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Flowering, floral visitors and climatic drivers of reproductive phenology of two endangered magnolias from neotropical Andean forests

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Abstract

Depressed fruit and seed production and scant seedling recruitment appear to be imperiling natural populations of Colombian *Magnolia* species. From February 2015 to April 2017, we recorded data on flowering, pollen limitation, floral visitors, and the reproductive phenology of *Magnolia jardinensis* and *Magnolia yarumalensis*, two threatened and endemic species from Colombian Andean cloud forests. Both species are protogynous with anthesis lasting two consecutive days—with *M. jardinensis* flowers opening in the evening, while *M. yarumalensis* flowers open early in the morning. Fruit abortion in both bagged flowers and open pollination experiments suggests that both species exhibit considerable reproductive limitations. There was a lower percentage of pollen germination in *M. jardinensis* (3–26%) than in *M. yarumalensis* (60–86%), suggesting more limitations in the former species. *Magnolia yarumalensis* was a prolific fruit producer compared to *M. jardinensis*, which produced only one ripe fruit during the study, in spite of its high production of floral buds. Despite sharing the same locality, these species did not share pollinators, thus further increasing their vulnerability to extinction. Floral buds in both species were negatively correlated with minimum temperature and precipitation, while ripe fruits in both species were positively correlated with increases in precipitation and temperature. Our results provide information about how climate change could affect the various phenophases of these species. Knowledge about reproductive phenology and life history traits such as flowering and pollinators is crucial for specific and efficient conservation efforts to protect these endangered species under the current climate change scenarios.

KEYWORDS

cloud forests, flowering, *Magnolia*, pollinators, threatened species

1 | INTRODUCTION

The family of Magnoliaceae, with ca. 350 species of trees and shrubs, is a highly threatened group with 50% of species near extinction in the wild around the world, particularly in the Neotropics, the second center of Magnoliaceae diversity (Rivers, Beech, Murphy, & Oldfield, 2016; Vázquez-García et al., 2016). Neotropical Magnoliaceae consists of a single

genus, *Magnolia*, with ca. 170 species in three sections and three subsections (Vázquez-García et al., 2016). This region shows an extraordinarily high species richness and high endemism for Magnoliaceae characterized by a marked pattern of allopatric speciation, but many species are extremely rare with small population sizes (Vázquez-García et al., 2016). In South America, Colombia has the greatest number of *Magnolia* species with 39 species reported, of which 28 species belong to section Talauma—including four species of the recently described subsection Chocotalauma—and 11 species belong to section Splendentes—previously known as subsection Dugandiodendron (Aguilar-Cano, Mendoza-Cifuentes, & Ayala-Joya, 2018; Cogollo-Pacheco, Hoyos-Gómez, & Serna-González, 2019; Pérez, Arroyo, Neill, & Vázquez-García, 2016; Serna-González, 2005; Vázquez-García et al., 2017; Wang et al., 2020). Unfortunately, most Colombian species (95%) are considered endangered due to overexploitation, habitat loss, and forest fragmentation (Rivers et al., 2016). In 2018, 197,159 ha were deforested in Colombia (Instituto de Hidrología, Meteorología y Estudios Ambientales [IDEAM], 2019), mainly for land use change, which has been recognized as a primary driver of biodiversity loss (Pimm et al., 2014). Because *Magnolia* species in Colombia are both endemic and very rare, they were chosen as a conservation priority group by the National Strategy for Plant Conservation (Samper & García, 2001). One of the least-known aspects related to the current conservation status of the Colombian species is their reproductive phenology. Flower bud abortion, low year-round seed production, and low seedling recruitment (López-A, Bock, & Bedoya, 2008; Gómez, 2010; Serna-González & Urrego-Giraldo, 2016), as well as the effects of climatic seasonality on their reproduction, are all issues that have been poorly characterized and are of concern. Thus, improvement of this knowledge is needed to develop successful conservation efforts.

Studies on tropical plant phenology have shown the influence of rainfall, light availability, and temperature on the production of leaves and reproductive structures (Pau, Cordell, Ostertag, Inman, & Sack, 2020). Notably, reproductive phenology and floral cycles of Colombian *Magnolia* species have not been fully recorded (Serna-González & Urrego-Giraldo, 2016). In this study, we chose two endangered Colombian *Magnolia* species that cohabit in Andean remnant cloud forests. *Magnolia jardinensis* (section Talauma) is an endemic and critically endangered (CR) species (International Union for Conservation of Nature [IUCN], 2017) with a spatial distribution restricted to a few isolated trees (Rivers et al., 2016; Serna, Velásquez, & Cogollo, 2009; Velásquez & Serna, 2005) located in the Northwestern Andean Colombian region. To date, this species has only been found in the municipality of Jardín. Wood overexploitation in the last 20 years has led to its near extinction. *Magnolia yarumalensis* (section Splendentes) is an endangered species (EN) (IUCN, 2017) whose populations are more extensive and found throughout a wider distribution range, but they are still restricted to small forest remnants in different disturbance regimes and exhibit low seedling recruitment (Gómez, 2010; Velásquez & Serna, 2005). We recorded reproductive phenophases, floral developmental stages, and floral visitors for both species from February 2015 to April 2017 to answer the following questions: (a) Do these species share the same floral traits and floral visitors? (b) Do they have reproductive limitations? (c) How do climate variables influence their reproductive phenology over two consecutive years? (d) And finally, what are the implications for conservation regarding these particular traits? We performed pollination experiments and gathered useful data for the conservation and management of these highly endangered tree species during this time.

2 | MATERIALS AND METHODS

2.1 | Study area

This study was performed in remnant forest patches from the Western Andean cordillera between 2,000–2,600 m.a.s.l. (Jardín municipality, Antioquia province, Colombia, Figure 1) with trees of both *Magnolia* species.

According to climatic data from February 2015 to April 2017 (Figure 2) provided by the governmental authorities (IDEAM, 2017), monthly average maximum temperature values (26–28°C) were recorded in August (2015–2016) and April 2017 (29.2°C), while the average of minimum temperatures was registered in April and September 2015 and August 2016 (7°C). The average highest values of monthly precipitation were recorded in March 2015 (247 mm), April and December 2016 (244–361 mm), and April 2017 (253.4 mm), exhibiting a bimodal distribution as reported for the tropical Andes (Espinoza et al., 2020). Monthly average values of cloudiness were considerably high year-round (6–7 oktas). Trees of *M. jardinensis* can reach a height of up to 25 m and a diameter at breast height (DBH) of 60 cm, while trees of *M. yarumalensis* can reach a height of up to 30 m and a DBH of 70 cm. After a thorough search in the field sampling site, only four *M. jardinensis* trees were found. However, only two trees were used for phenology assessment because the other two were either too tall to climb for periodic floral observation or too young to produce reproductive structures. At the same study site, five *M. yarumalensis* trees with relatively accessible locations were climbed every month for phenological records.

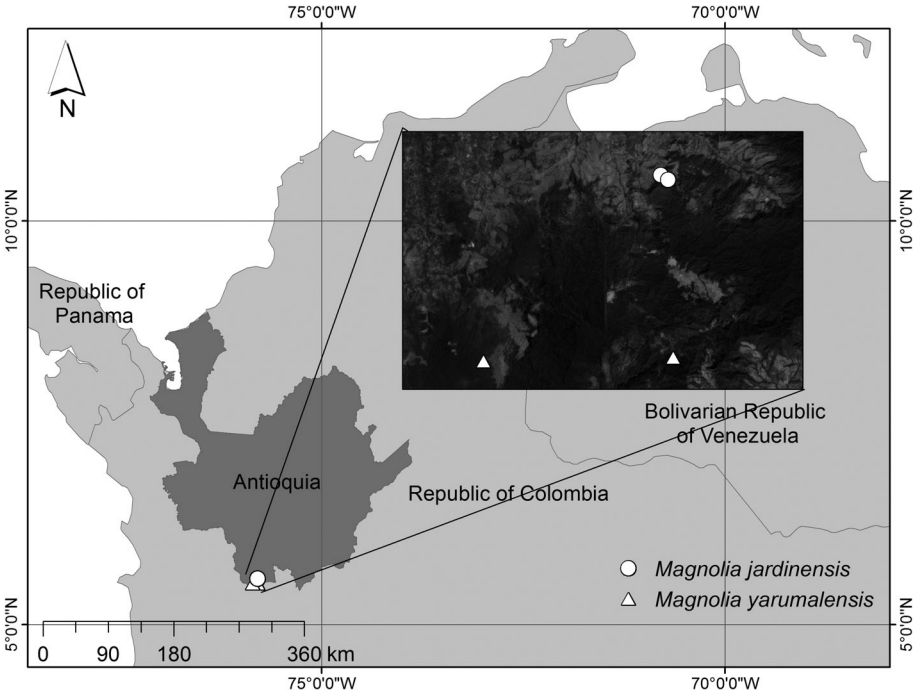

FIGURE 1 Sampling location of *Magnolia jardinensis* and *Magnolia yarumalensis* in Jardín municipality (Antioquia province, Colombia). Source: www.sasgis.org

