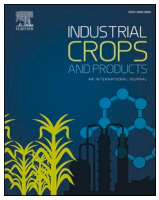
Industrial Crops & Products 192 (2023) 116118

Contents lists available at ScienceDirect

Industrial Crops & Products

journal homepage: www.elsevier.com/locate/indcrop



Crop phenology and floral induction in different *Artemisia annua* L. genotypes

José Abramo Marchese [a,*,1], Jorge F.S. Ferreira [b,2], Rita Maria Moraes [c,3], Franck E. Dayan [d,4], Michelle F.F. Rodrigues [a,5], Jorge Jamhour [a,6], Lucas Vinicius Dallacorte [a,7]

[a] *Agronomy Department, Federal University of Technology – Paraná, Pato Branco, PR, Brazil*
[b] *US Salinity Laboratory (USDA-ARS), Riverside, CA, USA*
[c] *National Center for Natural Products Research, School of Pharmacy, The University of Mississippi, Oxford, USA*
[d] *Department of Agricultural Biology, Colorado State University, Fort Collins, CO, USA*

ARTICLE INFO

Keywords:
Sweet wormwood
Ontogeny
Photoperiod
Breeding
Artemisinin

ABSTRACT

Artemisia annua L. (sweet wormwood or artemisia), the only commercial source of the antimalarial artemisinin (ART), has genotypes with a wide range of ART concentrations and varying blooming periods. This makes the cross-pollination of high-ART genotypes difficult unless the flowering of selected parents is synchronized. In this study, we evaluated the phenological pattern of artemisia to establish a phenological scale that will allow successful cross-pollination and breeding. The scale was based on daily observations and photographs of the plant at specific time intervals and was divided into two distinct phases: the vegetative phase (with five stages) and the reproductive phase (with 12 stages). Although short days induce flowering, our experiments with Chinese and Vietnamese genotypes of artemisia demonstrated that genotypes from different geographic origins are better synchronized on both short days and low temperatures, with the Chinese genotype requiring only short days to flower and the Vietnamese genotype requiring short days and low/moderate temperatures to reach full flowering. We also noticed that Chinese plants flowering under short days can be reversed to the vegetative stage if plants are exposed to long photoperiod early enough. Finally, plants growing under long days, in the vegetative phase, have higher densities of glandular trichomes and higher concentrations of ART when compared to plants grown under short days and induced to flower. These observations suggest that the further from the inductive photoperiod the transplantation to the field occurs, the higher the concentration and yield of ART.

1. Introduction

According to the US/IBP Phenology Committee, "phenology is the study of the timing of recurring biological events, the causes of their timing with regard to biotic and abiotic forces, and the interrelation among phases of the same or different species" (Lieth, 2013). Phenological observations and calendars have been used in agriculture since thousands of years ago in both China and Rome. The term "phenology"

Abbreviations: ACT, Artemisinin Combination Therapy; ART, Artemisinin; CP, Critical Photoperiod; CPQBA, Pluridisciplinary Center for Chemical, Biological, and Agricultural Research, Campinas State University - SP; DAT, Days After Transplantation; DC, Planting date of cuttings; DM, Dry Mass; FL, Date when 50% + 1% of the plants started the emission of flower buds; GACP, Good Agricultural and Collection Practice; IDP, Intermediate-Day Plants; LD, Long Day; PC, Photoinductive Cycles; SD, Short Day; SDP, Short Day Plant; TD, Date of transplant to the field; US/IBP, International Biological Program of United States of America; WHO, World Health Organization; PD, Planting Date.

* Corresponding author.
E-mail address: abramo@utfpr.edu.br (J.A. Marchese).
[1] ORCID Id: 0000–0002-9010–3403
[2] ORCID Id: 0000–0003-4550–6761
[3] ORCID Id: 0000–0003-4209–8192
[4] ORCID Id: 0000–0001-6964–2499
[5] ORCID Id: 0000–0001-9071–9622
[6] ORCID Id: 0000–0001-9186–0556
[7] ORCID Id: 0000–0001-9145–7679

https://doi.org/10.1016/j.indcrop.2022.116118
Received 15 September 2022; Received in revised form 18 November 2022; Accepted 8 December 2022
Available online 15 December 2022
0926-6690/© 2022 Elsevier B.V. All rights reserved.

was first proposed by the Belgian botanist Charles Morren in 1853, but the father of modern plant phenology and phenological observation networks is the Swedish botanist Carolus Linnaeus (or Carl von Linné) (Hopp, 1974).

Knowledge of crop phenology is central in plant sciences. It is a useful tool for scheduling agricultural practices such as irrigation, fertilization, pesticide application, and harvest; it is also the basis for the construction of crop growth simulation models and a reference for assessing crop performance under variable inputs and conditions (Archontoulis et al., 2010; Ferrer-Blanco et al., 2022; Nazarpour and Yadegari, 2021).

Despite the worldwide importance of *Artemisia annua* L. as the source of the raw material for antimalarial drugs (Rodrigues et al., 2019; Soni et al., 2022), its crop phenology or developmental stages have not yet been defined or described in detail. WHO (2006) has published a monograph on good agricultural and collection practices (GACP) for artemisia (WHO, 2006), which describes the growth cycle of artemisia from sowing to harvest and seed set. However, the phenological scale is weak on plant developmental details and lacks illustrations. Some authors report a few stages of the plant, such as: vegetative, pre-flowering, and full flowering (Jain et al., 1996); vegetative, early flowering, and late flowering stages (Gupta et al., 2002); pre-flowering, full bloom, and post-flowering stages (Baraldi et al., 2008); vegetative, pre-bloom, bloom, and post-bloom stages (Rana et al., 2013); vegetative, bud, and flower stages (Ranjbar et al., 2015); bolting, budding, full flowering, and post-flowering (Towler and Weathers, 2015); or vegetative phase, flowering stage, and seed formation stage (Jeremić et al., 2022). Nevertheless, these descriptions do not offer a complete phenological scale with details of plant development and most of them lack illustrations. Thus, the development of complete and illustrated descriptions for the growth stages of artemisia based on field and greenhouse observations is paramount for artemisia breeding and research.

A. annua, from the Asteraceae family, originates from Asia and is the major commercial source of ART, a sesquiterpene lactone that is extracted, purified, and derivatized to manufacture Artemisinin Combination Therapies (ACTs) for the treatment of malaria (Alam and Albalawi, 2020; Brisibe et al., 2012; Ferreira et al., 2018; Towler and Weathers, 2015). Besides its antimalarial activity, ART has proved to be effective against some types of cancer (Efferth, 2005; Ivanescu and Corciova, 2014; Mondal and Chatterji, 2015; Posner et al., 1999; Singh and Lai, 2004), but its anticancer use has yet to match its reputation as an antimalarial medicine. In addition, plant extracts are reported to inhibit the replication of SARS-CoV-2 *in vitro* (Nair et al., 2021). The secondary or specialized metabolites responsible for *A. annua* biological activities, such as ART, vary in their accumulation depending on plant organ and plant phenological phase, especially in the transition between vegetative and reproductive stages as observed by Towler and Weathers (2015), Ferreira et al. (2018), and Li et al. (2020).

Artemisia is a species with C3 photosynthetic metabolism (Marchese et al., 2005a) and has a short-day requirement for flowering (Ferreira et al., 1995a). Photoperiod-sensitive plants have their cycle/development conditioned to the critical photoperiod (CP), which for Chinese genotypes of artemisia tested in Indiana, USA, was approximately 13 h with plants flowering two weeks after induction (Ferreira et al., 1995a). During the blooming phase, the growth stops, the internodes elongate, and the leaves are replaced by flowers (Marchese et al., 2005b).

The onset of the *Artemisia* blooming stage is very important for successful outcrossing and breeding programs and to obtain large quantities of viable seeds of high vigor. Synchronization of flowering of different genotypes is important so that the release of pollen and stigma receptivity of parental lines coincide (Ferreira et al., 1995b; Wetzstein et al., 2014). For instance, early-blooming Chinese and late-blooming Brazilian plants were successfully crossed under field conditions in Indiana (USA) by synchronizing their flowering under short photoperiod by simply bagging plants of both genotypes with a black opaque plastic bag (painted white outside to reduce heat absorption) to reduce their photoperiod perception to less than 13 h, based on information published by Ferreira et al. (1995a). After crossing, the plants redirect their photosynthetic assimilates to seed formation, starting the senescence stage of an annual species. On the other hand, under long days (non-inductive photoperiod), plants remain vegetative and internode production is linear as plants grow indefinitely (Ferreira et al., 1995a; Marchese et al., 2005b). Even though the elaboration of a detailed phenological chart is challenging due to these intrinsic characteristics of artemisia, its relevance is remarkable, especially in determining the harvest time for shoot biomass and the right time for outcrossing.

