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Performance of the 'Anna' apple (*Malus domestica* Borkh.) in Tropical Highlands: A review

Desempeño del manzano 'Anna' (*Malus domestica* Borkh.) en el trópico de altura: Una revisión

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ARTICLE DATA

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ABSTRACT

The 'Anna' apple is a variety of low requirements of winter chill (250 to 300 chilling hours ≤7.2°C). This apple has essential health benefits and remarkable adaptive potential in tropical and subtropical areas affected by climate change. Thus, this review presents the significance of the 'Anna' apple cultivation, the phenological and eco-physiological modifications, and the current state of agronomic management when continuous crops are managed in tropical highlands. The production of this apple in tropical highlands has outstanding potential to obtain cyclical or continuous harvests (two harvests per year) in certain areas with specific environmental conditions, implementing a particular management system. In plantations, it is crucial to carry out some agronomic practices during the reproductive phenology so that the apple tree does not enter into an endodormancy. These are water stress - defoliation – tie-down branches, and the application of dormancy-breaking agents (flower-inducing compounds). In Colombia, 'Anna' variety was introduced in 1985 and is grown in areas with temperatures between 14 and 20°C, altitudes between 1700 and 2800 meters above sea level (m a.s.l.), with bimodal and monomodal rain regimes, and solar brightness between 800 and 2000 hours a year. The harvest is between 100 to 120 days after anthesis, with firmness values of 38.38N, a soluble solids content of 8.58°Brix, and total titratable acidity of 0.7% of the fruit. This documentation indicates a good production with great potential in terms of growth and development, earliness, and quality of the 'Anna' apple tree in Colombian highlands.

Keywords: Ecophysiology; endodormancy; nutrition; pomology; predormancy.

RESUMEN

La manzana 'Anna' es una variedad de bajo requerimiento térmico (250 a 300 horas frío ≤7,2⁰C). Esta posee beneficios importantes para la salud y tiene un gran potencial en zonas tropicales y subtropicales,

afectadas por el cambio climático. En esta revisión, se pretende presentar la importancia del cultivo de manzano 'Anna', los cambios fenológicos y ecofisiológicos, y el estado actual en el manejo agronómico cuando se trabajan cosechas continuas en condiciones del trópico alto. En zonas altas tropicales, esta variedad de manzano presenta dos cosechas en un año en zonas con condiciones ambientales específicas e implementando un manejo particular. Bajo la producción de estas cosechas continuas, se realizan algunas prácticas agronómicas durante la fenología productiva para que el manzano no ingrese a una endodormancia, dichas prácticas son estrés hídrico - defoliación – agobio de ramas y aplicación de compensadores de frío (inductores de brotación floral). En Colombia, esta variedad fue introducida en 1985 y se cultiva bajo condiciones de temperaturas entre 14 y 20°C, de 1700 a 2800 metros sobre el nivel del mar (m.s.n.m.), precipitación bimodal y monomodal, y brillo solar entre 800 y 2000 horas al año. El desarrollo del fruto y la cosecha es entre 100 a 120 días después de la antesis, con valores de firmeza de 38.38 N, contenido de sólidos solubles de 8.58°Brix y acidez total titulable de 0.7% del fruto. Esta documentación indica una producción favorable y con gran potencial en cuanto al crecimiento y desarrollo, precocidad y calidad del manzano 'Anna' en zonas altas colombianas.

Palabras clave: Ecofisiología; endodormancia; nutrición; pomología; predormancia.

INTRODUCTION

The apple tree, a deciduous fruit tree, grows in temperate, tropical, and subtropical zones. The cultivar 'Anna' is self-sterile, diploid (2n) originating in Israel (subtropical), obtained from 'Adassin Red' × 'Golden Delicious' (Andersen & Crocker, 2009) cultivars. It requires low chilling hours less than 7.2°C and display modifications in peripheral tissues that maintain internal cellular homeostasis (Voronkov *et al*., 2019), making it a potentially cultivable variety in the Colombian tropical highlands.

In Colombia, the varieties 'Anna' and 'Dorsett Golden' (pollinator of 'Anna') were introduced in 1985 to replace some old commercial cultivars (Puentes, 2006). The national production of the apple tree in 2019 reached 5053t in 613 ha harvested with an average yield of 8.24t/ha (FAOSTAT, 2021). The Department of Boyacá is the leading producer of deciduous fruit trees because of its agro-climatic conditions and highland tropics, where 'Anna' is the predominant apple variety, located in the municipalities of Tibaná, Duitama and Nuevo Colón (Miranda & Carranza, 2013). As a result of the climatic conditions and low chilling requirement, specific agronomic management has been proposed to grow and develop the apple tree, where two harvests can be obtained yearly (Fischer, 1993a).

The consumption of 'Anna' apples has considerable benefits for human health, such as anti-cancer properties, preventing heart disease, eliminating bacteria, and improving the quality of the lungs (Skinner *et al*., 2018) thanks to the content of phenolic compounds (antioxidant substances). Therefore, the quality of the fruit is a fundamental requirement for consumers (Rojas-Candelas *et al*., 2021). Navarro *et al*. (2018) reported 41 polyphenolic compounds in the exocarp and mesocarp of 'Anna' fruits, with a total phenol content of 619.9 and 576mg/GAE g, respectively. Other vital substances in 'Anna' apples include volatile compounds since, unlike in other varieties, they impart better aroma and lead to use in infusions as a body relaxant and to relieve insomnia. Lurie *et al*. (2002) identified an extensive range of aromas, wherein acetate esters (hexyl acetate, 2-methyl butyl acetate), alcohols (hexanol), and aldehydes (2-hexenal) stand out.

