular system is of closed collateral type of arrangement.

The stem cross-sections are illustrated in Figure 2. Across all treatments, the stem is circular to nearly quadrangular in cross-section and is covered with non-glandular trichomes. The epidermis consists of a one-layer oval to rectangular cells covered by a thin cuticle. The thick layer of the epidermis was evident in the transverse stem section of *T. grandiflora*. Also, the species have a simple internal structure characteristic with regards to the presence of a thick epidermal layer. There is a dense amount of trichomes in stem and its average thickness is at 82.29 (µm), as shown in Appendix VIII and Figure 2.

DISCUSSION

Growth Response of *Thunbergia grandiflora* on Varying Shading Intensity

In the T4 (38.25 lx) and T3 (113.50 lx) treatments, *T. grandiflora* tends to grow slowly compared to other treatments. The light is insufficient for the wildlings to grow and proliferate. As shown in Appendix IV, samples from the treatment did not grow as fast as the samples from the other treatments. In addition, discoloration of leaves was also persistent in samples of this treatment in the form of chlorosis, necrosis, and silver spots. Leaves became scaly and whitish. Most of the leaves turn out to be wilted even during their developmental stage. Stems were thin, causing them to improperly loop in the stick or even grasp

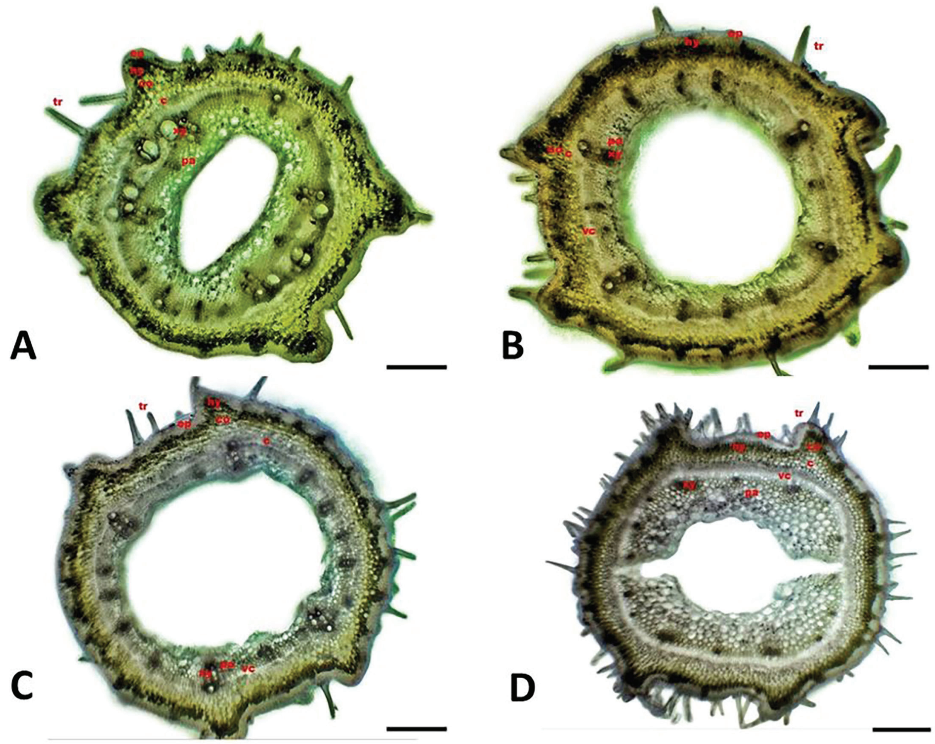


Figure 2. Transverse section of the stem of wildlings of *Thunbergia grandiflora* from (A) the Macolod soil series, (B) Lipa soil series, (C) Luisiana soil series, and (D) Bantog soil treatments using freehand technique under 400x. Abbr.: ep – epidermis, hy – hypodermis, co – collenchyma, c – cortex, xy – xylem, pc – parenchyma cells, and hp – hallow pith. Bar represents 197.35 µm, 142 µm, 146.81 µm, and 152.43 µm, respectively.

on the stick provided. The most notable response from the treatments was the dullness of color, which is due to a lack of chlorophyll content in the leaves. The T4 condition interrupts the growth performance of the plant but may still be able to survive under such unfavorable conditions. According to Bossdorf (2013), these characteristics were inherent to invasive plants and therefore created the hypotheses of "enemy release." Disturbance typically causes fluctuations in the growth of plants with decreased available resources, such as light and nutrients. Davis *et al.* (2000) reported that such disturbance in resource availability tends to favor invasive species.

