ABSTRACT

Flowering is an amazing, integrated and complex process of multifatorial control, which due to its great importance for agriculture has been extensively studied worldwide. Pineapple is one of the main crops on which many research works about this subject have been carried out, and it was the first one to have artificially induced flowering. However, even with all those studies, pineapple flowering is not yet characterized in many aspects. The main point for the comprehension of the physiologic aspects of flowering initiation is the identification of the factors involved in the differentiation of the apical meristem into floral primordium, as well as how these factors exert their action. This work aimed to describe the flowering process of pineapple plants, including notions about general flowering physiology, pineapple inflorescence, natural and artificial flowering. Flowering relation to several chemical substances which are involved with the vegetative growth of the plant are also discussed, in order to bring out more light on its underlying mechanisms, and also to help in crop management.

Key words: Ananas comosus var. comosus, inflorescence, flowering control, plant growth regulators.

RESUMO

A floração é um processo integrado, de natureza complexa. Em decorrência de sua grande importância para as plantas, muitos trabalhos têm sido desenvolvidos em todo o mundo sobre o assunto. O abacaxi é uma das principais culturas sobre cuja floração têm sido feitas diversas pesquisas, tendo sido a primeira planta a ter o florescimento induzido artificialmente. Porém, mesmo com todos esses estudos, a floração do abacaxizeiro ainda não está bem caracterizada em muitos aspectos. O principal ponto da fisiologia do florescimento consiste em se entender os fatores que atuam na transformação do meristema vegetativo em primórdio floral, e de que forma exercem suas ações. Esta revisão procura descrever o processo do florescimento do abacaxizeiro, incluindo possíveis tecnologias alternativas de indução e inibição. São abordadas, também, noções gerais de fisiologia do florescimento e da inflorescência da planta, florescimentos natural e induzido, principalmente com relação a substâncias químicas endógenas e exógenas que possuem atividade reguladora do crescimento vegetativo da planta, para melhor entendimento dos seus mecanismos e, consequentemente, do manejo da cultura.

Palavras-chave: Ananas comosus var. comosus, floração, fatores ambientais, reguladores de crescimento.
1. INTRODUCTION

The pineapple *Ananas comosus* var. *comosus* (Coopens & Leal), is an herbaceous monocotyledonous perennial tropical plant, of the Bromeliaceae family. It is cultivated in more than 60 countries, where its fruit presents great demand and economical importance. In Brazil, it is the fifth more cultivated tropical fruit and represents a great option for cultivation in non traditional areas, such as the semi-arid region.

Pineapple plants require careful management and present some morphological and physiological aspects, which knowledge facilitates the understanding of its flowering mechanisms. Among those aspects is the apical meristem, that differentiates into leaves during the vegetative phase, but later undergoes transformations, originating the inflorescence, and thereafter retakes its vegetative activity, producing the crown of the fruit.

The transition from the vegetative phase to flowering is very important for the plants, since flowering is the first step of the sexual reproduction (Bernier et al., 1993), resulting in fruit production, which is the main objective for economical exploration of the plants. Thus, several studies aiming to understand how that transition is controlled have been accomplished in the last decades, in several species, generating a great amount of information (Bernier et al., 1981; Havelange and Bernier, 1983; Bernier, 1988; Evans and King, 1988; Bagnall, 1992).

The objective of this work is to review the flowering process in the pineapple culture, mainly the involvement of some chemical substances which are related to the vegetative growth, in order to better understand its mechanisms and, consequently, help in the management of the crop.

2. NOTIONS ON THE PHYSIOLOGY OF THE FLORAL DIFFERENTIATION

Flowering is an unique and integrated process, of very complex nature and multifactorial control, that has been studied extensively, from ecophysiology to biophysics aspects (Bernier et al., 1981a,b; Bernier et al., 1993; Kinet et al., 1981; Kinet, 1993). Most of the plants react to environmental signals regulating the transition into flowering, since all individuals of a given species have to bloom synchronously for the success of crossings and, also because they should complete sexual reproduction under favorable external conditions (Bernier et al., 1993). In general, natural flowering is stimulated by regular seasonal changes of climatic conditions, such as photoperiodism, thermoperiodism and water balance. Such changes are sensed by different organs of the plant: the photoperiod by the leaves; the temperature by all parts of the plant, although low temperatures are preferably sensed by the stem apex; and the water deficit by the roots (Bernier et al., 1993). In general, the presence of at least one leaf on the plant is necessary for the perception of photoperiodic stimuli (Traub et al., 1939; Wareing and Phillips, 1981; Bernier, 1988). According to Lang (1965), floral initiation delimits the transition between vegetative growth and reproductive phase in seeded plants, being, therefore, an outstanding event in their lives. Flowers are nothing more than modified branches and leaves, produced by modified branch meristems, in other words, the floral primordia.

It is known that the meristem receives from other plant parts permanently a group of signs of variable intensity that are favorable to the production of structures, sometimes vegetative, other times reproductive (Miginiac, 1979). The central subject of the floral initiation physiology consists of understand which factors act in the transformation of the stem apex into a floral primordium and how they play their role (Lang, 1965). The knowledge of those signals has a fundamental and practical importance for a more rational crop exploration. According to Bernier (1988), the main environmental factors responsible for floral induction are the photoperiod (daylength – hours of light) and temperature (vernalization – cold effect). Yet according to that author, it is required that the plant reaches an adequate developmental stage to be induced to flower, being necessary that the leaves capture photoperiodic signals. Once totally accomplished the differentiation of the caulinar meristem into floral primordium, the latter becomes unable to retake the vegetative growth. That is why vegetative growth and reproductive development in plants are events considered mutually exclusive.

In general, two phases are observed during flowering: floral initiation and floral development. In the first one, it is necessary that some events take place in the stem apex and lateral buds, in an irreversible way – with the initiation of flowering, the first step for the formation of flowers, denominated “evocation” (Kinet et al., 1981).

