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Original Paper

Reproductive strategies of the *Macroptilium lathyroides* (Papilionoideae: Phaseoleae) explain the success of ruderal species in anthropized environments

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Abstract

Ruderal plants are important they are used for animal (*e.g.*, beekeeping/pasture) and human food. Many of these plants present multiple reproductive strategies that ensure that they remain in disturbed environments. Therefore, we investigated the sexual reproduction and regeneration by regrowth of the forage ruderal *Macroptillium lathyroides* in an anthropized area to support management of this species after cutting or grazing and determine its requirements for seed production and conditions for commercial use. We assessed the occurrence of reproduction through regeneration and species dependence on pollinators. *M. lathyroides* has an axial underground system capable of regrowth but not propagation post-cut. Its flowers last about eight hours and are papilionate, asymmetrical, hermaphrodite, nectariferous, vinaceous and diurnal. They present secondary pollen that is transferred to the trichomes of the style. The species is self-compatible and presents spontaneous self-pollination. The small bee *Exomalopsis* cf. *auropilosa,* was the only pollinator since it activated the brush-type pollination mechanism while gathering nectar/pollen. The species depends on seeds to propagate or maintain a seed bank, since all plants do not regrow after cutting. Thus, sexual reproduction is necessary, but pollinators are not since it is not pollinator independent.

Key words: asymmetric flower, autonomous self-pollination, brush type pollen release, *Exomlaopsis* bee, vegetative propagation.

Resumo

Plantas ruderais são importante componente da diversidade urbana, mas principalmente do ecossistema agrícola, como fonte de ração animal (e.g., apicultura, forragem, pasto) e na alimentação humana. As plantas ruderais costumam apresentar várias estratégias reprodutivas que ajudam a garantir sua perpetuação em ambientes perturbados (*e.g.*, cultivos). Investigamos a reprodução sexuada e regeneração por rebrota da forrageira *Macroptillium lathyroides* em área antropizada para auxiliar no manejo da espécie após corte ou pastejo e seus requisitos para produção de sementes em áreas antropizadas e otimizar as condições de uso econômico dessa espécie. Avaliamos a ocorrência de reprodução vegetativa por regeneração (rebrota), dependência de polinizadores e presença de polinizadores na área de estudo. *M. lathyroides* possui um sistema axial subterrâneo capaz de regenerar, mas não de propagação vegetativa. Suas flores duram cerca de oito horas e são papilionadas, assimétricas, hermafroditas, nectaríferas, vináceas e diurnas. Elas têm apresentação secundária de pólen, que é apresentado por tricomas do estilete. A espécie é autocompatível e apresenta autopolinização espontânea (autônoma). A pequena abelha *Exomalopsis* cf. *auropilosa* foi o único polinizador que ativa o mecanismo de polinização (tipo escova) enquanto coleta néctar e pólen. A reprodução por rebrota é a estratégia importante para manter as plantas em ambientes perturbados ou após pastejo. No entanto, a espécie depende de sementes para propagação ou manutenção de um banco de sementes, uma vez que nem todas são capazes de rebrota. Assim, a reprodução sexual através da polinização é necessária, embora *M. lathyroides* independe de polinizador para produção de sementes.

Palavras-chave: flores assimétricas, autopolinização autônoma, liberação de pólen tipo escova, *Exomalopsis*, propagação vegetativa.

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Introduction

Through the process of natural selection, species evolve reproductive strategies that are likely to maximise their persistence (Norman *et al.* 2005). Plants in environments that are subject to disturbances (*i.e.*, mechanisms that limit plant biomass through destruction) (sensu Grime 1977) present ruderal strategies in which natural selection is likely to favour genotypes with rapid growth and early reproduction, thus increasing the probability that sufficient offspring will be produced for the survival and re-establishment of the population (Grime 1977, 1988). However, reproductive success in disturbed environments where pollination can be uncertain is a common problem that many lineages of seed plants face (Ai *et al.* 2013). Thus, many ruderal species have developed several reproductive strategies to manage unfavourable pollination conditions such as mechanisms that guarantee self-pollination and/or self-fertilization, reducing their dependence on pollinators for seed production (James 1984).

Many ruderal species are important food sources for domesticated animals (*e.g.*, beekeeping, fodder, pasture) and humans (Soares Filho *et al.* 2016 and cited references). Numerous native Leguminosae species are ruderals and are used as forage for cattle because of their high protein content, which improves the animal's performance compared to those that fed exclusively on grass pastures (Borges *et al.* 2019). Among the legumes, diverse *Macroptilium* species are ruderals and are used as forage, green manure and in medicine (Freitas *et al.* 2011; Borges *et al.* 2019). *Macroptilium* (Benth.) Urb. (Papilionoideae, Phaseoleae) contains about 20 Neotropical herbaceous species distributed from the southwestern United States to northern Argentina and southern Uruguay (Delgado-Salinas and Lewis 2008). Some *Macroptilium* species occur in open habitats and dry climates and, therefore, are efficient forage plants in semi-arid environments around the world (*e.g.*, Africa, South America) (Macharia *et al.* 2010; Borges *et al.* 2019).

For *Macroptilium* species, there have been relatively few studies regarding sexual reproduction and reproduction by regrowth has been recorded only for *M. atropurpureum* (Jones 1974; McDonalds and Clements 2005). *Macroptilium* species have papilionoid asymmetric nectariferous flowers characterized by long wing-petals that are longer than the vexillum (or standard) and keel petals. During anthesis, the upwardly directed left-wing petal replaces the vexillum (Delgado-Salinas and Lewis 2008). These flowers are pollinated by medium/large bees of the genera *Bombus, Centris* and *Euglossa* (Apidae), as well as those of the Andrenidae, Halictidae and Megachilidae families, which can activate the specialized pollination mechanism, i.e., trigger the brush-type mechanism with secondary pollen presentation by style brush (Lavin and Delgado 1990; Brizuela *et al.* 1993; Hoc *et al.* 2003; Vieira *et al.* 2002).

