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Phenological documentation of *Lantana camara* L. using modified BBCH scale in relation to climatic variables

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Abstract

Lantana camara L. (Verbenaceae) is cultivated as an ornamental and hedge plant in many countries which is native to American tropics. It's introduction to the Indian subcontinent dates back around 200 years ago. It is an invasive alien species that has a negative impact on native biodiversity. It is evident that management of *L. camara* is crucial for the conservation of biodiversity. Studying its phenological characteristics as they adapt to environmental circumstances through time and space will aid in the development of management goals and strategies. This study uses BBCH scale firstly to describe the phenology of *L. camara*, which is represented by nine Phenological Growth Stages (PGS) in response to environmental conditions during a 32-months period in Dehradun, Uttarakhand, representing its growth. To standardise morphological traits and the phenological observation, photographs of certain significant developmental stages in addition to the descriptions have been illustrated. Researchers can utilise this uniform labelling method as a tool to help with weed management efforts. Phenological studies of this invasive weed species may be employed for tracking the gradual impact of climate change on biodiversity and its effect on the key phenological events in the lifecycle.

Keywords

BBCH scale, invasive species, phenological growth stages, phenology, *Lantana camara*

Introduction

Invasive alien plant species (IAPS) like *Parthenium hysterophorus, Lantana camara, Hyptis suaveolens*, *Eichhornia crassipes* and *Prosopis juliflora* are amongst the most critical reasons for biodiversity loss. *L. camara* is listed in world's most aggressive invasive species. They change the ecosystem's structure and function, affecting ecological services (1). Invasive species are opportunistic and have higher phenological sensitivity, allowing them to change their phenologies in response to environmental changes (2); thus, IAPS has a broad ecological amplitude. These characteristics enable invasive species to expand their distribution range (3), while the native species may suffer from changes in climatic conditions.

Phenology is the study of the patterns of periodic occurrences in the life cycle of a species (4). Phenology influences the number of distribution of species, ecological services, food webs, global water and carbon cycles (5). Phenological study in the biological sciences is concerned with transitions between organisms recurring developmental or behavioural stages (6).

Changes in temperature and precipitation, in turn, can affect phenology (7, 8). Plant invasion is inextricably linked to phenology, a critical contemporary topic in the biological sciences (9, 10). Understanding weed phenology helps us better understand its impact on the environment. Exploring the phenological patterns exhibited by IAPS is essential to determine their range of spread and develop better management techniques for their control (11–13).

The acronym BBCH scale (Biologische Bundesanstalt, Bundessortenamt and CHemishe Industrie) is a phenological coding system that is simplified, standardized and generally acknowledged. Plant development phases using the decimal code was provided for the first time (14) and later it was suggested and characterized in (15). A book detailing the phenology of twenty-seven plant species was published using the expanded BBCH scale (16). The BBCH scale has already been approved as a standard for species protection and phenological monitoring by the European and Mediterranean Plant Protection Organization (EPPOC), the Global Phenological Monitoring Network, and the European Phenology Network, encouraging its usage globally. The BBCH scale is a two- or three-digit decimal code that describes a plant's full life cycle in a methodical manner. The BBCH scale uses decimal coding to divide a plant's growth into 9 Principal Growth Stages (PGS). PGS and Secondary Growth Stages (SGS) are indicated by the first and second digits of the two-digit code, with ordinal values ranging from 0 to 9. Previously the BBCH scale was only limited to be used for agricultural purposes. However, it is currently being used to track invasive alien plant phenology like *Sapium sebiferum, Parthenium hysterophorus* (17, 18).