Currently, the cultivation of 'Anna' apple trees faces difficulties in highland tropics affecting yields. The lack of apple tree commerce and

research is due to low production concerning domestic demand, leading to the import of other varieties and affecting the economy of local production systems (Patiño & Miranda, 2013). In addition, lack of knowledge of the producers about the management of continuous harvests for this apple tree prevents the increase of the yield.

Given the importance and productive potential of 'Anna' apple tree crops in the production systems of the highland tropics, this review was carried out to show the advances that have been made in this crop.

Botany and Morphology. The common apple tree or domestic apple tree (*Malus Domestica* Borkh.) belongs to the family Rosaceae, subfamily Maloideae (Pomoideae), and genus Malus, from which interspecific hybrids of *Malus pumilia* Mill., *M. baccata* (L.) Borkh., *M. sieversii* (Ledeb.) *M. Roem*, *M. prunifolia* (Willd.) Borkh. and *M. silvestris* (L.) Mill. are derived from Europe and Asia (Luby, 2003). The apple crop has been classified as one of the most widely distributed and commercialized deciduous trees globally (Skinner *et al*., 2018).

The 'Anna' apple tree is a deciduous tree whose height depends on the rootstock's vigor (Jackson & Palmer, 2010). In the highland tropics, it can reach a height of 2 m with a rootstock of medium vigor and up to 20 m with a rootstock propagated by seed (Casierra-Posada, 2012). The growth habit of this apple tree varies from rounded to pyramidal in the crown but, under tropical conditions, the growth of branches tends to be vertical and straight due to the effect of acute apical dominance (Cárdenas & Fischer, 2013). Apple blossoms can start both in the terminal buds and axillary shoots, depending on when they are cultivated. The 'Anna' apple is characterized by flowering in buds on the lateral shoots, making it a precocious and prolific variety (Campos, 2013). Apple tree flowers are hermaphroditic and have a medium peduncle, a corolla with five white petals mottled with pink, a calyx with five sepals, five pistils, and twenty yellow stamens (Jackson & Palmer, 2010). These flowers are organized in a simple top-type inflorescence, made up of five epigene hermaphrodite flowers, where, in most cases, the middle flower is the first to position itself in a terminal shape and is the most developed in size and quality (Dennis, 2003; Casierra-Posada, 2012).

The variety 'Anna' fruit is a pommel with a red peel where it receives sunlight and yellow-greenish otherwise on the other side. Its pulp is white with a crunchy texture and a sweet and slightly acid flavor. It is elongated, with a medium-size and average weight of 180g (Campos, 2013). Its seeds come from five carpels formed from tissues in the mesocarp and receptacle (Jackson & Palmer, 2010).

Ecophysiology. Deciduous fruit trees are native to temperate and subtropical zones; that is to say, they are adapted to strongly marked and different climatic seasons. Climatic and environmental conditions highly influence the production of these fruit trees under tropical conditions since they are little changed throughout the year (Fischer *et al*., 2016), which can hinder the development and production of these species. In addition, global climate change is a new challenge for tropical producer that alters constant climatic variables. According to Ortiz-Bobea *et al*. (2021), the global mean temperature increased by 1.2°C from 1960

to 2020 and is predicted to increase in the 21st century by 3.2°C (UNEP, 2019) which will have a significant impact on vegetation and affect the agro-ecosystems. For example, the highlands subtropical zone of Uttarakhand (India) is affected by global warming and traditionally has several apple trees, but now, they no longer produce because of changes in phenology and poor bud sprouting. The best alternative was implementing the 'Anna' variety because of its low chilling requirement (Nautiyal *et al*., 2020).

In the tropics, this variety has been managed with different techniques to obtain two crops a year (continuous cropping) (Fischer, 1993a) that depend on specific environmental conditions in some areas of the highland tropics.

Light, solar radiation and photoperiod. Plants typically capture light energy from solar radiation to synthesize photo-assimilates and gains in biomass production (Lal, 2018). Plants can distinguish the effect of light through three components: light intensity, light quality, and hours of light per day (photoperiod). However, photoperiod does not drastically affect deciduous trees grown under tropical conditions because of minimal variation (Casierra-Posada *et al*., 2008).

In Colombian areas where the 'Anna' apple is grown, the hours of direct sunlight vary between 800 and 2000 hours per year (Fischer, 2013), which represents the light intensity and has a very sensitive relationship with the growth and development of a tree. The low light intensity can affect the induction of flower buds, differentiation, fruit set, shape, size, color, and quality of deciduous fruits (Fischer & Orduz-Rodríguez, 2012). However, low lighting can increase sprouting and break 'Anna' apple tree dormancy because of the degradation of growth inhibitors (Fischer, 1992). On the contrary, high intensity can cause light stress, which induces damage to photosystem II (PS II) and degrades the D1 protein, leading to photoinhibition (Lal, 2018). Likewise, if the apple tree is at rest, an increase in the temperature of bud tissue because of high luminosity will make it challenging to generate new shoots (Fischer, 2013). In pomáceas, a light level is needed within the tree canopy of no less than 30% (Dussi, 2007). To improve these levels, horizontal bending (crowding) is used on the primary branches, which allows a greater incidence of light on the flower buds and a higher photosynthetic rate of the lower leaves.