Macroptilium lathyroides (L.) Urb. (wild bush bean) is a Brazilian native, annual or bi-annual species, that can regenerate through the seed bank and not demanding in terms of soil fertility and drainage (Ferreira *et al.* 2004). Popularly known as "Feijão-de-Pombinha" or "Feijão-do-Campo", originates in the tropical South America and was introduced in tropical and subtropical India, Australia, Africa, and Southeast North America (Barbosa 1986). It is also a ruderal species that is commonly found in urban environments (*e.g.*, García-Lahera 2016; Deng and Jim 2017) and, like other *Macroptilium* species, has ornamental potential on lawns or flowerbeds due to its small size and showy flowers (Stumpf *et al.* 2009).

Considering the economic potential of *M. lathyroides*, the fact that it is a ruderal and that it reproduces mainly by seeds, herein we investigated some aspects of its sexual (breeding and pollination system) and regeneration by regrowth in an environment subject to disturbances. Our aim was to understand the reproductive strategies that this species uses to maintain and reproduce in an anthropized environment. Specifically, we aimed to verify if: (i) *M. lathyroides* plants reproduce by regeneration (regrowth) after cutting; (ii) this species is pollinator-dependent; (iii) its "specialized" flowers are visited by bees that can activate its sophisticated trigger mechanism (brush-type) that is essential to pollinate these flowers in the disturbed environment. Our data will help manage this species after cutting or grazing, determine its requirements, limitations and/or conditions for seed production in anthropized areas and optimize conditions for the commercial use/production of this species.

Materials and Methods

Study area

We performed the study in April 2017 and January-May 2018 in two distinct open/large environments at the campus of the Universidade Federal de Mato Grosso do Sul - UFMS, Campo Grande, MS (20º27' S, 54º37' W; 530m) (Fig. 1).

The climate of the study area is Aw according to the Köppen classification, with tropical dry winters, a rainy season from October to March (summer), and a drier and cooler period from April to September (winter), with slight water deficit in July (Köppen apud Alvares *et al.* 2013). Average annual rainfall in the region is approximately 1400 mm, and the average annual temperature ranges from 21 to 26° C (Vilas Boas *et al.* 2013). We evaluated a ~20m radius in three areas colonized by *M. lathyroides* (Fig. 1). At the study site, the colonizing species were invasive grasses (*Brachiaria* spp.) and *M. lathyroides*, which were sometimes found near concrete on the University (See Fig. S1, available on supplementary material <https://doi.org/10.6084/m9.figshare.16879807.v1>). We evaluated three areas with three patches (sets of plants) each area, totaling nine patches of the *M. lathyroides*.

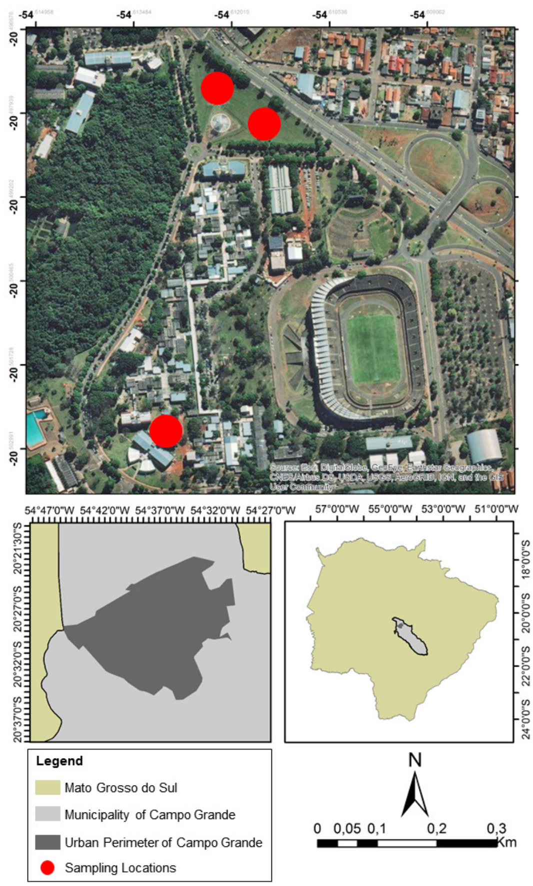

Figure 1 – Collection and sampling points (in red circles - groups of plants) of *Macroptilium lathyroides* at the Universidade Federal de Mato Grosso do Sul, Campo Grande campus, state of Mato Grosso do Sul, in Brazil.

