

1. Refs. &#x201C;Garrido et al. (2017), Murgueito et al. (2012), Murgueitio et al. (2015), Bacab (2013), Murgueitio and Giraldo (2009), Navas (2010), Bacab et al. (2013), Jose et al. (2019), Calle and Murgueitio (2008), Mauricio et al. (2017), Ruiz et al. (2014), Barrett (2014), Lloyd (1979), Fenster et al. (2004)&#x201D; are cited in text but not provided in the reference list. Please provide details in the list or delete the citation from the text.

I have added missing citations and removed others. The quote from Garrido et al. in 2017 was accidentally included twice in the literature; this was a mistake I made at the beginning.

2. Rios et al. (2016) has been changed to Rios et al. (2006) as per reference list. Please check if okay.

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Ríos N, Cárdenas AY, Andrade HJ, Ibrahim M, Jiménez F, Sancho F, Ramírez E, Reyes B, Woo A (2007) Escorrentía superficial e infiltración en sistemas ganaderos convencionales y silvopastoriles en el trópico subhúmedo de Nicaragua y Costa Rica. *Agroforestería en las Américas* 45:66–71.

3. References &#x201C;Char&#x00E1; et al. (2017), L&#x00F3;pez-S&#x00E1;ez et al. (2007), Ministerio de Consumo/EC-JRC (2022), Solorio (2013)&#x201D; were not cited anywhere in the text. Please provide in text citation.

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## Sexual Reproduction in *Tithonia diversifolia* and the Implications for Its Use in Intensive Silvopastoral Systems

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### Abstract

Intensive Silvopastoral Systems (ISPS) in Latin America represent a sustainable alternative to intensive livestock production as part of nature-based solutions to reduce climate change, increase the economic value of the livestock enterprise and thus provide better living standards to small-scale producers. The intentional integration of different vegetation strata (grass, herbaceous, shrubs, palms and trees) and livestock with intensive management promotes an increase in available forage biomass, improves soil quality, fosters a greater diversity of organisms and biological interactions and improves animal welfare. The use of *Tithonia diversifolia* in animal production in Latin America and the Caribbean has increased in recent years as it represents an alternative protein-rich forage for livestock, while also providing a source rich in nectar and pollen for insects and increasing cattle rancher's income as a result of higher productivity. The propagation of *T. diversifolia* has usually been carried out in a vegetative way, which results in higher implementation costs, reduces the genetic variability of crops and produces plants with weaker and more superficial roots. Reproduction via seeds would optimize its implementation in ISPS, which highlights the importance of identifying the viability of seeds from crosses between different individuals (outcross-pollination) or within the same individual (self-pollination). The aim of this work was to measure differences in the proportion of fruits/seeds produced from different hand-pollination experiments. In addition, I quantified the visitation rate of flower visitors as an indicator of potential pollinators. The results indicate that *T. diversifolia* is a self-incompatible species and requires the presence of pollinators, which ensure efficient pollen transfer among plants (allogamous), for its reproduction. The studied population was visited by 46 morphospecies of insects, one of which is classified as vulnerable in the red list of species. I conclude that to acquire a higher percentage of viable seeds for implementation in ISPS, the presence of genetically distinct individuals and the presence of pollinators is essential. I highlight the importance of this species as a source of nectar and pollen for

pollinating insects, as well as increasing spatial heterogeneity, which could help to mitigate the current decline in insect populations.

## Keywords

Asteraceae  
Hand-pollinations  
Incompatibility system  
Nature-based solutions  
Pollinators  
Sustainable livestock

## 15.1. Introduction

**Cattle** raising is a very old human activity that began with the domestication of sheep and goats in the Neolithic. Its importance has been captured since ancient times in Egyptian hieroglyphs dating back to 4500 years BC (Fig. 15.1).

