AN ABSTRACT OF THE THESIS OF

Ursula K. Schuch for the degree of Doctor of Philosophy

in Horticulture presented on October 4, 1990.

Title: Physiology of Flowering in Coffea arabica L.: Role of Growth Regulators and Water Relations

Abstract approved:
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Coffee trees growing in the Kona district of Hawaii were treated with different concentrations of either BA, GA_3, or Promalin, and pruned at the time of flowering. No significant differences in the flowering and fruit ripening pattern were found. High concentrations (100 mg/l) of growth regulators decreased fruit fresh weight. Pruning yielded the lowest number of fruit and flowers, and caused branch dieback. Fruit set was between 46 and 83% for the two years studied.

Flower buds from trees growing in the field that were >4 mm, but not developed to the candle stage at the time of GA_3 treatment, reached anthesis 20 days earlier than the controls. Their development was independent of rainfall, unlike the controls. Fruit from buds that had been treated at the >4 mm or the candle stage ripened more synchronously and earlier than the control. Buds smaller than 4 mm did not respond to GA_3 treatment.

A threshold leaf water potential of -2.7 MPa, and flower bud water potential of about -4.0 MPa was necessary to overcome dormancy of flower buds in greenhouse grown trees. GA_3 stimulated anthesis in some plants that were stressed to leaf water potentials of -2.1 MPa. Ethylene evolution of flower buds where dormancy had been broken with water stress was low, compared to dormant flower buds. At anthesis, ethylene evolution reached highest levels. Free and conjugated IAA levels in flower buds changed markedly after the dormancy breaking stimulus. Doubling of fresh and dry weight of flower buds occurred 3 to 5 days after water stressed plants were rehydrated. It was preceded by an increase in free IAA, and a tripling in the rate of water uptake from 1 to 3 days after rehydrating water stressed plants. Throughout the development, the largest percentage of IAA was present in the conjugated form.

Physiology of Flowering in *Coffea arabica* L.: Role of Growth Regulators and Water Relations

by

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A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Completed October 4, 1990

Commencement June 1991

APPROVED:

Professor of Horticulture in charge of major

Chairman of Department of Horticulture

Dean of Graduate School

Date thesis is presented: October 4, 1990

Presented by: Ursula K. Schuch

Acknowledgement

Thanks are due to many people who helped me materialize my goal of finishing this degree. My major professor Les Fuchigami gave me the opportunity and the support to work on the 'coffee project'. I am grateful to Mike Nagao for his guidance and encouragement during my stay in Hawaii and throughout my thesis work. Special thanks to Anita Miller for taking the time and effort to teach me some of her expertise. I like to thank Tony Chen who was always available for helpful discussions and advice.

Thanks to my fellow graduate students and co-workers for friendship, support, and memorable experiences. Thanks to all my friends who made my stay in the islands unforgettable.

To Shu Tan my sincerest thanks for providing the best home. Your encouragement, motivation, and good example contributed much to my growth.

I gratefully acknowledge the assistance of Judy Yoshimoto and Elodie Ho-A in Hawaii. Financial support for this research was provided in part by the U.S. Department of Agriculture under CSRS Special Grant No. 88-34135-3604, managed by the Pacific Basin Advisory Group.

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Physiology of Flowering in *Coffea arabica* L.: Role of Growth Regulators and Water Relations

Chapter 1

Introduction

In the Kona district of Hawaii coffee has been grown for 150 years and exported since 1845 (Dean 1939). Early reports focused on how the environment affects growth and yield of the coffee plant (Beaumont 1939). Information on vegetative and reproductive development of *Coffea arabica* L., the most important commercial species of coffee, is now available for the major coffee producing areas in the world and has been reviewed thoroughly within the past 15 years (Gopal and Vasudeva 1973, Nunes 1976, Maestri and Barros 1977, Barros et al. 1978, Gopal and Clowes and Allison 1982, Cannell 1985).

Growers in Hawaii and other areas are increasingly interested in mechanizing coffee harvest. Labor costs for harvesting account for 70% of the total variable costs for coffee production according to a 1986 study (Yokoyama et al. 1989). Currently coffee is hand-picked when the fruit are mature which is a costly and labor intensive practice. Harvesting is spread over many months (September to January), because unsynchronized flowering results in uneven fruit ripening. An alternative to repeated hand picking of the ripe fruit becomes more important as demand for seasonal labor is increasingly difficult to meet.

Flower initiation and development are two separate processes (Alvim 1973) that are triggered by different environmental factors. The mechanism of flower initiation and development are still not completely understood, although considerable research has been devoted to these topics. Both processes offer possibilities for manipulation in order to synchronize flowering.

The cause of several flowering peaks that occur over a prolonged period of time originates during flower initiation. Young coffee plants are known to have a critical short photoperiod between 13 and 14 hr. Daylength less than this period causes the reproductive meristems to initiate flowers with a higher intensity as daylength shortens (Piringer and Borthwick 1955). In Hawaii (20°N latitude), the annual photoperiod fluctuates between 10.9 and 13.3 hr (Camargo 1985), exposing coffee plants to almost year-round inductive conditions. In Hawaii, however, flower induction is thought to begin in August as days are shortening (Bullock 1980, Camargo 1985). In areas closer to the equator where annual changes in photoperiod are minimal, factors other than photoperiod, such as mild drought and low temperatures, are found to be associated with flower induction (Barros et al. 1978).

Flower buds undergo several periods of dormancy during their development (Clowes and Allison 1982). Dormancy can be imposed by unfavorable growing conditions, called quiescence, from initiation until flower buds have reached

a length of 4 to 6 mm in length. When this stage is reached, flower buds enter true dormancy, which has been associated with high levels of abscisic acid (ABA) and low levels of gibberellic acids (GA) (Browning et al. 1970, Browning 1973a). During true dormancy the xylem connection in the peduncle of flower buds is poorly developed (Mes 1957). A period of water stress is necessary to break dormancy, similar to chilling requirements of dormant temperate plants (Alvim 1960). The balance between ABA and GA is reported to change in favor of GA when dormancy is broken and plants are rewatered after the stress period. Browning (1973a) concluded that free GA is released from a bound form within the bud when the plants are rehydrated after water stress.

Flower initiation can be manipulated by inhibiting the early period of initiation. In younger plants which are sensitive to photoperiod, extending the natural daylight or interrupting the dark phase will inhibit flower initiation for several weeks (Piringer and Borthwick 1955, Wormer and Gituanja 1970). Older plants under field conditions respond slowly to photoperiod and flower initiation is not delayed until 6 months after the beginning of long day treatment (Cannell 1972).

The objective of this research is to synchronize flowering by manipulating flower initiation and development. With a synchronized flowering pattern one

could expect a synchronized harvesting pattern, making the use of a mechanical harvester feasible. Also, in areas that are inaccessible to large equipment, growers could have the option to accelerate or delay flowering in different parts of a plantation. Flowering and harvesting peaks could then be staggered in time throughout a plantation or area. The advantage of such a management strategy is that a smaller, but steady force of workers could be employed to harvest an evenly ripened, synchronized crop.

The main focus of this research is on flower development from the period when flower buds are visible in the leaf axil until anthesis. Emphasis is placed on the period of flower bud dormancy.

Field trials were conducted for two growing seasons in Hawaii to evaluate different growth regulators and pruning treatment to synchronize flowering and harvesting. In one of the field experiments the stage of development at which flower buds are responsive to GA_3 treatment is identified. It was shown that if flower buds are treated with GA_3 when they are at the 4 mm stage or just before anthesis, flowering and fruit development can be synchronized (Schuch et al. 1990).

Studies with younger plants that were grown in the greenhouse and in growth chambers focused on the interaction between water stress and GA_3, and their effects on flower bud development and anthesis. The effect of

water stress on the coffee plant, ethylene evolution and ion leakage were determined.

Water uptake into the flower bud is inhibited by a poor vascular connection when flower buds are dormant (Mes 1957). Auxin is the primary regulator of xylogenesis (Aloni 1988), however, the relationship between flower bud development and endogenous indole acetic acid (IAA) in coffee flower buds has not been studied with sensitive methods. One working hypothesis is that the poor vascular connection during flower bud dormancy is associated with low levels of free IAA. Once dormancy is broken, free IAA levels increase to stimulate the rapid development of the vascular system in the peduncle. Free and conjugated IAA levels were measured to determine if the bound IAA might serve as storage and conversion form to be readily activated as needed. By using a xylem transported dye, a method was developed to quantify water uptake by dormant flower buds, and buds that develop to anthesis.

Chapter 2

Review of literature

I. INTRODUCTION

Coffea arabica L., the most important species for commercial coffee production and is in the botanical family *Rubiaceae* (Charrier and Berthaud 1985). Indigenous to the highlands of Ethiopia, the coffee plant was first discovered about AD 850 and cultivated in Arabia (Smith 1985). From the 17th to the 19th century coffee was introduced to many subtropical and tropical countries.

II. PHENOLOGY

Based on its flowering pattern, coffee is classified as a gregarious species, where individual plants flower simultaneously over extended areas and within a short time (Cannell 1985). Coffee can be found growing in two major growing regions that are distinguishable by their periodicity of seasonal growth. In the equatorial regions (e.g. Kenya, Tanzania, central Colombia) two cycles of shoot elongation and flowering occur annually, regulated by two distinct wet/dry cycles. Shoots grow slowly during the cool/dry cycle, suggesting an additional influence of temperature (Maestri and Barros 1977). In non-equatorial areas (India, Ethiopia, Central America, Brazil, Hawaii) the coffee plant produces a single cycle of vegetative and reproductive growth (Cannell 1985, Vasudeva and Ramaiah 1979). Shoot elongation coincides with increasing

daylength, the onset of a wet period after drought, and warmer temperatures, but the responses to these environmental factors interact and vary by region (Clowes and Allison 1982).

1. THE FLOWER BUD

Flower buds have been studied in detail by Moens (1963, 1965). Flower primordia are thought to originate in the node of the branch tip at the time when newly formed leaves separate from the terminal bud (Alvim 1973). In *Coffea canephora* the primordia is characterized by a larger size and a flattening and broadening of the central dome (Moens 1967). Catalase activity in the leaves subtending the buds is low when buds are undifferentiated and increases when floral activity begins (Boyer 1969). Soon after initiation, flower buds are only visible with a microscope and may be used to distinguish between vegetative and reproductive buds.

Axillary buds grow in serial rows on primary and/or secondary lateral branches, and can develop into either more branches, inflorescences, or remain undifferentiated (Wormer and Gituanja 1970). As many as five buds can be formed in a leaf axil, and each bud can develop into inflorescences containing up to four flowers (Mes 1957). The number of inflorescences and flowers produced depends on the growing conditions.

Under inductive conditions, the first of the series of

buds will be induced first, followed sequentially by the next buds that will be florally determined (Mes 1957). This sequence of development is retained throughout flowering. At an early stage, when the flower buds are first visible in the leaf axil, they are covered with a gummy substance (Wormer and Gituanja 1970). The gum-like substance disappears gradually as the buds grow to a length of 4 mm when they reach a period of dormancy.

2. PHYSIOLOGY OF FLOWERING

Two distinct physiological processes in flowering of coffee are flower bud initiation and flower bud development to anthesis (Alvim 1973). These processes appear to be regulated by different environmental factors.

A. Flower bud initiation

The induction of flowering in coffee is triggered by short photoperiods. The critical photoperiod for coffee younger than two years is reported to be between 13 and 14 hr (Piringer and Borthwick 1955). One exception is the *C. arabica* variety 'Semperflorens', which is reported to be day neutral and flowers under photoperiods ranging from 8 to 24 hr (Went 1957). Plants that were 28 months old and received 18 hr supplemental light responded with more flower initiation than control plants (Monaco et al. 1978). When different varieties such as 'Catuai Amarelo', 'Acaia', and 'Mundo Novo' hybrids are treated with short and long photoperiods, responses in growth and branch production

differ widely between progenies (Soendahl et al. 1977). Adult C. arabica plants under field conditions in Kenya show little changes in flowering pattern when supplemented with light to either extend daylength or interrupt the night (Cannell 1972). According to Cannell (1972), seasonal growth and flowering cycles cannot be attributed to changes in daylength, since differences in photoperiod are too small in the coffee growing regions. Rather, he suggests an ontogenetic drift, where young trees respond as short day plants, but lose this trait as they mature. In general, plants become more sensitive to photoperiod changes with age (Vince-Prue 1975).

Flower initiation was stimulated at day/night temperatures of 23/17°C, and inhibited at high (30°C) and low (17°) temperatures (Mes 1957, Went 1957). Subsequent flower development was hastened at higher, and retarded at lower temperatures. Abnormal flowers (star flowers) or flower abortion was frequently observed when plants were grown at 30/24°C day/night temperatures.

Under natural conditions coffee trees growing near the equator are induced throughout the year (Wormer and Gituanja 1970). Lower temperatures, and a moderate drought seem to be associated with flower initiation, but the flowering pattern is not under the control of a single environmental factor (Barros et al. 1977).

Flower initiation is delayed by repeated application of GA_3 and has changed the seasonal fruiting pattern in

Kenya (Cannell 1971). Auxins have been proposed to retard flower induction, and ethephon, cytokinins, and growth retardants are suggested to improve flower induction (Cambrony and Snoeck 1983).

B. Flower bud development

After initiation, coffee flower buds grow slowly for several months to a length of about 4 to 6 mm when they stop growing and enter a period of dormancy (Mes 1957). Researchers believed that this dormancy, which can last from a few weeks to a few months (Alvim 1960), was a form of quiescence, imposed by unfavorable growing conditions (Rees 1964, Mes 1957). Quantification of the hormone balance in flower buds and xylem sap before and after dormancy led to the conclusion that the arrested growth is a true dormancy (Browning 1973a,b).

1. Temperature

Temperature affects the rate of bud growth before and after anthesis. Anthesis occurs in 8 days at 30/24°C, in 9 days at 23/24°C, in 10 days at 26/20°C, and in 11 days at 23/17°C after dormancy is broken and regrowth stimulated (Mes 1957). Reddy (1979) found that after dormancy was broken, flower buds reached anthesis faster if they were exposed to temperatures above 27°C compared to lower temperatures. Low temperatures such as 18°C prevent further growth of flower buds after dormancy is broken with water stress and plants are rewatered. Higher temperatures

favor the formation of starflowers, in which petals remain small, fleshy and stiff, and fail to set fruits (Mes 1957). Some authors claim that a drop in temperature is important in breaking flower bud dormancy, but no unequivocal experimental evidence has shown that cooling alone can break dormancy (Barros et al. 1978).

2. Water relations

Water relations are important factors in overcoming dormancy and in the growth of coffee flower buds. A period of drought is required to break dormancy, and after dormancy is broken rain or irrigation stimulates the flowers to develop to anthesis (Porteres 1946, Piringer and Borthwick 1955, Alvim 1960, Magalhaes and Angelocci 1976). While irrigation is believed by some researchers to overcome quiescence (Porteres 1946, Mes 1957, Gopal and Vasudeva 1973), others believe that true dormancy is broken by a dry period and regrowth stimulated by irrigation. Many treatments that simulate rainfall can stimulate coffee flowers to develop to anthesis. Ground irrigation (Porteres 1946, Piringer and Borthwick 1955, Alvim 1960, Pagacz 1959, Clowes and Wilson 1974, Magalhaes and Angelocci 1976), overhead irrigation (Mes 1957, Browning 1973a,b), immersion of the basal end of cut branches in water (Mes 1957, Went 1957, Mathew and Chokkana 1961, Rees 1964), immersion of branches from intact plants in water (Mes 1957, Alvim 1958, 1960), and direct application of

water to flower buds on intact plants and cut branches (Astegiano et al. 1988) have been shown to provide the regrowth stimulus for development of flower buds to anthesis. Trees that never experience drying conditions have flower buds that do not open or develop abnormal flowers (Piringer and Borthwick 1955, Pagacz 1959, van der Veen 1968, Alvim 1973, Humphrey and Ballantine 1974). Alvim (1960) suggested the term "hydroperiodism" to describe plants that require water stress to break dormancy under tropical conditions. Thus water stress has an effect similar to chilling which is required to break dormancy of temperate zone plants. He further proposed that flower buds of coffee have two phases of rest; the first phase is true dormancy, controlled by high levels of growth inhibitors, which decrease during periods of drought; and the second phase is a quiescence, resulting from inadequate water supply Alvim (1973).

Plants growing in a nutrient solution in Brazil (Franco 1940), and regularly watered plants in the field in Nigeria (Rees 1964) and in the greenhouse in Costa Rica (Alvim 1973) flowered following rains at the same time as coffee in surrounding fields. Alvim (1960), however, suggested that high transpiration rates can cause moisture stress even in irrigated trees or plants growing in nutrient solution.

Mahalhaes and Angelocci (1976) found that dormancy was broken only in plants when flower buds and leaves reached a

water potential below -1.4 and -1.2 MPa. These results suggest that water stress in the flower bud itself is necessary, and that dormancy release might be triggered by a sudden rise in the water potential of the flower bud. The hypothesis that water flows from the buds to the subtending leaf during drought, and in the opposite direction after irrigation, is not supported by observations that show flowering occurs even when subtending leaves are removed (Mes 1957, Browning 1973a, Astegiano et al. 1988). The subtending leaf enhances mobilization of water to the flower buds (Astegiano et al. 1988), and anthesis occurs more readily if leaves are present (Raju et al. 1975). Dormant flower buds are known to have a low water content (Mes 1957, Frederico and Maestri 1970, Gopal and Vasudeva 1973, Janardhan et el. 1977), which could result from the high apoplastic resistance to the flow of water (Browning and Fisher 1975, Astegiano et al. 1988) and the poor vascular connection in the peduncle (Mes 1957).

3. Plant growth regulators

Plant hormones appear to play an important role during the reproductive development of coffee, especially during the period of flower bud dormancy. Exogenous gibberellins could replace the stimulus for flower bud development to anthesis following the breaking of dormancy with water stress (Alvim 1958, 1960; van der Veen 1968, Browning 1975a). During this period either irrigation or rainfall

cause an increase in endogenous GA in flower buds before a rapid increase in bud fresh and dry weight (Browning 1973a). However, the levels of free GA in the xylem sap remain unchanged before and after irrigation. Browning (1973a) concluded that GA is the causal stimulus for dormancy release in coffee flower buds. Attempts to prevent dormancy release by application of GA biosynthesis inhibitors have failed, suggesting that *de novo* biosynthesis of GA does not occur at the time of dormancy release, but that bound, inactive forms of GA or precursors are activated with dormancy release.

Endogenous abscisic acid (ABA) remains stable from the period dormancy is released until it increases 4 days before anthesis (Browning 1973a). ABA was extracted from flower buds before and after dormancy was broken, and 75% of the inhibitory activity in the acidic extract was attributed to ABA (Browning et al. 1970). Exogenous ABA can counteract the stimulus provided by GA if ABA is applied either before irrigation (van der Veen 1968) or rainfall (Browning 1975a) to relieve water stress. With increasing duration of water stress higher concentrations of ABA are necessary to prevent flowering. After 12 weeks of drought high ABA concentration has little inhibitory effect (Browning 1975a). Exogenous ABA cannot inhibit the stimulatory effect of exogenous GA, suggesting that ABA plays a role in controlling the equilibrium between bound and free GA in the flower bud. Browning (1975a) suggests

that after flower buds reach a threshold water potential they produce large quantities of ABA, which inhibits further development of the poorly developed xylem.

The balance between GA and ABA appears to influence normal development after floral growth resumes. When plants are treated with 200 ppm ABA and remain dormant for 3-4 months, they either deteriorate or develop into starflowers (van der Veen 1968). In plants kept dormant by regular watering, abnormal flowers can be prevented by applying GA sprays. When ABA treated buds are supplied with varying amounts of GA, the occurrence of abnormal flowers depends on the ratio of GA to ABA that is applied (Browning 1975a). If GA inhibitors are applied when growth resumes, abnormal flowers are formed, however, exogenous GA causes normal flower development. The smaller starflowers are not affected by GA sprays, whereas the larger starflowers with the corolla slightly open so that stigmas are exposed, develop into normal fruits after GA application (Alvim 1958). In greenhouse grown coffee plants premature flowering with elongated styles and stigma is reported after plants have been sprayed with GA (Cambrony and Snoeck 1983).