UV radiation has a directly proportional relationship with altitude. When apple trees receive high levels of UV rays, the flavonoid pathway is affected, and the levels of anthocyanins, flavonols, and proanthocyanidins in the pommel peel are influenced, regulated by the expression of the *FLS*, *HY5*, and *MYB10/MYB22* genes (Henry-Kirk *et al*., 2018), these are considered genes involved in the synthesis of phenolic compounds of plant species. A study in the Colombian highland tropics demonstrated a positive correlation between the intensity of UV radiation, altitude, and total anthocyanin contents in 'Anna' apple fruits (Gómez, 2019). It is also important to note that continuous harvesting in the tropics occurs because the leaves are photosynthetically active and receive solar radiation for approximately 11 months, unlike in temperate or subtropical zones where this period is only between 6 and 7 months, which allows them to increase carbohydrate reserves for two harvests (Erez, 2000).

Temperature and altitude. Temperature is one of the variables that are more influenced by global warming and one of the most important for the growth and development of plants (Fischer *et al*., 2022). It influences the speed of physiological processes since it affects the kinetic energy of enzymatic activity (Fischer *et al*., 2016). Under tropical conditions, the temperature is relatively constant throughout the year, and, in 'Anna' apple trees, the average daytime temperature is between 14 and 20°C, facilitating forced production. 18°C is the temperature where the highest growth, flowering, and fruit development occurs (Fischer, 1992) because this variety needs 5731 growing degree days (> 10°C for fruit trees) to have full flowering (El-Agamy *et al*., 2001; Fischer and Orduz-Rodríguez, 2012), which is a low value for cultivars of this species. For minimum or night temperatures, the 'Anna' apple tree is cultivated in Colombian areas where minimum temperatures reach 6.4°C (Fischer, 1993b), which are in optimal ranges for the cultivar's physiology because it requires between 250 to 300 chilling hours (El-Agamy *et al*., 2001).

Westwood (1993) stated that each place and variety needs a minimum altitude to reach enough chilling hours (≤7.2°C). In this regard, commercial 'Anna' apple crops are planted in Colombia at altitudes between 1700 and 2800 m a.s.l., i.e., in the highland tropics (Fischer, 2013). The influence of this factor on 'Anna' apple fruits has been studied at the tropic and subtropical level (Gómez, 2019; Karagiannis *et al*., 2020), and it has been found that, at high altitudes, the apple peel accumulates a higher content of anthocyanins, phenolic compounds, and carbohydrates but lower values of glutamic acid and proteins.

The apple tree resists high temperatures, unlike other deciduous trees, since its reproductive buds can open even at 28°C (Erez, 2000). Casierra-Posada & Quintero (2001) reported that the internal temperature of 'Anna' apple tree buds in the tropics varies according to the day, where a sunny day can have an internal temperature of the bud that is 2.3°C higher than the air, and cloudy and rainy days see a temperature that is higher by 0.57°C. At night, there were no differences. However, a high temperature in the tropics indicates a few chilling hours, so the apple tree is affected since it may suffer irregularity in the opening of the buds, especially in vegetative buds, and insufficient lateral budding (Fischer, 2013). On the other hand, this author noted that a low temperature in the tropics can indicate frost (temperatures below 0°C), which generally occur at altitudes above 2300 m a.s.l., where the 'Anna' apple is grown. In summary, the apple tree yield potential in the highland tropics depends on the presence of a long growing season with moderate temperatures, combined with relatively cool nights to avoid insufficiency of chilling hours or frost damage. This damage is associated with the quality of the fruit because, in apple trees, in the last four weeks before harvest, the temperature must be high during the day but low at night to have good pigmentation, also causing a conical elongated fruit shape (Fischer, 1993c).

Water and Irrigation. The apple tree should be planted in areas with a minimum of 700mm/year. The rain can be monomodal (one rainy season during the year means one harvest per year) or bimodal (two rainy seasons during the year confers two harvests per year) (Fischer, 2013). In monomodal areas (700 to 1000mm/year), there is an accumulation of cold and humidity (as a result of the rainy season) during the dormancy of

the apple trees, which is reflected in good fruit quality at harvest. Bimodal climatic zones (1400 to 2000mm/year) make it possible to obtain two crops with the doubled amount of water; however, the plants can decrease fruit quality if the variety has medium or high chill requirements (Fischer, 1993a). This situation does not occur in the 'Anna' variety since it requires fewer chilling hours, making it potentially cultivable in these areas (Fischer, 2013). High rain intensity with clay and compacted soils can cause waterlogging. If rain occurs in phenological stages such as flowering or during fruit setting, one can witness the fall of flowers, appearance of fungi, and fruit cracking (Fischer and Orduz-Rodríguez, 2012).

An irrigation system that supplies the water needed in the different phenological stages is an essential technique in apple trees because of its advantages in the efficient use of water, the possibility of using fertigation, and a reduction in labor. In deciduous trees, the drip irrigation system, the most common one in the highland tropics, should be used since it presents better coefficients of uniformity in the application of water than sprinkler irrigation systems (Vélez & Álvarez-Herrera, 2012). In the experimental farm Tunguavita (Paipa, Boyacá), for the production of the apple tree 'Anna' it is recommended to use 16 mm hoses with four (4) drippers per plant and flow rates of 6 l/h and, additionally, implement two (2) nebulizers due to its excellent coverage in perimeter (Hernando Paipilla, personal communication, 2021). On the other hand, Soliman *et al*. (2018) studied controlled deficit irrigation under subtropical conditions in 'Anna' apples and found that applying 12,972m^3/tree/year of water obtained a savings of 25% and fruit set and yield that were similar to those of irrigated trees without water restrictions.