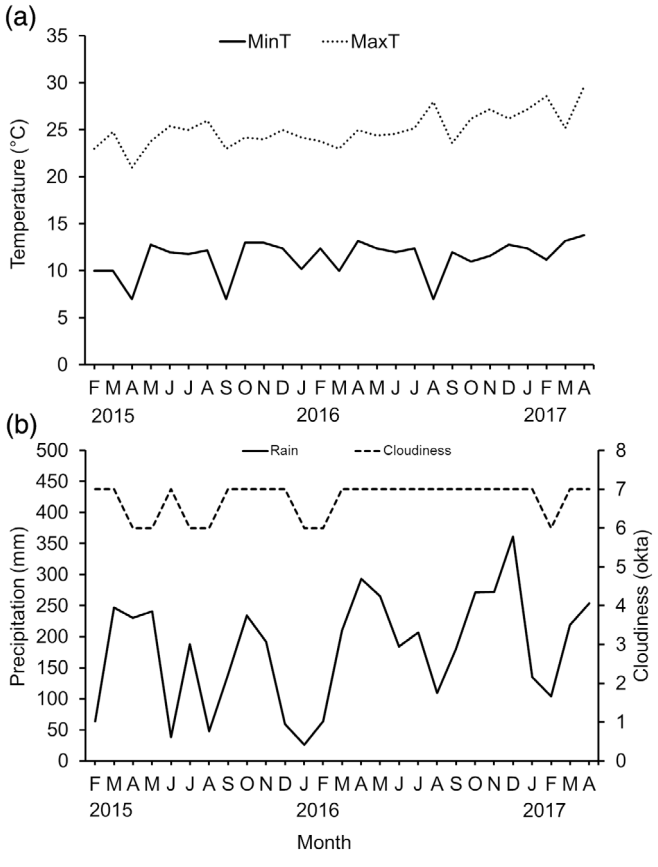

FIGURE 2 Distribution of (a) monthly extreme temperatures and (b) monthly precipitation and cloudiness from February 2015 to April 2017. MaxT, maximum temperatures; MinT, minimum temperatures

2.2 | Floral stages and observation of floral visitors

Direct observations were made and sampling of flowers was performed by tree climbing. Fifteen flowers per species ($n = 30$) were collected to describe different flowering stages in detail. Anthesis was recorded in the field with digital scouting Bushnell Trophy Cameras attached to tree branches of both species. Cameras were programmed to take pictures of floral buds and flowers every 5 min over two consecutive days. To assess stigmatic receptivity in the field, drops of 3% hydrogen peroxide (H_2O_2) solution were dripped on the stigmas to observe bubble formation, which is an indicator of current receptivity (Dafni, 1992; Li, Pang, & Saunders, 2016). For pollination experiments, 24 developing floral buds were selected per species ($n = 48$) in May 2016 and January 2017. For each species, 12 of the buds were tagged and enclosed within tulle bags to induce self-pollination, while the other 12 were simply tagged (for open pollination). The percentage of reproductive success was estimated based on the number of fruits that produced at least one seed. To estimate the percentage of pollen germination, we collected pollen grains by gathering flowers of both species at the female stage and waited for anther dehiscence. Pollen grains were then spread on slides with germination medium containing 5% sucrose and agar (Kameneva & Koksheeva, 2013). Pollen germination percentages were estimated 1 day later in the

laboratory, according to the number of germinated and non-germinated pollen grains, through direct observations under a light microscope. The experiments were performed in triplicate for each species. Seeds were collected in September 2015 for germination experiments in sterile substrate of soil and sand at a ratio of 2:1 and contrasting light treatments: darkness and full exposure. The experiments were performed at the Piedras Blancas station from Universidad Nacional de Colombia, located at 2,400 m.a.s.l., with a monthly temperature average of 15°C (Pérez & Ramírez, 2005). Insects were collected in pistillate and staminate stages of both species' flowers. Visitors of *M. jardinensis* were collected from one or two flowers in April, May, September, and October 2015, June, August, and December 2016, and January 2017 ($n = 12$) from 11:00 to 19:00. Visitors of *M. yarumalensis* were collected from flowers of one or two trees in September and October 2015, June and December 2016, and January, March, and April 2017 from 07:00 to 14:00 ($n = 12$). In order to clarify the possible role of floral visitors, a second sampling was performed to collect insects individually to study their pollen loads on flowers in pistillate and staminate stages from trees of both species (*M. jardinensis*, $n = 23$; *M. yarumalensis*, $n = 24$) in July, September, and November 2019 and January, February, and March 2020 in the same time ranges. Flowers were enclosed in plastic bags as they were cut from the branches to trap the insects, which were then transferred to tubes with 70% ethyl alcohol to preserve them before identification. Criteria such as abundance, visit frequency, the coincidence of the visits with the sexual stages of the flowers, insect activity, and pollen loads were used to classify floral insects as visitors, predators, pollinators, casual pollinators, or herbivores (Irvine & Armstrong, 1990; Li et al., 2016; Núñez-Avellaneda & Rojas-Robles, 2008). The experts John Cesar Neita (Instituto Alexander von Humboldt, Colombia) and Jan Klimaszewski (Canadian Forest Service, Natural Resources Canada) identified the insects. The samples were added to the collection of the Museo Entomológico Francisco Luis Gallego from Universidad Nacional de Colombia, Medellín campus (http://ciencias.medellin.unal.edu.co/museos/entomologico/index.php/). Additionally, petals were collected to obtain 5 g of dried material from each species to analyze nutritional composition. Analyses to measure the percentages of dry weight of carbohydrates, proteins, and ash content were performed at the Laboratorio de Análisis Químico y Bromatológico, and the percentage of dry weight of lipid content was analyzed at the Laboratorio de Separaciones Químicas, both laboratories from Universidad Nacional de Colombia, Medellín campus.

2.3 | Reproductive phenology

Two *M. jardinensis* trees and five *M. yarumalensis* trees were visited once a month from February 2015 to April 2017 to record their reproductive phenophases. A phenological data matrix was built separately for each analyzed species that included five phenophases: floral buds with bracts (FlBudBr), floral buds without bracts (FlBud), flowers (either female or male stages), unripe fruits (UnripeFr), and ripe fruits (RipeFr). Each phenophase was analyzed separately using Oriana – Circular Statistics for Windows (Version 4), n.d. Months were converted into angles, with intervals of 30° wide to calculate the mean angle (μ) or mean date, referring to the time of the year around which the phenological activity is most concentrated, and vector r, which indicates the intensity of concentration (0 to 1) around the mean angle. Vector r can be considered as a measure of the seasonality degree. The Rayleigh test was applied to indicate the significance ($p < 0.05$) of the mean angle (Dahua-Machoa, 2018; Müller & Schmitt, 2017). Each monthly measure was regarded as a sample. An environmental data matrix was used to relate the phenology of both species to the climate. The variables included were monthly precipitation, cloudiness, and maximum and minimum temperatures.

The normality of both phenological and environmental datasets was assessed using the Shapiro–Wilk test. As not the entire set of variables showed normality, a non-metric multidimensional scaling (NMDS) approach was used to detect the ordination pattern of both species' phenological data separately. The Bray–Curtis dissimilarity index was used to build the distance matrices as input for the NMDS analysis with three dimensions (k = 3). The analyses were performed using the "metaMDS" function of the Vegan library in R v3.6.1 (Oksanen et al., 2018). After standardizing (media = 0, $SD = 1$), the climatic explanatory variables were incorporated into the ordination process employing the Envfit function also of the vegan library (Oksanen et al., 2018) to assess the potential relationship between phenophases (two NMDS ordination axes) and regressed vectors of precipitation, cloudiness, and minimum and maximum temperatures.