Although ART peak accumulation occurs at the beginning of flowering (Marchese et al., 2005b; Towler and Weathers, 2015) or full flowering (Ferreira et al., 1995a) for low-ART (0.2%) Chinese clones, high-ART (0.5–1.5%) late-flowering clones of Brazilian and Swiss origin, and early-flowering Chinese clones high in ART (0.7%) and dihydroartemisinic acid (>1.2%), all reached their ART peak in early September in West Virginia, approximately one month before the onset of flowering for Brazilian and Swiss clones (Ferreira et al., 2018). This indicates that the ART peak accumulation is associated with the photoperiod, and not with flowering, as previously thought. Therefore, observations associated with the identification of the phases of greatest agronomic interest of artemisia are fundamental to the management of the crop and for seed production and synchronization under short photoperiod and require detailed knowledge of the phenology of this species. Field evaluation of genotypes of commercial interest, when accompanied by High-performance Liquid Chromatography (HPLC) analysis, will establish the best time for both maximum leaf biomass and ART accumulation for ART commercial production in any geographic area (Ferreira et al., 2018). It is worth noting that artemisia flowers early and does not accumulate sufficient biomass when cultivated in latitudes close to the Equator (Ferreira et al., 2005).

The purpose of this research was to evaluate the phenological pattern of *Artemisia annua* L., and to establish a detailed phenological scale for agronomic recommendations and breeding of the species. In addition, the effect of temperature in conjunction with different photoperiods on the induction of artemisia flowering and other parameters of floral development were assessed, considering the hypothesis that ambient temperature could also influence flowering. Finally, the photoperiod effect on the ART peak in artemisia was verified and compared to the phenological phase effect.

2. Materials and methods

2.1. Phenological scale study

The plants grew inside Quonset huts at the Federal Technological University of Paraná (UTFPR), Campus of Pato Branco, PR, Brazil (26°11' S, 52°36' W and 760 m of altitude using artificial light to provide over 13 h of light/day to avoid flowering for the first 40 d. The phenological scale study monitored the development of artemisia cv. Artemis (Mediplant, Switzerland), grown in six pots containing 3 kg of sterilized soil and fertilized with 2.0 g of NPK (8−20−20). As seeds germinated, the seedlings were observed and photographed daily. After 40 d, we interrupted the artificial illumination and 20 d later the plants started to flower. The internodes were counted, and plants were photographed every other day. The codes assigned had scales from 1 to 17. The results with codes and description of phenological stages are reported in Table 1 while the images can be seen in Fig. 1.

2.2. Environmental requirements for flowering

2.2.1. Flowering control by photoperiod and temperature

The experiments were carried out in photoperiodic chambers, inside Quonset huts, without temperature control, located at the UTFPR (26°11' S, 52°36' W). Two genotypes of artemisia with different geographical origins were used in the experiments. The first genotype

Table 1
Developmental stages of *Artemisia annua* L. cv. Artemis.

STAGE CODES	DESCRIPTION	PLATE FIGURES
VEGETATIVE PHASE		
Stage 01	Emergence	A
Stage 02	Emission of 1st pair of true leaves	B
Stage 03	Emission of 2nd pair of true leaves	C
Stage 04	Vegetative growth (require >13.5 h light/day)	D, E, F
Stage 05	The transition from vegetative growth to blooming (under short days - <13 h light/day)	G
REPRODUCTIVE PHASE		
Stage 06	Emission of flower buds	H
Stage 07	Formation of capitula	I, J
Stage 08	Open capitulum	K
Stage 09	Ray florets starting anthesis	L
Stage 10	Disc florets starting anthesis	M
Stage 11	Growing of achenes	N
Stage 12	Early filling of achenes	O
Stage 13	Growth stages of achenes	P
Stage 14	Mature achene (hard and vitreous)	Q
Stage 15	Senescent capitula	R
Stage 16	Empty bracts	S
Stage 17	Full plant senescence	T

denominated Vietnamese (CPQBA 2/39 ×1 V), was developed by a breeding program done by the Centro de Pesquisas Químicas, Biológicas e Agrícolas from Campinas State University (CPQBA/UNICAMP), SP, Brazil, with parents from Vietnamese plants (Magalhães et al., 1997), and a second genotype denominated Chinese originated from low-ART Chinese plants, provided by the Departments of Horticulture and Landscape Architecture from Purdue University, USA, also used by Ferreira et al. (1995b). The clones were obtained by cuttings from only one matrix plant of each genotype. Both clones and matrix plants were maintained under a 15-h photoperiod to avoid flowering. After the cuttings had rooted, they were transplanted into pots containing 3000 cm^3 of a substrate (Plantmax® plus soil).

The first experiment was carried out from October 20 to February 15 during the Brazilian spring/summer season. 30 cloned plants of each genotype, 30 cm in height, were selected randomly and submitted to six photoperiods: 7, 9, 11, 13, 15, and 17 h of light/day. Six plants from each treatment were tagged and used to determine the number of photoinductive cycles (24 h) and flowering plants (%). The remainder of the 24 plants within each chamber (a total of 30) were used to determine artemisinin concentration (see topic 2.5.1). Plants were exposed to 7 h of natural light, with the photoperiod extended inside photoperiodic chambers, using 40 W fluorescent lamps (1.6 μmol m^{-2} s^{-1}) and a 100 W incandescent lamp (1.6 μmol m^{-2} s^{-1}), supplying a photon flow density of about 3.5 μmol m^{-2} s^{-1}. Plants were checked daily for flowering. During the experiment (56 d), the daily average temperatures recorded were of 37 ºC (max.) and of 19 ºC (min.).

Considering the results of the first experiment and the hypothesis that ambient temperature can influence flowering, another experiment was carried out from February 29 to June 26 during the Brazilian autumn/winter in the same place, only with the Vietnamese genotype (2/39 ×1 V hybrid), during 56 d, with 6 plants in each treatment, the same parameters used to evaluate the first experiment were used in the second. The daily average temperatures had a maximum of 29 ºC and a minimum of 13 ºC.

2.2.2. Different planting dates and flowering

The experiment was conducted in the Experimental Area of UTFPR (26º11' S, 52º36' W). For this study, we used plants obtained by cuttings from the Vietnamese genotype (CPQBA 2/39 ×1 V). The cuttings were kept in tubes with a rooting substrate (Plantmax®) for approximately 30 d and taken to the field at six different planting dates (PD): Sep 23 (PD1), Nov 20 (PD2); Jan 16 (PD3), Feb 07 (PD4), Mar 05 (PD5) and Jul 10 (PD6). Each replication or experimental plot consisted of 25 plants, with spacing between plants of 0.6 m in the planting row and 1 m between the rows. The maximum and minimum temperature and precipitation accumulation registered during the experiments in Pato Branco, PR, Brazil, were obtained from Meteorological Station SIMEPAR/IDR (See Supplementary Figure 1).

The evaluation of the emission of flower buds was performed daily. When 50 + 1% of the plants had flower bud emission, the nine central plants of the plot were harvested to determine the dry mass of leaves and stems (LDM and SDM, respectively), leaf/stem ratio, and ART concentration (mg g^{-1} LDM).

2.3. Tissue-culture plants and reversible flowering

Tissue-culture plants were obtained from greenhouse Chinese clones, sterilized, and grown in a hormone-free medium in GA-7 Magenta vessels, as this procedure avoided plant vitrification and allowed maximum root growth and ART production in planta. Cultures and light conditions were the same as specified elsewhere (Ferreira et al., 1995b). Greenhouse plants were generated from cuttings of pre-cloned field plants and maintained under 16-h photoperiod provided by high-pressure sodium lamps (1000 Watts). Plants kept under 12-h day-length flowered after 2–3 weeks of exposure to short days. These plants were transferred to 16-h day-length in a Quonset-type greenhouse with extra light provided by high-pressure sodium lamps of 1000 Watts until they reverted to the vegetative stage to test if flowering was reversible. Plants were kept under long days during the winter until transferred to the field in the spring.

2.4. Number of capitate glands versus day-length

This experiment was conducted in a greenhouse at the University of Mississippi Experimental Station, Oxford, MS, USA (34º24'N and 89º24'W). Seedlings from seeds of the Brazilian access CPQBA 23/9 × 1 V (Vietnamese) 50 days old and grown under natural photoperiod (34º24′ N) plus 6 h of artificial light to avoid flowering were transplanted to pots filled with growing media (SUN GRO®) plus 3.8 g of the slow-release fertilizer OSMOCOTE® (N 14% - P 14% - K 14%). And after 1 week, 48 pots were transferred from the greenhouse to growth chambers (CONVIRON® CMP4030) and 24 plants were placed inside a chamber with a photoperiod of 10 h, and other 24 plants were placed inside a chamber with a photoperiod of 16 h. The temperature and PAR (photosynthetically active radiation) inside both chambers were approximately 20 ºC and 280 μmol m^{-2} s^{-1}, respectively. After a period of 32 d inside the chambers, the plants were transferred back to the greenhouse and 8 plants from each day-length treatment were used for microscopic analysis to compare the number of capitate glands. Fully expanded leaves were harvested from the middle third of the plant canopy and prepared in FAA with glycerin. For microscopic comparison of the number of capitate glands, 4 replicates per plant were examined under a Nikon Eclipse 600 microscope with a fluorescence source. Digital photomicrographs were taken using a Kodak camera attached to the microscope. Samples were analyzed under UVEX 450–490 (20 × magnifications).

