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IDENTIFICATION OF THE PHENOLOGICAL GROWTH STAGES OF *RHODOMYRTUS TOMENTOSA* VAR. *TOMENTOSA* USING THE BIOLOGISCHE BUNDESANSTALT, BUNDESSORTENAMT AND CHEMICAL INDUSTRY (BBCH) SCALE

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Rhodomyrtus tomentosa var. *tomentosa* is a multipurpose flowering shrub in the Myrtaceae family. Naturally, this species thrives in the secondary forest areas and is cultivated as a garden plant due to its attractive flowers. The Biologische Bundesanstalt, Bundessortenamt and Chemical Industry (BBCH) scale was used to identify the phenological growth stages of *R. tomentosa.* A phenological observation on the organs of the plants was made at the Kepong Botanic Gardens, Forest Research Institute Malaysia, Kepong, Selangor. Branches with various developing plant organs were tagged to identify the principal and secondary growth stages and were photographed using digital camera. The observation was carried out daily for five months. Eight principal growth stages were recognised for *R. tomentosa*, namely, bud and leaf development, shoot elongation, flower emergence, flowering, fruit development, fruit maturity or ripening, and senescence beginning of dormancy. These principal stages were described and supported with images and growth duration. The developed BBCH scale for *R. tomentosa* will be a useful guide in improving landscape management and provide valuable information for scientific communications.

Keywords: Rose myrtle, Myrtaceae, ornamental plant, growth period, urban landscape

INTRODUCTION

Rhodomyrtus tomentosa var. *tomentosa* (Myrtaceae) is an attractive flowering shrub endemic to Malaysia, Indonesia, the Philippines, India, southern China and Japan. This species is best-known as rose myrtle or *kemunting* by the locals. It grows up to 2–4 m tall and thrives in sandy soil of degraded open areas, riversides and shorelines (Latiff 1992, Xie et al. 2021). The presence of this species can be associated with the lower soil pH of the area (Wei et al. 2009). In Malaysia, *R. tomentosa* is also grown as a garden plant due to its attractive flowers.

This species produces distinctive five-petalled flowers that measure about 2.5–3.0 cm in diameter, are purplish pink in colour, and grow in solitary or clusters of two or three. Nectar-feeding insects are attracted to the blossom, which adds greater landscaping value. It produces edible ellipsoid berries with a persistent calyx (Vo & Ngo 2019). The green skin turns purple when the fruit is ripe. The sweet, juicy and aromatic purple pulp of the ripe fruits can be consumed fresh or used to make pies and jams (Latiff 1992). The fruits contain numerous deltoid seeds, which are 1.5 mm in diameter (Yang et al. 2010) and dispersed by fruit-eating birds and ants (Wei et al. 2004). This shrub can be easily propagated through stem cuttings, which is a faster propagation technique compared with seed germination (Wong 2008).

Rhodomyrtus tomentosa has traditionally been utilised to treat various discomforts (Ong & Nordiana 1999, Do 2001, Lim 2012). The leaves, roots, and fruits are used to alleviate digestive disorders and its decoction can be used externally to wash and clean wounds (Parnell & Chantaranothai 2002). It also has significant antibacterial activities (Salni et al. 2002, Voravuthikunchai et al. 2007, Limsuwan et al. 2009) and is effective as an anti-biofilm agent, antifungal, antioxidant, antidiarrheal, and osteogenic (Jeenkeawpieam et al. 2012, Jeong et al. 2013, Wu et al. 2015).

Phenology is a study of changes in the timing of recurring life-cycle stages, driven by environmental forces, and how interacting species respond to changes in timing within an ecosystem (Lieth 1974, Liang 2019). It examines the changes in the timing of seasonal events, for instance leaf bud emergence, flowering, fruiting and dormancy that are closely associated with temperature or day length. The interval between growth phases is known as 'phenological stages', describing the association between the developmental changes of plants and the environment, such as the occurrence of rainfall or temperature changes. Information on the phenological growth stages of valuable plants is vital for proper management to improve growth and flowering potential besides the observation of flora–fauna relationship. Phenological changes will have cascading effects on dependent herbivores, nectarivores, and frugivores within ecosystems (Butt et al. 2015, Sheldon 2019). For instance, the pattern of advanced *Vaccinium macrocarpon* flowering over time coupled with increased temperature has implications on the relationship between the plant and its insect associate, *Lycaena epixanthe* (Ellwood et al. 2013). Thus, by knowing the phenological growth stages of certain plant species, the occurrences of specific faunas are predictable.

