Original article

Optimizing agronomic practices for pineapple (*Ananas comosus* (L). Merr. 'MD-2' cultivar) production based on growth stages

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Summary

Introduction – **Descriptions of the developmental stages of crops are necessary for optimal scientific communication and technology transfer between researchers, technicians, and farmers. Although pineapple cultivation is an intensive technical activity, there is no precise description of the crop's phenology. The purpose of this study is to accurately describe visually identifiable vegetative (V) and reproductive (R) phenological stages (PS) during the development of the pineapple plant.** *Materials and methods* – **Plants of the cultivar 'MD-2' were planted in three different environments in Costa Rica. The same substrate and management practices were applied in the experimental plots. The V-stages were described based on the 5/13 phyllotaxy of the pineapple plant, while the R-stages were defined according to standard names commonly found in the literature but more precisely and systematically.** *Results and discussion* – **Five vegetative and six reproductive (PS) were proposed and developed using the cultivar 'MD-2'. These stages can be used to describe the life cycle of 'Smooth Cayenne' and other cultivars that are its offspring, such as 'CO2' and 'MD-2', that are of great economic importance worldwide.** *Conclusion* – **The discrete and visual identification of the (V) and (R) life cycle stages of the pineapple plant will provide a more precise basis on which to schedule cultural practices that require precision. This identification of stages will improve scientific communication and technology transfer between researchers, technicians, and growers. It will also provide a basis for proposing improvements for pineapple production and the development of computational models for growth prediction.**

Keywords
phenology, phyllotaxy, vegetative, reproductive, forcing

Significance of this study

What is already known on this subject?

- Until now, no functional phenological stages have been described that can serve as a standard and precise description for growth and development of pineapple.

What are the new findings?

- Functional and precise phenological vegetative and reproductive stages are proposed as guidance for scientific communication, planning and timing of cultural practices in pineapple.

What is the expected impact on horticulture?

- A precise reference on the phenological stages in pineapple improves the understanding of scientific communication and is essential for extension recommendations that are based on the timing of cultural practices.

Introduction

Pineapple production is an intense activity. For companies that produce fresh fruit for export, the daily cultivation processes range from preparation of the land to harvest of the fruit, which is complex for overall crop management. The probability of human error is high due to the number of agricultural activities that are required daily for optimal growth and development. Therefore, precise recordkeeping and follow-up of the different cultivation practices at different growth stages are fundamental. Despite this complexity of requirements related to activities at each stage, the existing definitions of phenological vegetative and reproductive stages in pineapple are lacking, insufficient, incomplete, or they were developed for other purposes that do not lend themselves to practical agronomic use.

The description of the developmental stages for some crops has been used successfully for scheduling cultural practices, such as irrigation, the application of fertilizers, insecticides, fungicides, and growth regulators, and for harvest. They are also fundamental for other scientific disciplines related to agronomy, such as plant physiology, phytopathology, and entomology to understand and describe physiological phenomena and the periods for greater predisposition or susceptibility to pests and diseases.

One of the objectives of scientific research is to describe precise methodological information so that experiments can be replicated and verified by other researchers. In scientific research on pineapple, it is often difficult to define precisely when a sample was taken or when a treatment was applied precisely. For example, in a study of organic acids related to CAM metabolism, Rainha *et al.* (2016) reported taking samples used in the research before flowering, during flowering, and after flowering, leaving it unclear precisely when the

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samples were actually collected. Therefore, it must be assumed that any vegetative stage before, during, or after flowering leads to similar results. Similarly, Zhang *et al*. (2023) reported that a treatment to improve the quality of the 'MD-2' fruit in China was carried out 40 days after fading of the flower. However, the term "fading" is ambiguous, so replicating that experiment is difficult. In an experiment carried out in Brazil on fertilization with sewage sludge for the fertilization of soil cultivated with pineapple (Mota *et al*., 2021), it is indicated that a dose of sludge equivalent to 15 g of inorganic fertilizer was applied in the vegetative phase. If the development of the plant was relevant to define the moment of the application, with the information provided it is not really known when the fertilization was applied.

For many years the importance of using standardized terminology by personnel involved in the production of a crop when discussing the development of the plant has been established. For example, Fehr *et al*. (1971) state that in soybean production the descriptions are intended to be objective and precise so that minimal variation will occur among persons identifying the stage of a plant. By the other hand Liang (2019) mentions that in phenology currently standard observational protocols are usually needed to reduce observer biases and ensure broad data comparability.

An internationally accepted scale for recording the growth stages of plant species is clearly desirable. Zadoks *et al*. (1974) established a scale specifically for cereals, and later their approach was used to develop the BBCH scales, an acronym derived from the German "Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie" (Meier, 2018), meaning the Biological Federal Institute, Federal Plant Variety Office, and Chemical Industry, the names of the participants that were originally interested in the development of a nomenclature of phenological stages for plants. BBCH scales have been developed for different crop species where similar growth stages of each plant receive the same code, which helps to systematize the nomenclature and facilitates scientific communication.

The precise identification of a phenological stage for a crop depends both on the uniformity of the plant population and on the precision of the characterization of each phenological stage. For peanut, Boote (1982) established that a certain stage has been reached when 50% of the sampled plants have achieved the characteristic feature of the stage.

From a practical point of view, the determination of a phenological stage depends on the objective sought. Given that in intensive pineapple cultivation there are some cultural practices and agronomic decisions that require a high level of precision and others where a reasonable level of precision is enough, a scale or description of phenological stages must support the most critical requirements.