2.5. Quantification of artemisinin

2.5.1. Experiments carried out in Brazil

ART levels in leaves were quantified using the high-performance liquid chromatography technique with ultraviolet detection (HPLC-UV), described by Marchese et al. (2001a) and adapted from Zhao and Zeng (1986). HPLC/UV consisted of a WATERS chromatographer model M-45. The separation was carried out in one WATERS/NOVA-PAK® Cl8/4 pm column (3.9 ×150 mm). ART quantification was done by UV detection after derivatization of ART to Q260, which absorbs light under the wavelength of 260 nm, through a WATERS detector MODEL 481, according to Zhao and Zeng (1986).

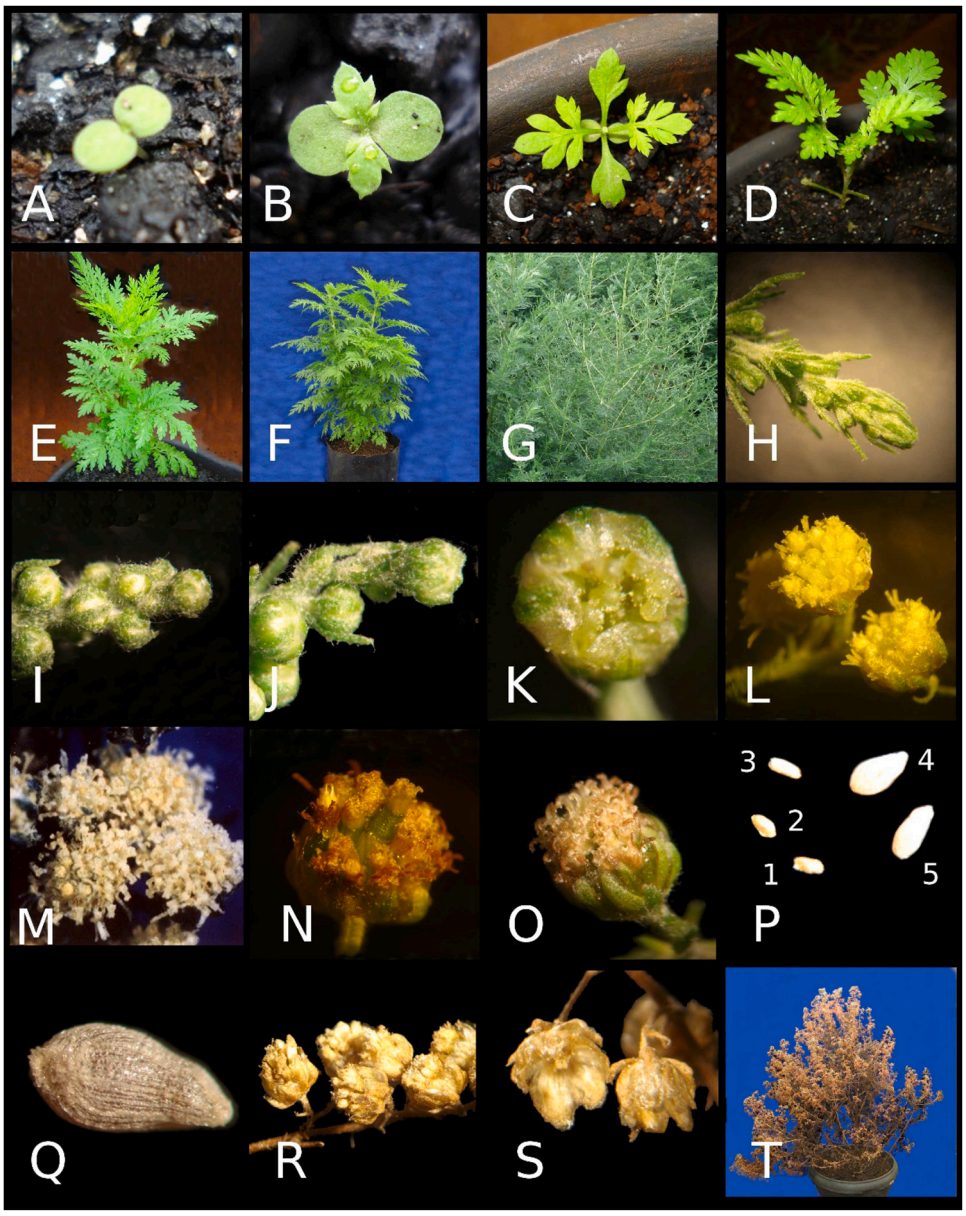


Fig. 1. Identification chart of different phenological stages of *A. annua* from germination to full plant senescence. A description of the stages is shown in Table 1 (Vegetative phases from A to G, and Reproductive phases from H to T).

2.5.2. Experiments carried out in the USA

Dried leaves were grounded and approximately 100 mg were transferred to centrifuge tubes, followed by 5 mL of acetonitrile. All samples were homogenized using a polytron for 30 s at 20,000 RPM. These extracts were centrifuged, the supernatants were filtered through a 0.45 μm nylon membrane filter, and transferred to HPLC vials for injection (Marchese et al., 2001b). The HPLC-ELSD analysis was performed using a Waters (Milford, MA, USA) Module liquid chromatograph interfaced with a UV detector and a Sedex (SEDERE, Alfortville, France) model 55 ELSD operated at 30 ºC and 2.0 bar of nitrogen. A Phenomenex column Luna 18(2) (250 ×4.6 mm; 3 μm particle size) was used at ambient temperature. The mobile phase consisted of a mix of 0.1% TFA (A), methanol (B), and acetonitrile (C) at a flow rate of 0.8 mL/minute with the gradient elution going from 35%A:25% B:40%C to 5%A:0%B:95%C from 0 to 30 min. At the end, the column was washed with 5%A/95%B for 5 min; then re-equilibrated with 35% A:25%B:40%C for 15 min before the next injection. After the injection of 10 μL of the sample, data were collected and analyzed using the Waters Millennium 32 software.

3. Results

3.1. Phenological scale

The crop phenology or phenological scale of the development of artemisia was divided into two distinct phases: vegetative and reproductive (Fig. 1). The vegetative phase was divided into five sub-phases or stages ranging from emergence to the cessation of vegetative growth. The reproductive phase was divided into 12 sub-phases or stages, which range from the emission of flower buds to complete plant senescence. In West Virginia, the plants that flowered in September (Chinese clone) had mature seeds in October, 30–45 d after the anthesis. If seeds were not collected, they fell to the ground. Although temperatures dropped to zero centigrade in the field, plants were not affected by snow or cold and the seeds that fell off the plants germinated without any problem in the spring. Seeds germinate better if uncovered (or

lightly covered) by soil, as they do in natural settings (from the authors). Fresh seeds germinated (Fig. 1A) and reach stages B and C within 6–10 d of being in contact with moist soil. Approximately 2 months after seeding, plants reach stage E and were approximately 25 cm in height and ready to be transplanted to the field. Field plants in West Virginia reached vegetative maturity (end of stage F) and had 1.8–2.0 m in height (maximum biomass accumulation), and reached the peak in ART in early September, regardless of their geographic origin (Brazil, China, or Switzerland) or proximity to the blooming stage, considered as being from the appearance of flower buds (H) to anthesis (M). The florets in the capitulum are either pistillate (female, ray) or hermaphroditic (disk) florets. The pistillate florets are at the margin of the capitulum and open first (Fig. 1L) and the disk florets are at the center and open next. It is important to note that at anthesis, pollen grains are released when stigmas are not yet receptive (or vice-versa). After anthesis and cross-pollination by wind, the achenes fill up and eventually mature (N – Q). Once the achenes (seeds) are mature they can be collected (between stages Q and R) as viable seeds. After those stages, the capitula started to senesce (R) and the seeds fell off (S). After the S stage, senescence sets in (T), and the plant is considered dead.