L. camara is a major IAPS of the Verbenaceae family with over 650 cultivars found in more than 60 countries (19, 20). The species is spreading in the forests worldwide due to canopy opening from deforestation and forest degradation (21, 22). The species is known to have a significant influence on agriculture and natural habitats. It spreads quickly in the fallow land, producing dense clusters. It can grow in any soil (sandy, clayey, loamy and rocky soils). Although it is also found at middle altitudes up to 600 m or more, it is common in the plains. Invasive weed like *L. camara* has a tremendous competitive ability. They outcompete native species by having great dispersion capacity, fast reproduction and the ability to adjust physiologically to new circumstances. In addition, *L. camara* also releases allelochemicals that play a crucial role in promoting its invasiveness (23, 24). The species release allelochemicals like "sesquiterpenes, flavonoids, triterpenes and phenolic compounds" in the rhizosphere of soil which may alter the growth of native species. Allelochemicals can change the content of growth regulators or trigger aberrations in numerous phytohormones, inhibiting plant growth and development, such as seed germination and seedling growth (25, 26). It is evident by some studies that allelochemicals affect the growth of various crops; mainly inhibiting roots.

L. camara was introduced to India as an ornamental species in many hybrid forms, and it has developed into an enigmatic complex during the last 200 years (27). The species grows as a woody bush, forms a dense thicket, and spread as a scrambling shrub on the forest floor. The Adaptive plasticity of *L. camara* is helping the species to expand its niche and infiltrate biogeographically diverse places in a short period raising concerns for biodiversity conservation and habitat management (28). Moreover, traditional approaches to tackle this weed have failed over time. Mechanical control provides a temporary solution to this problem, while biotic and chemical control methods have drawbacks. Mechanical control with crop competition through native species was proposed as a control measure for *L. camara* (29). To select the species for crop competition, understanding the phenology of the invasive and native species becomes very important (30). Plant population biology research is essential where traditional weed science techniques have failed to control weeds. This study investigates the phenological stages of *L. camara* following the BBCH scale in response to climatic factors over 32 months. However, the plant is a perennial species and would have survived under natural conditions.

Materials and Methods

Experimental Site

The experiment was conducted in Forest Research Institute (FRI) campus, located at Dehradun district (30° 20'10.8"N 77°59'24.3"E, and altitude of 644 m) of Uttarakhand, India. Dehradun valley lies in the north-western part of Uttarakhand (Garhwal region), located in the Himalayas and Shivalik's foothills. The climate of Dehradun is subtropical, humid with four distinct seasons: Winter (December to February) (High/Low: 22/7.33) in (°C); Summer (March to May) (High/Low: 32.33/17) in (°C), Monsoon (June to September) (High/Low: 32/22.5) in (°C), and Postmonsoon (October-November) (High/Low: 28/13.5) in (°C), with an average yearly rainfall of about 2051 mm. The climate in Dehradun is mild and moderate. Summers receive significantly more rainfall than winters. Dehradun receives 70-80 % of precipitation between June to September. Dehradun has fertile alluvial soil with sandy, clayey and rocky components. During the study, meteorological observations were acquired from the climate-observatory of Forest Ecology and Climate Change Division, FRI.

Methodology/ Experimental Design

Mature fruits of *L. camara* were harvested from the blooming branches of fully developed plants growing in the Doon valley at different altitudes from 35 different locations (Fig. 1). Fresh seeds weighing 100 gm (approx.) were gathered from each site and planted in separate pots (Diameter: 24 cm, Depth: 27 cm). The seeds weighed around 12-15 mg each. The pots were kept weed-free and watered at regular intervals. For further studies, only four individuals from each site were maintained in the pots. After germination, these seedlings were tagged to record the phenological phases. The number of plants decreased to 67 by the time the plants reached the flowering stage. Observations were