Nutrition. For 'Anna' apple trees to have two harvests in one year, the producer must supply the nutritional requirements at the right time and in sufficient quantities. One of the tools studied in 'Anna' apple trees is a nutritional diagnosis based on a foliar analysis since these values are the result of a relationship between the agro-climatic factors, variety, state of development, and management (Casierra-Posada, 2012). Casierra-Posada *et al*. (2003a) examined the nutritional status of branches, leaves, and fruits on 'Anna' apple trees to analyze the incidence of nitrogen (N), phosphorus (P), potassium (K^+), calcium (Ca^{2+}), magnesium (Mg^{2+}), sulfur (S) and some minor elements regarding the days after flowering (daf). In the branches, all nutrients presented a higher concentration at the beginning than at the end of the season, except for iron (Fe) and copper (Cu), which may indicate that this variety does not require a large amount of these elements and had good availability in the soil.

In the leaves, the concentration of N, B, Zn, Mg^{2+}, P and S increased in the first 46 daf and decreased in the days close to harvest, which was related to the pre-harvest metabolic process of fruit formation. In the fruits, the Ca^{2+}, Fe, and Cu increased in concentration during development, and Cu increased because it is an active ingredient in defoliants and protective products commonly applied in apple tree cultivation (Casierra-Posada *et al*., 2003a). Minor elements are necessary for apple trees; for example, a boron deficiency can cause bitter fruits and the internal decomposition of apples (Andersen & Crocker, 2009). In the 'Anna' apple tree, a zinc deficiency can cause "small leaf' disease and become unfruitful or produce only tiny fruits (Jackson & Palmer, 2010). The terminals may die, and spurs lose their leaves

and become unfruitful or produce only tiny fruits. Likewise, minor elements can affect production and physiology (Table 1). Other studies on 'Anna' apple trees have suggested that nutritional relationships also depend on irrigation and the state of development of the tree (Abdel-Sattar & Kotb, 2021).

The balance of nutrients within plants must also be considered since there may be synergism and antagonism between nutrients. Casierra-Posada *et al*. (2004) stated that the relationships and interactions between nutrients directly affect the behavior and quality of postharvest fruits.

In the 'Anna' apple tree, several fertilization options for nutritional management have been reported both in the tropics and in the subtropics. Table 1 shows the studies on form and types of fertilization, best compounds, and concentrations that have shown better yields in this variety. It is necessary to highlight that fertilization and irrigation for the 'Anna' apple tree should be done just after harvest and before defoliation for continuous harvests since they stimulate this process and advance harvesting (Fischer, 1993b).

Rootstock and Propagation. The 'Anna' apple tree is a vigorous plant and highly susceptible to the fungus *Venturia inaequalis* (Cooke) Winter and the woolly aphid (*Erisoma lanigerum* Hausm.), making it necessary to graft it into a pattern with dwarfed and rustic characteristics (Casierra-Posada, 2012). Studies in the highland tropics and subtropical conditions have reported that grafting 'Anna' on the MM-106 rootstock has the advantages of reducing vigor, accelerating growth (earliness), reducing woolly aphid attacks, presenting a larger trunk diameter, requiring low oxygen levels in the soil (5% O_2). Moreover, responding better to applications of hydrogenated cyanamide (Schwarz, 1993, Andersen & Crocker, 2009; Fischer & Orduz-Rodríguez, 2012; El-Sabagh *et al*., 2012). However, Casierra-Posada (2012) observed

Table 1. Results obtained with different types of fertilization in *Malus domestica* Borkh. cv. 'Anna' in the tropics and subtropics.

Type of fertilization	Applied substances	Results or yields	Source
Foliar fertilization for vegetative growth in seedlings	0.8 g/L of MICRO NATE (0.15% Cu – 0.20% Zn – 2% Mn - 0.4% B - 9.3% Fe – 2% Mg - 0.10% Mo)	230.4 leaf/plant 53.34% dry matter in leaves	Alkamed *et al*. (2020) (subtropics)
Soil organic fertilization	8 kg/tree of effective microorganisms (EM) in the form of Bokashi	46.65kg apples/tree	Sahain *et al*. (2007) (subtropics)
Foliar organic fertilization	30 cm^3/tree of humic acids	36kg apples/tree	Morsey *et al*. (2015) (subtropics)
Foliar fertilization with Zn and B	300 mg/L of B as borax and 1.5 g/L Zn as chelate (other complete nutrients)	54kg apples/tree	Kassem *et al*. (2016) (subtropics)
Soil fertilization	500 – 1500 g/tree (according to the apple tree age) of 10-20-20 NPK + 20 – 30 g/tree of B + 500 g/tree of dolomitic lime	61.5kg apples/tree	Casierra-Posada (1992) (tropical highland)

the appearance of root nodules (burrknots) in the MM-106 pattern in the highland tropics, which is a physiological disorder of these rootstocks that limits the transport of fluids from the vascular system of the plant and allows the entry of phytopathogens, rendering the plant unproductive. Another problem with this pattern is that it is very susceptible to flooding because of *Phytophthora cactorum* (Lebert and Cohn) Schröt fungus (Campos, 2013). On the other hand, another rootstock commonly used in the subtropics and with good potential for use in the tropics is MM-111, since it shows good results in terms of fruit weight and volume, resistance to *P. cactorum,* and high vigor when grafted with 'Anna' (Ikinci & Bolat, 2019).