With much pressure from light suppression, it can be noted that T4 and T3 responses are examples that can be accounted for bioinvasiveness, like from Baker's study (1965) that proposed to explain the concept of "ideal weed" in which traits are most linked to other ruderal plants like *T. grandiflora*. The superior growth responses exhibited by the samples despite some modifications still explain the performance of invasive plants and their continued growth, notwithstanding disturbance. Also, lowering the light intensity can disturb its growth in terms of height and diameter.

Growth Response of *Thunbergia grandiflora* on Varying Soil Series

The difference observed between the Luisiana and Bantog soil series implies that soil pH has a significant effect on the increment of RCD of *T. grandiflora.* According to Jose *et al.* (2013), invasive plants tend to thrive well on acidic soil pH. This has been proven in the experiment. The reason for this is because acidic soils tend to lower the availability of nutrients, leading to the decreased occurrence of native species, thus reducing the events of the competition. Invasive species still thrive on these areas because species of this kind have wide adaptive capacities. The soil textural type of the Bantog series can also be the factor that can affect the behavior of the plants. The soil textural type in Bantog differs from that in Macolod. This implies that *T. grandiflora* can grow and survive even in soils with poor OM content or even in highly eroded soil.

In the case of the Luisiana soil series with strong acidic soil, the mean RCD increases (Appendix Vb): as the pH decreases, the RCD increases the most. *Thunbergia grandiflora* can thrive even on extremely acidic soil. However, regardless of the specific response mechanism, invasive species tend to exhibit strong competitive effects, particularly on species within their invaded range. This is because invasive species are known for their aggressive growth characteristics.

In addition, the result of soil analysis showed that OM content across all treatments on soil series ranged only from 1.38–3.62% (Table 1). This range is even lower than 5%, which is the usual OM content of typical productive soil. The minimum value was observed in the Luisiana soil series, while the maximum value was observed in the Macolod soil series. Despite this low level of OM, the highest growth in terms of height and RCD was observed in the Luisiana soil series.

Leaf and Stem Anatomical Characteristics Grown in Different Soil Series

The presence of thick epidermal layer in this study is useful as, according to Meyer and Lavergne (2004) and Li *et al.* (2009), this will help the species to survive and withstand extreme drought condition. Also, the presence of a thicker cuticle on the leaf helps the plant from excess water loss through evapotranspiration (O'Neil 2010). According to Oliveira (2017), invasive species exhibit a thicker cuticle and trichomes, which can reduce transpiration. *T. grandiflora* has a thicker epidermal layer in the adaxial surface (62.86 μm) than that of in the abaxial surface (32.65 μm) (Appendix VII). This is similar to the epidermal characteristics reported in some species of the Acanthaceae family (Fatimah and Tripps 2012). Except in the Macolod soil series, the presence of non-glandular trichomes on both sides of the leaf in all other treatments was also remarkable. Caedo *et al*. (2014) observed two types of trichomes in their study, namely the sessile glandular trichome with panduriform (axe-shaped) head and unicellular non-glandular trichome on the epidermal surface of the *T. grandiflora*. There are many functions of trichomes that vary from different conditions. Various studies reported that leaf trichomes provide resistance against harm, especially from animals and herbivores. Further, these trichomes, especially the glandular ones, have head cells that secrete poisonous secondary metabolites (Gonzales *et al.* 2008). The ones in *T. grandifolia* is non-poisonous as the lack of calcium oxalate crystals within its inner leaf cells removes the risk of toxicity (Franceschi and Nakata 2005). Trichomes could enhance the water storage of the plant. The species was known to be a sun-loving vine species. These dense trichomes could help the species provide shade and trap a layer of still air over the leaf surface to retain a humid layer and reduce water loss by evaporation while the stomata are open. In this context, the species can thrive even in areas with full or high light intensity conditions. Trichomes are also known for their role in protecting plants against the strong wind (O'Neil 2010) and light on the underlying tissues (Dickinson 2000; Folorunso and Awosode 2013). These trichomes reduce excessive evaporation that might lead to desiccation and severe disruption of photosynthetic function. Further, non-glandular trichomes would also help reduce the risk from fungal spores' germination – especially in warm, humid conditions – and prevented the species from being infected. These, among others, are the characters reported for the invasive species and they are responsible for their aggressiveness and xerophytic nature.