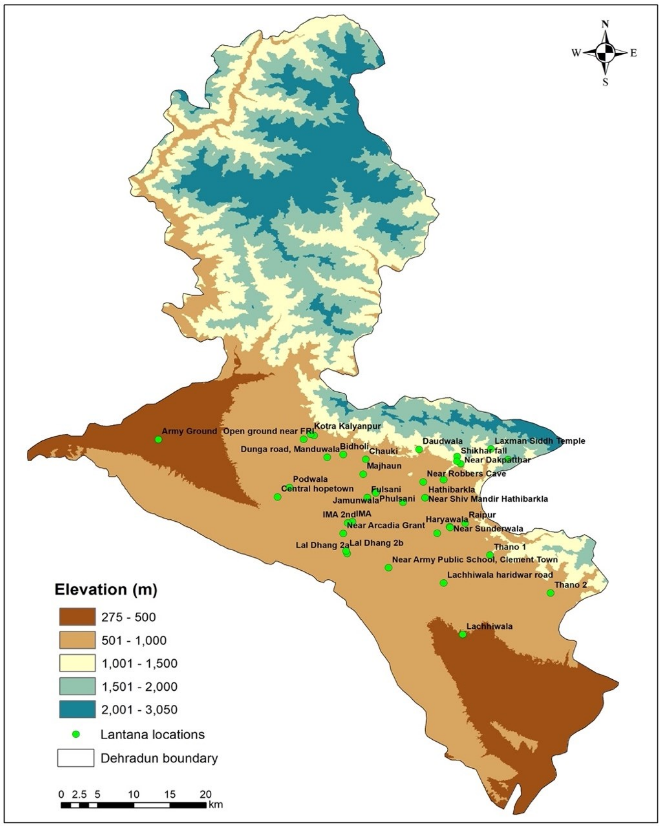

Fig. 1. Map showing locations from where seeds were collected in Dehradun, Uttarakhand, India.

recorded at every alternate day, along with photo-documentation of various phenophases. The modified BBCH scale proposed (12, 13) was used to create a phenological chart that followed 0-9 PGS.

Soil parameters for the pots used for experiment is as follows: water holding capacity = 35.65- 53.5%, pH = 5.9-6.5, total nitrogen = 0.08-0.13% and phosphorus = 0.001-0.006%. Meteorological data for humidity, rainfall, temperature, rainy days and average sun duration was recorded. The monthly average for the meteorological parameters was determined and arranged as per seasons for analysis. BBCH scale is depicted by two-digit numerical coding i.e. PGS (0–9). PGSs correspond to 10 plant development stages with designated codes. Germination/ vegetative bud development is denoted by (0), leaf development is denoted by (1), shoot development is denoted by (3), inflorescence emergence is denoted by (5), flowering is denoted by (6), fruit development is denoted by (7), fruit and seed maturity is denoted by (8), and senescence or beginning of dormancy is denoted by (9). Photographs of *L. camara* (shot by Nikon D750, Nikkor 24-120 mm) with unique identifiable phenological growth stages were chosen and arranged to represent the year-round phenological events of the species.

We recorded qualitative and quantitative changes in plant growth and development, as well as the onset, duration and end of phases, before finalizing a phenophase. Table 2 shows a list of *L. camara* phenological phases arranged by number codes in ascending order, and Fig. 2 shows a pictorial guide with codes.

Results and Discussion

We utilised sightings and recordings of *L .camara* phenological phases to figure out how the plant progressed during its life cycle (Fig. 2). The two-digit BBCH coding system was used to characterize the phenology of *L. camara*. External morphological characteristics that may be easily

Table 2. Description of the phenological growth stages of *L. camara* as per modified BBCH scale

Code	Description
Principal Growth Stage 0: Germination, Sprouting, Bud Development	
00	Dry seed (seed dressing takes place at stage 00)
Principal Growth Stage 1: Leaf Development (Main Shoot)	
12	Two true leaves, leaf pairs, or whorls unfolded
13	Three true leaves, leaf pairs, or whorls unfolded
14	Four true leaves, leaf pairs, or whorls unfolded
15	Five true leaves, leaf pairs, or whorls unfolded
16	Six true leaves, leaf pairs, or whorls unfolded
Principal Growth Stage 2: Formation of Lateral Shoot	
21	First lateral Shoot Visible
Principal Growth Stage 3: Stem elongation/shoot development (main shoot)	
30	Beginning of Stem Elongation
31	One visibly extended internode
Principal growth stage 5: Inflorescence emergence (main shoot) / heading	
51	Inflorescence or flower bud visible
55	First individual flowers visible (still closed)
59	First flower petals visible (in petalled forms)
Principal growth stage 6: Flowering (main shoot)	
60	First flowers open (sporadically)
65	Full flowering: 50% of flowers open
69	End of flowering: fruit set visible