The Biologische Bundesanstalt, Bundessortenamt and Chemical Industry (BBCH) scale has been extensively used to describe various crops (Böttcher et al. 2016, Sosa-Zuniga et al. 2017, Feldmann & Rutikanga 2021). However, only a few phenological growth scales have been documented for landscape plants, such as *Rosa* sp. (Meier et al. 2009), *Xanthostemon chrysanthus* (Ahmad Nazarudin et al. 2012), *Acca sellowiana* (Ramírez & Kallarackal 2018), and *Sapindus mukorossi* (Zhao et al. 2019). Recently, the growth pattern of *R. tomentosa* has been documented, representing various altitude gradients in the Silent Valley National Park, Kerala, India (Adarsh et al. 2018). Therefore, our study aimed to add detailed information on the growth and development of *R. tomentosa* by developing the BBCH scale and the duration of each growth stage of the species for better documentation and future investigation.

MATERIALS AND METHODS

Study location and plant materials

Seven existing *R. tomentosa* var. *tomentosa* plants growing in Kepong Botanic Gardens, Forest Research Institute Malaysia (FRIM), Kepong, Selangor, Malaysia (3° 13' N, 101° 37' E) were monitored for identification of growth stages. These shrubs were approximately seven years old and had flowered in previous years. The average height and canopy diameter of the plants were 1.5–2.5 m and 2.0–2.5 m respectively. The study site has an average daily temperature of 27 °C, annual rainfall of 2500 mm, and approximately 76% humidity. The soil is categorised as fine to medium sandy clay.

At the beginning of the study, 20 branches with various developing plant organs were tagged for each shrub. The organs were observed from 8:00 to 11:00 am daily from March till July 2021 to identify principal and secondary growth stages and were photographed using digital camera. Fruit nets were used to protect tagged fruits from birds and insects.

Climatological data, daily rainfall and temperature were obtained from the nearest weather station of the Malaysian Meteorological Department located at FRIM. The weekly rainfall and mean weekly temperature during the study are shown in Figure 1.

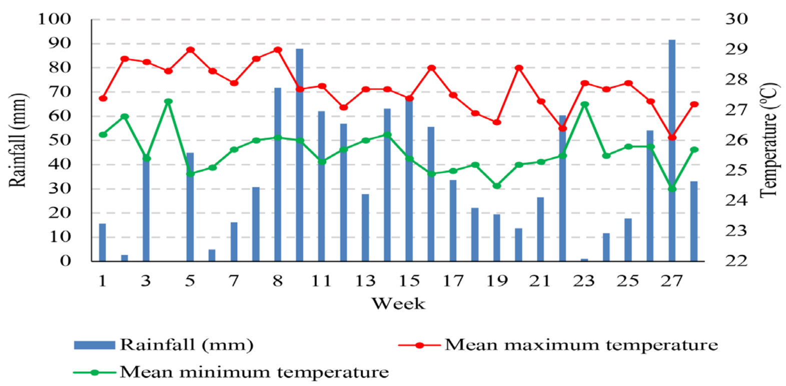

Figure 1 Weekly rainfall and mean weekly temperature of the study site (February–August 2022)

The BBCH scale

The coding of phenological growth stages of *R. tomentosa* were determined and interpreted using the BBCH scale (Meier 2001). The scale was segmented into 10 identifiable developmental phases numbered from 0 to 9, representing the 10 principal growth stages, and each stage was in turn subdivided in 10 secondary growth stages also designated 0 to 9. The secondary growth stages correspond to the growth progress within each principal growth stage and are described with ordinal numbers or percentage values. The combination of both principal and secondary growth stages provides a two-digit numerical code. In some cases, a diagonal stroke was applied if there were two developmental stages occurring concomitantly, e.g. the concurrent development of leaf and flower emergence. The duration (days) of each identified principal growth stage of *R. tomentosa* was also determined.