Zhang *et al*. (2016) published a BBCH scale for pineapple using the 'Comte de Paris' cultivar as a reference. According to Sanewski *et al*. (2018), the 'Comte de Paris' cultivar is very different from the 'Smooth Cayenne', 'MD-2' and 'CO2' cultivars, for which there is currently a large distribution worldwide. One notable example of these differences is the growth of suckers before flowering in the 'Comte de Paris' cultivar, which never happens for the cultivars 'MD-2' or 'CO2'. Additionally, the description of each stage is not precise enough to determine when exactly it occurs. Therefore, the BBCH scale proposed by Zhang *et al*. (2016) for pineapple is not useful for the most important cultivars that are currently cultivated.

More than 75 years ago, Okimoto (1948) and Krauss (1949) provided a detailed description of the anatomy and growth of the vegetative and reproductive organs of the pineapple plant, using the 'Smooth Cayenne' cultivar. These studies are applicable for the most important current cultivars, including 'MD-2' and 'CO2'. However, most studies are histological in nature, with a detailed description of the macroscopic morphology, vascular anatomy, and histology of the internal structures of the leaves, stem, buds, inflorescence, and fruit. Microscopic information on the development of the inflorescence has been presented by Bartholomew and Sanewski (2018), while the morphology, anatomy, and taxonomy have been addressed by d'Eeckenbrugge and Leal (2018). However, microscopic descriptions are not helpful for practical agronomic use.

The goal of those who develop scales or standards to define phenological stages for agronomic applications is to propose precise and objective descriptions of discrete visual phenological events of the morphological and physiological development of the plant, normally separating them into vegetative and reproductive phenological or growth and development stages (Boote, 1982).

The objective of the current study was to design descriptions of developmental stages for pineapple 'MD-2' based on visually identifiable vegetative (V-stages) and reproductive (R-stages) events. The application of these descriptions is expected to improve scientific communication among pineapple researchers and to facilitate communication between technicians and operators to carry out cultural practices at precise times. As a result of this precision, better planning, more rational use of agricultural inputs and better use of resources (labor, machinery and equipment) will lead to improved development and a higher productivity of pineapple and, therefore, a greater economic and environmental sustainability of pineapple production. Based on this approach, this study also provides recommendations for agronomic use with the defined V-stages and R-stages and expected benefits of their use.

Materials and methods

In order to develop the growth stages for pineapple, plants of the 'MD-2' pineapple cultivar were grown in three contrasting environments in Costa Rica, specifically at three elevations, *i.e*., 90 m a.s.l., 174 m a.s.l. and 1,573 m a.s.l. These three locations are referred to as Warm Zone (WZ), Typic Zone (TZ), and Cool Zone (CZ), respectively. At these three sites, the cultivar 'MD-2' was cultivated at three different planting dates, dividing the year into quarters starting in January 2021. The average rainfall and cumulative, maximum and minimum temperature averages, the average relative humidity and the average and total cumulative solar irradiance of each site are presented in Table 1, where a comparison of means is also shown through an ANOVA per study site of the indicated climate variables. Soil moisture, which can be a limiting factor due to insufficient rainfall, was controlled by applying weekly irrigation equivalent of 43.1 mm. The plants were grown in culture bags using the same soil as a substrate, *i.e*., a sandy clay soil with a pH 4.45, to limit the factors that can impact growth. The spatial arrangement of the plants in the experimental plots resulted in plant density of 1.33 plants m^{-2}. Crop management following the standards of the Costa Rican agroindustry was applied on all experimental plots.

Each planting within each site consisted of 40 plants. For each planting date per zone, 10 plants were selected for a periodic weekly follow-up of the phyllotaxy and to determine with precision the moment when the vegetative phenological

Table 1. Air temperature, relative humidity and solar radiation for the different zones during the study of phenological stages of development in pineapple cultivar 'MD-2'. ANOVA test.

Zone	Temperature†			Relative humidity (average %)	Solar radiation‡ (Mj m^{-2} day^{-1})	Rainfall* (mm)
	Minimum	Maximum	Average			
	°C					
CZ	14.5 (9.3–17.0)a	20.7 (16.8–24.2)a	17.5 (14.6–20.3)a	86.5a	12.2 (4,540–4,378)a	243 (2,593–3,256)
WZ	21.3 (14.2–25.9)b	33.1 (26.6–38.1)b	26.9 (23.5–30.1)b	82.1b	19.5 (7,305–6,937)b	137 (1,394–1,887)
TZ	21.7 (15.1–24.3)c	30.2 (22.4–34.4)c	25.7 (20.7–28.9)c	90.4c	14.1 (5,045–5,263)c	242 (2,950–2,859)

† Average of daily data over 2 years and, in brackets, range minimum and maximum of total data.
‡ Average of daily total over 2 years and, in brackets, cumulative total of year 1 and year 2.
* Average monthly over 2 years and, in brackets, cumulative total of year 1 and year 2.
Equal letters within each column represent non-significant differences for $p < 0.05$.

stages were reached. These were considered to be fulfilled when at least 50% of the plants reached the specified stage. The same criteria were used to evaluate the scope of the reproductive phenological stages.

Sucker seed was used, since it is the most widely used in the commercial cultivation of 'MD-2' pineapple. An illustrated description of pineapple seed types can be reviewed at Py *et al.* (1987). In all cases, sucker seed harvested the same day was used and sown the next day. Seed weight is relevant in commercial pineapple cultivation because it is directly related to the productivity and the duration of the crop cycle. A fresh weight between 600 and 650 grams was used, as it is the weight preferred by the top producers in Costa Rica. The seed was obtained from the same seed plot from a company known for its high technological level within the Costa Rican agroindustry.