Principal growth stage 7: Development of fruit	
71	Fruits begin to develop
79	Nearly all fruits have reached the final size standard for the species and location.
Principal growth stage 8: Ripening or maturity of fruit and seed	
81	Beginning of ripening or fruit colouration
89	Fully ripe
Principal growth stage 9: Senescence, the beginning of dormancy	
97	End of leaf fall, plants or above-ground parts dead or dormant

viewed, numbered, or quantified and expressed in ordinal values were used in the description (Table 2).

The primary developmental phases of the plant did not follow a predetermined order and may be unrelated or coincidental. As stated by PGS 0, the life cycle began with hypogeal seed germination (Stage 00; Fig. 2) and emergence of seedling through the soil surface (germination). It usually germinates in two weeks, exhibiting 86% germination. Because this activity took place underground, it was not possible to capture or document it. The seedling grows its first genuine leaves 20 to 40 days following emergence. There are pair of inflorescences at the leaf axils, with larger leaves at the base becoming larger and smaller, younger leaves emerging in the centre.

The average temperature during the years 2019 and 2020 was 21.94 °C and 20.57 °C. S2 season of 2019 and 2020 showed an average of 29.27 °C and 26.95 °C, whereas relative humidity was the lowest, i.e. 58.66 % and 56.66% respectively in the same season (Table 1).

The average temperature during S2 of 2019 was

Fig. 2. Different Phenological Growth Stages (PGS) were observed in *L. camara* according to the modified BBCH scale. Images of different stages are based on selection of a particular stage (best-suited) from the 67 pots under observation.

29.27 °C (the warmest of all seasons) and 26.95 °C was recorded for 2020. Humidity in the same season was 58.66 % for 2019 and 56.66 % for 2020 (Table 1). S3 showed variation in humidity from S2 with 86 % and 78 % for subsequent years, i.e. 2019 and 2020. S1 recorded minimum temperature viz. 9.35 °C in 2019 and 6.12 °C in 2020. The rainfall pattern in 2019 was slightly more (1623.31 mm) when compared to 2020 rainfall data (1448.12 mm) (Table 1). The

Table 1. Mean monthly climatic data observed during the study period

Months	Max. Temp.	Min. Temp.	Avg. Temp.	Humidity	Rainfall	Rainy Days	Sun Duration
				2018			
September	28.40	20.31	24.36	82	174.11	19	8.9
October	25.40	13.72	19.56	76	28.44	1	10
November	22.61	8.35	15.48	72	11.43	1	10.2
December	21.44	5.63	13.54	91	5.32	2	10.8
				2019			
January	21.74	5.32	13.53	83	28.71	7	8.5
February	25.73	8.91	17.32	69	49.46	13	10.1
March	33.21	13.82	23.52	53	50.31	7	11
April	35.90	16.11	26.01	49	26.81	5	11.3
May	35.80	28.82	32.31	74	49.54	7	8.4
June	35.51	23.49	29.50	82	159.31	6	7.9
July	29.80	22.41	26.11	89	535.70	29	8.2
August	28.63	21.61	25.12	85	503.74	30	9.5
September	27.46	19.88	23.67	76	195.70	28	9.4

