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Research Article

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Posted Date: November 1st, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3425432/v1>

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ABSTRACT

The gall wasp *Ophelimus migdanorum* represents a pest risk for several *Eucalyptus* species established in Colombia. Biological development and damage could be conditioned by the climate and, in the tropical Andes, by precipitation. We studied gall phenology, population fluctuation, and leaf damage caused by *O. migdanorum* and its parasitoid *Closterocerus chamaeleon* over five months at two sites with contrasting rainfall in peri-urban areas of Bogotá, Colombia. Gall phenology and foliar damage were evaluated on ten trees per site for six months. We evaluated gall phenology through characterization of the gall size and color and the wasps' development stage. We also evaluated gall phenology based on the affected leaf area and the gall's density on the leaf blade. Likewise, the individuals found in five attractive traps at each site were quantified biweekly to record the population fluctuation. The effect of precipitation on wasp frequency and gall density between sites and sampling dates was compared with the Chi² test, while the relationship with precipitation was evaluated with the Kruskal-Wallis test. Only females of the gall wasp and its parasitoid were observed during the evaluation, which showed multivoltine behavior. Six developmental stages of the galls were differentiated, each having typical coloration. Drier microclimates favored the gall's size, stages development frequency, population trend, and foliar damage. A general trend indicates a greater abundance of the parasitoid *C. chamaeleon* than *O. migdanorum*. Our study indicates that in places with rainy microclimates, there's a less damaging effect of *O. migdanorum* on *E. globulus*.

Keywords: *Ophelimus*, *Closterocerus*, *Eucalyptus*, gall phenology, population trend, foliar damage.

INTRODUCTION

Gall-inducing foliage insects represent one of the main threats to reforestation with species of the *Eucalyptus* genus outside their native range (Borowiec et al. 2019; Hurley et al. 2016). In particular, gall wasps of the genera *Leptocybe* and *Ophelimus* have been dispersing in the last decade in the Middle East, Europe and America (Dhahri et al. 2010; Molina-Mercader et al. 2019a; Protasov et al. 2007; Pujade -Villar & Riba-Flinch 2004; Raman & Withers 2003). These wasps may alter the development of host plants, affecting processes such as growth, reproduction and photosynthetic activity (Cuevas-Reyes et al. 2006; Fernandes et al. 2010; Heredia & Ruiz 2011; Larson 1998). Trees with a high density of leaf galls may present premature leaf abscission, which can cause death in trees sensitive to defoliation. (Cuevas-Reyes et al. 2006; Fernandes et al. 2010; Heredia & Ruiz 2011; Larson 1998; Mendel et al. 2017; Raman & Withers 2003).

Variations in the behavior of gall wasps in *Eucalyptus* have been related to the host species and climatic factors of the new establishment site (Hurley et al. 2016; Nyeko et al. 2010; Pinzón et al. 2002). For example, the damage caused by *Leptocybe invasa* Fisher & Lasalle has been associated with precipitation and temperature in Sri Lanka, Uganda and Kenya (Nyeko et al. 2010; Udagedara & Karunaratne 2014). Likewise, variations in the life cycle and the population trend of *Ophelimus* spp. have been evidenced. in Israel, New Zealand, Jordan and Iraq as a function of climate seasonality related to temperature and relative humidity (Al-Sweedi et al. 2020; Ghabeish & Araj 2016; Protasov et al. 2007; Pujade-Villar & Riba-Flinch 2004; Raman & Withers 2003).

In particular, *Ophelimus migdanorum* Molina-Mercader was reported for the first time in Valparaíso (Chile) in 2019, causing damage to *E. globulus* and *E. camaldulensis* trees (Molina-Mercader et al. 2019, 2019a). The first studies on this gall-living species were focused on its taxonomic identification, morphological description and report of its possible biocontrol agent *Closterocerus chamaeleon*. In 2020, *O. migdanorum* was reported for the first time in the northern Andes, associated with *E. globulus* trees in Bogotá (Pinzón-Florián 2020). Its presence indicates the introduction of the first gall-inducing phytophagous species in the tropical Andes and suggests a threat to areas planted with *Eucalyptus* spp.

In Colombia, approximately 58,000 ha is planted with *Eucalyptus* spp. (PROFOR 2017) in different systems (plantations, agroforestry systems, and isolated trees), and an increase in that area is expected due to the reforestation goals set by the national government (UPRA 2015). In Bogotá, approximately 45,000 *Eucalyptus*

spp. are planted in public spaces (Botanical Garden of Bogotá 2022), not considering the extensive areas established on the eastern hills. The presence of *O. migdanorum* in the Sabana de Bogotá is considered important since the climatic conditions in terms of temperature are relatively homogeneous throughout the year, and there are variations in precipitation that could influence the development and successful establishment of these parasites in *Eucalyptus* trees.

Based on the above, our objective was to recognize the possible effect of precipitation on the phenology, population trend, and damage levels of *O. migdanorum* and its parasitoid *C. chamaeleon* on *E. globulus*. Likewise, to identify variations in the sizes of the galls, the frequency of the development stages of the wasps, the population trend and the foliar areas affected, product of contrasting precipitation conditions in two peri-urban areas of Bogotá.

MATERIALS AND METHODS

Study area

The study was carried out in two peri-urban areas of Bogotá established with *Eucalyptus globulus* plantations. The selection criteria of the sites were as follows: 1) similar establishment and management conditions (more than 20 years of establishment, DCH between 50 and 60 cm, heights between six and 10 m and without silvicultural management); 2) presence of more than 1000 trees with active galls caused by *O. migdanorum*; and 3) different precipitation conditions, one dry microclimate and the other humid. The definition of the microclimate was made based on the Caldas-Lang climate classification map of the department of Cundinamarca (IDEAM 2010).

The sampling area, defined as a humid microclimate, was located in Quebrada La Vieja (QV) (4° 38' 59.309" N, 74° 2' 48.534" W), located in the Oriental Forest Protective Reserve of Bogotá, in the locality of Chapinero. Rainfall presents a bimodal-seasonal regime, with maximums in February and November (between 261.1 and 265.7 mm/month) and minimums in June (46 mm/month); the average temperature is 16.1°C, and the average relative humidity is 77% (Cantillo Higuera & Cuellar 2013). According to the Caldas-Lang climate classification, this site has a cold semi-humid climate, with rainfall between 1,000 and 1,500 mm/year and average temperatures between 12 and 16°C (IDEAM 2010). During the sampling time, rainfall varied between

51.4 and 273.1 mm/month; a dry period was recorded during the month of March, and two wet periods were recorded, one at the end of November and the other at the beginning of May. The meteorological data were obtained from the UAN Circunvalar (Universidad Antonio Nariño) and Colegio Simón Rodríguez Sede C stations, belonging to the Hydrometeorological Network of the District Institute for Risk Management and Climate Change (IDIGER).