Through such structure, some of the energy reaching the leaf surface can enter the inner cell layers and eventually take advantage of this by developing thick layers of photosynthetic palisade mesophyll cells. Observed in the study of leaves of invasive species by Thomas (2001) was a direct relationship between light intensity and development of palisade tissue. The palisade cells invariably more strongly developed and compacted on the side where the leaf was more exposed to sun and other environmental factors. The same structure was reported in the study of Lothelier (2013).

The cystolith, known to occur in all species under family Acanthaceae, develops from the epidermal layer and is also visible serving as their defense mechanism – cystoliths appear as crystal cells of the species containing toxic substances (O'Neil 2010). Remarkable is the multi-layered collenchyma cells in the hypodermis. The cortex is composed of 8–12 layers of irregular oval to round parenchymatous cells with intercellular spaces.

The presence of sclerenchymatous tissues in the stem is evident. These tissues form a cap-like structure around the vascular tissues. This can contribute to the resilience of invasive species against cavitation, embolism, and implosion (Folorunso and Awosode 2013). According to Hacke *et al.* (2001), sclerenchymatous tissues may act to strengthen vessel walls that can somehow increase the resistance of the species to cavitation without a necessary change in vessel thickness or lumen diameter. It causes undue of wilting in leaves because these tissues are adapted to withstand both compressive and tensile stresses in plants (Jarvis 2012). In a similar study of Caedo *et al.* (2014) on the anatomy of the young stem of *T. grandiflora*, the cortex consists of three to seven layers of storied collenchyma ground tissue beside several layers of sclerenchyma towards the single cutinized epidermal layer. The increased epidermal thickness and the decreased thickness of the parenchyma may contribute to water saving (Oliveira 2017).

The phloem and xylem are next to each other (Figure 2). The phloem cells are small and polygonal. Wide rays are visible in the xylem tissues. The xylem tissue was located further towards the pith that is hallowed. Xylem is present in compact groups with relatively larger xylem vessels scattered at intervals near the interxylary phloem, characteristic of the genus *Thunbergia* (Caedo *et al.* 2014). Larger xylem pores have been discovered in the freehand section of *T. grandiflora* that indicates the ability to conduct water through the conduit pit membrane and – according to Folorunso and Awosode (2013) – the wider the xylem pores, the more the capacity to conduct water *via* conduit pit. Wider xylem pores provide support and strength to the stem during stressful situations, which can help the species thrive even in adverse environmental conditions such as acidic soil and intense soil and atmosphere temperature (Hacke *et al.* 2001). The third type of vessel restriction is characterized by *Thunbergia* sp. in which vessels are large and are sheathed by imperforate tracheary elements together with all xylem tissues. These were observed in the examination of the stem of *T. grandiflora*. The cambium with 3–6 irregular oval to round cells is also distinguishable.