RESULTS

Principal growth stages

In total, eight principal growth stages were exhibited by *R. tomentosa*. Four principal growth stages were recorded for the vegetative phase, and four stages were observed in the reproductive phase (Table 1). However, the other two principal growth stages, namely, stage 2 (formation of side shoot) and stage 4 (development of harvestable vegetative plant parts) are not included because these stages are not applicable to *R. tomentosa*.

Stage 0 (bud development)

For this stage, the BBCH scale describes the morphology of the bud. The traits considered included the stage of bud swelling and the appearance of green scales and leaf tips. For instance, BBCH code 01 was assigned when the bud began to swell (Table 1, Figure 2a). Later, the green leaf tips became visible, indicating the end of the bud development (BBCH code 09, Table 1, Figure 2b). Bud development (stage 0) in *R. tomentosa* took two weeks to complete, which was about 7.69% of the whole duration of the vegetative stage (Table 2).

Stage 1 (leaf development)

For leaf development, the code for secondary growth refers to the leaf colour intensity and percentage of leaf size compared with the size of mature leaves. Stage 1 started when the first leaves separated and continued with leaves unfolding (Table 1, Figure 2c). BBCH code 13 was assigned when the observed leaf reached 30% of size compared with mature leaves (Table 1, Figure 2d). The leathery, oblong leaves were oppositely arranged with three distinct longitudinal main veins. The light green young leaves gradually turned to darker green towards maturity. At stage 15, the second pair of leaves was unfolding when the size of the first pair of leaves was about 50% compared with the size of mature leaf (Table 1, Figure 2e). BBCH code 19 was given when the first pair of leaves was about 90% of the final size, with a darker green shade. A pair of *R. tomentosa* leaves took 18 days to fully expand and mature (Table 2). The length and width of the mature leaves measured 5–6 and 2–3 cm respectively.

Stage 3 (shoot elongation)

The continuous production of new pairs of leaves and increase in internode number led to shoot elongation (stage 3). As the shoot elongated, axillary bud above the leaf petiole often emerged concomitantly (Figure 2f), forming the side shoot (stage 2). Shoot elongation (stage 3) was considered complete when it had stopped expanding and no more internodes developed. The length of elongated shoot compared with the final shoot length was taken into account when identifying secondary growth stages of stage 3. For instance, if the shoot had developed about 80% of its final length, BBCH code 38 was assigned (Table 1, Figure 2g). The shoot elongation progress was sometimes parallel to the development of lateral shoot (stage 2). However, stage 2 was excluded because the study only considered the primary shoot development. The shoot took 5 months to fully elongate (Table 2).

Stage 5 (flower emergence)

Stage 5 was based on the morphological changes in the plant organs. The increase in flower bud

Table 1 Description of the principal growth stages of *Rhodomyrtus tomentosa* according to the BBCH scale

BBCH code	Description
Principal growth stage 0: Bud development	
00	Bud dormancy
01	Beginning of leaf bud swelling (Figure 2a)
03	End of leaf bud swelling, green scales visible
05	50% of the green scales separated
08	Green scales fully separated
09	Green leaf tips visible (Figure 2b)
Principal growth stage 1: Leaf development	
10	First leaves separating
11	Visible leaves unfolded, leaves are light green (Figure 2c)
12	Initial increase in size and leaf colour intensity
13	Leaf about 30% of final size (Figure 2d)
15	Leaf about 50% of final size, second pair of leaves unfold (Figure 2e)
16	Leaf about 60% of final size, more leaves visible
19	Leaves fully expanded and darker green
Principal growth stage 3: Shoot elongation	
31	Beginning of shoot elongation
32	Shoot length about 20% of final length
33	Shoot length about 30% of final length (Figure 2f)
35	Shoot length about 50% of final length
38	Shoot length about 80% of final length (Figure 2g)
39	Shoot length about 90% of final length
Principal growth stage 5: Flower emergence	
51	Beginning of flower bud swelling
52	Flower bud with sepals visible (Figure 2h)
53	Flower bud about 30% of final size
55	Flower bud about 50% of final size
58	Flower bud about 80% of final size, pinkish petals visible (Figure 2i)
59	Flower bud about 90% of final size but still closed, pink petals visible (Figure 2j)
Principal growth stage 6: Flowering	
60	First flower bud opening, beginning of petals separation
61	10% of the first flower bud open, petal separation and elongation, stigma and stamens visible
64	40% of the first flower open (Figure 2k)
68	80% of the first flower open
69	More than 90% of the first flower open, petal colour changed from pink to white, end of flowering, beginning of fruit growth (Figure 2l and 1m)
Principal growth stage 7: Fruit development	
70	Fruit set; petals, stigma, and stamens dried and fell off (Figure 2n)
72	Fruit about 20% of final size
73	Fruit about 30% of final size (Figure 2o)
75	Fruit about 50% of final size
78	Fruit about 80% of final size (Figure 2p)
79	Fruit about 90% of final size, fruit skin green in colour
Principal growth stage 8: Fruit maturity or ripening	
81	Beginning of fruit ripening or coloration (Figure 2qA)
83	Fruit skin turned to light purple (Figure 2qB)
87	Velvety skin with darker colour (Figure 2r)
88	Fruit skin dark purple and shiny (Figure 2s)
89	Fruit fully mature and ripe, fruit dropped due to abscission of fruit pedicel
Principal growth stage 9: Senescence, beginning of dormancy	
91	Shoot growth completed, foliage fully green
92	Leaf colour changed from green to yellowish, beginning of senescence
95	50% of the leaf turned to yellow (Figure 2t)
97	Abscission of the old leaves, beginning of dormancy