**Livestock production** is considered, along with agriculture, the first great economic revolution in history, as it represented an important step towards a sedentary lifestyle, as well as a vital source of protein (milk and meat), with a consequent growth in human populations (Renfrew and Bahn 1993). Mediterranean Dehesa or Montado, provide examples of **livestock production** where human interventions in ecosystems can be sustainable over time, with some of these sites dating from ca. 4500–3300 BC (Garrido et al. AQ1 2017; Ferraz-de-Oliveira et al. 2016). These semi-natural ecosystems (according to the legal context of the European Union) are characterized by complex biotic interactions and an appropriate balance between tree cover and different, complementary uses in the understory (i.e., between forest and agricultural uses), as well as grazing by different types of animals (**cattle**, sheep, goats and pigs) (Fig. 15.1). This ancient socio-ecological system involves various tangible material values, such as access to wild products (acorns, chestnuts, almonds, olives, cork, edible mushrooms, wood) or agro-tourism. Dehesas are an example that illustrates how livestock breeding can be done under the protection of trees while maintaining higher biodiversity compared with extensive **cattle** ranching. Indeed, Dehesas serve as a model ecosystem in ecological restoration (Gann et al. 2019).

### Fig. 15.1

(a) Domestication of **cattle** represented in an Egyptian Hieroglyph approximately 4500 BC. (b, c) Spanish Dehesa with *Quercus ilex*, *Q. suber* as tree strata and different shrubs and pastures as understory strata for the production of sheep (merino breed) and **cattle** (retinto x limousine). (Photos R. Santos-Gally)



In contrast, the tropics of America continue to lose forests at alarming rates. For example, wet and dry forest have been lost at a rate of between 3.8 (Achard et al. 2014) and 4.88 (Baccini et al. 2012) million ha/year, with a mean annual deforestation rate of 0.49% (Achard et al. 2014). This pressure on ecosystems is exerted in part due to conventional cattle ranching practices, which are developed in extensive areas of pasture without tree cover (Herrero et al. 2016), subjected to continuous overgrazing of low protein forages with low digestibility (Herrero et al. 2013). All this contributes to low stocking rates ( $\pm 0.6$  Animal Units  $\text{ha}^{-1}$ ), which means that tropical livestock production registers poor levels of productivity and competitiveness (González et al. 2015). Under such conditions, large areas of forest are continuously converted to new grasslands when pastures become unproductive due to soil erosion as a result of overgrazing, little moisture retention, and high temperatures due to lack of shade. Another factor that contributes to land transformation in the tropics is the booming trade in feed for meat production. For example, meat, compared to other products of the European basic basket, contributes to more than 50% of the transformation of land for feed production (Crenna et al. 2019; Ministerio de Consumo/EC-JRC 2022). Cattle ranching conducted in this way has been recognized as one of the main contributors to global environmental problems, including deforestation, climate change and biodiversity loss (Herrero and Thornton 2013).

Driven by the growing demand for animal protein (Valin et al. 2014), the growth of the human population, and the increase in per capita income (Alexandratos and Bruinsma 2012), Latin America and the Caribbean lost approximately 2.8 million  $\text{ha}$  year<sup>-1</sup> of forest cover between 2010 and 2018 (FRA 2020 RSS). If the trend in animal protein consumption continues, by 2050 a total of 517 million heads of cattle and buffalo are expected in the American tropics (Alexandratos and Bruinsma 2012). In such scenario, silvopastoral systems (SPS) are a necessary and sustainable alternative to increase the profitability of livestock production, freeing areas not suitable for livestock production for restoration, and thus allowing to protect some of the great biodiversity accumulated in the tropics.

Silvopastoral systems consist of a well-designed combination of different vegetation strata such as grasses, herbs, shrubs, trees and/or palms. The combination of these different vegetation strata promotes a more efficient use of solar energy in the conversion of food biomass, which can also add nutrients to the soil through the decomposition of leaves, the filtration of