3 | RESULTS

3.1 | Floral stages

Regarding floral stages, floral buds of *M. jardinensis* developed over approximately 2 months. The buds were covered by three pubescent bracts. The last bract abscission

occurred 1 day before flower opening (Figure 3a). At 10:00 the next day, the flower was still tightly closed, indicating that it was still in the pre-pistillate stage. Then, sepals (3) started to separate around 13:00 (Figure 3b), and petals (6–8) started to open 1 h later. Then, the pistillate stage started around 16:00 when sepals began to reflex, and the petals moved until being completely open at 18:00 (Figure 3c) with the sepals completely reflexed, exposing curled and papillose stigmas. The flower, possibly activated by thermogenesis, began to emit sweet floral scents, attracting floral visitors, particularly tiny flies posing on the petals (Dieringer et al., 1999; Seymour et al., 2009). Beetles began visiting the stigmas 1 h later. Petals started to close again around 18:30 and 1 h later the petals were completely closed while the sepals remained reflexed (Figure 3d). The following day, the flower was observed at the pre-staminate stage with the petals still closed during the morning with beetles (*Cyclocephala concolor*) inside. The staminate stage started with the opening of petals around 16:00, and few minutes later (16:20), stamens began to dehisce. At 17:00, petals were completely open at the staminate stage with the stigmas retracted to the receptacle and all stamens detached from the receptacle (Figure 3e). Insects began arriving at about 14:00, attracted by floral scents and possibly by the thermogenic environment within the floral chamber. At 18:00 the petals moved back to a more erect position and remained that way until wilting and eventually detaching from the receptacle (Figure 3f). The following 3 days, petals remained in this position until withering and eventually detaching from the receptacle. Although the pistillate stage lasted ca. 2 h from 16:00 to 18:00 with petals fully opened, stigmatic receptivity of *M. jardinensis* lasted ca. 7 h, starting at 13:00 with the beginning of petals opening, until 20:00 when petals were closed. The staminate stage lasted ca. 2 h from 16:00 to 18:00 the second day. There were no significant differences in sepal and petal dimensions between female and male stages ($F_{1,7} = 1.10$, $p = 0.37$). Fruit formation required approximately 2 months (6 months since the formation of floral bud with bracts). The only mature fruit observed during this study was composed of 10 carpels, most with one seed per carpel. Upon dehiscence, the external carpel walls remained adhered to the receptacle, and the open carpels exposed 10 black seeds, each covered by a red sarcotesta (Figure 3g).

As in *M. jardinensis*, *M. yarumalensis* floral buds developed over 2 months. However, buds in this species were covered by two bracts (Figure 4a). After bract abscission, the pre-pistillate stage started with the beginning of the gradual separation of sepals (3) early in the morning, around 4:30. Then, petals (7) started to open, creating small spaces between the unfolding petals, which provide access for a few insects, attracted by the floral scents at that time (Figure 4b). Around 7:00, petals were completely open at the pistillate stage, exhibiting curled and papillose stigmas (Figure 4c). Sepals remained open at 9:30 while petals started to close. The flower was completely closed about noon. Several insects (mainly beetles from subfamily Aleocharinae) were found inside the closed flower to contact the stigmas. The flower was completely closed with sepals still horizontally arranged around 15:00 at the pre-staminate stage (Figure 4d). The following day, petals started to open around 5:00, attracting insects probably by emitting sweet floral scents, and were completely open at the male stage around 7:00 with anthers releasing pollen grains while stigmas remained curled but brownish and not receptive at the staminate stage, with beetles inside (Figure 4e). Finally,

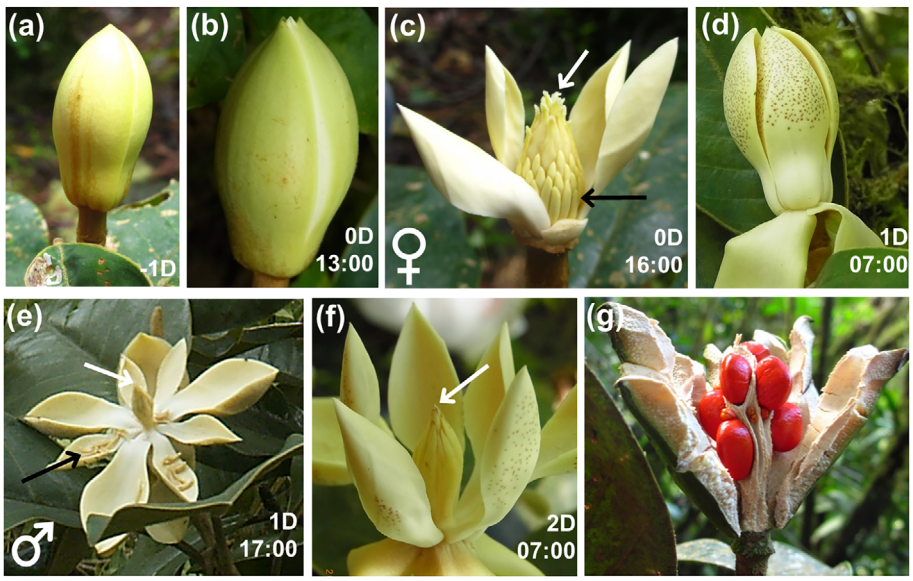

FIGURE 3 Floral development stages of *Magnolia jardinensis* (section Talauma). (a) Floral bud stage 1 day before opening (−1D). (b) Floral bud starting to open (0D, pre-pistillate). (c) First flower opening on the evening of the first day (0D, pistillate). (d) Flower closed in the morning of the next day (1D, pre-staminate). (e) Flower reopened at the evening of the next day with dehiscent anthers (1D, staminate). (f) Flower still reopened without stamens (2D, post-staminate). (g) Ripe fruit with red seeds. Black arrows, stamens; white arrows, stigmas

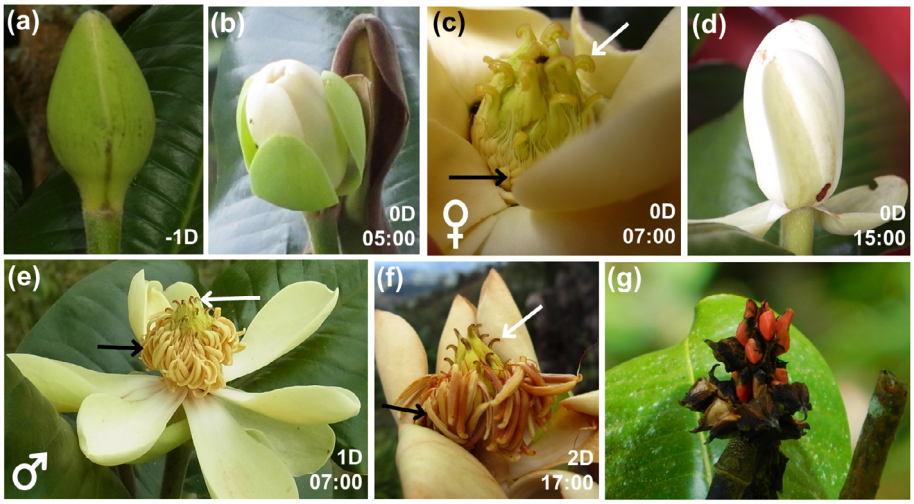

FIGURE 4 Floral development stages of *Magnolia yarumalensis* (section Spendentes). (a) Floral bud stage 1 day before opening (−1D). (b) Floral bud starting to open (0D, pre-pistillate). (c) First flower opening on the evening of the first day (0D, pistillate). (d) Flower closed in the evening of the first day (0D, pre-staminate). (e) Flower reopened in the morning of the following day with dehiscent anthers (1D, staminate). (f) Flower still reopened holding empty stamens (2D, post-staminate). (g) Ripe fruit exhibiting seeds. Black arrows, stamens; white arrows, stigmas. Note that stamens remain into the flower, via their log thread-like connective apices, which are still embedded in the gynoecium, typical of section Dugandiodendron

the petals began to move to a more erect position again at 8:00, and around 9:00, the petals started to be slightly erect around the receptacle until withering several days later (Figure 4f). Although the pistillate stage lasted ca. 4 h, from 7:30 to 11:30, stigmatic receptivity of *M. yarumalensis* lasted ca. 10 h from ca. 5:00 until 15:00. The staminate stage lasted ca. 2 h from 6:00 to 8:00. Floral asynchrony was observed in this species as well, with flowers at female and male stages observed at the same time within the trees. Fruits were formed with 13 (11–16) carpels containing one or two seeds per carpel with an average of 17 seeds per fruit ($n = 30$). Formation of fruits took approximately 4 months from the time that buds with bracts became visible. The external wall of the carpels collapsed separately when the fruits ripened, exposing brown seeds (12–24 seeds per fruit) with a reddish sarcotesta (Figure 4g). A two-way ANOVA test showed significant differences between sizes of sepals and petals of *M. yarumalensis* ($F_{1,7} = 10.49$, $p = 0.0478$).