The interaction between GA and ABA is suggested to play a major role in controlling flower bud dormancy (Browning 1975a). During water stress ABA levels cannot decline, otherwise buds that would open after prolonged stress without the proper stimulus would be abnormal.

During water stress there may be a build up of GA in the buds, but an additional stimulus is still required, and may be a xylem transported factor such as cytokinin. Browning suggests that initially ABA prevents GA production, which is for growth. Once active growth is stimulated, GA is required to overcome the inhibitory effect of ABA on bud development.

Cytokinin content in xylem sap and in flower buds increases after dormancy is released, whereas cytokinin levels in the xylem sap of unirrigated trees remains low (Browning 1973b). Some of the cytokinin seems to be produced within the bud, however, the wood itself and the developing vascular cambium are other possible sources. Exogenous cytokinin can replace the regrowth stimulus, but only if plants are subjected to prior water stress (Browning 1973a). It is speculated that GA in the buds may determine the sink strength for cytokinins from the xylem sap, which would explain why exogenous GA alone can break dormancy in some cases, independent of the status of soil moisture (Browning 1973a).

Endogenous IAA is found at high levels in developing buds and decreases before they reach dormancy (Janardhan et al. 1977). In plants that experience a drought period, prior to rain showers which stimulate regrowth of buds, IAA levels increase slightly, and then decrease as flowers reach anthesis. Exogenous application of auxins like IAA, IBA, NAA, 2,4-D (Alvim 1958, van der Veen 1968, Bullock

1980) cannot stimulate growth of dormant flower buds.

The cambium of *Abies balsamea* is known to have a rest period (Little and Bonga 1974). Exogenous IAA application stimulates cambium activity when shoots are in the quiescent state, but has no effect when applied during deep rest (Little 1981). Auxin content seems to control the intensity of mitosis, but not the onset and cessation of cambial activity (Lachaud 1989). During bud and seed dormancy auxins seem to have no regulatory function (Powell 1988).

III. VASCULAR DEVELOPMENT

The xylem connection in the peduncle of dormant coffee flower buds is poorly developed (Mes 1957). During dormancy, water uptake into the flower bud is severely restricted and causes lower water potentials in the flower bud compared to the leaf (Magalhaes and Angelocci 1976). After bud dormancy is broken with water stress, the xylem develops rapidly and allows water to move without restrictions into the developing flower bud (Astegiano et al. 1988). Xylogenesis occurs within two to three days, and it is suspected that hormones are associated with this rapid change.

1. GROWTH REGULATORS

IAA is the main limiting and controlling factor for both, phloem and xylem differentiation (Sachs 1981, Aloni 1987, 1988). Vascular differentiation depends on a

continuous flux of signals that determine the polarity of tissue (Sachs 1981). Differentiation is a gradual, long term response of the cells to the actual flux of signals.

Xylem does not differentiate in the absence of phloem, but phloem often develops without xylem, particularly at low auxin levels (Aloni 1988). From leaves to roots an increase in vessel size and a decrease in vessel density is due to decreased auxin concentration.

Fiber differentiation requires auxin and gibberellin, and in their presence cytokinin can become a limiting factor (Aloni 1987). When IAA and GA_3 are applied simultaneously, high IAA/low GA_3 concentrations favored xylem formation, whereas low IAA/high GA_3 levels promoted phloem production. Xylogenesis in callus of soybean cultures is induced by exogenous application of IBA plus either kinetin, transzeatin, BA or GA_3 (Ackermann and van Staden 1988). Concentration and timing of GA application is critical to induce xylem in lettuce pith cultures (Pearce et al. 1987). NAA and GA_3 enhance phloem differentiation more than xylem development, which leads to the hypothesis that tracheid differentiation in _Pinus_ is limited by another factor that is produced only in young needles, but not in old ones (Ewers and Aloni 1985). Cultured explants of artichoke require auxin for cell proliferation early in the culture, and cytokinin for xylogenesis at a later stage (Phillips 1987). A strong interaction among leaf expansion, internodal elongation and

xylem development is related to the net mobile auxin from expanding leaves in poplar (DeGroote and Larson 1984).

Ethylene may regulate xylem differentiation in lettuce by inducing wall-bound peroxidase activity during lignin synthesis (Miller et al. 1985). Ethylene precusors enhance protein phosphorylation and xylogenesis, whereas ethylene inhibitors can prevent these processes (Koritsas 1988). Xylem differentiation in _Lactua_ occurs only when IAA is applied together with ethylene or kinetin, suggesting that ethylene can substitute qualitatively for cytokinins, and that both ethylene and auxin are required for xylogenesis (Miller et al. 1984).

2. FREE AND CONJUGATE AUXIN METABOLISM

The response of plants to IAA application is well studied. However, the relation between endogenous IAA and plant response and the regulation of IAA and its metabolites are poorly understood (Reinecke and Bandurski 1988). Rather than looking at single events that might involve auxins, researchers focus increasingly on the inputs into and outputs from the IAA pool. Inputs include _de novo_ synthesis from tryptophan or other non-indolylic precursors, conjugate hydrolysis of esters and amides, and IAA transport. Outputs occur via oxidative catabolism, conjugate synthesis, and the use of IAA in growth, which is thought to be linked to its destruction or conjugation.

A GA-controlled racemization of L to D-tryptophan may

control the synthesis of IAA from the large pool of tryptophan. However, de novo biosynthesis of IAA is also demonstrated from precursors other than tryptophan.

In bound or conjugated IAA a molecule is covalently bound to the auxin (Cohen and Bandurski 1982). The ester conjugate IAA-myo-inositol, and the amide conjugate IAA-aspartate occur naturally. Leaves are suggested as the site of synthesis of the indole nucleus, and enzyme systems for conjugation are identified in developing seeds. IAA is produced in Zea mays kernels, and the esters, not as previously believed the tryptophan, are the source of IAA. Exogenous application of auxin, cytokinin, and ethylene affects conjugate synthesis. IAA seems to be metabolized to IAA-aspartate or IAA-glucose; NAA, 2,4-D and IBA are converted to aspartate and glutamate conjugates. Cytokinin reduces conjugation when IAA or 2,4-D are present. Light and ethylene treatment stimulate auxin conjugation when IAA is provided. IAA conjugates are used increasingly in tissue culture where they slowly release IAA, and may target the IAA to particular tissues or cell organelles at the required rate (Reinecke and Bandurski 1988).

IAA catabolism occurs most frequently by a peroxidase catalyzed oxidative decarboxylation, but rarely by oxidation without decarboxylation (Reinecke and Bandurski 1988).

Endogenous and applied auxins are found in the

vascular system, moving more rapidly than by polar transport (Rubery 1988). Gradients of water potential or osmotic potential determine the vascular transport of IAA, whereas the distribution of protein carriers is responsible for the polar transport of IAA. The ester form of IAA seems to be the preferred transport for conjugates (Cohen and Bandurski 1982). Experimental evidence suggests that IAA movement within the plant symplast may be regulated by metabolic gating of the plasmodesmatal connections of the plant symplast (Reinecke and Bandurski 1988).

A summary of the role of IAA conjugates shows their role in the transport of IAA, storage and subsequent reuse, protection from enzymatic destruction, and for the homeostatic control of IAA concentrations in the plant (Cohen and Bandurski 1982). Further, it is proposed that the rate of auxin conjugate hydrolysis determines the free IAA concentration within the plant tissue. Changes in conjugated IAA levels compared to free IAA during the development and maturation of bean seeds suggest that the conjugates serve as storage form of auxin in the seed (Bialek and Cohen 1989). IBA is found in potato, tobacco, pea, and one maize cultivar in free and conjugated form, but not at all developmental stages (Epstein et al. 1989). Its promotive effect on rooting suggests that IBA might be a natural rhizocaline, a compound that acts together with IAA in forming adventitious roots.

Chapter 3

Effect of benzyladenine, gibberellic acid, Promalin, and pruning on flower and fruit development in *Coffea arabica* L.

Abstract

To determine the effect of growth regulators and pruning on synchronizing flowering and ripening, coffee trees in the Kona district of Hawaii were treated at the beginning of the 1988 and 1989 flowering season with benzyladenine (BA), gibberellic acid GA_3 (GA), and Promalin (PR). No differences between treatments were found for flowering, but the percentage of flowering at anthesis differed between months. Flowering in 1988 occurred in three peaks, one per month from Mar to May. In 1989, the majority of flowering occurred in Feb, and slowly decreased until Jun. Largest flowering peaks were always associated with high rainfalls during or just before the month of major flowering. The total number of flowers and fruit on a per tree and per node basis was lower in 1988 compared to 1989, probably due to a dry spring in 1987. Fruit ripening differed between treatments in 1988 and between months in both years. Fruit fresh weight of flowers treated prior to anthesis with 100 mg/l BA, GA, or PR was lowest among the treatments. Fruit fresh weight decreased during the last month of harvest. Pruning of three apical nodes of primary lateral branches in Feb caused delays in flowering, reduced flower and fruit number per tree, and caused branch die-

back. Vegetative growth was most vigorous from Mar to Jun in 1988, and from Apr to Aug in 1989. In general, months with higher rainfall stimulated more shoot growth.

Introduction

With increasing labor costs for harvesting, coffee growers are looking towards mechanizing the harvesting process. Currently, mechanical harvesting is impractical due to non-uniform maturation of fruit. Synchronizing flowering and subsequently the ripening process are prerequisites for the efficient use of a mechanical harvester.

Most of the studies with growth regulators have been conducted mainly with gibberellins or cytokinins at the time of anthesis. In these experiments changes in the pattern of flowering or ripening are reported. Although effects are apparent, it is often unclear at what stage of flower and fruit development these effects are expressed. In earlier studies (Alvim 1958, van der Veen 1968, Browning 1975a), the effect of growth regulators on fruit set, an important parameter of yield, is not reported. Vegetative growth determines the potential yield in the following growing season and is often affected by environmental conditions, the presence of fruit, and growth regulators (Cannell 1985).

Gibberellic acid (GA_3) enhances coffee flower buds to develop to anthesis if the plants have received some water

stress (Alvim 1960, van der Veen 1968, Browning 1975a, Cambrony and Snoeck 1983). GA_3 stimulated and synchronized flowering and fruit ripening only if buds larger than either 4 mm or at the candle stage were treated (Chap. 4). Repeated low concentration sprays of Promalin and benzyladenine synchronize harvesting when applied at the time of flowering (Bullock 1980).

The objectives of this study were to determine the effects of GA_3, BA, PR, and pruning on flowering, fruit ripening, fruit set, fruit fresh weight, and vegetative growth, and to determine whether any of the treatments has a potential for use in synchronizing flowering and fruit ripening of coffee in the field.

Materials and Methods

Coffea arabica L. cv. Guatemalan were 18 months old when they were planted in January 1986 in Kainaliu, in the Kona district of Hawaii, at an elevation of 640 m. A few of the trees had produced a first light crop in fall 1987.

First year (1988) study. The following plant growth regulator treatments were applied: benzyladenine (BA), gibberellic acid GA_3 (GA), and Promalin (PR) at concentrations of 50 and 100 mg/l each. Control treatments were sprayed with distilled water. The spray solution contained the growth regulator, 0.2% Tween 20, and distilled water. Each treatment was applied to tagged branches to run-off with a handsprayer on 22 Jan, 3 Feb,

and 25 Feb, 1988. Pruning (PRN) treatment consisted of removing the three apical nodes of a primary lateral branch.

Number of flowers at anthesis were counted at 3 to 6 day intervals from 25 Jan until 29 Jun, 1988. The average number of days to flowering were counted from 22 Jan on and were calculated as:

$$\text{Days to flowering} = \frac{\text{sum (flowers * day)}}{\text{total flowers}}$$

Harvesting of mature, fully red fruit started at the end of Aug 1988 until Jan 1989 at biweekly intervals. Since only a few fruit were ripe in Sep, they were added to the fruit that were harvested in Oct. The number of days to harvest was calculated similar to days to flowering, except fruit was substituted for flowers. Oct 1 was designated as day 1 for harvesting.

Fruit set was calculated as:

$$\text{Fruit set (\%)} = \frac{\text{total fruit * 100}}{\text{total flowers}}$$

Fruit were picked when they had turned completely red and before they started to dry. The samples were collected in ventilated plastic bags and placed in a cooler on ice for transport to the laboratory. The following day fruit fresh weight was determined by dividing the total fruit fresh weight of each treatment by the number of fruit per treatment.

Vegetative growth was measured from Mar to Dec 1988 on

8 untreated trees. Three branches, located in the middle part of the tree and bearing flowers and fruit similar to the trees used in the experiment, were tagged and measured at monthly intervals.

The experiment was analyzed as a completely randomized design with 5 replicate trees per treatment and 3 branches per tree as subsamples. Treatments were compared for significant differences by analysis of variance, means were compared with Tukey's Studentized Range Test.

Second year (1989) study: On 10 Feb, 1989 each of the following treatments were applied to two branches of ten trees: BA at 25 and 50 mg/l, GA at 25 mg/l, PR at 25 mg/l, and a control spray with distilled water and 0.2 % Tween 20. The solutions were formulated and applied as described above.

Flowering was recorded from 17 Feb until the end of Jun 1989, and days to flowering were calculated starting on Feb 10. Mature fruit was harvested from the end of Aug until the end of Dec. Similar to the previous year's study, the fruit that were collected in Aug were added to the Sep samples. All variables were calculated as in the first year study. The data was analyzed as a complete randomized block design, with 10 trees as blocks.

Results

The parameters that were measured for the first and second year study and their significance in the analysis of variance are summarized in Table 3.1. The data of the two concentrations of each growth regulator were combined for the analysis of flowering and harvesting on a monthly basis. The general pattern of flowering, harvesting, vegetative growth, as well as rainfall and temperature at the study site for the 2 years of experiments are shown in Figure 3.1A-C.

First year (1988) study

Flowering: Three flowering peaks (9 Mar, 14 Apr, and 9 May, 1988) were observed for all treatments. Average days to flowering, or the number of days from 22 Jan, 1988 until 50% of the total flowers were at anthesis, did not differ significantly between treatments for any of the three treatment times (Table 3.1). However, the largest differences between control and growth regulators were found on plants that were treated with PR100, GA100, and PR50 on 22 Jan (Table 3.2). Of the treatments that were applied on 3 Feb, 1988, BA50 treated trees flowered on the average 12 days earlier than the control trees. The largest delay in flowering, 14 days compared to control, was found when plants were pruned on 25 Feb, 1988.

When flowering was expressed on a monthly basis as percentage of the total flowers, PR and GA enhanced early flowering during Feb and Mar compared to the control

treatment on 22 Jan (Figure 3.2). For plants that were treated on 3 Feb, 1988, BA enhanced early flowering so that by Mar 49 % of the total flowers had bloomed, compared to 40% of the control. The pruning treatment on 25 Feb resulted in a delay in flowering, and more than half of the total flowers bloomed during May.

The number of flowers per node ranged from 2.4 to 3.2, and the overall mean for all treatments combined were 2.8, 2.9, and 2.9 for the treatment dates 22 Jan, 3 Feb, and 25 Feb, 1988, respectively.

The average total number of flowers from three branches per tree and treatment ranged between 455 and 798. The overall means were 619, 629, and 690 flowers per tree for the three treatment dates. Pruning yielded the lowest number of flowers per tree with 497, 455, and 540 for the three treatment dates.

Harvest: Average days to harvesting for GA100, PR100, and PR50 were 15, 14, and 11 days less, respectively, compared to the control for plants that were treated on 22 Jan, 1988. The BA50 treatment on 3 Feb was harvested 13 days earlier compared to the control. In contrast, pruning treatment on 25 Feb delayed harvest by 13 days compared to the control.

Up to 62% of fruit were harvested during the months of Oct and Nov of trees that had received BA, GA, or PR on 22 Jan, compared to 43% of fruit harvested in the control (Figure 3.3.). Harvesting of fruit in pruned trees and 3

Feb BA treatment was 40% during Oct, compared to 23% of the control. For trees treated on Feb 25, 48% of fruit from the pruned treatment was harvested in December, compared to 29% of the control.

The average number of fruit per node ranged between 1.7 and 2.6, with the overall mean of 1.9, 2.1, and 2.3, respectively, for the 22 Jan, 3 Feb, and 25 Feb treatment dates. The average total number of fruit per tree was 332, 347, and 382 when collapsed for all treatments for each of the three treatment dates, respectively. Pruned branches had the poorest yield with 280, 220, and 322 fruit per tree for the three treatment dates, respectively.

Fruit set was consistent among all treatments and treatment dates, and varied between 46 and 66%. The overall averages for the three treatment dates were 53, 56, and 56%.

Fruit fresh weight differed among treatments at all three treatment dates. The pruning treatment on Jan 22 yielded the fruit with the highest fresh weight among the treatments (Figure 3.4). A significant interaction between treatment and month of harvest was found for the 3 and 25 Feb treatment dates. Fruit of trees that had been treated with high concentrations of PR and GA, and one pruning treatment had low fruit weight during the months of Sep and Oct (Figure 3.4). Regardless of the treatment, all fruit decreased in weight by Jan.

Vegetative growth: Lateral branches in the flower and

fruit bearing zone grew most vigorous from Mar to Jun 1988, between 4 to 8 cm per month (Figure 3.5). From Jun to Oct branches grew less than 2 cm per month and by Nov and Dec no more branch extension was measured.

Second year (1989) study

Flowering: None of the parameters measured showed significant differences between treatments. Flowering showed several peaks, the largest one on 24 Feb, followed by progressively decreasing peaks on 21 Mar, 3 Apr, 5 May, and 15 Jun, 1989. The average number of days until 50% of the total flowers had reached anthesis ranged from 41 to 49 days. Fifty percent of the flower buds that were treated with BA50 bloomed after 41 days compared to 47 days for the control. The largest difference between treatments was found in Feb, where 43% of the BA50 treated flowers and 34% of the control flowers bloomed (Figure 3.6). The control treatment had 329 flowers per tree the highest number, whereas BA50 and PR 25 had 220 flowers per tree, the lowest number. For all treatments, the average number of flowers per node ranged from 6.1 to 7.6.

Harvest: The average number of days until 50% of the harvest was collected was between 54 and 57 for all treatments. The amount of fruit that was harvested on a monthly basis was very similar for all treatments and the total monthly harvest is shown in Figure 3.7. The number of fruit per tree was highest for the control treatment (263) and lowest for the BA50 and PR25 treatments (158 and

166). On the average, between 4.7 and 5.3 fruit per node were counted.

Fruit set ranged between 72 and 83% with no differences between treatments. Fruit fresh weight was not affected by treatments, but differed between months. In the first two months of harvest fruit were heavier than during the last two months (Figure 3.6).

Vegetative growth: Vegetative growth of the lateral branches that were treated with different growth regulators showed no significant differences. The average monthly growth for all treatments was most vigorous from Apr to Aug 1989 (Figure 3.1C). Shoot elongation decreased consistently during fall.

Discussion

The results suggests that PR, GA, and BA at concentrations of 100 mg/l may have the potential of enhancing early flowering although no significant differences in flowering were found between treatments. Differences between growth regulator treatments and control became smaller for the second and third treatment date, suggesting that factors other than supplemental growth regulators determined flower development. The importance of timing the application of growth regulators was found in another study, where only flower buds larger than 4 mm were responsive to GA_3 and bloomed independent of rainfall (Chap. 4).

The largest flowering peaks in 1988 and 1989 occurred at the time of highest rainfall. During Apr 1988, the highest amount of rain was recorded, and on 9 May the largest flowering peak was observed. Feb 1989 was the month with the most precipitation, and the largest flowering peak. BA 50 stimulated earlier flowering in 1989 by 6 days, but did not affect fruit ripening. It appears that rainfall is the major trigger to induce flowering, and that large rainfalls will override the effect of growth regulator applications. Similar observations are reported in Costa Rica, where GA_3 application showed no effects when applied during the rainy season (Alvim 1973). In months with little rain, growth regulators stimulated the development to anthesis within 10 to 14 days (Alvim 1958, van der Veen 1968).