It was noticed that parenchyma cells are scattered all over the ring-type xylem cells, radiating horizontally. Obaton (1960) reported the presence of a few smaller vessels in addition to larger vessels in some Acanthaceae species. These vessel groupings are sheathed by libriform cells called suberized parenchyma. This is supported by the example of Carlquist and Zona (1987) that this pattern has not been recognized as a form of the vessel but of a type of restriction in groups together with xylem and parenchyma tissues (*e.g. T. grandiflora*). Further, cross-sections of the young stem of *T. grandiflora* revealed several layers of non-storied parenchyma in its pith, which forms a ring serving as the innermost layer. Trippsand Fekadu (2014) stated that Acanthaceae species have large, thin-walled cells comprising the parenchymatous pith in the stem center. *T. grandiflora* has parenchyma cells that are closely packed for effective conduction of water and nutrients. According to Folorunso and Awosode (2013), invasive species have more parenchyma cells than that of non-invasive species. Parenchyma cells play an important role in the repair of damaged cells by continuously dividing throughout the plant's life. This allows the plant to heal itself, especially against injury (Hacke *et al.* 2001).

The absence of pith tissue being for added mechanical flexibility is necessary for vines (Caedo *et al.* 2014). As the stem matures, its conductive capability is vastly increased with much larger xylem vessels, which are lignified with age. The stem of *T. grandiflora* was observed to be quite rigid, just like other Acanthaceae species despite the herbaceous habits. This is evident in the presence of thick collenchyma tissue, which enhances rigidity (O'Neil 2010; Leroux 2012).

CONCLUSION

Skyflower (*Thunbergia grandiflora* R.) is an evergreen vine and is introduced in the Philippines as an ornamental plant from the family Acanthaceae. More recently, it is now uncontrolled due to its weedy and prolific growth characteristics. The species is widely smothering lower slopes of MMFR, including those near streams and open areas. Some trees were already killed by suppression of *T. grandiflora*.

Pot experiments on the effect of different shading intensity and soil series were conducted to determine the invasive growth characteristics of the species. Results from the conducted pot experiments on the effect of shading and soil series revealed that *T. grandiflora* has high height and RCD under full light exposure. It can also grow on soil with low organic matter and strongly acidic. Its leaf and stem anatomical structures also suggest the ability of the species to adapt to certain harsh conditions through the presence of dense non-glandular trichomes and well developed mechanical and storage providing tissues and crystals. Therefore, *T. grandiflora* has the characteristics that can give the species all the means to grow in different types of environments and even in conditions that are unfavorable to the growth of other plants. Hence, the species can be invasive, and the extent may depend on the prevailing conditions in the site. Consequently, the invasive characteristics can be suppressed by controlling the shade and soil conditions during the wildling stage of the species. The species can be bioinvasive and the extent may depend on the prevailing conditions in the site. However, further researches on the species' root physiological and anatomical responses to other biotic and abiotic factors in the environment are recommended. Implications of knowing the invasiveness of *T. grandiflora* will help us identify possible strategies to limit the spread of the species, including early detection of the occurrence of species, avoiding disturbance in the area, and identifying species as biocontrol.

NOTE ON APPENDICES

The complete appendices section of the study is accessible at http://philjournsci.dost.gov.ph

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APPENDICES

Appendix I. ANOVA on the effects of light on height of wildings of *Thunbergia grandiflora*.

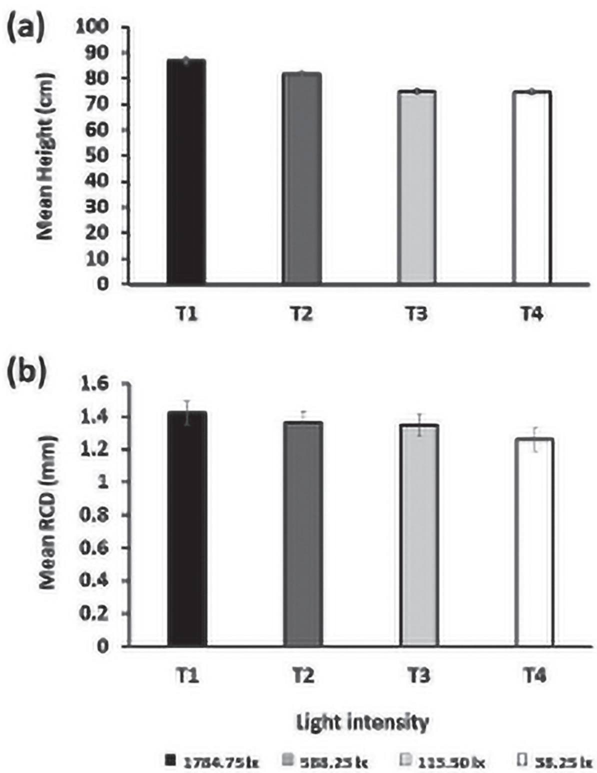
Source of variation	df	Sum of squares	Mean squares	Fc
Treatment	3	5369	1789.7	2.022*
Residuals	96	84976	885.2	
Total	99	90345		

