EFFECTS OF FLOREL® AND PROMALIN® APPLICATIONS ON BRANCHING, FLOWERING, AND FRUITING IN JATROPHA (Jatropha curcas L.)

By

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A THESIS PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2012
To my family and friends
ACKNOWLEDGMENTS

I sincerely thank God for giving me the blessing of life and for allowing me to go through this journey. I thank my mother Aldamanza Pinheiro Costa and my father Roberto Osmar Costa for their unconditional love and for supporting me regardless of the situation. I extend my gratitude to my sister Ellen Pinheiro Costa, for her love, patience and for being my role model of friendship. I thank Martinho Junior for his company and for all his help in my life.

I would like to thank my supervisory committee, Dr. Wagner Vendrame, Dr. Kimberly Moore, and Dr. Jonathan Crane for their academic guidance and unending support during my graduate research. I thank Dr. Bruce Schaffer for his statistical support and comments. A special acknowledgement to Dr. Wagner Vendrame and Dr. Silvia Nietsche for their instruction, assistance, and for encouraging me to follow my ideals. I sincerely thank Regina Rieckenberg and Valent Biosciences Corporation™ for providing Promalin® for this study. I also thank Alba Myers and Maria Salinas for their technical assistance.
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Jatropha (Jatropha curcas L.) is a pantropical species widely distributed in Central and South America that has been identified for biofuel production. However, jatropha is still undomesticated. Among several factors that limit commercial yields in jatropha are the limited branching and lack of yield uniformity. This study aimed at evaluating the effects of ethephon (Florel®, FL) and a combination of 6-benzyladenine (BA) and gibberellic acid isomers A₄ + A₇ (GA₄ +7) (Promalin®, PR) on branch induction, and subsequent flowering and fruit production in jatropha. Dormant (without leaves) and actively growing plants (with leaves) were sprayed with single applications of FL or PR. In general, plants with leaves performed better than plants without leaves. Jatropha plants sprayed with FL or PR had no increase in plant size, number of branches per plant, number of inflorescences per plant, number of female flowers per inflorescence, fruit set, male to female flower ratio, number of fruits per plant, fruit weight, fruit yield, number of seeds per plant, seed yield, number of seeds per fruit, seed fresh weight, 100-seed weight, and seed size (length, width, and thickness). However, the total number of flowers per inflorescence in plants treated with FL was reduced as FL
concentration increased due to a decrease in the number of male flowers per inflorescence. It was observed that PR applications increased seed dry weight as concentration increased. Therefore, FL and PR seem to affect flowering and seed production to a greater extent than branching. This study also provided invaluable information on sample size requirements on which to base future studies of the effects of foliar PGR applications on jatropha.
Jatropha (Jatropha curcas L.) is a pantropical species widely distributed in Central and South America that has been identified for biofuel production. The oil extracted from the seeds produces superior quality biodiesel over fossil diesel and biodiesel from other agronomic crops (Azam et al., 2005; Fairless, 2007). Furthermore, the high quality oil has been successfully tested for use as bio jet fuel, meeting European and American quality standards (Lu et al., 2009; Openshaw, 2000).

However, jatropha is still undomesticated and not considered a commercial crop due to the lack of breeding and genetic improvement, as well as the lack of specific cultivation practices (Francis et al., 2005; Rao, 2006). Most existing plantations were initiated from seeds derived from wild plants and therefore yields are variable (Carels, 2009; Fairless, 2007; King et al., 2009) and productivity non-uniform (Achten et al., 2008; Kant and Wu, 2011).

Seed and oil yield in jatropha are affected by several factors, including genetics (Kaushik et al., 2007; Laviola et al., 2010; Mohapatra and Panda, 2010), age of the plant (Jongschaap et al., 2007; Ouwens et al., 2007), field site characteristics, such as rainfall, and soil type and fertility (Francis et al., 2005; Ouwens et al., 2007), and agronomic practices, such as plant spacing, pruning, irrigation, and fertilization (Behera et al., 2010; Gosh et al., 2011). Seed and oil production is also dependent on female flower production and seed set (Jongschaap et al., 2007). Therefore, high male to female ratio is considered one of the factors limiting yield in this species (Raju and Ezradanam, 2002; Wu et al., 2011). Because inflorescences are formed terminally on the branches, poor branching is considered another limitation to high yield (Carels,
Kuvel (2006) reported that the overall oil yield could be improved by increasing the total number of branches bearing fruits per plant. Plant growth regulators (PGRs) have been used to modify plant architecture (Bell et al., 1997; Elfving and Visser, 2006; Hayashi et al., 2001; Mackay et al., 2007). Studies of exogenous applications of PGRs show that they can induce growth responses for large-scale production, increasing growth and yield in a variety of crops such as jatropha, Zea mays L. (corn), and Gossypium hirsutum L (cotton) (Abdelgadir et al., 2010; Biles and Cothren, 2001; Shekoofa and Emam, 2008). Ethephon is labeled as a branching compound that decomposes to ethylene within plant tissues. It is commonly used to reduce stem elongation, increase lateral branching, and manipulate flowering initiation. Ethephon is known to improve branching on Dendranthema x morifolium Tzvelev (chrysanthemum) (Kher et al., 1974), Petunia hybrida Vilm. (petunia) (Tjia and Buxton, 1977), Impatiens balsamina L. (impatiens) (Tamari et al., 1998), and Glycine max L. (soybean) (Campos et al., 2009). A combination of 6-benzyladenine (BA) with gibberellic acid isomers A₄ + A₇ (GA₄+₇) has been used to increase shoot elongation and flower production, as well as to release buds from apical dominance, and to promote lateral bud break and branch development. Several studies have reported that exogenous applications of BA + GA₄+₇ induces branching in a number of woody landscape plants (Keever and Foster, 1990), Euphorbia lathyris (caper spurge) (Preece, 1990), Simmondsia chinesis (Link) Schneider (jojoba) (Ravetta and Palzkill, 1992), Pyrus communis L. (pear) (Jacyna et al., 1994), Malus domestica Borkh. (apple) (Ouellette et al., 1996), and Prunus avium L. (sweet cherry) (Jacyna and Puchala, 2004).
Studies on the use of PGRs to induce branching in jatropha are very limited. Increased branching in jatropha could increase fruit production. The objective of this study was to evaluate the effects of ethephon and BA + GA₄₊₇ applications on branch induction, and subsequent flowering and fruit production in jatropha.
Jatropha curcas L. (jatropha) belongs to the Euphorbiaceae family and is a diploid species with \(2n = 22\) (Ambrosi et al., 2010). Jatropha is a perennial small tree or large shrub with a life expectancy of up to 50 years (van der Putt, 2010). Plants usually reach 3 to 5 m, but according to Divakara (2010), under favorable conditions it may grow as tall as 8 to 10 m.

Jatropha is monoecious and is pollinated by insects (Raju and Ezradanam, 2002). As a deciduous plant, it sheds its leaves in the dry season (Heller, 1996; Kumar and Sharma, 2008). In this dormant stage, photosynthesis is performed by the stem (van der Putten et al., 2010). Leaves are 5 to 7 lobed with an alternate arrangement and branches contain latex (Heller, 1996). Plant architecture varies from plants that branch low on the trunk or plants with a main trunk with no or few branches (van der Putten et al., 2010). Generally, jatropha has a taproot and four lateral roots. The deep taproot provides stabilization and exploits moisture deep in the soil profile whereas the lateral roots are important for nutrient and water uptake. The extensive lateral roots help reduce soil erosion as they promote soil cohesion (Reubens et al., 2011).

The panicle-type inflorescences are formed terminally on the branches (Jongschaap et al., 2007). Flowers are usually unisexual with female and male flowers occurring in the same inflorescence (Jongschaap et al., 2007; Raju and Ezradanam, 2002). Female flowers occupy central sites in the inflorescence and they are surrounded by male flowers that are usually present in greater numbers (Jongschaap et
al., 2007). However, hermaphrodite flowers do occur occasionally (Laviola pers. comm.; Pan and Xu, 2010). These flowers are capable of self-pollination (Kumar and Sharma, 2008). Inflorescences with exclusively female flowers have also been observed (Foild et al., 1996; Nietsche pers. comm.). The inflorescences may be classified into three types depending on the number of female flowers per inflorescence (Wu et al., 2011). The number of female flowers and the male to female flower ratio on female-type inflorescences range from 16:5 to 15:1, whereas in middle-type inflorescences these values range from 7.2 to 20:1. All flowers in the male-type inflorescences are male flowers (Wu et al., 2011).