Treatments that stimulated early flowering in Mar 1988 generally had the highest percentage of harvestable fruit in Oct. The ranking of average days to flowering and harvesting shows that treatments which stimulated earlier flowering also stimulated earlier harvest, and treatments that delayed flowering also delayed harvest. Other studies reported that GA_3 applied to dormant flower buds could stimulate flowering without irrigation after the plants had experienced a dry period (Alvim 1958, van der Veen 1968, Browning 1975a). When multiple sprays of $GA_{4,7}$ or $GA_{4,7}$+BA were applied to dormant flower buds, yield was concentrated in Oct and Nov, whereas control plants were harvested from

Oct to Feb (Bullock 1980). Unfortunately, these studies have no reports on the pattern of both, flowering and harvesting. However, it seems reasonable to conclude that the effect of growth regulators like GA, BA, and PR, applied to dormant flower buds, are carried over from flowering to ripening of coffee.

The proportions of flowering and ripening during 1988 were similar when calculated over all treatments (Fig. 3.1A). In contrast, in 1989 the pattern of flowering and ripening differed considerably. The major harvest could have been expected by Sep, if flowering and fruit ripening would have retained the same pattern. Flowers that were at anthesis in Feb, would have their maximum gain in fresh weight and maximum expansion 6 to 16 weeks later (Cannell 1985). The normal development was most probably retarded by the dry conditions in May, and caused a one month delay of the major ripening peak.

The number of flowers and the number of fruit on a per tree and per node basis were larger for 1989 compared to the previous year. Much of the annual variability in coffee yield in Kona is attributed to fluctuation in the Feb to Jun rainfall during the previous year (Dean 1939). From Feb to Jun 1987 the total rainfall was only 47% of the sum of rainfall from Feb to Jun 1988. Vegetative growth during a growing season determines the following year's production potential (Beaumont 1939), and the low flower

and fruit production in 1988 may have been due to poor vegetative growth during the previous dry spring. Production of flowers and fruit might have also increased due to maturity of trees, which had their first commercially usable crop in 1988.

Flowering and ripening were most synchronized by the pruning treatment in late Feb 1988, but pruned branches yielded consistently the lowest number of fruit per tree. By Aug/Sep 1988 branches that had been pruned started to die back, but the ripening fruit was supported to maturity. Die-back can be caused by defoliation of new growth or shading, since shoot extension is dependent of the supply of assimilates from adjacent leaves (Clowes and Allison 1982). Pruning decreased the potential number of nodes with flowers by removal of terminal nodes, and the branch was not able to compensate for the loss of terminal vegetative growth by forming secondary laterals, which we had expected. Fruit set, however, was not affected, and it is known that fruit can import assimilates from other parts of the trees, whereas shoot extension is dependent on adjacent healthy leaves (Clowes and Allison 1982). Although pruning, if done at the right time, appears promising for synchronizing flowering and fruit ripening, the detrimental effect of branch dieback and thus the reduction of future production area, lead to the conclusion that pruning lateral branches in Jan and Feb cannot be recommended.

Fruit set in 1988 ranged from 46 to 66% and was similar to another study which was conducted in the same year in a nearby location at lower elevation (Chap 4). Fruit set in 1989 ranged between 72 to 83%. Only irrigation and fertilizer studies in Kenya were reported to have similarly high fruit set (Huxley and Ismail 1969). Young trees often exhibit the potential problem of overbearing since they set more fruit than they can sustain (Cannell 1985). Shoot dieback or biennial bearing through physiological exhaustion are possible dangers if the crop severely depletes carbohydrate reserves of the tree (Clowes and Allison 1982). Fruit set in arabica coffee can vary between 20 to 80%, depending on how favorable growing conditions are during fruit development (Reddy and Srinivasan 1979). Fruit shedding occurs 12 to 15 weeks after flowering and at that time final fruit set is determined (Cannell 1985). Growth conditions at the study site were favorable for fruit set in terms of temperature, moisture, and nutrient supply.

Fruit fresh weight was reduced by high concentrations of PR and GA, and fruit size of these treatments was visibly smaller in Sep and Oct. Since only fruit at the Sep and Oct harvest had low fruit weight and were smaller in size, it seems that only flower buds that had been treated just before anthesis were affected. Flower buds which bloomed in April or May and were thus harvested in

Nov or Dec remained unaffected by the growth regulator. The cause for reduced fresh weight remains unknown, because we did not explore the fresh weight of the different fruit components. Decreased weight could be due to lower water content, change in ratio of seed to pericarp, or reduction of seed and pericarp volume (Cannell 1985). Bean size is mainly determined by the tree water status during the period of rapid pericarp growth 6 to 16 weeks after flowering (Ramaiah and Vasudeva 1969). Repeated sprays with 100 ppm GA_3 produced lighter seed, but a single application had no effect (Cannell 1971). In both years, fruit weight decreased during the later harvest months. This might be due to lower water availability during the time of maximum fruit growth and the final months of development. It is also likely that assimilates become less available for fruit that develops towards the end of the season (Cannell 1985). However, fruit can be reduced in size because assimilates can be diverted from wood, leaves, and even from the fruit pericarp, but the bean size remains the same (Huxley 1970, Cannell 1974).

Vegetative growth was not affected by the different growth regulator treatments. Repeated sprays of 100 or 50 ppm GA_3 did not affect branch elongation of coffee, either because the aqueous solution is not adequately absorbed by the leaves, concentration of GA_3 was not high enough, or GA_3 is not a limiting factor for shoot growth (Cannell 1971). In conjunction with irrigation or rainfall GA_3 can

further stimulate shoot growth (Browning 1975b). Maximum vegetative growth coincided with the flowering season in both years, which is reported for many coffee growing areas (Clowes and Allison 1982, Browning and Fisher 1975, Ramaiah and Venkataramanan 1985). In 1988, shoot extension and flowering coincided with high rainfall. In 1989 vegetative growth remained vigorous until Aug, two months after flowering was completed. Rainfall in 1989 was higher from Jul until Oct and probably contributed to more growth compared to the previous year. May 1989 was a relatively dry month, but shoot extension was still vigorous. A factor independent from soil moisture must be responsible to stimulate continuous growth flushes without the rain (Browning 1975b). Inherent shoot growth periodicity, supra-optimum tissue temperatures and daytime water stress due to high evaporative demand, and fruit development which make assimilates unavailable for shoot growth might be responsible for discontinuous and slow shoot elongation during summer (Cannell 1985).

Table 3.1. Parameters measured from coffee (*Coffea arabica* L.) trees that were treated with growth regulators [a] or pruned[b], and their significance in analysis of variance.

Parameters measured	--- 1988 ---- treatm.	month	--- 1989 ---- treatm.	month
I. Flowering				
1. Days to anthesis	NS[c]	-	NS	NS
2. Flowers (%) per month	NS	*	NS	*
3. Flowers per node	NS	-	NS	-
4. Flowers per tree	NS	-	NS	-
II. Harvesting				
1. Days to harvest	NS	-	NS	-
2. Fruit (%) per month	*	*	NS	*
3. Fruit per node	NS	-	NS	-
4. Fruit per tree	NS	-	NS	-
5. Fruit fresh weight	*	*	NS	*
6. Fruit set (%)	NS	-	NS	-
III. Vegetative growth				
1. Branch growth	NS	*	NS	*

[a] growth regulators: benzyladenine, GA_3, and Promalin
[b] three apical nodes of primary lateral branches removed
[c] NS=non-significant ($p>0.05$), *=significant $p<0.05$.

Table 3.2. Average days to flowering and harvesting for coffee (Coffea arabica L.) trees that were treated with growth regulators[a] or pruned[b] on Jan 22, Feb 3, and Feb 25, 1988.

Treatment concentr. (mg/l)	Average days to flowering[c]		
	Treatment time		
	Jan 22	Feb 3	Feb 25
Control	93.9	86.0	84.7
Pruning	90.6	89.6	99.3
BA50	97.4	73.8	88.3
BA100	92.4	79.5	82.7
GA50	88.8	82.4	90.3
GA100	80.4	89.3	80.4
PR50	81.2	90.3	76.7
PR100	77.3	88.1	85.1

Treatment concentr. (mg/l)	Average days to harvest[d]		
	Treatment time		
	Jan 22	Feb 3	Feb 25
Control	62.1	59.8	48.5
Pruning	57.7	50.3	61.3
BA50	60.7	47.1	53.7
BA100	60.5	46.3	50.7
GA50	62.8	53.1	56.7
GA100	47.4	59.6	46.7
PR50	51.3	59.4	47.3
PR100	47.5	54.2	55.7

[a] growth regulators: benzyladenine, GA_3, and Promalin
[b] three apical nodes of primary lateral branches removed
[c] Counted from Jan 22, 1988 [d] Counted from Oct 1, 1988

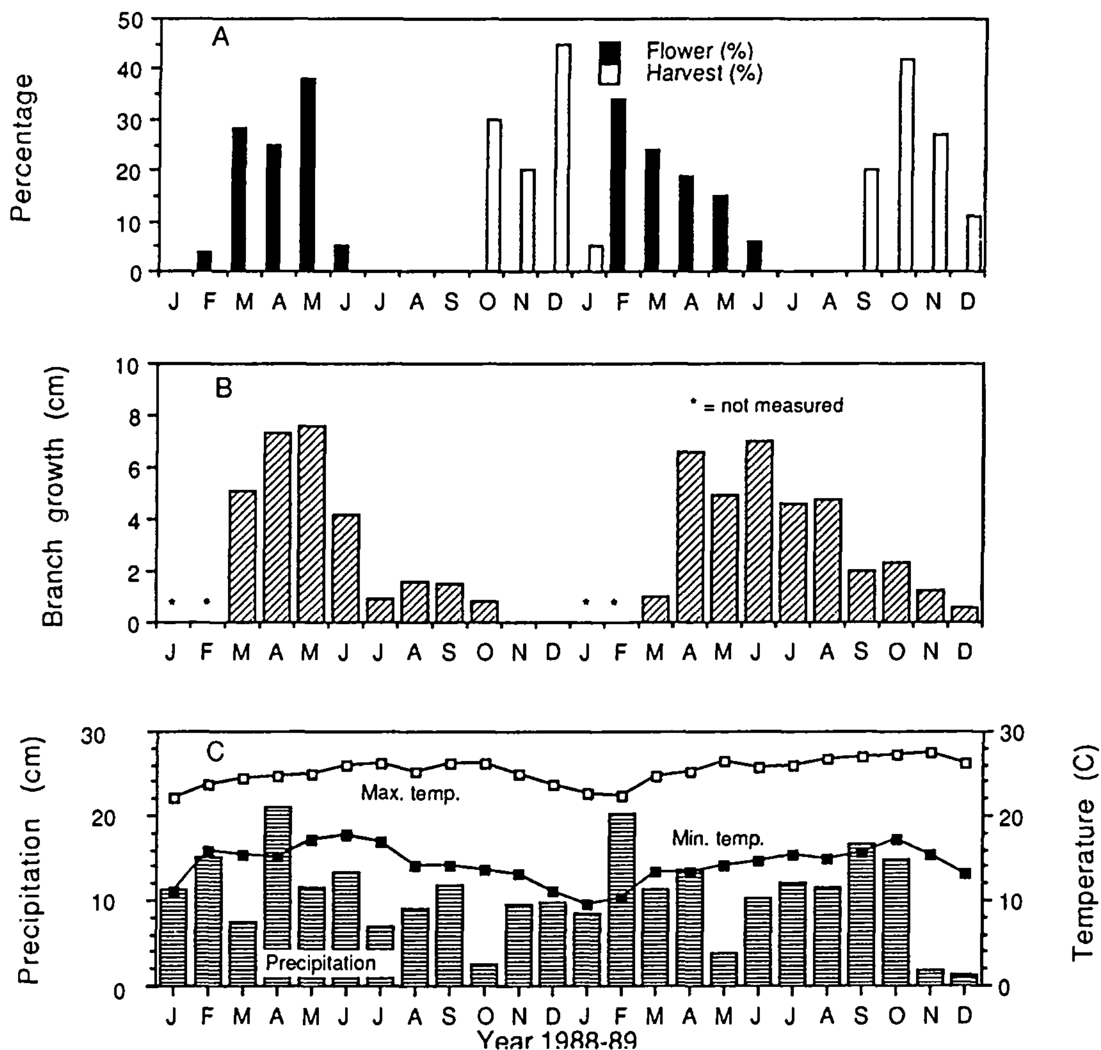

Figure 3.1. Summary of reproductive (A) and vegetative (B) development of coffee (<u>Coffea arabica</u> L.) trees treated with various growth regulators or pruned in Jan and Feb 1988 and in Feb 1989. Monthly rainfall and mean minimum and maximum temperature at the study site (C).

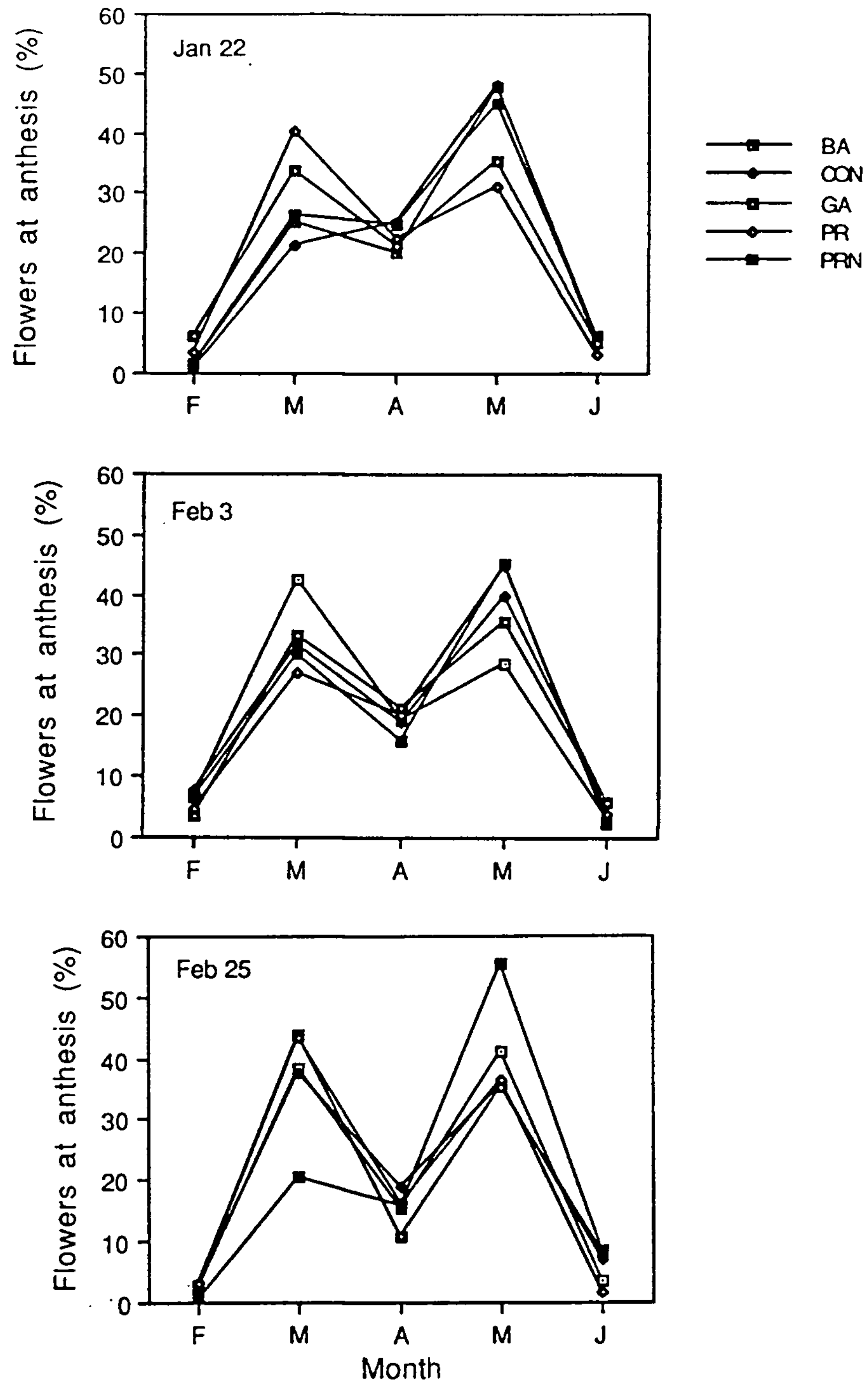

Figure 3.2. Percentage of flowers at anthesis of coffee (Coffea arabica L.) trees treated with either growth regulators (BA, GA, PR) or pruned on 22 Jan, 3 Feb, and 25 Feb, 1988.

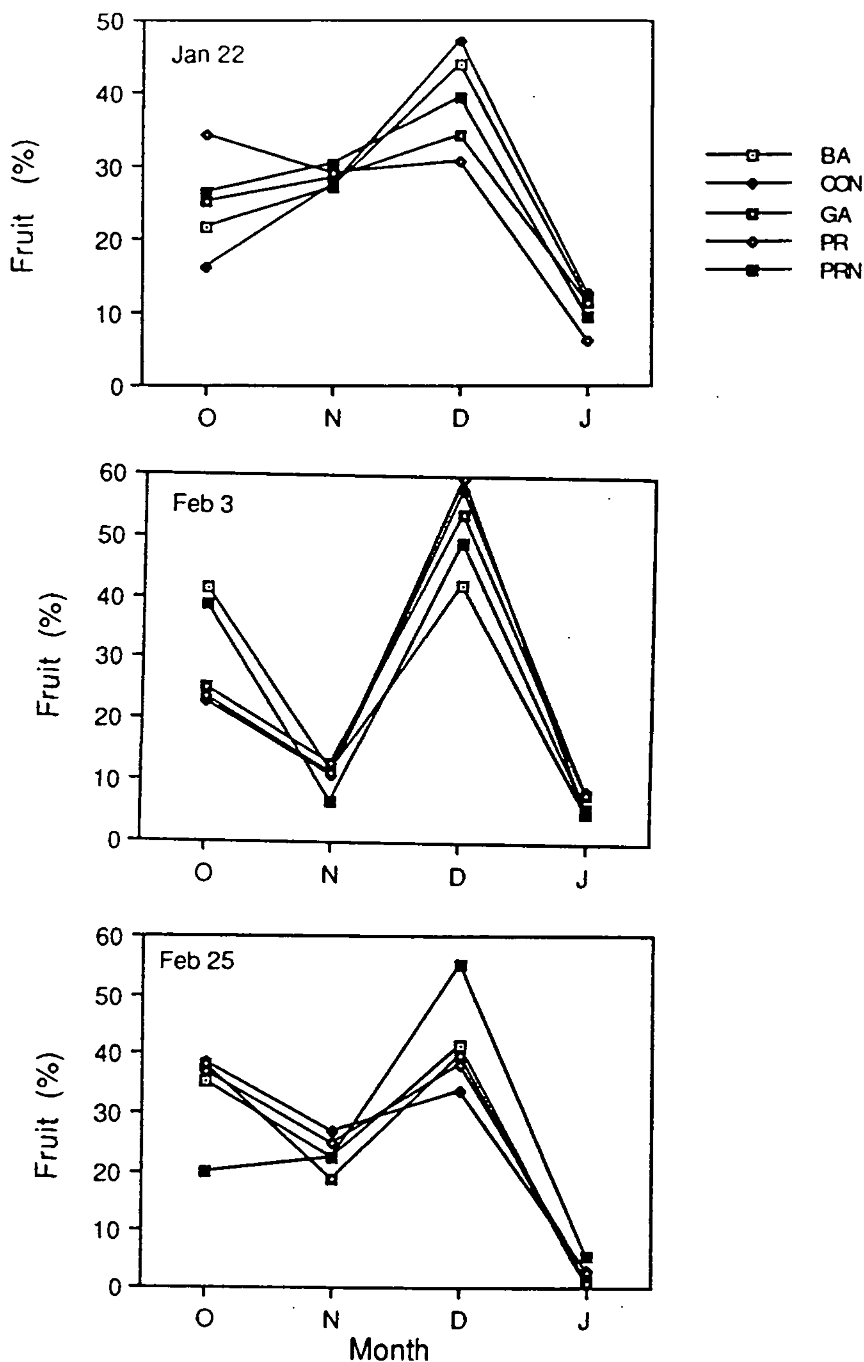

Figure 3.3. Fruit harvested per month of coffee (<u>Coffea arabica</u> L.) trees treated with either growth regulators (BA, GA, PR) or pruned on 22 Jan, 3 Feb, and 25 Feb, 1988.