3.2 | Pollination and pollen germination experiments

In open-pollination experiments, most *M. jardinensis* flowers (58%) were aborted, impeding the formation of fruits (8%), while in bagged flowers, half of the flowers formed fruit, but finally, all were aborted (Figure 5). In contrast, in *M. yarumalensis*, no flowers were aborted, and initial fruit formation was high in both open pollinated (100%) and bagged flowers (67%) in our experiments. However, fruit abortion reached 58% (7 fruits) for open-pollinated and 67% (8 fruits) for bagged flowers in this species (Figure 5). The pollination experiments for bagged flowers indicated that neither species was able to produce fertile fruits, suggesting possible parthenocarpy.

Pollen viability of *M. jardinensis* was lower than that of *M. yarumalensis*. Germination of pollen grain ranged from 3.2 to 25.9% in *M. jardinensis* and from 66.9 to 86.0% in *M. yarumalensis*. Even though bagged flowers in the pollination experiments often formed fruits in both species, none of these fruits produced any seeds. Thus, all seeds were obtained from fruits produced by open pollination. For germination experiments, 320 seeds from ripe fruits of *M. yarumalensis* were collected in September 2015. No significant differences in germination percentage were found between full exposure and darkness treatments (13 vs. 11%, respectively, $p > 0.05$). All seeds (10) collected from the only fertile fruit of *M. jardinensis* germinated in a moist towel within plastic bags after storage at 4°C for 1 month. The germinated seedlings were transplanted in Medellín (1,500 m.a.s.l.), but no cotyledons opened, and only two seedlings reached 5 cm in height. All seedlings of *M. jardinensis* eventually died.

3.3 | Floral visitors

As the trees were very tall, flowers were obtained by climbing every tree of both species, and the insects were

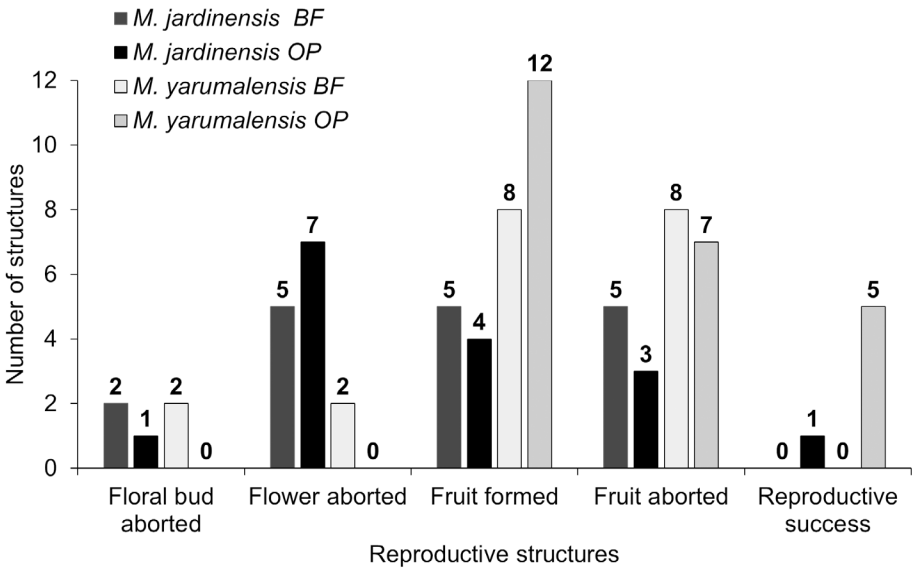

FIGURE 5 Pollination experiments of *Magnolia jardinensis* and *Magnolia yarumalensis*. BF, bagged flowers (by enclosing floral buds with tulle to avoid the entrance of floral visitors); OP, open pollination in which floral buds were labeled and observed periodically (12 flowers per treatment/species). The number above bars indicates the total of each reproductive structure (floral buds, flowers, and fruits) that was aborted or formed at the end of the experiment

collected from 12 flowers per species in both pistillate and staminate floral stages from April 2015 and April 2017. To verify pollen loads, insects were collected from 23 flowers in *M. jardinensis* and 24 flowers in *M. yarumalensis* between July 2019 and March 2020. Insects associated with each *Magnolia* species were collected and observed under a light stereomicroscope (16 species in *M. jardinensis* and 21 species in *M. yarumalensis*) (Table 1).

According to our observations, *C. concolor* beetles were found between 11:00 and 13:00 within the closed flower, suggesting that the individuals entered the flower before the closing of petals. These beetles remained within the flowers of *M. jardinensis* through the night, feeding on pollen and other flower parts without significantly damaging the flowers. As we observed pollen on their bodies in both female and male stages of flowers (Figure 6a,b; Table 1), they were considered to be the effective pollinators of *M. jardinensis*. Insects of one species of rove beetles from genus *Phloeonomus* were found enclosed in the floral chamber in both floral stages having contact with stamens and stigmas and consuming pollen. Because pollen was observed on their bodies only during the second sampling period, we classified them as casual pollinators.

Most flies, such as *Cyphomyia* sp., Phoridae spp., *Coenosopsia* sp., and *Monardia* sp. (Figures 6d–h), were considered visitors based on their behavior. Beetles from tribe *Bothrosternini* found in flowers of this species (Figure 6q) were considered visitors because they were observed posing on petals without eating any flower resource. The most abundant insects collected in flowers of *M. jardinensis* were little flies (*Psychoda* sp.) that started to arrive at the petals from 16:00 until 18:00 at the pistillate stage and from 14:00 to 16:00 at the staminate stage, apparently attracted by sweetish floral scents (Figure 6k). However, they rarely entered the flower and usually remained on the external surface of the petals. The following day, several dots were observed on the petals (approximately 380–400 tiny markings per petal, Figure 6l) corresponding to eggs oviposited by these flies. Tiny larvae were observed (using a magnifying glass) growing inside petal tissues present on the forest floor (Figure 6m). Flowers were not affected by these flies, while larvae of a moth (Stenomidae, Figure 6p) perforated the petals to gain entry to the flower and consume stigmatic tissues (Figure 6n,o). Bees such as *Apis* sp. (Figure 6r) and *Plebeia* sp. (Figure 6s) were found foraging pollen only in the female stage and without pollen grains on their bodies, so they were considered as visitors while pollen loads were observed on the bodies of *Partamona* sp. in both floral stages, so this species was classified as a casual pollinator.

In *M. yarumalensis*, small beetles that were collected within the flowers early in the morning until 14:00 (Figure 7a,b) from genus *Hoplandria* (Figure 7c) were identified as pollinators, as pollen grains were found on their bodies and the insects remained for several hours in the flowers at both female and male stages. Much the same beetles, *Polylobus* sp. (Figure 7d), exhibited similar behavior, but they were absent in male floral stages, and pollen loads were not found in their bodies, so this species was classified as a visitor. Beetles such as *Diabrotica balteata* (Figure 7e), *Zischkaita* sp. (Figure 7f), and other species from Chrysomelidae (Figure 7g–j) and Coccinellidae (Figure 7l) were considered visitors, except one species of Curculionidae (Figure 7k) that was recorded eating floral tissues in both floral stages, so it was classified as herbivorous. Other hymenoptera insects such as Formicidae (Figure 7m,n), *Crematogaster*