The sampling area, defined as a dry microclimate, was located on the property called Bajo Teusacá (BT) (4° 56' 18.388" N, 73° 58' 54.847" W), owned by the Bogotá Water and Sewer Company (EAAB), located in the Bogotá savanna, municipality of Sopó. The precipitation presented a bimodal regime, with maximums in May and October (between 80 and 110 mm/month), an average temperature of 13.8 °C and a relative humidity of 77.8% (CAR 2017; Garcia Quiñones 2019). According to the Caldas-Lang climate classification, this site has a cold semi-arid climate, with rainfall between 500 and 1,000 mm/year and average temperatures between 12 and 16°C (IDEAM 2010). During the sampling, rainfall varied between 36.4 and 92.3 mm/month, and two dry periods were recorded at the end of February and April and two wet periods, one in mid-November and the other at the end of March. The rainfall information was obtained from the rainfall station of the TIBITOC water treatment plant, owned by the EAAB, the closest to the sampling site, approximately 4 km away.

Data collection

Data collection in the field was carried out from November 15th, 2020, to June 12th, 2021. Monitoring the phenology of the galls and the damage to the leaves was carried out monthly, while the population trend was carried out every two weeks. Likewise, the data collection methodologies were different for the three objectives. It should be noted that in January and part of February 2021, no information was collected due to the impossibility of accessing the properties due to mobility and confinement restrictions, guidelines established by the national and regional authorities due to the emergency decreed by COVID-19.

Gall phenology

Gall phenology was monitored monthly from November 15th, 2020, to May 1st, 2021. Ten *E. globulus* trees with evidence of damage caused by *O. migdanorum* were selected at each site (Mendel et al. 2017). Every month for five months, a 40-60 cm long branch per tree, with 20 leaves, was collected at a height between three

and five meters from the ground. Each branch was stored in Ziploc-type polyethylene bags with absorbent paper and marked with the sampling date, site and tree number. The samples were kept for a maximum period of 20 days under ambient conditions (temperature between 8 and 15 °C, relative humidity between 8,0 and 90%), constantly changing the absorbent paper to prevent the development of fungi.

To characterize the phenology of the galls, part of the methodology used by Garcia et al. (2019) and Ghabeish & Araj (2016) was applied. Thirty galls were characterized on each branch, whose selection was systematic and consisted of choosing leaves number 1, 5, 9, 13 and 17 of each branch, folding each leaf in half and choosing the first three galls from the middle toward the apex and from the middle toward the base, for a total of 6 galls per leaf; when the leaves did not have galls, the next leaf was selected.

To monitor gall development, beginning after oviposition and recognized by size and coloration, each gall was photographed (with a stereomicroscope and camera) and categorized according to a color scale elaborated during the project. The galls were dissected to record the developmental stage of the wasps and to identify (when possible) the species (*O. migdanorum* or *C. chamaeleon*). Subsequently, with the help of ZEN Lite software (from the ZEISS Discovery V8 Stereomicroscope) and the photographic record, the gall perimeter was measured. In this way, observations were made of 30 galls per sample, 10 samples per site and 5 repetitions, and a total of 3,000 galls were characterized.

Population trend

The population trend of *O. migdanorum* and its parasitoid *C. chamaeleon*, understood as the variation in the number of wasps in a given time, was carried out every two weeks from November 15th, 2020, to June 12th, 2021. Part of the methodology used by Ghabeish & Araj (2016), Udagedara & Karunaratne (2014) and Zhu et al. (2012) was followed, and five attractant traps were installed per site, marked with the trap number and hung between two trees at approximately 1.8 m above the ground (Mendel et al. 2017). At each site, 10 repetitions were performed, for a total of 100 observations.

Green attractant plastic traps were used due to the attraction that this color exerts on *Ophelimus* and their parasitoids (Mendel et al. 2007; Protasov et al. 2007). Vegetable oil was used as an adhesive because of the size of the wasps (approximately 1 mm). The traps were replaced by others with the same characteristics every

fifteen days and taken to the Forest Health Laboratory of Francisco José de Caldas District University. The individuals in each trap were separated using a stereoscope and counted by species. The material of the dissections and the traps was kept in vials with 85% ethanol by sampling date and is now part of the Colección Entomológica Forestal Universidad Distrital Francisco José de Caldas.

Estimation of affected gall leaf area

To estimate the damage caused by *O. migdanorum* in the leaves of *E. globulus*, the same samples collected for the evaluation of the phenology were used. In this case, the 20 leaves of each branch collected per tree on each sampling were examined. Each leaf was folded in half, where a 1 cm² template was located, and the photographic record was taken with a camera and stereomicroscope (Al-Sweedi et al. 2020; Protasov et al. 2007). Subsequently, with the software IMAGE J (free to download) and ZEN Lite (from the ZEISS Discovery V8 Stereomicroscope), the number of inviable and viable galls registered in 1 cm² was counted, and the area of the galls was measured to obtain the density of galls and affected area per cm². To estimate the affectation of the foliage, 10 branches per site were analyzed, 20 leaves per branch, during over 5 months (5 repetitions) at two sites, and a total of 2.000 observations were made to develop this objective.

Analysis of data

To characterize the phenology of galls as a function of rainfall, the following variables were considered: gall coloration, gall size, development stage frequency, and rainfall (mm/month). On the other hand, for the population trend, abundance by species and precipitation (mm/fortnightly) were considered. Finally, to estimate leaf damage, the gall density per cm² and the area occupied by the galls on the leaf blade were used.

To quantify the frequency of the development stages of each gall by species, a descriptive analysis of the percentage of galls for each stage of development was carried out by sampling and by color category compared between sites. To compare the frequencies of development stages at each sampling date between sites, Fisher's and Chi² tests were performed. Likewise, to identify and compare variations in the size of the galls, the Kruskal–Wallis test was performed (taking into account that the data are not normal) between development stages and between sites. To estimate the possible effect of precipitation on the frequency of each stage of development of the galls in the sampling sites, it was compared using the Kruskal–Wallis test per sampling (1, 2, 3, 4 and 5).

For the population trend study, an initial descriptive analysis of the absolute abundance of wasps was carried out by species by site. A Poisson distribution analysis was performed to identify the effect of precipitation on the wasp population trend, considering that the data obtained corresponded to counts.

Finally, a descriptive analysis of the gall density and the area affected by sampling by site was carried out to estimate the leaf area affected by galls on the leaves. Subsequently, to compare between sampling dates and between sites, the Kruskal–Wallis test was performed. All analyses were performed in R version 3.6.2 (R Core Team, 2013). The significance applied in the study was $\alpha = 0.05$.