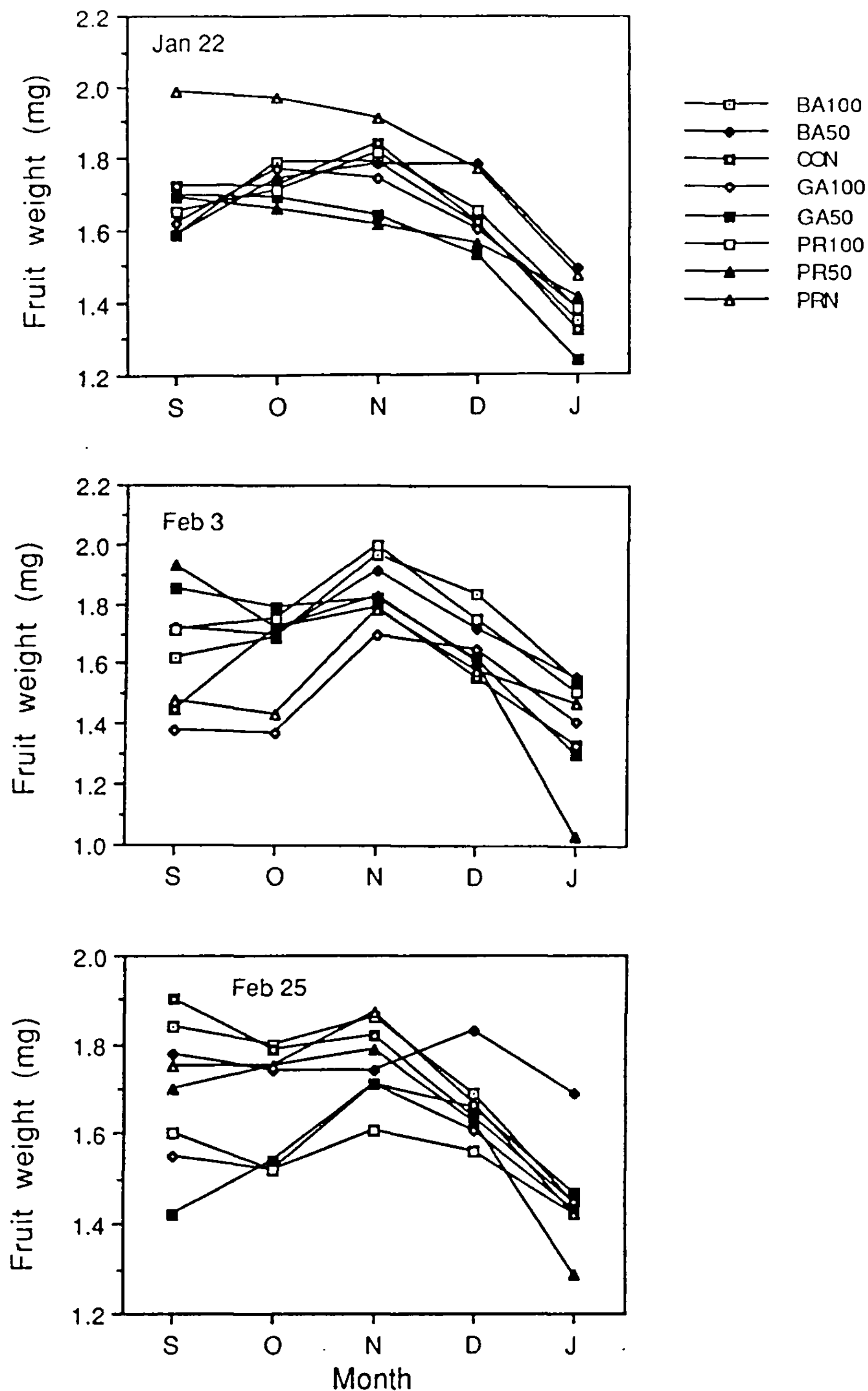

Figure 3.4. Fresh weight of fruit that were harvested from coffee (Coffea arabica L.) trees in 1988. Trees treated with growth regulators (BA, GA, PR) or pruned on 22 Jan, 3 Feb, and 25 Feb, 1988.

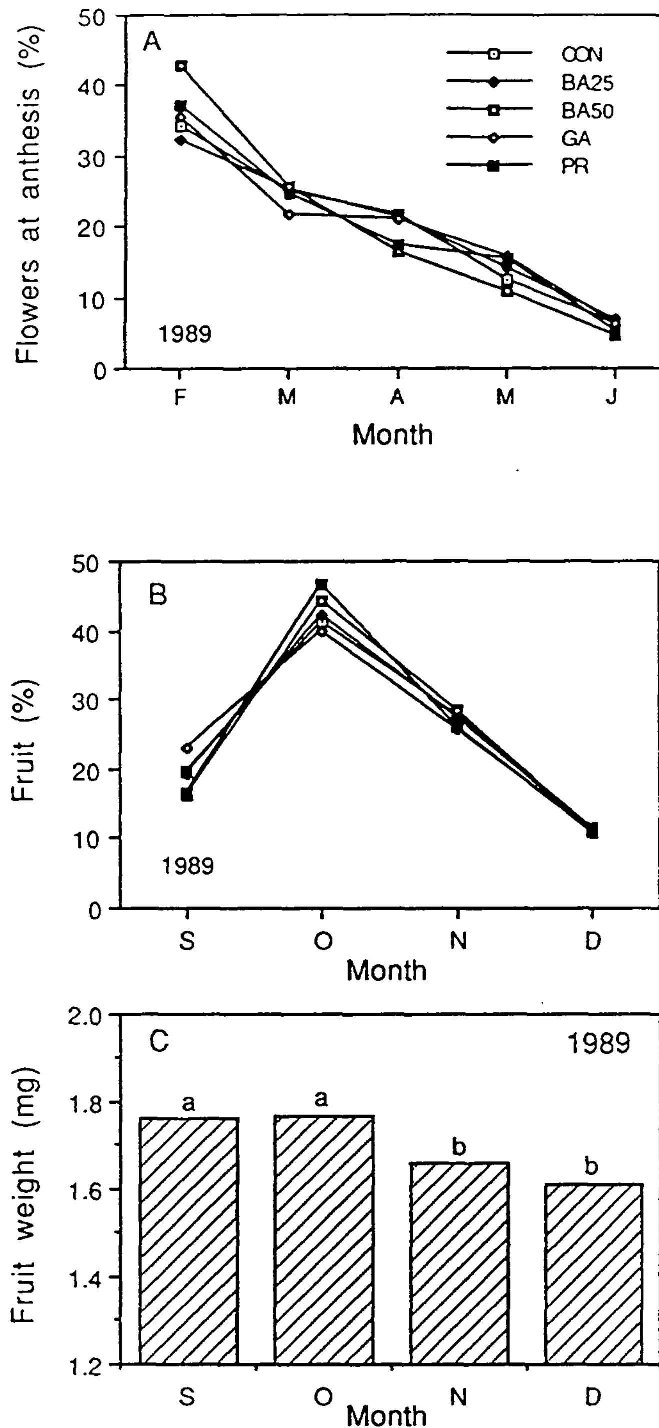

Figure 3.5. Flowering (A) and harvesting (B) and fruit fresh weight (C) of coffee (*Coffea arabica* L.) trees that were treated with growth regulators (BA, GA, PR) on 10 Feb, 1989.

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Chapter 4

Gibberellic acid causes earlier flowering and synchronizes fruit ripening of coffee

Abstract

The effect of 100 mg l^{-1} gibberellic acid (GA_3) on flowering and fruit ripening synchrony, fruit set, fruit fresh weight, and vegetative growth were studied for different size classes of coffee (Coffea arabica L. cv. Guatemalan) flower buds. Flower buds that were > 4 mm, but not developed to the candle stage at the time of GA_3 treatment, reached anthesis 20 days earlier than the controls, and their development was independent of precipitation, unlike the controls. Fruit from buds that were treated with GA_3 at the candle stage showed earlier and more synchronous ripening than the control, although no differences in flowering were found during anthesis. Buds that were smaller than 4 mm at the time of treatment did not respond to GA_3 applications. Treatment with GA_3 did not affect fruit set, fresh weight of fruits, or vegetative shoot growth.

Introduction

Flowering of coffee (Coffea arabica L. cv. Guatemalan) in the Kona district of Hawaii occurs from February to June. The prolonged flowering results in an extended harvesting period, from August to December, since fruits mature at different times. Since coffee is hand-picked, a

very time- and cost- intensive practice, chemical treatments to synchronize flowering and fruit ripening could allow mechanical harvesting to become feasible and thereby reduce labor costs.

Coffee flower buds stop growing and enter a period of dormancy when they reach a length of about 4 mm (Cannell 1985). External and internal morphology of the dormant bud indicate that floral organ formation has been completed (Cueto et al. 1984), and dormancy induction appears to be due to high levels of abscisic acid (Browning 1973). A period of water stress will break dormancy, and rehydration of the plant will stimulate anthesis within 8 to 10 days (Mes 1957, Alvim 1960, Magalhaes and Angelocci 1976). Exogenous GA can replace the period of water stress and induce bud growth (Alvim 1958, Browning 1973).

Coffee fruits undergo cell division 6-8 weeks after fertilization, but grow very little in weight or volume during that period (Cannell 1985). This is believed to be a form of true dormancy of fruit growth which is accompanied by high levels of ABA and low levels of free GA (Opile 1979).

The objectives of this study were to determine if GA_3 application could synchronize flowering and fruit ripening in coffee, and to determine if the GA_3 effect on flowering and ripening is dependent on the stage of floral bud development at the time of treatment.

Materials and Methods

Five-year old coffee trees, with an average height of 2.6 m and an average stem diameter of 4.9 cm at 20 cm above ground, growing at the Kona Experiment Station in Kainaliu, Hawaii, were used for this experiment. On Jan 27, 1988 flower buds were separated into four bud size classes based upon length: < 2 mm, 2-4 mm, > 4 mm, and the candle stage at which buds are > 10 mm just prior to anthesis. In buds at the candle stage dormancy has been broken, whereas buds > 4 mm may or may not be dormant. On each of 10 trees, buds were thinned to a single size class, resulting in a similar number of buds on one branch. GA_3 (Sigma) at 100 mg l^{-1} with 1% Tween 20 was applied to runoff to the thinned branches of 5 trees. The branches on 5 control trees, thinned to bud size classes as above, were sprayed with a solution of distilled water and 1% Tween 20.

The number of flowers at anthesis were counted at 4 to 7 day intervals from Feb to Aug 1988. Yield data was collected by counting fully red fruits at 2 week intervals from Aug 1988 to Feb 1989. Flowering and yield data were subtotaled for each calendar month, and expressed as a percentage of the total flowers and total fruits harvested, respectively. Means of GA_3 treated and control trees for each bud size class were compared on a monthly basis with a t-test.

Mature ripe fruits were hand-picked and refrigerated at 5°C. Fruit fresh weights were recorded one day after

picking at each harvest date for each bud size class. Branch lengths were measured at the time of treatment and after 12 months. Growth is reported as the mean of 20 branches. Fruit set, the percentage of flowers that developed into harvestable fruits, and vegetative growth of GA_3 treated and control branches were compared with a t-test. Precipitation and temperature were recorded at the study site during the main flowering period.

Results and Discussion

GA_3 significantly enhanced early anthesis of coffee flower buds that were > 4 mm at the time of treatment (Fig. 4.1). Early flowering resulted in earlier fruit ripening. For GA_3 treated branches 70% of the fruits ripened in Sep (Fig 4.2). In contrast, buds > 4 mm on control branches flowered much later, with 81% flowering in Mar. However, only 54% of the fruits on the control branches were harvestable during Oct, and fruit were picked throughout Nov to Dec. The control plants flowered about 10 days after a major rain (9cm/day), while GA_3 treated buds reached anthesis 20 days prior the controls, before measurable rainfall occurred.

The flowering pattern is similar to that reported by Alvim (1958), in which buds reached anthesis within 9-10 days of GA_3 applications at concentrations higher than 10 or 20 mg l^{-1}. Other experiments showed that GA_3 had more of an effect on plants that had been subjected to water

stress (Alvim 1960). Breaking of flower bud dormancy has been demonstrated to be an antagonism between ABA levels that inhibit blossoming, and GA levels that induce anthesis (Browning 1975). After water-stressed plants were rehydrated, GA increased several fold in magnitude, whereas ABA levels remained unchanged (Browning 1973). It was concluded that ABA might regulate the equilibrium between free and bound gibberellin (Browning 1973, 1975).

No differences between GA_3 treatment and control were apparent at the time of flowering for buds that were treated at the candle stage (Fig. 4.1). However, significant differences between treatments were observed in their yield pattern (Fig. 4.2). Fruit from GA_3-treated buds ripened in a more concentrated period (64% in Aug) compared to the controls in which ripening was spread from Aug to Dec. Even though flowering of the buds that were treated at the candle stage was concentrated in Feb, this synchronization was lost by the time of harvesting.

For buds smaller than 4 mm at the time of treatment, no differences between GA_3 treated and control branches in flowering and ripening were found. Thus the effect of GA_3 on flowering and fruit ripening is dependent on the stage of floral bud development.

Fruit set ranged from 42 to 67% with no differences between GA_3 treatment and control. This is comparable to values reported in a study of flower production and fruit set of several varieties of arabica coffee under South

Indian conditions (Reddy and Srinivasan 1979). Premature fruit drop can be attributed to adverse climatic factors, low auxin content, carbohydrate deficiency, and a cultivar specific variation in fruit retention (Reddy and Srinivasan 1979, Kumar 1982, Ramaiah and Venkataramanan 1985).

Fruit fresh weight ranged between 1.67 and 1.83 g. As reported by other researchers (Cannell 1971) no differences were found between GA_3 treatment and controls. Fruit size, and thus fresh weight, is largely dependent on the amount of rainfall or irrigation during the phase of rapid fruit growth. Fruit reach maximum volume 6-16 weeks after flowering (Cannell 1985) which coincides with the period of rapid increase in fresh weight to about 90% of the final weight (Ramaiah and Venkataramanan 1985).

There was no significant difference in shoot growth between control and GA treatment, which averaged 125 and 147 cm respectively. Other studies also found no increase in branch length with a single application of 100 mg l^{-1} GA_3, however, multiple applications resulted in a significant branch extension (Cannell 1971).

This study demonstrated that GA_3 treatments may have a potential for synchronizing flowering and ripening of coffee. *The application time is critical since flowering* and ripening responses to GA_3 are dependent on the morphological floral bud development. If buds larger than 4 mm in length are sprayed with GA_3, flowering and fruit ripening are enhanced.

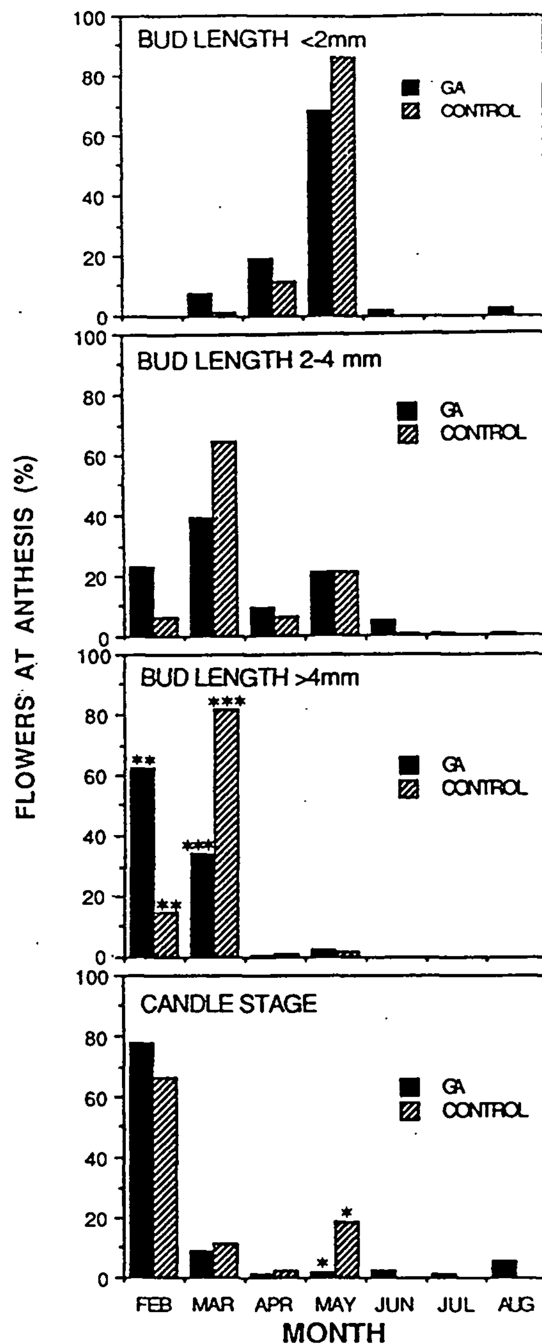

Figure 4.1. Effect of 100 mg l^{-1} GA_3 on anthesis of coffee (<u>Coffea arabica</u> L.). Flower buds were <2 mm, 2-4mm, >4 mm, and at the candle stage at the time of application, Jan 27, 1988. (t-test comparing GA_3 to control for mean differences each month significant at * $p<0.05$, ** $p<0.01$, and *** $p<0.001$).

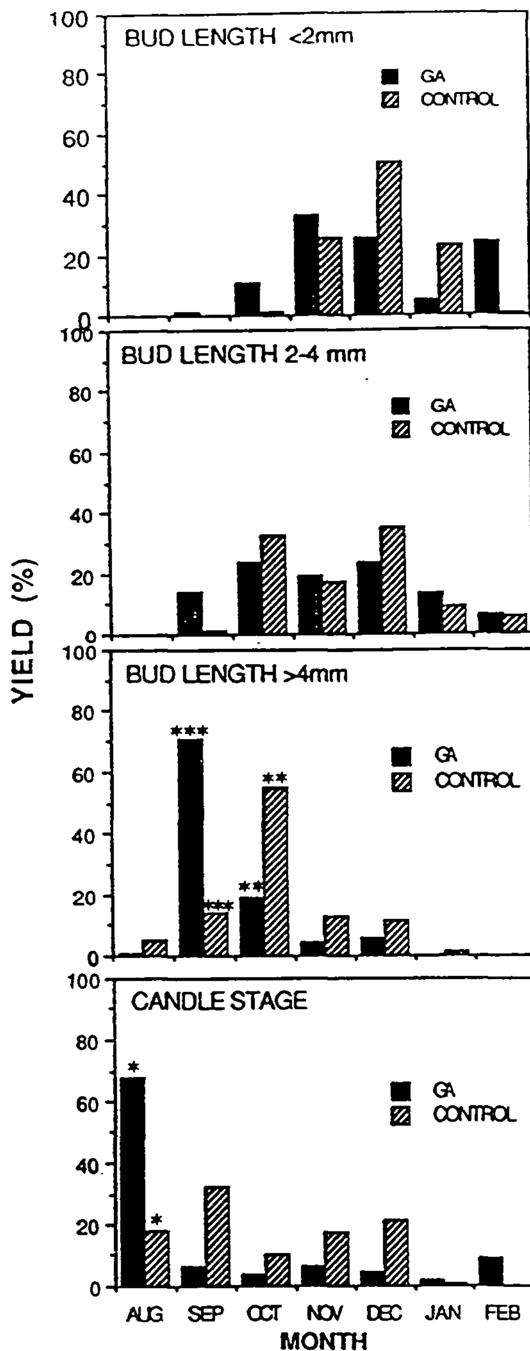

Figure 4.2. Effect of 100 mg l^{-1} GA_3 on ripening of coffee (<u>Coffea arabica</u> L.). Flower buds were <2 mm, 2-4mm, >4 mm, and at the candle stage at the time of application, Jan 27, 1988. (t-test comparing GA_3 to control for mean differences each month significant at * $p<0.05$, ** $p<0.01$, and *** $p<0.001$).