Morphology and floral biology

In the field, we counted the number of flowers per inflorescence (n = 201 inflorescences, five sets), per sets of plants (n = 4), and measured peduncle length (n = 30 inflorescences). We studied floral morphology (*e.g.*, shape, size, colour, resource) in the field and/or the laboratory using fresh (in 70% alcohol) flowers. Floral diameter (width, length), floral tube length and the length of stamens and style/stigma was measured with a digital caliper and the number of ovules per ovary (n = 20 fresh flowers, 4 per sets of plants) were counted. We tested pollen viability in pre-anthesis flowers fixed in FAA 70% with aceto-carmine (n = 50, 4–5 per sets of plants) (Dafni 1992). Stigmatic receptivity was investigated by the presence of exudates on the stigma surface using a handheld magnifying glass (Luppenbrille) and a stereomicroscope. The volume and solute concentration of the nectar were measured from pre-anthesis bagged flowers (n = 15 flowers, 3 per plant) before 16h30 using a graduated microsyringe (10µl) and a digital pocket refractometer (0 + 53 Brix, Pocket Pal-1 Atago model), respectively. We also verified the presence of odour by placing fresh flowers (n = 30, 10 per cluster) in a Petri dish, refrigerating them for about 10–20 minutes, heating them up between our hands for 1–2 minutes and then opening them up to allow scent emission. The floral longevity was evaluated from tagged pre-anthesis flowers (n = 30, 10 per sets of plants) until they wilted. We deposited a voucher of *M. lathyroides* in the CGMS Herbarium (CGMS 70201).

Floral visitors

We observed floral visitors of *M. lathyroides* (phytocentric sampling) in April 2017 (10 hours, two sets of plants) and January and March 2018 (6.5 hours, four sets of plants), between 08h00 and 17h00, for a total of 16.5 hours. We recorded visitor type, behaviour and visit frequency (number of visit bouts/total hours of observations) and floral resource gathered (nectar, pollen). We defined a bout as a continuous visit to any number of flowers. We recorded floral visitor behaviour through notes, photographs and/or video recordings. When possible, we collected floral visitors with nets, plastic vials, and/or waterproof bags; measured total body length with digital caliper; and analyzed the presence and place of *M. lathyroides* pollen deposition on visitor's bodies under a stereomicroscope. We performed zoocentric sampling to check for *M. latyroides* pollen deposition sites on the body by removing pollen, when present, with glycerine gelatin fuchsine (Dafni 1992) and mounting it on slides to quantify pollen load using optical microscopy. We counted 200 pollen grains per specimen from 1 to 10 specimens, depending on the floral visitor. We mounted specimens on entomological pins and partially identified them. Specimens will be deposited into the Zoological Collection at the UFMS (ZUFMS).

Breeding system

In January and March 2018, we tested the reproductive sexual system of *M. lathyroides.* We selected the following four treatments: Autonomous Self-Pollination ASP (18 flowers in January + 10 flowers in March = 28 flowers); Hand Self-Pollination HSP (19 flowers in January + 5 flowers in March = 24 flowers); Hand Cross Pollination HCP (10 flowers in March), and Natural Control NC (21 flowers in January + 10 flowers in March = 31 flowers) (Oliveira and Sigrist 2008). Every month, the number of flowers in each treatment was added for data screening and statistical tests. We evaluated the number of fruits per flower in the sets of plants and the percentage of fruiting for eight consecutive days. We followed the development of fruits (and abortion rate) and collected them after 14 days to count the seeds in the laboratory. With the seeds, we evaluated the volume size in mm^3 (length x width x thickness) and fertility (number of seeds per fruit: average number of ovules, Ferreira *et al.* 2018) from seven fruits of each treatment. To verify embryo viability, we immersed seeds in distilled water for two hours, removed the tegument, cut seeds in half, and placed them in 0.075% aqueous solution of tetrazolium chloride for 45 minutes. We considered viable seeds as those with a reddish embryo (modified from Brasil 2009).

We also calculated the Index of Autonomous fruit/seed set (IAS = % fruiting/seed after ASP/% fruiting after HCP), Self-Incompatibility Index (ISI = % fruiting/seed after HSP/% fruiting after intraspecific HCP) and Reproductive Efficacy (RE = % fruiting/seed on NC/% fruiting after intraspecific HCP) (Zapata & Arroyo 1978; Lloyd & Schoen 1992). We considered a population to be non-autogamous with IAS < 0.3, partially autogamous ≥ 0.3–0.6 and autogamous > 0.6–1. We considered a compatible population to have ISI ≥ 1, partially compatible < 1–0.2 and self-incompatible < 0.2 (Fachardo & Sigrist 2019). Since the variables seed volume and number of fruits had abnormal distribution, we used Kruskal-Wallis test (Ref) and Dunn's post-hoc test with adjustment of the p value to compare the means. The fecundity, embryo viability and abortion rate had normal distribution, so we used one-way ANOVA (Zar 2010) and Tukey post-hoc test when necessary. We considered 5% significance for tests. All tests and the graphics were carried out in R software (R Development Core Team 2016; version 3.3.3).

Regeneration by regrowth

We experimentally tested potential of regenerating *M. lathyroides* in April-May 2018 by cutting at ground level. *M. lathyroides* is cut by University employees who maintain the green areas (invasive grasses). We selected 30 adult plants in three 3.5m × 4m plots that we observed for five weeks (35 days). Then, we evaluated the formation of new green shoots on its stem/root (vegetative buds), new leaves and stem size (Fig. 2a-c). To determine whether *M. lathyroides* could produce new plants through its roots, we collected all the plants for further examination.

Figure 2 – a. *Macroptilium lathyroides* plant. b. subterranean system of excavated plants (red arrow). c. detail of vegetative buds (red circles). Scale = 3 cm.