RESULTS

This is the first study conducted for *Ophelimus migdanorum* and its parasitoid *Closterocerus chamaeleon* in the tropical Andes, focused on gall phenology, population trends, and leaf damage. Females of the gall wasp *O. migdanorum* and its parasitoid *C. chamaeleon* were present throughout the studied period at the two sites analyzed. The variations in the frequency of the wasps were influenced both by the microclimates of each site and by the rainfall fluctuation. Additionally, the gall size, the fluctuation of the frequency of the development stages, the abundance of the wasps, and the affectation of the leaves were positively correlated with the site with less precipitation. Likewise, the galls induced by *O. migdanorum* presented differences in coloration and size, in accordance with the stage of development of the wasp.

Gall phenology

The galls caused by *O. migdanorum* on *E. globulus* leaves were located in different parts of the trees and presented various shapes and colors. Circular, oval, and asymmetrical galls were observed on the upper surface, underside, main veins, leaf edges, petioles, and stems. In addition to the localized increase in the volume of the leaf tissue, changes in the coloration of the tissue were observed, where six categories were defined: 1) galls without color changes: they correspond to galls that preserve the original coloration of the affected tissue; 2) yellow–green galls: with colors between light green and yellow-green on the leaf blade and light yellow on the veins and petiole; 3) olive galls: galls yellow-green to olive; 4) brown galls: tissues with colors between light brown and dark brown; 5) beige galls: tissues with colors between light and dark beige; and 6) dark red galls: varying between dark red and maroon colors (Fig 1).

Five stages of process development of galls and wasps were differentiated. Three stages corresponded to differentiable stages of wasp development: larvae (three instars), pupae and pre-emergent adults, while the remaining stages correspond to a previous stage (nonviable postures) and a later stage (adult emergence holes) of wasp development. The wasp eggs were not differentiated on the tissue, which corresponds to the first stage of immature development. The nonviable postures refer to galls in which the deformation of the tissue began after the posture, but the insect development was not completed. Adult emergence holes are indicative of the successful development of wasps. Additionally, galls with remains of unidentifiable tissue and gums were found and referred to in the analysis as unidentified material (Fig 2).

Changes in gall coloration were associated with the developmental stages of the wasps. Although different stages were recorded in galls with various colorations, each stage was predominantly associated with one coloration. At both sites, immature stages (larvae and pupae) were most frequently found in yellow–green galls (49 and 43%, respectively), followed by galls without a change in coloration (27% each). Pre-emergent adults were recorded mostly in olive galls (37%), while galls with adult emergence holes had a brown tone (88%). Non-viable postures were more frequent in beige galls (88%), and unidentified material was mainly recorded in brown and dark red galls (46 and 18%, respectively) (Table 1 and Supplementary Table 2).

Table 1 Frequency of development stages of *O. migdanorum* and its parasitoid *C. chamaeleon*, by gall color category, by site, of the entire sampling (BT: Bajo Teusaca, QV: Quebrada Vieja; NCC: No color changes; BrG: Brown galls; YGG: Yellow green galls; BeG: Beige galls; OIG: Olive galls and DRG: Dark red galls)

Development stages of <i>Ophelimus migdanorum</i> and <i>Closterocerus. chamaeleon</i>	Site	Gall color category in <i>E. globulus</i>						Total galls
		NCC	BrG	YGG	BeG	OIG	DRG	
Larvae	BT	145	4	260	2	110	10	531
	QV	45	5	89	1	32	9	181
	TOTAL	190	9	349	3	142	19	712
Pupae	BT	43	-	74	1	43	4	165
	QV	22	-	37	-	12	3	74
	TOTAL	65	-	111	1	55	7	239
Preemergent adults	BT	13	1	11	3	19	3	50
	QV	3	1	4	-	4	1	13
	TOTAL	16	2	15	3	23	4	63
Adult emergence holes	BT	5	480	1	-	29	4	519
	QV	7	162	4	-	21	18	212
	TOTAL	12	642	5	-	50	22	731
Nonviable postures	BT	31	-	2	50	5	-	88
	QV	50	2	5	751	16	2	826
	TOTAL	81	2	7	801	21	2	914
Unidentified material	BT	10	78	20	9	11	19	147
	QV	33	79	26	12	2	42	194
	TOTAL	43	157	46	21	13	61	341