water by roots, climate buffering, among others AQ2 (Ríos et al. 2006; Murgueitio et al. 2013). This process of changing conventional agricultural practices (i.e. pasture monoculture), including a reduced dependence from external inputs (i.e. agrochemicals, food supplements) as well as the diversification of multipurpose species, can be considered a component within the “solutions based on nature”. The tree cover favors the thermoregulation of cattle which, together with a better diet, translates into an improvement in milk and/or meat production, and in decreased methane emissions due to better health of the cattle, as a consequence of a more balanced diet and a less stressful environment (Broom et al. 2013; Calle et al. 2012; Chará et al. 2019). A silvopastoral system consisting of restoration plots of the native vegetation (e.g. in riverside and areas with slope not suitable for cattle ranching) and pastures enriched with forage trees would allow to increase plant cover, carbon fixation, as well as the reestablishment of ecological evolutionary processes and of the ecosystem services characteristic of the humid tropical forest of the region. The establishment of SPS would result in greater human well-being, both directly (increased production of food, wood of commercial value, food for livestock) and indirectly (improvement in ecosystem services such as biological control of pests, zoonotic disease outbreaks, crop pollination, regulation of water flow, reduction of soil erosion and protection from winds) (Fig. 15.2). Therefore, SPS constitute actions to protect, sustainably manage, and restore natural or modified ecosystems, addressing societal challenges (i.e. climate change, food and water security) effectively and adaptively, while providing human well-being and biodiversity benefits (Cohen-Shacham et al. 2016). SPS as nature-based solutions support sustainable socioeconomic development (Maes and Jacobs 2017), providing more productive and diverse agroforestry arrangements that provide animal welfare, contributing to climate change mitigation (Murgueitio et al. 2011).

**Fig. 15.2**

Ecosystem services produced in Silvopastoral Systems (Design and Photos by R. Santos-Gally)



In particular, intensive silvopastoral systems (ISPS) represent innovative “solutions” consisting of the arrangement of different vegetation strata (see SPS above) and where the stratum of high-protein forage plants (high N and P content) is

planted in high densities within the paddocks. These plants are used for direct browsing by **cattle**, which considerably improves their protein intake. The use of electric fences for **cattle** rotation and permanent access to water within the paddocks is also important. The species currently used for the shrub strata within ISPS in Latin America and the Caribbean are *Leucaena leucocephala*, *Tithonia diversifolia* and *Guazuma ulmifolia* (Murgueitio et al. 2015). Planted at high densities (between 10,000 and 40,000 ha<sup>-1</sup>) within pastures, *L. leucocephala* facilitates high nitrogen fixation and transfer, while *T. diversifolia* favors the solubilization of phosphorus in acid soils, thus benefiting associated grasses (Ojeniyi et al. 2012; González 2013; Bacab et al. 2013). In ISPS, **cattle** feed better thanks to efficient and quality grazing on protein-rich forage. Animals suffer less heat stress, since the temperature in wooded paddocks can drop between 4 and 8 °C compared with open pasture areas, and the distances needed to access water or food are reduced through the presence of mobile drinkers and more biomass fodder. These arrangements in ISPS can result in an increase of five to ten times the amount of meat production, and up to an additional 80% in the volume of milk produced compared to conventional pastures (Thornton and Herrero 2010; Murgueitio and Giraldo 2009; Navas 2010; González 2013; Bacab et al. 2013; Sanchez-Santana et al. 2018; Chará et al. 2019; Jose et al. 2019; Murgueitio et al. 2019). In addition, intensive livestock rotation results in an increase in stocking rate per ha that is four to five times higher than what is achieved in extensive livestock farming (Murgueitio et al. 2019). Expenditures on external inputs, such as fertilizers, can be reduced to zero due to the higher nitrogen fixation and other nutrients provided by forage shrubs (González 2013; Murgueitio et al. 2019). Forage biomass is also increased by up to 47% compared to that of a pasture monoculture, thus reducing the need for feed supplementation by more than half (González 2013; Calle et al. 2012). The multiple benefits that high-protein forage species provide to ISPS show the importance of ongoing agronomic and reproductive biology research.