Some theories were formulated to explain flowering, based on several researches carried out along decades, involving aspects on physiology, genetics (related to the sensibility of plants to climatic factors) and adaptation. Studies involving grafting in plants sensible to photoperiod were the base for
Chailachjan, mentioned by Min (1995), to postulate the existence of a hypothetical flowering hormone, called “florigen”. It was also raised the possibility of existing a floral inhibitor, the “antiflorigen”, that would act as an antagonist to the “florigen”. In the case of plants that bloom under the effect of vernalization, another product – the “vernalin” – was associated with the “florigen” and flowering, as discussed by Lang (1965). Among the theories of internal control of flowering induction have been mentioned: the “florigen/antiflorigen” concept; a model of multifactorial control; the hypothesis of the deviation of nutrients, and electric signals (Bernier, 1988). From those, the first two are more accepted, even though there are no definitive proofs. However, Bernier et al. (1993) presented results that support the multifactorial control. According to O’Neel (1992), any explanation about the control mechanisms of flowering by photoperiod should consider the presence not only of promoters but also of inhibitors, what is in agreement with the model of control of the “evocation” proposed by Bernier et al. (1981b). According to these authors, the factors are not the same for different species, and can be synthesized in leaves, roots, stem apex and other organs. If just one factor is absent, the process will not continue, but in general all of them are present under inductive conditions. Some evidences show that flowering in the meristem may consist of several phases, each one being activated individually (Bernier, 1992). However, a lot of questions still need to be clarified.

3. PINEAPPLE FLOWERING

The floral differentiation of pineapple plants has another singularity: it can be triggered artificially, by chemical substances, that are also related to the natural flowering. The flowering process of pineapple plants may be better understood if the life cycle is known. This cycle takes from 12 to 30 months until the first fructescence is produced, depending on environmental conditions and crop management. This cycle can be divided into three phases (Cunha, 1998): a) vegetative phase – involves the period from planting to floral differentiation; b) reproductive phase (flowering and fruiting) – goes from floral differentiation to fruit maturation; c) propagative phase – begins at the productive phase, but continues after the fruit is harvested, involving slips and suckers development until their harvest for planting. Among those phases, the less flexible is the reproductive phase, regardless flowering is naturally or artificially induced. Several factors influence the crop cycle, such as climatic conditions, mineral nutrition, type and weight of planting material and planting time (Gowing, 1961; Mitchell, 1962; Gaillard, 1969; Reinhardt and Cunha, 1982a; Cunha et al., 1993). The susceptibility of the pineapple plant to natural or artificial flowering is related to the age or size of the plant. The first one is related to induction by environmental factors, and the second by means of the use of chemical products, in general plant growth regulators. In both cases, there is the involvement of hormones synthesized by the plant, such as indolacetic acid (IAA) and ethylene, the latter being the real inducer factor (Burg and Burg, 1966). Ethylene is synthesized through a sequential action of the enzymes ACC synthase and ACC oxidase (Yuri et al., 2005). The pineapple crop is commercially explored due to the possibility of controlling the flowering, favoring off-season fruit harvests – when fruit prices are higher, or the production throughout the year.

The natural flowering of pineapple is a very complex process which presents several inconveniences. Pineapple plants tend to flower from the late fall to early winter, although it may also happen in other seasons, depending on the region. Natural flowering can result in serious losses to growers all over the world, because it affects crop management, fruit harvest and sale, reducing incomes for plant and ratoon crops. These losses become worse if flowering is precocious, when plants are not enough developed to produce fruits with commercial value. This fact has been shown both in commercial and experimental plantations. Besides, differences in the susceptibility of plants to flowering have been observed. In general, the largest plants are more susceptible. In spite of that, it has also been observed that small plants can be susceptible to natural and artificial inductive flowering signals. Being also a function of environmental conditions, natural flowering varies from year to year according to the seasons and producing regions, and its occurrence increases in areas of higher altitudes and latitudes. In the main producing regions of the world the occurrence of natural flowering varies from 20% to 80% (Reinhardt et al., 1986; Scott, 1993; Barbosa, 1997; Rebolledo-Martínez et al., 1997).

3.1 Pineapple inflorescence – botanical description, growth and development

The pineapple inflorescence is formed by a group of sessile flowers that are connected with their subtending bracts and among each ather around an axis – the core, which is an elongation of the peduncle, aligned in eight spiral rows, presenting a 8/21 phyllotaxy (Okimoto, 1948).
According to this author, the flowers are hermaphrodite, presenting three sepals, three petals, six stamens and an inferior, tricarpellate and trilocular ovary, with three nectariferous glands separating the loculi. Pineapple flowers are formed by the same meristem that originates the leaves, located in the apex of the stem. Flowering involves the transition from the differentiation of vegetative structures to the formation of an inflorescence at the apical meristem of the stem (Clark and Kerns, 1942). The first evidence of a morphological change in this meristem is the expansion of the diameter of the disk tissue, with the initial formation of the stalk (peduncle) and of the first flower, which occurs when the meristem exhibits the maximum diameter. This process can be observed about four days after the floral differentiation, by a longitudinal cut of the stem apex (Py and Silvy, 1954). The flowers do not open or mature at the same time, and blossoming proceeds spirally upwards along the inflorescence stalk, with one to several flowers opening each day (just during the first morning hours), during three to four weeks (Okimoto, 1948; Sideris and Krauss, 1938).

Two important aspects of inflorescence development can be distinguished. The first one occurs, approximately, two months after differentiation, and corresponds to the stop of growth of the stalk, the opening of the first flowers and the beginning of the crown growth. The other one occurs around 15 days before harvesting, corresponding to the stop of crown development and wilting of the stalk, with an important flow of sugars to the growing fruit (Teisson, 1973).

3.2 Natural flowering

Factors involved

Basically, the initiation of pineapple flowering depends on the physiological state and nutritional reserve of the plant, day length and temperature (Bartholomew and Malezieux, 1994). According to these authors, a minimum difference between day and night temperatures is necessary to elicit natural flowering, in addition to the effect of short days. Drought stress may also stimulate floral differentiation in areas where changes in photoperiodism and temperature are small (tropical areas). Those authors also reported the occurrence of natural flowering in Hawaii from December to January, a period of minimum temperatures dropping, below 15 °C, usually at night. Also, they mention that plants submitted to constant temperature of 25 °C exhibit high flowering rates at photoperiod of eight hours, as compared to photoperiod of 16 hours. Friend and Lydon (1979) observed that the vegetative growth of ‘Smooth Cayenne’ pineapple plants increased in the same photoperiod interval, during which the crassulacean acid metabolism (CAM) also started to operate. Those authors concluded that the flowering of the pineapple crop is controlled by the photoperiod, being not directly influenced by the dry weight of the plant nor the CAM metabolism. Min and Bartholomew (1997) observed that the production of ethylene and the activity of the ethylene biosynthetic enzyme (ACC oxidase) in the stem and in leaf tissues of pineapple plants cultivated at 30/30 °C (day/night) were lower than that of plants grown at 30/20 °C.