Male flowers are generally more abundant than female flowers in jatropha. Thus, there is a high male to female flower ratio in this species. Typical ratios of 25:1 and 29:1 have been reported (Wu et al., 2011; Raju and Ezradanam, 2002, however, Rao et al. (2008) found ratios could be as low as 7.2:1 and as high as 33:1.

Fruits are ellipsoidal, trilocular and they are formed in bunches after pollination (Heller, 1996). Approximately 10 fruits are produced per inflorescence (Kumar and Sharma, 2008). Fruits are about 40 mm long (van der Putten et al., 2010). The number of seeds per fruit is variable, ranging from 1 to 4, but fruits with three seeds are common (Makkar et al., 2008). Seed maturation occurs 3 to 4 months after flowering (Heller, 1996; Kumar and Sharma, 2008), when there is a change in color of the fruits from green to yellow-brown (Achten et al., 2008). Seeds are black and are on average 18 mm long, 12 mm wide, 10 mm thick, and have a fresh weight from 0.5 and 0.8 g. The seed shell accounts for 37% of its weight and the kernel accounts for about 63% of its weight (van der Putten et al., 2010). Dry seeds have about 7% moisture content and 30
to 40% oil (Foidl et al., 1996; Francis et al., 2005; Islam et al., 2011; van der Putten et al., 2010).

**Center of Origin**

Jatropha is a tropical plant that produces seeds with high oil content suitable for biodiesel production and has received a lot of attention as an alternative source to fossil diesel (Achten et al., 2008; Augustus et al., 2002; Azam et al., 2005; Foidl et al., 1996; Francis et al., 2005).

There is some controversy regarding its center of origin. Francis et al. (2005) reported that jatropha is native to South America. Similarly, Carels (2009) reported that its center of origin is tropical South America and that jatropha migrated to Central America. However, most authorities believe the center of origin of jatropha is Mexico and Central America, from where it was dispersed to different parts of the world by Portuguese sailors (Heller, 1996).

Jatropha now grows in tropical and subtropical regions worldwide (Jongschaap et al., 2007; van der Putten et al., 2010), including Africa, Latin and North America, and Asia (Heller, 1996).

**Distribution and Ecological Requirements**

Jatropha is a drought tolerant plant that establish quickly and is capable of growing in a wide range of conditions (Openshaw, 2000; van der Putten et al., 2010). It is best adapted to arid and semi-arid regions with average annual rainfall ranging from 300 to 1000 mm and altitude ranging from 0 to 500 m (Heller, 1996). However, jatropha has been reported to grow in areas with average annual rainfall of 3000 mm and up to 1800 m above sea level (Foidl et al., 1996). In general, it is thought jatropha has a low water requirement and that during the dry season or drought it sheds it leaves to reduce
transpiration and avoid drought stress (Biswas et al., 2006; Gour, 2006; Kumar and Sharma, 2008). As leaves abscise they may accumulate around the base of the plant forming mulch, which increases soil fertility (Gour, 2006; Kumar and Sharma, 2008; Singh et al., 2006).

Jatropha is adapted to areas with an average annual temperature range of 20°C to 28°C (Heller, 1996). However, jatropha is very sensitive to low or freezing temperatures and plants are damaged by frost. In contrast, high temperatures may decrease plant yield (Gour, 2006). Jatropha is a day neutral species and plants become dormant in response to dry soil conditions and/or cool temperatures (Heller, 1996).

Jatropha may grow on sandy, saline and gravelly soils, but not in wetlands (Gour, 2006). This species grows better in well-drained and well-aerated soils with a pH from 5.5 and 8.5 (Franken and Nielsen, 2010). Plants can survive in drier regions and in soils with low fertility (Foidl et al., 1996; Heller, 1996), but growth, flower and seed production are limited, as they are influenced by soil nutrient content and soil moisture (Openshaw, 2000). Soils with nutrient deficiency may lead to flower abortion and poor seed development (Jongschaap et al., 2007; Openshaw, 2000). According to Gour (2006) and Shukla (2006), growth and yield can be improved when plants are cultivated with proper irrigation and fertilizer inputs.

**Uses**

Jatropha is known for being a multipurpose plant (Jongschaap et al., 2007; Openshaw, 2000). The fruit and seeds of most jatropha accessions are toxic (Kumar and Sharma, 2008) and because of this jatropha is not edible or browsed by animals. For this reason, it is widely used as hedge and living fence to protect agricultural fields
(Heller, 1996; Islam et al., 2011; Jongschaap et al., 2007; Openshaw, 2000; van der Putten et al., 2010). This species is widely used to conserve and restore soil fertility in degraded areas, for erosion prevention, and erosion control (Heller, 1996; Islam et al., 2011; Jongschaap et al., 2007; Openshaw, 2000). Jatropha cultivation is very attractive because of its ability of growing on marginal lands and not compete with food crops (Francis et al., 2005). Additionally, as the flowers attract bees, jatropha may be used in honey production (Heller, 1996).

There are multiple uses for the different parts of the plant. Leaves contain anti-inflammatory substances and therefore, they are used in traditional medicines and for veterinary purposes (Heller, 1996; Jongschaap et al., 2007; Oskoueian et al., 2011). For example, decoction of the leaves is used for coughs as well as an antiseptic after birth (Heller, 1996). They are also used as fertilizer (Jongschaap et al., 2007).

The latex can be extracted from leaves and stems and it is used in the dye production process (Islam et al., 2011). Alkaloids present in the latex (jatrophine, jatrophan, jatrophone and curcain) are believed to have anticancer effects (Gour, 2006). The latex is also known for its antimicrobial properties, coagulating activity (Heller, 1996), and antioxidant and anti-inflammatory effects (Oskoueian et al., 2011). Jatropha has been used in medicine for wound healing (Islam et al., 2011; Jongschaap et al., 2007), and as a remedy for alopecia, burns, eczema, and yellow fever (Gour, 2006).

The bark contains tannin, which can be used to treat leather (Openshaw, 2000). It also contains a dark blue dye, which is used for coloring cloth, fishing nets and lines (Islam et al., 2011).
From the wood, charcoal can be produced as a fuel option (Kumar and Sharma, 2008). The wood can be used as firewood as well (Islam et al., 2011; Openshaw, 2000). However, according to Kumar and Sharma (2008), because jatropha wood is very light and burns too fast and is not a popular source of fuel.

Roots are used for dye production as well as in medicine (Islam et al, 2011). In a recent study performed by Oskoueian et al. (2011), they suggested that the roots could be a source of anti-cancer agents. Anti-inflammatory properties of jatropha root powder were also reported by Mujumdar and Misar (2004).

Fruit hulls are used for fuel production and as fertilizer (Gübitz et al., 1999; Islam et al., 2011; Jongschaap et al., 2007; van der Putten et al., 2010). Furthermore, fruit hulls have anti-inflammatory properties and they are used for biogas production (Gübitz et al., 1999).

The toxic properties of jatropha seeds is due to curcin, phorbol esters, trypsin inhibitors, lectins and phytates (van der Putten et al., 2010). However, after proper detoxification, seeds or seedcake can be used as animal feed (Heller, 1996; Islam et al., 2011; Jongschaap et al., 2007; Openshaw, 2000). Seed shells can serve as combustibles/ burning material for producing more energy, as well as fertilizer (Gübitz et al., 1999; Heller, 1996; Islam et al., 2011; Jongschaap et al., 2007). The seed cake can be used in the production of biogas and its residue used as fertilizer (Gübitz et al., 1999; Islam et al., 2011) or entirely used as fertilizer due to its high nutrient content (Heller, 1996; Jongschaap et al., 2007; van der Putten et al., 2010). This kind of recycling process maintains the soil productivity (van der Putten et al., 2010) and reduces the inputs for jatropha production and for other crops (Achten et al., 2008).
The oil of jatropha seeds is viscous and is used as lubricant, for soap and candle manufacturing, lighting and cooking oil, as well as in the cosmetic industry (Jongschaap et al., 2007; Kumar and Sharma, 2008). Phorbol esters presented in the oil are believed to be responsible for its insecticidal (Heller, 1996) and molluscicidal properties (Liu et al., 1997). The oil is reported to have purgative properties, to be used in skin diseases, and to sooth pain (Heller, 1996).