TABLE 1 Floral visitors of *Magnolia jardinensis* and *Magnolia yarumalensis*

	Floral stage				Mean of pollen loads[a]		
	Female	Male	Abundace	Relative abundace (%)	Female	Male	Role
Magnolia jardinensis							
Coleoptera							
Bothrosternini sp.	x		4	1.03%			Visitor
Curculionidae sp. 1		x	1	0.26%			Herbivorous
Cyclocephala concolor	x	x	8	2.05%	67.00	74.67	Pollinator
Phloeonomus sp.	x	x	9	2.31%	0.50	0.60	Casual pollinator
Diptera							
Diptera sp. 1		x	1	0.26%			Visitor
Coenosopsia sp.		x	1	0.26%			Visitor
Phoridae sp. 1		x	1	0.26%			Visitor
Phoridae sp. 2		x	1	0.26%			Visitor
Psychoda sp. 1	x	x	354	90.77%			Herbivorous
Cyphomyia sp.		x	1	0.26%			Visitor
Monardia sp.		x	1	0.26%			Visitor
Lepidoptera							
Stenomidae sp.		x	1	0.26%			Herbivorous
Hemiptera							
Pentatomidae sp. 1		x	1	0.26%			Visitor
Hymenoptera							
Apis sp.	x		2	0.51%			Visitor
Plebeia sp.	x		2	0.51%			Visitor
Partamona sp.	x	x	2	0.51%	11.00		Casual pollinator
Magnolia yarumalensis							
Araneae							
Araneae sp. 1	x		1	0.14%			Predator
Coleoptera							
Chrysomellidae sp. 1	x		1	0.14%			Visitor
Chrysomellidae sp. 2	x		3	0.43%			Visitor
Chrysomellidae sp. 3	x		1	0.14%			Visitor
Chrysomellidae sp. 4		x	1	0.14%			Visitor
Zischkaita sp.		x	3	0.43%			Visitor
D. balteata			1	0.14%			Visitor
Coccinellidae sp. 1	x		2	0.28%			Visitor
Curculionidae sp. 2	x	x	3	0.43%			Herbivorous
Hoplandria sp.	x	x	609	86.75%	0.56	1.57	Pollinator
Polylobus sp.	x		58	8.26%			Visitor
Diptera							
Diptera sp. 2		x	2	0.28%			Visitor
Psychodidae sp. 2	x		8	1.14%			Herbivorous
Psychodidae sp. 3	x		1	0.14%			Herbivorous
Sciara sp.		x	1	0.14%			Visitor

TABLE 1 (Continued)

	Floral stage				Mean of pollen loads[a]		
	Female	Male	Abundace	Relative abundace (%)	Female	Male	Role
Ephemeroptera							
Baetidae sp. 1	x		1	0.14%			Visitor
Hymenoptera							
Trigona amalthea		x	1	0.14%			Visitor
Partamona sp.		x	1	0.14%		35.00	Visitor
Formicidae sp. 1	x		1	0.14%			Visitor
Formicidae sp. 2		x	1	0.14%			Visitor
Crematogaster sp.	x		2	0.28%			Visitor

[a]Pollen grains per individual.

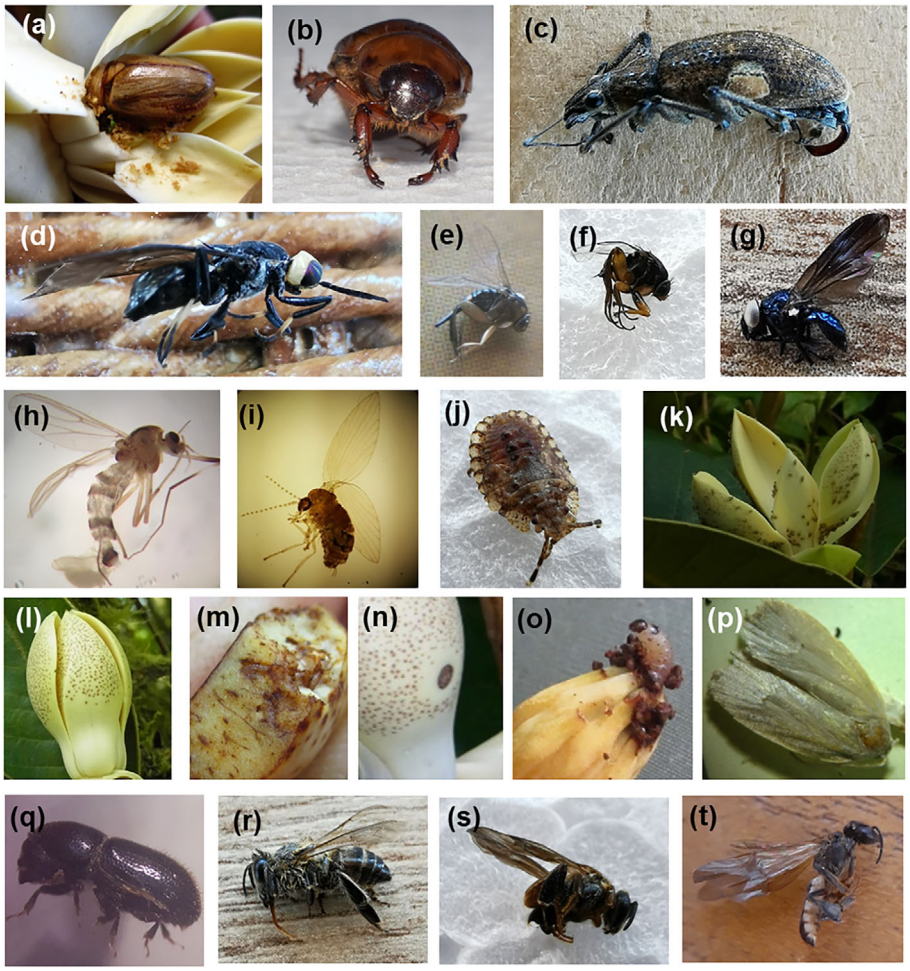

FIGURE 6 Floral visitors of *Magnolia jardinensis*. (a) *Cyclocephala concolor* within the flower. (b) *Cyclocephala concolor*. (c) Curculionidae sp. 1. (d) *Cyphomyia* sp. (e) Phoridae sp. 1. (f) Phoridae sp. 2. (g) *Coenosopsia* sp. (h) *Monardia* sp. (i) *Psychoda* sp. (j) Pentatomidae. (k) Flies of *Psychoda* sp. arriving on the petals from 14:00 to 18:00. (l) Marks on petals by *Psychoda* sp. (m) larvae of *Psycoda* sp. (n) Perforation of petals caused by a larva of Stenomidae. (o) Larva of Stenomidae eating the stigmas. (p) Adult of Stenomidae. (q) *Bothrosternini* sp. (r) *Apis* sp. (s) *Plebeia* sp. (t) *Partamona* sp

sp. (Figure 7o), *Trigona amalthea* (Figure 7p), and *Partamona* sp. (Figure 7q) were classified as visitors according to their behavior and time within the flower. Two species of Psychodidae were classified as herbivorous, exhibiting a similar behavior to that of *Psychoda* sp. in *M. jardinensis*, although no larvae were found on the petals. In contrast, *Sciara* sp. (Figure 6q) was recorded as a floral visitor.

Analyses of perianth nutritional content for both species showed that they include lipids, proteins, and sugars that could be related to the role of some floral visitors (Table 2). Sugar, protein, and ash contents were higher in

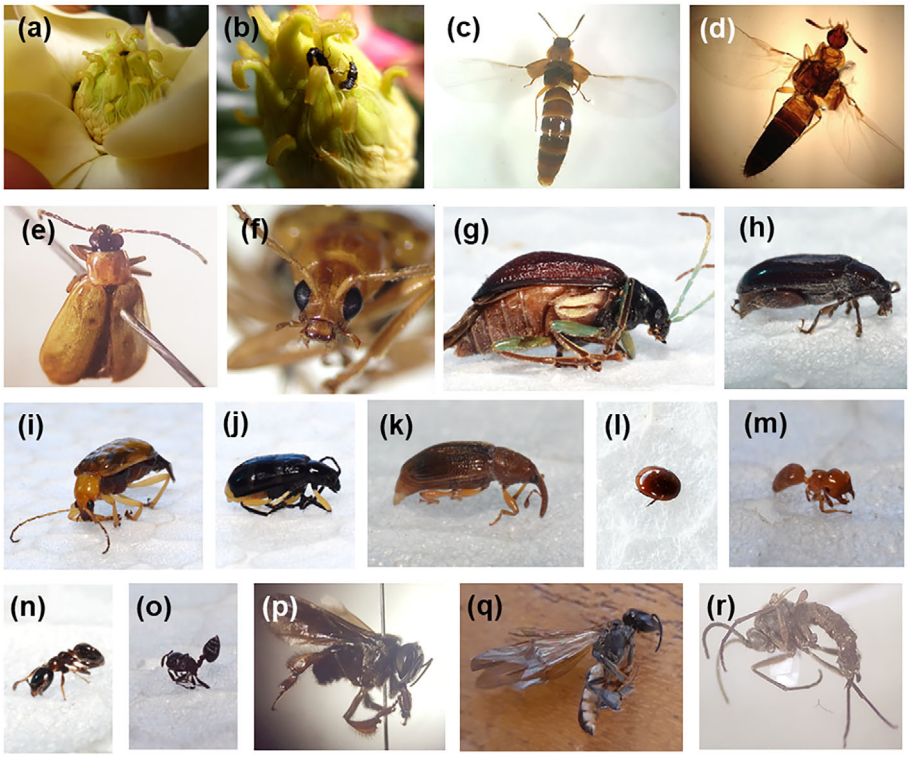

FIGURE 7 Floral visitors of *Magnolia yarumalensis*. (a, b) *Hoplandria* sp. around stigmas within flowers. (c) *Hoplandria* sp. (d) *Polylobus* sp. (e) *Diabrotica balteata*. (f) *Zischkaita* sp. (g) Chrysomelidae sp. 1. (h) Chrysomelidae sp. 2. (i) Chrysomelidae sp. 3. (j) Chrysomelidae sp. 4. (k) Curculionidae sp. 2. (l) Coccinellidae. (m) Formicidae sp. 1. (n) Formicidae sp. 2. (o) *Crematogaster* sp. (p) *Trigona amalthea*. (q) *Partamona* sp. (r) *Sciara* sp