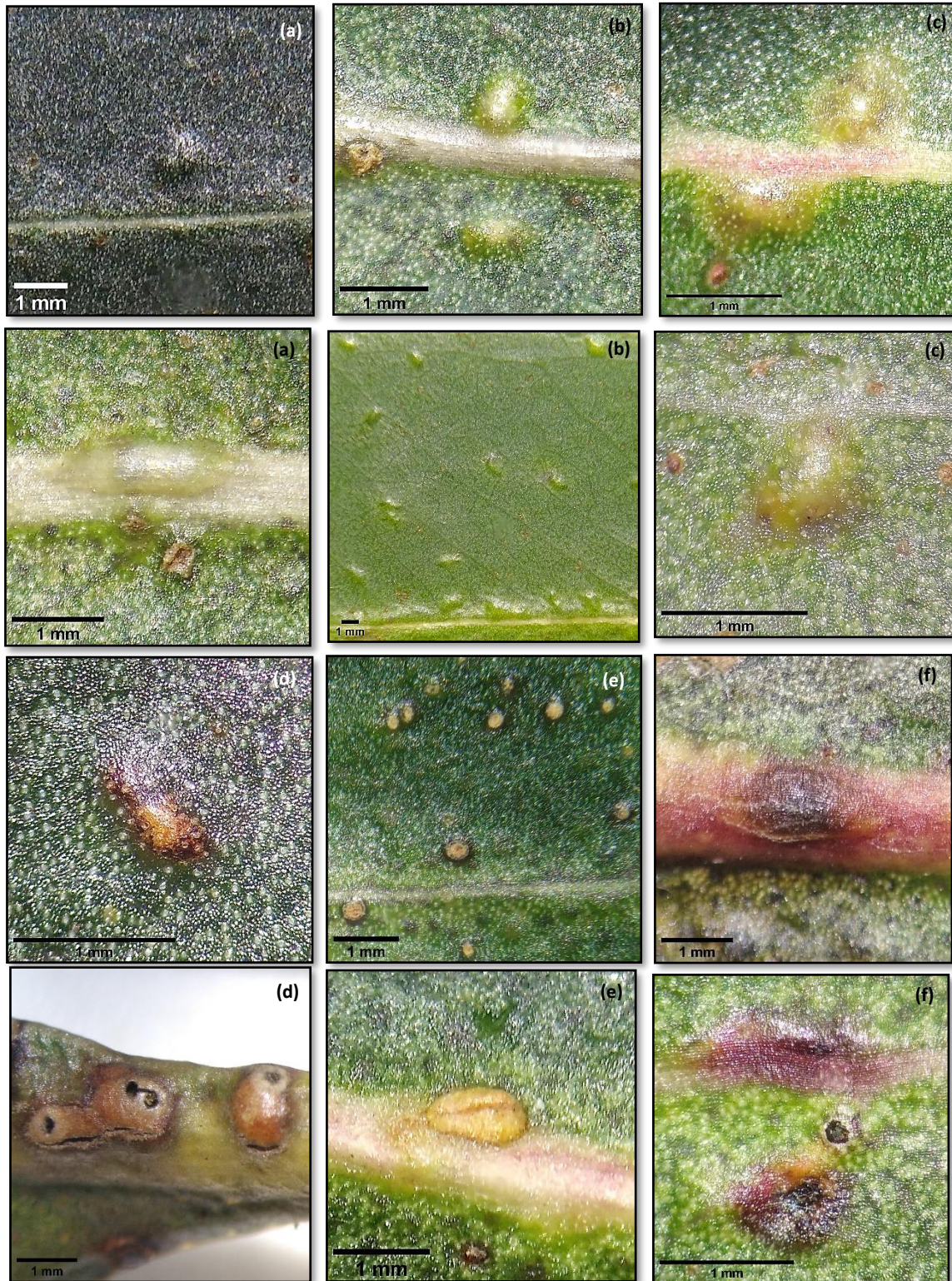


Fig 1 Gall color categories of *O. migdanorum* and its parasitoid *C. chamaeleon* on *E. globulus* leaves in Bajo Teusacá and Quebrada La Vieja (a: without color changes; b: yellow–green; c: olive; d: brown; e: beige and f: dark red)

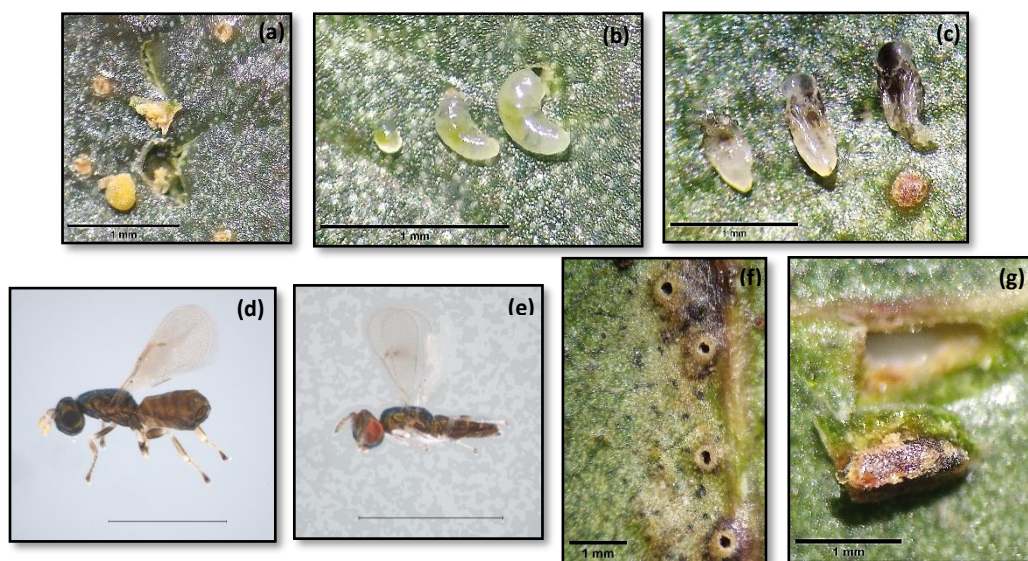


Fig 2 Development stages of *O. migdanorum* and its parasitoid *C. chamaeleon* associated with leaf galls of *E. globulus* in localities with different precipitation Bajo Teusacá and Quebrada La Vieja (a: Nonviable postures, b: Larvae, c: Pupae, d: Adult *O. migdanorum*, e: Adult *C. chamaeleon*, f: Adult emergence holes and g: Unidentified material)

The frequency of larvae, pupae, pre-emergent adults and adult emergence holes was higher in BT than in QV (Fig 3, Supplementary Table 3). The most abundant developmental stage in BT was the larval stage, while in QV, it was a non-viable stage (Fig 3, a and b). Emergence holes were the second most recorded stage at both sites, contrary to pre-emergent adults, which were the least abundant (Fig 3d). Within adults, *C. chamaeleon* was more abundant than *O. migdanorum* in all samplings in BT, while in QV, *O. migdanorum* was more abundant in three of the five samplings (Fig 3c, Supplementary Table 3).

The lowest viability of *O. migdanorum* postures was recorded in QV, corresponding to the most humid microclimate studied. There, between 40 and 60% of the postures did not develop into galls, while in BT, 87% were viable (Fig 3a). During the driest period, the lowest frequencies of non-viable laying (0 in BT and 45% in QV) and larvae (21% in BT and 7.3% in QV) were recorded at both sites. Particularly in QV, the lowest frequencies were also recorded for pupae (1%) and the absence of adults (Fig 3, c and d), while in BT, peaks were registered for pupae (16%) and emergence holes (46%) (Fig 3, c and a).