Most importantly, the seeds have high oil content, which can be used as a fuel for transportation (van der Putten et al., 2010). The oil can be blended with diesel (Foidl et al., 1996), but it is also suitable to be converted into biodiesel by transesterification (Gübitz et al., 1999; Jongschaap et al., 2007). Very little or no engine modification is required for an up to 20% blend of jatropha oil and diesel fuel (Kumar and Sharma, 2008; Kureel, 2006). Different studies demonstrated that chemical and physical properties of the jatropha biodiesel meet the standard requirements for biodiesel in Europe and the U.S. (Azam et al., 2005; Fairless, 2007; Tiwari et al., 2007).

**Propagation**

Jatropha can be propagated either by seeds or by cuttings. Propagation from seeds does not generate plants genetically uniform due to the cross-pollination. In contrast, uniform and identical true-to-type plants are obtained by vegetative propagation (Gour, 2006; Swamy and Singh, 2006).

Seeds can be directly sown in the field or pre-cultivated in nurseries/greenhouses prior to field transplanting (Heller, 1996; Openshaw, 2000). Jatropha seeds are orthodox and show primary (innate) dormancy, with high viability but low germination rates (Heller, 1996). Therefore, germination percentages may be improved by soaking the seeds in water overnight, as recommended by Singh et al. (2006). It takes from 7 to 8
days for pre-soaked seeds to germinate in hot weather whereas in cold and drier conditions it may take longer (Gour, 2006). In the latter situation, regular irrigation may be used as a way to improve seed germination (Singh et al., 2006).

Both transplant of seedlings (Carels, 2009) and direct seeding (Gour, 2006) must be done at the beginning of the rainy season. Seedlings can be transplanted 45 days after seeding (Gour, 2006; Singh et al., 2006). Survival of seeds by direct seeding is related to sowing time and sowing depth (Heller, 1996).

The advantage of propagating plants by cuttings is that it allows the cultivation of elite accessions. Cuttings can be planted directly in the field or they can be grown in nurseries/greenhouses for 2 months and transplanted to the field at the beginning of the rainy season (Carels, 2009). However, plants originated from cuttings do not develop a tap root and therefore, may show greater mortality during initial stage of development in drier areas (Gour, 2006) and are more susceptible to being blown over by strong winds (Heller, 1996).

Rooting potential and plant survival are highly influenced by cutting length, age and season (Gopale and Zunjarrao, 2011; Lima et al., 2010; Swamy and Singh, 2006; Tagliani et al. 2010). Appropriate rooting media, well drained and aerated may enhance rooting potential (Heller, 1996).

Differences in seed yield may occur depending on the propagation method used. Plants propagated by direct seed generally yield more than transplanted cuttings; plants propagated from seed have been recommended for establishing large-scale plantations and cultivation for oil production (Heller, 1996). Transplanted cuttings are recommended when growing plants for hedge and soil erosion control (Heller, 1996).
Canopy Management

Jatropha has not been domesticated to any great extent many selections have poor branching patterns. Because jatropha flowers and fruits at the branch terminals the non-improved plant architecture is considered one of the limitations for higher yields (Carels, 2009; Ouwens et al., 2007). Logically increasing the number of branches per plant could lead to an increase in the number of inflorescences, fruits, and as a consequence, increased seed yield (King et al., 2009; Kureel, 2006).

There are several ways to induce branching in plants. Pruning is a canopy management tool that increases branching through the removal of apical dominance, allowing lateral buds to grow. This ultimately results in stimulating inflorescence production and increased seed yield (Gour, 2006; Jongschaap et al., 2007; Openshaw, 2000). Pinching of the terminal branches at 6 months is highly recommended in order to induce branching. After pruning, jatropha trees are expected to have a minimum of 25 branches at the end of the first year and 35 to 40 branches at the end of the second year (Gour, 2006). However, sometimes large-scale pruning may not be convenient or cost-effective and in fact, heavy pruning can stimulate more vegetative growth, delaying crop onset (Gosh et al., 2011; Veinbrants and Miller, 1981). In situations like this, the use of plant growth regulators (PGRs) could substitute for pruning, making the process more efficient.

Flowering and Fruiting

Flowering occurs during the rainy season (Jongschaap et al., 2007) and the number of flowering spans is dependent on the plant, location and agro-climatic conditions (Gour, 2006). Some jatropha plants flower continuously throughout the year under proper moisture conditions (Gour, 2006; Singh et al., 2006).
The proportion of flowers in the same inflorescence may vary not only within accessions, but also according to the season (Wu et al., 2011). The proportion of male sites to female sites on an inflorescence is about 20:1, but male flowers are able to develop at the female sites whereas female flowers are not able to develop at male sites; this is the reason why the observed number of female flowers is usually lower than the expected number. As plant yield is dependent on female flower number, the predominant high male to female flower ratio in jatropha is considered one of the reasons for its low yield (Raju and Ezradanam, 2002; Shukla, 2006; Wu et al., 2011). Therefore, increasing the proportion of female flowers could be a means of increasing seed yield.

Variability in flowering characters is also related to soil fertility. According to Jongschaap et al. (2007) and Openshaw (2000), soil fertility plays an important role on flower development, determining whether they will succeed or not. Therefore, all the discussed variables (genetic make-up, season, and soil fertility), together or separately might explain the great variability in flower number and ratio reported in the literature.

Similarly to flower number and flower ratio, jatropha fruit and seed parameters show great variability. Cultural practices such as fertilization and irrigation as well as inherent genetics of an accession influence jatropha fruit and seed production (Gour, 2006; Singh et al., 2006Das et al., 2010; Rao et al, 2008). As fruit and seed traits are usually correlated to plant yield (Das et al., 2010; Ginwal et al., 2004; Kaushik et al., 2007; Rao et al, 2008), variations in fruit and seed weight, size and number are of great importance for understanding and reporting yields.
Harvesting

Fruits are mature when their peel turns from green to yellow in color (Achten et al., 2008), usually 2 to 3 months after fruit set (Islam et al., 2011). However, fruit maturity does not occur at the same time. In jatropha there is a lack of flowering synchronicity, i.e., flowers and fruits of different stages of development may be on the same branch and within the same tree. This makes harvesting difficult, labor intensive and expensive and successful development of mechanical harvesting doubtful (Achten et al., 2008; Jongschaap et al., 2007).

Fruit harvesting is also influenced by the climate of a particular production area and may vary due to seasonality (Kaushik et al., 2006). Harvesting may occur during 2 months in semi-arid conditions, or during the whole year in humid conditions (Gour, 2006).

Yield

Jatropha yield can be affected by soil, nutrients, and rainfall (Francis et al., 2005), plant age (Heller, 1996; Islam et al., 2011), crop management (Gour, 2006; Singh et al., 2006), and inherent genetics (Kaushik et al., 2007; Rao et al., 2008). Because jatropha grows in many different edaphic and climatic areas there is the potential for great variability in its yield (Jongschaap et al., 2007) and even between individual plants within a field (Francis et al, 2005). Thus, its annual seed yield has been reported to range from about 200 g to more than 2 kg per plant, and from 0.5 to 12 ton·ha\(^{-1}\) (Francis et al., 2005).

Jatropha starts yielding fruits and seeds in limited quantity at 9 months of age and after 5 years its potential annual seed yield can be 6 to 12 ton·ha\(^{-1}\) (Islam et al., 2011). In contrast, Gour (2006) reported that the jatropha predicted annual seed yield ranges
from 1 to 5 ton ha\(^{-1}\), with the current achievable annual seed yield of 2.5 ton ha\(^{-1}\). This agrees with the 2 to 3 ton ha\(^{-1}\) annual yield estimates by Francis et al. (2005) and Heller (1996). Annual yield target of 5 ton ha\(^{-1}\) could only be achieved on wastelands with optimal inputs or in sites with good soils and annual rainfall of 900 to 1200 mm (Foidl et al., 1996; Francis et al., 2005; Gour, 2006).

As indicated above, yields reported in the literature show great variability and are sometimes not clear. Incorrect extrapolation of annual yields from single plants to ha\(^{-1}\) yr\(^{-1}\) is one of the reasons for such yield variability and lack of coherence (Achten et al., 2008). According to Islam et al. (2011), one or a combination of several factors may affect yield variability reports. Unfortunately yields are sometimes reported in terms of number of fruits or seeds and unspecified dry or fresh weights. Also since jatropha is grown in such a wide range of climates and soils, reports that do not document the soil and climatic conditions or cultural practices make comparing data on yields difficult (Islam et al., 2011). Another source of confusion on reported yield data is the fact that some data do not specify if the values are related to fresh or dry weight, whole fruits, complete seeds with kernel, or the kernel itself (Jongschaap et al., 2007).