The determination of the vegetative growth stages (Table 2), with the exception of the first two stages, is based on the scope of phyllotaxy cycles. The pineapple phyllotaxy has been shown to be 5/13 (Ekern, 1968; Kerns *et al.*, 1936; Krauss, 1949), which means that 5 leaf turns can always be counted around the stem axis of the pineapple plant for a total of 13 leaves.

The determination of the vegetative stage V1 (root initiation) was carried out through non-destructive sampling, separating the plants from the soil and checking for compliance with the naked eye. For the determination of the V2 stage (emergence of the first new leaf), the smallest leaf visible in the leaf whorl was identified (using white water-based paint to paint the tip of this leaf at the time of planting). When a new leaf appeared after sowing, the V2 stage was considered finished. The scope of the other vegetative stages (V3 to V5) was determined according to the scope of phyllotaxy cycles as indicated in the previous paragraph. The technique and detailed parameterization of the evaluation are an integral part of the results.

The scope of the reproductive stages (R2 to R4) was determined by observing and characterizing flowering in the first and last fruitlet of the long spirals. The evaluation and characterization of stages R2 to R4 is an integral part of the results and will be explained in detail in this section. The scope of the R5 and R6 reproductive stages was determined by observing and describing the general and fundamental parameters in use in the agroindustry of fruit production for fresh consumption or export. Additionally, the translucency parameter in the R5 and R6 stages was clarified to standardize usage within the agro-chain.

The agronomic practice of forcing, *i.e.*, artificial induction of flowering, while not a phenological stage, is considered to be a main cultural practice in the industry as it determines the beginning of flowering. In this study, it was identified as R1, to indicate the induction of flowering.

The dates on which the described phenological stages were reached generate two response variables that are used

Table 2. Growth stage description for the pineapple cultivar 'MD-2'.

Stage No.	Stage title	Description
Vegetative stages		
V1	Root initiation (RI)	Beginning of root growth.
V2	First new leaf (FNL)	Leaf initiation and emergence of the first new leaf.
V3	Leaf cycle 1 (LC1)	13 leaves produced after sowing.
V4	Leaf cycle 2 (LC2)	26 leaves produced after sowing.
V5	Leaf cycle 3 (LC3)	39 leaves produced after sowing.
Reproductive stages		
R1	Forcing	Exogenous application of a growth regulator, strongly marks beginning of flowering in pineapple.
R2	Open heart (OH)	Beginning of emergence and consequent visual occurrence of the inflorescence in the rosette.
R3	Early anthesis (EA)	In the first fruitlet of at least one of the long spirals (in the base), the emergence of the petals with a length of approximately 3 to 4 mm is observed. Undifferentiated crown.
R4	Final anthesis (FA)	In the last fruitlet of at least one of the long spirals (in the top), the emergence of the petals with a length of approximately 3 to 4 mm is observed. Differentiated crown.
R5	Physiological maturity (PM)	This stage completes when the fruit presents 13 °Brix.
R6	Harvest maturity (HM)	The shell fruit has a partially yellow coloration.