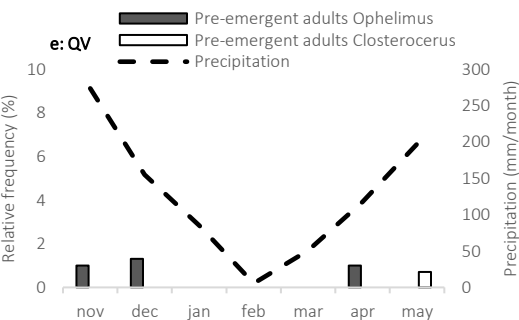
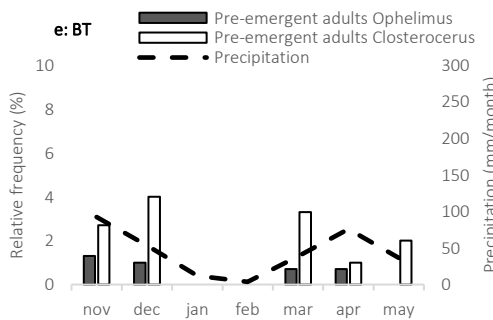
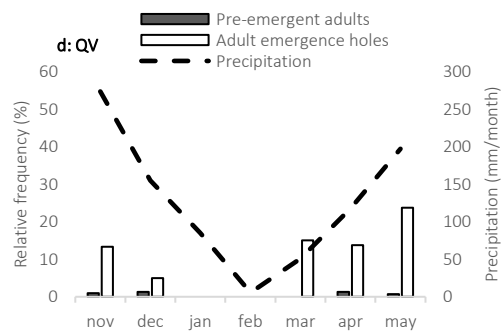
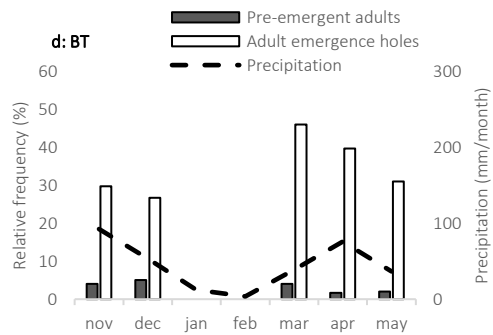
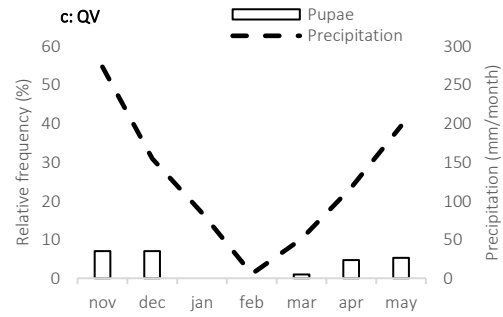
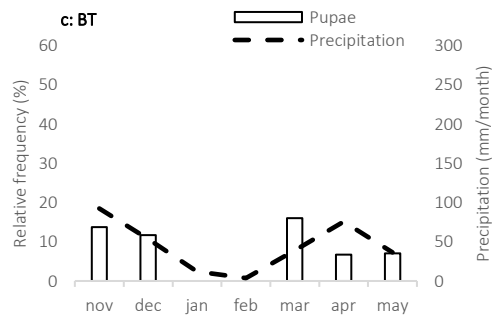
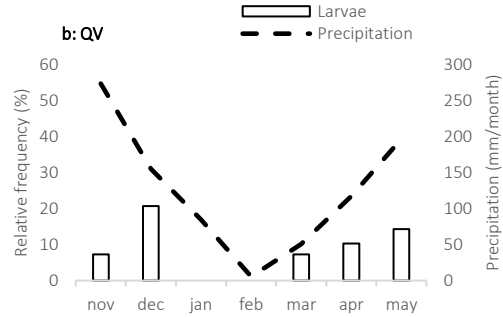
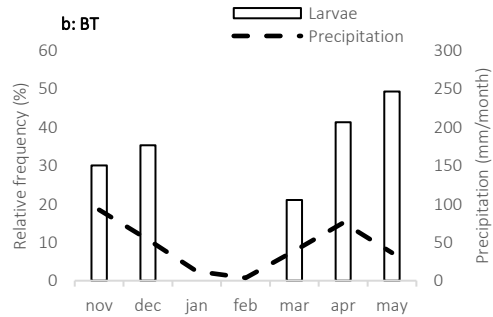
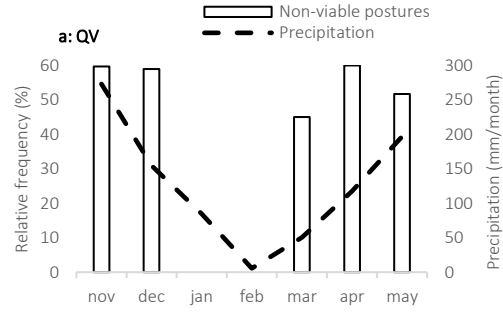
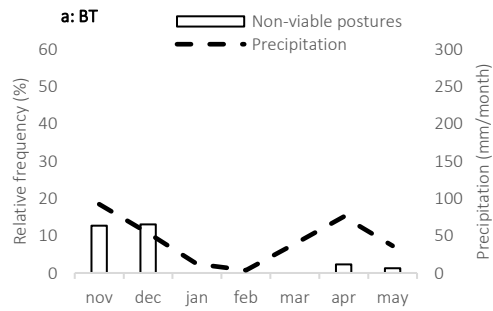


Fig 3 Relative frequency of the development stages of *O. migdanorum* and *C. chamaeleon* by sampling at two sites (Left: Bajo Teusacá (BT); Right: Quebrada La Vieja (QV)) with different precipitation parameters (a: non-viable postures; b: larvae; c: pupae; d: adults and emergence orifices; e: pre-emergent adults of *O. migdanorum* and *C. chamaeleon*). (In January and February, there was no sampling due to the pandemic)

The average size of all galls was 2.4 times larger in BT than in QV. On average, the area occupied by each gall in BT was 0.724 mm², while in QV, it was 0.306 mm². At both sites, emergence holes and pre-emergent adults corresponded to the largest galls on average, while non-viable galls and larval galls corresponded to the smallest galls. The galls with adult emergence holes recorded the most marked variations in size, with ranges of 2.5 and 1.9 in BT and QV, respectively. In contrast, galls with failed postures recorded narrower variations in size with ranges of 1.1 and 1.2 in BT and QV, respectively (Table 2).

Table 2 Average size (mm²) of galls induced by *O. migdanorum* on *E. globulus* foliage in Bajo Teusacá and Quebrada la Vieja by developmental stage. The values in parentheses correspond to the minimum value, maximum value and standard deviation. The p value corresponds to the significance of the comparison between sites using the Kruskal–Wallis test (df: 1)

Development stages of de <i>O. migdanorum</i> and <i>C. chamaeleon</i>	Average size		P
	Bajo Teusacá	Quebrada La Vieja	
Larvae	0,59 (0,2; 1,9; +/-0,3)	0,53 (0,1; 1,8; +/-0,2)	0.001299
Pupae	0,72 (0,2; 1,8; +/-0,3)	0,65 (0,2; 1,6; +/-0,3)	0.03604
Preemergent adults	0,8 (0,4; 1,9; +/-0,3)	0,60 (0,1; 1,5; +/-0,3)	0.04691
Adult emergence holes	0,96 (0,2; 2,8; +/-0,4)	0,72 (0; 2; +/-0,4)	2,74E-09
Nonviable postures	0,27 (0; 1,1; +/-0,2)	0,1 (0; 1,2; +/-0,2)	< 2.2e-16
Unidentified material	0,63 (0,1; 1,9; +/-0,3)	0,4 (0; 1,8; +/-0,3)	5,82E-12
TOTAL	0,72 (0; 2,8; +/-0,4)	0,31 (0; 2; +/-0,3)	< 2.2e-16

On the other hand, the average sizes of galls per stage of development varied in relation to the dry and rainy periods at each site. In BT during the rainiest period (November), the largest galls for pre-emergent adults and adult emergence holes were recorded, while larvae and failed postures corresponded to the smallest galls. In QV in December, when the rains began to decrease, the largest galls were recorded with larvae, pre-emerging adults and adult emergence holes, and in the driest period (March), the largest galls were recorded with pupae. (Fig 4).