## 15.2. Relevant Aspects of the Use of *Tithonia diversifolia*

*Tithonia diversifolia* has been introduced and propagated in most continents, mainly for ornamental use, green manure, erosion control and beekeeping. More recently, its use has increased as a forage species because of its high-protein content, with up to 28.8% of crude protein in its leaves and high content of P. In addition, it is suitable for different types of livestock (sheep, goats, pigs, **cattle**), has a wide edaphoclimatic **adaptation** and it regenerates acid soils (Calle and Murgueitio 2008; Mauricio et al. 2017). The reproduction of *T. diversifolia* in ISPS has been carried out mostly vegetatively (Ruiz et al. 2014), because the sowing of seeds resulted in low germination (Zapata Cadavid and Silva Tapasco 2016). However, different studies have shown low germination in seeds that were not stored and sown 15 days after collection, while those sown 4 months after being stored at room temperature (19 °C) had significantly higher germination success, greater than 90% (Santos-Gally et al. 2020). With these results, it has been possible to determine the presence of dormancy in *T. diversifolia* (Muoghalu and Chuba 2005; Wen 2015; Santos-Gally et al. 2020, but see Rodríguez et al. 2019, for a different view) and a likely explanation for the differences in reported germination success (Ruiz et al. 2018). However, seed dormancy might not be the only explanation for the observed variance in germination success, which could also be related to sexual reproduction, that is, the production of viable seeds after fertilization.

Sexual reproduction is important because it implies the transmission of genes from one generation to another and the combination of genes from different parentals. In hermaphroditic plants (presence of both sexes in the same individual) it can be carried out by selfing or by outcrossing (Barrett 2014). Two opposing forces determine the evolution of the first, the advantage of transmitting 50% of self-compatible genes and inbreeding depression. Inbreeding depression refers to the reduction in viability and/or fertility of offspring derived from selfing compared to offspring produced by interbreeding between genetically different individuals. Selfing provides reproductive assurance, especially in ecological situations where the number of possible mates is scarce (in a colonization process or bottleneck), or where there is a scarcity of **pollinators** (poor dispersion of pollen grains) (Jarne and Charlesworth 1993). Crossing between genetically distinct individuals (outcrossing) provides offspring with a different genetic load than the parents, that is, a new combination of alleles that may be beneficial in **adaptation** to changing conditions (Linhart and Grant 1996).

In flowering plants different strategies promote cross-pollination. Spatial (herkogamy) or temporal (dicogamy) separation of the sexual organs reduces the probability of self-pollination. The self-incompatibility system is another mechanism that prevents self-fertilization and is one of the most widespread in angiosperms (Barrett 2014). A self-incompatibility system combines physiological, genetic (diallelic), sporophytic and biochemical mechanisms to avoid selfing, thereby promoting exclusive fertilizations if pollen is successfully transferred between mates (Takayama and Isogai 2005) (Lloyd 1979). In allogamous species, the production of viable seeds would be determined by the transfer of pollen between genetically different mates. Determining the production of seeds by self or cross pollination allows us to determine the presence of an

**incompatibility system**. Because the importance of *T. diversifolia* for the implementation of ISPS through seeds, in this study we analyzed the proportion of flowers (namely florets in **Asteraceae**) that became fruits through different pollination treatments, specifically comparing the success of self-fertilization with that of outcrosses, to determine if *T. diversifolia* presents a self-incompatibility system.

## 15.3. Incompatibility System and Pollinators in *Tithonia diversifolia*

### 15.3.1. Study Species

*Tithonia diversifolia* (Hemsley) A. Gray is a perennial colonizing species in the **Asteraceae** family (La Duke [1982](#)). It occurs naturally from tropical Mexico to Central America. The species is frequently found within different ecosystems, mainly in tropical humid forests, semi-deciduous forests, and oak-pine forests. It is commonly found along light gaps, roadsides and anthropized places. It grows in different types of soils (clay, sand, silt) from 0 to 2500 masl. Inflorescence (capitulum) has an average of 12.4 ( $\pm$  0.38) ray sterile ligulate florets and 127.6 ( $\pm$  3.64) central fertile tubular florets. Each hermaphroditic floret has stamens adnate to the base of the corolla tube, free filaments, and fused anthers protruding from the apex of the corolla. The anthers dehisce before the style protrudes the anthers, so it is likely that the species presents protandry (pollen maturation precedes stigma maturation). Each capitulum produces two types of achenes, central ones with fused squamellae pappus, subequal awns and pubescent pericarp and ray glabrous achenes without awns (Santos-Gally et al. [2020](#)). Interestingly, the proportion of these two morphotypes varies between populations (Santos-Gally [2023](#)), and so does their dormancy. In general, awned achenes germinate faster and to a greater extent than ray achenes (Santos-Gally et al. [2020](#)). Dormancy is also reported in Africa and Asia (Muoghalu and Chuba [2005](#); Wen [2015](#)), where the species has been widely used as an ornamental plant or for soil recovery.