When discussing yield variability, it is important to understand that certain genotypes present greater number of seeds per fruit and greater seed shell mass, for example, resulting in higher seed yield as compared to genotypes with lower number of seeds per fruit and lower seed shell mass (Makkar et al., 2008). However, it has been already demonstrated that higher seed yield is not a synonymous of higher oil yield (Makkar et al., 2008). Thus, seed yield reports might not correctly reflect oil yield. Another important factor to be considered is that same genotypes may behave
differently under different environmental conditions and agro-practices. Unfortunately, data concerning jatropha yield performance are still limited (King et al., 2009) and there are no improved varieties available so far (Gour, 2006).

**Plant Growth Regulators**

Plant hormones consist of non-nutrient organic substances that are produced in the plants (Davies, 1987). Very small quantities of these hormones (10^{-4} M) are able to promote, inhibit, or change physiological and morphological processes in the plant tissues (Castro and Vieira, 2001). Agriculture has widely benefited from plant hormone use and manipulation, improving crop quality and reducing production time (Basra, 2000).

Gibberellins (GAs) are plant hormones known to affect seed germination (Andreoli and Khan, 1999; Groot and Karssen, 1987; Plummer and Bell, 1995; Renner et al., 2007; Valencia-Diaz and Montana, 2003), seed dormancy (Dissanayake et al., 2010; Nicolás et al., 1996), stem elongation (Peng and Harberd, 1997; Yang et al., 1996), and sex determination (Irish, 1996). GAs are involved in flower development (Mielke et al., 2008; Prat et al., 2008) and they may induce precocious flowering, and inhibit or increase flowering depending on the species (Pharis and King, 1985). They are also reported to improve plant tolerance to drought stress (Botelho et al., 2001).

Cytokinins (CKs) may stimulate or inhibit different plant processes. They are produced in the roots and transported to different parts of the plant, regulating the partitioning of biomass between shoots and roots (Beck, 1996; Kuiper et al., 1988). CKs are reported to affect cell division, cell expansion (Kappler and Kristen, 1986; Stoynova-Bakalova et al., 2004), leaf expansion (Rahayu et al., 2005; Ulvskov et al., 1992), and to delay foliar senescence (Gan and Amasino, 1995; Wingler et al., 1998).
Its exogenous application is reported to break apical dominance (Cline, 1991) stimulating growth of lateral buds (Pillay and Railton, 1983; Turnbull et al., 1997). Cks are able to promote carbohydrate metabolism and to influence assimilate translocation to treated sites. Therefore, they play an important role in influencing or inhibiting flowering (Ogawa and King, 1979).

Ethylene is a gas produced in different parts of the plants, such as leaves, stems, flowers, roots, tubers, fruits, and seedlings (Yang and Hoffman, 1984). It stimulates elongation of vegetative and reproductive parts and inhibits hypocotyl elongation (Hopkins and Hüner, 2004). In addition, ethylene promotes fruit ripening (Adato and Gazit, 1974), and senescence and abscission of leaves, flowers and fruits (Hopkins and Hüner, 2004). It is synthetized as a response to environmental or biological stresses, especially in senescing or maturing tissues (Apelbaum and Yang, 1981).

Plant growth regulators (PGRs) are synthetic substances, which once applied exogenously, are able to affect plant development similarly to plant hormones, or to interfere in the biosynthesis, metabolism, or translocation of these hormones (Castro and Vieira, 2001).

PGRs can be applied directly to the plants (leaves, fruits, seeds) in order to increase yield, improve and facilitate crop harvesting, and its use has showed great potential on improving crop production in both qualitative and quantitative ways (Castro, 1998). Induced branching and flowering, and increased plant yield are some examples of processes known to be affected by the use of PGRs (Batlang et al., 2006; Darginavičienė et al., 2011; Hayashi et al., 2001).
Ethephon

Ethephon is a PGR that releases ethylene after breakdown as well as promoting ethylene production within the plant (Bondad, 1976). Therefore, it is expected that plants treated with ethephon would show responses similar to those caused by ethylene treatments (Yang, 1969). Ethephon is also referred in the literature as Ethrel, Florel, CEP, CEPA, 2-CEPA, ACP 66-329, Amchem 66-329, Amchem 68-62, Amchem 68-64, 2-chloroethanephosphonic acid, and 2-chloroethylphosphonic acid, among others.

Ethephon has been widely used in horticulture in order to produce dwarf plant and for growth suppression prior to shipping. It has been demonstrated that simple and multiple applications of ethephon retard stem elongation and reduce plant height, producing more compact plants with shorter internodes (Hayashi et al., 2001; Kher et al., 1974; Tamari et al., 1998; Tjia and Buxton, 1977).

Ethephon has been extensively used to induce branching in different crops including chrysanthemums, impatiens, and petunia (Hayashi et al., 2001; Kher et al., 1974; Tamari et al., 1998; Tjia and Buxton, 1977). According to (Hayashi et al., 2001), one of the benefits of using ethephon as a branch inducer is that it does not damage the apical meristem or growing point while promoting axillary shoot development. However, concentration of 1000 mg L\(^{-1}\) may be phytotoxic to branch tips of Coffea arabica L. (coffee) (Crisosto et al., 1991).

Ethephon can inhibit inflorescence and flower production (Nagao and Sakai, 1990), initiate precocious flowering (Kher, 1974), and cause young flower abortion (Tamari et al., 1998). Kher et al. (1974) reported that chrysanthemums cultivated in pots remained vegetative after being treated with ethephon at 2000 mg L\(^{-1}\). They observed an inhibitory effect of this chemical on floral bud formation in dissected plant
apices, demonstrating that ethephon at high concentrations could be used to prevent flower formation in this plant. In a study performed by Nagao and Sakai (1990) with Macadamia integrifolia Maiden & Betche (macadamia), no increase flowering was found due to ethephon applications of 100 and 500 mg L⁻¹. The authors also reported a decrease in the number of racemes with fruits per plant due to ethephon treatments. In another experiment, Tamari et al. (1998) reported reduced number of flowers in impatiens treated with ethephon at 200 to 800 mg L⁻¹ as compared to control plants. According to the authors, the reduced number of flowers was a result of bud abortion and not from the inhibition of flower development, as indicated above. Subhadrabandhu and Koo-Duang (1987) showed that Litchi chinensis Sonn. (lychee) flowering responses to ethephon applications are cultivar dependent. Plants were sprayed prior to normal flowering season and showed a reduction or increase in flower depending upon the cultivar. Increased flowering due to ethephon applications has also been reported in Mangifera indica L. (mango) and Ananas comosus L. (pineapple) (Bondad, 1976). Flowering stimulation following defoliation in Plumeria rubra L. (plumeria) after ethephon application was also reported by Criley (1995).

Ethephon has been shown to be effective on floral bud retention and on delaying flowering without eliminating flowering for the entire season (Rahemi and Ramezanian, 2007; Sauco et al., 1991; Tjia and Buxton, 1977). Flowering date manipulation is extremely useful in horticulture as it allows for shipping after normal flowering date of the crop (Hayashi et al., 2001). Synchronization of flowering, fruit ripening and fruit abscission has been reported for several crops such as Vitis sp. (grape) and Ananas comosus L. (pineapple) (Bondad, 1976), coffee (Crisosto et al., 1991), macadamia
(Nagao and Sakai, 1988; Trueman et al., 2002), and *Pistacia vera* L. (pistachio) (Rahemi and Ramezanian, 2007). According to these studies, the use of ethephon could be of great importance facilitating harvesting operations and reducing costs with harvesting of late varieties.

Plant yield response to ethephon has been widely researched, especially for cereal crops. Yield increase due to lodging prevention in *Hordeum vulgare* L. (barley) and *Triticale* hexaploid L. (triticale) has been demonstrated by Dahnous et al. (1982). However, even when lodging does not occur, yield components can be positively or negatively affected, as showed by Moes and Stobbe (1991). Despite the increase in the number of spikes per plant, these authors found a reduction in the number of kernels per spike, which decreased grain yield in barley. Similarly, Norberg et al. (1987) reported that ethephon decreased lodging in corn plants, but did not increase, and sometimes even reduced grain yield. The benefits of ethephon on improving plant yield seem to be more pronounced when plants are cultivated under stress conditions. Shekoofa and Emam (2008) demonstrated increasing grain yield in corn plants in response to increasing ethephon rates at high plant densities and under water stress conditions.