Some authors are unanimous in affirm that the period between planting and fruit maturation is a function of the type and weight or size of the planting material (Gaillard, 1969; Teisson, 1972; Reinhardt et al., 1986). Other authors point out that the age of the plant at the period favorable for floral induction is also related to the process, in addition to environmental factors and cultural treatments that affect the vegetative growth of the plants (Gowing, 1961; Mitchell, 1962; Dalldorff, 1978; Friend and Lydon, 1979; Mekers and De Proft, 1983; Cunha et al., 1993).

Studies carried out to determine the environmental factors involved in natural floral differentiation have shown that this process is related, largely, to the shortening of the day, as well as to low temperature, mainly at night, and low irradiance due to cloudiness (Nightingale, 1942; Van Overbeek and Cruzado, 1948; Gowing, 1961; Teisson, 1972; Friend and Lydon, 1979; Reinhardt et al., 1986). According to Teiwes and Grüneberg (1963), the climatic requirements are characterized by the pineapple great sensibility to frost and very intense solar radiation. Despite not demanding cold climate, temperatures below 17-15 °C promote natural flowering (Bartholomew and Malezieux, 1994). Saneowski et al. (1998) observed a 100% of natural flowering in pineapple plants maintained under 20 °C for 10 to 12 weeks.

According to these authors, although the direct effect of the low temperature on natural flowering is not known, low temperatures and shortening of the day probably cause an increase of ethylene production in the apical meristem and in the basal white portion of the leaf, and thus stimulating flowering. Evidences also demonstrated that low night temperature increases the level of free auxin in the plant, leading to flowering and reducing the demand for short days (Van Overbeek and Cruzado, 1948; Gowing, 1958). However, the direct effect of the low temperature is not yet elucidated (Saneowski et al., 1998).
In Ivory Coast, located at 4° N, where the change in daylength is very small (just about 36 minutes), with little or none seasonal variation in temperature, the stimulus for natural flowering may occur in response to the reduction of the hours of solar radiation (BARTHOLOMEW and KADZIMIN, 1977), and to the slightly lower temperatures, during the months of August and December-January (BARTHOLOMEW and MALÉZIEUX, 1994).

Due to those facts, the pineapple is considered as a quantitative but not an obligate short-day plant, that depends on the cumulative effect of those days (GOWING, 1961; FRIEND and LYDON, 1979; BARTHOLOMEW and MALÉZIEUX, 1994). However, not all the varieties behave equally in relation to the floral stimuli, and some of them are more and others less sensible (VAN OVERBEEK AND CRUZADO, 1948; PY, 1968; BARTHOLOMEW and KADZIMIN, 1977).

The natural flowering of pineapple, besides being influenced by climatic factors, undergoes the effects of the rate of development of the plant, being necessary that the plant reaches an appropriate size – the ontogenetic maturity, to answer to the environmental stimuli (LACOEUILHE, 1975; BARTHOLOMEW and KADZIMIN, 1977), as it was also observed in ornamental Bromeliaceae by MEKERS and DE PROFT (1983). The referred minimum size is reached in shorter periods under favorable conditions, than where the growth is retarded by lack of nutrients and water and by low temperature (BARTHOLOMEW and MALÉZIEUX, 1994). In practice, however, it has been observed that even small plants present some capacity to be induced under favorable floral stimuli, either natural or artificial (CUNHA, 1989b). Plant maturity for flowering is always correlated with the capacity of the plant in converting the exogenous 1-aminocyclopropane-1-carboxylic acid (ACC) into ethylene (Dr. GREEF et al., 1983). SANEWSKI et al. (1998) observed that the rate of ACC (precursor of ethylene) increased about 40% in the winter, when the low average temperature reached 14.5 °C. Plants that had already formed the inflorescence exhibited a much higher ACC oxidase activity in leaves and in the stem than plants in vegetative stage (MIN, 1995). In that case, the ethylene production in leaves was also higher.

Based on the fact that pineapple flowering can be artificially induced by the application of several chemical substances that stimulate the production and/or activity of the ethylene, it can be said, hypothetically, that the natural flowering is driven by the ethylene produced endogenously or by changes in the susceptibility of the plant to this product or both (MIN and BARTHOLOMEW, 1993). Similarly to what happens with other crops, an elevated rate of vegetative growth can inhibit or delay pineapple flowering, by reducing its sensibility to the floral stimuli (EVANS, 1959; WEE and NG, 1968; GAILLARD, 1969). It seems that the balance between the vegetative stage and reproduction (flowering) of pineapple tends towards the first. And, once the plant has reached an appropriate size to become susceptible to the floral stimuli, the environmental factors that promote flowering are those that tend to reduce the rate of vegetative growth, such as reduction in nutrients, water supply, temperature, daylength and solar radiation (BARTHOLOMEW and KADZIMIN, 1977). The younger is the plant, the lower will be its answer to the flowering promoting factors (natural and artificial). Thus, nitrogen fertilization and irrigation, by favoring the vegetative growth of plants, could contribute to inhibit flowering (NIGHTINGALE, 1942; PY and GUYOT, 1970). REINHARDT and CUNHA (1982b), however, did not observe any influence of nitrogen nutrition on the efficiency of artificial induction of flowering. As regarding irrigation, BARTHOLOMEW and MALÉZIEUX (1994) indicated that, inversely, as the growth rate of plants increases in response to water supply, in areas where drought periods are long, natural flowering is anticipated by the increase of plant size. Similar result was observed by Almeida et al. (2000), studying the effect of irrigation on the 'Pêrola' pineapple cycle, when increase of water laminae contributed to anticipate flowering and fruiting, shortening the crop cycle by 22 days. However, when environmental conditions are changing (from low to high solar radiation) or after a cold stress or soon after a replanting or transportation of the planting material, flowering becomes unpredictable and irregular (MEKERS and DE PROFT, 1983).