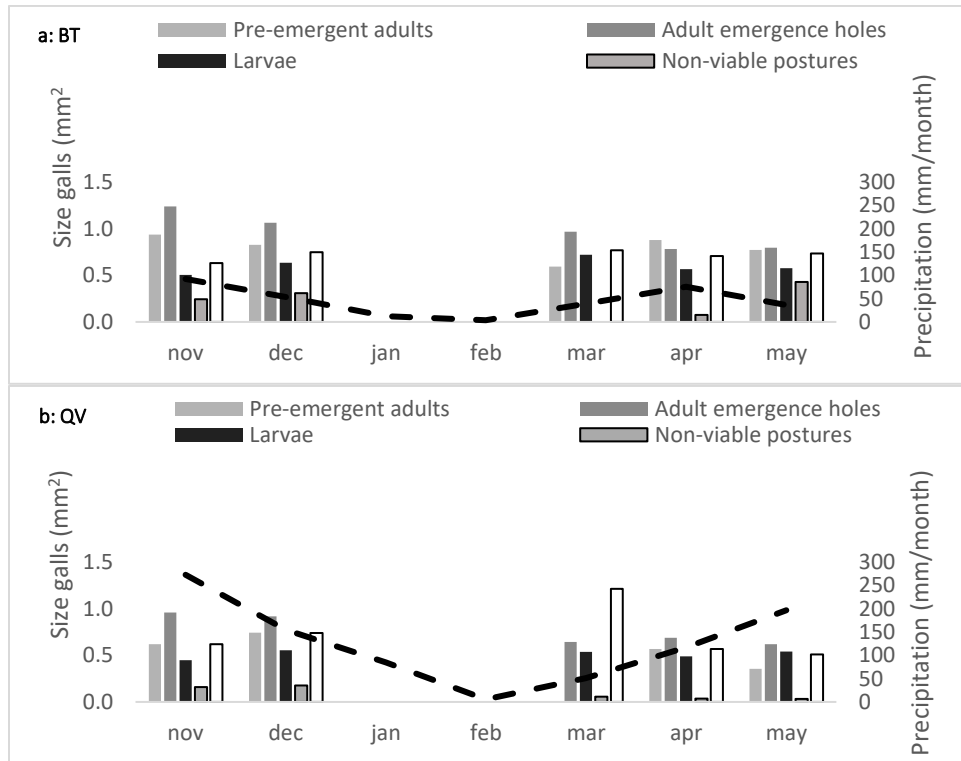


Fig 4 Average gall size by developmental stage of *O. migdanorum* and *C. chamaeleon* by sampling at two sites (a: Bajo Teusacá (BT) and b: Quebrada La Vieja (QV)) with different precipitation parameters

Population trend

In both QV and BT, the presence of the different stages of development of *O. migdanorum* and *C. chamaeleon* was recorded throughout the period studied (Fig 3 and 5). At BT, adults of both species were recorded during the study, while at QV, at least one of the two species was recorded, except in May, when no individuals of either species were collected (Fig 3e and 5). Both *O. migdanorum* and *C. chamaeleon* were more abundant in BT (site with a dry microclimate) than in QV (precipitation ($F(1,gl) = -2.831$, $p: 0.00464$). In 80% of the samples in BT, *C. chamaeleon* was the most abundant, while in QV, no marked trend was observed ($F(1,df) = 1.654$, $p: 0.09806$) (Fig 5).

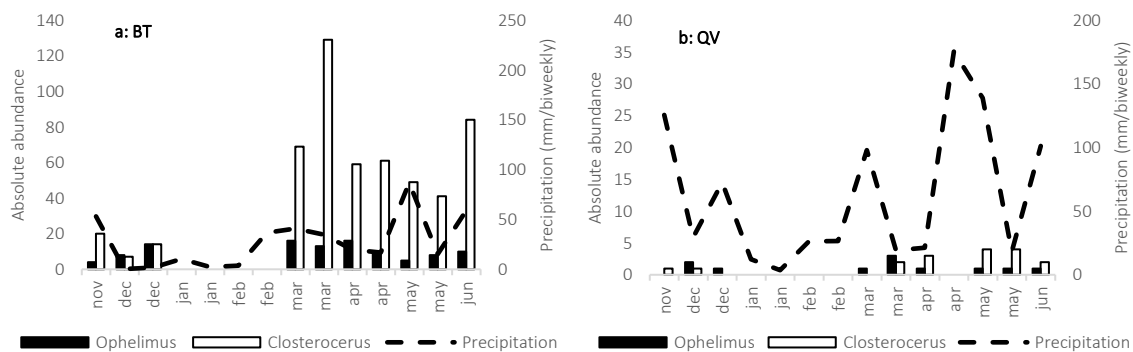


Fig 5 Trend of *O. migdanorum* and *C. chamaeleon* by sampling at two sites (a: Bajo Teusacá (BT) and b: Quebrada La Vieja (QV)) with different precipitation parameters

Damage to leaves

On average, the total leaf area affected by *O. migdanorum* in *E. globulus* leaves was 1.4 mm²/cm². The average leaf area affected in QV was 1.46 mm²/cm² and fluctuated between 0.112 and 7.250 mm²/cm², while in BT, it was 1.41 mm²/cm² and fluctuated between 0.044 and 8.159 mm²/cm² ($F(1,df)= 6.6593$, $p: 0.009864$). However, the average leaf area affected by the development of viable galls was greater in BT (1.07 mm²/cm²) than in QV (0.41 mm²/cm²), while the damage from nonviable galls was greater in QV (1.42 mm²/cm²) than in BT (1.17 mm²/cm²) (Fig 6). During the wettest period (November) in QV, the largest affected area was recorded on average (2.1 mm²/cm²), while in BT (1.8 mm²/cm²), it was recorded in the driest period (February). No important variations were observed between samplings at the same site, but there were differences between sites, except in April, with 1.2 mm²/cm² of average damage at both sites (Fig 6 and Supplementary Table 4).

On the other hand, gall density did not present important variations between the sampled sites or the samples from the same site. However, the tendency showed a higher gall density at the site with a humid microclimate (QV) ($F(1,df)= 2.3965$, $p: 0.1216$). In BT, the density varied between 1 and 96 galls/cm², while in QV, it varied between 3 and 115 galls/cm². The highest gall density was recorded in December in BT (37 galls/cm²), while in QV, it was recorded in November (32 galls/cm²) (Fig 7 and Supplementary Table 5).