### 15.3.2. Field Sampling and Study Site

Seeds were collected from a population composed of approximately 200 individuals, near Catemaco, Veracruz in southern Mexico. Heads were harvested from several plants. The seeds of each individual were separated and labeled. The average distance between sampled plants was more than 10 m, to reduce the probability of sampling related individuals. The seeds were stored for 4 months in dry conditions at room temperature (15–20 °C). Seeds were germinated on the surface of the soil in trays with a lid (20 × 15 cm) and commercial soil (a combination of oak leaves, peat and vermiculite) and then transplanted to a greenhouse until plants reached a height of 30 cm. In July 2019, 50 plants from 50 different individuals were transplanted to an experimental plot (10 × 10 m<sup>2</sup>), approximately 20 km from the site where seeds were collected. The site where seeds were planted is found within a tropical lowland moist forest with an average rainfall of 2000–4000 mm and an annual mean temperature of 24 °C (Gutierrez-García and Ricker [2011](#)). Plants were sown at a distance of 2 m. In March 2020 manual pollination treatments were carried out to determine the **incompatibility system** of the species.

### 15.3.3. Hand-Pollination Experiments

**Hand-pollination** was performed in the field to determine whether *T. diversifolia* presents an **incompatibility system**. I applied two **hand-pollination** treatments to 6519 florets from 29 individuals: self-pollination and cross-pollination, and 12,254 florets from 29 and 9 individuals for control and autonomous self-pollination, respectively. Florets for the cross-pollination treatments were emasculated before anthers dehisced. I randomly assigned each of the four treatments to four capitula at different positions in the plant. The number of replicates per treatment was balanced across individuals, except for autonomous self-pollination where only nine individuals were used (as in Hernandez-Marquez et al. [2022](#)).

Capitula were marked and bagged with exclusion nets (0.1 mm pore size) avoiding possible contamination by pollen from other individuals. I performed **hand-pollinations** daily for 3 days on bagged capitula with pollen from a random donor to perform cross-pollinations, which consisted of transferring pollen to all open florets within the capitulum. Self-pollination involved no emasculated florets, which were hand-pollinated using pollen obtained from the same floret. In both treatments floret buds surrounding the pollinated florets were removed, to avoid confusion when collecting the achenes. Open-pollinated control involved capitula tagged with florets that opened on the days preceding the experiment or while it was taking place, and these florets remained available to visitors throughout the experiment. With the fruit/seeds produced by

these capitula, I can determine if there is pollen limitation in the population or if manual pollinations were effective, which would be indicated by a lack of significant differences between cross and controls. Finally, a total of 25 capitula were bagged to determine if the plants can produce fruits through unmanipulated (automatic) self-pollination. Fruits were harvested 3–4 weeks after pollination, at which point I counted the number of achenes with seed and aborted seed (empty achene). I used a beta regression model with binomial distribution and logit link functions to test the effect of different treatments on the fruit set (florets to achene number). I included treatment as a categorical explanatory variable. For both analyses, I used the package `betareg` (Simas and Rocha 2006) in (R Team 2018). The ratio of the averages of self- and cross-pollination treatments was used to measure the self-compatibility index proposed by Becerra and Lloyd (1992), indicating self-incompatibility when values are equal to or lower than 0.75.

### 15.3.4. Pollinators

Pollinator censuses were carried out in the same plot as the pollination experiment. The site is within the region where the species occurs naturally and the observations of insect visitation were during the flowering peak in the experimental plot and for three consecutive days in March 2020. Diurnal observations were recorded for a total of  $14 \text{ h}^{-1}$  of diurnal pollinator censuses. Observation periods of 5-min were initiated at 10.00 h and continued until 18.00 h, when diurnal pollinator visitation declined markedly. Observers were rotated randomly among plots, changing every 5 min. For each flower capitulum, observers recorded the visiting species and if visits were legitimate (e.g. the body of the visitor came in contact with anthers and/or stigma) (as in Hernandez-Marquez et al. 2022). The number of flower heads observed was also recorded for each observation bout. To identify the most representative pollinators, three people captured specimens on the third day of observations during the peak of activity and individuals were later identified in the laboratory. I calculated the visitation rate (number of visits per 5 min) by the number of capitula in the plot and the importance value of the pollinator based on the visitation rate per capitulum.