Results

Floral morphology and biology

In the *M. lathyroides* plants, one to two flowers opened daily per inflorescence (1.3 ± 0.5) with 30–300 flowers (155.5 ± 122.8) per sets of plants. Long peduncle (17.9 ± 4.6 cm) and rachis (7.2 ± 2.7 cm) elevate the flowers above the foliage. Flowers are hermaphrodite, nectariferous, diplostemonous, diurnal, and longer (22.4 ± 4.2 mm) than they are wide (15 ± 2.9 mm) (Fig. 3a). The calyx is gamosepalous, tubular, pinkish and partially surrounds the base of floral tube, which is 11.9 (± 1) mm long (Fig. 3a) and formed by the base of the vexillum and staminal column (or sheath) that delimit the nectariferous chamber. The corolla has unguiculate petals with a vinaceous limb (Fig. 3a) and cream or greenish claw. Vexillum is on the left, opposite the keel (Fig. 3a), and its base does not fully cover the staminal column, thus leaving the nectariferous chamber partially exposed (Fig. 3c-d). Wings are sculpted at the base of the limb (Fig. 3b) next to the zone of adnation with the keel. The androecium is diadelphous and coiled as a keel, with nine stamens united into a staminal sheath, which is adnate to the wings and keel claws, and one free vexillary stamen. Anthers are basifixed, rimose, slightly elongate and yellow, and produce pollen with high viability (94.2 ± 4.2%). The gynoecium is 18.5 (± 3.4) mm long and ends in a terminal discoid stigma covered by a fine cuticle. Style is filiform, green, thickened at the apex and coiled as a keel; it possesses a short pollen brush formed by hyaline trichomes below the stigma (Fig. 3c), where the pollen is deposited before the flower opens.

The ovary contains 15–21 ovules (17.6 ± 1.7). The nectariferous tissue located around the ovary base produces a small amount of nectar (8.6 ± 2.1 μl) that accumulates in a nectariferous chamber inside the floral tube and has a solute concentration of 18.1% (± 3.0%). Flowers last about eight hours. During pre-anthesis, the anthers open and release pollen on the stylar brush (Fig. 3c); the stigma is receptive, and nectar accumulates in the nectariferous chamber. Most flowers are open at 09h00 (82%), with a small percentage finishing wing expansion (11%) or remaining closed (7%). All flowers (100%) are open by 10h00 and emit a "grass-like" odour while functional. Wings wilt and close around 17h00–17h30, closing the entrance to the floral tube.

Floral visitors

Flowers of *Macroptilium lathyroides* were visited by five small (≤ 12mm in length) bee species (sensu Frankie *et al.* 1983) and one species of butterfly (Lycaenidae), which collected nectar and/or pollen. *Exomalopsis* cf. *auropilosa* was the most frequent floral visitor (Tab. 1, Fig. 3d-e), being recorded in both study periods and in the most clusters. When visiting *M. macroptilium* flower, *E.* cf. *auropilosa* lands on the lower (right) wing, enters the floral tube and pushes the keel, displacing (separation) the wings and vexillum. At this point, the style slides out of the keel, exposing the stigma and

Table 1 – Floral visitors sampled in plants of *Macroptilium lathyroides* at the campus of the Universidade Federal de Mato Grosso do Sul, Campo Grande, Brazil. Visit frequency (number of visits bouts/total hours of observations) and % de pollen (average of all individuals).

Floral visitor (number of collected specimens)	Body length (mm) $\bar{X}$ ± SD	Visit frequency	*Macroptilium* pollen load (%)		
			head	thorax abdomen	posterior leg
Hymenoptera - Bee					
Augochloropsis sp. (1)	10.1	0.20	95.0	72.0	83.7
Exomalopsis cf. *auropilosa* Spinola, 1853 (5)	6.7 ± 0.4	0.85	82.1	76.5	74.6
Paratrigona lineata (Lepeletier) (4)	4.7 ± 0.5	0.40	100	100	23.9
Trigona spinipes Fabr. (5)	6.2 ± 0.6	0.40	78.9	78.4	58.9
Halictidae (2)	9.5 ± 0.8	0.20	62.5	0	0
Lepidoptera - Butterfly					
Lycaenidae	-	0.40	0	0	0