3.2. Effect of environment on plant development and flowering

3.2.1. Flowering control by photoperiod and temperature

Tables 2 and 3 show two different genotypes of artemisia with different requirements when exposed to a range of day length and temperature. During the spring/summer experiment (average temperatures of 37 °C (max) and 19 °C (min)), 100% of the Chinese plants flowered in approximately 2 weeks (14.7 photoinductive cycles (PC) of 24 h) after being submitted to short day (SD) treatments. The critical photoperiod (CP) for the Chinese genotype was between 13 and 15 h (Table 2). However, plants under long day (LD) treatments remained vegetative during the 56 d of the experiment. However, the Vietnamese genotype (Table 2) behaved differently from the Chinese genotype. Under SD, only 27.8% of plants emitted floral buds, starting from the fourth week (29.3 PC) of induction. Under LD treatments, plants remained vegetative. Branches of vegetatively cloned Chinese plants grown at 27 °C in a greenhouse that received 8 h of natural light before being covered with a black cloth and supplemented with incandescent light (40 W, 4.8 μmol. m^{-2}. s^{-1}) to complete 10, 12, 16, 20, and 24 h of light per day, according to Ferreira et al. (1995a) were photographed (Fig. 2). Considering the results of the first experiment, another experiment with the Vietnamese genotype was set up during the autumn/-winter (temperatures: 29 °C (max) and 13 °C (min)) combining photoperiod with temperature (Table 3). After five weeks (36.7 PC) of inductive short photoperiod, 94.44% of the plants bloomed, while plants under LD treatments remained vegetative. For the Vietnamese genotype, the CP was between 11 and 13 h (Tables 2 and 3).

Table 2
Effect of different photoperiods in the flowering induction of plants of two *A. annua* genotypes (Chinese and Vietnamese) cultivated inside photoperiodic chambers[a].

Photoperiod	Nº photoinductive cycles (24 h) necessary for floral initiation		Plants flourished (%)	
	Chinese	Vietnamese	Chinese	Vietnamese
Short days:				
7 h	14	30	100	33.3
9 h	14	24	100	33.3
11 h	14	34	100	16.7
13 h	17	–	100	–
[2]*Short days*	14.7 ± 2.58	29.3 ± 3.46	100 ± 0	27.8 ± 9.58
[3]*Long days*	–	–	–	–

[a] Average temperatures: 37 °C (max) and 19 °C (min). [2]Average of the short days treatments (inductive photoperiod) 7, 9, 11, and 13 h for the Chinese genotype, and 7, 9, and 11 h for the Vietnamese genotype. [3]Average of the long days treatments (no-inductive photoperiod) 15 and 17 h for the Chinese genotype and 13, 15, and 17 h for the Vietnamese genotype.

Table 3
Different photoperiods and temperatures affect the flowering induction of the Vietnamese genotype of *A. annua* cultivated inside photoperiodic chambers[a].

Photoperiod	Nº photoinductive cycles (24 h) necessary for floral initiation		Plants flourished (%)	
	37 °C/19 °C[1]	29 °C/13 °C[2]	37 °C/19 °C[1]	29 °C/13 °C[2]
Short days:				
7 h	30	34	33.3	100
9 h	24	32	33.3	100
11 h	34	44	16.7	83.33
[3]*Short days*	29.3 ± 5.03	36.7 ± 6.43	27.8 ± 9.58	94.44 ± 9.62
[3]*Long days*	–	–	–	–

[a] Average temperatures inside the photoperiodic chambers from October 20 to February 15: 37 °C (max) and 19 °C (min). [2]Average temperatures inside the growth chambers from February 29th to June 26th: 29 °C (max) and 13 °C (min). [3] Average of the Long days treatments 15 and 17 h (no-inductive photoperiod), and the average of the Short days treatments 7, 9, and 11 h (inductive photoperiod).

Fig. 2. *A. annua* Chinese clones were induced to flower under short photoperiods of 8, 10, and 12 h or kept vegetative with continuous growth at 16, 20, and 24 h photoperiod induced by incandescent lamps (60 W).

3.2.2. Tissue-culture plants and reversible flowering

As additional information, plants propagated by tissue culture, in a hormone-free medium, according to Ferreira et al. (1994), and maintained under tissue culture for over a year lost their apical dominance (Fig. 3A). These plants did not flower under short photoperiods (Fig. 3B) unless they recovered their apical dominance (Fig. 3B, insert). Also, cuttings from mature plants, propagated under short photoperiods and kept in a greenhouse at the end of August/early September in West Virginia (USA), flowered (Fig. 3C) under short days. These plants reverted to vegetative growth if placed under a 16-h photoperiod, and if flowering was not too advanced (Fig. 3D, E).

3.2.3. Different planting dates and flowering

In the field experiment done in Pato Branco, PR, southern Brazil, the PC found for the Vietnamese genotype was approximately 28 cycles or days (Fig. 4), and the CP occurred on Feb 07, which day-length was 13.07 h, that is, CP= 13.07 h (Fig. 4). Seedlings transplanted to the field between September and February, PD1 to PD4, flowered only in March (Table 4). On the other hand, PD5 and PD6 were transplanted within the inductive photoperiod and had their flowering 28 and 31 d after transplanting, respectively (Table 4). Regarding the dry mass accumulation of leaves, stems, leaves + stems, and ART concentration and yield of the Vietnamese genotype, the planting season that resulted in the significantly highest yields was TD1 (Table 5).

The results observed in the experiment carried out in a growth chamber resulted in plants with the highest number of glandular trichomes (Fig. 5-A, C) and the highest ART concentrations (Fig. 5-B) in plants in the vegetative stage and growing under long days, in

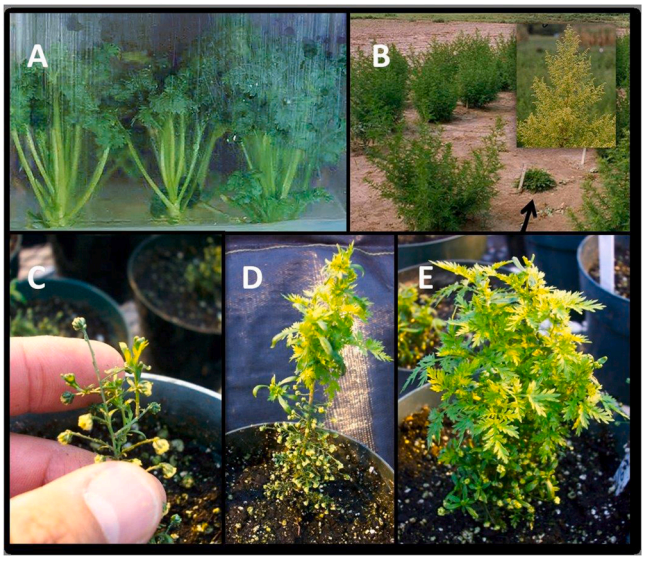


Fig. 3. Chinese *A. annua* under tissue culture (TC) (A), field (B), and greenhouse (C-E) conditions. Plants that lost apical dominance under TC (A), grew prostrate in the field (black arrow) and never flowered (B). Cuttings of mature field plants (August in West Virginia) flowered under the short days of September (C) but reverted to vegetative growth under a 16 h photoperiod (D and E).

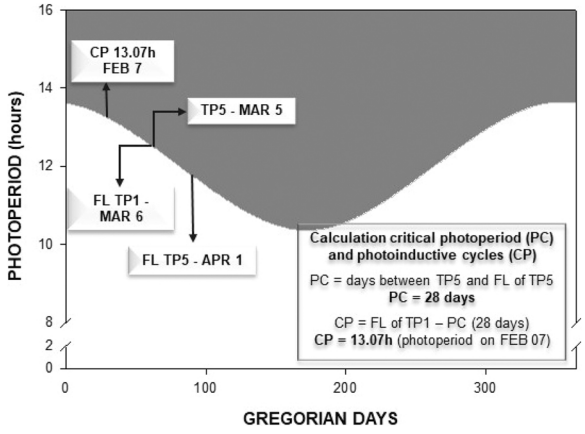


Fig. 4. Annual photoperiod in Pato Branco-PR, Brazil (latitude 26°07' S, longitude 52°41' W, altitude of 760 m) and characterization of *A. annua* flowering (Vietnamese genotype). CP = critical photoperiod; PC = number of photoinductive cycles. TP = transplanted to the field [TP1 = 1st TP (Sept 1st year); and TP5 = 5th TP (March 2nd year)]; FL = flower bud emission (when 50% +1% plants started the emission of flower buds).

comparison to those plants grown under short days, under inductive photoperiod (Fig. 5-A, C).

4. Discussion

Commercial *Artemisia annua* crops are the sole source of ART worldwide. The establishment of the phenological stage with the simultaneous highest accumulation of leaf biomass and ART is important for effective crop management and high yield of ART. In the literature, there are mainly two different views on the phenological phase coinciding with ART peak during plant development. Some authors stated that the peak of ART production occurred just before plant flowering (Stage 05, Table 1/Image G, Fig. 1) (Acton et al., 1985; Chan et al., 1995; Liersch et al., 1986; Woerdenbag et al., 1994, 1991) or during the floral-bud stage (Stage 06, Table 1/Image H, Fig. 1) (Towler and Weathers, 2015; Zhang et al., 2006), while others reported that ART peak occurred during flowering (Stages 07–09, Table 1/Images I, J, K and L, Fig. 1) (Baraldi et al., 2008; Ferreira et al., 1995a; Ferreira and Janick, 1996; Morales et al., 1993; Pras et al., 1991; Singh et al., 1988).