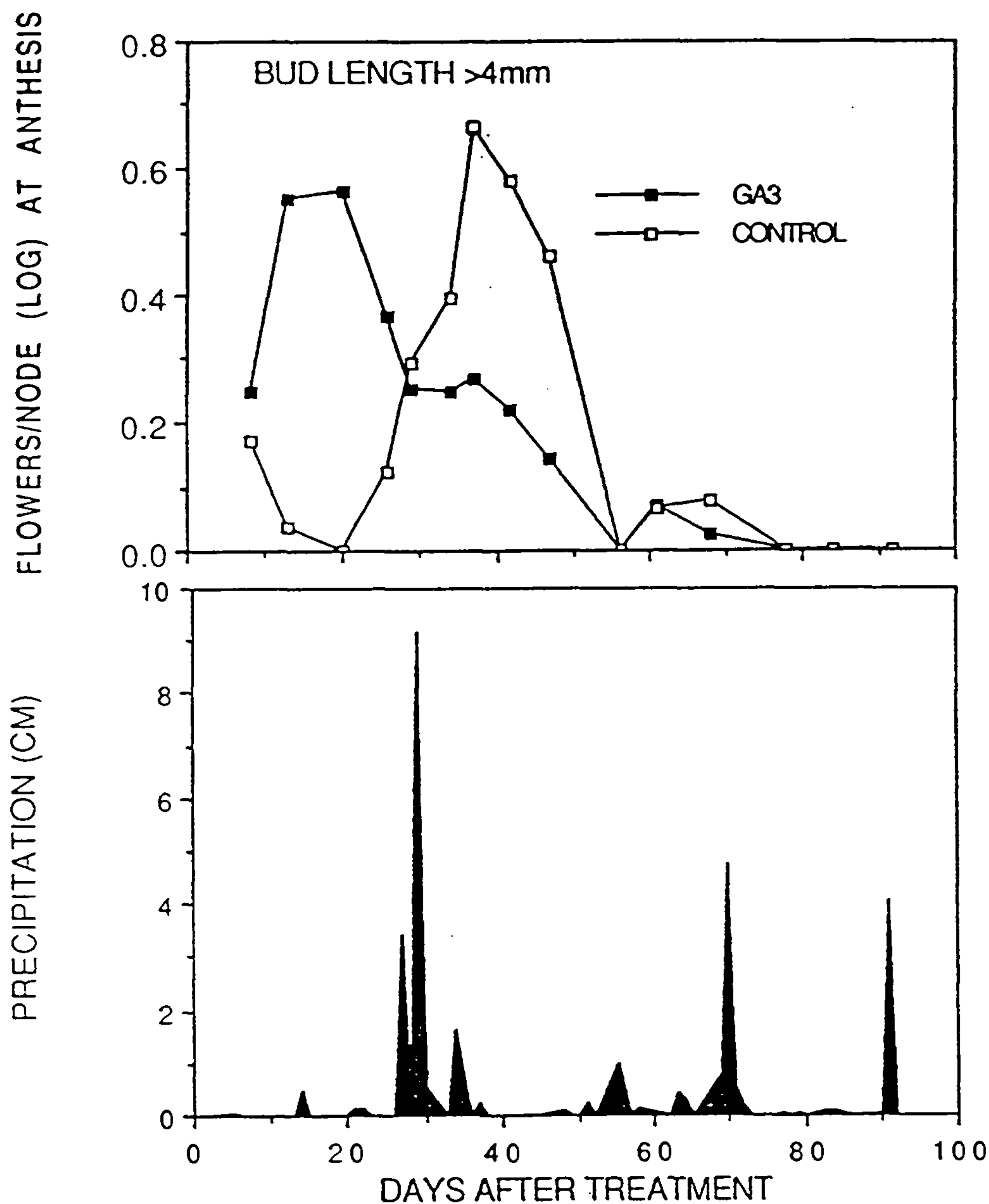

Figure 4.3. Anthesis of the coffee (Coffea arabica L. cv. Guatemalan) flowers treated with GA_3 until 100 days after treatment, and corresponding precipitation at the study site. Flower buds were > 4 mm at the time of treatment, Jan 27, 1988. Mean minimum and maximum temperatures were 16°C and 23.5°C.

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Chapter 5

Responses of *Coffea arabica* L. to breaking flower bud dormancy with water stress and gibberellic acid

Abstract

To determine how water stress and gibberellic acid (GA_3) affect the breaking of dormant flower buds (4 mm stage) in coffee (*Coffea arabica* L.), dormancy, water potential, ethylene production, and ion leakage of flower buds and leaf disks were examined between the period from release from water stress until anthesis. In the first experiment water was withheld until the trees reached leaf water potentials (WP) of -1.2, -1.75, -2.65, or -3.5 MPa. In the second experiment, trees were treated with 0, 50, 100, and 200 mg/l GA_3 and kept well watered. In experiment three, water was withheld until plants reached WP of -0.6, -1.3, -2.1, and -3.0 MPa, and each tree was treated with 0, 50, and 100 mg/l GA_3. Anthesis was stimulated within 9 days after rewatering trees that had experienced leaf WP of < -2.65 MPa, and flower bud WP of about -4.0 MPa. Trees exposed to -2.1 MPa flowered partially when GA_3 was applied. At low water stress conditions, increasing GA_3 concentrations induced partial flowering. Ethylene evolution of flower buds where dormancy had been broken with water stress was less than that in dormant buds. Severe water stress treatments and GA_3 application increased ethylene evolution of leaf disks. Ion leakage of flower buds and leaf tissue were increased by water stress.

Ethylene production and ion leakage of flower buds were highest at anthesis. When plants were stressed beyond the threshold leaf and flower bud WP, dormant flower buds of coffee at the 4 mm stage developed to anthesis; GA_3 could partially compensate for insufficient water stress. After water stress, dormant and non-dormant flower buds at the 4 mm stage could be distinguished based on their ethylene evolution.

Introduction

Flower buds of coffee become dormant after reaching 4 to 6 mm in length (Mes 1957). Water stress breaks dormancy and is considered mandatory for normal flower development (Alvim 1960, Piringer and Borthwick 1955). After dormancy is broken, irrigation or exogenous gibberellic acid (GA_3) can stimulate development of the flowers to anthesis (Alvim 1958, van der Veen 1968, Browning 1975). Magalhaes and Angelocci (1976) found that dormancy is broken and flowering is induced when coffee plants are subjected to water stress with leaf water potentials (WP) less than -1.2 MPa. Water movement into a flower bud occurs with increasing water stress and is enhanced by the presence of a leaf subtending the flower bud, compared to defoliated nodes (Astegiano et al. 1988).

Environmental conditions or chemical agents that produce near lethal stress can break bud dormancy in temperate woody plants (Fuchigami and Nee 1987). Increased

membrane permeability and ethylene production are related to the degree of stress and breaking of rest in crabapple and red-osier dogwood (Nee 1986). An increase in ethylene production with increasing water loss was observed in plum leaves (Kobayashi et al. 1981). Increased ion leakage coincided with peak ethylene production in rhododendron leaf disks, suggesting the onset of membrane disintegration (Harber and Fuchigami 1986).

In a previous field study we found that when buds at the 4 mm stage are treated with 100 mg/l GA_3, they flower regardless of precipitation (Chap. 4). Since the exposure of water stress in the field could not be controlled and was not monitored, the effects of different degrees of water stress and GA_3 alone, and their interaction need further investigation in a controlled environment.

The first objective of this study was to determine the effects of water stress and GA_3 on flower bud dormancy of coffee and on flower development to anthesis. Secondly, ethylene evolution and membrane permeability of flower buds and leaf tissue were determined after treatments that release bud dormancy and stimulate development to anthesis.

Materials and Methods

Coffee (Coffea arabica L. cv. Guatemalan) trees were grown in 4 l plastic pots in a mixture of pumice, peat, and soil (2:1:1 v/v) for 2 years in a greenhouse under day/night temperatures of 25/19°C and natural photoperiod

(Oregon, 44°N latitude). Plants with flower buds 4 mm in length and smaller were transferred to a growth chamber with an 8 hr photoperiod (600 umol $m^{-2}s^{-1}$) and day/night temperatures of 26/23 ($\pm$2)^{o}C and acclimated for 3 weeks before the experiment. Plants were irrigated every other day.

Experiment I. Water was withheld until the plants attained leaf WP of -1.2, -1.75, -2.65, and -3.5 MPa. When the desired WP were attained (day 0), plants were watered until the soil was saturated, thereafter, the pots were irrigated daily with 500 ml of water. Each water-stress treatment was applied to three trees in a completely randomized design. Days to anthesis and percentage of 10 selected nodes per tree that reached anthesis were recorded. Leaf and flower bud WP, ethylene evolution, and ion leakage were determined 0, 3, 6, and 9 days after re-irrigation. Leaf and bud samples from replicate trees were pooled.

Experiment II. Four branches were selected on 10 trees and on each branch 0, 50, 100, or 200 mg/l GA_3 plus 0.2% Tween 20 was applied with a brush. Trees were irrigated with 500 ml of water daily. Ethylene evolution of two flower buds per tree and treatment was determined every 5 days until 20 days after GA_3 application. Number of flowers and nodes at anthesis on 5 selected nodes per treatment on each tree were recorded. The data were

analyzed as a randomized block design with one tree representing a block.

Experiment III. Water was withheld until plants attained leaf WP of -0.6, -1.3, -2.1, and -3.0 MPa. At rewatering (day 0), 0, 50, and 100 mg/l of GA_3 plus 0.2% Tween 20, was applied with a brush to lateral branches. The experiment was a split-plot design with water stress as the main effect applied to three trees each, and each GA_3 concentration applied to two branches per tree as the subeffect. Days to anthesis, the percentage of 5 nodes per treatment combination on each tree at anthesis, and flower bud length were recorded. Leaf WP, and ethylene production, and ion leakage of flower buds and leaf disks were determined 0, 3, 6, and 9 days after rewatering. Leaf and flower-bud samples were pooled for each treatment combination from replicate trees.

Water potential measurements. Leaf WP was estimated by determining xylem WP of the most recent fully expanded leaf pair on a lateral branch in the upper half of the canopy with a pressure chamber (PMS, Corvallis, Oregon). Water potential of flower buds was measured with a thermocouple psychrometer (Decagon Devices, Pullman, Washington). Flower buds were cut at the base of the pedicel, and 2 buds were equilibrated in thermocouple cups for 2 hr before the measurements were taken. At each sampling time the psychrometer was calibrated with two or three standard solutions, and flower buds, collected from

the same treatments, were pooled and five replicate measurements were taken.

Ethylene measurements. Flower buds and 10 mm leaf disks were incubated with a drop of water in 5-ml syringes for 2 hrs in the dark at room temperature (22-24°C). A 1.0 ml sample was injected in a Gow-Mac Series 580 (Gow-Mac Instrument, Bridgewater, New Jersey) gas chromatograph with a flame ionization detector and activated alumina column. Column, detector, and injector temperatures were 80, 100, and 90° C, respectively. Flow rates for helium and hydrogen were 25 ml/min, and 200 ml/min for air. Five to six replications were measured for each treatment combination and sampling time.

Ion leakage. Flower buds and leaf disks used for ethylene determination were incubated in stoppered vials with 3 ml of double-distilled water, placed on a shaker for 24 hr, and initial ion leakage measured with a conductivity meter. The final leakage was determined after vials were placed in a 60°C waterbath for 2 hr to kill the plant tissue, and incubated for another 24 hr on the shaker. Percent leakage was calculated as the ratio of initial to final values. At each sampling time, five replications were measured for each treatment combination.

Results

Experiment I. Plants that attained leaf WP of -2.65 and -3.5 MPa reached anthesis within 9 days after rewatering, whereas those exposed to -1.2 and -1.75 MPa leaf WP did not flower (Figure 5.1). Flower buds that developed to anthesis (-2.65 and -3.5 MPa leaf WP treatments) had a WP of about -4.0 MPa before plants were irrigated (Figure 5.1). Three days after irrigation all treatments had equilibrated to flower-bud WP around -2.5 MPa, and thereafter ranged between -1.8 and -2.7 MPa.

For all experiments, daily watering or re-irrigation of plants resulted in leaf WP between -0.6 and -1.1 MPa in all treatments and sampling times.

Flower buds on plants that were stressed to -2.65 and -3.5 MPa and developed to anthesis produced less ethylene at 0, 3, and 6 days after re-irrigation compared to buds on plants receiving lower water stress (Figure 5.2). Ethylene evolution at day 9 (anthesis) increased sharply to levels equal or above the level produced by buds that did not flower. Leaf disks from plants that were stressed to -2.65 and -3.5 MPa produced more ethylene at day 0, 3, and 6 than disks from plants in the -1.2 and -1.75 MPa treatments (Table 5.1).

Ion leakage of flower buds and leaf disks was generally higher in plants exposed to greater water stress, but the effect was not consistent (Table 5.1). At anthesis flower buds exhibited the highest amount of ion leakage,

but at other earlier sampling times ion leakage ranged between 3 and 10%.

Experiment II. Branches that received the highest GA_3 concentration (200 mg/l) developed the highest percentage of flowering nodes and the most flowers per node at anthesis 10 days after treatment (Table 5.2). Abnormal flower buds occurred on all treatments, either as starflowers that opened when they were still 4 mm in length or smaller (van der Veen 1968), or flower buds 4 mm in length that remained closed with the style elongating beyond the petals (Huxley and Isamil 1969). In either case flowers aborted within two weeks after opening or style elongation. In flowers treated with the highest GA_3 concentration, the style elongated to 8-12 mm, whereas styles from the other treatments elongated less.

GA_3 did not influence ethylene production of flower buds, except on day 15, where the 100 and 200 mg/l GA_3-treated buds evolved 6.6 and 7.8 nl ethylene per g fresh weight (FW)/hr ethylene compared to the 0 and 50 mg/l GA_3-treated buds that produced 5.1 and 5.3 nl ethylene per g FW/hr. The mean ethylene evolution of flower buds ranged between 4.4 and 7.8 nl ethylene per g FW/hr for all samples.

Experiment III. Plants stressed to leaf WP of -3.0 MPa flowered within 8 days after rewatering, regardless of the GA_3 concentration they received (Table 5.3). A small

percentage, 10 and 23%, of plants stressed to -2.1 MPa flowered within 8 days when 50 and 100 mg/liter GA_3 was applied. Anthesis occurred within 28 days after rewatering for some trees that had received water stress of -0.6 and -1.3 MPa (Table 5.3).

Flower buds of plants that were stressed to -3.0 MPa produced significantly (p=0.001) less ethylene on day 0, 3, and 6, and more ethylene on day 9 than flower buds of plants that were exposed to leaf WP greater than -2.1 MPa (Figure 5.3). GA_3 treatment did not influence ethylene production of flower buds. On day 9 there was a significant (p=0.05) interaction between WP and GA_3 treatment affecting ethylene evolution.

Ethylene evolution of leaf disks was significantly (p=0.05) influenced by WP and GA_3 treatment, however, there were no significant interactions when means were tested for differences at each sampling day. Ethylene evolution of the GA_3-treated leaf disks was always higher than the control, but the difference decreased over time (Table 5.4).

Ion leakage of flower buds and leaf disks was significantly (p=0.05) affected by leaf WP in the analysis of variance at all sampling dates (Table 5.4). For ethylene evolution of leaf disks a significant interaction between leaf WP and GA_3 was found. Ion leakage of flower buds at day 0 was twice as high for the -2.1 and -3.0 MPa treatments, compared to the lower water stress treatments.

Flower buds that reached anthesis had the highest ion leakage at that time (Table 5.4). At other sampling times, ion leakage of < -2.1 MPa treatments ranged between 3 and 10%. In contrast, ion leakage of flower buds and leaf disks of trees stressed to -3.0 MPa reached 11 and 14%.

When plants were exposed to water stress, flower buds were between 4 and 6 mm long. Three days after rewatering, flower buds that developed to anthesis had expanded to 9 to 12 mm, and doubled this length in the next 3 days. Flower buds were up to 25 mm long prior to their anthesis. Flower buds treated with GA_3 tended to expand slightly faster than untreated ones, but differences were not statistically significant. Flower buds that remained dormant did not lengthen.

Discussion

Studies on coffee flowering have indicated that water stress is required for flower buds to break dormancy and develop to anthesis. Flowering observed 8 to 10 days after rewatering of plants indicates that flower bud dormancy had been broken by water stress, as reported by Piringer and Borthwick (1955), Alvim (1960), and van der Veen (1968). Magalhaes and Angelocci (1976) found that threshold leaf WP of about -1.2 MPa and flower bud WP of -1.4 MPa was the minimum water stress necessary to induce flowering. In the experiment reported here, flower bud WP of about -4.0 MPa and leaf WP of -2.6 MPa was associated with breaking of

dormancy. Under a daily watering regime, leaf WP of unstressed or re-watered trees were between -0.6 and -1.1 MPa in this study, whereas -1.2 MPa leaf WP in Magalhaes and Angelocci's report is sufficient to break dormancy in coffee flower buds. Differences might be due to the time of sampling, position of sampling, plant cultivar and plant age.

Some plants stressed to a leaf WP of -2.1 MPa flowered partially 8 days after rewatering, but only when treated with GA_3, suggesting that GA_3 in combination with mild water stress can stimulate anthesis, probably by compensating for low endogenous GA. These results confirm Alvim's (1958) observations that GA_3 was more effective in breaking dormancy in water stressed than in unstressed coffee plants, and that GA_3 application during the rainy season did not affect flowering. Browning (1973) suggested that a rise in endogenous GA content is the stimulus for releasing dormant coffee flower buds. He showed that after bud dormancy was broken with water stress and regrowth stimulated by irrigation or rain, the endogenous GA concentration in flower buds increased significantly before buds gained fresh weight. Since GA biosynthesis inhibitors did not prevent dormancy release, he suggested that GA is converted from a bound form to a free, active form at the time of release from dormancy. We also found that paclobutrazol, a GA biosynthesis inhibitor, when applied

prior to water stress as a soil drench in 500 ml of water at concentrations of 0, 5, 50, and 100 mg per pot, did not inhibit flowering after plants were released from dormancy (data not shown).

In addition to soil moisture water-stress, high temperatures and humidities hastened dormancy release (Reddy, 1979). Differences in transpiration and soil moisture content between plants result in different levels of water stress, and can lead to varying dormancy status and different responsiveness to GA in flower buds. Factors that favor release from dormancy might accumulate, similar to chilling accumulation observed in dormant plants in temperate zones (Alvim 1960). This might be the case in our experiments and can explain some of the variable responses to GA in plants exposed to water stress that is inadequate to break dormancy. The accumulation might also explain the sporadic blossoming of unstressed plants as observed by Astegiano et al. (1988). Ga_3 might provide the required stimulus for continued bud development after water stress, or compensate for water stress that is inadequate for dormancy breaking, as indicated by increased blossoming with higher GA concentrations in less stressed trees. Irrigation or rain triggers the increase in endogenous GA (Browning 1973), effects of exogenously applied GA_3 to stimulate bud development become apparent only in the absence of irrigation or rain.

Cambrony and Snoeck (1983) reported flowers with

elongated styles and stigmata when greenhouse grown coffee plants were sprayed with GA, similar to our observations. Mes (1957) suggests that high temperatures and high transpiration rates promote this floral atrophy. Observations in Kenya suggest that heavy rain during the period of rapid flower bud enlargement may cause starflowers or deviations from normal flowers, however, the role of water deficits in causing atrophied flowers needs more study (Huxley and Ismail 1969). A combination of GA_3 and high temperatures during noon hours in the greenhouse might have disrupted the normal growth of flower buds in this study.

Ethylene evolution of dormant flower buds was always higher than in those in which dormancy had been broken by water stress. The severe water stress required to remove dormancy did not increase ethylene production, an unexpected result, since in general ethylene evolution increases in stressed tissues (Abeles 1973). Compared to coffe flower buds, tight orchid buds produce relatively high concentrations of ethylene throughout their development (Yip and Hew 1988). The physiological stage of flower buds determines ethylene evolution, which was found to vary considerably between orchid genera (Goh et al. 1985). Ethylene production increases rapidly at pollination (Reid 1988). Increased ethylene production was observed in our experiments at anthesis and may be due to

pollination. The difference in ethylene production of dormant and nondormant coffee flower buds is an important finding because one can now separate physiological stages that cannot be distinguished by morphology or anatomy.

Throughout the experiment, ethylene evolution of leaf disks was highest from severely water-stressed plants. Similarly, Kobayashi et al. (1981) showed that ethylene production increased in water-stressed plum leaves as moisture loss increased. The general pattern that tissues increase ethylene production under stress has been confirmed in this study for leaf disks of coffee plants, but the opposite was found for flower buds.