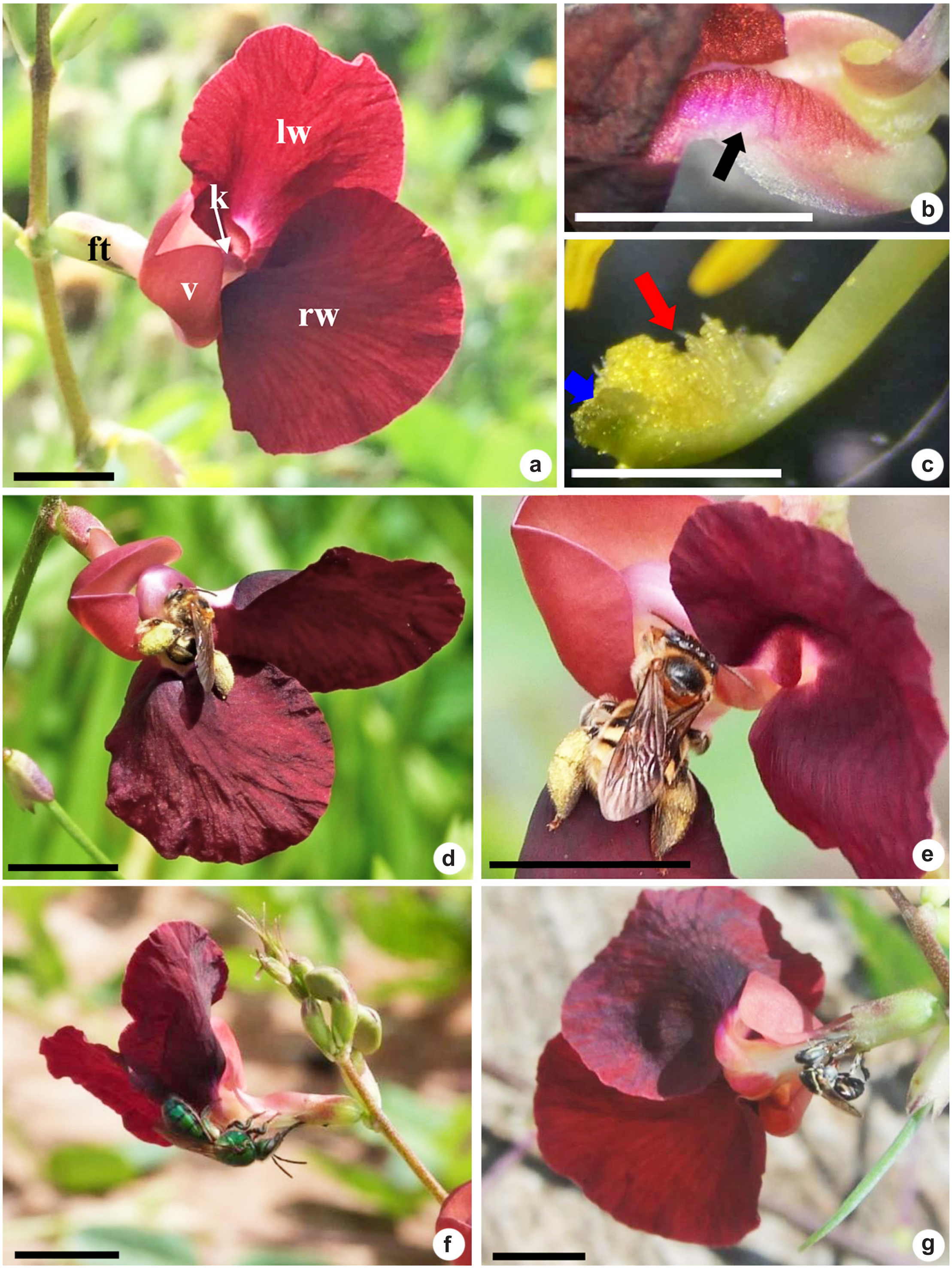

Figure 3 – a-g. *Macroptilium lathyroides* – a. flower in frontal view (ft = floral tube; k = keel; lw = left wing; rw = right wing; v = vexillum); b. sculpture at the base of the wing limb (black arrow); c. stigma (blue arrow) and stylar pollen brush at anthesis (red arrow); d-e. *Exomalopsis* sp. collecting pollen (d.) and nectar (e.) in *M. lathyroides* flower after activating the brush-type trigger mechanism; f-g. bees thieving nectar by inserting their proboscis in the "exposed/unprotected" portion of the nectariferous chamber – f. *Augochloropsis* sp.; g. *Paratrigona lineata*. Scales: a = 10 mm; b, d-g = 5 mm; c = 1 mm.

the style brush that contact the lateral-ventral portion of the bee's head and eventually thorax (Fig. 3d-e). After 6–15 seconds, the bee leaves the flower and the style-stigma return to their original position inside the keel. Then, the bee might visit other *M. lathyroides* flowers (2–6), visit adjacent flowers of other ruderal species or leave the location. This bee collects nectar with its proboscis (Fig. 3d) or gathers pollen with its anterior legs. The other floral visitors steal nectar by accessing the nectariferous chamber from the outside near the calyx (bees) (Fig. 3f-g) or landing on the lower wing and inserting their proboscis into the entrance of the floral tube (ants), without triggering the pollination mechanism. Nevertheless, all bee species analyzed had high percentages of *M. lathyroides* pollen on their head, thorax-abdomen and/or posterior legs (Tab. 1), indicating that, in some way, these bee species might contact the style pollen brush.

Breeding system

Fruit was recorded in all treatments during both periods (Fig. 4a) and the fruiting percentage was 32-35% (Kruskal-Wallis chi-squared = 18.195, df = 3). The final percentage of fruiting was around 35.5%, and the HSP treatment obtained a higher final percentage (45%, $p<0.01$, Fig. 4a). The other treatments showed no significant differences. The number of fruits aborted over the 14 days was higher in NC and ASP (n=20, Fig. 4b), with no differences between them ($p>0.05$). However, the final percentage of abortion was significantly higher in HCP, followed by NC and ASP (Fig. 4c). As a result, HSP and NC treatments had the highest number of seeds, followed by HCP and ASP (Fig. 5a).

Seed size was smaller in HCP and larger for other treatments (Fig. 5b), but fertility was higher for NC (Fig. 5c), with no difference between the other treatments. Therefore, the viability of the embryo was also greater, followed by HSP/ASP and HCP (Fig. 5d). Values of IAS were 1.07 for fruit set and 0.6 for seed set. ISI value for fruit set was1.52 and 1.03 for seed set. For RE values, fruit set was 1.18 and seed set was 1.21.

Regeneration by regrowth

Macroptilium lathyroides has an axial underground system that does not originate new plants from the lateral roots but regrows after cutting. Vegetative bud formation (Fig. 6) was recorded only in the first week on 14 plants (46.7%) (1–13 buds per plant, average = 5.3 ± standard deviation = 3.7). Sprouts and leaves (mainly simple) were recorded from the first week to the end of the experiment (Fig. 6). Most plants with regrowth exhibited considerable stem growth and/or new leaf formation from the third week onward (Fig. 6).