Figure 2 Phenological development stages of *Rhodomyrtus tomentosa* according to the BBCH scale

size and separation of sepals were key attributes monitored. The final size of the flower bud was approximately 0.8–1.0 cm. *Rhodomyrtus tomentosa* frequently showed synchronised growth between the emergence of flower and leaf development. Therefore, this point was described using a diagonal stroke. For instance, when the leaf size was about 40% compared with the mature leaves, and the flower bud with sepals was visible, the suitable BBCH code was 14/52 (Table 1, Figure 2h). In stage 5, BBCH code 58 referred to the continuous emergence of flower bud with 80% separation of sepals and the pinkish petals were visible (Table 1, Figure 2i). BBCH code 59 indicated a brighter colour of petal which evidenced when the flower bud reached 90% of its final size (Table 1, Figure 2j). The total time required for the species to complete stage 5 was about 29 days or 28.43% of the total reproductive timeline (Table 2).

Stage 6 (flowering)

As the flower bud increased in size, the pink petals of the flower became more visible, indicating the transition of flower bud emergence to flowering (stage 6). The BBCH scale considers the percentage of open flowers of the main shoot, from the first open flower bud (60) until the end of flowering (69), instead of percentage of opening of each flower. Petal discoloration was also used as indicator of flower maturity. The changes in the morphological appearance and the colour of the flower were noticeable throughout the blooming period. On the first day, about 40% of the first flower opened, while the petals elongated (Figure 2k), showing their filaments and styles. The pink petals gradually opened and the style became erect as the flower bloomed (Table 1, Figure 2l). The diameter of the full bloom measured approximately 2.5–3.0 cm. The petals turned white, whereas the stamens and style appeared pale pink on the second day (Table 1, Figure 2m). Subsequently, the flower deteriorated, and the petals, style, and stamens dried and fell off, marking senescence of the flower (Table 1, Figure 2n). A period of 43 days (42.16% of the reproductive timeline) was required for the flowering period of *R. tomentosa*, starting from flower bud emergence through the stage when the petals changed from pink to white.

Stage 7 (fruit development)

Stage 7 started when the petals, style, and stamens dried and fell off (Table 1, Figure 2n). Codes for the secondary growth stages were associated with the changes in fruit size compared with the final fruit size. For example, BBCH codes 73 and 78 were when the sizes of the fruits were 30 and 80% respectively compared with the size of mature fruit (Table 1, Figures 1o and 1p). The fruits stopped growing when maturity stage was achieved and the skin was green in colour.