Ethephon is sometimes used as a defoliant in order to increase subsequent flowering production (Criley, 1995) or to delay bloom (Crisosto et al., 1990). However, depending on the species and application time, fruit set and fruit yield can be either reduced or increased (Crisosto et al., 1990). In macadamia, ethephon is usually applied in order to promote fruit abscission. Although leaf abscission occurred, Trueman (2003)
demonstrated that ethephon applications at 1200 mg L\(^{-1}\) for 2 consecutive years were successful on increasing fruit yield by the third year.

Seed and oil yield can be influenced by ethephon application as well and they vary among species and cultivars, as discussed above. For example, ethephon applications caused seed yield to decrease in *Panicum maximum* Jacq (guinea grass) (Joaquín et al., 2007), whereas in *Brassica napus* L. (rapeseed) seed yield was increased due to crude fat accumulation (Darginavičienė et al., 2011). Similarly, El-Keltawi and Croteau (1986) reported that ethephon at 250 mg L\(^{-1}\) reduced *Mentha piperita* L. (peppermint) oil yield, whereas the same concentration slightly increased oil yield in *Salvia officinalis* L. (sage).

Studies on the effects of ethephon in jatropha are limited. Augustus et al. (2002) reported the ability of ethephon to increase the percentage of hydrocarbon content of the seeds from 3.9% to 5% when applied at 15 mg L\(^{-1}\). More recently, Joshi et al. (2011) demonstrated the effects of ethephon on growth, flowering and yield parameters of jatropha when using 100 to 150 mg L\(^{-1}\). Ethephon increased jatropha collar diameter and reduced plant canopy spread and height as concentration increased. Ethephon reduced the length of flowering initiation after application, increased the number of inflorescences per plant, and the numbers of male and female flowers per inflorescence. Ethephon was also successful in reducing the male to female flower ratio, and fruit and seed yield were reported to increase as concentration increased.

**BA + GA\(_{4+7}\)**

**BA + GA\(_{4+7}\)** is a mixture of the cytokinin 6-benzyladenine (BA) with gibberellic acid isomers GA\(_{4+7}\). It is found as a mixture of 1.8% BA + 1.8% GA\(_{4+7}\) (*Fascination, Perlan, Progerbalin, Promalin,* or 1.8% BA + 0.18% GA\(_{4+7}\) (*Accel*).
This PGR is labeled for inducing branching in several species as BA induced partial release of apical dominance promoting lateral bud initiation while GA$_{4+7}$ enhanced further growth stimulating cell elongation (Ali and Fletcher, 1971; Rossi et al., 2004). Branching of young trees is highly desirable as it reduces the production cycle and increases plant productivity (Lawes et al., 1997). In many species there is a correlation between the number of branches per plant and plant flower potential, and between the numbers of branches and fruits per plant. In *Asclepsias tuberosa* L. (butterflyweed), for example, greater number of branches leads to increased number of flowers (Wyatt, 1980). Similarly, Cramer and Wehner (2000) reported greater number of fruits in *Cucumis sativus* L. (cucumber) plants with higher number of branches. Branching is also important in horticulture in order to produce high quality and marketable plants. The efficiency of BA + GA$_{4+7}$ as a branch inducer has been widely documented in bearing fruit plants such as apple, pear, and sweet cherry (Cody et al., 1985; Edgerton, 1983; Elfving and Visser, 2006; Jacyna and Buczek, 2008; Jacyna et al., 1994; Ouellette et al., 1996; Rossi et al., 2004), as well as in ornamentals such as *Pelargonium x hortorum* L.H. Bailey (geranium), *Vinca minor* L. (periwinkle), and *Rhododendron* sp. (azalea) (Foley and Keever, 1992; Foley and Keever, 1993; Keever and Foster, 1990). However, hard to branch species or cultivars may require bark injury (Elfving and Visser, 2007), the use of surfactants (Elfving and Visser, 2009), a mixture of BA + GA$_{4+7}$ with latex (Jacyna and Puchala, 2004), or multiple applications of BA + GA$_{4+7}$ (Preece, 1990).

As a consequence of BA + GA$_{4+7}$ applications, plants show branch elongation as well as an increase in height (Bell et al., 1997; Keever and Foster, 1990; Ranwala and
Miller, 1999). BA + GA_{4+7} applications have also proved to be effective in increasing growth rate and plant dry weight of the bioenergy crop caper spurge as concentration increased from 150 to 1200 mg L^{-1} (Preece, 1990). However, some species may show height reduction at high BA + GA_{4+7} concentrations (Keever and Foster, 1990; Rossi et al., 2004).

BA + GA_{4+7} have been reported to increase flowering in several species, such as cucumber, Zantedeschia ‘Galaxy’ (Calla lily) and jojoba (Batlang et al., 2006; Funnel et al., 1992; Ravetta and Palzkill, 1992). It is possible that the reduced apical dominance promoted by BA stimulates shoot development, increasing shoot responsiveness to GA_{4+7} to promote flowering (Funnel et al., 1992). Flowering can be increased either by stimulating buds within a shoot to flower (Ravetta and Palzkill, 1992) or by the increase in the number of shoots producing flowers (Funnel et al., 1992).

Flowering can be anticipated or delayed by BA + GA_{4+7} treatments, as reported by Grzesik and Rudnicki (1985). According to these authors, single application of BA + GA_{4+7} at 1000 mg L^{-1} promoted early blooming in Forsythia x intermedia ‘Spectabilis’ (forsythia), whereas three applications at same concentrations delayed flowering.

BA + GA_{4+7} also have the potential to improve yield by increasing different yield components (number of flowers, fruits and seeds, fruit set, and fruit and seed size). Batlang (2008) reported no increase in Capsicum annuum L. (hot pepper) plant height, number of branches, or fruits per plant due to BA + GA_{4+7} applications at 10, 15, 20, or 25 mg L^{-1}. However, fruit yield was increased by PGR application due to fruit size increase. In contrast, BA + GA_{4+7} were efficient in increasing Phaseolus vulgaris (snap bean) yield by increasing pod size, number of pods per plant, and number of pods per
hectare (Emongor, 2007). In cucumber, plants treated with BA + GA$_{4+7}$ showed higher yield due to the greater number of flowers produced, increased fruit number and fruit size as compared to control plants (Batlang et al., 2006).

There are no reports in the literature about the effects of BA + GA$_{4+7}$ in jatropha growth, flowering and yield parameters. However, the use of BA in jatropha has been reported (Abdelgadir et al., 2009). Single foliar applications of BA increased branching in jatropha both in the greenhouse and in field conditions. Therefore, it has been suggested that BA applications could substitute manual pruning, which is time-consuming and labor-intensive. In the year following BA application, an increase in the number of flowers per plant, fruits per bunch, and fruit size was found, but no increase in fruit set or seed weight (Abdelgadir et al., 2010). In contrast, jatropha seed yield has been reported to increase when BA was applied directly to the inflorescences (Pan and Xu, 2011). There was an increase in the total number of flowers per inflorescence as well as in the number of female flowers per inflorescence. Greater number of female flowers and the induction of bisexual flowers lead to increased number of fruits, and consequently higher seed yield.
CHAPTER 3  
MATERIAL AND METHODS  

Plant Material and Site Characteristics  

Plants for the experiment were selected from a jatropha field plot at the University of Florida’s Tropical Research and Educational Center (TREC) (25°50'N and 80°50'W, 3.8 m above sea level), in Homestead, FL. Homestead has a marine subtropical climate with average precipitation of 1473 mm per year. The mean annual temperature is 24°C with average maximum and minimum temperature of 29°C and 19°C, respectively (Crane et al., 2010). The soil is classified as a Krome very gravelly loam (Li, 2001). This type of soil is calcareous, very shallow, and well drained with limerock up to the soil surface, pH 7.4 to 8.4, low organic matter content, and low nutrient content (Li, 2001).  

Plants selected for this study consisted of 2-year-old plants. Seeds originated from a jatropha accession from India were germinated in a greenhouse and planted in the field at TREC on June 25, 2009. Plants were spaced 2.4 m (in-row) by 3.7 m (between-rows). During prolonged dry periods plants were irrigated every other day with a microsprinkler system (98 L·h⁻¹) throughout the year. Plants were fertilized monthly with 100 g (July through December) to 200 g (January through April) of 6N–5P₂O₅–15K₂O fertilizer (Atlantic FEC – Fertilizer & Chemical Co., Homestead, FL).  