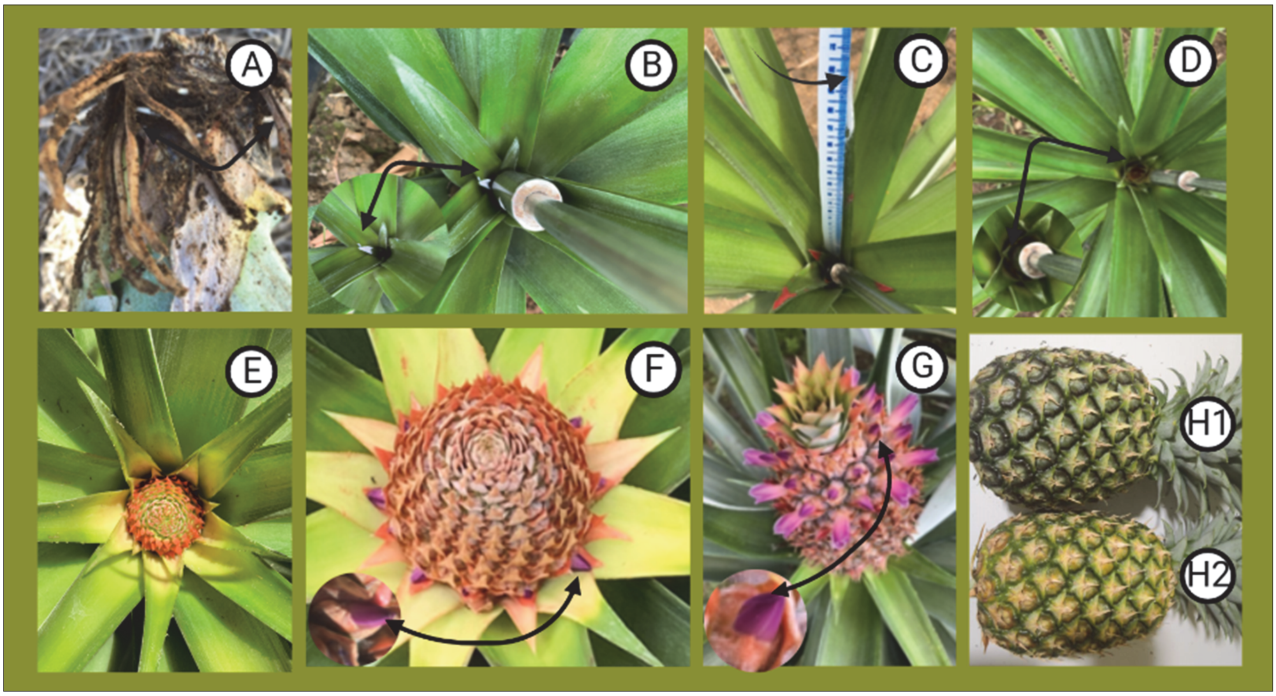

FIGURE 1. Phenological stages of the pineapple cultivar 'MD-2'. A) Root initiation, the arrows in the picture point to the white root tips. B) Leaf initiation and emergence of the first new leaf; the arrow in the picture points to the identification of the reference leaf at the time of planting. C) Leaf cycle 1, 2 and 3 show the leaf cycle identification methodology; the arrow in the picture points to the first leaf of the developing leaf cycle (tip painted white) and corresponds to the reference leaf for leaf count events. D) Open heart, the diameter of the cylinder and the diameter of the emerging inflorescence are the same. E) Red cone lies between Open heart and Early anthesis. F) Early anthesis, at least one of the long spirals presents the emergence of petals with a length between 3 and 4 mm in the first fruitlets. G) Final anthesis, at least one of the long spirals presents the emergence of the petals with a length of 3 and 4 mm in the last fruitlet, the crown has differentiated. H1) Physiological maturity, 13 °Brix degrees external appearance is green. H2) Harvest partially yellow color, in the export agroindustry it is usually due to the practice of degreening.

to statistically demonstrate the botanical validity of the described phenological stages. The experiment consisted of a completely randomized multi-site experimental design. The response variables were defined as the Days After Planting (DAP) in which each of the phenological stages are reached and the Growing Degree Days (GDD) necessary to reach each of the phenological stages. The statistical analysis was carried out using the SAS software, through a statistical model of fixed effects (fixed effect of the Zone and fixed effect of the Phenological Stage) and the interaction of the Zone by the Phenological Stage using GLIMMIX procedure. In all cases, the comparison of means was made based on a LSMEANS (PDIFF) test using the software indicated above, grouping the different phenological stages into V-stages and R-stages due to the base temperature differences that are used to calculate the GDDs (13 °C and 2 °C, respectively). More information about the methodology used to estimate the GDD or Daily Thermal Time (DTT) using ALOHA model available in DSSAT 4.8 (Hoogenboom *et al.*, 2021) can be found in Zhang and Bartholomew (1993).

Results and discussion

The V-stages and R-stages for cultivar 'MD-2' allows the methodology and phenological stages that will be described and explained below to be used by the most important cultivars grown throughout the world. This not only includes the cultivar 'MD-2', but also the cultivar 'CO2' which is sibling of 'MD-2', as well as the cultivar 'Smooth Cayenne', which contributed the largest parental component of the two previous cultivars (Sanewski *et al.*, 2018).

Given the importance that the identification of these phenological stages has for future research, the agroindustry, technicians, and pineapple farmers, the techniques and equipment used are provided along with a description, when applicable, so that the information serves as a diagnostic tool.

Vegetative growth stages

The phyllotaxy 5/13 in the emission of leaves of the pineapple plant was demonstrated in this study. This represents botanical evidence of the validity of using the phyllotaxis cycles as a method of evaluating the phenological development of the pineapple plant in a systematic and objective way. The phyllotaxy was demonstrated since identifying the smallest leaf in the whorl in the central part of the vegetative seed of the pineapple, the 14th leaf that emerges in the whorl will occur in the same position and exactly 13 leaves will have been produced. Consequently, leaf number 14 is leaf number 1 of the following leaf cycle.

The defined phenological V-stages are also supported by statistical means, since the data were collected in contrasting natural environments, especially with respect to temperature and solar radiation. There are statistical differences ($p < 0.05$) in Days After Planting (DAP) between study sites to reach the plants each phenological stage, but when the Growing Degree Days (GDD) are analyzed, there are no statistical differences ($p > 0.05$), which means that the leaf cycles are completed systematically according to thermal time for each site (Table 3).

A schematic representation of the advancement of the phenological stages in a typical Costa Rican environment can

Table 3. Duration of the individual phenological stages in Days After Planting (DAP) and Growing Degree Days (GDD) of the pineapple cultivar 'MD-2' grown in Costa Rica, at the different experimental sites. The initials n.f. means that natural flowering occurred before the stage and e.q. means the stage was reached simultaneously with the previous stage. Average data of three replicates. LSMEANS (PDIFF) test.

Stages	WZ	TZ	CZ	WZ	TZ	CZ
	Average DAP†			Average GDD‡		
V-stages						
V1	7 A	8 A	21 A	103 a	106 a	90 a
V2	7 B	12 B	30 A	e.q.	37 a	34 a
V3	100 B	105 B	315 A	1,220 a	1,152 a	1,167 a
V4	165 A	177 A	n.f.	834 a	857 a	n.f.
V5	228 B	258 A	n.f.	839 a	881 a	n.f.
R-stages						
R1	Moment of forcing is a technical criterion					
R2	267 B	256 B	424 A	853 a	946 a	840 a
R3	284 B	272 B	452 A	439 a	441 a	438 a
R4	307 B	291 B	493 A	606 a	556 a	667 a
R5	384 B	366 B	624 A	1,970 a	2,049 a	2,039 a
R6	388 A	370 A	e.q.	110 a	107 a	e.q.

† Comparison of means for DAP variable in the study sites. Same capital letters represent non-significant differences, $p < 0.05$.
‡ Comparison of means for GDD variable in the study sites. Same lowercase letters represent non-significant differences, $p < 0.05$.
Note that in the GDD variable the letter in all cases is a lower case "a", because there are no statistical differences between the GDDs by phenological stage.