Stage 8 (fruit maturity or ripening)

The changes in the skin colour of fruit were the criterion used to describe fruit maturity or

Table 2 The growth period of vegetative and reproductive stages in *R. tomentosa* according to the BBCH scale

Vegetative growth (BBCH code)	Duration (days)*
Bud development (01–09)	14 ± 0.995
Leaf development (10–19)	18 ± 0.649
Shoot elongation (31–39)	150 ± 1.518
Timeline (days)	182
Reproductive growth (BBCH code)	Duration (days)*
Flower emergence (51–59)	29 ± 1.124
Flowering (60–69)	14 ± 0.801
Fruit development (70–79)	45 ± 1.276
Fruit maturity or ripening (81–89)	14 ± 0.865
Timeline (days)	102

*Mean value ± standard deviation

ripening. BBCH code 81 corresponded to a 10% increase in fruit colour compared with the final colour of ripe fruit (Table 1, Figure 2qA). The skin colour of the fruit was green when young and turned to light purple at maturity (Table 1, Figure 2qB). The velvety fruit measured about 1.5–2.0 cm at mature stage, with persistent sepals at the tip (Table 1, Figure 2r). BBCH code 88 (Table 1, Figure 2s) showed a fully ripe fruit with darker purple and shiny skin. In BBCH code 89, the fruit fell due to the abscission of the fruit pedicel. Based on our observations, this species required 8–9 weeks to complete the fruit stage, starting from fruit set to full maturation and ripeness (Tables 1 and 2). Stage 8 took 13.73% of the total timeline in the reproductive phase.

Stage 9 (senescence, beginning of dormancy)

Stage 9 started when no further growth increments were observed. At this stage, the changes in leaf colour were taken into account when determining the BBCH codes. The green leaf colour became yellowish as a result of chlorophyll degradation (Table 1, Figure 2t). The yellowing of the leaf was the main characteristic of the senescence of the leaf that eventually abscise. Later, the shrubs went through a dormancy stage with less emergence of new buds relative to the period when water availability in the soil was higher.

Growth duration

The vegetative and reproductive stages of *R. tomentosa* required 182 and 102 days respectively (Table 2). In the vegetative stage, the development of the buds required 14 days, while the leaf took 18 days to mature. The remaining 150 days of the entire vegetative stage were needed for the shoot elongation. Meanwhile, in the reproductive stage, the flowering was completed in 43 days, from stage 51 (swelling of the flower bud) to stage 69 (the change of petal colour from pink to white), where the flowering ended and the fruit growth began. However, the flowers only bloom for 2–3 days and the final fruit size was achieved after 45 days, whereby it took 14 days for the fruit to mature and be fully ripe.

DISCUSSION

Detailed information on phenological changes in the growth of certain plant species is essential for various purposes, including determining the appropriate time for cultural practices, annual assessment of plant growth associated with environmental changes, and facilitating scientific communication between different disciplines. These descriptions assist in the proper prediction of upcoming events and performances of the species. Our study provides additional documentation on how the BBCH scale is used for a multipurpose shrub, *R. tomentosa*, which is grown as a garden plant in tropical conditions. Eight principal growth stages were identified for *R. tomentosa*. Two principal growth stages, i.e. stages 2 and 4, were excluded as they were not applicable to *R. tomentosa*. Stage 2 (side shoot formation) in trees and shrubs was not taken into account due to their extensive branching, and it is not covered by the BBCH scale. Finn (2007) suggested that side shoot formation can best be described using architectural models of trees.

The phenological growth of *R. tomentosa* begins with bud swells. In general, the bud starts to swell when appropriate environmental factors are present. Temperature, soil moisture and photoperiod are the most essential environmental factors that stimulate plant growth (Neil & Wu 2006, Wilkie et al. 2008), while endogenous factors such as growth stages, photosynthates and phytohormones ensure the formation of specific plant organs (Wilkie et al. 2008). Thus, both environmental and endogenous factors promote the change of meristematic tissue in the bud as a leaf or flower (Hanke et al. 2007, Ahmad Nazarudin & Tsan 2018).

The growth of vegetative parts in *R. tomentosa* occurred continuously and axillary buds emerge frequently forming the side shoots. Stage 3 of *Psidium guajava* and *X. chrysanthus* comprises growth of the lower pair of leaves succeeded by another pair of leaves (Salazar et al. 2006, Ahmad Nazarudin & Tsan 2018). Among the eight principal growth stages identified in *R. tomentosa*, shoot elongation required the longest duration (150 days) to reach its final length, i.e. 82.42% of the entire vegetative growth timeline (Table 2). The final length of shoot was achieved when there was no other additional development of nodes observed.