In different producing areas, the rates of natural flowering are quite variable, but rates of 5% to 10% are very common. In Mexico, where this phenomenon is most important, depending on the climatic conditions, those rates may reach 20% (REBOLLEDOMARTÍNEZ et al., 1997), while in Australia, in some years, they vary from 50% to 70% (SCOTT, 1993). In Brazil, the precocious flowering has become quite frequent in all producing areas, with rates up to 80% (BARBOSA, 1997). In studies at the ‘Recôncavo Baiano’, REINHARDT et al. (1986) reported that natural flowering occurred in different seasons and for long periods, with peaks in the months of March/April (49.9%), May/June (88.9%) and November/December (77.4%), tending to concentrate in the middle of the year (Figure 1). In Hawaii, the natural induction represents now an occasional problem since the production of fruits throughout the year became a common practice (BARTHOLOMEW, 1996).
According to Scott (1993), favorable environmental conditions can accelerate the growth rate of the suckers on the mother-plant, in such a manner that the incidence of precocious natural flowering in the ratoon crop may reach rates of 50% to 70%.

In relation to the planting material, variations observed on natural flowering are mainly due to differences in nutritional content and physiological state among its several types. The most precocious is the sucker and the latest one the crown, while the slip presents an intermediate behavior (Reyes, 1997). Giacomelli et al. (1984) observed that the plant cycle was influenced by the mass of planting material, with suckers of 700-800 g producing fruits much earlier than the ones of 300-400 g.

All those aspects lead to the conclusion that natural flowering is still one of the main problems of the pineapple crop, and that it can occur even in plantations carefully planned and managed in order to avoid this incidence.

Natural Flowering Control

Generally, the prevention of flowering in crops can be done by several methods: a) interrupting the night or dark period with light; b) increasing the temperature; c) pruning leaves and branches; d) cutting water supply; e) applying appropriate chemical products (Nickell, 1982). In the case of pineapple, precocious natural flowering can be controlled or at least minimized by adopting the following measures: a) planting suckers, slips or crowns that reach an appropriate size/weight before flowering or at the beginning of the period favorable for natural differentiation; b) the use of planting material that may go through the period of natural induction without having reached enough vigor to answer to environmental stimuli; c) an appropriate management of the crop, in order to turn the plants less sensible to natural flowering inducers; d) or, finally, to carry out artificial induction and thus avoiding the effects of climatic factors (Cunha, 1998). Another important method is the use of plants less sensible to natural flowering stimuli, because as mentioned previously there are differences among varieties. Botella et al. (2000) are trying to produce transgenic pineapple plants that carry sense and antisense copies of the gene of ACC synthase, in order to reduce its expression and suppress precocious natural flowering. The main objective of that work is to obtain plants that do not produce ethylene in response to the reduction of temperature, thereby inhibiting natural flowering.
Studies on transgenic tomato plants and mutants demonstrated this possibility (Hamilton et al., 1990; Klee and Romano, 1994; Lanahan et al., 1994; Bowler and Chua, 1994). Yuri et al. (2002) cloned a pineapple ACC sintase gene (ACACS1), obtained from its apical meristem, which is activated by environmental conditions that induce natural flowering. According to these authors, plants containing ACACS1 with sense orientation capable of inhibiting the gene by antisense-suppression mechanisms, have already been developed.

According to Bartholomew (1996), pineapple natural flowering can be minimized by growing plants at the best possible conditions and by planting only small slips and suckers which are less susceptible to natural induction.

In many horticultural crops, chance of inhibiting, inducing or delaying flowering, which leads to reduction in economical benefits, is already a fact. The promotion of flowering is practiced on pineapple and other Bromeliaceae, while the inhibition or delay of floral initiation is accomplished in many other crops, such as peach, almond and some flowering plants (Nickell, 1982). Wang (1987) reported that the delay in flowering of apple, pear, plum and cherry is beneficial for avoiding losses due to occasional spring frosts. This delay was reached using aminoetoxyvinilglycine (AVG), an inhibitor of the ACC synthase. The first product used for prevention of flowering was the maleic hidrazide on sugar-cane (whose translocation occurs through the xylem (Lever, 1986), ethylene, under the form of 2-chloroetilfosfonic acid, has been used in sugar-cane crop, at the doses of two liters per hectare, to avoid natural flowering.

Pinon (1986 and 1990) reported that natural flowering was causing many problems to pineapple crops in Martinique, making harvesting and the ratoon crop difficult to manage. In his studies, only one product showed some inhibiting effect (the silver nitrate), but its high costs and high number of applications (up to seven) turned its practical use unviable. Another product that had some effect on flowering was the thiurea, but it caused phytotoxicity. Millar-Watt (1981) had already observed that the silver nitrate at 1.000 mg L⁻¹, applied three times at intervals of 30 days, reduced natural flowering to 27%, against 57% in the control, the same occurring when applied few hours before the artificial induction with ethephon (Sanford and Bartholomew, 1981).

Preliminary studies performed by Cunha (1989b) in Bahia, Brazil, showed the viability of the use of some growth regulators for inhibiting flowering of pineapple. NAA (400 mg L⁻¹) presented the best result – only 5% to 13% of flowering (induced with calcium carbide), perhaps due to its competitive action in reducing the level of the natural auxin in the stem apex (Castro, 1986). Clark and Kerns (1942), Gowing (1961), Millar-Watt (1981) and Sampaio et al. (1998) reported that NAA, at high concentrations and in several applications, inhibited flowering of pineapple. Similar results were observed on Aechmea victoriana (Meckers and De Proft, 1983). Scott (1993) succeeded to reduce the occurrence of precocious flowering on pineapple, from 48.5% to 8.2%, using 2-(3-chlorofenoxi) propionic acid (50 mg L⁻¹), and from 55.2% to 28.5%, with paclobutrazol (160 mg L⁻¹). Rebolledo-Martínez et al. (1997), also using 2-(3-chlorofenoxi) propionic acid (100 mg L⁻¹), in three applications, at intervals of 15 days, obtained inhibition of precocious flowering by 76% and 82% in ‘Smooth Cayenne’ pineapple plantings with 33 and 46 thousand plants per hectare, respectively. In the control treatment, flowering reached 95%, at low density, and 82%, at high one. The best results observed for the higher density may be due to the slower plant growth rate under conditions of higher density. For Rebolledo-Martínez et al. (2000), younger plants are more sensitive to flowering inhibition treatments.