October	25.95	12.62	19.29	60	14.64	9	8.3
November	21.31	7.39	14.35	50	9.572	5	9.1
December	20.48	4.62	12.55	50	8.561	3	7.8
				2020			
January	19.32	3.61	11.47	85	37.75	17	8.4
February	22.40	5.65	14.03	76	64.50	11	9.3
March	26.21	9.12	17.67	60	45.55	14	10.8
April	32.53	13.33	22.93	50	28.50	12	11.5
May	35.53	16.82	26.06	50	42.53	12	11.8
June	34.40	29.41	31.91	70	137.40	15	10.6
July	30.50	22.64	26.57	75	399.54	28	8.1
August	29.70	22.33	26.02	70	494.53	31	7.5
September	25.20	19.72	23.56	89	167.80	20	8.6
October	22.80	12.37	18.79	80	19.46	0	9.7
November	21.90	7.61	15.21	74	10.54	7	9.4
December	20.46	4.23	12.64	84	23.70	2	8.6
				2021			
January	25.31	4.82	12.64	75	51.54	4	7.3
February	34.53	9.35	17.33	73	60.43	3	9.3
March	36.91	12.45	23.49	59	54.51	6	10.7
April	30.57	15.37	25.34	42	23.60	10	8.9
May	36.91	26.46	31.69	47	48.35	19	12.3
June	30.57	24.63	27.60	64	139.53	20	11.9

plant restarts its vegetative and reproductive growth when favourable environmental conditions are in place. Humidity showed variation in S4 of 2020 (26.98 %) compared to 2019 (68.33 %).

The study period lasted for 32 months in which S4 data was recorded in 2018, which didn't show much variation compared to 2019 and 2020 S4 data. However, humidity and average sun duration were comparatively lower, i.e. 53.33% and 8.4 hrs. The data collected in 2021 was for S1 and S2, which only showed significant variation in humidity (26.98 %) when compared to the 2019 (68.33 %) and 2020 (53.33 %) data. The average sun duration was 8.4 hrs in 2019 and 9.23 hrs in 2020 (Table 1). Many phenophases can be seen in the same plant simultaneously, as presented in Fig. 3.

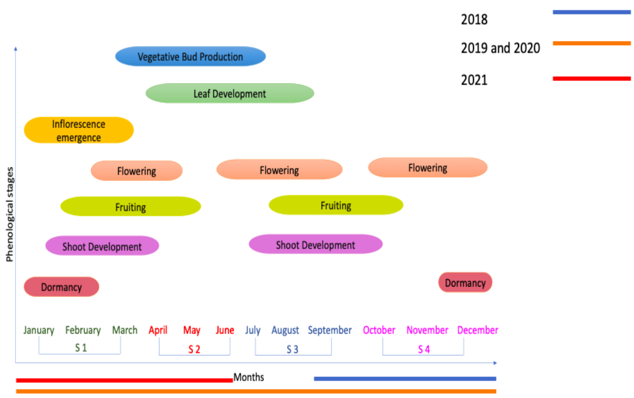

Fig. 3. Phenophases of *L. camara* in different seasons S1, S2, S3 and S4 respectively.

Dry seeds (00) are shown in PGS Stage 0 (Fig. 2). PGS 1 exhibits five-leaf development stages viz. 12, 13, 14, 15 and 16 (Fig. 2). The earliest genuine leaves (12/ 13) were opposite and elliptical. The average shoot growth rate was 19 cm /year and leafing period lasts for 5-6 months in *L. camara*.

Stage (21) of PGS 2 elucidates the initial lateral shoot. Stages 30 and 31 of PGS 3 revealed the start of stem elongation as well as one clearly lengthened internode. The primary inflorescence started as a terminal umbel contained between the leaf axils at the end of the stem. The primary stem grew longer as the terminal umbel grew larger. Secondary inflorescences began to grow in the axils of the lower leaves of the stem, resulting in lateral branches. In the same way, when secondary inflorescence grew, lateral branches became longer, resulting in higher-order inflorescence and branches. Because the plant does not replicate vegetatively, PGS 4 (vegetative propagation) was removed.