I identified the five primary functional groups: bees, butterflies, flies, beetles, and bugs. Each group presents distinct taxonomic, morphological, and behavioral characteristics. Using these functional groups and their visitation rate, a pollinator importance value was calculated, to define whether the pollination system was specialist or generalist. Each visit was counted if the pollinator contacted the sexual organs (Hernandez-Marquez et al. 2022). Although this measure does not include quantification of pollen removed and/or deposited on stigmas, it can be used as a proxy for pollinator efficiency (Armbruster and Herzig 1984). An index to compare the importance of functional groups was obtained from Martén-Rodríguez et al. (2009). I standardized each value by dividing by the sum of the importance values of all functional groups of pollinators. The range of the index is from 0 to 1. Following Fenster et al. (2004) and Martén-Rodríguez et al. (2009), I considered the pollination system of *T. diversifolia* as specialized when the importance index of the primary pollinator functional group was higher than 75% and generalized when none of the functional groups of pollinators had importance values equal or higher than 75%. Due to the presence of the non-native species *Apis mellifera*, I calculated the importance index of the functional group with and without this species, to determine a possible effect of the bee on the index.

## 15.4. Results

### 15.4.1. Hand-Pollination Experiments

The proportion of florets converted to fruits differed significantly between treatments (Table 15.1). Autonomous and self-pollination showed significantly lower success compared with cross and control pollination ( $P < 0.0001$ ). The mean proportion of fruits produced by cross-pollinations was 65.31%, whereas for self-pollination it was 36.2%, while for autonomous self-pollination it was 2% (Fig. 15.3). Under natural conditions (control treatment) I found that the proportion of florets was 74.72% and there was a non-significant difference with cross-pollination treatment ( $P = 0.85$ ).

**Table 15.1**

Beta regression model results of the effect of treatment on fruit-set following self-pollination, outcross-pollination, control (intercept), and autonomous self-pollination on *Tithonia diversifolia*

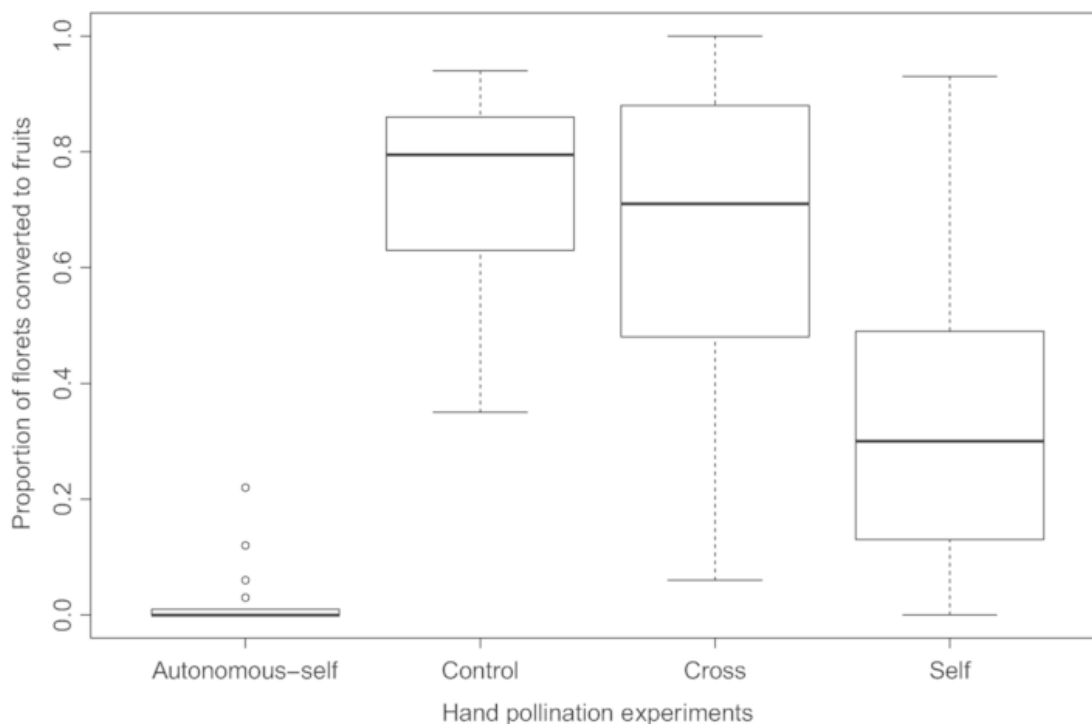
	Estimate	S.E.	z	p
Intercept	0.80	0.12	6.88	<0.000