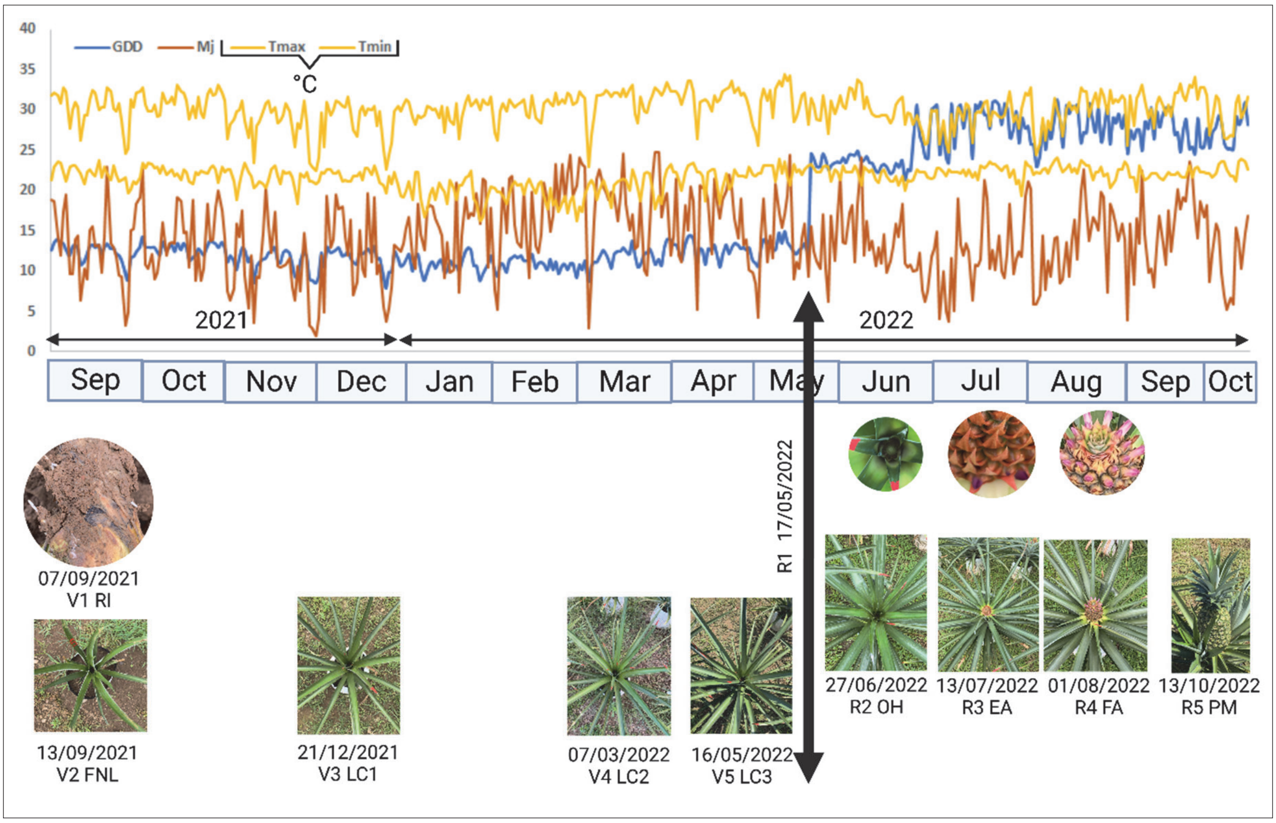

Figure 2. Moments in which the phenological stages of the pineapple cultivar 'MD-2' are reached throughout the growing season of a typical Costa Rican environment (TZ). Sowing in January 2021, forcing in May 2022 and harvest in October 2022. Mj: mean Mj m^{-2} day^{-1}. GDD: mean daily thermal time units. Note that after forcing, there is an increase in GDD units, which is due to the change in base temperature (Tbase). Tbase of V-stages = 13 °C and Tbase of R-stages = 2 °C.

be seen in Figure 2. Note the change in GDD that occurs after forcing, which is mainly due to the effect of a different base temperature on plant development and infructescence.

V1: Root Initiation (RI): Initiation corresponds to the moment after sowing when one or more white root tips are visible emerging from the stem. We found that it is impossible to objectively, practically, and easily review this stage without separating the plant from the soil. However, this is the only stage that has such a drawback. We determined that non-destructive sampling is a necessary and viable option for this stage, since it is related to the type, age, and quality of the seed, which can be considered a growth limiting factor and can already become evident at this stage.

The stage is considered reached when white root tips are observed (Figure 1A). Otherwise, a new sampling must be scheduled and the procedure repeated after 3 to 7 days.

An example picture of *root initiation* stage is presented in Figure 1A. Once the plant has been sampled, it can be replanted without negative effects on growth, provided that the sampling is done in the state shown in Figure 1A.

This stage is mainly dependent on soil temperature and available soil moisture. There is no dormancy in the pineapple vegetative seed, but the start of rooting is affected by the quality of the seed, which, among other factors, depends on the dry matter content of the stem consisting mainly of starch (Bartholomew, 2018; Vásquez-Jiménez *et al*., 2018). For this reason, the evaluation of this stage has implications and agronomic diagnostic scope of the quality and homogeneity of the seed, which can be transcendental in the management and continuous improvement of the pineapple crop.

V2: Leaf initiation and emergence of the first new leaf (FNL): We found that it is not possible to determine with certainty and precision this phenological stage without an initial reference mark. Therefore, to determine this phenological stage, as well as the different leaf cycles, it was necessary to build and implement a tool and to use water-based paint and a plastic measuring tape with graduations in cm.