The most severely water-stressed plants showed the highest amount of ion leakage from flower buds and leaf disks at the end of the water stress treatment and before irrigation was applied. An increase in ion leakage was found in severely dehydrated plum leaves, when 50% or more moisture was lost (Kobayashi et al. 1981). Similar observations were made in tissues that were damaged by freezing stress (Harber and Fuchigami 1986). Fluctuating values for flower-bud and leaf tissue indicate that changes in ion leakage were reversible, and that the initial high values for severely stressed tissue caused no permanent damage to membrane permeability and did not result in cell death. Once flower buds broke dormancy and continued to develop to anthesis, they grew rapidly, and ion leakage increased, reaching a peak at anthesis.

These experiments show that a threshold leaf and bud water potential were necessary to break dormancy in coffee flower buds. In flower buds where dormancy was not completely broken by water stress, GA_3 stimulated development to anthesis, probably by compensating for a lack in endogenous GA. Ethylene evolution was found to be a useful parameter to distinguish between dormant and nondormant buds.

Table 5.1. Ethylene evolution of leaf disks and ion leakage of flower buds and leaf disks from trees that were water stressed (experiment I).

Leaf WP[z] (MPa)	Ethylene evolution (nl/g FW/hr) Days after treatment				Leaf WP (MPa)	Ion leakage (%) Days after treatment			
	0	3	6	9		0	3	6	9
	Leaf disks					Flower buds			
-1.2	0.8 ab[y]	0.6 a	0.8 a	0.8 a	-1.2	7.0 a	5.4 a	7.0 a	4.9 a
-1.75	0.5 a	0.6 a	0.7 a	1.0 a	-1.75	5.1 a	3.1 a	5.8 a	5.0 a
-2.65	1.1 ab	1.6 b	1.3 b	0.8 a	-2.65	6.2 a	10.3 b	8.3 a	39.1 b
-3.5	1.6 b	1.3 b	1.3 b	2.3 b	-3.5	10.5 a	4.7 a	21.9 b	53.6 c
						Leaf disks			
					-1.2	5.2 a	7.4b	10.5 b	5.8 a
					-1.75	6.3 a	5.7a	8.8 ab	9.4 b
					-2.65	7.1 a	8.3b	8.4 ab	7.8 ab
					-3.5	10.4 b	5.2a	7.6 a	8.8 b

[z] WP is leaf water potential at day 0, when trees were re-irrigated.
[y] Means within a column are separated by Tukey's Studentized Range Test (p=0.05).

Table 5.2. Anthesis of coffee flower-buds that were treated with different concentrations of GA_3 (experiment II).

GA_3 (mg/l)	Number of flowering nodes (%)	Flowers/node at anthesis
0	34 a[z]	1.0 a
50	40 a	1.4 a
100	36 a	0.9 a
200	64 a	2.6 b

[z] Mean separation within columns based on Tukey's Studentized Range Test (p=0.01). Each mean represents 10 replications.

Table 5.3. Percentage of nodes[z] with flowers at anthesis 8 and 28 days[y] after plants at different water potentials (WP) were treated with GA_3 (experiment III).

WP (MPa)	GA_3 (mg/l)	Flowering nodes (%) after 8 days	28 days
-0.6	0	0	10.0 (5.8)
	50	0	26.7 (17.7)
	100	0	30.0 (19.8)
-1.3	0	0	0
	50	0	0
	100	0	10.0 (9.9)
-2.1	0	0	0
	50	10.0 (5.8)	10.0 (5.8)
	100	23.3 (14.5)	29.3 (17.6)
-3.0	0	93.3 (6.7)	93.3 (6.7)
	50	96.7 (3.3)	96.7 (3.3)
	100	100.0	100.0

[z] Each mean (standard error) is calculated from 10 nodes replicated on 3 trees.
[y] Cumulative percent are presented after 28 days.

Table 5.4. Ethylene evolution of leaf disks and ion leakage of flower buds and leaf disks from coffee trees that were water stressed and received GA_3 treatments (experiment III).

Treatments Leaf WP[y] (MPa)	Ethylene evolution[z] (nl/g FW/hr)				Treatment Leaf WP (MPa)	Ion leakage (%) Days after treatment			
	0	3	6	9		0	3	6	9
	Leaf disks					Flower buds			
-0.6	1.01[x]a	0.7 a	1.0 a	0.6 ab	-0.6	6.6 a	6.9 a	5.8 a	4.9 a
-1.2	0.4 b	1.9 b	0.8 a	0.5 a	-1.2	4.8 a	7.4 a	8.7 ab	4.6 a
-2.1	0.7 ab	1.1 ab	1.1 a	0.7 ab	-2.1	9.8 b	6.0 a	8.8 ab	16.8 b
-3.0	0.9 a	1.5 ab	1.2 a	0.9 b	-3.0	11.3 b	10.4 b	10.2 b	57.9 c
GA_3						Leaf disks			
0	1.0	0.9 a	0.6 a	0.5 a	-0.6	8.8 a	6.3 a	9.8 bc	8.6 ab
50	-	1.2 ab	1.1 ab	0.7 ab	-1.2	5.6 a	7.9 ab	11.1 c	7.7 ab
100	-	1.8 b	1.3 b	0.8 b	-2.1	8.0 a	7.4 ab	8.6 bc	7.2 a
					-3.0	14.3 b	8.6 b	7.5 a	9.0 b

[z] Means are pooled by main effects and represent 15 and 20 replications for WP and GA, respectively.

[y] WP is leaf water potential at day 0, when trees were re-irrigated and GA_3 was applied.

[x] Means within a column are separated by Tukey's Studentized Range Test ($p=0.05$).

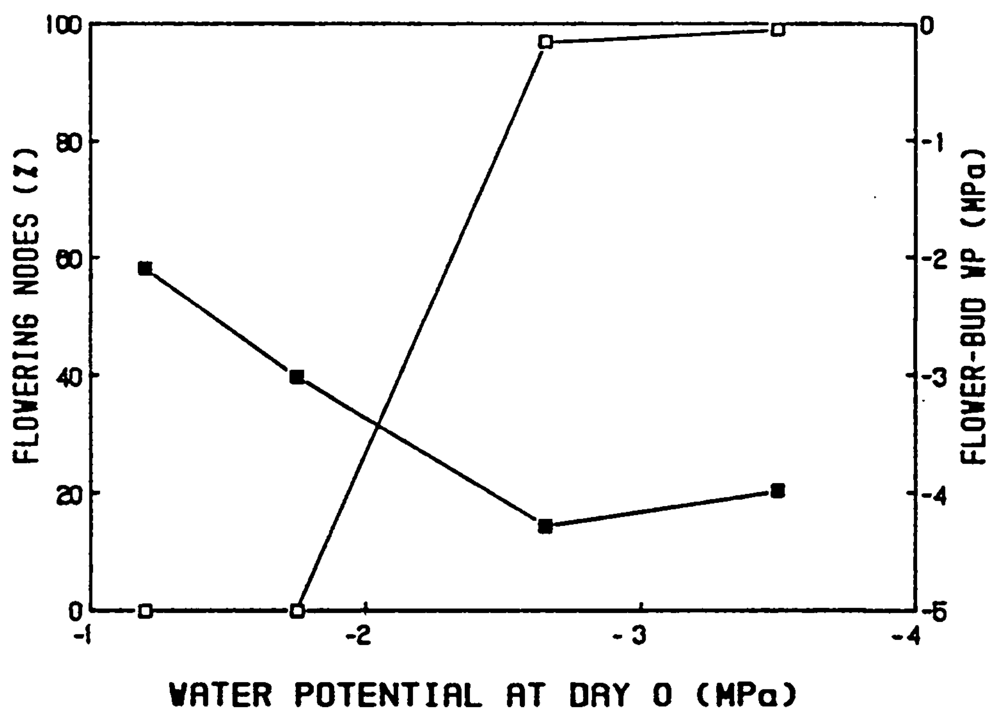

Figure 5.1. Relationship between leaf water potential (WP) at day 0 and anthesis (open squares) and flower-bud WP (closed squares) after water stress (experiment I). Leaf and flower-bud WP were measured at the time of rewatering of stressed plants. Flowering occurred 9 days after rewatering.

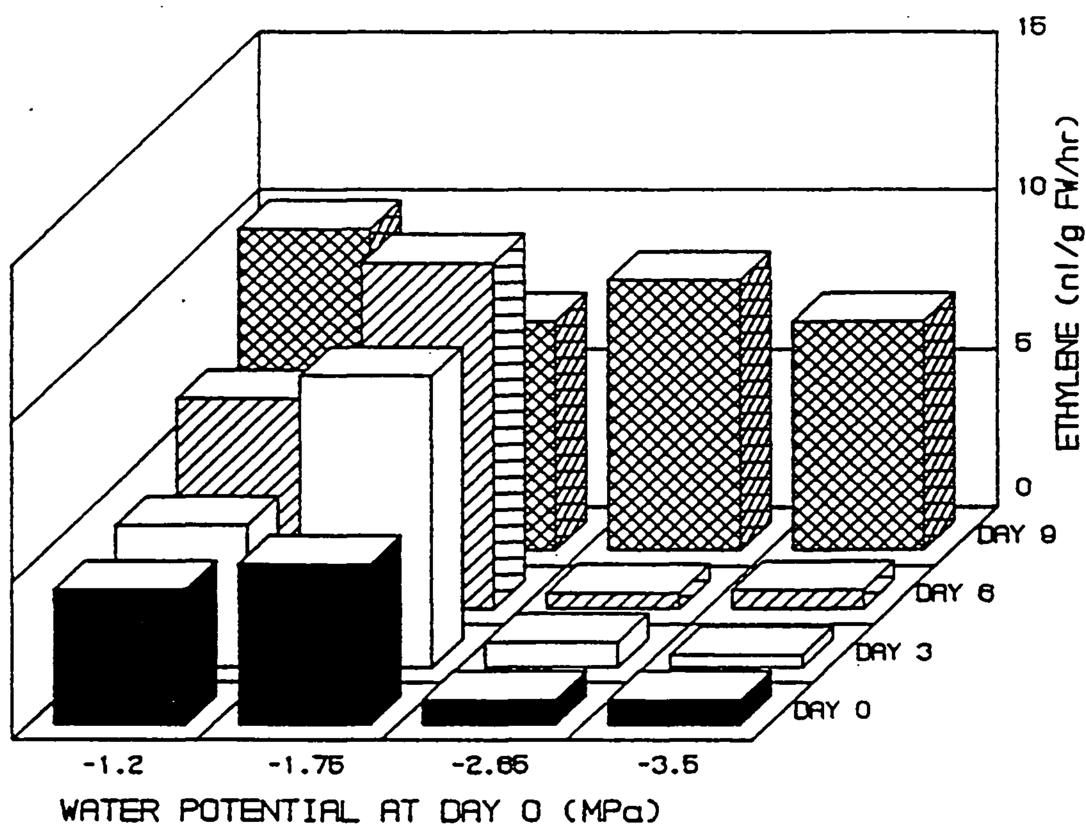

Figure 5.2. Effect of water stress on ethylene evolution of flower buds 0, 3, 6, and 9 days after re-irrigation (experiment I). Each mean represents 5 replications.

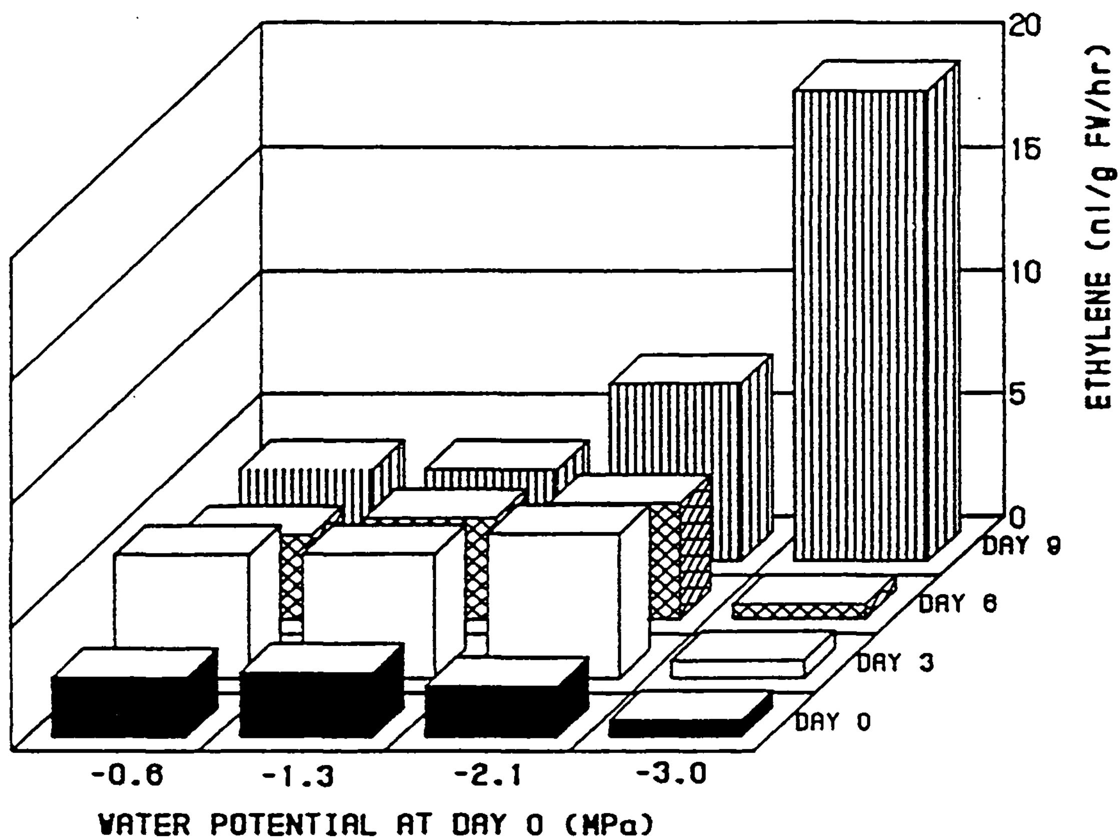

Figure 5.3. Effect of water stress and GA_3 on ethylene evolution of flower buds 0, 3, 6, and 9 days after re-irrigation (experiment III). Means are pooled over GA_3 treatments (0, 50, 100 mg/l), each bar represents 20 replications.

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Chapter 6

Water uptake and ethylene after breaking dormancy in flower buds of coffee

Abstract

To determine the relationship among dormancy, water uptake, and ethylene evolution in flower buds of coffee, a xylem specific dye was used to quantify the uptake of water after dormancy had been broken with water stress. Water uptake of non-stressed flower buds was variable, in stressed flower buds the rate of uptake tripled from day 1 to day 3 and preceded the doubling of fresh weight of buds. Lateral branches that were placed in the dye solution or water and treated at either 4 or 21°C and with either 0 or 200 mg/l GA_3 flowered, regardless of the treatment. Highest ethylene evolution was associated with non-stressed flower buds that had taken up large amounts of water. Ethylene evolution was high in buds at the 4 mm stage and low in buds that were 12-15 mm long and developing to anthesis, regardless of whether they had taken up water or not. To determine whether ethylene releasing agent or inhibitors can stimulate anthesis, ethephon, AVG, and silverthiosulfate were applied to 4 mm non-stressed flower buds. Ethephon at 1,000 mg/l was phytotoxic, increased ethylene evolution of flower buds and caused leaf and bud abscission and branch dieback. AVG and silverthiosulfate did not affect ethylene evolution and flowering.

Introduction

Water potential of the coffee plant and the flower bud are known to play an important role from the time that flower buds reach dormancy until they continue to develop to anthesis (Alvim 1958, 1960; van der Veen 1968, Browning 1973). Xylem development in the peduncle is poor when the buds enter dormancy (Mes 1957), until dormancy is broken by subjecting plants to water stress (Alvim 1958, 1960) or a sudden temperature drop (Browning 1973). Two to three days after a regrowth stimulus such as irrigation or gibberellic acid application (Alvim 1958, van der Veen 1968, Browning 1973), the vascular system develops rapidly to maintain adequate water supply for the rapidly growing bud (Mes 1957).

A threshold leaf water potential of -1.2 MPa was found as prerequisite for flower buds to respond to irrigation and develop to anthesis (Magalhaes and Angelocci 1976). Leaf and flower bud water potentials of -2.6 and -4.0 MPa were necessary for plants to reach anthesis after irrigation. Small amounts of tritiated water were taken up by flower buds of non-stressed plants, but the uptake doubled right after plants were water stressed, and reached a steady level 4 days after re-irrigation (Astegiano et al. 1988). Water uptake was enhanced if the subtending leaf was present, whereas in buds where the subtending leaf was removed, uptake was considerably lower. Calcium, considered to be transported mainly in the apoplast, was

not taken up at all into flower buds of non-stressed plants, but was readily imported into flower buds of stressed plants (Astegiano et al. 1988), leading to the conclusion that water in dormant plants enters the bud via the symplast as suggested earlier by Mes (1957).

In temperate woody plants, conditions that produce stress were shown to break dormancy (Fuchigami and Nee 1987). Increased ethylene production is often found as the result of stress (Reid 1988), and was directly related to increased water loss in plum leaves (Kobayashi et al. 1981). Ethylene production of coffee flower buds is contrary to the previously established pattern. Dormant flower buds produce high levels of ethylene, whereas after a severe water stress which breaks dormancy the ethylene evolution is very low until it increases again at anthesis (Chap. 5).

In the experiments reported here, we studied the relationship between dormancy, water uptake, temperature, and ethylene evolution of flower buds of coffee. The dye azosulfamide, transported in the xylem (Ashworth 1982), was used to quantify the uptake of water after dormancy was broken with water stress, and the relationship between dye uptake and ethylene evolution was determined. The question of how ethylene is involved in dormancy and the breaking mechanism was addressed by applying ethephon, an ethylene releasing agent, AVG (aminoethoxyvinylglycine), an ACC (1-

aminocyclopropane-1-carboxylic acid) biosynthesis inhibitor, and silverthiosulfate, an ethylene action inhibitor (McKeon and Yang 1987).

Materials and Methods

Dye uptake

Thirty-month-old greenhouse grown coffee (Coffea arabica cv. Guatemalan) plants with dormant flower buds (4-6 mm lengths) were withheld from watering until their leaf water potential was below -2.6 MPa, which is known to break flower bud dormancy (Chap. 5). At 2 day intervals, starting the day after rewatering until anthesis, branches with the mature dormant buds were cut and placed either in water or 1% aqueous azosulfamide solution. Buds were harvested at 1, 3, 6, 12, and 24 hr after exposure to water or azosulfamide, weighed, and homogenized in distilled water. The samples were centrifuged for 5 min at 1,000 rpm. Aliquots of the supernatant were diluted and absorbance at 503 nm was measured with a Shimadzu UV-120 spectrophotometer. Absorbance (A) was expressed per gram fresh weight; the difference between the control branches in water and the branches in dye was calculated as relative absorbance percent with reference to the control (A dye - A control)*100/A control. At each sampling time 4 replications were processed. Regressions were calculated for the rate of uptake for each sampling date.

Dye/ temperature/ gibberellic acid study

Lateral branches with dormant flower buds were cut and immediately placed in either azosulfamide solution or water. Branches were exposed to 4°C for 2 hr and then transferred to room temperature, control branches remained at room temperature. Half of each dye/temperature treatment combination was then sprayed with either 200 mg/l gibberellic acid (GA_3) plus 0.1% Tween 20 or with distilled water. Each treatment combination was replicated with 7 branches, and the data was analyzed as a factorial design.

Application of ethylene promoter and inhibitors

Ethephon was applied with a handsprayer to run-off in a concentration of 1,000 mg/l with 0.2% Tween 20. Eight trees were selected and two to three branches were isolated and sprayed with ethephon, and the same number of branches per tree were sprayed with water.

AVG at a concentration of 200 mg/l, and the commercial mix of Silflor/50 carnation conditioner was prepared as advised on the label to apply silverthiosulfate. All solutions were prepared with distilled water and 0.2% Tween 20 and applied with a brush to the dormant buds. Ten trees were selected and five nodes per tree were treated with each AVG and silverthiosulfate.