Two types of branches, i.e. flushing and flowering branches, were frequently found to develop simultaneously in a single *R. tomentosa* plant. Previous studies have also revealed that more than one principal growth stage occur in parallel for tropical plants. For instance, the concomitant growth of flushing and flowering branches of the ornamental plant *X. chrysanthus* allows the plant to flower all year round (Ahmad Nazarudin et al. 2014). Other studies also show a similar trend in plant growth, where the synchronisation of flowering and fruiting have been observed across diverse groups of the Asian dipterocarps (Sakai 2002, Bawa et al. 2003). The BBCH scale is helpful to elucidate the progress of synchronous growth stages. For instance, the stage 14/52 in *R. tomentosa* showed that the leaf had expanded about 40% compared to the final leaf size, while the flower bud with sepals was visible (Table 1, Figure 2h). It was also observed that the leaves and flower buds emerged profusely from week 8 to 16 (April to May 2021) due to increased volume of precipitation and reduced daily temperature (Figure 1). Similarly, the flowering in *X. chrysanthus* increased with greater volume of rainfall in March to April (Ahmad Nazarudin & Tsan 2018). This indicates that dryness or precipitation has profound influence on the emergence of leaves and flower buds of the species grown in tropical conditions.

Our study showed that the flowering period of *R. tomentosa* was approximately six weeks, although Adarsh et al. (2018) reported it to be 16 weeks. The difference in the climatic conditions of the study sites could be the main influence of the variation in flowering periods. However, the lifespan of the flower in both study locations was similar (between 2 to 3 days). Although the blooming is short, asynchronous flowering occurs, making this species attractive in the landscapes. A similar condition occurs in *A. sellowiana*, where flowering remains asynchronous over the year, though the main flowering seasons are observed in February and September (Ramírez & Kallarackal 2018).

Rhodomyrtus tomentosa required 8–9 weeks to complete the fruit stage, starting from fruit set to full maturation and ripeness of the fruit (Table 2). This result is in line with Adarsh et al. (2018). The fully ripe fruits are usually eaten by birds or ants, spreading the seeds to other places. *Rhodomyrtus tomentosa* is also important as a food source for other faunas such as butterflies and bees besides its aesthetics and valuable healing properties.

Stage 9 began as the shoot stopped growing. The fully green leaves became yellow over time as the chlorophyll degraded (Figure 2t). Yellowing of leaf is a sign of ageing prior to leaf abscission. Leaf senescence defines the final stage of a developmental phase, which is a degenerative process, and occurs in a coordinated manner over time (Gan & Amasino 1997, Lim et al. 2007). Dormancy of tropical woody species is not obvious compared with the species grown in temperate climates. In general, reduced emergence of leaf buds indicates the dormant stage of the tropical species.

The BBCH scale is useful for identifying the optimal time for fertilisation, pests and disease controls, and trimming frequency required for the species. In addition, the scale can be beneficial to predict the appropriate pollination stages of the flowers and the relationship between growth stages and potential pollinators. It is also helpful in predicting fauna assemblages that are influenced by the specific phenological growth stages, namely, flushing, flowering or fruiting. Finally, the scale can also be applied for screening bioactive compounds in suitable growth stages when required.

CONCLUSION

The developed BBCH scale, timing and specific measurements for *R. tomentosa* were based on the observation of the plant growing under the climatic conditions of the year and the study site. However, general description of the growth stages can be used in different places and climatic conditions. In total, eight principal growth stages were observed as specified by the BBCH scale: stage 0 (bud development), stage 1 (leaf development), stage 3 (shoot elongation), stage 5 (flower emergence), stage 6 (flowering), stage 7 (fruit development), stage 8 (maturity or ripening of fruit), and stage 9 (senescence, beginning of dormancy). Simultaneous growth of the vegetative and reproductive phases frequently occurs in *R. tomentosa*. This species took 182 and 102 days to complete the vegetative and reproductive cycles respectively. The flowering period of *R. tomentosa* was 43 days, including the 2- to 3-day lifespan of an individual flower. The developed phenological growth stages with defined growth periods can be used to improve both horticulture and landscape managements of the species.

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