At the initiation of treatments in May 2011, several plants had set leaves after breaking winter dormancy (plants with leaves) while others did not have any new leaves (plants without leaves) (Fig. 3-1). A total of 64 plants were selected for the experiment; 32 with leaves and 32 without leaves. Plants were not pruned prior to the experiment initiation.
Plant Growth Regulators

Treatments consisted of spray applications of ethephon [Florel® (FL); Monterey Lawn and Garden Products, Inc., Fresno, CA] and a combination of 6-benzyladenine (BA) and gibberellic acid isomers (GA$_{4+7}$) [Promalin® (PR); Valent Biosciences™ Corporation, Walnut Creek, CA] to plants with leaves and plants without leaves. Ethephon was applied at concentrations of 0, 500, 1000, or 2000 mg L$^{-1}$ and BA + GA$_{4+7}$ was applied at concentrations of 0, 250, 500, or 1000 mg L$^{-1}$. Tween® 20 (Merck, Germany) 0.1% (v/v) was added to the solutions as a surfactant. The pH of FL and PR solutions was adjusted to 4.5 and, 6.0 respectively, as recommended by the manufacturers. Plants (stem and canopy) were entirely sprayed with 600 mL of solution per plant per application using a hand sprayer. Control plants were sprayed with 600 mL of water. Immediately after sunrise on 27 May 2011, a single application for each PGR was made.

As per manufacturers recommendations, FL and PR should be applied to actively growing plants. Plants with leaves were actively growing at PGR application time and had already started to flower and initiate fruit production. In contrast, plants without leaves were dormant at the time of PGR applications.

Morphological Measurements

Plants were monitored from May through November 2011. In November 2011, final plant size (cm) and number of branches per plant were recorded. Plant height (cm) was measured as the distance between soil surface and the tip of the main stem. Plant canopy (cm) was measured in two perpendicular directions (width1 and width2). Plant size (cm) was calculated by (height + width1 + width2)/3, as suggested by Keever.
Number of all branches longer than 3 cm was recorded (Abdelgadir et al., 2010).

Flowering and fruiting data was collected from May through November 2011. Parameters evaluated included total number of inflorescences per plant, percentage of inflorescence set, number of flowers per inflorescence (total, female and male), male to female flower ratio, number of fruits per bunch, percentage of fruit set, total number of fruits per plant, fruit yield (g), total number of seeds per plant, seed yield (g), individual fruit fresh weight (g), number of seeds per fruit, seed fresh weight (g), seed dry weight (g), 100-seed weight (g), and seed length (mm), thickness (mm) and width (mm).

Total number of inflorescences per plant was recorded monthly. Inflorescence set was given as the percentage of inflorescences with fruits per treatment. Number of flowers per inflorescence (total, male, and female flowers), male to female flower ratio, number of fruits per bunch, and percentage of fruit set were given as the mean of 8 inflorescences randomly selected on each plant, a total of 32 inflorescences per treatment. Fruit set was determined as the percentage of fruits over the number of female flowers per inflorescence. Fruit set was estimated one month after first inflorescence flower has opened. Total number of fruits per plant was recorded by harvesting and counting all fruits from inflorescences formed after PGR application. Fruits were manually harvested when they started to mature. Total number of seeds per plant was recorded by manually opening all fruits from each plant and counting the number of seeds. Fruit yield was recorded as the whole fruit fresh weight of all fruits harvested per plant. Seed yield was recorded as the whole seed dry weight of all seeds harvested per plant, after seeds were oven dried for 48 hours at 70°C. Fruits fresh
weight, number of seeds per fruit, seed fresh and dry weight, and seed length, thickness and width were given as the mean of 50 fruits or seeds randomly selected per plant, a total of 200 fruits or seeds per treatment. To calculate 100-seed weight, 100 seeds from each plant were randomly selected and weighted.

**Experimental Design and Data Analysis**

Four plants per treatment combination [leaf presence (2) x PGR (2) x PGR concentrations (4)] were laid out in a completely randomized design. Analysis of variance was performed to evaluate possible interactions between leaf presence and concentrations. Regression analysis was performed to evaluate significant linear and quadratic concentration relationships for each PGR. Plants with leaves and without leaves were combined for regression analysis for all variables where no interaction leaf presence by concentration was detected. Differences between plants with leaves and without leaves were accessed using a Student’s t-test. Statistical analyzes were performed using the SAS Software (SAS Institute Inc., Cary, NC, USA).
Figure 3-1. *Jatropha curcas* L. plants at the time of PGR application. A total of 64 plants were sprayed; 32 plants that were actively growing (with leaves), and 32 plants that were still under winter dormancy (without leaves).
CHAPTER 4
RESULTS AND DISCUSSION

Within 4 hours of FL and PR foliar applications, a light rain (0.80 mm) occurred at TREC, with a total of 15.8 mm for the day (Florida Automated Weather Network, 2011). Both PR and FL might have had their activity reduced due to rainfall within 6 to 24 hours of application. Therefore plants were monitored daily to confirm any potential reduced activity of both PGRs. Plants sprayed with FL at 1000 and 2000 mg L\(^{-1}\) showed leaf, inflorescence, and fruit yellowing, and leaf abscission beginning on the third day until the seventh day after application (Fig. 4-1). Leaf burn was also observed for all FL concentrations (Fig 4-1). Ethylene effects similar to these have been described by Hopkins and Hüner (2004). Plants sprayed with PR at 500 and 1000 mg L\(^{-1}\) showed a slight leaf curling and leaf drop 4 days after application (Fig. 4-2). Therefore, the light rain appeared not to have a significant effect on the activity of either PGR.

Overall, plants with leaves showed significantly greater height, plant width and thus, greater plant size for each PGR at all concentrations.

Specifically for FL applications, differences were observed for plants with leaves, which had greater number of fruits per plant, number of seeds per plant, fruit yield and seed yield as compared to plants without leaves (Table 1). For PR applications, the number of seeds per fruit was significantly increased for plants without leaves compared to plants with leaves (Table 1). Inflorescence set was significantly greater for plants without leaves treated with PR at 250 mg L\(^{-1}\) (\(P = 0.036\)) and 1000 mg L\(^{-1}\) (\(P = 0.029\)) whereas plants with leaves showed greater but marginal significant difference on the number of inflorescences with fruit set at 500 mg L\(^{-1}\) (\(P = 0.046\)) (Table 2).
Despite the greater inflorescence set for plants without leaves sprayed with PR at 250 mg L\(^{-1}\) and 1000 mg L\(^{-1}\), no differences were observed in fruit and seed number. Possibly plants without leaves had a lower number of female flowers or a higher percentage of fruit abortion than plants with leaves. This would result in lower fruit set in plants without leaves, therefore comparable to that of plants with leaves. The lower number of female flowers has been reported to limit yield in jatropha (Raju and Ezradanam, 2002; Wu et al., 2011). In a similar manner, despite no differences in inflorescence set for plants with or without leaves treated with FL, plants with leaves had greater number of fruits, and seeds, and greater fruit and seed yield as compared to plants without leaves. The number of female flowers and fruit abortion might explain those results. However, data on flowering and potential fruit abortion was not collected for plants without leaves. We expected to see continuous flowering for both plants with and without leaves. However, flowering for plants without leaves was concentrated within the month of June and after that very few inflorescences were produced. Therefore, the numbers of inflorescences collected was not sufficient for statistical analysis and the data was not included.

There was no increase in the number of branches, inflorescences, fruits and seeds due to FL or PR applications at all concentrations tested (\(P > 0.05\)). However, for plants treated with FL there was a linear response to PGR concentration for the number of flowers and for the number of male flowers per inflorescence. As FL concentration increased, there was a reduction in both the numbers of flowers (\(P = 0.024\)) and male flowers per inflorescence (\(P = 0.021\)) (Fig. 4-3). This could explain why plants treated with FL showed lower mean male to female flower ratio (25.3:1) as compared to plants
treated with PR (39.7:1). It is possible that ethephon reduced the formation of flowers (Kher, 1974; Nagao and Sakai, 1990) and/or increased the abortion of young flowers (Tamari et al., 1998).