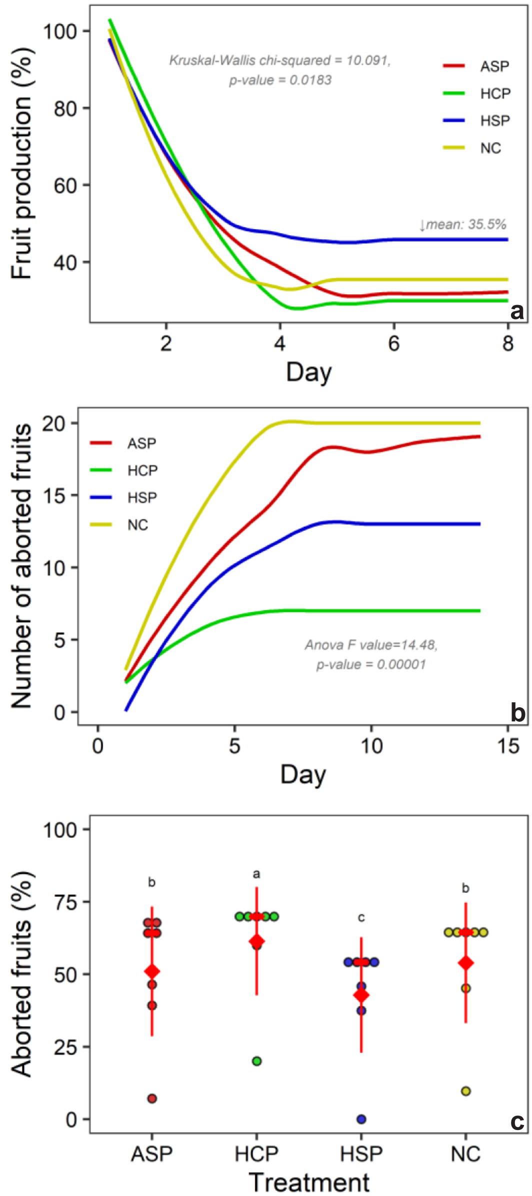

Figure 4 – a. Percentage of fruit production. b. number of fruits aborted. c. percentage of aborted fruits. (For each treatment sampled in *Macroptilium lathyroides* [Leguminosae, Papilionoideae]). Different post hoc letters between treatments indicate statistical differences ($P < 0.05$).

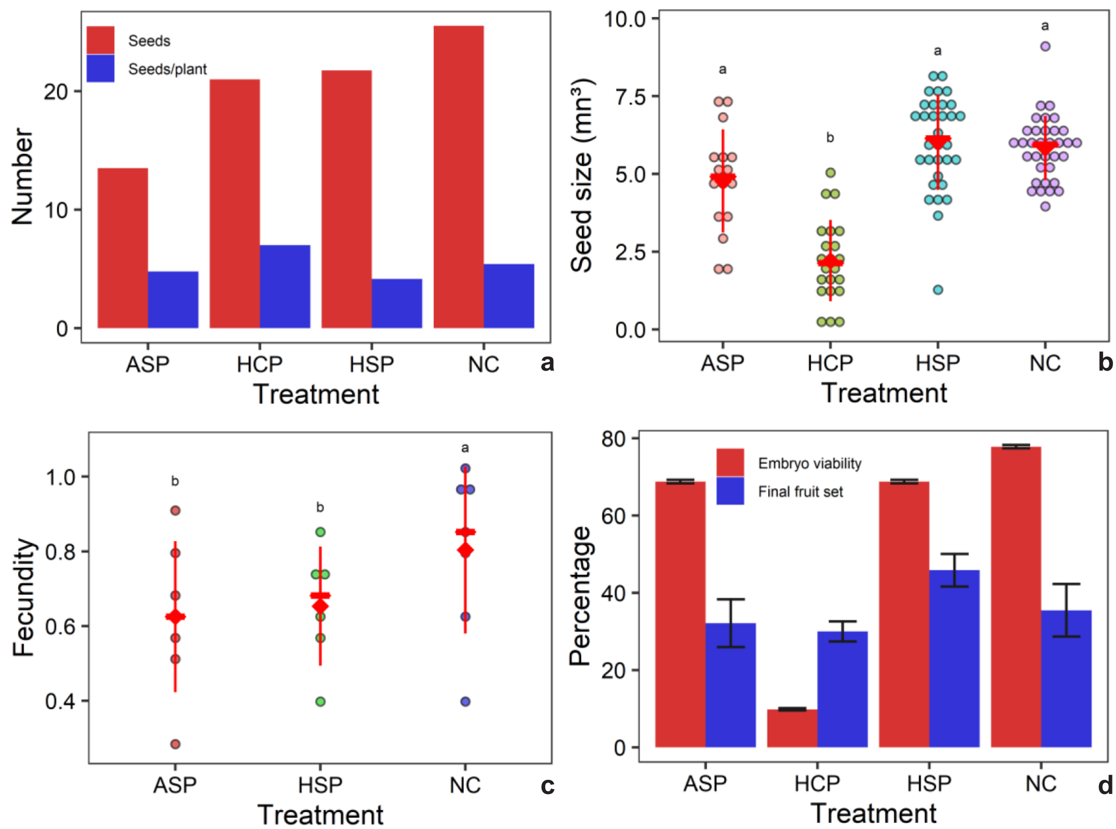

Figure 5 – a. Number of seeds and seed per plant; b. seed size; c. fecundity; d. percentage of embryo viability and final fruit set. (For each treatment sampled in *Macroptilium lathyroides* [Leguminosae, Papilionoideae]). Different post hoc letters between treatments indicate statistical differences (P < 0.05).