Autonomous self-pollination	-3.55	0.255	-13.89	<0.000
Cross-pollination	0.03	0.18	0.191	0.85
Self-pollination	-1.64	0.166	-9.89	<0.000

**Fig. 15.3**

The box plot shows the median for the fruit-set of four hand-pollination treatments conducted in *Tithonia diversifolia*, as well as the lower (Q1) and upper (Q3) quartiles, representing measures within the 9–95 percentile range



The value of the Self Compatibility Index was lower than 0.75 (SCI = 0.55) indicating that the species present a self-incompatible system.

### 15.4.2. Visitation Rate of Pollinators

A total of 895 insect pollinator visits were observed across 14.0 h of observations in March 2020. A total of 46 morphospecies were observed visiting the flowers of *T. diversifolia*. (Diptera, 15 spp.; Lepidoptera, 12 spp.; Hymenoptera, 11 spp.; Hemiptera, 6 sp.; Coleoptera, 2 spp). *Apis mellifera*, native bees and butterflies were the most abundant pollinators (Table 15.2). Based on these observations, I consider that *Tithonia diversifolia* presents a generalist pollination system, where all functional groups of pollinators presented importance indices <75%. Importance values were (in descending order) 32.5% for Hymenoptera (with *A. mellifera* 90%), 31.7% for Lepidoptera, 18.7% for Diptera, 15.4% for Hemiptera and 1.6% for Coleoptera.

**Table 15.2**

Total number of pollinators that visited *Tithonia diversifolia* inflorescence

Order	Species name	Total number of visits/5 min/Number of flowers
Short-tongued insect:		
Coleoptera		
	Coleoptera sp. 1	0.2
	Coleoptera sp. 2	0.2
Diptera		
	Muscidae sp. 1	0.4



	Muscidae sp. 2	0.2
	Muscidae sp. 3	0.2
	Muscidae sp. 4	0.2
	Muscidae sp. 5	0.2
	Muscidae sp. 6	0.2
	Syrphidae sp. 1	0.4
	Syrphidae sp. 2	0.6
	Syrphidae sp. 3	0.4
	Syrphidae sp. 4	0.2
	Syrphidae sp. 5	0.2
	Syrphidae sp. 6	0.4
	Syrphidae sp. 7	0.4
	Syrphidae sp. 8	0.4
	<i>Eristalis</i> sp. 1	0.4
Hemiptera		
	sp. 1	2.6
	sp. 2	0.2
	sp. 3	0.2
	sp. 4	0.2
	sp. 5	0.2
	sp. 6	0.4
Hymenoptera		
	<i>Apis mellifera</i>	154.2
	<i>Scaptotrigona</i> sp.	0.4
	<i>Bombus medius</i>	5.6
	<i>Exomalopsis</i> sp.	0.2
	Apidae sp.	0.2
	<i>Lasioglossum</i> sp.	0.2
	<i>Augochlora</i> sp.	0.2
	Scoliidae sp.	0.4
	Vespidae sp. 1	0.2
	Vespidae sp. 2	0.4
	Vespidae sp. 3	0.2
Long-tongued insects:		
Lepidoptera		
	<i>Urbanus</i> sp.	4.2
	sp. 1	0.2
	sp. 2	0.2
	sp. 3	0.2
	sp. 4	0.4
	sp. 5	1.4
	sp. 6	0.2
	sp. 7	0.2
	sp. 8	0.2
	sp. 9	0.2
	sp. 10	0.2
	sp. 11	0.2