In the Colombian highland tropics, propagation with bank layering or marking is widely used to obtain the rootstocks MM-106 and MM-111, providing an approximate multiplication rate of 8:1 every eight months, depending on the environmental conditions of the area (Casierra-Posada, 2012). However, the rootstock MM-111 lacks mother plants in the tropics, so it is recommended to include it in different propagation programs. Studies carried out in the Colombian highland tropics on the propagation of the MM-106 rootstock showed that, by using patterns with four shoots/plant in the mound layer, new rootstocks with greater length, size, leaves, and quality are obtained (Pulido & Rodriguez, 2018). These same authors reported that, when applying 50 ppm of gibberellic acid (GA), the MM-106 patterns had a higher dry and fresh weight of the stem and root, increasing the production of shoots. Acero & Sosa (2018) confirmed more vigorous stems in MM-106 rootstocks when applying doses of GA and reported that 20g/plant of mycorrhizaes improved the quality, height, number of roots, and foliar area of these rootstocks.

Management and Phenology. The 'Anna' apple tree is precocious compared to other varieties in the highland tropics since it manages to complete fruit development in 120 days (Casierra-Posada, 2012). The crop stages proceed from harvest to harvest as follows: harvest - leaf fall - dormancy - flower induction - flower differentiation - pollination - growth and development of fruits - harvest.

Floral induction and differentiation are the most critical physiological stages to manage for continuous crops (Casierra-Posada, 2012). These metabolic processes must occur early after harvest, that is, in pre-dormancy before the tree enters root dormancy (deep dormancy) (Fischer, 1993a). The floral induction of the apple tree occurs when the longitudinal vegetative growth of branches ceases in the middle third of fruit development and may not occur because of excess available nitrogen (Fischer, 1993b). In addition, complete differentiation of the flower bud can last between 4 to 13 weeks (Buban & Faust, 1982).

In deciduous trees, a fully differentiated bud during the vegetative cycle will not sprout as long as there are leaves in total growth since they produce inhibitory hormones, such as abscisic acid; for this reason, leaf drop or defoliation of these fruit trees is necessary. Another influencing factor in the formation of flower buds of this species is the leaf-fruit ratio, which for the apple tree ranges between 20 and 50:1 (Fischer, 1993b), making fruit thinning in the resting season important. In addition, Dennis (2003) stated that if you want to achieve early, high and uniform sprouting, it is necessary to avoid conditions

of high temperatures and non-uniform light intensities within the canopy, and it must be taken into account the use of not vigorous rootstocks, the subjection to hydric stress, defoliation, branch bending, fruit thinning and reduction of nitrogen fertilization that can advance phenology.

In the tropics, as a result of the climatic conditions (bimodal climate), a low chilling requirement and great adaptability from the apple tree 'Anna,' and specific agronomic management has been proposed for the growth and development of the apple tree, where two continuous harvests can be obtained in one year, that is, a forced production (Fischer, 1992; Fischer, 1993a; Casierra-Posada, 1993). This management consists of the following agronomic practices: harvest - water stress - defoliation - pruning and tie-down of branches - application of chemical products - bud sprouting - pollination - fruit growth and development - harvest

Management in Forced Production

Water stress - Water suppression. A technique commonly implemented by apple growers in the tropics to produce continuous crops is induced droughts during rest, which occurs after the harvest since the trees are without fruits and require less water (Fischer, 2013). The purpose of this process is to increase bud sprouting since droughts reduce vegetative growth, suspend apical dominance, and promote differentiation of existing buds (Fischer, 1993a). This same author also stated that the timing and duration of this technique are essential for apple trees since a very early drought can cause narrower flowers, slower floral development, and irregular budding. It should be done just after harvest and should last about four weeks. Studies carried out in Caldas (Colombia) on water deficits in 'Anna' apple trees reported that stress of 15 days after harvest combined with the bending of branches and an application of Dormex® (hydrogen cyanamide) showed the highest sprouting percentage (52%) of horizontal buds under tropical conditions (Gonzáles & Aristizábal, 1998).

This technique is also used in subtropical climates, as Abdel-Sattar & Kotb (2021) reported, who found that, during the dormant season of 'Anna' apple trees, a 45-day drought increased physiological parameters, such as the number of open flowers, percentage of floral induction, fruit set, and yield per tree, and an increase in fruit quality parameters such as firmness, total soluble solids (TSS), total sugars, and anthocyanin content.

Defoliation and leaf fall. The loss of leaves is a fundamental process in continuous harvests, which generates a decrease in the concentration of abscisic acid, which increases the concentration of gibberellic acid and cytokinins in reproductive buds and advances sprouting (Casierra-Posada, 2012). In temperate zones, this phenomenon occurs naturally; however, under constant conditions of moderate temperatures and tropical photoperiods, trees do not defoliate 100% (Pinzón *et al*., 2014), so manual defoliation must be induced. Chemical compounds used as defoliants in 'Anna' apple trees include copper sulfate (5%), magnesium chlorate (0.72%), urea (12%), and Koccide 101® (2.5%) (Fischer, 1993a).