The tool is a stainless-steel cylinder with a 19 mm diameter and 50 mm length welded to a holding rod that is 10 mm in diameter and 500 mm long. The cylinder was delicately slid into the whorl of the bud, and white paint was used to mark the tip of the last leaf (smallest leaf) protruding from one side of the cylinder. The cylinder selects the smallest leaf in a consistent and systematic way.

The elongation of this first leaf of each leaf cycle (reference leaf) may also optionally be monitored. With a sufficient amount of data, a relationship can be found between the length of this leaf and the accumulated number of leaves in each leaf cycle. A plastic measuring tape with centimeter graduations (see Figure 1C) slides over the blade of the leaf to take measurements at each sampling event.

This selected leaf will be considered as the *reference leaf*, from which the subsequent leaves that emerge or grow in the whorl will be counted. The leaf should be identified and marked at planting time. Either weekly or at agronomically viable time intervals, the cylinder is slid again to check if a new leaf is protruding from the side of the cylinder. When a new leaf does emerge, the tip is painted with a white water-based paint, and the phenological stage “leaf initiation and emergence of the first new leaf” has ended, *i.e.*, the first leaf of leaf cycle 1 has been identified. An example picture and the methodology are presented in Figure 1B**.** This leaf initiation stage always occurs at or a few days after the initiation of the root, depending on the number of heat units (GDD) that accumulate per day at the cultivation site.

With good quality seed and warm or typical pineapple production environments, root initiation and the emergence of the first new leaf can occur simultaneously under an optimum temperature, adequate soil moisture, and a high starch content in the seed stem. These conditions were found in the WZ site (Table 3) favored by a higher temperature compared to the other sites (Table 1). These conditions are the necessary stimuli for both root initiation and leaf growth. Occasionally, these stages occur only a few days apart. The effect of soil temperature and soil moisture on the growth of the pineapple plant has been described in other studies (Bartholomew, 2018; Vásquez-Jiménez *et al*., 2018). In the process of monitoring and applying the methodology, we found that both stage V1 and V2 can be diagnostic traits of seed quality and the technical management of seed homogenization.

V3: Leaf cycle 1 (LC1): We established that this phenological stage is reached when 13 leaves have emerged after sowing (1 leaf with the tip painted white and the following 12 leaves with the tip painted red). After identification of the first new leaf (with the tip painted white), for the following leaves the tip of each leaf is painted with red paint. The methodology for determining this stage is the same as described earlier for the V2 stage.

An example picture is shown in Figure 1C. The first leaf cycle is reached more slowly than the following leaf cycles due to the establishment of the plants in the field “transplant effect” (Py *et al*., 1987) and due to the greater demand for assimilates during this period while the pineapple plant still has a small leaf area, since the efficiency of this process depends on the photosynthesis rate and the interception of solar radiation (João *et al*., 2014). Due to this slow initial growth (see LC1 in a typical phenological development in Figure 2), the diagnostic methodology for identification and counting of new leaves has a minimum precision of 7 days. From a practical point of view this means that weekly evaluations are recommended to evaluate the emergence of new leaves and to count the phyllotaxis cycles.

V4: Leaf cycle 2 (LC2): This phenological stage is reached when 26 leaves have emerged after sowing. The second leaf cycle is reached faster than the first because the plant has already passed the establishment period in the field and has a robust root system, while there are 13 more leaves compared to the V3 stage.

Because the ‘MD-2’ pineapple plant does not develop vegetative structures other than roots, leaves, and the main stem before the induction of flowering, the assimilates that are produced can easily support the development of a new whorl of leaves that emerges from the apex of the stem, with some of the surplus assimilates stored in the stem (Zhang and Bartholomew, 1997).

In areas where there is an incidence of natural flowering and the season is conducive, natural flowering may occur during the middle of this leaf cycle or later, for example for site TZ. It is important to note that if natural flowering occurs, the production of new leaves is definitively interrupted (Bartholomew and Sanewski, 2018), and as a result, no more leaf cycles are produced in these plants (see CZ site in Table 3).

The elongation of the leaves stops when a new leaf cycle is initiated, *i.e.*, for every 13 leaves that emerge, one leaf completes its full length. Leaf number 14 marks the completion of the elongation of leaf number 1, leaf number 15 marks the completion of the elongation of leaf number 2, and so on.

However, we found that leaf measurements may differ up to about 10 cm from the time a cycle is completed to time of harvest. This difference may be due in part to the fact that the leaf sheath continues to open as the plant flowers.

V5: Leaf cycle 3 (LC3): This phenological stage is reached when 39 emerged leaves have been counted after sowing. Again, this leaf cycle is reached faster than leaf cycle 2. It is important to keep in mind that if the tracking is done correctly, the tip of the leaf 1, leaf 14, leaf 27 and leaf 40, will always be pointing in the same direction. We determined that this can have a practical utility at the field level to count leaf cycles with only the first marked reference leaf.

It is important to note that a plant could be induced naturally before LC3, or that a grower could artificially induce flowering at or before leaf cycle 3. However, if the plant is not induced naturally or if it is not induced artificially, the plant can continue to increase the number of leaves and, consequently, its leaf cycles. Seed with a lower weight than the one used in this study could require more than 3 leaf cycles to reach forcing with agronomically correct conditions.

Only three leaf cycles are described here because for the specific type and weight of the seed used in this study, after the third leaf cycle, if the plant is not induced (forcing) the harvest index decreases below the economic interests of production.