Plants treated with PR showed a linear relationship between concentration and seed dry weight. As PR concentration increased, seed dry weight also increased ($P = 0.030$) (Fig. 4-4). It is possible that the increase in seed dry weight was due to assimilate mobilization into the seeds caused by cytokinins and gibberellins. As a result of cell division and enlargement caused by these compounds, more sites for assimilate deposition become available, increasing the accumulation of dry matter (Brenner et al., 1988). Gibberelins have been shown to be related to early and late seed development and their concentration is usually correlated to seed fresh weight accumulation in different species (Pharis and King, 1985). Similarly, BA is known for its ability to allocate nutrients to sites of application (Crosby et al., 1981). Increased seed dry weight due to PR application supports the hypothesis that gibberellins and cytokinins could play an important role on improving seed yield.

The lack of a branching response to PGR applications could be related to other parameters such as plant age, PGR concentrations, and the number of applications. Effects on branching, plant height and number of inflorescences per plant have been shown to vary among several herbaceous species due to single and multiple ethephon applications at 500 or 1000 mg L$^{-1}$ (Hayashi et al., 2001). Keever and Foster (1990) reported lack of branching response for several woody species when treated with single applications of BA at 125 to 1000 mg L$^{-1}$. Higher BA concentrations as well as PR at 2000 to 5000 mg L$^{-1}$ promoted greater number of shoots in most studied species.
However, even higher PR concentrations did not increase shoot count in *Ternstroemia gymnanthera* Wight & Arn. (cleyera) and *Raphiolepis indica* L. (Indian hawthorn) (Keever and Foster, 1990) or in azalea (Bell et al., 1997). Single BA applications to 5-month-old and 1-year-old jatropha plants have been reported to promote branching both in greenhouse and field conditions at 2700 mg L\(^{-1}\) (Abdelgadir et al., 2009). It is likely that the concentrations used in our study were not sufficiently high to promote branching in 2-year-old jatropha plants when applied as a single foliar treatment, and under South Florida environmental conditions.

Repeated applications might have resulted in more branching effects. Preece (1990) reported no significant differences in lateral shoot number and length for *Euphorbia lathyris* L. (Caper spurge) treated with a single application of PR from 150 to 1200 mg L\(^{-1}\). However, multiple PR applications stimulated branching. Similar results were reported for *Jatropha intergerrima* N. von Jacquin (jatropha) in response to cyclanilide applications (Mackay et al., 2007).

The method of PGR application might also have affected the results in this study. BA applied directly to forming inflorescences was effective in inducing development of bisexual flowers, increasing the number of female flowers per inflorescence, and increasing seed yield (Pan and Xu, 2010).

The low response to PGR treatments could also be related to the genotype effect, i.e., an accession with both dormant and actively growing plants at the time of PGR applications. Such genotype effect is probably responsible for the lack of significant differences for most variables, although some trends were observed (Figs. 4-5 and 4-6).
Due to large variance observed within control plants, which could be greater than treatment effects, we decided to estimate sample size for specific variables using control plants. Sample size estimate was calculated in order to access the projected sample size required to see a 5 and 10% difference at $P = 0.05$. Sample size estimate was calculated as

$$n = \frac{(Z_\alpha^2)(v_s)}{(d^2)(\bar{x}^2)},$$

where $n$ is the required sample size, $Z_\alpha$ is the value of the standardized normal variate corresponding to $\alpha$, $v_s$ is the sampling variance, $\bar{x}$ is the sample mean value, and $d$ is the margin of error of expressed as a fraction of the plot mean (Little and Hills, 1978).

Adequate sample sizes are important to increase the probability of detecting a statistically significant differences among treatment means. Our results showed that the predicted sample sizes required for all variables evaluated needed to be larger than those used in this study (Table 4-3). Therefore, although sample sizes in the present study were probably too small to see significant differences, data collected provided useful information for future studies of PGR application effects on jatropha growth and development. Based in these findings, we suggest greater number of repetitions for field studies performed with jatropha plants originated from seeds. It is possible that a greater number of repetitions per treatment would have found a response to the FL and PR treatments on jatropha branching, flowering, and fruiting parameters.
Table 4-1. Comparison of growth, flower, fruit and seed variables between *Jatropha curcas* L. plants with leaves and plants without leaves.

<table>
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<th>Ethephon No leaves</th>
<th>Ethephon With leaves</th>
<th>BA + GA&lt;sub&gt;4+7&lt;/sub&gt; No leaves</th>
<th>BA + GA&lt;sub&gt;4+7&lt;/sub&gt; With leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. branches</td>
<td>70.44 b</td>
<td>74.75 a</td>
<td>65.56 a</td>
<td>79.25 a</td>
</tr>
<tr>
<td>Plant size (cm)</td>
<td>227.9 b</td>
<td>262.0 a</td>
<td>239.5 b</td>
<td>279.4 a</td>
</tr>
<tr>
<td>No. Inflorescences per plant</td>
<td>38.81 a</td>
<td>41.19 a</td>
<td>47.56 a</td>
<td>38.63 a</td>
</tr>
<tr>
<td>Inflorescence set (%)</td>
<td>58.39 a</td>
<td>48.68 a</td>
<td>*z</td>
<td>*</td>
</tr>
<tr>
<td>No. fruit per plant</td>
<td>62.44 b</td>
<td>95.5 a</td>
<td>79.81 a</td>
<td>77.13 a</td>
</tr>
<tr>
<td>Fruit fresh yield per plant (g)</td>
<td>695.17 b</td>
<td>1216.47 a</td>
<td>904.25 a</td>
<td>887.02 a</td>
</tr>
<tr>
<td>No. seeds per plant</td>
<td>163.1 b</td>
<td>263.6 a</td>
<td>216.7 a</td>
<td>198.3 a</td>
</tr>
<tr>
<td>Seed dry yield per plant (g)</td>
<td>69.26 b</td>
<td>129.8 a</td>
<td>94.75 a</td>
<td>99.32 a</td>
</tr>
<tr>
<td>Fruit fresh weight (g)&lt;sup&gt;y&lt;/sup&gt;</td>
<td>10.87 a</td>
<td>12.13 a</td>
<td>11.71 a</td>
<td>11.78 a</td>
</tr>
<tr>
<td>No. seeds per fruit&lt;sup&gt;y&lt;/sup&gt;</td>
<td>2.69 a</td>
<td>2.68 a</td>
<td>2.78 a</td>
<td>2.39 b</td>
</tr>
<tr>
<td>Seed fresh weight (g)&lt;sup&gt;y&lt;/sup&gt;</td>
<td>0.92 a</td>
<td>0.97 a</td>
<td>0.96 a</td>
<td>0.98 a</td>
</tr>
<tr>
<td>Seed dry weight (g)&lt;sup&gt;y&lt;/sup&gt;</td>
<td>0.45 a</td>
<td>0.47 a</td>
<td>0.48 a</td>
<td>0.53 a</td>
</tr>
<tr>
<td>100-seed weight (g)</td>
<td>41.79 a</td>
<td>43.42 a</td>
<td>45.07 a</td>
<td>47.09 a</td>
</tr>
<tr>
<td>Seed length (mm)&lt;sup&gt;y&lt;/sup&gt;</td>
<td>17.09 a</td>
<td>16.98 a</td>
<td>17.26 a</td>
<td>16.42 a</td>
</tr>
<tr>
<td>Seed thickness (mm)&lt;sup&gt;y&lt;/sup&gt;</td>
<td>8.14 a</td>
<td>8.19 a</td>
<td>8.26 a</td>
<td>8.09 a</td>
</tr>
<tr>
<td>Seed width (mm)&lt;sup&gt;y&lt;/sup&gt;</td>
<td>10.62 a</td>
<td>10.77 a</td>
<td>10.68 a</td>
<td>10.36 a</td>
</tr>
</tbody>
</table>

Plants were treated with ethephon [Florel<sup>®</sup> (FL)] and BA + GA<sub>4+7</sub> [(Promalin<sup>®</sup> (PR)]. Mean separation within columns (separately for Florel and Promalin) by Student’s t-test. Different letters indicate a significant difference (P < 0.05, n = 16). <sup>z</sup> Values not presented due to interactions found for leaves by rate for this variable (see Table 4-2); <sup>y</sup> Mean of 50 fruits or seeds per plant.
Table 4-2. Comparison of inflorescence set (%) between *Jatropha curcas* L. plants with leaves and plants without leaves.