Ethylene evolution

Flower buds were incubated with a drop of water in 5 ml syringes for 2 hr in the dark at room temperature (22-24°C). Samples of 1 ml were injected in a gas

chromatograph (Gow-Mac Series 580) with flame ionization detector and activated alumina column. Column, detector, and injector temperatures were 80, 100, and 90°C, respectively. Six replications were measured for each treatment unless otherwise noted.

Results and Discussion

Dye uptake

Absorbance of 1% azosulfamide solution peaked at 503 nm (Figure 6.1A). The extract of buds that had only been exposed to water showed a slow decrease in absorbance when scanned from 400 to 600 nm (Figure 6.1B). The extract of buds that were exposed to the dye solution showed an absorbance peak at 503 nm, similar to the pure compound, confirming that absorbance at 503 nm was from the dye.

Dye uptake at day 1, one day after plants were irrigated after water stress, was very slow for the first 12 hr (Figure 6.2A), suggesting that the xylem at this time is poorly developed and restricts water movement (Mes 1957). A rapid increase in dye uptake after 24 hr suggests that xylem differentiation is occurring. This indicates that flower buds on detached branches continue their development, which is confirmed by the observation that after 7 days blossoming occurs.

From day 3 until day 7, one day before anthesis, dye uptake increased rapidly (Figure 6.2B-D), indicating that the water conducting elements in the pedicel must be

completely established. A noticeable leveling off of uptake occurs at the 24 hr sampling time. This could indicate a possible clogging of xylem elements by the dye molecules. This phenomenon was most pronounced at the 24 h sampling on day 7, which was also the only time when control buds had a higher fresh weight than buds that were exposed to the dye solution for 24 hr.

Thus, to calculate the rate of uptake, only the measurements from 1 to 12 hr were used in linear regression (Figure 6.3). The rate of uptake was highly variable for non-stressed flower buds as some of them absorbed dye readily, whereas others, even after 12 hr, showed no sign of uptake. One day after irrigation, uptake in branches from stressed plants was still variable and slow. Within the following two days, uptake more than tripled and preceded the doubling of fresh weight. By day 5 the rate of dye uptake reached a peak and leveled off by day 7.

Assuming that the movement of azosulfamide in the xylem is proportional to the rate of water uptake into the bud, it is apparent that water uptake is limited in non-stressed flower buds and in buds of plants that were released from stress for 1 day. By day 3, the xylem elements in the pedicel are established (Mes 1957). The increase in the rate of water uptake from day 3 might not be exclusively due to an increase in functional xylem elements, but might be due to the increased evaporative

demand of the rapidly growing flower bud. Increased respiration was also observed during flower bud development. Buds at the 4 mm non-stressed stage evolved low CO_2 with 17.4 nl/g FW/hr, whereas stressed flower buds 7 days after re-irrigation or at anthesis evolved 31.7 and 35.2 nl/g FW/hr CO_2.

Dye/temperature/GA study

High amounts of ethylene were measured from non-stressed flower buds that had taken up large amounts of water during 4 hr exposure to azosulfamide solution (Table 6.1.). Flower buds that had taken up little water, and flower buds that were placed in water evolved smaller amounts of ethylene. Leaf disks exposed to azosulfamide showed fully red veins, indicating that the dye was readily taken up with the water, but ethylene evolution for leaf disks was the same for leaves that were exposed to water or azosulfamide (Table 6.1).

It is possible that when large quantities of azosulfamide were taken up by the flower bud, increased ethylene evolution was stimulated. However, in leaf disks azosulfamide did not cause increased ethylene production. Flower buds that readily absorbed the dye might have already a functioning xylem connection and might be at a different physiological stage than buds that absorbed only small amounts of dye, and ethylene production might not be connected with the presence of azosulfamide, but may reflect a different stage of dormancy in the flower bud.

Ethylene evolution was not affected by azosulfamide, temperature, or GA_3 when determined three days after the treatments. However, when flower buds were classified according to their length in either the 4-6 mm group or the 12-15 mm group, ethylene evolution was 17.4 and 1.9 nl/g FW/hr, respectively. At this time neither azosulfamide solution, temperature or GA_3 treatment influenced ethylene evolution, but the dormancy status determined that flower buds that were elongating and developing to anthesis evolved small amounts of ethylene, whereas buds still dormant evolved high levels as was found in previous studies (Chap. 5).

Flowering occurred after 7 days on all branches, regardless of the treatment, and no differences between treatments were found. Detached branches, whether stressed or non-stressed, were reported to flower in other studies (Astegiano et al, 1988, Mes 1957). Blossoming was not dependent on whether flower buds could take up dye or not before any treatment. Flower buds that excluded dye and remained green or just turned pink at the base as well as buds that turned red within a few hours could either develop to anthesis or remain at the 4 mm stage. The fact that dormant flower buds allowed the azosulfamide solution to pass through the xylem into the bud, indicates that dormancy is not always associated with a poor vascular connection. There might be different degrees or stages of

dormancy one of which might be the development of the xylem in the flower pedicel. However, additional signals might be required to remove inhibitors, or activate stimulators to completely overcome flower bud dormancy. Signals that have this capacity are probably triggered when a branch is cut from an intact plant, for flowering is always triggered in detached branches. The nature of the stimuli, however, is still unclear.

Ethylene promoter and inhibitors

Ethylene evolution, measured three days after application of ethephon more than doubled compared to the control (Figure 6.4). Silverthiosulfate and AVG did not lower ethylene evolution substantially (Figure 6.4). Flowering was observed only on two out of 50 nodes that were treated with AVG; none of the other treatments stimulated anthesis. Ethephon was toxic and caused leaf and flower bud abscission, and branch dieback.

These results suggest that AVG and silverthiosulfate should be applied at higher concentrations and/or repeatedly to test whether ethylene is involved in inhibiting the breaking of dormancy. Ethephon should be used in concentrations lower than 1,000 mg/l for further studies.

Table 6.1. Ethylene evolution of non-stressed flower buds and leaf disks that were placed for 4 hr in either azosulfamide or water. Means are the average of 6 determinations.

Treatment	Leaf disks	Flower buds	
	--- Ethylene (nl/g FW/hr)---		Petal color
Water	1.15 a	7.52 a	green
Azosulfamide	1.40 a	2.10 a	pink
		14.61 b	red

Means followed by the same letter are not significantly different by Tukey's Studentized Range Test P=0.05.

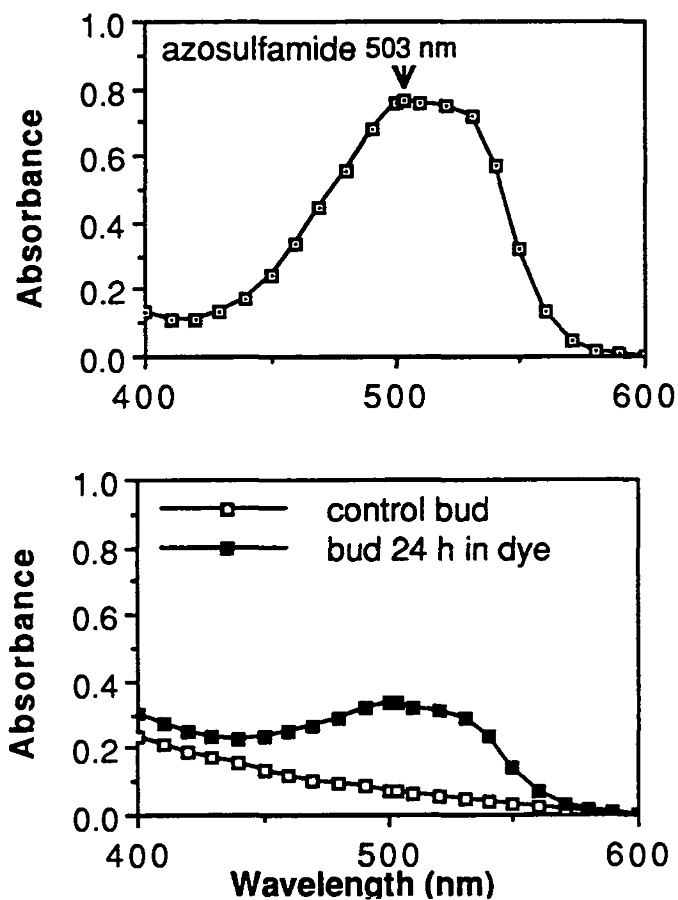

Figure 6.1. Absorbance of azosulfamide (A) and extracts of coffee flower buds from branches that were placed for 24 hr in either water or azosulfamide (B).

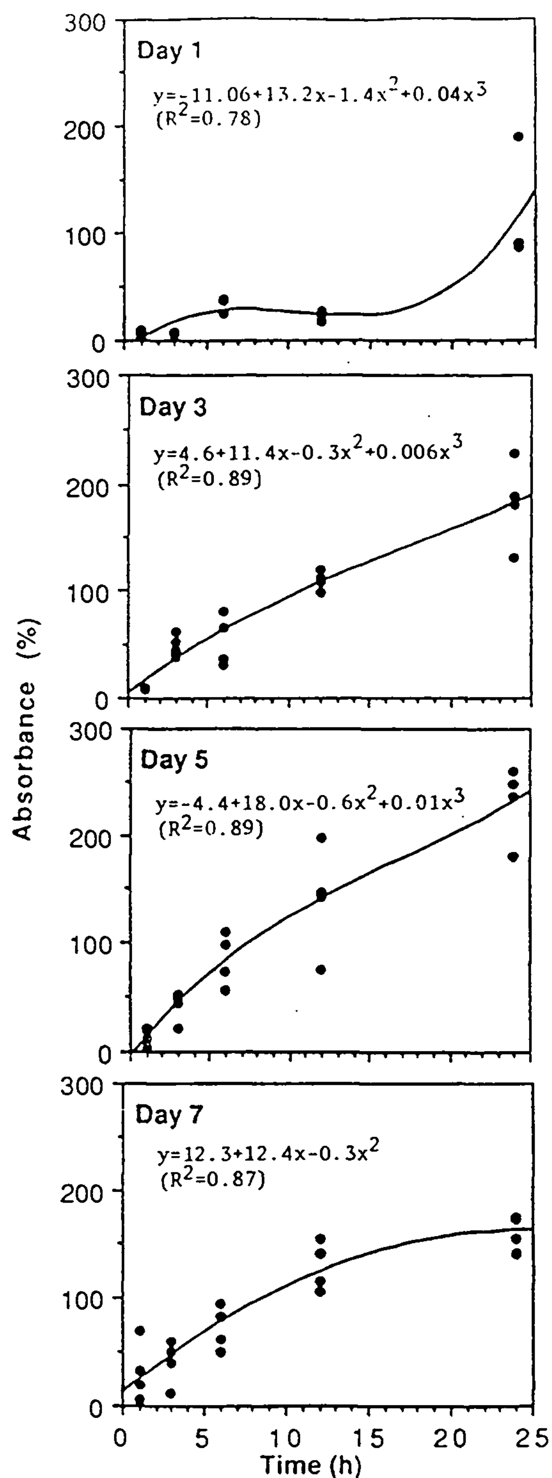

Figure 6.2. Changes in absorbance of coffee flower buds that were developing to anthesis. Absorbance (A) change (%) was calculated as (A dye - A control)/A control.

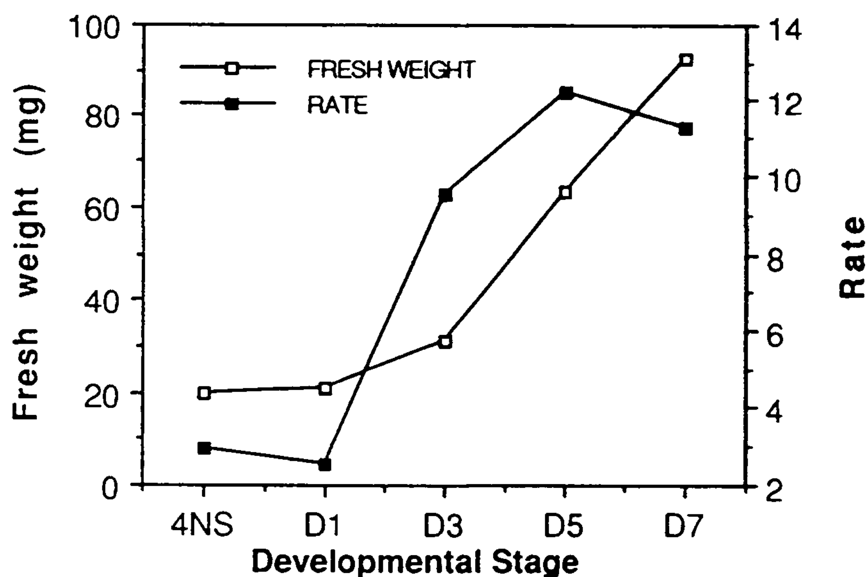

Figure 6.3. Fresh weight and rate of azosulfamide uptake at different stages of developing coffee flower buds. 4NS= 4mm non-stressed; D1, D3, D5, D7 = 1, 3, 5, and 7 days after release from dormancy and re-irrigation.

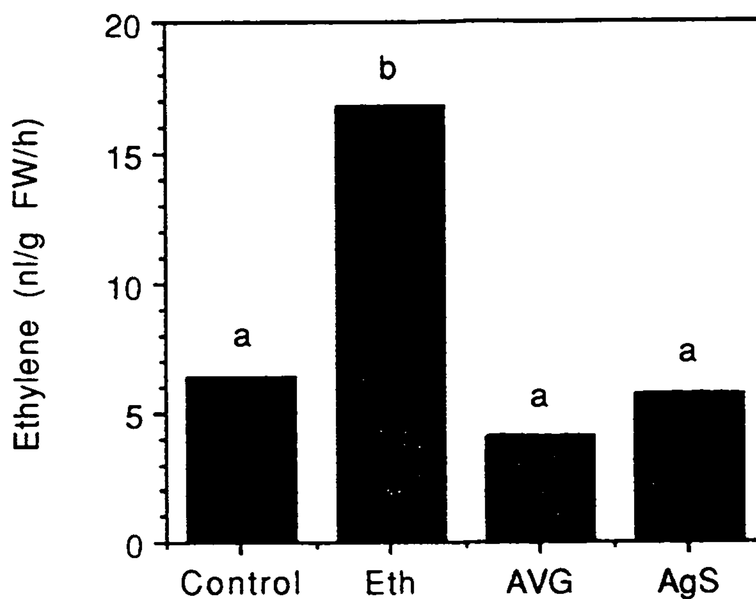

Figure 6.4. Ethylene evolution of flower buds three days after they were treated with water (control), ethephon (Eth), AVG, or silverthiosulfate (AgS).

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Chapter 7

Levels of free and conjugated IAA in developing flower buds of *Coffea arabica* L.

Abstract

Free and conjugated IAA levels and ethylene evolution were quantified during the development of the reproductive buds of *Coffea arabica* L. Free, ester, and amide IAA levels were measured by gas chromatography-mass spectrometry-selective ion monitoring using an isotope dilution assay with [$^{13}C_6$]IAA as an internal standard. Throughout the development, the largest percentage of IAA was present in conjugated form and the dominant conjugate was in the form of amide IAA. After dormancy was broken, the largest changes in free and conjugated IAA levels preceded the doubling of fresh and dry weight of the flower buds. The percentage of amide-IAA increased immediately after the water stress treatment. One day after water stress and rehydration of plants, the percentage and concentration of free IAA increased three-fold in the flower buds. Exogenous auxin failed to promote flowering of dormant buds. Ethylene evolution was high from dormant buds. After the water stress, the rates of ethylene evolution dropped to the lowest level. At anthesis, ethylene rates were again high. Auxin and ethylene evolution were independent. Free IAA was related to growth rate and xylem development in coffee flower buds. IAA conjugates could serve as storage and transport forms of auxin in the

developing coffee flower bud.

Abbreviations: IAA, indole-3-acetic acid; HPLC, high performance liquid chromatography; GC-MS-SIM, gas chromatography-mass spectrometry-selected ion monitoring; FW, fresh weight; IBA, indole butyric acid; NAA, naphthalene acetic acid.

Introduction

In *Coffea arabica* L., inflorescences develop over a period of several months after induction, until flower buds reach a corolla length of about 4 mm. At this stage, the flower buds become dormant and generally require a period of water stress to break dormancy, followed by watering or exogenous gibberellic acid or cytokinin to promote further development to anthesis (Alvim 1958, 1960; van der Veen 1968, Browning 1973b). Gibberellin levels in the xylem sap remain unchanged. However, gibberellin increases immediately after dormancy release until the flowers start to expand, and then decreases. Changes in the cytokinin content of bud and xylem sap increase, induced by watering, during the growth period (Browning 1973b). Endogenous abscisic acid is high in dormant buds, and decreases once buds continue to develop to anthesis (Browning 1973a).

In the dormant flower buds, microspores are fully developed, but the xylem connections between the floral structure and the branch are poorly developed. After dormancy is broken and growth resumes, the xylem in the peduncle develops profusely (Mes 1957). A lack of xylem continuity in dormant flower buds was also observed in

peach, apricot, and several Prunus species (Ashworth and Rowse, 1982).

Vascular differentiation depends on a continuous flux of signals, of which auxin is the primary (Sachs 1981, Aloni 1987). Xylogenesis has been studied in many species in response to phytohormone application or incorporation of phytohormones into the media of tissue cultured plants. Although cytokinin, gibberellin, or ethylene could enhance xylem differentiation, IAA, NAA, or IBA are identified as essential compounds prerequisite for differentiation (Shininger 1971, DeGroote and Larson 1984, Sachs 1981, Phillips 1987, Ackermann and van Staden 1988).

Recent research on the involvement of auxins in many growth promoting processes has focused on the relationship between endogenous IAA and its metabolites, and on inputs and outputs from the total IAA pool (Reinecke and Bandurski 1988). IAA conjugates are ubiquitous (Bandurski and Schulze 1977) and involved in IAA transport, storage and subsequent release, protection from enzymatic destruction, and in the homeostatic control of IAA concentrations in the plant (Cohen and Bandurski 1982). Changes in conjugated IAA levels compared to free IAA suggest that IAA conjugates serve as storage forms of auxin in developing seeds (Bialek and Cohen 1989). Application of IAA conjugates to stems of beans showed that differential growth was quantitatively related to the degree of hydrolysis of the conjugate

(Bialek et al. 1983). These findings support the hypothesis that the rate of conjugate hydrolysis in part determines the IAA concentration in the tissue (Bialek et al. 1983, Cohen and Bandurski 1982).

Changes in IAA associated with flower bud development of coffee were investigated earlier by estimating IAA levels colorimetrically (Janardhan et al. 1977). In this study, we quantify the amounts of free and conjugated IAA in reproductive buds of coffee during various stages of development, using GC-MS. We relate changes in free and conjugated IAA levels to physiological changes during flower bud development and xylem differentiation from bud dormancy to anthesis. In addition, we determine the effect of exogenous auxin on dormant flower buds.

Materials and Methods

Coffee (*Coffea arabica* L. cv. Guatemalan) trees were grown for 2 years in a greenhouse (25/19°C, natural photoperiod in Oregon, 44°N) in 4 l plastic pots in a mixture of pumice, peat, and soil (2:1:1: v/v). Plants with flower buds of 4 mm long and smaller were transferred to a growth chamber with 8 hr photoperiod (600 umol $m^{-2}s^{-1}$) and day/night temperatures of 26/23($\pm$2)^{o}C for three weeks before the experiment began. Plants were irrigated every other day. To stress plants with dormant flower buds at the 4 mm stage, water was withheld until the latest fully emerged leaf pair had reached a xylem water-potential

between -2.6 to -3.0 MPa, which is known to break flower bud dormancy (Chap. 5). Water potential was measured with a pressure chamber. After the desired stress levels were reached, the plants were rehydrated, and thereafter irrigated daily. Flower buds of various developmental stages (Table 7.1) were excised, fresh and dry weights recorded, and the tissue was immediately frozen in liquid nitrogen and stored at -70°C until auxin was determined. Developmental stages of flower buds are abbreviated as follows: <2 = buds smaller than 2 mm in length, 2-4 = 2-4 mm bud length, 4 NS = non-stressed 4 mm buds and irrigated daily, 4 STR = water stressed 4 mm buds; D1, D3, D5, D7, D8/9 = 1, 3, 5, 7, 8, or 9 days after water stress treatment and rehydration, irrigated daily.