Species	Lipids	Sugar	Protein	Ash	Source
Magnolia jardinensis	0.72	8.68	19	9	This work
Magnolia yarumalensis	3.25	11.81	9.2	3.8	This work
Magnolia tamaulipana	9.2		13.7	7.1	Dieringer et al. (1999)

TABLE 2 Percentage of dry weight of nutritional content in petals and sepals of *Magnolia* species under study

sepal and petals of *M. jardinensis* (6) compared to *M. yarumalensis* (7).

3.4 | Reproductive phenology

Five phenophases were assessed every month from February 2015 to March 2017: floral buds with bracts, floral buds without bracts, flowers, unripe fruits, and ripe fruits (Figures 8 and 9). The production of floral buds with bracts occurred nearly year-round in this study (except May and October 2015), reaching up to 28 in a single tree in October 2016 (Figure 8a). Floral buds without bracts were observed during all months, but they were not as abundant as the former phenophase, reaching up to 6 in April 2015 (Figure 8b). Flowering of *M. jardinensis* occurred year-round (except November 2015 and 2016). However, it was more pronounced in October 2015 and July 2016 (6 flowers) and February 2017 (8 flowers, Figure 8c). Flower production was low compared to the abundant production of floral buds with bracts (up to 28 in a single tree in October 2016). Unripe fruits were observed mainly in September and December 2015, January, September, and December 2016, and January 2017, when up to four fruits per tree were observed (Figure 8d). It is noteworthy that only two ripe fruits were observed from all floral buds over a span of 27 months and only one fully ripe fruit was observed in December 2016 (Figure 8e). While the production of unripe and ripe fruits was related to higher values of temperatures, precipitation, and cloudiness, the floral stages developed under lower values of these variables as shown by the NMDS ordination (Figure 10a).

Trees of *M. yarumalensis* produced floral buds with bracts all months except September 2015 (Figure 9a), while floral buds without bracts were absent in September and October 2016 and March 2017 (Figure 9b). Flowers were observed year-round, reaching a maximum of seven flowers in May and June 2016 and February 2017 (Figure 9c). In contrast to the former species, unripe fruits were observed almost the whole year, being most abundant in September 2015 with 33 fruits and in August 2016 with 25 fruits (Figure 9d). On average, the production of

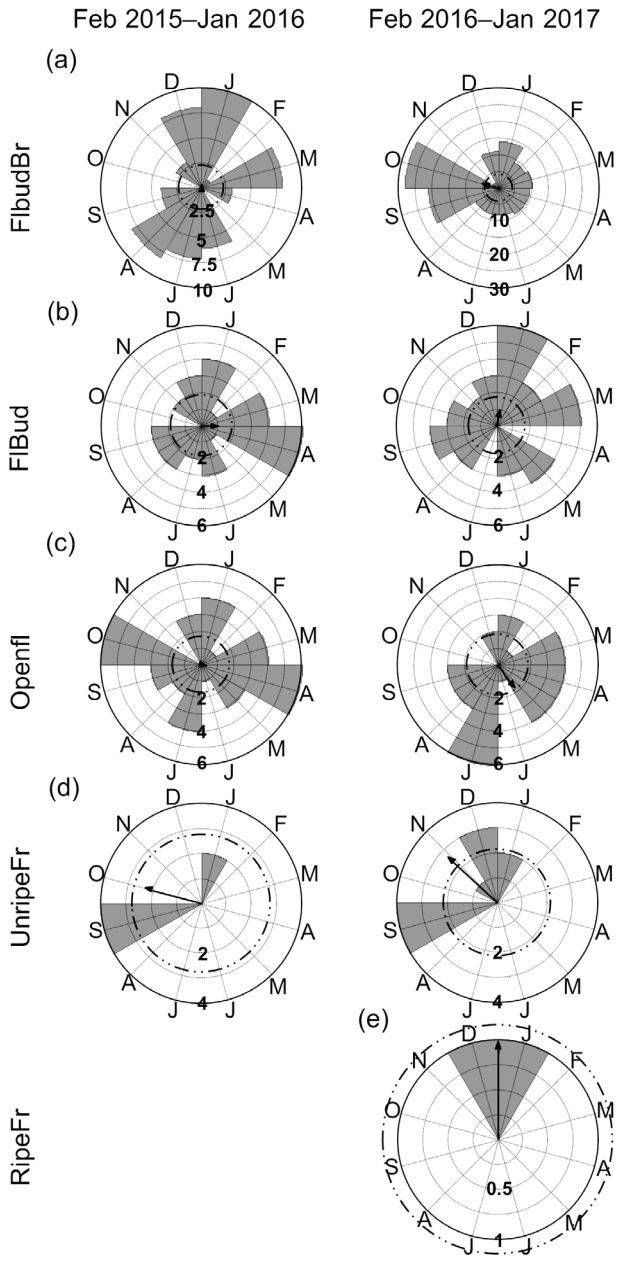

FIGURE 8 Variation in the temporal occurrence of five phenophases of *Magnolia jardinensis*. (a) Floral buds with bracts (FlbudBr). (b) Floral buds without bracts (FlBud). (c) Open flowers (Openfl). (d) Unripe fruits (UnripeFr). (e) Ripe fruits (RipeFr). The direction of vector r represents mean angles; the length (0 to 1) indicates the concentration around the mean angle. Mean angles are significant when vector r crosses the dashed line (Rayleigh test $p < 0.05$)

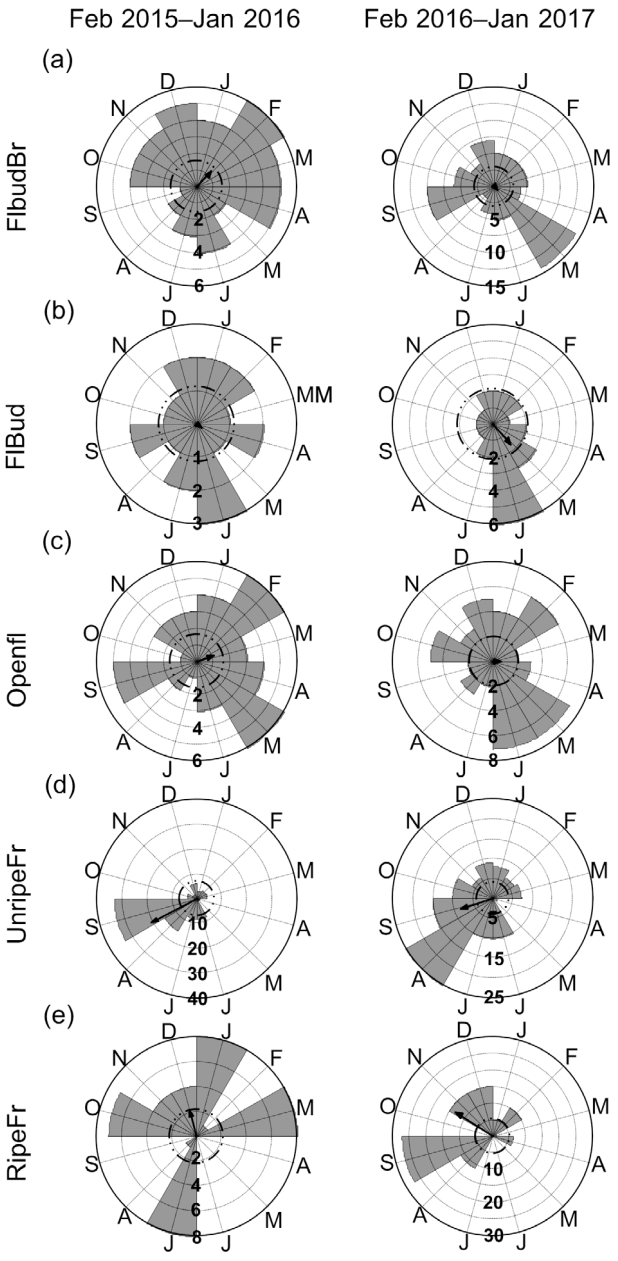

FIGURE 9 Variation in the temporal occurrence of five phenophases of *Magnolia yarumalensis*. (a) Floral buds with bracts (FlbudBr). (b) Floral buds without bracts (FlBud). (c) Open flowers (Openfl). (d) Unripe fruits (UnripeFr). (e) Ripe fruits (RipeFr). The direction of vector r represents mean angles; the length (0 to 1) indicates the concentration around the mean angle. Mean angles are significant when the r vector crosses the dashed line (Rayleigh test $p < 0.05$)