In our experiment, carried out in photoperiodic chambers, we found the ART peak occurred two weeks after the emission of flower buds (Stage 06, Table 1/Image H, Fig. 1) in Vietnamese plants (Fig. 6). Several authors have associated ART peak with flowering based on field-grown plants (Ferreira et al., 1995b; Morales et al., 1993; Pras et al., 1991; Singh et al., 1988). However, in West Virginia, USA, all Brazilian, Chinese, and Swiss plants reached their ART peak in early September, before any signs of flowering were observed, indicating that ART peak is associated with the photoperiod, not with the phenological stage (Ferreira et al., 2018). This agrees with others studies that had previously stated that there may not be a link between flowering and ART biosynthesis (Marchese et al., 2005b; Wang et al., 2007, 2004). Wang et al. (2004) transferred the flowering-promoting factor1 (gene fpf1) from *Arabidopsis thaliana* into artemisia via *Agrobacterium tumefaciens*. Under short-day conditions, the flowering time of fpf1 transgenic plants was about 20 d earlier than the non-transformed plants; however, no significant differences were detected in ART content between the flowering transgenic plants and the non-flowering non-transgenic plants. Wang (et al. (2007) also transferred the early flowering gene CONSTANS (CO) from *A.thaliana* into artemisia. Although the flowering time of the CO transgenic plant was about 2 weeks earlier than that of the non-transgenic plant under short day conditions, no significant difference in ART concentration was found between flowering transgenic plants and non-flowering non-transgenic plants. Although these results under laboratory conditions indicated that flowering was not a necessary factor for increasing ART concentration, the field data obtained in West Virginia with genotypes from different origins made that clear (Ferreira et al., 2018). Thus, once elite genotypes are identified for maximum biomass and ART production, the knowledge of their requirements for flowering synchronization and phenological stages is important for their breeding to generate high-ART genotypes.

In addition to flowering, other stages of development are important for the agronomic management of the crop. After fertilization at sowing, at stage 03 and the beginning of stage 04 (Image C, D, E, and F, Fig. 1), when the plants are in the vegetative growth phase, nitrogen fertilizer should be applied, contributing to a greater vegetative development of the plants. On stage 09 (Image L, Fig. 1), the peripheral ray florets open, but they are female flowers with no pollen to provide. Shortly after that, hermaphroditic disc florets open and give the capitulum a yellow tinge. The yellow color is provided by the pollen inside of the transparent anthers of disc florets as these hermaphroditic florets are ready to dehisce (open) and release the pollen into the air (Image M, Fig. 1). Stage 14 (Image Q, Fig. 1) is when the achenes are vitreous (glassy) and is the recommended stage for seed harvest.

Chinese and Vietnamese genotypes of artemisia showed different requirements when exposed to a range of day lengths and temperatures. In an experiment with average temperatures of 37 °C (max) and 19 °C (min), 100% of the Chinese plants flowered approximately 2 weeks (PC 14.7) after being submitted to SD treatments, while the Vietnamese plants behaved differently from the Chinese ones and only a few plants, 27.8%, emitted floral buds approximately 4 weeks (PC 29.3) after being submitted to SD treatments. However, both Chinese and Vietnamese plants remained vegetative under long-day (LD) treatments (Table 2). Due to these observations, the Chinese and Vietnamese genotypes behaved as determinate SDP, qualitative or obligatory for short days. This data confirms earlier reports by Ferreira et al. (1995a), (1995b). Considering that just short photoperiod was not enough for the Vietnamese genotype to get competence to flower, we hypothesized that lower/mild temperatures could help induce flowering. Thus, another experiment with this Vietnamese genotype was set up under 29 °C (max) and 13 °C (min) combining photoperiods with temperatures (Table 3). After approximately five weeks of inductive short photoperiod, 94.44%

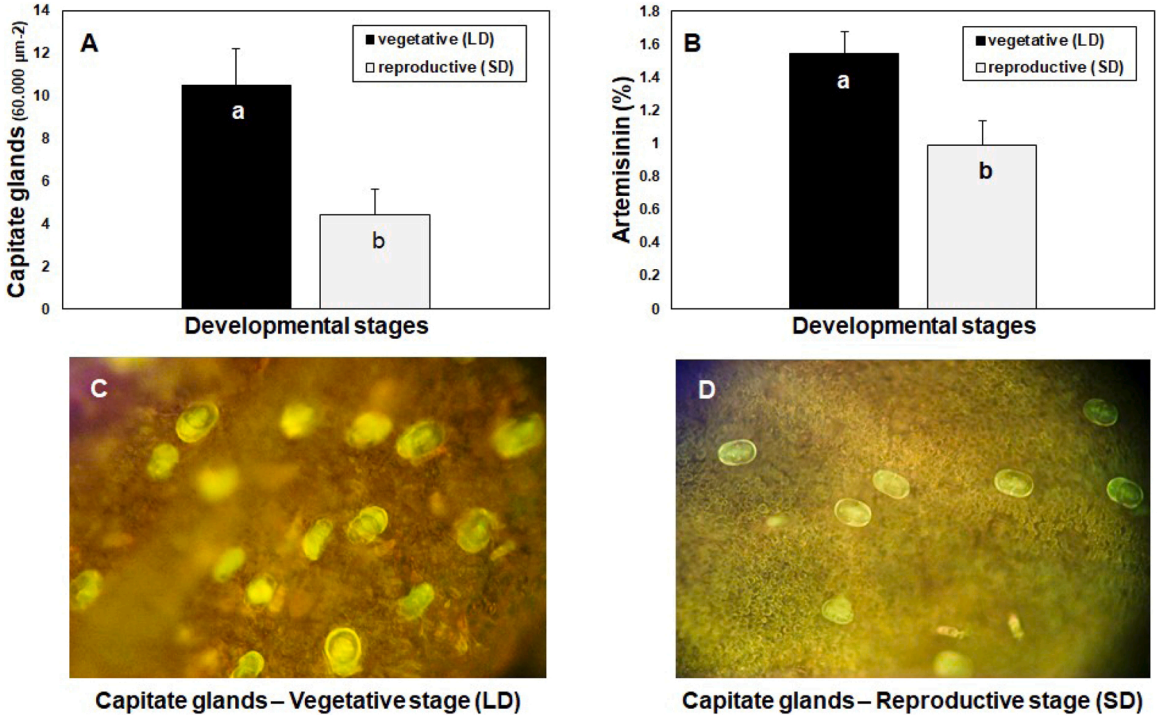


Fig. 5. Effect of short-days – SD (10 h of light) and long-days – LD (16 h of light) on the number of leaf capitate glands (A) and artemisinin concentration (B) of *A. annua* Vietnamese plants cultivated inside growth chambers. Leaf capitate glands in the vegetative stage (C) and reproductive stage (D). Columns marked with the same letter are not significantly different by the 5% Bonferroni test.

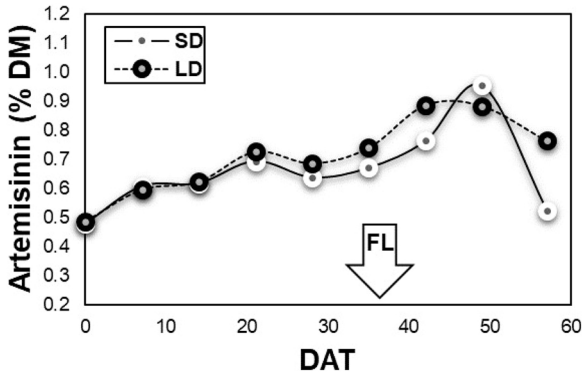


Fig. 6. Effect of short-days – SD (pooled data from treatments with 7, 9, and 11 h of light) and long-days – LD (pooled data from treatments with 13, 15, and 17 h of light) on artemisinin content of *A. annua* Vietnamese plants cultivated inside growth chambers. FL = flower bud emission in SD (36.7 DAT). DAT = days after transplanting.

of the plants bloomed, while plants under LD remained vegetative. For the Vietnamese genotype plants, the critical photoperiod (CP) was between 11 and 13 h (Tables 2 and 3) regardless of the temperatures under which the experiments were performed, while the CP for the Chinese genotype was between 13 and 15 h (Table 2). The inductive photoperiod for this Chinese genotype agrees with the previously established inductive photoperiod of 13.3 h in Indiana, USA (Ferreira et al., 1995a). Considering these results, the Vietnamese genotype is also classified as a determinate SDP, qualitative or obligatory for short days, requiring lower/mild temperatures to reach full flowering. Low temperature-dependent flowering regulation, has been characterized recently and temperature also regulates the Flowering Locus T (FT) in leaves (Song et al., 2013). It is known that FT genes are responsible for the response of plants to photoperiod, and signal the plant to start the reproductive stage, acting as florigens (Liu et al., 2018). Lv et al. (2018) report that short days triggered the induction of gene AaFT2 in artemisia, resulting in flowering, and when they inhibited this gene, flowering was delayed, resulting in a longer growing season.