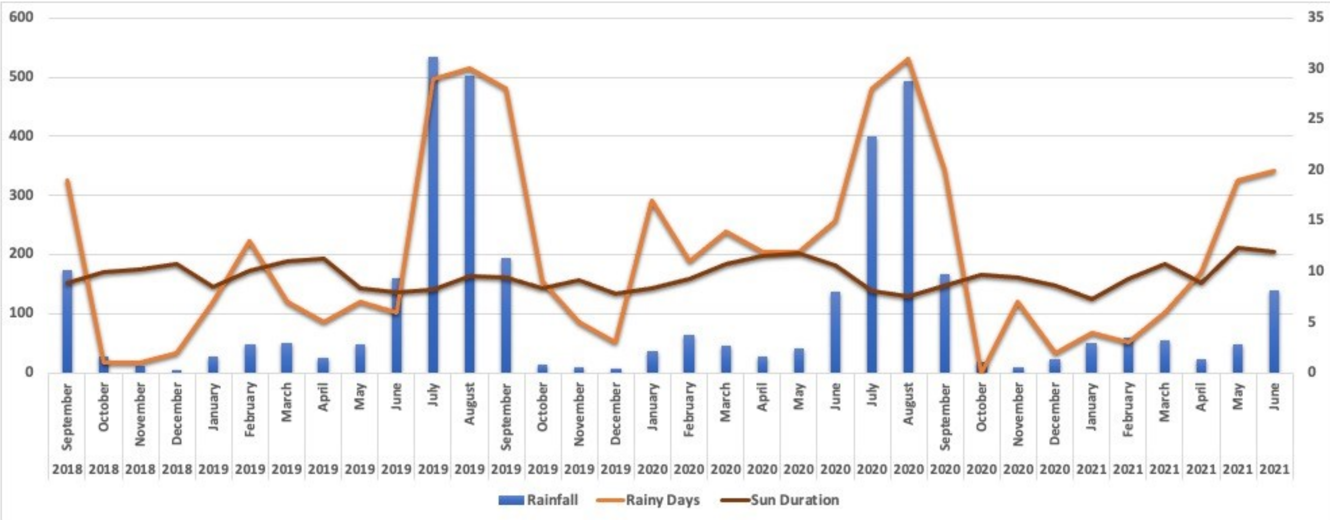

Fig. 4. (a) Graph showing average Rainfall (mm), Number of Rainy days and Sun duration (hrs) in each month.

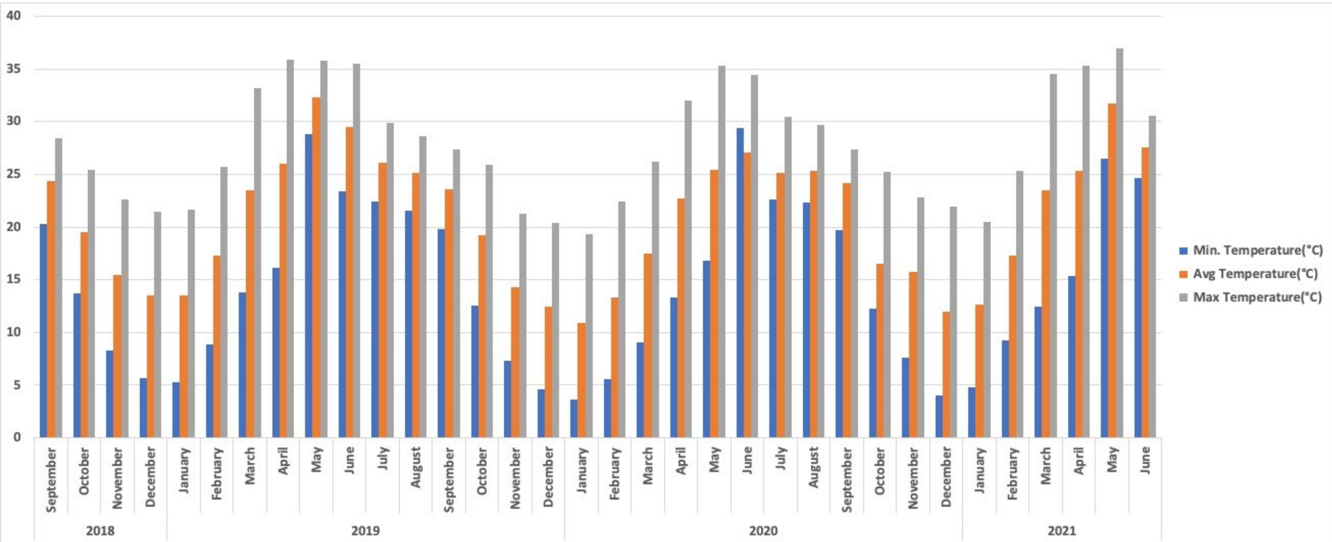

Fig. 4. (b) Minimum, Maximum and Average Temperature during the study period of 32 months.