Defoliation is directly related to the time it is carried out after harvest. If it is carried out very early, it can inhibit floral induction because of a lack of accumulated carbohydrates, nitrogen, and phosphorus

(Fischer & Lüdders, 1995; Álvaro Castro, personal communication, 2021). On the contrary, if it is done too late, sprouting is reduced because the apple tree goes into deep dormancy (Erez, 2000). Fischer (2013) proposed that the exact moment after harvest to start defoliation in apple trees under tropical conditions is when the color of the leaf blade turns from green to yellow, and the natural leaf fall begins. Similarly, Casierra-Posada *et al*. (2008) evaluated the most appropriate time to defoliate 'Anna' apple trees in the Colombian highlands. They concluded that two weeks after harvest is the appropriate time to do so when the highest bud sprouting occurs, which gives time for the translocation and absorption of organic compounds for the next harvest, similar to that reported by Mohamed (2008) for subtropical conditions. This author found that defoliation treatments in 'Anna' apple trees obtained a higher fruit set, sprouting, and yield than those treated with the Dormex® chilling compensator for two seasons in a row. In any case, systems with one harvest per year also need to defoliate trees if the leaves do not fall naturally and completely.

Pruning and tie-down. For continuous harvest, severe pruning should not be carried out since sprouting, and the growth of many buds could be stimulated, which demand large reserves, reduce sprouting, and delay harvests (Fischer *et al*., 2010) because pruning stimulates the accumulation of gibberellins, cytokinins, and auxins in bud sprouting. In addition, pruned plants adapt better to prolonged droughts (He & Schupp, 2018). During the growth and development of 'Anna' apple trees, the formation of a modified open glass canopy is widespread and enables the formation of a central axis. However, pruning seeks to reduce the growth of new branches with vertical growths, pruning three buds that would later induce spurs (Fischer, 2013). Thus, they are stored for the following season instead of consuming the photoassimilates for vegetative growth. Likewise, Fischer (2013) stated that pruning should be done after defoliation for continuous harvests to facilitate the previous translocation of nutrients from the leaves to the plant.

In young 'Anna' apple trees, the technique of tying lateral branches to a horizontal position is a crucial practice that reduces vegetative development, induces precocity, and allows solar radiation to reach the lower buds (Fischer, 1993a; Dussi, 2007). Gonzáles & Aristizábal (1998) reported that the bending of branches produced a positive effect in terms of bud sprouting.

Dormancy and chilling hours. The precise agro-climatic conditions that make forced harvests possible are particular characteristics of the highland tropics and promote the cultivation of 'Anna' apple trees. However, it should be clarified that physiologically speaking, when a plant enters dormancy, the external growth in any plant structure that contains a meristem is temporarily suspended (Lang *et al*., 1987). This mechanism serves as an adaptability mechanism to withstand cold winters in temperate zones.

'Anna' apple trees under continuous harvests do not enter endodormancy, so it is not a break from rest but rather a suppression of rest because the flower buds differ shortly after harvest (thanks to the conditions of the site, variety, and techniques), and the tree is in pre-dormancy (Westwood, 1993). The chemical substances applied after irrigation, defoliation, pruning, and tying stimulate the sprouting of buds; in addition, they advance

flowering and harvest. It should be clarified that, when implementing continuous harvests in apple trees and applying chemical or exogenous products to the plant, they are not cold compensators because they are added when the plant is in a state of pre-dormancy and not endodormancy.

In the tropics, quantifying chilling hours is best done with the Utah model of Richardson *et al*. (1974) because it includes the effect of hours that pass 7.2°C and does not consider the chilling hours received the night before when the daytime temperatures are too high. Even though 'Anna' apple trees have a low requirement for chill hours (250-300), the measurement system for these hours is not relevant in the production of continuous crops since the plants do not go into endodormancy (Westwood, 1993).

When handling one harvest per year in 'Anna' apple trees, it is advisable to do so in climates with a monomodal rainy season because of the accumulation of cold and low humidity for the dormancy state of the plants (Fischer, 1993a). With this production type, the apple tree does enter endodormancy, so the applied substances will function as dormancy-breaking agents or break the rest period.

Bud sprouting - Dormant break or suppress. the development of flower buds in 'Anna' apple trees is regulated by the *MdFLC* gene, which responds positively to cold exposure during endodormancy, as compared to other cultivars (Nishiyama *et al*., 2019). In the highland tropics, continuous harvests do not need applications of dormancy-breaking agents. However, some substances increase bud sprouting and suppress endodormancy (Fischer, 1992; Gonzáles & Aristizábal, 1998). Applications are not strictly necessary for bimodal zones or even in monomodal zones in the tropics; the application of substances that help break the internal rest of plants is essential (Figure 1).

In the tropics, according to Fischer (1993a), concentrations of 0.50% to 0.75% of hydrogenated cyanamide (Dormex®) + 2% to 3% of mineral oil are used to break dormancy and improve sprouting in the 'Anna' variety since the latter increases the anaerobic respiratory rate, producing ethanol and acetaldehyde, which increase sprouting, similar to the report by Gonzáles & Aristizábal (1998). Dormex® applications are used because of its effect on plants, where cyanamide inhibits catalase, caused by the reaction of the nitrile group with thiols and hematin of the enzyme. This leads to an overflow of hydrogen peroxide (H_2O_2) and causes the oxidation of nicotinamide adenine dinucleotide (NADH) to NAD +, an essential component of the glucose-6-dehydrogenase coenzyme, which initiates the oxidative pathway of pentose phosphate (PPP) in glucose, a standard process for the growth and early fruiting of shoots (Amberger, 2013; Wang *et al*., 2021).

Potassium nitrate (KNO_3) acts on the budding of apple tree buds. It should be applied in pre-dormancy states, mainly promoting flower buds with 5 to 7% concentrations in the tropics (Fischer, 1993a). On the other hand, by applying KNO_3 at the beginning of dormancy, vegetative buds are prevented from entering endodormancy (Erez *et al*., 1998).