Flowering time is regulated by many kinds of signaling pathways, including photoperiod, temperature, plant age, gibberellins, shading, and other factors. Ferreira et al. (1995a) pointed out that some *A.annua* plants disseminated by seeds in their experiments, even under an 8 h photoperiod, flowered only after two months, when they outgrew the juvenile stage and reached maturity. Nevertheless, in our experiments we used cuttings of adult plants for both genotypes (Vietnamese and Chinese genotypes), bypassing the juvenility phase. However, they maintained the differences between genotypes for the number of PC required to flower (Table 2). In addition, interestingly, tissue-culture plants that lost their apical dominance (Fig. 3A) did not flower under short photoperiods (Fig. 3B) unless they recovered their apical dominance (Fig. 3B, insert), indicating that the photoinductive signal is perceived by the apical meristem. Also, rooted cuttings from mature plants flowered if propagated under short photoperiods at the end of August/early September in West Virginia (USA) (Fig. 3C) but reverted to vegetative growth if placed under a 16 h photoperiod. This was not reported before and indicates that the flowering induction is reversible by long photoperiod if flowering is not too advanced (Fig. 3D, E).

In the field experiment, the photoinductive cycles (PC) for the Vietnamese artemisia was approximately 28 cycles or days (Fig. 4), very close to the average number of PC found in the photoperiodic chamber experiments (Table 3). The approximate CP for the Vietnamese genotype occurred on Feb 07, which day length was 13.07 h (Fig. 4). This result agrees with data obtained previously under field conditions for the Chinese genotype (CP = 13.3 h) (Ferreira et al., 1995a). Seedlings transplanted to the field between September (1st year), and February (2nd year) PD1 to PD4, flowered in March (2nd year) (Table 4), due to the inductive period having started in February (2nd year), since the CP of 13.07 h was determined on February 7th (2nd year). In turn, PD5 and PD6 were transplanted within the inductive photoperiod (March 2nd year) and had their flowering 28 and 31 d after transplanting,

Table 4
Planting, transplanting, and flowering dates for the Vietnamese genotype of *A. annua* in Pato Branco-PR, Brazil (26°07′ S and 52°41′ W – 760 m altitude).

Month	1st date	2nd date	3rd date	4th date	5th date	6th date
Aug. 1st year	DC 18 *	–	–	–	–	–
Sept. 1st year	TD 23	–	–	–	–	–
Oct.1st year	–	DC 21	–	–	–	–
Nov. 1st year	–	TD 20	DC 25	–	–	–
Dec. 1st year	–	–	–	DC 15	–	–
Jan. 2nd year	–	–	TD 16	–	DC 20	–
Feb. 2nd year	–	–	–	TD 07	–	–
Mar. 2nd year	FL 06	FL 10	FL 19	FL 19	TD 05	–
Apr. 2nd year	–	–	–	–	FL 01	–
May 2nd year	–	–	–	–	–	DC 15
June 2nd year	–	–	–	–	–	–
July 2nd year	–	–	–	–	–	TD 10
Aug. 2nd year	–	–	–	–	–	FL 09

DC = planting date of cuttings; TD = date of transplant to the field; FL = date when 50% + 1% of the plants started the emission of flower buds, and also the date of harvest. *Month day.

respectively (Table 4 and Fig. 4). Under the inductive photoperiod, the number of days from transplanting to flowering (when 50% +1% of the plants started emitting flower buds) is the number of photoinductive cycles (PC). In this experiment, at the end of February and beginning of March of the 2nd year, the plants from the 1st to the 4th planting time (TD1-TD4) began to flower, being the ideal time to transplant the 5th planting time (TD5) under the inductive photoperiod and determine the PC number, which was the number days from transplanting to flowering (28 cycles or days). To find out the approximate critical photoperiod (CP), go back in the calendar 28 days from March 6th, when the 1st planting time (TD1) bloomed, achieving on February 7, whose day length is 13.07 h (this is CP) (Fig. 4). These results indicate the need for approximately 28 photoinductive cycles after CP of 13.07 h (which occur on February 7th), for the transition from the vegetative to the reproductive phase (emission of flower buds, Stage 06, Table 1/Image H, Fig. 1) which will be at the beginning of March, when the plants should be harvested due the ART peak. Regarding the dry mass yield of leaves (LDM), stems (SDM), leaves + stems (TDM), and ART yield for the Vietnamese genotype, the transplanting date (TD) that significantly reached the highest yields was the TD1 (Table 5), due to the longest time of permanence of the plants vegetating in the field before the emission of flower buds (Stage 06, Table 1/Image H, Fig. 1), 165 d from transplanting to harvest, September 23rd to March 06th, respectively. The plants transplanted on November 20th, January 1st, and February 07th, respectively TD2, TD3, and TD4, ended up flowering in March (late summer), along with those of the 1st planting time (TD1), and consequently, they remained less time vegetating in the field and presented lower phytomass and ART yields. On the other hand, plants from TD5 and TD6 were 4–5 weeks under inductive photoperiod in the field, flowered quickly, and presented the lowest yields of the entire experiment (Table 4). These results determine that the transplanting of seedlings to the field for the coordinates 26°07′S and 52°41′W should be done primarily in the last third of September or in early October (early spring), to achieve a higher yield of leaves dry mass and ART. Planting in the first two-thirds of September is not recommended due to the risk of frost damage. Plants from the TD6, transplanted on July 10th and harvested on August 09th had leaf damage due to the frosts that occurred at that time.

Yet, in the field experiment, (Table 5), interestingly, ART concentrations reached mid-levels between the first transplant date (TD1) and the last (TD6), respectively, 165 DAT and 31 DAT. ART concentration was highly correlated with DAT ($r = 0.87$; $p \leq 0.077$). Plants transplanted to the field very close to the inductive photoperiod had a short vegetative period, developing few fully developed leaves and presenting low biomass, while those that remained vegetating in the field for a longer period under long days developed a greater number of fully developed leaves and accumulated greater biomass than plants transplanted close to the inductive photoperiod. A possible explanation for a higher concentration of ART in plants that vegetated for a longer period is the greater number of mature or fully developed leaves, which in turn had a higher density of glandular trichomes than young leaves. Results observed by us in an experiment conducted in a growth chamber reinforce this argument, where we found the highest number of glandular trichomes (Fig. 5-A) and the highest ART concentrations (Fig. 5-B) in plants in the vegetative stage growing under long days, in comparison to those grown under short days, under inductive photoperiods. In general, the density of glandular secretory trichomes in artemisia is often positively correlated with ART content (Xiao et al., 2016; Yan et al., 2017).

This study used a description of the developmental steps of artemisia to introduce a detailed phenological scale based on visual observations. The identification of these phases is meant to facilitate the management and implementation of practices for this species, such as fertilization, crossing, leaf harvest for ART/essential oil extraction, and seed harvest. When hermaphroditic florets reach anthesis, they release pollen at a different time than when stigmas are receptive, and that mechanism probably evolved to foster cross-pollination over selfing. Thus, the establishment of the local day length required for flowering and the knowledge of flower phenological stages are very important for the synchronization of flowering and the crossing of high-ART genotypes that would naturally flower at different times if not synchronized by short photoperiods. Our results and our previous experience selecting desired genotypes, strongly suggest that maintenance of desired clones should be done in a greenhouse and not under tissue culture conditions, which may affect inductive photoperiod perception. Regarding the ART

Table 5
Yield of dry phytomass and artemisinin % (g/100 g DM), leaf/stem ratio, and artemisinin concentration of *A. annua*'s Vietnamese genotype, under different transplanting dates: TD1 (165 DAT); TD2 (111 DAT); TD3 (63 DAT); TD4 (41 DAT); TD5 (28 DAT); TD6 (31 DAT).