Discussion

Floral visitors and pollinators

Only one floral visitor, the small bee *Exomalopsis* cf. *auropilosa*, was able to (potentially) pollinate *M. lathyroides* flowers, since it legitimately collected nectar and pollen (i.e. activated the brush-type trigger mechanism) and was recorded in most sampled plants throughout the two-year study period. This differs from the pattern observed for Papilionoideae species with large asymmetric flowers (≥ 20mm) (Etcheverry *et al.* 2012) that are generally pollinated by medium to large bees (> 12mm) (sensu Frankie *et al.* 1983) of diverse genera (*e.g.*, *Bombus*, *Centris*, *Euglossa*, *Xylocopa*) (Brizuela *et al.* 1993; Vieira *et al.* 2002; Etcheverry & Vogel 2018). However, there are visitation records for different *Exomalopsis* species in flowers of *M. lathyroides* and other genera of Fabaceae in the French West Indies (Meurgey, 2016), as well as in soybean (*Glycine max*) (Chiari *et al.* 2005), hot pepper (*Capsicum annum*) (Raw 2000) and tomato (*Solanum lycopersicum*) crops (Silva-Neto *et al.* 2017) in Brazil.

Brush-type pollen presentation has arisen independently in six tribes of Papilionoideae and it is common among species with asymmetric flowers (*i.e.*, loss of bilateral symmetry) (Endress 1994; Etcheverry *et al.* 2008b). The brush-type mechanism of *Macroptilium* species, in which pollen is transferred from the anthers to the trichomes of the style (secondary pollen presentation), might be associated with an efficient pollination system that ensures deposition of conspecific pollen grains on the parts of a pollinators' body that cannot be groomed. Therefore, this mechanism could reduce pollen waste and, consequently, make pollen unavailable to pollinators so flowers behave as typical nectar flowers (Etcheverry *et al.* 2012). This does not seem to work for the *M. lathyroides* flowers because *E.* cf. *auropilosa* also collected pollen during its visits. The small size of this bee probably favours active pollen gathering in

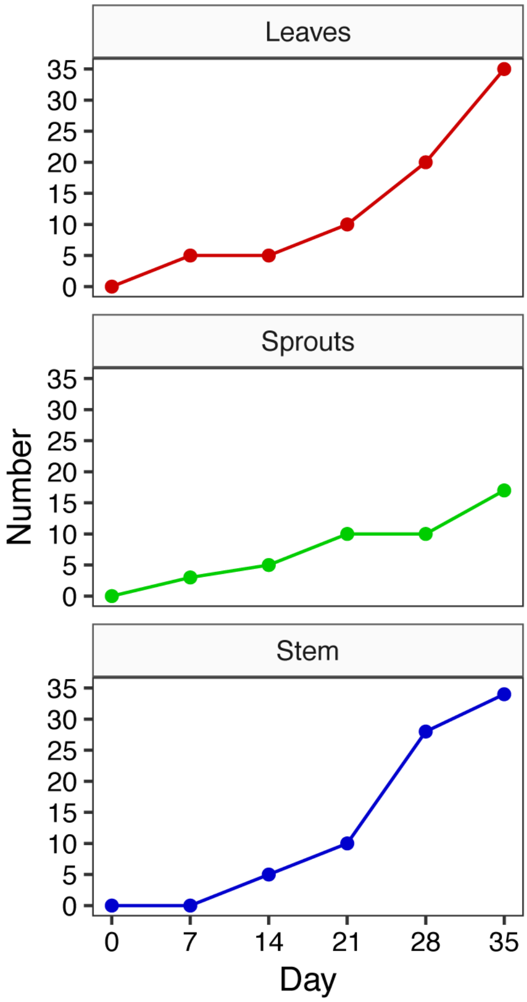

Figure 6 – Number of sprouts and leaves and stem length recorded for five weeks (04/26 to 05/24/2018) in *Macroptilium lathyroides* plants (n = 14) which shoot was removed.

the large nectariferous flower of *M. lathyroides*, as it can support its anterior and middle legs in the sculpture of the lower (right) wing and in the vexillum (Fig. 3b,d-e). In a comparative study of the floral morphology of Papilionoideae species in Argentina, the sculptures of the *Macroptilium* spp. flowers were found to be deeper in the right-wing, which serves as a landing area for pollinators (German 2014 *apud* Etcheverry & Vogel 2018).

The highly elaborated floral morphology (asymmetric) of *M. lathyroides* does not provide mechanical barriers that delimit access to nectar. The vexillum does not completely cover the staminal column, leaving the nectariferous chamber partially exposed, thus allowing nectar to be stolen. In fact, the other floral visitors (bees, butterfly) should be considered nectar thieves according to their visiting behaviour (phytocentric sampling). However, the high percentage of *M. lathyroides* pollen on the body (head, thorax-abdomen, legs) of all bee species collected (zoocentric sampling) suggests that some of these hymenopterans contacted the style's pollen brush. Nectar theft by bees and butterflies that cannot activate the trigger mechanism and/or perforate the base of the floral tube have been reported for *M. bracteatum* and *M. atropurpureum* (Brizuela *et al.* 1993; Vieira *et al.* 2002).

The trigger mechanism releases pollen when the staminal column is exposed due to pressure from a floral visitor or pollinator landing on the wings and keel to access nectar accumulated at the base of the flower (Arroyo 1981). This mechanism help economize nectar, and sometimes pollen, and seems to have allowed the development of even more specialized relationships between papilionoid flowers and their pollinators (Endress 1994; Aronne *et al.* 2012). However, some *Macroptilium* species do not depend on pollinators since they have spontaneous self-pollination and self-compatible systems (Brizuela *et al.* 1993; Hoc *et al.* 2003; Etcheverry *et al.* 2008b and references therein; Vieira *et al.* 2002).