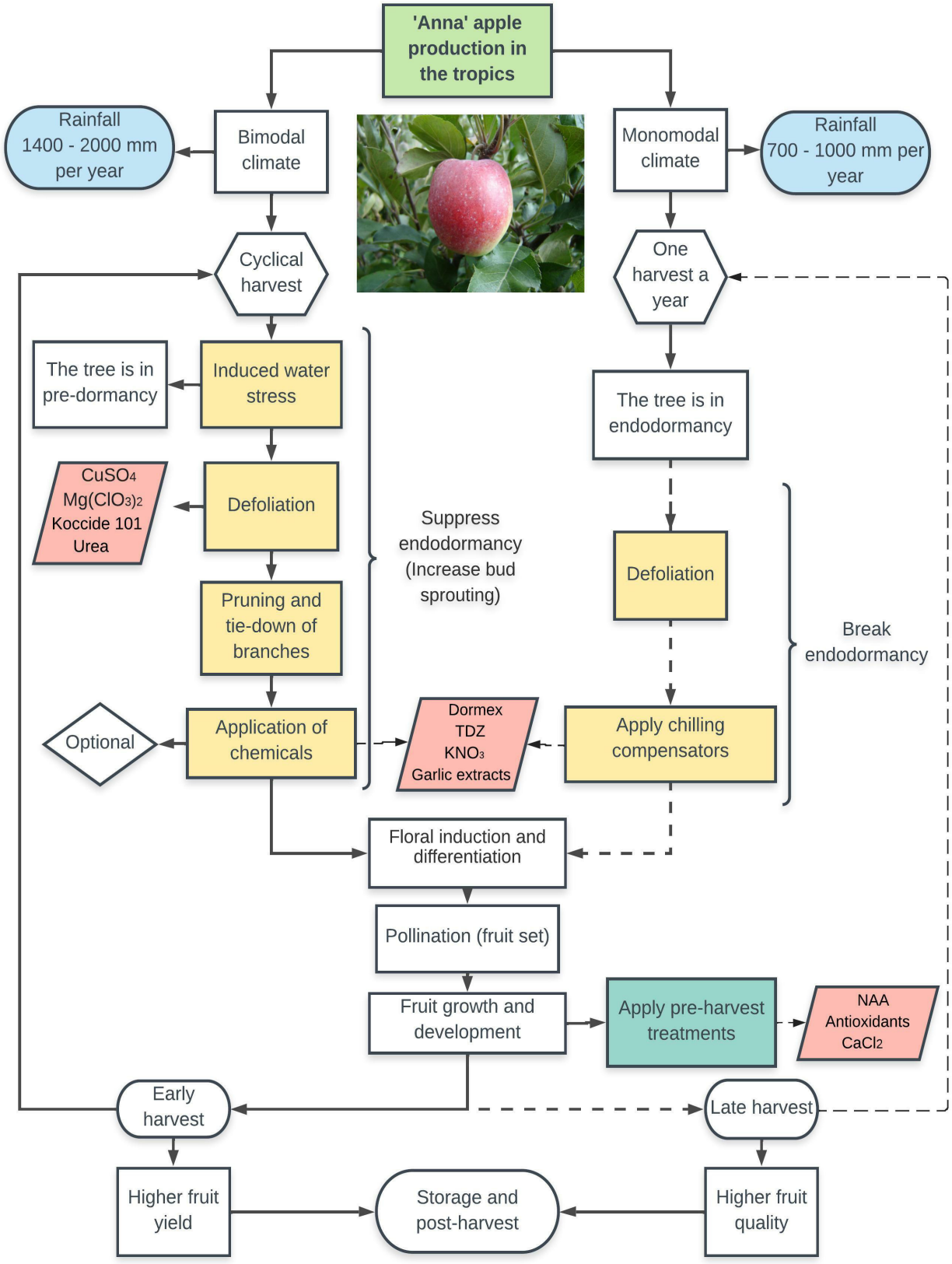

Figure 1. Management of continuous harvests and one harvest per year in the 'Anna' apple tree in the highland tropics.

Thidiazuron (TDZ) is a cytokinin analog used to increase bud sprouting and reduce chilling requirements. In Colombia, the application of TDZ has been reported in peach trees, promoting sprouting and accelerating regrowth in old branches with concentrations of 250mg/L (Fischer, 1993a). Steffens & Stutte (1989) reported that using concentrations of 750µM of TDZ in shoots of apple tree 'Anna' before entering endodormancy decreased the cold units and promoted the sprouting of flower buds.

Under subtropical conditions, El-Yazal *et al*. (2014b) analyzed the effect of Dormex®, potassium nitrate (KNO_3), calcium nitrate, mineral oil, and thiourea on the breakdown of dormancy and the protein-hormonal relationship in 'Anna' apple tree branches and found that all substances increased early sprouting. However, Dormex® had a higher percentage of sprouting, fruit set, gibberellic acid, indole 3-acetic acid, arginine, and proline and reduced abscisic acid content, which visualized the latency-hormone relationship within the physiology of apple trees. On the other hand, when 150ml/L of garlic extract and 200ml/L of onion extract were applied in dormancy, the early floral budding, fruit set, growth, yield, and quality of 'Anna' apple trees increased. However, there was a reduction in total phenols in the fruit (Rady & El-Yazal, 2014; El-Yazal & Rady, 2014a). In addition, it has also been shown that applications of gibberellic acid (GA_3) and 1-naphthalene acetic acid (NAA) break dormancy and favor budding, flowering, yield, and quality in 'Anna' fruits (Aristizábal *et al*., 1998; Mostafa & Saleh, 2006).