There are evidences that the effect of paclobutrazol, reducing vegetative growth, is due to the interruption of gibberelin synthesis, by inhibition of the oxidation of the kaurene to kaurenolic acid, whose translocation occurs through the xylem (Lever, 1986; Early and Martin, 1989; Burondkar and Gunjate, 1991). When applied on the leaves, the portion really used is the one deposited on the apical bud or on the tender tissues located just below the bud. According to Hillier (1991), decrease in gibberelin content in the sub-apical meristem stops vegetative growth, initiating reproductive development and flowering. Lever (1986) informed that the paclobutrazol, besides retarding plant growth, contributes to reproductive development, formation of buds, production and growth of fruits, and reflects the productivity of fruit trees. Similar effects were also observed in apple (Williams, 1982), peach (Marini, 1987), and walnut pecan (Wood, 1988), as well as in plum, pear and mango (Burondkar and Gunjate, 1991). In mango crop, due to its effect on reduction of vegetative growth favoring the formation of a smaller canopy, paclobutrazol is used to promote and to anticipate flowering, in order to avoid the bi-annual or alternate bearing and to allow off-season harvesting (Burondkar and Gunjate, 1991; Charnovichit and Tongumpai, 1991; Hillier, 1991).
Bartholomew and Min (1996), studying the effects of the environment on growth, flowering and fruiting of pineapple plants, observed that the application of paclobutrazol and uniconazole delayed or inhibited flowering in a significant way. Ethylene production by the white basal tissue of leaves was inhibited by the products mentioned, one to two months after the treatment, probably being one of the factors responsible for flowering delay. According to these authors, under controlled conditions (plants in pots) the products uniconazole, paclobutrazol and 2-(3-chlorofenoxi) propionic acid also inhibited natural flowering, and the first two products are more efficient. However, the inhibition of vegetative growth was a side effect of unknown consequences.

Sampaio et al. (1997) mentioned that the fertilization with nitrogen, such as foliar application of urea, did not affect natural flowering of pineapple. Barbosa et al. (1998) observed that paclobutrazol was the only product to show a significant effect, when applied in June – in Bahia, Brazil, inhibiting up to 82.8% the flowering of pineapple ‘Pérola’. According to these authors, 2-(3-chlorofenoxi) propionic acid showed inhibitory effects, but caused some morphological anomalies in plants (torsion of the foliar rosette and formation of adventitious roots in leaves, initiating from the vascular bunches), while urea and mepiquat chloride did not have any inhibitory effect. Studies performed in Hawaii, by Taniguchi (1999), showed that tebuconazole and propaconazole – growth regulators of the group of triazols with fungicide action and efficient in the control of Chalara paradoxa, inhibited natural flowering of pineapple. Those products are active at low concentrations and are not phytotoxic (Davis et al., 1988).

Both 2-(3-chlorofenoxi) propionic acid and paclobutrazol, in concentrations varying from 90 to 240 mg L$^{-1}$, inhibited significantly natural flowering of pineapple ‘Pérola’, with the rates varying, respectively, from 90.9% to 94.4% and from 67.9% to 78.5% (Cunha, 2001; Cunha et al., 2005). This was possibly due to the reduction of vegetative growth, as shown by the reduction of the “D” leaf length. Scott (1993) attributed the inhibition of flowering of ‘Smooth Cayenne’ pineapple to the reduction of plant biomass, more than to a direct interference of 2-(3-chlorofenoxi) propionic acid in the process of floral differentiation. Min (1995) related the effect of 2-(3-chlorofenoxi) propionic acid as an inhibitor of pineapple flowering to its action as an auxin, favoring ethylene production by the plant stem. Analyzing the contents of 1-aminoclopropane-1-carboxilic acid (ACC – precursor of ethylene) and of 1-(malonylamino) ciclopropane-1-carboxilic acid (MACC) in tissues of plants treated with 2-(3-chlorofenoxi) propionic acid, the author observed an increase of ethylene production, concluding that the mechanisms by which the 2-(3-chlorofenoxi) propionic acid inhibits flowering requires more studies.

Grossman et al. (1989) observed a reduction of 70% in ethylene production in barley and Brassica napus, after five hours, when treated with growth inhibitors – like uniconazole. This decrease was followed by an increase or maintenance of constant ACC and MACC levels, suggesting an inhibition of ACC conversion to ethylene. Min (1995) pointed out that the way of action of auxins as inhibitors of flowering, when applied at high concentrations is not yet understood.

### 3.3 Artificial flowering of pineapple

#### History and advantages

The treatment for floral induction (TFI) of pineapple using appropriate chemical substances – growth regulators or phytohormones – is known for a long time, because this crop fits quite well for this practice (Rodrigues, 1932; Cooper, 1942; Das, 1964, 1965; Das et al., 1965; Burg and Burg, 1966; Dutta, 1966; Cooke and Randall, 1968; Jorgensen, 1969; Guyot and Py, 1970; Raúl Salazar and Danilo Rios, 1971; Bondad, 1973; Norman, 1975; Dallдорf, 1979; Onaha et al., 1983; Cunha, 1998; Maita et al., 1998). The TFI involves technological, economical and social advantages such as: a) rational use of land; b) fructification uniformity and harvest concentration, with cost reduction; c) regular and constant supply of fruits for the industry and the fresh fruit market, and during more favorable commercial periods, without affecting fruit quality; d) easier control of pests and diseases, since flowering can be induced during periods, when the inoculum potential is the least; e) control of fruit weight and size, according to demands of the consumer market; f) increase of the crop revenue, due to a larger number of harvested fruits per area; g) better distribution of labor force and easier administration of the property; h) possibility of exploration of a second or ratoon crop (Cunha et al., 1994). However, some problems may rise as a result of an inadequate application of floral inducers: small fruits with large crowns; elongation and bending of the peduncle; reduction in the number of slips and suckers per plant; damages and deformations to the fruits (very round or conical ones) (Cunha, 1998). All of these reduce drastically the revenue of the crop.
To anticipate and uniformize pineapple flowering has always been a challenge in order to reduce production costs, shorten the relatively long cycle – more than 15 months that the plant requires to floral differentiation – and concentrate fruit harvest within the plantation, which may last up to ten to 12 months.