ripe fruits was higher in 2016 and 2017 (seven fruits per year) compared to 2015 (three fruits per year). A maximum of eight ripe fruits per tree was observed in February, April, and August 2015, while in 2016, trees produced 28 fruits in September and 15 in November and December, and in 2017, trees produced 18 fruits in February (Figure 9e). When comparing reproductive phenophases of both species, we found that most *M. jardinensis* flowers were aborted, while most of the flowers of *M. yarumalensis* remained on the tree and eventually formed fruits. In *M. yarumalensis*, in contrast to the former species, open flowers and unripe fruits were displayed during months with lower values of precipitation, temperature, and cloudiness, while floral buds and ripe

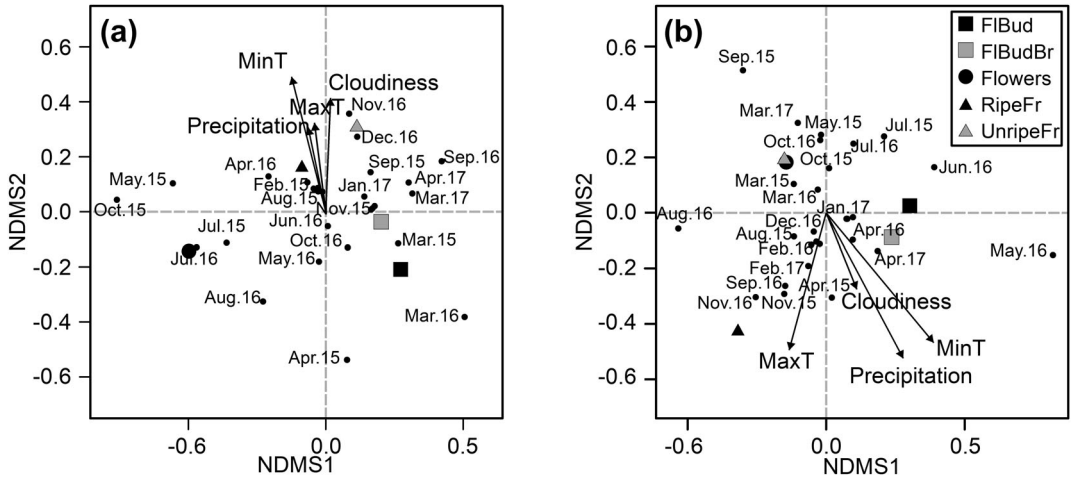

FIGURE 10 Non-metric multidimensional scaling (NMDS) ordination of the temporal pattern of the reproductive phenophases of (a) *Magnolia jardinensis* (bray-stress: 0.073) and (b) *Magnolia yarumalensis* (bray-stress: 0.078) and the climate variables minimum temperature (MinT), maximum temperature (MaxT), cloudiness, and precipitation. FlBud, floral buds; FlBudBr, floral buds with bracts; RipeFr, ripe fruits; UnripeFr, unripe fruits

fruit production increased with the values of these climate features as shown by the NMDS ordination (Figure 10b).

3.5 | Climate influence on reproductive phenology

The results of the NMDS ordination are represented in Figure 10. The analysis showed a good fit for both species with stress values of 0.073 and 0.078 for *M. jardinensis* and *M. yarumalensis*, respectively. For *M. jardinensis*, the first axis (NMDS 1) showed a clear separation between flower buds (with and without bracts) from open flowers. While the flower buds were positively related to the first axis (NMDS 1), the open flowers were negatively related to this axis. Instead, fruit production (unripe and ripe fruits) was positively related to the second axis (NMDS2), and flowering was negatively related to the second axis. While the floral phases were related to lower values of precipitation, temperature, and cloudiness, fruit production was directly related to higher values of these climate variables (Figure 10a). In *M. yarumalensis*, floral buds (with and without bracts) were positively related with the first axis (NMDS 1). Open flowers and fruiting phases were negatively related to the first axis, but these phenophases were separated along the second axis (NMDS 2).

In contrast to the former species, in *M. yarumalensis* the peaks of unripe fruits and open flowers were rather synchronous and showed a positive relation with the second axis and a negative relation with precipitation, cloudiness, and both temperatures. Ripe fruits were negatively related with the second axis (NMDS 2) and positively with all these climate variables. Despite the good fit of the NMDS in both species and the relationship with climate variables shown by the Envfit, only the minimum temperature was significantly and positively related with both fruiting phenophases along the second axis of *M. jardinensis* (NMDS 2). This relationship was corroborated by the linear model built between the first axis of the NMDS and climate variables, where after a backward selection of variables, the minimum temperature was the only significant variable ($t = 3.01$, $p \leq 0.006$). In *M. yarumalensis*, any climate variable showed a significant correlation with the first two NMDS axes. However, a linear model between the third axis and climate variables showed a significant correlation with precipitation, cloudiness, and minimum temperature.

Floral buds with and without bracts increased at the end of the dry season in *M. jardinensis* (Figure 10), characterized by low monthly precipitation levels, and when minimum temperatures were not extreme, particularly in March of the 3 years. However, a decrease of floral buds with bracts was exhibited in *M. yarumalensis* when the lowest monthly values of minimum temperatures occurred (September 2015 and August 2016), while those of *M. jardinensis* decreased a month after the occurrence of those minimum temperatures. Plain flowering of both species increased when monthly values of maximum and minimum temperatures were relatively constant (around 12–13°C of minimum temperatures and 24–25°C of maximum temperatures). The unripe fruit production was not synchronous between both species, while *M. yarumalensis* exhibited an increase of unripe fruits when the lowest monthly minimum temperatures were registered after having constant temperature values during 4 months (from May to August in

2015–2016 and from April to July in 2016–2017). In *M. jardinensis*, both unripe and ripe fruits increased with minimum temperature values. The number of ripe fruits in *M. yarumalensis* increased with higher values of maximum temperatures.

4 | DISCUSSION

4.1 | Floral stages, pollination, and pollen experiments

Given that pistillate and staminate stages were available simultaneously within the same tree in both species, fertilization of flowers by pollen from other flowers of the same plant (geitonogamy) may occur (Lloyd & Webb, 1986). Fruit abortion observed in both *Magnolia* species is possibly related to low genetic variability (Pansarin & Pansarin, 2017). Notwithstanding, other Colombian *Magnolia* species have exhibited high genetic variability despite their rarity and endemism (López-A et al., 2008).

Although both species formed fruits without pollinators (which were aborted before fully ripening), the capacity of unfertilized flowers to form ripe fruits is not completely ruled out. Further studies need to be performed to determine the possibility of parthenocarpy in the studied species as an adaptative advantage during suboptimal pollination conditions, mainly in polyploid species (Picarella & Mazzucato, 2019). Parthenocarpy is also regarded as a defensive strategy against seed predation, damages in reproductive organs, or pollen limitation (Ramos-Ordoñez et al., 2008). Exceptionally, parthenocarpy in *M. jardinensis* could respond to the stigma destruction by feeding larvae (Solomon, 1980).