<table>
<thead>
<tr>
<th>BA + GA&lt;sub&gt;4+7&lt;/sub&gt; concentration (mg L&lt;sup&gt;-1&lt;/sup&gt;)</th>
<th>No leaves</th>
<th>With leaves</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>55.45 a</td>
<td>42.20 a</td>
</tr>
<tr>
<td>250</td>
<td>58.57 a</td>
<td>39.48 b</td>
</tr>
<tr>
<td>500</td>
<td>48.40 b</td>
<td>65.30 a</td>
</tr>
<tr>
<td>1000</td>
<td>56.05 a</td>
<td>40.73 b</td>
</tr>
</tbody>
</table>

Plants were treated with BA + GA<sub>4+7</sub> [Promalin® (PR)] at 0, 250, 500, and 1000 mg L<sup>-1</sup>. Mean separation within columns by Student's t-test. Different letters indicate significant difference (P < 0.05, n = 4).
Table 4-3. Variability and sample size estimates for *Jatropha curcas* L. grown at the Tropical Research and Education Center (TREC) in Homestead, FL.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Variance</th>
<th>Sample size 5%</th>
<th>Sample size 10%</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>No. branches per plant</td>
<td>75.86</td>
<td>483.13</td>
<td>128.97</td>
<td>32.24</td>
<td>plants per treatment</td>
</tr>
<tr>
<td>Plant size (cm)</td>
<td>254.47</td>
<td>902.59</td>
<td>21.42</td>
<td>5.35</td>
<td>plants per treatment</td>
</tr>
<tr>
<td>No. inflorescences per plant</td>
<td>43.94</td>
<td>347.53</td>
<td>276.63</td>
<td>69.16</td>
<td>plants per treatment</td>
</tr>
<tr>
<td>No. fruit per plant</td>
<td>75.25</td>
<td>2146.47</td>
<td>582.48</td>
<td>145.62</td>
<td>plants per treatment</td>
</tr>
<tr>
<td>Fruit fresh yield per plant (g)</td>
<td>961.51</td>
<td>253593.84</td>
<td>421.51</td>
<td>105.38</td>
<td>plants per treatment</td>
</tr>
<tr>
<td>No. seeds per plant</td>
<td>206.44</td>
<td>15884.80</td>
<td>572.77</td>
<td>143.19</td>
<td>plants per treatment</td>
</tr>
<tr>
<td>Seed dry yield per plant (g)</td>
<td>99.43</td>
<td>3809.91</td>
<td>592.18</td>
<td>148.04</td>
<td>plants per treatment</td>
</tr>
<tr>
<td>No. flowers per inflorescence</td>
<td>98.70</td>
<td>308.64</td>
<td>48.68</td>
<td>12.17</td>
<td>inflorescences per plant</td>
</tr>
<tr>
<td>No. female flowers per inflorescence</td>
<td>4.00</td>
<td>1.91</td>
<td>183.33</td>
<td>45.83</td>
<td>inflorescences per plant</td>
</tr>
<tr>
<td>No. male flowers per inflorescence</td>
<td>94.80</td>
<td>324.11</td>
<td>55.42</td>
<td>13.85</td>
<td>inflorescences per plant</td>
</tr>
<tr>
<td>Male to female flower ratio</td>
<td>31.80</td>
<td>800.91</td>
<td>1217.04</td>
<td>304.26</td>
<td>plant</td>
</tr>
<tr>
<td>Fruit fresh weight (g)</td>
<td>10.87</td>
<td>21.46</td>
<td>279.12</td>
<td>69.78</td>
<td>fruit per plant</td>
</tr>
<tr>
<td>Seed fresh weight (g)</td>
<td>0.86</td>
<td>0.13</td>
<td>288.17</td>
<td>72.04</td>
<td>seeds per plant</td>
</tr>
<tr>
<td>Seed dry weight (g)</td>
<td>0.44</td>
<td>0.03</td>
<td>302.03</td>
<td>75.51</td>
<td>seeds per plant</td>
</tr>
</tbody>
</table>

Sample size estimation used a 5% and 10% detectable difference at P = 0.05. Values are mean, variance and sample size estimates, respectively for 16 plants sprayed with water (control).  

- Mean of 4 plants per treatment;  
- Mean of 8 inflorescences per plant;  
- Mean of 50 fruits or seeds per plant.
Figure 4-1. Effects of ethephon (Florel®; FL) on *Jatropha curcas* L. leaves and fruits. A) Leaf yellowing and leaf drop. B) Fruit yellowing. Effects were observed five days after ethephon application at 1000 to 2000 mg L⁻¹.
Figure 4-2. Effects of BA + GA$_{4+7}$ (Promalin®; PR) on *Jatropha curcas* L. leaves. A) Leaf curling. B) Leaf drop. Effects were observed five days after BA + GA$_{4+7}$ application at 500 to 1000 mg L$^{-1}$. 


Figure 4-3. Flower characteristics in *Jatropha curcas* L. plants treated with ethephon (Florel®, FL). A) Number of flowers per inflorescence. B) Number of male flowers per inflorescence. Concentrations of ethephon used were 0, 500, 1000, and 2000 mg L$^{-1}$. Plants were treated in May 2011 (n = 4). Values are means of 8 inflorescences randomly selected for each plant, with a total of 32 inflorescences per treatment.
Figure 4-4. Seed dry weight (g) in *Jatropha curcas* L. plants treated with BA + GA<sub>4+7</sub> (Promalin®, PR). Concentrations of BA + GA<sub>4+7</sub> used were 0, 250, 500, and 1000 mg·L<sup>-1</sup>. Plants were treated in May 2011 (n = 8). Values are mean of 50 seeds randomly selected per plant, with a total of 400 seeds per treatment.
Figure 4-5. Effects of ethephon (Florel\textsuperscript{\textregistered}; FL) on flowering and yield variables of \textit{Jatropha curcas} L. A) Number of inflorescences per plant. B) Seed dry weight (g). Concentrations of ethephon used were 0, 500, 1000, and 2000 mg L\textsuperscript{-1}. Plants were treated in May 2011 (n = 8). Number of inflorescences per plant was estimated monthly by recording the number of all inflorescences per plant. Values are mean of 50 seeds randomly selected per plant, with a total of 400 seeds per treatment.
Figure 4-6. Effects of BA + GA₄+₇ (Promalin®; PR) on yield variables of Jatropha curcas L. A) Number of fruits per plant. B) Number of seeds per plant. C) Fruit yield (g). D) Seed yield (g). Concentrations of BA + GA₄+₇ used were 0, 250, 500, and 1000 mg L⁻¹. Plants were treated in May 2011 (n= 8). Number of fruits per plant was recorded after harvesting all fruits from inflorescences formed after PGR application. Fruits were manually harvested when they changed color from green to yellow. Number of seeds per plant was recorded by manually opening all fruits from each plant and counting the number of seeds. Fruit and seed yields were recorded as the whole fruit fresh weight and seed dry weight of all fruits and seeds harvested per plant.
CHAPTER 5
CONCLUSION

Studies on the use of PGRs to promote branching and improve yield in jatropha are either very limited or nonexistent. For FL and PR, no recommendations exist for the optimum concentrations to be used in jatropha cultivation. Therefore, this study represents the first assessment performed to address the effects of FL and PR on branching in jatropha.

Despite a poor correlation coefficient, FL and PR at the concentrations tested appeared to have a greater effect on flower and seed production than in branching stimulation. However, treatment effects were also affected by genotype, as variability was observed within individuals from the same accession.

Further studies are necessary to clarify the responses of FL and PR applications in jatropha. Clonal material should be used to avoid the potential genetic variability. Single and multiple foliar applications, as well as higher product concentrations should also be considered. Product applications to forming inflorescences under South Florida conditions should also be included in future studies.

Although the number of plants available in the field limited this study, it provided important and relevant information currently lacking on the use of selected PRGs, namely FL and PR to improve jatropha yields.
LIST OF REFERENCES


BIOGRAPHICAL SKETCH

Anne Pinheiro Costa was born in Teófilo Otoni, Minas Gerais, Brazil, in 1981. She received a bachelor’s degree in biology in 2005, from the Universidade Federal de Uberlândia, in Minas Gerais, Brazil. After her graduation, Anne worked in a greenhouse in New Hampshire for 10 months. In 2006, Anne completed a specialization course in environmental management and she enrolled in another undergraduate course in agricultural engineering at the Universidade Federal de Uberlândia. Anne was admitted as a graduate student by the University of Florida and started her Master of Science in the Department of Environmental Horticulture in 2010. Her career goal is to earn a position as a lead researcher in a private company.