According to Rodrigues, 1932, smoking was the first procedure used for artificial induction of pineapple flowering, what must happened in the XIX century (in 1885), in Azores Island, and was an accidental discovery. However, only in the decade of 20’ was discovered that the smoke agent that initiated the flowering was the gas ethylene, an unsaturated hydrocarbon.

Since that time, many studies have been done, and several other substances were found capable of inducing flowering, such as auxins and other similar compounds (Van Overbeek, 1945; Groszmann, 1948; Green, 1963; Das et al., 1965; Randhawa et al., 1970; Teisson, 1979; Soler, 1985). Later, in the decade of 30’, growers in Hawaii started to use ethylene directly to promote floral induction of pineapple, as well as acetylene. During the years 40’, it was demonstrated that auxins could also cause flowering of pineapple, and therefore, growers started to use α-naphthalene acetic acid (NAA) (Nickell, 1994). It is not only flowering, but also other physiological processes that can be affected by the use of plant growth regulators.

Based on these results and on the recognition of ethylene as an important plant growth regulator, mainly as a stimulator of physiological process of maturation of fruits (Warner and Leopold, 1969), it is currently accepted that flowering of pineapple is much related to this substance. However, despite its involvement with many physiological processes in plants, the pathway that ethylene uses in those processes and in natural flowering of pineapple and other Bromeliaceae is still not fully known (De Greef et al., 1983; Yang, 1987).

These authors mentioned that maturity for flowering of Aechmea victoriana (an ornamental Bromeliaceae) is correlated with the capacity of the plant to convert the 1-aminociclopropane-1-carboxilic acid (ACC) into ethylene. However no relationship between ethylene production and plant size was demonstrated (Min, 1995). Burg and Burg (1966) did not observe ethylene in pineapple plants with eight months old. According to Botella et al. (2000), ethylene could be responsible for natural flowering of pineapple since low temperature stimulates its biosynthesis, which is regulated by the enzyme ACC sintase.

Substances used and performance

So far, several identified growth regulators are efficient in initiating pineapple flowering. Among these products, the most commercially used are: α-naphthalenic acid (NAA), β-naphthlenic acid (BNA), indolbutiric acid (IBA), 2,4-dichlorofenoxiacetic acid (2,4-D), succinic acid, 2-chloroeltilosfonic acid (ethephon), ethylene (C2H4), acetylene (C2H2), calcium carbide (CaC2), hydroxietilhidrazine (HOH) and β-hydroxietilhidrazine (BOH). However, only a few are used, such as the ethylene, acetylene, calcium carbide and ethephon. In Brazil, the most commonly applied is the calcium carbide (precursor of acetylene), perhaps for being cheaper and of easy handling, but since the decade of 70’, the ethephon had its use spread out. Cooke and Randall (1968) recommended ethephon as an agent of flowering in the pineapple crop, in spite of the fact that its efficiency can be modified by some external factors, as suggested by PY and Guyot (1970). These authors indicated that rain fall and high temperature may have a negative action on that products performance.

It is believed that plant growth regulators act by promoting an increase of ethylene content inside the plant, more precisely in the meristematic zone (Burg and Burg, 1966), where the absorption of the products is faster. There, due to the more intense cellular activity, the stem apex is more sensitive to effects of endogenous auxin. Before being able to carry on its action, ethylene needs to be synthesized by the plant or exogenously supplied (Yang, 1987).

In relation to other hormones, it is thought that ethylene links itself to a receptor molecule, resulting in an active complex that elicits a series of reactions, including modifications in the expression of genes, leading to a wide diversity of physiological effects. Yet according to Yang (1987), the plant response to ethylene can be modified, by control or regulation of the level of this product in tissues through: 1) its addition or removal; 2) stimulation or inhibition of ethylene biosynthesis in tissues; 3) modulation of the receptor concentration; and 4) manipulation of the expression of its dependent gene. Biochemically, ethylene production is controlled by the concentration of the 1-aminociclopropane-1-carboxilic acid (ACC), by the activity of the ethylene biosynthesis enzyme – ACC oxidase (Yang and Hoffman, 1984; Kende, 1989 and 1993), and by the ACC sintase, the primary factor that limits the production of ACC (Min and Bartholomew, 1993).

The steps of ethylene biosynthesis are the following (Kende, 1989):
AHMED and BORA (1987) reported that pineapple flowering occurred in response to the increase of sequential metabolites (sugars, proteins, ascorbic and nucleic acids) in the apical bud, what can be caused by the application of some plant growth regulators at the right concentration and time. It was also observed some structural changes in the stem apex, which is transformed into an inflorescence. DAS BISWAS et al. (1983) reported increases in ethylene level in the caulinar apex, in response to the use of flowering inducers, independent of their application time, but more intensively when applied in June and decreasing until January.

Pineapple flowering is not only related to a series of external factors (daylength, temperature, solar radiation), but also to some internal ones (hormones produced by the own plant). Among those, are the auxins, mainly the indoleacetic acid (IAA), an endogenous plant auxin, which requires high concentrations – 1.000 to 2.000 mg L\(^{-1}\) (LANG, 1965). BURG and BURG (1966) used the pineapple to clarify some apparent contradictions of the interaction “auxin-ethylene” as flowering inducers. According to them, a high concentration of IAA exists in the apical meristem of the plant, which favors or promotes flowering. In that way, in order to induce flowering is necessary to apply substances that can alter the level of IAA in the meristem, which should stay within a specific concentration range during some time. GOWING (1956) assumed that the effect of synthetic auxins is based on the translocation of endogenous auxin (IAA) from its activity sites to the apical meristem of the plant. The IAA, despite being active, is hindered of acting “in loco” by phenolic inhibitors.