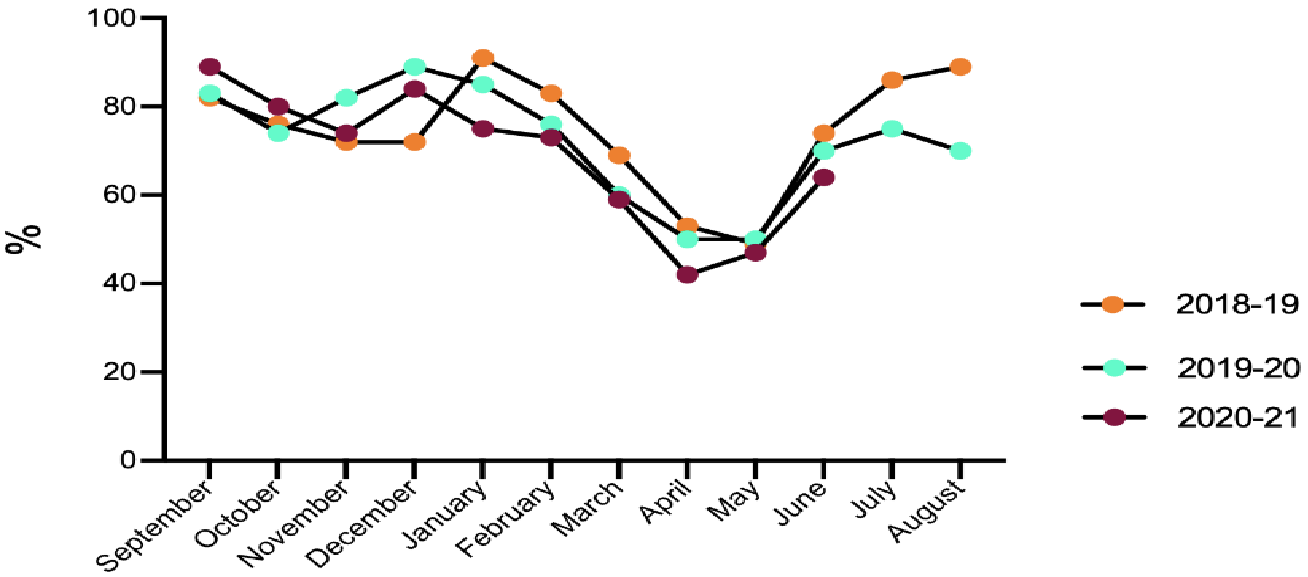

Fig. 4. (c) Humidity in percentage (%) during the study period.

Stages 51 (inflorescence or flower bud visible), 55 (first individual blooms seen (still unopened), and 59 (first flower petals visible) are illustrated in PGS 5. (Fig. 2). As a result, subsequent growth proceeds in a basipetal way along the stem and lateral branches, resulting in more lateral and larger branches, and also the formation of a terminal umbel. Flowers were observed on the plants throughout the year, with some gaps as presented in Fig. 2. The relevance of photoperiodic phenomena in flowering initiation, the average day length (in hrs) was 9.86 in S1, 9.2 in

S2, 9.03 in S3 and 8.4 in S4 for 2019 and 9.56 during S1, 11.36 during S2, 8.06 during S3, and 9.36 during S4 in 2020. PGS 6 depicted three Stages 60, 65 and 69 related to flowering, as illustrated in Fig. 2. Generally, fruit ripening occurs two weeks after flowering, and mature fruits can be seen until November.