Finally, it is important to emphasize the agronomic relevance of the phyllotaxis cycles (or leaf count) to evaluate and compare the vigor between plantations or between treatments. Pineapple "D leaf" is often used as a parameter to evaluate and compare the growth and vigor of plants in different treatments, for example Wang *et al*. (2011) used the weight of "D leaf" as part of the variables to evaluate the response in the plant to different treatments used to control soil pests. Usman *et al*. (2015) used characteristics of the "D leaf" to develop a model to predict the fresh weight of the pineapple plant and as a criterion to determine the opportune moment of forcing. The problem with the estimation of the fresh weight of the pineapple plant or variables associated with the "D leaf" to predict the weight of the plant is that, being a succulent CAM plant, about one third of the volume of the leaves is water storage tissue, and when it is depleted by drought it can be replenished after sufficient rain (Bartholomew, 2018). In such cases, the plant weight differs before and after rain, although the dry matter is the same. Therefore, the number of leaves produced after sowing is a more robust variable to evaluate the growth of the pineapple plant.

Reproductive growth stages

A total of six reproductive phenological stages were defined, with R1 corresponding to forcing.

Growth of the infructescence of the pineapple could be observed from one day to the next, from Open heart to Final anthesis (see Figure 2). After Final anthesis, the changes are much less noticeable to the naked eye. For this reason, precise and easily identifiable points were defined so that they could be used as reference points for projection and prediction of the dates of physiological maturity and harvest maturity by agroindustry users. The two final phenological stages are included mainly because of their relevance to the pineapple agroindustry. In all cases, the R-stages were statistically confirmed similar to the V-stages. There were no statistical differences ($p > 0.05$) in GDD (Table 3), which confirms that the phenological R-stages described are completed systematically according to the specific daily thermal time of the site where the plants are being grown.

Natural Flowering (NF) is reported as one of the main problems of the pineapple agroindustry (Bartholomew, 2014), while the artificial induction of flowering is considered one of the most relevant agronomic practices in the pine agroindustry (Min and Bartholomew, 1993). For these reasons, the precise definition of reproductive phenological stages is a valuable tool for dating natural and artificial flowering events. It can also be used for scheduling phytosanitary management activities and setting the harvest date for mature fruits.

R1: Forcing: termination of leaf production by exogenous application of a growth regulator, and initiation of the transition to reproductive growth. This stage does not really correspond to a phenological stage itself, since the application of ethylene is an event through an operation based on a technical decision. This stage is a clear distinction for the beginning of the reproductive phase, and is, therefore, the first stage of the reproductive phenological stages. For a typical pineapple production environment, for a seed size as indicated in the methodology and an average technological management of the crop, the induction of flowering could be conducted between leaf cycle 2 and leaf cycle 3, with the upper limit of flowering induction in leaf cycle 3.

R2: Open heart (OH): We define this stage as the beginning of the emergence and visual detection of the inflorescence. The estimation of the harvest date of the fruit, whether it is fruit from natural flowering or fruit from forcing, is relevant for pineapple producers. The weather can influence the harvest date (Malezieux *et al*., 1994), and for this reason, the precise visual definition of this stage and the following ones can be used for estimating the harvest date.

This stage is complete when the tool described in V2 is gently slid over the emerging inflorescence and the cylinder snugly enters the last whorl of leaves and rests on the inflorescence so that the diameter of the cylinder (19 mm) is approximately the same diameter as the inflorescence in emergence. An example picture is shown in Figure 1D; the inflorescence is still white and quite flat on top (cone not differentiated).

Due to the rapid growth of pineapple infructescence and the defined phenological monitoring traits, a precision of 1 day is achieved in the determination of the reproductive phenological stages described in this study.

R3: Early anthesis (EA) or beginning of flowering. Difficulties resulting from different characteristics, lengths, and colors during the opening of the flowers can be overcome by defining the stage at a time when there is little probability of confusion. Therefore, early anthesis is reached when petals approximately 3 to 4 mm in length resembling pencil points are observed in the first fruitlets of at least one of the long spirals and the fruit crown is not differentiated. An example picture is shown in Figure 1F.

The phenological stage prior to *early anthesis* is commonly known in the Costa Rican pineapple agroindustry as "red cone", and it can be easily identified with the naked eye. However, the inflorescence "red cone" can last for several days without obvious changes, so the precise definition of when it starts and ends is difficult. Therefore, it was not included as a phenological stage, but it obviously occurs between *open heart* and *early anthesis* (Figure 1E). The characteristic "red cone" condition begins several days after *open heart* and often is still called "red cone" even a few days after *early anthesis*. The stage "red cone" is, thus, an imprecise phenological stage, but under the indicated caveats it could eventually be useful for some scientific or agronomic purposes that do not require too much precision.

R4: Final anthesis (FA) or end of flowering. The determination of this stage is similar to the previous phenological stage, except that instead of evaluating the first fruitlet of the spirals, the final fruitlet is evaluated when the crown of the fruit has already started to differentiate (Figure 1G).

Depending on the vigor of the plants, the weather conditions during the vegetative development stages, the consequent allocation of photosynthate to the stem, and the prevailing weather conditions after *final anthesis*, the plants may develop vegetative buds at the base of the fruit (seed slip), at the base of the fruit stalk (hapa seed), or on the stem of the plant (sucker seed) (Py *et al*., 1987).

The number of propagules can vary according to the vigor of the plant, available photosynthates, and the size and strength of the sinks. All combinations are possible, from none to one of each, several of each, several of only one type, *etc*.

The fruit is the main sink (Bartholomew and Sanewski, 2018), but in *final anthesis* it is still small, so the propagules can develop. Slip buds were found that swelled up to 3 cm but then did not continue their growth. Due to all the possible combinations, the systematic production of these propagules cannot be characterized as a phenological stage. However, bud initiation of propagules, if it occurs,) occurs just after *final anthesis*.