## 15.5. Implications of *Tithonia diversifolia* Reproductive System in the Implementation of ISPS

The results from the studied population indicate that *Tithonia diversifolia* is self-incompatible, although the self-incompatibility system is not perfect and there is moderate self-compatibility. Self-compatibility varies continuously, with some plants more self-fertile than others (Fig. 15.3), and thus such variation might also be present among populations of the same species (Cheptou et al. 2000). Because *T. diversifolia* is a good colonizer, commonly found in disturbed remnants of forest vegetation or roadsides, a plausible explanation for the transition from outcrossing to selfing would be the advantage of selfing individuals over outcrossing ones when mates or pollinators are scarce, known as the reproductive assurance hypothesis (Stebbins 1957). The autonomous self-pollination treatment resulted in seed production of less than 2%, indicating that *T. diversifolia* requires the presence of pollinators to carry out fertilization. What implications do these results have for the establishment of *T. diversifolia* in ISPS?

The presence of a self-incompatible system highlights the importance of having genetically different individuals to obtain the largest number of seeds. If we choose to establish a plot of *T. diversifolia* plants to obtain seeds, it is highly advisable to obtain seeds from a natural population that is as large as possible and choose seeds from individuals that are between 10 and 15 m away, with the purpose of favoring genets. It is also important to avoid collecting seeds from nearby individuals, so as to maximize the diversity of parental individuals for sexual reproduction and seed production and minimize the effects of inbreeding depression. Although in this specific study I did not analyze the presence of inbreeding depression, in self-incompatible species (e.g. *Raphanus sativus* and *Leontodon autumnalis*) there is evidence of a decrease in seed production in plants that are produced from self-pollination (Nason and Ellstrand 1995; Picó and Koubek 2003). Plants from crosses between relatives may present negative effects from reduced genetic variation, which can be expressed in reduced seed production, germination or growth (Cheptou et al. 2000).

Given the interest in *T. diversifolia* as a species with high forage potential, the results of this experiment suggest that care must be taken when establishing intensive forage shrub lines, to avoid negative effects due to inbreeding given that the success of seed production via selfing although low is not null. Vegetative reproduction is likely to result in a loss of genetic variability which could have undesired effects on growth and seed production. It is important in the future to investigate if decreased genetic variability could also impact forage quality. The pollination study indicates that the production of seeds via outcrossing, or through natural pollination, is more than 50% higher than through selfing, therefore the seeds that come from natural populations within the studied region can be used for future ISPS establishment. These results also highlight the importance of natural pollinators for seed production and successful reproduction.

Nectar and pollen production in ISPS is of crucial importance in the current pollinator decline. This crisis is reflected in the decrease in the number of pollinating insects (both wild bees and honey producers), which produce a significant amount of the foods consumed by humans because of their pollination services. In 2005, the annual contribution of pollinating insects to agriculture was estimated at US\$153 billion (Gallai et al. 2009). The decline of insects can lead to a crisis in food production that would lead to an economic and environmental crisis. It is estimated that 87% of all plants on the planet depend on pollination for fruit production (Ollerton et al. 2011). *T. diversifolia* is a generalist species that contribute with nectar and pollen for 46 species in the studied population. Although an exotic bee (*A. mellifera*) was the most frequent visitor, in a hypothetical absence of this species, the second insect with the highest visit rate was *Bombus medius*, a native species registered as threatened according to the IUCN.

Although honeybees (*Apis mellifera*) are not the only or most important insect that contributes to the production of fruits, it is true that they provide us with another very important food, which is honey. In Mexico, deforestation due to changes in land use has contributed to the reduction of food resources for honeybees. Currently, the purchase of sugar to feed bees to compensate the scarcity of natural food sources increase production costs thus reducing the profitability of beekeeping (Magaña and Leyva 2011). In addition to sugars, nectar contains amino acids, vitamins and minerals that are essential for bee health. In this sense, the ISPS through the trees and shrubs within the paddocks, become refuge sites and feeding areas for pollinating insects.

### Acknowledgements

A. Hernández-Marquez, G. Franco, M.P. Escamilla Herrera and M. Muñoz for assistance in field and laboratory work. A.

González-Voyer commented on early drafts of the manuscript and field assistance. Félix López Xólot for assistance in fruit collection. This work was supported by funding from Cátedras-CONACyT and CONACyT 319075, the latter during analysis and writing.

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