PGS 7 (Development of fruit) has 2 Stages 71 and 79 (Fig. 2) followed by 81 and 89 of PGS 8 that shows the beginning of ripening in fruits (81) and fully ripened fruits (89) (Fig. 2). PGS 9 (senescence) was the last stage of the scale, when the leaves, stem and branches began to yellow and dry at the plant's base. Although the plant is an evergreen perennial, it was observed that the development of new leaves is inhibited at this time, and the plant undergoes in dormancy stage for a brief period. The plant is a perennial species and continues to survive after the completion of the study. It's possible that if the right environmental circumstances are in place, the respective plant's life cycle will extend beyond.

Through purposeful imports by Europeans almost two centuries ago, *L. camara* expanded beyond its geographical limits: The West Indies and Central and South America. *L. camara*'s wide tolerance of edaphic and climatic conditions has also contributed to its naturalization and invasiveness in its introduced habitats (29). The presence of adequate moisture and light aids the growth of *L. camara* and duration of the life cycle changed with respect to climatic factors. This shade-tolerant woody scandent shrub may scramble up into trees and reach a height of 6 m. The decimal codes generated for *L. camara* corresponded to BBCH developmental stages except for the PGS 4 stage, i.e. vegetative reproduction.

Studies on the *L. camara* germination ecology reveal that no clear environmental conditions may prevent it from germinating (23). As a result, the plant may thrive in plenty throughout the year, giving it a significant competitive edge against natural species. The study revealed that Season S3 comprises four Principal Growth Stages, i.e. Leaf formation, flowering, fruiting and shoot development. In the same way, the plant reached its maximum height (173.58 cm) and number of branches i.e. 7 in S4.

The findings may hold important implications for studies of IAPS and invasion biology (32). Knowledge of the ecology of invasive plant species, as well as timely interventions to suppress them, is often required for the restoration of damaged areas (33, 34). Changes in climatic circumstances, particularly temperature, have a significant impact on phenological growth patterns. It may be inferred that the weed prefers the optimal temperature and high humidity for growth, although it can live in almost any climate at the study site. The present study provides data for the observed phenology of *L. camara* under humid subtropical conditions of Dehradun. Comparative phenology of *L. camara* across its distribution area can give insight into the species phenotypic plasticity, as well as assist to explain variations in the species invasive potential in response to environmental variables. Studies on comparative phenology of the species will also help forecast the impact of changing climatic variables on species distribution. Mechanical control practices for *L. camara* suggest uprooting of the individuals before the onset of seeds. Though germination of the species is observed only during monsoon, it was observed that the plant produces seeds throughout the year. Hence, there will be sufficient seed-bank in the soil even after uprooting to generate a new population. Therefore, revegetating the reclaimed land from *L. camara* with native species is essential to control the re-emergence of *L. camara* (35, 36).

Conclusion

This research established a standardized method for characterizing the phenology of *L. camara*. Morphological factors were used to code essential phenological features related to vegetative development and flowering. The phenology of *L. camara* changed in response to shifting temperature and humidity conditions, but no obvious climatic condition inhibited its germination or flowering, according to the findings. Variability in *L. camara* phenological features in response to changing environmental circumstances reflect the weed's acclimatisation capacity, which allows it to grow widely in non-native locations. Comparison between native and invasive flowering phenology will be helpful in controlling invasiveness. This paper reports the first use of the BBCH numerical coding scheme to *L. camara*, which could be useful to scientific investigators. It may ease research problems amongst *L. camara* researchers in different areas of the world. More significantly, this description is relevant to *L. camara* growing in tropical and subtropical zones pan India (mainly pasture lands), temperate regions and India's protected forest regions. This system of consistent labelling will be a useful tool for designing scientific eradication experiments for this invasive plant.

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Authors contributions

AK performed the experiments, and prepared the manuscript. SS designed the experiments and helped in drafting the manuscript. HB and RK participated in helping with the experimental setup and data collection. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

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