The ethephon (2-chloroetilfosfonic acid) is degraded when reaching the internal plant tissues, releasing then ethylene, chlorate and phosphate ions. This degradation is enhanced when the pH of the solution is higher than four (alkaline range) (MAYNARD and SWAN, 1963; DE WILDE, 1971), because ethylene is stable in aqueous solution with low values of pH (acid range). The reactions of release of ethylene by ethephon (1) and of acetylene by calcium carbide (2) are the following:

1. \[
\text{Cl} - \text{CH}_2 - \text{CH}_2 - \text{PO}_3\text{H}_2 + \text{OH}^- \leftrightarrow \text{CH}_2 = \text{CH}_2 + \text{Cl} + \text{H}_3\text{PO}_4
\]

**Ethephon**

2. \[
\text{CaC}_2 + 2\text{H}_2\text{O} \leftrightarrow \text{C}_2\text{H}_2 + \text{Ca(OH)}_2
\]

**Calcium carbide**

**Acetylene**

1-aminocyclopropane-1-carboxilic acid

[Involved enzymes: (a) AdoMet sintetase; (b) ACC sinthase; (c) ACC oxidase].
CASTRO (1986) believes that ethylene makes the tissues of the vegetative apex more sensitive to endogenous auxins. However, the artificial flower induction caused by ethephon is not always uniform (RANDBWA and IYER, 1978; CUNHA 1989a, 1998), as may be observed in areas and periods of high temperature, mainly at night (MIN, 1995; TURNBULL et al., 1999). According to TURNBULL et al. (1993, 1999), local high temperature may be the cause of partial or total failure of the induction with ethephon, by determining a fast drying of its solution on the surface of the leaves, mainly when applied during very hot days. The absorption of this product is very dependent on the ambient temperature and relative humidity, the pH of the inducer solution and the surface where the drops of the solution are deposited. Similar response is observed when the plant is under an active and fast growth period.

Taking into account that the floral differentiation of the pineapple is a physiological answer to the increasing of ethylene content in the apical meristem and that ethephon releases ethylene during degradation (BURG and BURG, 1966), it should be considered the importance that modifications in its concentration and the intensity of factors that influence its degradation exert on the inducer action of that product. Initially, are mentioned those factors that affect the concentration of the product before its absorption by the plant, such as the application method, that interferes directly on the interception of the product by the plant; the rain, that dilutes or drags the solution from the leaves (PY and GUYOT, 1970); the high temperature, causing the kinetic decomposition of the product, with losses of ethylene (BIDDLE et al., 1976); the wind, that drags the droplets before they are absorbed by the plant; and the solar radiation, although in a smaller scale, since the product is relatively stable in the presence of light (DE WILDE, 1971). LOPEZ DE VELÉZ and CUNHA (1983) schematized the influence of those several factors (Figure 2).

Figure 2. Schematic representation of factors that influence the efficiency of ethephon as floral inducer. (Source: LOPEZ DE VELÉZ and CUNHA, 1983)
Several studies have demonstrated drastic effects of the inducer solution pH, application site and environmental conditions (temperature, relative humidity) on the absorption, translocation and degradation of ethylene in the plant (Turnbull et al., 1993). López de Vélez and Cunha (1983) idealized an hypothetical curve of action of coadjuvant products on the performance of ethephon, in which the initial phase indicates concentrations that do not initiate the process of floral differentiation (perhaps because they are too low); the second phase corresponds to the range where it is possible to influence the inducer action with the increase of the pH and the addition of urea to the solution; in the third phase, it becomes almost useless to add coadjuvants due to poor increase of the efficiency of ethephon; the last phase is characterized by a range of concentrations where the efficiency of the ethephon almost is not altered with the use of coadjuvants (Figure 3).

Once the product contacts the leaves, it is confronted with factors that hinder its absorption, especially physical barriers of the cuticular, cellulosic and waxy layers and the abundant trichomes (Noogle and Fritz, 1976). Such obstacles exert a relevant role, because the absorption/dilution process through the cuticle is in general much more important than the penetration by the stolices of the stomata, which are full with water vapor or gases. The highest absorption of ethylene by the pineapple plant occurs through the lower leaf surface – the basal achlorophyllous tissue (Turnbull et al., 1993). Due to this fact, those authors consider important that the inducer application should be driven to the center of the leaf rosette, resulting in an accumulation of the solution at the leaf axil, and allowing a larger period of contact of the product with the abaxial epidermis close to the stem apex. Entering into the cytoplasmic stream, the factors that influence the speed of ethephon degradation acquire great importance, among which the pH of the cytoplasm (Maynard and Swan, 1963). Edgerton and Blanpied (1968) demonstrated that the largest rate of ethylene release in aqueous solution occurs in the pH range between 5.0 and 7.0. Studying the effect of different pH values on the degradation of ethephon in solution and in etiolated tissue, Warner and Leopold (1969) observed the following: in solution, the release of ethephon increased linearly up to the pH 9.0 (maximum value tested), while in the tissue the release rate almost doubled with the elevation of the pH value from 4.0 to 6.0. According to Biddle et al. (1976), the speed of degradation of ethephon depends on the fraction that is in the dianion shape, being essentially found in that state in pH 9.0.

Plant response to the use of floral inducers is very fast, having been demonstrated that within four days after the application of the product it can already be observed the beginning of differentiation, by a longitudinal cut of the stem apex. In that zone is noticed a swelling of the apical meristem, with an increase of the diameter of the meristematic area, which ceases of producing leaf primordia, as occurs during the vegetative stage, forming the inflorescence (Kerns et al., 1936). Such fact may also be confirmed by pulling out one of the central leaves of the rosette and observing its base. If it is red, it is a signal that flowering was already initiated. Depending on environmental conditions, starting from 40-50 days after the induction treatment, the inflorescence emerges in the center of the leaf rosette. The first sign of meristem differentiation into a floral primordium is the immediate increase of mitotic activity of the cells below the central zone or the most apical/distal portion of the vegetative meristem (Lang, 1965).

As it happens with natural flowering, the effect of artificial induction treatments vary according to the environment, and the vigor, growth rate and type of the planting material. Suckers are more easily induced to flowering than slips and crowns. This fact was demonstrated in a test on precocious induction of planting materials of several sizes (20 to 47 cm), when it was observed a gradient in susceptibility to flowering of pineapple ‘Perola’, with the larger suckers and slips being more sensitive (Cunha, 1989b).

**Application of floral inducers**

Floral inducers differ in relation to the mode of application and efficiency: calcium carbide, acetylene and 2,4-D are applied in solution into the center of the leaf rosette; ethylene and BNA are used.