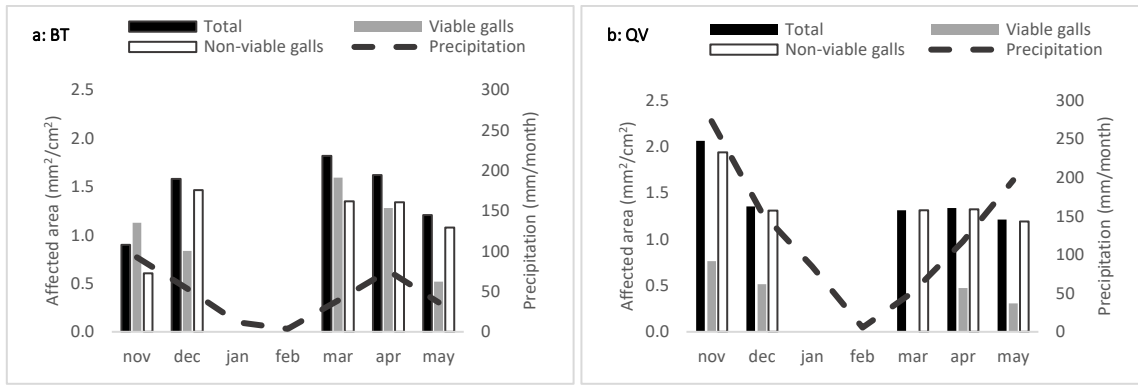


Fig 6 Average leaf area affected by *O. migdanorum* in 1 cm^2 of *E. globulus* leaf blade per sampling at two sites (a: Bajo Teusacá (BT) and b: Quebrada La Vieja (QV)) with different precipitation parameters

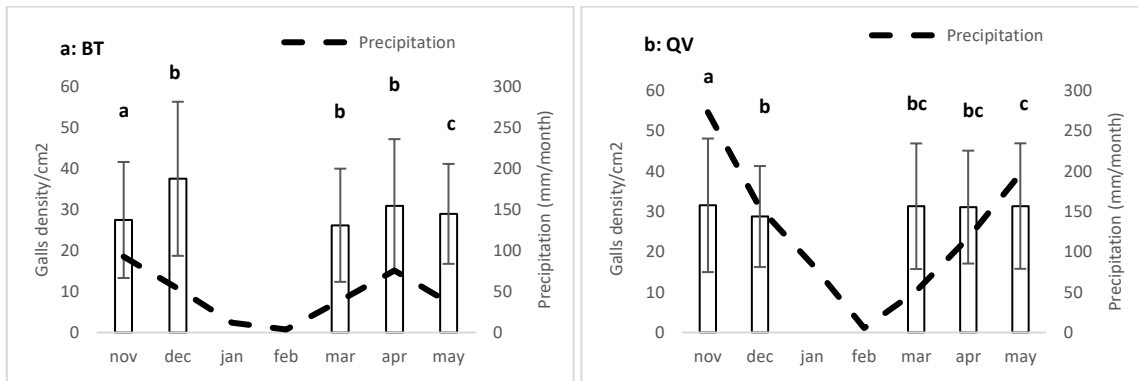


Fig 7 Average density of galls caused by *O. migdanorum* in 1 cm^2 of leaf blade of *E. globulus* by sampling at two sites (a: Bajo Teusacá (BT) and b: Quebrada La Vieja (QV)) with different precipitation parameters. The standard deviation and similarities between sampling by sites are shown (with lowercase letters)

DISCUSSION

Our study suggests that *O. migdanorum* and *C. chamaeleon* develop better in sites with dry rather than humid microclimates. Differences were observed between the compared sites in gall sizes, frequencies of development stages and population fluctuation. However, the variation in gall coloration and shape during wasp development was similar in both microclimates. Additionally, the average affected leaf area was similar at both sites, with differences between sites and sampling, associated with the presence of viable and non-viable galls. The differentiation between wasp species was possible only in the pre-emerging adult stage because in the larval and pupal stages, nonobvious morphological differences were shown, possibly because they belonged to the same family (Eulophidae).

Gall phenology

The galls induced by *O. migdanorum* recorded in this study are similar to those described for other species of the genus *Ophelimus*, with some particularities. The galls of *O. migdanorum* presented varied shapes (circular, oval and asymmetrical). They were located on the upper surface, underside, leaf blade, petioles and stems (Molina-Mercader et al. 2019a). *Ophelimus* sp. induces galls of an asymmetrical shape and are located only on one side of the leaf blade. The galls induced by *O. mediterraneus* are round and located on the upper part of the leaves, and those of *O. maskelli* are ellipsoid and can be found at various points on the leaf blade (Borowiec et al. 2019; Garcia et al. 2019; Molina-Mercader et al. 2019; Protasov et al. 2007). This variability, both in the shape of the galls and in the type of tissue induced by *O. migdanorum* in *E. globulus* in Quebrada La Vieja and Bajo Teusacá, may be presented as a mechanism of adaptation to the environmental conditions of the site (Ramos et al. 2019; Wool 1977) or as an enhanced defense against its biological controller (Stone & Schönrogge 2003).

Likewise, the galls coloration is a characteristic shared with other species of the genus *Ophelimus*, with some differences. Although several authors agree with greenish colorations at the beginning of gall development (Guedes et al. 2023; Protasov et al. 2007; Withers et al. 2000), the trend is that the galls of *O. migdanorum* are yellowish green and brown (Guedes et al. 2023), while those induced by *O. maskelli* are reddish and purple, and those of *Ophelimus* sp. and *O. mediterraneus* have brown tones (Borowiec et al. 2019; Garcia et al. 2019; Molina-Mercader et al. 2019; Protasov et al. 2007). In this sense, the variation in gall coloration may be associated with the host species and the level of exposure to the sun (Protasov et al. 2007), which suggests that the characteristics recorded for *O. migdanorum* could change if the mentioned conditions vary.

On the other hand, microclimatic conditions seem to affect the initial development of wasps. However, at both sites, three larval instars, pupae, pre-emerging adults and adult exit holes were recorded, similar to other species of the genus *Ophelimus* (Borowiec et al. 2019; Garcia et al. 2019; Protasov et al. 2007). To differentiate, in this study, non-viable postures were included as a stage in the development process of galls and wasps, corresponding to galls that apparently did not develop. According to the results, the humid microclimate condition in Quebrada La Vieja may cause lower development viability of the wasps, represented by a greater number of non-viable galls and a lower frequency of developed states compared to Bajo Teusacá.

Nevertheless, the changes on the coloration of the galls as the wasps develop are not new for this group. Greenish and yellow–green colorations in galls with initial stages of wasps have also been recorded in galls induced by *O. migdanorum* in Chile (Guedes et al. 2023), *O. maskelli* in Israel (Protasov et al. 2007) and *O. eucalypti* in New Zealand (Withers et al. 2000). Likewise, the brown galls called senescent in Chile are the same ones referred to as galls with adult emergence holes in this study, found frequently in brown color.

In the same way, the variation in the size of the galls was related to the stages of development of the wasps and the microclimates of the sites studied. The largest galls corresponded to the most advanced stages of development of the insect in Bajo Teusacá. This suggests that conditions in Quebrada la Vieja can influence the viability of the galls, as well as their size and therefore the development of the wasps. However, variations in the size of the galls induced by *O. migdanorum* have also been related to the type of tissue in which the gall develops (Guedes et al. 2023), data not recorded in this study. Similarly, larger galls than *E. globulus* have been reported in other *Eucalyptus* species induced by *Ophelimus* spp. (Branco et al. 2009; Garcia et al. 2019; Protasov et al. 2007), suggesting that the size of the galls induced by *O. migdanorum* can vary in other hosts.