R5: physiological maturity (PA): This stage is not observable with the naked eye. Because pineapple is a non-climacteric fruit, the determination of physiological maturity is relevant to ensure the quality of the fruit (Ikram *et al*., 2020). For fresh export pineapple, this stage must be sampled, *e.g*., destructive sampling, and the brix degrees and *internal color* determined, which is a common practice in the pineapple agroindustry. For practical purposes, this stage is completed when the fruit presents 13 °Brix (field measurements with a handheld refractometer degrees brix or similar), and a uniform yellow color (desirable) or defined sections with different intensities or shades of yellow color can be seen in all the pineapple flesh after making a longitudinal cut to the fruit. Also at physiological maturity, the practice of degreening occurs, which involves the application of ethephon for cosmetic purposes to improve the external appearance (color) of the fruit for the final consumer (Figure 1H1).

The term translucency is widely used in the pineapple agroindustry to describe fresh fruit for export as an important subjective evaluation variable of the physiological maturity of the fruit. In this case, the concept is used incorrectly to characterize grades of *internal color* of the flesh fruit with required quality. The same concept is used to characterize a physiological problem that leads to the rejection of the fruit, applying the concept of translucency correctly. This concept was originated in Hawaii, where important studies from the Pineapple Research Institute were devoted to or involved the study of translucency for canned fruit. In Hawaii, the term translucency, *i.e*., the ability of a body to let light pass through it, was correctly used to refer to pineapple that produces a high recovery or a high fruit yield for canned fruit (fancy-slices = fruit with translucency), mostly for 'Smooth Cayenne'. However, some levels of translucency can cause losses in canned fruit because the fruit does not withstand automated peeling. In other words, translucency of the pineapple fruit was utilized as a maturity index and a quality attribute desirable for processing (Sinha *et al*., 2012).

Translucency in 'Smooth Cayenne' as a fruit for fresh consumption was described as a physiological disorder in Hawaii by Chen and Paull (2001), indicating that it is a fruit whose flesh shows water soaking and has low porosity. Recently, Chen *et al*. (2023) described translucency as a physiological disease for seven cultivars that are used for consumption as fresh fruit, including 'MD-2', ('Tainong 4', 'Tainong 17', 'Tainong 21', 'Tainong 23', 'MD-2', 'Smooth Cayenne' and 'Comte de Paris').

The internal differences in the pineapple flesh between cultivars of fresh export fruits and canned fruit are essential to understand and use the concepts correctly. In export fruit, translucency shortens the shelf life, and it is undesirable. However, in canned fruit, translucency favors the high recovery of fancy slices, and it is desirable. The ambiguity exists only in the fresh fruit export agroindustry, and it often generates ambiguous differences of opinion between producers and fresh fruit buying/exporting companies.

The ambiguity or confusion would not exist if, in the pineapple for fresh consumption, when evaluating the internal condition of the fruit, only the term translucency is used to qualify the fruit with this physiological disorder and *internal color* of the pineapple flesh to diagnose the degree of physiological maturity.

R6: Harvest maturity: This stage is reached when the fruit is partially yellow, after degreening that imparts a partial yellow coloration (Figure 1H2). Various color charts have been developed by fresh fruit trading companies. Producers must respond to these colors according to their contracts. Organic producers and producers who trade the fruit locally do not carry out degreening, so they must wait for the fruit to acquire this color naturally.

Limitations and future directions

The limitations of the phenological stages described include the following in the V-stages. For V1, the need to separate the plant from the soil to check the white root tips, which forces this monitoring to be carried out at a maximum of 7 days after sowing to avoid damage to the root system. Waiting longer could cause the root to develop too much, thus affecting the growth of the plant. To diagnose from V2 onwards, it is necessary to have marked the smallest leaf visible in the bud at the time of planting (reference leaf). However, with good planning this can be done on one monitoring station, *i.e*., a group of monitoring plants or stationary plot, for each individual plot. To monitor the emergence of new leaves, it is necessary to have a tool like the one indicated in V2. However, the measurements used are commercial measurements of the materials used in its construction, so it does not represent a major inconvenience.

The leaf cycle identification methodology is applicable for any seed size. However, it is important to specify the seed size used in any experiment or treatment reported in technical papers or scientific articles where this methodology is used to identify pineapple phenological stages. Since the type and weight of the seed have a significant influence on the duration of the pineapple crop cycle (Haroldo *et al*., 2018), more than three leaf cycles could occur if low weight seed is used.

Regarding the R-stages, for Final anthesis the number of long-whorl fruitlets can be different between fruits. It is important to remember that the stage is fulfilled when 50% of the plant population has reached this reproductive stage. A smaller percentage could improve the estimation of harvest date of small fruits, *i.e*., fruits with fewer fruitlets, but doing so would lead to an early prediction of the harvest date of large fruits, *i.e*., fruits with more fruitlets, and *vice versa*.

Finally, since the prediction of phenological stages is the basis of computational models for crop growth prediction, the description of pineapple phenological stages could be used to create growth prediction models or improve existing ones.

Conclusions

The first two vegetative stages, *i.e.*, V1 and V2, can be used to evaluate the phenological progress of the crop and also allow for diagnosing the quality and uniformity of the seed.

The leaf emission monitoring methodology used to determine the phyllotaxis cycles has a minimum precision of 7 days.

The methodology defined for the determination of reproductive phenological stages has a precision of one day.

The phyllotaxis cycles allow to objectively characterize the vegetative phenological development of the pineapple plant, while the different traits used for characterizing the pineapple infructescence growth, provides several opportunities for improvement of crop management for growers' fields and use to define the application of agricultural practices with clarity and precision.

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