The low germination percentage of pollen grains in *M. jardinensis* (only up to 26% germination) compared to *M. yarumalensis* (up to 86%) may indicate that pollen grain viability could be affecting its reproductive success as reported for other *Magnolia* species (Chen et al., 2016; Hirayama et al., 2005; Wang et al., 2010). *Magnolia jardinensis* contains up to 5,000 pollen grains per anther, so lack of pollen does not appear to be the problem. However, stamens were collected during the pre-staminate stage (at 14:00 when the inner petals were still closed prior to anther dehiscence), which suggests the possibility that the pollen grains had not fully matured since the stamens had been prematurely removed from the warm (thermogenic) environment of the closed flower. Similarly, in *M. denudata* lower pollen germination was encountered after researchers mitigated thermogenetic warming by deliberately preventing petals from closing during the post-pistillate stage (Liu et al., 2017). However, the pollen grain viability of *M. yarumalensis* was high despite being collected at the pre-staminate stage. Considering that both *Magnolia* species in this study share similar climatic and habitat conditions, further studies need to be performed to establish the causes of low pollen grain viability in *M. jardinensis*. Similar differences in timing of floral stages have been recorded in other nocturnal (i.e., evening-opening) *Magnolia* species including from sections Gynopodium (Chen et al., 2016), Magnolia (Figlar, 2019; Losada et al., 2014; Thien, 1974), and Talauma (Gottsberger et al., 2012; Figlar, 2019), and also in other diurnal (i.e., morning-opening) species including from section Yulania (Liu et al., 2017; Wang et al., 2014). Additional species from sections Splendentes and Talauma need to be assessed to determine if the other species in those sections exhibit morning-opening traits as in *M. yarumalensis* or evening-opening traits as in *M. jardinensis*. It is suggested that this trait (diurnal vs. nocturnal opening) could be useful in the further classification of Neotropical *Magnolia* species. This trait could also be associated with a specific plant–pollinator interaction, considering the behavior of different pollinators recorded for *Magnolia* species (Table S1), and could have implications for conservation according to the resilience capability of insects in light of disturbance processes (Sharma & Armstrong, 2013).

4.2 | Floral visitors

Besides *C. concolor* (Scarabaeidae) recorded here in flowers of *M. jardinensis*, three other species of this genus—*Cyclocephala jalapensis* (Dieringer & Espinosa, 1994), *Cyclocephala caelestis* (Dieringer et al., 1999), and *Cyclocephala literata* (Gottsberger et al., 2012)—have been reported as being effective pollinators of at least four other *Magnolia* species from the Americas. Cantharophily (pollination by beetles) is thought to be the most common pollination syndrome in Magnoliales, as documented for other basal families such as Annonaceae (Gottsberger, 2012; Teichert et al., 2012), Nymphaceae (Seymour & Matthews, 2006), Myristicaceae (Sharma & Armstrong, 2013), Winteraceae (Marquinez et al., 2009), and monocots such as Araceae (Maia et al., 2010). Flowers of these families present abundant production of pollen grains, floral chambers, and thermogenesis (Wang & Zhang, 2015), key to attract these beetles. Several floral visitors have been recorded for *Magnolia* species, reaching up to eight different insect species (i.e., *Magnolia sinica*; Chen et al., 2016; see Table S1). Nevertheless, only one or two species of Coleoptera are identified as effective pollinators for each *Magnolia* species, which is the case for the two *Magnolia* species included in this study as in other *Magnolia* from Central America (Dieringer et al., 1999; Dieringer & Espinosa, 1994; Gottsberger et al.,

2012). Particularly in this study, despite the close proximity of trees in both species, each *Magnolia* species was associated with different community assemblies, while just one bee species, *Partamona* sp., visited both *Magnolia* species.

Generalized pollination is typical of high mountain species where pollinator availability is unpredictable (Funamoto & Sugiura, 2020). Notwithstanding, these two *Magnolia* species that cohabit in remnant Andean cloud forests do not share their floral visitors, underscoring their highly specialized insect relationships. *Magnolia jardinensis* flowers open at night while *M. yarumalensis* flowers open in the daytime, suggesting that one set of floral visitors is mostly nocturnal and probably endothermic, such as *Cyclocephala* beetles (Zermoglio et al., 2018; Seymour et al., 2009), while the other set of visitors is more active in the daytime. A relationship with specific pollinators is a relevant aspect in the understanding of plant speciation processes (Swarts & Dixon, 2009). In addition, as in other mutualistic interactions, the vulnerability to extinction is also higher because the loss of one species might cause the extinction of their associated ones (Smith & Smith, 2007). On the other hand, Aleocharinae beetles are usually present in forest litter. However, *Polylobus* sp. seems to be adapted for feeding of pollen grains in flowers of Andean cloud forests (Klimaszewski & Sturm, 1991), so the interaction of *M. yarumalensis* with these specialized beetles should be further studied. Interestingly, *Polylobus* sp. was almost absent in the latest sampling, suggesting that probably abiotic factors could be related to the abundance of floral visitors' assemblies, as well as flowers. Likewise, Coleoptera species of the genera *Diabrotica* and *Bothrosternini* found in the studied *Magnolia* flowers have been recorded as herbivores in other plant species, although we did not identify such behavior. Thus, more collections and observations are needed to clarify their role in the ecology of *Magnolia* species.

4.3 | Reproductive phenology and climate

According to our results, minimum temperature influenced reproductive phenophases of both *M. jardinensis* and *M. yarumalensis*, although only significantly for the former species. The number of floral buds (with and without bracts) for both species was negatively correlated with the minimum monthly temperature and precipitation. However, both species were not synchronous since the decrease in the number of buds that coincided with the lowest temperature and precipitation values in *M. yarumalensis* occurred a month later than in *M. jardinensis*. While these minimum temperatures and precipitation triggered the formation of unripe fruits in *M. yarumalensis*, the number of ripe fruits in both species increased during months of higher precipitation and temperature. The unripe fruits of *M. jardinensis* also increased with increases in these climate variables. In contrast, open flowers did not show a clear relationship with climate variables in both species.

In the context of climate change, an increase in maximum and minimum temperatures could affect reproductive phenology in *M. jardinensis* and *M. yarumalensis*, possibly causing a decline in the formation of flowers buds in these, as well as in other neotropical *Magnolia* species. Climate projections show increased warming in the tropical Andes—with more pronounced warming at higher elevations—and reductions in cloud cover, resulting in increases in hours of sun exposure (Anderson et al., 2011). In our study, monthly maximum temperatures ranged from 23 to 29.6°C. Higher values would lead to a decrease of floral buds, thus reducing flower production in these two species as well as other Andean *Magnolia* species.

Although the effects of climate change in tropical ecosystems do not appear to be as significant as in temperate ecosystems (Thackeray et al., 2010), a reduction in horizontal precipitation (fog interception by tree canopies in cloud forests) could lead to decreased moisture availability, with consequences for many species adapted to narrow elevational ranges on steep slopes in cloud forests (Anderson et al., 2011). An increase of 1.5°C from global warming is projected to cause increases in the frequency, intensity, or amount of heavy precipitation, particularly in the cloud forests of the Andes (Anderson et al., 2011; Hoegh-Guldberg et al., 2018). Such conditions could to lead changes in phenology patterns and increase extinction risks (Hoegh-Guldberg et al., 2018). According to our data, an increase of heavy precipitation would probably negatively affect the production of fruits and flower buds in *M. yarumalensis* and *M. jardinensis*, respectively. In addition, climate change can influence plant–animal interactions, affecting the timing of plant and animal reproductive cycles and thus mutualistic interactions (Cerdeira-Morellato et al., 2016; Visser, 2016). Thus, these *Magnolia* species may be more prone to extinction by altering the availability of resources for specific pollinators such as flowers that occur when maximum and minimum temperatures do not exhibit extreme values. Moreover, the warmer temperatures could possibly alter the beetles' behavior such that they become less prone to seek out the reward of thermogenic warmth inside the floral chamber of *Magnolia* flowers (Seymour et al., 2009). Nevertheless, these changes are still unpredictable.

5 | CONCLUSIONS

This research suggests that the studied tropical *Magnolia* species *M. jardinensis* and *M. yarumalensis* show

specialized floral cycles related to specific pollinators that guarantee greater efficiency in fertilization and, therefore, in the formation of fruits and seeds. However, such specialization might lead to an evolutionary dead-end if the specific pollinator disappears. This work provides novel information about reproductive phenology and floral biology of two highly endangered *Magnolia* species from neotropical cloud forests. Of the two species in the study, *M. jardinensis* appears to be more prone to extinction than *M. yarumalensis*. Meager fruit production in this species is related to several factors such as floral bud abortion and limited pollen germination, probably associated with increases in minimum temperature and precipitation related to current global warming besides forest cover changes with direct effects on tree populations and indirect effects on pollinators. Unquestionably, several *Magnolia* species from the Neotropics exhibit scarce and small populations and should be prioritized for conservation. Concrete actions for conservation, such as hand pollination in natural populations, fruit monitoring to time-optimal seed collection for propagation, seedling reintroduction and forest facilitation to recover natural distributions, monthly monitoring, and genetic studies to evaluate the genetic variability of these species, will all improve the chances for their reproductive success and survival.

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