Transplant date	[1]DAT	----------Artemisinin ----------	[2]DM leaves	DM stems	DM total	[2] leaf/stem ratio
		% DM	---------- Kg ha^{-1} ----------			
Sep. 23	165	0.99 ± 0.02 A	30.38 ± 5.1 A	3,045.1 ± 509.12 A	14,974.9 ± 3042.00 A	18,020.0 ± 4010.83 A	0.21 ± 0.02 B
Nov. 20	111	1.05 ± 0.05 A	17.20 ± 2.18 B	1,643.9 ± 243.55 B	5,383.2 ± 1141.88 B	7,027.10 ± 1548.46 B	0.32 ± 0.04 B
Jan. 16	63	0.81 ± 0.05 B	4.55 ± 0.45 C	561.3 ± 73.61 C	864.8 ± 134.69 C	1,426.10 ± 228.96 C	0.67 ± 0.03 B
Feb. 07	41	0.53 ± 0.02 C	0.87 ± 0.16 C	161.5 ± 26.17 C	186.9 ± 27.93 C	348.40 ± 57.68 C	0.87 ± 0.05 B
Mar. 05	28	0.50 ± 0.11 C	0.10 ± 0.01 C	20.9 ± 5.34 C	32.1 ± 8.89 C	71.70 ± 21.67 C	1.46 ± 0.24 A
July 10	31	0.55 ± 0.05 C	0.22 ± 0.05 C	39.6 ±11.60 C	14.6 ± 2.72 C	35.57 ± 5.33 C	1.35 ± 0.29 A

[1]DAT = days after transplantation; [2]DM = dry mass; ** Values followed by the same capital letter in the column do not differ significantly from each other by the Bonferroni test at 5%.

peak, our results differ from many earlier reports that flowering coincides with ART peak levels but agree with a previous report that photoperiod (e.g., early September in West Virginia, USA) may be the main factor that dictates ART peak in *Artemisia annua*. We have also demonstrated that flowering induction in artemisia genotypes from different geographic origins varies under the same photoperiod and temperature conditions and may require both photoperiodic and temperature induction to achieve successfully synchronized flowering. In addition, we showed that among the genotypes of the artemisia species, classified as determinate short-day plants - SDP, qualitative or obligatory for short days, the Vietnamese genotype also required low temperatures (quantitative or facultative) to reach full bloom, and that flowering under short days can be reversed if plants are exposed to long photoperiod early enough. Finally, we observed that plants growing on long days, in the vegetative phase, have higher densities of glandular trichomes and higher concentrations of ART when compared to plants grown under short days and induced to flower, suggesting that the further from the inductive photoperiod the plants are transplanted to the field, the higher the content and yield of ART.

5. Conclusions

i) This study has established a detailed *A.annua* phenological scale based on daily observations and photographs of the plant at specific time intervals and was divided into two distinct phases: vegetative phase (with five stages) and reproductive phase (with 12 stages); ii) Flowering induction in *A.annua* genotypes from different geographic origins varies under the same photoperiod and temperature conditions and may require both photoperiodic and low-temperature induction to achieve successfully synchronized flowering. Chinese genotype of *A.annua* requires only short days to flower while the Vietnamese genotype requires short days with the addition of low/moderate temperatures to reach full flowering; iii) Chinese *A.annua* plants flowering under short days can be reversed to the vegetative stage if plants are exposed to long photoperiod early enough; iv) Regarding the peak of ART, our results indicate that the photoperiod may be the main factor that dictates ART peak in *A. annua*; v) The Vietnamese genotype needs approximately 28 photoinductive cycles under 13.07 h or less for the transition from the vegetative to the reproductive phase (emission of flower buds); and vi) *A.annua* plants growing on long days, in the vegetative phase, have higher densities of glandular trichomes and higher concentrations of ART when compared to plants grown on short days.

Funding

These studies were supported by grants from National Council for Scientific and Technological Development (CNPq, Brazil) under projects 320.0364/2004–9 and #481.146/2010–5 and Fundação Araucária de Apoio ao Desenvolvimento Científico e Tecnológico do Paraná under project #14991. LVD and MFFR received master's, and doctoral scholarships from Coordination for the Improvement of Higher Education Personnel (CAPES, Brazil).

CRediT authorship contribution statement

José Abramo Marchese: Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Funding acquisition. **Jorge F.S. Ferreira:** Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Funding acquisition. **Rita Maria Moraes:** Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Funding acquisition. **Franck E. Dayan:** Conceptualization, Methodology, Investigation, Data curation, Formal analysis, Funding acquisition. **Lucas Vinicius Dallacorte**: editing and revision, making of graphs, and figures. **Michelle F.F. Rodrigues**: editing and revision, making of graphs, and figures. **Jorge Jamhour**: editing and revision, making of graphs, and figures. José Abramo Marchese and Jorge F.S. Ferreira contributed intellectually, in an equivalent way, in the discussion and writing of the article.

Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. The authors declare that this study received funding from The University of Mississippi (National Center for Natural Products Research), the United States Department of Agriculture (Natural Products Utilization Research Unit), and the Federal University of Technology – Paraná. The funders were not involved in the study design, collection, analysis, interpretation of data, the writing of this article, or the decision to submit it for publication.

Data Availability

The data that has been used is confidential.

Acknowledgments

The authors are in debt to Xavier Simonet (Mediplant, Switzerland), Pedro Magalhães (CPQBA/UNICAMP), and Kevin Mak (China) for supplying the seeds of *A. annua*. We thank Mauricio Capelezzo and Daniele Galvan for the preparation and organization of artemisia's photos. We are also grateful for the financial and infrastructure support provided by The University of Mississippi (National Center for Natural Products Research), the United States Department of Agriculture (Natural Products Utilization Research Unit), and the Federal University of Technology – Paraná.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.indcrop.2022.116118.

References

Acton, N., Klayman, D.L., Rollman, I.J., 1985. Reductive electrochemical HPLC assay for artemisinin (qinghaosu). Planta Med 51, 445–446.

Alam, P., Albalawi, T.H., 2020. 2020. In vitro alteration of artemisinin biosynthesis in *Artemisia annua* L during treatment with methyl jasmonate. Trop. J. Pharm. Res. 19, 33–37.

Archontoulis, S.V., Struik, P.C., Vos, J., Danalatos, N.G., 2010. Phenological growth stages of *Cynara cardunculus*: codification and description according to the BBCH scale. Ann. Appl. Biol. 156, 253–270.

Baraldi, R., Isacchi, B., Predieri, S., Marconi, G., Vincieri, F.F., Bilia, A.R., 2008. Distribution of artemisinin and bioactive flavonoids from *Artemisia annua* L. during plant growth. Biochem. Syst. 36, 340–348.

Brisibe, E.A., Udensi, O., Chukwurah, P.N., de Magalhães, P.M., Figueira, G.M., Ferreira, J.F.S., 2012. Adaptation and agronomic performance of *Artemisia annua* L. under lowland humid tropical conditions. Ind. Crops Prod. 39, 190–197.

Chan, K., Teo, C., Jinadasa, S., Yuen, K., 1995. Selection of high artemisinin yielding *Artemisia annua*. Planta Med. 61, 285–287.

Efferth, T., 2005. Mechanistic perspectives for 1, 2, 4-trioxanes in anti-cancer therapy. Drug Resist. Upd 8, 85–97.

Ferreira, J., Simon, J., Janick, J., 1995a. Developmental studies of *Artemisia annua*: flowering and Artemisinin production under greenhouse and field conditions. Planta Med. 61, 167–170. https://doi.org/10.1055/s-2006-958040.

Ferreira, J., Simon, J., Janick, J., 1995b. Relationship of Artemisinin content of tissue-cultured, greenhouse-grown, and field-grown plants of *Artemisia annua*. Planta Med. 61, 351–355. https://doi.org/10.1055/s-2006-958098.

Ferreira, J.F.S., Janick, J., 1996. Distribution of artemisinin in *Artemisia annua*. Prog. N. Crops 579–584.

Ferreira, J.F.S., Laughlin, J.C., Delabays, N., Magalhães, P.M., 1994. Cultivation and genetics of *Artemisia annua* L. for increased production of the antimalarial artemisinin. Plant Genet. Resour. 3, 206–229.

Ferreira, J.F.S., Laughlin, J.C., Delabays, N., Magalhães, P.M., 2005. Cultivation and genetics of *Artemisia annua* L. for increased production of the antimalarial artemisinin. Plant Genet. Resour. 3, 206–229.

Ferreira, J.F.S., Benedito, V.A., Sandhu, D., Marchese, J.A., Liu, S., 2018. Seasonal and differential sesquiterpene accumulation in *Artemisia annua* suggest selection based on both artemisinin and dihydroartemisinic acid may increase artemisinin in planta. Front. Plant Sci. 9, 1096.

Ferrer-Blanco, C., Hormaza, J.I., Lora, J., 2022. Phenological growth stages of "pawpaw"[*Asimina triloba* (L.) Dunal, Annonaceae] according to the BBCH scale. Sci. Hortic. 295, 110853.

Gupta, S.K., Singh, P., Bajpai, P., Ram, G., Singh, D., Gupta, M.M., Jain, D.C., Khanuja, S. P., Kumar, S., 2002. Morphogenetic variation for artemisinin and volatile oil in *Artemisia annua*. Industrial Crops and Products 16, 217–224. https://doi.org/10.1016/S0926-6690(02)00049-3.

Hopp, R.J., 1974. In: Phenology and Seasonality Modeling. Phenology and Seasonality Modeling. Springer, Berlin, Heidelberg, pp. 25–43.

Zhao, S., Zeng, M.Y., 1986. Application of precolumn reaction to high-performance in liquid chromatography of qinghaosu in animal plasma. Analytic. Chem. 58, 289–292.

Ivanescu, B., Corciova, A., 2014. Artemisinin in cancer therapy. *Artemisia annua* -. Pharmacology and Biotechnology 9783642410, 205–227. https://doi.org/10.1007/978-3-642-41027-7_12.