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Figure 3 - Hypothetical model of action of coadjuvant products on the performance of ethephon, in the process of floral differentiation of pineapple. (Source: López de Vélez and Cunha, 1983)
sprayed on the plants, while ethephon, NAA and BOH are applied either into the center of the rosette or on the whole plant. NAA is more efficient when applied around to the period of natural differentiation (Gowing, 1961; Das, 1964), what is generally true for all treatments of artificial induction. Calcium carbide may be applied in solid form (granulated or powder, 0.5-1.0 g plant\(^{-1}\)) during rainy periods, or as a solution prepared with 350-400 g of CaC\(_2\) dissolved in 100 liters of cold and clean water (30-50 mL plant\(^{-1}\)) in dry seasons. When appropriately applied, CaC\(_2\) may reach an efficiency of 100% (Singh and Rameshwar, 1974; Cunha, 1998).

In relation to ethephon, the recommended concentration is up to 4.000 mg L\(^{-1}\), that is, one to four liters of the commercial product for 1000 liters of water. The decrease of the acidity of the inducer solution to a pH 8 or 10 increases the efficiency of ethephon, making possible the use of reduced amounts of the product, once the release of ethylene is favored in alkaline solutions (Dass et al., 1975, 1976; Lopez de Velez and Cunha, 1983). The increase in pH may be achieved by the use of some alkalinizing substances, such as CaCO\(_3\), Na\(_2\)(CO\(_3\)) and Ca(OH)\(_2\). Cunha (1989a) obtained good results adding 35 g of Ca(OH)\(_2\) to 100 liters of the inducer solution, raising the pH to 10. In this case, the recommended concentration may be reduced to 25 or 100 mg L\(^{-1}\), being applied from 30 to 50 mL of the solution per plant, what results in more than 90% of efficiency in the induction of flowering. The addition of urea at concentrations of 2% and 3% (2-3 kg 100\(^{-1}\) liters) increases even more the efficiency of the artificial induction (Fahl et al., 1981; Reinhardt and Cunha, 1982a). Dass et al. (1976) reported positive results in flowering induction by using 10 mg L\(^{-1}\) of ethephon and 2% of urea. Hazarika and Mohan (1998) observed that ethephon at 25 mg L\(^{-1}\) + CaCO\(_3\) (0.04%) + urea (2%) increased the flowering percentage and reduced the periods until the emergence of the inflorescence and maturation of the fruit of ‘Kew’ pineapple.

Ethylene may also be applied directly to induce pineapple flowering, being preferred in mechanized plantations, due to its known efficiency and beneficial effects on the inflorescence, fruit quality and planting material production (Py and Silvy, 1954). However, ethylene use is restricted, because it is a gaseous substance and requires specific equipment for application, which is viable only in mechanized plantations. This operation consists on total spraying of the plants with a saturated solution of that gas, obtained by the injection, under pressure, of ethylene from a cylinder, into a tank containing cold water. The amount of ethylene recommended is about 800 g ha\(^{-1}\) per application, where the volume of water (6.000 to 8.000 liters) and the homogeneous distribution of the solution on the plants are very important aspects to be considered (Derickke, 1974). In order to facilitate the diffusion of ethylene in the water and, therefore, its efficiency, it is recommended to add a coadjuvant substance to the solution – activated charcoal (0.5%o to 1.0%), or bentonita (1.0%).

The time of plant growth regulators application is very important, and it should be done preferably at night, from 8 pm to 5 am, or during cloudy days (Aldrich and Nakasone, 1975; Abutiate, 1977; Cunha and Reinhardt, 1986). The greatest efficiency observed with night applications may be the result of a larger concentration of ethylene in the tissues of the plant under those conditions and/or to a better absorption of the applied product. This could be explained by the fact that pineapple is a plant that exhibits, alternatively, the crassulacean acid metabolism (CAM), characterized by assimilation of CO\(_2\) and stomata opening predominantly at night. It is important that the stomata stay open for a period of about four to six hours after the application of the inducer (Glennie, 1979). Turnbull et al. (1993) recommended that floral inducers sprays should be performed at high volumes, starting from the end of the afternoon, avoiding hot days in order to reduce failures in the artificial flowering. The environment temperature during the application of the products is also very important, being not recommended to be superior to 26-28 °C. According to Glennie (1979), high diurnal temperature causes an intense decarboxilation, elevating too much the level of CO\(_2\), which is a powerful inhibitor of ethylene, and contributing to inhibit flowering or to reduce the efficiency of the artificial induction.

Some products require repetition of the application in order to have a greater efficiency, what is usually made two to three days after the first application, as it is the case for ethylene and NAA (Cunha, 1998). However, in relation to ethephon, repetition is not necessary, unless it rains within six hours after its application, the same being true for calcium carbide.

Considering that those substances, when used as inducers, do just initiate the flowering process, without having any effect on the duration of the reproductive phase, their application should be planned in agreement with the period planned to harvest the crop, what means, usually within five to ten months before harvest, depending upon the environmental conditions (Cunha et al., 1994). Usually, the artificial floral induction should be done before the period favorable to natural flowering, unless the main purpose is to uniformize the flowering already initiated. In these situations, the inducers may be used in smaller concentrations (Cunha, 1998).
Knowing that a positive/linear correlation exists between the size or weight of the plant and the weight of its fruit (TAN, 1969; CHAN and LEE, 2000), the induction of small or immature plants will reduce the crop revenue significantly. That is because smaller plants with smaller foliar area will produce smaller fruits and less vigorous suckers, what may even affect plants with smaller foliar area will produce smaller fruit weight of its fruit (TAN, 1969; CHAN and LEE, 2000), the exists between the size or weight of the plant and the weight of its fruit (TAN, 1969; CHAN and LEE, 2000), the.

Otherwise, it is known that a plant at active growth phase does not respond well to the treatment of artificial induction. The same is true when environmental conditions are adverse to flowering, like a severe water stress, which paralyzes the plant growth, or after a very dry period alternated with a rainy one, due to the retaking of plant growth (BARTHOLOMEW and KADZIMIN, 1977). Those cases may require higher concentrations of the inducers. Though, the use of very high concentrations of growth regulators is not recommended, in order to avoid physiological alterations in the plant or fruit damage. Usually, the treatment of floral induction should be carried out when the pineapple reaches seven to 15 months after planting, depending on the cultivar, on the crop management and on the geographic region.

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