*Denotes significance at ά = 5%

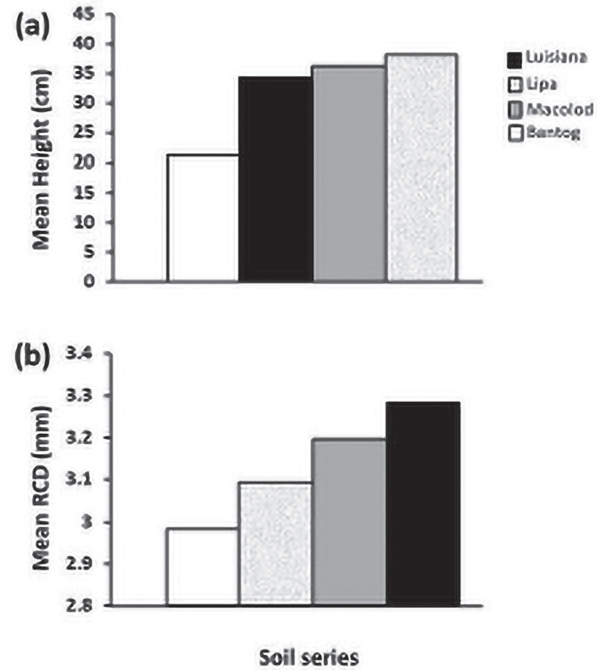
Appendix II. ANOVA on the effects of light on root collar diameter of wildings of *Thunbergia grandiflora*.

Source of variation	df	Sum of squares	Mean squares	Fc
Treatment	3	14.34	4.781	6.182*
Residuals	96	74.24	0.773	
Total	99	88.58		

*Denotes significance at ά = 5%



Appendix III. (a) Mean height (cm) and (b) mean RCD (mm) of wildings of *Thunbergia grandiflora* in the four different shading intensity (lx) treatments. T1 – control, full light (1784.75 lx); T2 – partially exposed to sunlight (588.23 lx); T3 – partially shaded (113.50 lx); and T4 – fully shaded (38.25 lx).



Appendix V. (a) Mean height (cm) and (b) mean RCD (mm) of wildlings of *Thunbergia grandiflora* grown in varying soil series.