Jain, D.C., Mathur, A.K., Gupta, M.M., Singh, A.K., Verma, R.K., Gupta, A.P., Kumar, S., 1996. Isolation of high artemisinin-yielding clones of *Artemisia annua*. Phytochemistry 43, 993–1001. https://doi.org/10.1016/S0031-9422(96)00369-X.

Jeremić, J.S., Godevac, D., Ivanovic, S., Simić, K., Trendafilova, A., Aćimović, M., Vljević, S.M., 2022. HPTLC-based metabolomics for the investigation of metabolic changes during plant development: The case study of *Artemisia annua*. J. Serb. Chem. Soc 87, 1–8. https://doi.org/10.2298/JSC210507007S.

Li, Y., Kong, D., Fu, Y., Sussman, M.R., Wu, H., 2020. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. Plant Physiol. Biochem. 148, 80–89. https://doi.org/10.1016/j.plaphy.2020.01.006.

Liersch, R., Soicke, H., Stehr, C., Tüllner, H.-U., 1986. Formation of artemisinin in *Artemisia annua* during one vegetation period. Planta Med 52, 287–390.

Lieth, H., 2013. Phenology and Seasonality Modeling. Springer Science & Business Media.

Liu, W., Jiang, B., Ma, L., Zhang, S., Zhai, H., Xu, X., 2018. Functional diversification of Flowering Locus T homologs in soybean: GmFT1a and GmFT2a/5a have opposite roles in controlling flowering and maturation. N. Phytol. 217, 1335–1345.

Lv, Z., Zhang, L., Chen, L., Zhang, F., Tang, K., 2018. The *Artemisia annua* FLOWERING LOCUS T Homolog 2, AaFT2, is a key regulator of flowering time. Plant Physiol. Biochem. 126, 197–200.

Magalhães, P.M., de, Delabays, N., Sartoratto, A., 1997. New hybrid lines of the antimalarial species *Artemisia annua* L. guarantee its growth in Brazil. Ciênc. cult. (São Paulo) 413–5.

Marchese, J.A., Rehder, V.L.G., Sartorato, A., 2001a. A comparison of thin layer chromatography and high performance liquid chromatography for artemisinin analyses Revis. Plantas Med. 4, 81–87.

Marchese, J.A., Rehder, V.L.G., Sartoratto, A., 2001b. Quantificação de artemisinina em *Artemisia annua* L.–Uma comparação entre as técnicas de cromatografia em camada delgada com detecção densiométrica e cromatografia líquida de alta eficiência com detecção no ultravioleta. Rev. Bras. De. Plantas Med. 4, 81–87.

Marchese, J.A., Broetto, F., Ming, L.C., Ducatti, C., Rodella, R.A., Ventrella, M.C., 2005a. Carbon isotope composition and leaf anatomy as a tool to characterize the photosynthetic mechanism of *Artemisia annua* L. Braz. J. Plant Physiol. 17, 187–190.

Marchese, J.A., Broetto, F., Ming, L.C., Rehder, V.L.G., et al., 2005b. External application of artemisinin in the induction of the flowering in *Artemisia annua* L. Revis. Plantas Med. 7, 12–16.

Mondal, A., Chatterji, U., 2015. Artemisinin represses telomerase subunits and induces apoptosis in HPV-39 infected human cervical cancer cells. J. Cell. Biochem. 116, 1968–1981. https://doi.org/10.1002/jcb.25152.

Morales, M.R., Charles, D.J., Simon, J.E., 1993. Seasonal accumulation of artemisinin in *Artemisia annua* L. Acta Hortic. 344, 416–420.

Nair, M.S., Huang, Y., Fidock, D.A., Polyak, S.J., Wagoner, J., Towler, M.J., 2021. *Artemisia annua* L. extracts inhibit the in vitro replication of SARS-CoV-2 and two of its variants. J. Ethnopharmacol. 274.

Nazarpour, M.R., Yadegari, M., 2021. Ecophytochemical survey in three species of *Artemisia* L. Afected by different climate and phenological stages in Khuzestan Province. Eco-Phytochem. J. Med. Plants 9, 71–90. https://doi.org/10.30495/ejmp.2021.694470.

Posner, G.H., Ploypradith, P., Parker, M.H., O'Dowd, H., Woo, S.-H., Northrop, J., 1999. Antimalarial, antiproliferative, and antitumor activities of artemisinin-derived, chemically robust, trioxane dimers. J. Med. Chem. 42, 4275–4280.

Pras, N., Visser, J.F., Batterman, S., Woerdenbag, H.J., Malingré, T.M., Lugt, C.B., 1991. Laboratory selection of *Artemisia annua* L. for high artemisinin yielding types. Phytochem. Anal. 2, 80–83.

Rana, V., Abirami, S., Blázquez, M.A., Maiti, S., 2013. Essential oil composition of *Artemisia annua* L. at different growth stages. Journal os Spices and Aromatic Crops 22, 181–187.

Ranjbar, M., Naghavi, M.R., Alizadeh, H., Soltanloo, H., 2015. Expression of artemisinin biosynthesis genes in eight *Artemisia* species at three developmental stages. Industrial Crops and Products 76, 836–843. https://doi.org/10.1016/j.indcrop.2015.07.077.

Rodrigues, M.F.F., Sousa, I.M.O., Vardanega, R., Nogueira, G.C., Meireles, M.A.A., Foglio, M.A., Marchese, J.A., 2019. Techno-economic evaluation of artemisinin extraction from *Artemisia annua* L. using supercritical carbon dioxide. Ind. Crops Prod. 132, 336–343. https://doi.org/10.1016/j.indcrop.2019.02.049.

Singh, A., Vishwakarma, R., Husain, A., 1988. Evalutation of *Artemisia annua* strains for higher artemisinin production. Planta Med. 54, 475–476.

Singh, N.P., Lai, H.C., 2004. Artemisinin induces apoptosis in human cancer cells. Anticancer Res. 24, 2277–2280.

Song, Y.H., Ito, S., Imaizumi, T., 2013. Flowering time regulation: photoperiod- and temperature-sensing in leaves. Trends Plant Sci. 18, 575–583.

Soni, R., Shankar, G., Mukhopadhyay, P., Gupta, V., 2022. A concise review on *Artemisia annua* L.: a major source of diverse medicinal compounds. Ind. Crops Prod. 184, 115072 https://doi.org/10.1016/j.indcrop.2022.115072.

Towler, M.J., Weathers, P.J., 2015. Variations in key artemisinic and other metabolites throughout plant development in *Artemisia annua* L. for potential therapeutic use. Ind. Crops Prod. 67, 185–191.

Wang, H., Ge, L., Ye, H.-C., Chong, Liy, B.-Y., Li, G.-F., 2004. Studies on the effects of fpf1 gene on *Artemisia annua* flowering time and on the linkage between flowering and artemisinin biosynthesis. Planta Med. 70, 347–352.

Wang, H., Liu, Y., Chong, K., Liu, B.Y., Ye, H.C., Li, Z.Q., 2007. Earlier flowering induced by over-expression of CO gene does not accompany increase of artemisinin biosynthesis in *Artemisia annua*. Plant Biol. 9, 442–446.

Wetzstein, H.Y., Porter, J.A., Janick, J., Ferreira, J.F.S., 2014. Flower morphology and floral sequence in *Artemisia annua* (Asteraceae) 1. Americ. J. Bot. 101, 875–885.

WHO, 2006. WHO monograph on good agricultural and collection practices (GACP) for *Artemisia annua* L. WHO Library Cataloguing-in-Publication Data, Switzerland.

Woerdenbag, H., Pras, N., Chan, N., Bang, B., Bos, R., van Uden, W., 1994. Artemisinin, related sesquiterpenes, and essential oil in *Artemisia annua* during a vegetation period in Vietnam. Planta Med. 60, 272–275.

Woerdenbag, H.J., Pras, N., Bos, R., Visser, J.F., Hendriks, H., Malingré, T.M., 1991. Analysis of artemisinin and related sesquiterpenoids from *Artemisia annua* L. by combined gas chromatography/mass spectrometry. Phytochem. Anal. 2, 215–219.

Xiao, L., Tan L., Z., H., 2016. *Artemisia annua* glandular secretory trichomes: the biofactory of antimalarial agent artemisinin. Sci. Bull. 61, 26–36.

Yan, T., Chen, M., Shen, Q., Li, L., Fu, X., Pan, Q., 2017. HOMEODOMAIN PROTEIN 1 is required for jasmonate-mediated glandular trichome initiation in *Artemisia annua*. N. Phytol. 213, 1145–1155.

Zhang, L., Ye, H.-C., Li, G.-F., 2006. Effect of development stage on the artemisinin content and the sequence characterized amplified region (scar) marker of high-artemisinin yielding strains of *Artemisia annua* L. J. Integr. Plant Biol. 48, 1054–1062.