IAA extraction and purification

The extraction procedure of Chen et al. (1988) was followed with a few modifications. The frozen flower bud tissue (approximately 2.5 g FW) was homogenized in a Waring blender with 15 ml of 65% 2-propanol in 0.2 M imidazole buffer (pH 7.0). At homogenization, 1 ug of $^{13}C_6$-(benzene ring)-IAA and approximately 120,000 dpm ^{3}H-IAA were added as internal standards. The plant extract was allowed to equilibrate for one hr at 4°C, and was then centrifuged at 1700 rpm for 5 min. The 2-propanol was vacuum evaporated, and the extract was centrifuged for another 5 min. The supernatant was divided into three equal parts to determine free IAA, free plus ester, and total IAA. Two or three

replications of each sample were processed.

For free IAA analysis, the supernatant was diluted 10 times with double-distilled water and applied to two stacked, preconditioned amino anion exchange columns (Baker-10 SPE 3 ml). The ion exchange columns were preconditioned with hexane, methanol, distilled water, and 0.02 M imidazole buffer (pH 7.0). After application of the extract, the columns were washed with 6 ml each of ethyl acetate, acetonitrile, and methanol. The IAA was eluted with 6 ml of methanol containing 2% (v/v) acetic acid. The eluent was evaporated to dryness in a rotary evaporator and the residue resuspended in 250 ul of 35% methanol-water with 1% (v/v) acetic acid. The IAA was further purified by HPLC using a Beckman Ultrasphere XL C-18 reverse phase column (7.5 cm x 4.6 mm with guard cartridge). The mobile phase was 35% methanol with 1% acetic acid at a flow rate of 1 ml/min. After HPLC, the radioactive fractions were pooled, evaporated to dryness, and resuspended in 100 ul of methanol. The extract was methylated with ethereal diazomethane, evaporated under nitrogen, and resuspended in 35 ul of ethyl acetate (Cohen 1984). The methylated extract was analyzed for IAA with GC-MS-SIM using the isotope dilution techniques (Rittenberg and Foster 1940, Cohen et al. 1986).

To determine IAA in free plus ester form, an equal volume of 2N NaOH was added to the supernatant, and allowed

to hydrolyze for 1 hr at room temperature. After dilution, the pH was adjusted to 2.5, and the solution was passed through two stacked C-18 columns (Baker-10 Spe 3 ml) which had been preconditioned with 6 ml each of hexane, methanol, water, and 1% acetic acid. The C-18 column was washed with 6 ml of water, and IAA eluted with 6 ml of acetonitrile. The eluent was diluted to 10% acetonitrile dilution with 0.02 M imidazole buffer, pH 7.0. The solution was applied to two stacked, preconditioned amino columns, and further processed as described for free IAA.

To determine total IAA, which includes free and conjugated IAA, the third aliquot of supernatant was diluted with water, and NaOH was added for a 7 N solution. The extract was hydrolyzed in a capped PFTE vial under water-saturated nitrogen gas for 3 hr at 100°C, diluted with water, adjusted to pH 2.5, and centrifuged for 10 min. The supernatant was further prepared as described for ester IAA.

<u>Auxin application</u>

NAA at concentrations of 0, 10^{-4}, 10^{-5}, and 10^{-6} M, or IAA-glycine or IAA-alanine at 10^{-2}, 10^{-3}, and 10^{-4} M in aqueous solution with 0.2% Tween 20 were applied with a brush to flower buds at the 4 mm stage. Ten trees were selected, and each concentration/treatment combination was applied to 10 nodes per tree in a completely randomized design. Plants were watered regularly, and flowering was evaluated.

Determination of ethylene evolution

Flower buds were incubated with a drop of water in 5 ml syringes for 2 h in the dark at room temperature (22-24°C). Samples of 1 ml were injected into a Gow-Mac Series 580 gas chromatograph with flame ionization detector and activated alumina column. Column, detector, and injector temperatures were 80, 100, and 90°C.

Evaluation of xylem development

Branches with flower buds of different stages were cut and placed either in water or 1% aqueous solution of azosulfamide. Buds (4 replications) were harvested at 1, 3, 6, and 12 h after exposure to water or asozulfamide, weighed, and homogenized in distilled water. The samples were centrifuged for 5 min at 1,000 rpm. Aliquots of the supernatant were diluted and absorbance at 503 nm was measured with a Shimadzu UV-160 spectrophotometer.

Absorbance (A) was expressed per gram fresh weight. The percent change in absorbance relative to the water (A dye - A water/A water). Linear regressions with the intercept at the origin were calculated for the rate of dye uptake for each stage of flower development.

Results

Dormant buds, whose dormancy was maintained by regular watering, slowly increased in fresh and dry weight from 13 to 27 mg and 5 to 9 mg, respectively, over a period of several weeks (Table 7.1). When dormancy was broken by

withholding water, the largest increase in fresh and dry weight, 105 and 109%, occurred between 3 and 5 days after plants were re-irrigated (Figure 7.1A).

Ethylene evolution of flower buds changed at different developmental stages (Figure 7.1B). Ethylene production was low (0.8 nl g^{-1}FW h^{-1}) during the early development, up to a bud length of 4 mm , and in rapidly expanding flower buds where dormancy had been broken. Dormant buds at the 4 mm stage produced higher levels of ethylene, and were exceeded in ethylene production only by flowers at anthesis.

Total IAA concentration of flower buds was highest in the < 2 mm stage of (Figure 7.2A), and decreased by almost 50% when the buds reached the 4 mm dormant stage. Free and amide IAA followed the same pattern, and all three forms of IAA showed a steep increase at day 3, followed by a rapid drop at day 5. Ester IAA concentration was similar to levels of free IAA during the early bud development, and remained below 5 ng g^{-1} FW during the most active enlargement of the flowers.

IAA content increased steadily as the buds grew from the 2 mm stage to the 4 mm stage (Figure 7.2B). Total IAA content remained stable from the time buds were dormant until D1. Amide content increased and free and ester content decreased from stressed to water saturated trees. From D1 to D7, changes in IAA per bud follow the same trend

as changes in IAA on a fresh weight basis (Figure 7.2B). A high correlation was found between weight and IAA content per bud. Correlation coefficients (r^2) between fresh and dry weight, and free, ester, amide, and total IAA per bud were between 0.77 and 0.81 ($p < 0.05$) and between 0.85 and 0.91 ($p < 0.01$), respectively.

The distribution of free and conjugate IAA changed throughout the floral development. IAA conjugates were always the predominant form of auxins in the flower buds, and amide IAA continuously comprised the highest percentage of the conjugates (Figure 7.2C). A rapid increase in the relative amide levels occurred from the time buds were stressed until D1, and was followed by a steep decline. Free IAA, expressed on a percentage basis, followed the same pattern with a lag of two days in the peak levels. This increase in free IAA preceded the doubling of fresh and dry weight (Figure 7.1A). Throughout flower bud development, the percentage of ester IAA was low, fluctuating between 13 and 25% until buds were exposed to stress, and then dropping to levels around 10% until anthesis.

The relative rate of azosulfamide uptake was highly variable for non-stressed flower buds (Table 7.2) as some of them absorbed dye readily whereas others, even after 12 hr, showed no sign of uptake. At D1, uptake was still variable and slow. Within the following two days (D3), the uptake rate more than tripled, reached a peak by D5, and

leveled off by D7. The probability levels shows, that the rate of uptake is significantly different from 0. The increasing rate corresponds to xylem development in the peduncle, which enables water to move into the rapidly developing bud.

Discussion

During the earliest stage (< 2 mm), flower buds contained high levels of free and amide IAA that might be required for cell differentiation and elongation of newly emerging inflorescences (Figure 7.2A). High IAA levels were also reported during early stages of coffee flower development by Janardhan et al. (1977). However, their estimates of IAA exceeded ours by more than 1,000 times and may have been due to the less specific method for auxin analysis. Growth was slow until the buds reached the 4 mm stage and IAA levels concomitantly decreased. Dormant buds still gained fresh and dry weight, similar to slow increases in the weight reported for dormant buds in deciduous fruit trees (Saure, 1985). The increase in total IAA content when plants were water stressed was due to an increase of amide and free IAA.

The largest percentage change in free and conjugated IAA from D1 to D3 were associated with the onset of rapid flower growth (Figure 7.1A, 7.2C). The increase in fresh weight of 4 mm buds when stress was released by irrigation indicates that the cells of flower buds were expanding by

taking up water. Assuming that azosulfamide movement in the xylem of the peduncle is proportional to the rate of water uptake, it is apparent that water uptake is limited in non-stressed flower buds and in buds at D1. Growth may be impeded by the lack of water-conducting elements in non-stressed and D1 flower buds, as indicated by a low uptake rate. This confirms the study of Mes (1957), who reported that xylem vessels in the peduncle of flower buds are poorly developed until 3 days after dormancy, where it is almost fully differentiated. The increase in the rate of water uptake from D3 to D5 may not be exclusively due to an increase in functional xylem elements, but might originate in the increased evaporative demand of the rapidly growing flower bud. The rapid decrease in amide and ester content and concentration from D3 to D5 corresponds to the doubling of fresh and dry weight during that time. The conjugates may have been hydrolyzed and free IAA could contribute to final xylogenesis and cell elongation in the buds (Cohen and Bandurski 1982, Cleland 1988).

The decrease of amide percentage from D1 to D3 may be attributed to mobilization and utilization of free IAA for xylem development (Sachs 1981, Aloni 1987, 1988). The rapid increase in the absolute content of amide and free IAA from D1 to D3 could be a result of increased de novo biosynthesis of free and conjugated IAA, and at the same time, conversion of the conjugates to free IAA (Cohen and

Bandurski 1982, Reinecke and Bandurski 1988). At D3, when the growth rate was most rapid, the variation of total, amide, and free IAA content was very large. Rapid changes in the rates of IAA biosynthesis, conjugation, and hydrolysis may be occurring at the same time.

Before D3, increases in IAA were most likely due to de novo biosynthesis and hydrolysis of conjugate auxins within the bud, because polar basipetal auxin transport would prevent the import of IAA from other parts of the plant, and the poor vascular connection would inhibit movement through xylem (Rubery 1988). After D3, however, the intact vascular connection could serve as transport channel for an influx of auxin by massflow from other parts of the plant to the flower bud, which is a strong sink.

From D5 to D7, total IAA content increased slightly with little changes in relative free and conjugate IAA content. This might indicate that a steady state of auxin biosynthesis, conjugation, hydrolysis, and catabolism had been reached to support the slow increase in dry weight. The increase in fresh weight is mainly due to water uptake and is driven by increased transpiration of the rapidly enlarging flower. At this time, the xylem connection is fully developed, and auxin is no longer required for xylogenesis in the peduncle.

Relative and absolute ester IAA content remained stable at a low level from D1 to D7. Ester IAA is a purported transport form of IAA (Cohen and Bandurski 1982),

and might have such a rapid turnover in conjugation and hydrolysis that measurable levels are relatively stable. The majority of bound IAA occurs mainly as esters in cereals and predominantly as amides in legumes (Bandurski and Schulze 1977). This could indicate a preference for amides as IAA conjugates in coffee and probably related species. Ester IAA could have been hydrolyzed to free IAA during homogenization of the fresh tissue (Baraldi et al. 1988).

When flower buds at the 4 mm dormant stage were treated with different concentrations of NAA and the auxin conjugates, IAA-aspartate, IAA-alanine, and IAA-glycine, variable results were obtained. In one experiment, IAA-glycine and IAA-alanine promoted flowering in a few plants, but in a second experiment none of the treatments evoked a response. Auxin conjugates are used successfully in tissue culture (Reinecke and Bandurski 1988) and IBA-alanine increases rooting in olive cuttings compared to IAA or IBA (Epstein et al. 1989). In coffee, the use of IAA or NAA to break flower bud dormancy has failed thus far (van der Veen 1968, Browning 1973a). The lack of response to exogenous auxins may be due to poor uptake or photodestruction of the auxin. Another possibility is that the flower bud tissue is not sensitive to auxin at this stage (Trewavas 1981). Auxin was found at high levels in <u>Abies</u> <u>balsamea</u> and was transported readily during cambial rest, but cell

sensitivity to auxin was low (Lachaud 1989).

Ethylene biosynthesis is known to be stimulated by auxin and it acts to stimulate growth of buds, but like auxin, it is not considered to have a regulatory role in seed or bud dormancy (Powell 1988). Ethylene levels in coffee flower buds were low, except in dormant buds and in flowers at anthesis. The fact that ethylene evolution in dormant, non-stressed buds was higher than in stressed and rapidly expanding buds indicates that ethylene was not involved as a stimulator of growth (Musgrave and Walters 1973) or xylem differentiation (Miller et al. 1985).

In summary, most of IAA was found in conjugated forms, dominated by the amide form. Auxin content was related to growth rate. Large changes in free and bound IAA levels occurred at the peak of metabolic activity and preceded the doubling of fresh and dry weight, and a burst in xylem development. Thus, IAA conjugates seem to play a role in storage and transport of auxin in the developing flower bud, whereas free auxin seems to regulate xylem differentiation.

Table 7.1. Growth characteristics during development of coffee flower buds.

Developm. Stage	Corolla Length (mm)	No. of Buds g^{-1} Fresh Wt.	Physiological Stage of Flower Buds
<2	<2	170	Growth after initiation
2-4	2- 4	130	Active growth
4 NS	4- 6	38-79	Dormant, microspores are fully developed
4 STR	4- 6	72	Dormancy broken by water stress
D1	4- 7	48	Regrowth begins in buds where dormancy was broken
D3	8-10	32	Active bud expansion
D5	11-14	16	Active bud expansion
D7	18-22	8	Active bud expansion, candle stage
D8/9	21-25	8	Anthesis, pollination

Table 7.2. Relative rate of azosulfamide solution uptake in dormant and actively growing coffee buds.

Developmental Stage	Rate	Standard Error	P
4NS	2.93	0.97	0.0061
D1	2.55	0.45	0.0001
D3	9.51	0.61	0.0001
D5	12.26	0.98	0.0001
D7	11.30	0.86	0.0001

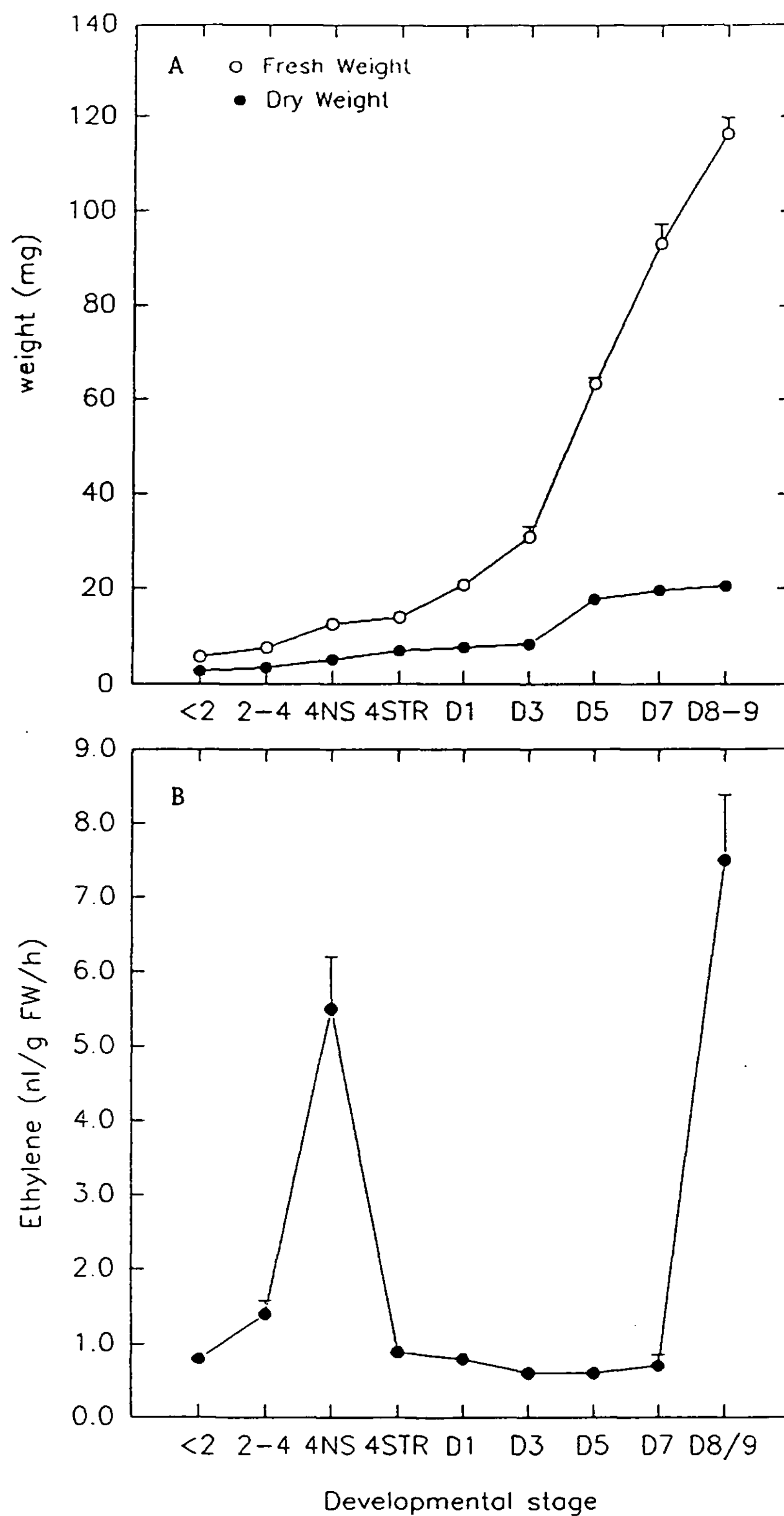

Figure 7.1. Changes in fresh and dry weight (A) and ethylene evolution (B) of developing coffee flower buds.

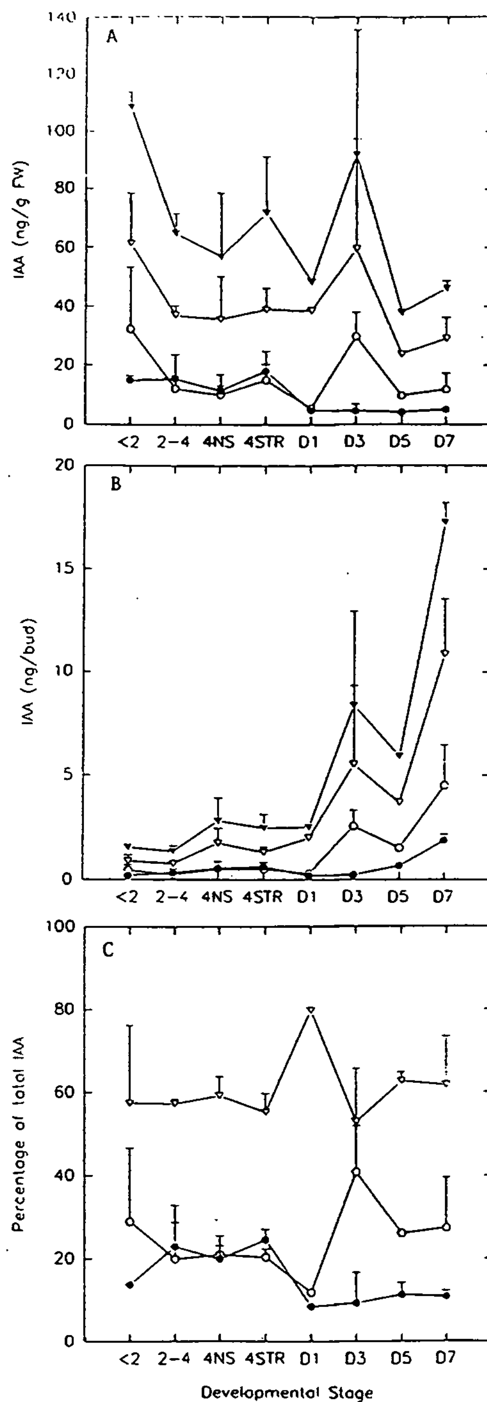

Figure7.2. Changes in total (▼), amide (▽), ester (●), and free (○) IAA concentration (A) and content (B) and percent of amide, ester, and free IAA (C).

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