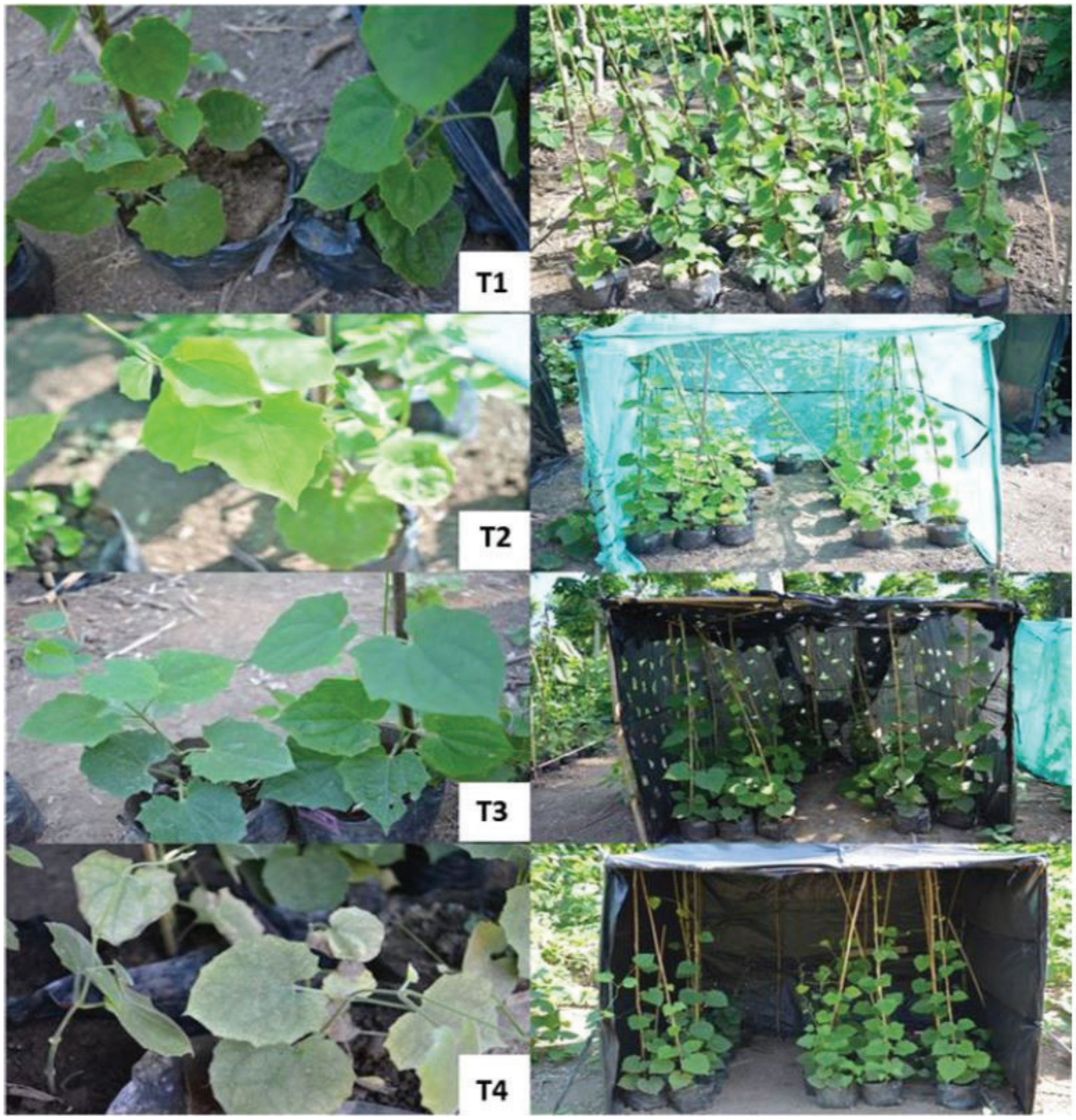
Appendix VI. ANOVA of the effects of soil series on root collar diameter of wildlings of *Thunbergia grandiflora.*

Source of variation	df	Sum of squares	Mean squares	Fc
Treatment	3	3.32	1.106	0.349*
Residuals	56	177.49	3.170	
Total	59	190.81		

*Denotes significance at ά = 5%

Appendix VII. Average thickness values of leaf anatomical tissues under 1000x magnification.

Leaf tissues	Average thickness (µm)
Trichome	128.32
Cuticle	11.52
Upper epidermis	62.86
Lower epidermis	32.65
Palisade mesophyll	61.53
Spongy mesophyll	127.12
Vascular bundle	237.11
Leaf blade	178.51
Midrib	379.19



Appendix IV. Experimental set-up of *Thunbergia grandiflora* with varying amounts of shading intensities: T1 – control, full light (1784.75 lx); T2 – partially exposed to sunlight (588.23 lx); T3 – partially shaded (113.50 lx); and T4 – fully shaded (38.25 lx).

Appendix VIII. Average thickness values of anatomical stem tissues under 400x magnification

Stem tissues	Average thickness (µm)
Trichome	82.29
Epidermis	15.24
Hypodermis	7.31
Collenchyma	16.24
Cortex	25.95
Xylem	142.00
Vascular cambium	71.88