Moreover, the presence of all developmental stages of *O. migdanorum* throughout the sampling suggests overlapping generations. Although other species of the genus *Ophelimus* in meridional and austral countries tend to have one to four generations per year (Al-Sweedi et al. 2020; Garcia et al. 2019; Ghabeish & Araj 2016; Mendel et al. 2007; Protasov et al. 2007), *O. migdanorum* showed multivoltine behavior in the perimeter areas of Bogotá. This condition has been observed in other tropical countries, such as Uganda and Kenya, where they found overlapping generations of *L. invasa* with adults throughout the year (Nyeko et al. 2010).

Population trend

The contrasting abundance values of *O. migdanorum* and its parasitoid *C. chamaeleon* in both sites suggest the influence of climatic factors on wasp populations. This is because other gall-induced *Eucalyptus* species, such as *O. maskelli* and *L. invasa*, have shown better development at higher temperatures (Al-Sweedi et al. 2020; Zhu et al. 2012) and in warmer areas (Ghabeish & Araj 2016; Mendel et al. 2004). Although it was not possible to obtain information on temperature and relative humidity at the sampled sites, it is suggested that greater precipitation at sites with similar temperatures could cause a lower thermal sensation in QV, which could affect

wasp populations. This could explain the abundance of wasps in the dry microclimate compared to the humid microclimate.

Likewise, the fact that *C. chamaeleon* was more abundant than *O. migdanorum* in most of the samplings suggests a successful adaptation of the parasitoid to tropical conditions. In addition, *C. chamaeleon* was significantly more frequent in phenology (Fig 3e) and population trend samplings (Fig 5) in Bajo Teusacá, suggesting a better adaptation to dry than humid micro-climatic conditions. On the other hand, the significant abundances of the biocontrol agent agree with reports in Portugal, where *C. chamaeleon* was more abundant than *O. maskelli* in the three sampled periods (Branco et al. 2009). However, it differs from reports in Chile, where *O. migdanorum* was more abundant than its parasitoid (Molina-Mercader et al. 2019a), both cases without previous anthropogenic releases.

Damage to leaves

The damage caused by *O. migdanorum* on *E. globulus* leaves was associated with the development of galls and wasps. Although on average the affected areas per site were greater in Quebrada la Vieja, in 60% of the sampling, the affected areas were greater in Bajo Teusacá, also with a greater frequency of galls viable and advanced stages of development. This suggests that dry microclimate conditions would favor damage to leaves. This coincides with reports in Uganda and Kenya, where another eucalyptus gall wasp (*Leptocybe invasa*) had greater infestations in hot and dry agroecological zones than in cold and humid zones (Nyeko et al. 2010).

In addition to the direct damage caused by the induction of galls by *O. migdanorum*, it is important to consider the physiological consequences on the tree. According to several authors, the induction of galls by insects affects processes such as growth, reproduction and variation in photosynthetic rates (Cuevas-Reyes et al. 2006; Fernandes et al. 2010; Heredia & Ruiz 2011; Larson 1998). This is because gall production requires more energy from the tree due to changes in metabolism and the production of defense substances, which could have implications for the yields of commercial plantations.

Although gall density did not show a marked trend between sites and samplings, the densities recorded in *E. globulus* were higher compared to other *Eucalyptus* species (Fig 7). The average gall densities recorded at both sites (30 galls/cm²) in *E. globulus* are higher than those reported in Karbala (Iraq) (19.87 galls/cm²) and in

Israel (between 11.5 and 36 galls/cm²) on *Eucalyptus camaldulensis* leaves, with temperatures of 10.64 and 24.5°C, respectively (Al-Sweedi et al. 2020; Protasov et al. 2007). However, it is unknown whether these studies also included viable and nonviable galls, as was done in this case.

Last, it is worth mentioning that although this study showed the relationship of precipitation in the development of wasps, other climatic and site variables cannot be discounted, such as the type of soil, the slope, the proximity to populated centers and the relationship with other plant species, which could influence the behavior of these species.

CONCLUSIONS AND RECOMMENDATIONS

Our results support the effect of climatic conditions (precipitation) on the development and level of damage of *Ophelimus migdanorum* and *Closterocerus chamaeleon* at new establishment sites. The site with less precipitation favored the development of galls and wasps, population abundance, and percentage of leaf area affected by *O. migdanorum*. At the two sites analyzed, populations of multivoltine females with overlapping generations were present. The size of the galls, the frequency of the development stages, and the abundance and of *O. migdanorum* and its parasitoid *C. chamaeleon* were positively influenced in sites with rainfall between 500 and 1,000 mm/year, where the coloration and size of the galls allow us to estimate the state of development of the wasps. The parasitoid *C. chamaeleon* has a high population level, which apparently limits the direct damage observed by the gall wasp on the foliage, estimated in 14% of the leaf area.

This study expands knowledge about the limitations of the establishment of *O. migdanorum* outside its place of origin. It highlights the presence of controlling agents such as *C. chamaeleon*, which could be considered an alternative within integrated pest management plans in the tropical Andes. Likewise, it guides the need for preventive (such as monitoring) and corrective management actions (chemical, biological, cultural control, etc.) and works as a basis for planning the establishment of forestry plantations or urban trees with *Eucalyptus* spp. To more broadly estimate the current and potential effect of *O. migdanorum* and its parasitoid *C. chamaeleon* on *Eucalyptus* spp. plantations, it is advisable to make comparisons between different eucalyptus species to evaluate susceptibility to damage, either by previously selecting eucalyptus species with the presence of *O. migdanorum* galls or inducing damage under controlled conditions. It is also recommended to consider the

evaluation of direct damage and its consequences on the growth rates of plants of different ages to establish if there is an economic impact.

ACKNOWLEDGEMENTS

To the Forest Health Laboratory of the Universidad Distrital Francisco José de Caldas their support in the research research development. To professor Giovanni Bogotá and reviewers of previous versions of the manuscript. To Alejandra Romero, Laboratory assistant, and Walter García for their support in the statistical analyses. To the Empresa de Acueducto y Alcantarillado de Bogota (EAAB-ESP), especially to Vladimir Gale, for the efforts made to facilitate the development of research on the company's property and obtaining rainfall information. To Nevardo Ardila and Jesús Ayala, forest rangers on the sampled properties, for their logistical support. To all the people who in one way or another contributed to the development of this study.

STATEMENTS AND DECLARATIONS

COMPETING INTERESTS AND FUNDING

The authors have no relevant financial or non-financial interests to disclose. No funding was received for conducting this study.

AUTHOR CONTRIBUTION

Angélica Lores-Medina and Olga Pinzón-Florián conceived the ideas and designed methodology. Angélica Lores-Medina performed the field work, laboratory and data analysis, and wrote the manuscript. Olga Pinzón-Florián commented and edited on the first draft. All authors contributed to the document and gave final approval for publication.

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