Breeding system and floral morphology

IAS and ISI references of Macroptillium lathyroides population indicate that species is self-compatible, showing greater fertility and number of viable embryos after Autonomous Self-pollination (ASP). However ASP has higher number of aborted fruits compared to cross-pollination and control. Pollinator independence of the *M. lathyroides* population may be advantageous in the study area (disturbed environment), since the species has flowers with elaborate pollination systems that depend on a pollinator activating the brush-type trigger mechanism (Lavin and Delgado 1990) (see below). Spontaneous selfing is an alternative way to set fruits and seeds without the pollinator intervention (Etcheverry *et al.* 2008a) and is important in the absence or reduction of pollinators which frequently occurs in disturbed and pasture environments (Ai *et al.* 2013).

Autonomous self-pollination has been recorded for species of several Leguminosae genera with forage potential, including *Macroptilium* spp., which presented ISF values > 1.0 and did not differ between treatments (*e.g.*, Etcheverry *et al.* 2008a).

This was also registered for *M. lathyroides* in January 2018, with spontaneous selfing possibly being favoured by the proximity of the style pollen brush and stigma (Fig. 4c), and the absence of dichogamy in flowers. Indeed, our data suggests that the fruit and seed production may be primarily originating from self-pollination in the study area, considering the small number of registered pollinators (only *E.* cf. *auropilosa*).

In the present study, the low fruit set percentage, embryo viability and/or seed size after cross-pollination in relation to the other treatments (March 2018) may be an effect of flower emasculation. Flower emasculation can interfere with floral development, as has been recorded for sweet cherry (*Prunus avium*), which accelerated ovule degeneration and reduced fruit set (Hedhly *et al.* 2009 and references therein). *M. lathyroides* it has axillary pseudo racemose inflorescences with long peduncle (Fig. 3a) and paired flowers on the distal portion of the rachis. Fruits are linear and autochoric legumes (Prabhukumar*et al.* 2016). For this reason, this species is an important forage during periods of scarcity and in the Brazilian Semi-arid region, where small farmers exploit it for animal feeding purposes (Ferreira *et al.* 2004; Borges *et al.* 2019).

Regeneration by regrowth

Subterranean structures or underground systems can have diverse functions in the asexual reproduction of seed plants, including the production of new individuals (*e.g.*, propagation, vegetative multiplication) and/or regeneration of existing individuals (regrowth) (Borges 2000). However, the underground structures of *M. lathyroides* function in regrowth since they can produce new shoots after the removal of aboveground biomass, which has also been reported for other species of the genus (*e.g.*, *M. atropurpureum*) (Jones 1974; 2014). This attribute can influence the population dynamics of *M. lathyroides* and is an important strategy for plant persistence after disturbance events, which are common in ruderal environments (*e.g.* the present study area), allowing regeneration of individual plants after aerial part are removed by cutting or grazing (Jones 1974, 2014; James 1984).

Although plant regeneration occurred relatively quickly (~ one month), more than half of the cut plants (53%) did not sprout. Such fact must be considered when using this species in grazing conditions. Furthermore, according to Jones (2014), herbaceous perennial legumes do not persist forever, therefore, new plants must be formed from either stolons or rhizomes or from seed for long-term maintenance of this type of legume in environments such as pastures. *M. atropurpureum* plants that are frequently pruned or grazed tend to die and be replaced from seed banks (Jones 1974; 2014). This is probably the case for *M. lathyroides* since it doesn't propagate (i.e., fragmentation and/or dispersion of new individuals from the mother plant), a feature that favors efficient occupation of an environment by a species. In this way, *M. lathyroides* seems to depend on the seed bank to regenerate new individuals, as well as for dispersal and occupation of new habitats or environments. Resprouting in *M. lathyroides* plants starts from vegetative buds. In some *Macroptilium* species, resprouting occurs throughout sub-surface vegetative buds and roots accumulate reserves (carbohydrates) that provide the necessary energy to promote regrowth (Montiel *et al.* 2012), which probably occurs in *M. lathyroides*. The xylopodium (woody subterranean organ that is sometimes slightly fleshy, derived from the roots) is present in many perennial herbaceous legumes, which enables plants to persist during adverse seasons when aerial parts disappear (Burkart 1952).

Ruderal species have evolved diverse reproductive strategies to establish themselves in new areas with selfing proven to be essential for the preservation of the genotype, as well as for ensuring reproductive success, even though it reduces genetic diversity (Sharma and Sharma 2019). *Macroptilium lathyroides* presents several reproductive strategies that allow plants to survive and/or maintain this species in disturbed environments, such as crop or ruderal areas. For example, the regrowth of the aerial part that enables plant regeneration after cutting or grazing. However, the species depends on seeds to propagate/multiply or maintain a seed bank, since not all plants regrow after cutting. Consequently, sexual reproduction (and pollination) is necessary, but pollinators are not because *M. lathyroides* spontaneously self-pollinates. Autonomous self-pollination is important since the large and asymmetric flowers of *M. lathyroides* present an elaborate pollinating mechanism (brush type) that depends on specialized pollinators (medium-large native bees) that are scarce in ruderal or crop environments (Etcheverry *et al.* 2008a). In fact, we recorded only one small native bee, *Exomalopsis* cf. *auropilosa*, as a pollinator of the *M. lathyroides* flowers in the study area, because it is able to contact the anther to receive pollen and to touch the stigma to transfer the pollen. However, this could be a positive result, since (*e.g.* crops) there are bee groups capable of pollinating the flowers of this species in urban environments.

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