Pollination. Most apple tree varieties are not self-pollinating, so cross-pollination is required, and more than one cultivar must be planted (Andersen & Crocker, 2009). In the Colombian highland tropics, the variety 'Dorsett Golden' is commonly used as a pollinator for 'Anna' at a ratio of 1:8, respectively (Casierra-Posada, 2012). The variety 'Ein Shemer' was introduced in these areas as a pollinator for 'Anna' (Fischer, 2013); however, 'Anna' sprouts earlier than 'Ein Shemer' because of the chilling hour requirement, which caused a failure in the flowering timing.

The biodiversity of pollinating agents in the tropics is significant for deciduous fruit trees since it considerably increases the percentages of fruit set. In Antioquia (Colombia), the diversity of pollinators in 'Anna' apple tree flowers was evaluated, where the entomophilic pollination increased the percentage of fruit set and the number of fruits per tree. Findings revealed six different orders of visiting insects in the flowers, mainly Hymenoptera, Diptera, and Lepidoptera, where the hymenopteran *Apis mellifera* L. stood out, which predominated in between 68 and 87% of the flowers in all the orchards evaluated in the highland tropics (Botero & Morales, 2000).

Harvest and Fruit Quality. Cepeda *et al*. (2021) determined the biochemical changes during fruit development, confirmed that growth is a simple sigmoid type, and managed to identify three growth phases: a first slow growth phase of 159 growing degrees day (GDD) at 30 days after anthesis (daa) with very little dry mass growth. A second phase with rapid growth of dry matter that ended at 823 GDD (90 daa); and, finally, a maturation phase that ends at harvest at 892 GDD (100 daa) and does not show a significant increase in dry mass. The latter was similar to that reported by Casierra-Posada *et al*. (2003b),

except for the time until harvest, which was 120 daa in the same area, indicating that the 'Anna' apple has a period from anthesis to harvest of 100 to 120 days that is influenced by environmental conditions. The thinning of fruits, foliar fertilization, and pre-harvest treatments should be carried out 30 dda to affect fruit growth. Casierra-Posada *et al*. (2003a) stated that the foliar fertilization of 'Anna' apple trees must be carried out before 46 daa since, at this point, the maximum concentration of nutrients occurs.

At harvest, the 'Anna' apple fruits had firmness values close to 38.38N, soluble solids contents of 8.58°Brix, and total titratable acidity of 0.7% (Cepeda *et al*., 2021), where firmness was used as an index of physiological maturity, and the harvest index was the time from anthesis to harvest. For continuous, staggered harvests and production throughout the year, 6 to 7 plots scheduled for fruit harvesting every month are recommended (Álvaro Castro, personal communication, 2021).

The 'Anna' apple is a climacteric fruit with high ethylene production, unlike other varieties (Singh *et al*., 2017), reducing storage time and making it essential to carry out pre-harvest methods to increase quality. Treatments that have been shown to be effective at increasing yield and improving fruit quality include foliar applications of naphthaleneacetic acid (NAA) 15 days after flowering with concentrations of 25mg/kg, which make it possible to increase the number of fruits per tree, mass, and pommel size (Aristizábal *et al*., 1998). Likewise, calcium chloride ($CaCl_2$) at 6% applied 45 days after anthesis increased acidity and firmness and decreased pH and degrees Brix (Holguín *et al*., 1998). Other substances studied in the subtropics, with promising results in the tropics for improving 'Anna' fruit quality, include boric acid, amino ethoxy vinyl glycine, p-coumaric acid, lyso-phosphatidylethanolamine, and some antioxidant compounds (Hafez & Haggag, 2007; Eissa *et al*., 2012; Kotb, 2019).

CONCLUSIONS

The 'Anna' apple tree has low chilling requirements and great productive potential for the Colombian tropical zones, and a high adaptive value to sites affected by climate change. In Colombia, the productive systems are located between 1700 to 2800 m a.s.l. (cold and moderate cold climate) with a positive correlation between altitude and exocarp pigmentation, accompanied by mild temperatures during the day (18°C) and cool nights that help in the formation and quality of the fruit.

Fertilization is carried out in the first 46 days after flowering when the plant takes the highest amount of elements, mainly N, P, S, B and Zn. Boron and zinc are essential for bud sprouting. Excess nitrogen fertilization affects floral differentiation. Thanks to its early and low vigor characteristics, the most widely used rootstock in the tropics is the MM-106.

In bimodal climates of the tropics, to obtain cyclical harvests and increase the sprouting of flower buds, water stress must be carried out 2 to 4 weeks after harvest, followed by defoliation 15 days after harvest. After defoliation, light pruning should stimulate growth regulators to increase bud sprouting and control tree reserves. The bending of branches should be carried out in young apple trees to increase the light levels within the

canopy and thus encourage buds sprouting. In addition, chemicals that increase flower bud sproutings, such as Dormex®, TDZ, or KNO_3, can be applied.

The cross-pollination of 'Anna' is carried out in the tropics with the variety 'Dorsett Golden', where the primary pollinating agent is the bee *Apis mellifera* L. The growth and development of the fruit occur during 100 to 120 days after anthesis, with firmness values of 38.38 N, soluble solids content of 8.58 °Brix, and total titratable acidity of 0.7% in the fruit. Preharvest treatments with NAA and $CaCl_2$ have increased productivity and fruit quality in these apple trees. It is recommended to open the investigation to other substances studied in the subtropics and with promising results in the tropics.

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