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Paclobutrazol Arrests Vegetative Growth and Unveils Unexpressed Yield Potential of *Jatropha curcas*

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Abstract Although the process for making EN 14214 grade *Jatropha* methyl ester (biodiesel) capable of running unmodified diesel engines in neat form has been demonstrated, getting higher seed yield from *Jatropha* shrubs in wastelands is critical to the success of *Jatropha* biodiesel. But, low productivity is inherent to many *Jatropha curcas* germplasms and raising large-scale plantations using such untested planting material can lead to wasteful expenditures. Unreliable and poor flowering and fruiting are important factors responsible for low productivity in the species. Although much is known about growth retardants applied to field and horticultural crops, their role in improving the seed productivity of *Jatropha* has never been explored. Here we report for the first time that paclobutrazol could be an extremely useful chemical, whose dose and time of application, if optimized, can significantly reduce unwanted vegetative growth, with concomitant improvement in yield and seed oil content of *Jatropha*. In the year following application of paclobutrazol, an unexpected increase in seed yield, as high as 1127% relative to controls, was obtained from one such unproductive *Jatropha* germplasm. We hypothesize that low seed production in this species may be a result of excess vegetative growth caused by an unfavorable endogenous hormonal configuration which competes with growth and development of flower, fruit, or seed. This undesired physiological state can be reversed by paclobutrazol application to achieve maximum oil yield from this energy shrub that holds great promise in the future.

Keywords *Jatropha curcas* · Biodiesel · Paclobutrazol · Seed yield · Oil content · Nutrient uptake

Introduction

Jatropha curcas is a shrub that could be a prospective source of biodiesel (Fairless 2007). Although the process for making EN 14214 grade *Jatropha* methyl ester capable of running unmodified diesel engines in neat form has been demonstrated by CSMCRI for the first time in 2004 (Fairless 2007; Ghosh and others 2007; Ghosh and others *vide* Patent No. WO2006043281-A1), getting a high seed yield from the *Jatropha* shrub is critical to success and calls for more agronomic research. Currently, reliable long-term predictions of *Jatropha* productivity on wastelands are largely absent but are required to make responsible decisions on investment (Jonschaap and others 2007). Results from our experimental plantations indicate that attaining seed yields of 2 t ha^{-1} $year^{-1}$ from mature plantations on wastelands was a realistic target, albeit using selected germplasm (Ghosh and others 2007). Unreliable and poor flowering and fruiting are important factors responsible for low productivity in the species (Kochhar and others 2005). Our past studies show that there is clear evidence of copious biomass generation in *Jatropha* but that does not correlate with seed productivity (Ghosh and others 2007) and there is a need to tilt the balance in favor of reproductive growth in the shrubs. Consequently, it was proposed to investigate the effects of paclobutrazol on

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growth, development, seed productivity, and oil content in *Jatropha*. A pilot-scale field trial was set up using 2-year-old *Jatropha* shrubs to which five paclobutrazol treatments, that is, none (control), 0.75, 1.00, 1.25, and 1.50 g a.i. (active ingredient) per meter of canopy diameter, were applied as soil drench. The randomized block design of the experiment allowed us to test the influence of varying paclobutrazol doses on the vegetative and reproductive behavior of *Jatropha* relative to untreated shrubs both during the year of application (2006–2007, Year 1) and the subsequent year (2007–2008, Year 2). No significant effect of foliar spray application, carried out as a separate trial (at 0, 125, 250, 375, and 500 ppm paclobutrazol), was evident and hence the results of only soil application are discussed in this article.

Table 1 Physicochemical properties of soil

Soil physicochemical properties (0–60 cm)	Values
Solum depth (cm)	88.9
Pebbles (%)	26.9
Textural class	Sandy loam
pH	7.3
EC (dS m^{-1})	0.2
CEC (cmol [p+] kg^{-1})	23.9
Organic carbon (%)	0.4
Available N (kg ha^{-1})	123.7
Available P (kg ha^{-1})	1.4
Available K (kg ha^{-1})	119.6
Available Cu (mg kg^{-1})	2.4
Available Fe (mg kg^{-1})	6.6
Available Mn (mg kg^{-1})	17.0
Available Zn (mg kg^{-1})	0.1

Materials and Methods

The pilot-scale experiment was set up at CSMCRI *Jatropha* Experimental Farm, Chorvadla (latitude 21°40′ N, longitude 71°46′ E; elevation 394 ft), located in the semi-arid part of the Gujarat State of India. Paclobutrazol treatments were applied as CULTAR® 23 SC [paclobutrazol 23% w/w SC (25% w/v), product of Syngenta Ltd., UK] at 0, 0.75, 1.00, 1.25, or 1.50 g a.i. (active ingredient) m^{-1} of canopy diameter on 2-year-old *Jatropha* shrubs of an accession that recorded comparatively lower seed productivity compared to other accessions. The experiment was laid out in a randomized block design (RBD) with five replications. The five treatments were allocated randomly among the shrubs within each of the replications. The treatments were given only once on 31 August 2006 and no treatments were applied to the shrubs in the subsequent year. The soil was well-drained sandy loam Entisols, classified according to the USDA soil taxonomy as isohyperthermic, mixed-loamy kaolinitic, lithic ustorthents with low fertility with the physicochemical properties given in Table 1.

Climate

The area receives rainfall during the South-West monsoon season. The mean annual rainfall (882 mm) received during 2004 through 2007 was 2.7 times less than the mean annual evapotranspiration (2371 mm) and was received in a yearly average of 47 rainy days.

Treatment Application

Fifty evidently unproductive shrubs (raised using untested planting material collected from the wild in northern Gujarat; mean seed yield less than 20 g $shrub^{-1}$ $year^{-1}$ during 2004–2005 and 2005–2006), were treated with paclobutrazol. Paclobutrazol was applied as a soil drench on half of the shrubs. Foliar spray treatments were applied to the remaining set of shrubs but this is not discussed here. The mean (±SEM) canopy diameter of the shrubs ($n = 25$) before the start of the experiment was 167 ± 4 cm. The treatments were mixed in 5 L water per shrub and one half of the quantity was poured at the base of the stem while the rest was poured uniformly in circular trenches in the soil (15 cm wide and 15 cm deep) around the base at a radial distance of 30 cm from it. The treatments were given only once in 2006–2007, whereas the observation data were recorded to assess the direct effect in 2006–2007 and residual effect in 2007–2008.

Agronomic Practices

The shrubs were transplanted during September 2004 at a spacing of 3 m × 2 m (density = 1667 shrubs ha^{-1}) and were not pruned until the completion of the experiment. Fertilizer was applied on a hectare basis at the rate of 40 kg N as urea (46% N), 30 kg P_2O_5 as single super phosphate (16% P_2O_5), and 20 kg K_2O as muriate of potash (60% K_2O) in addition to 2 t of de-oiled *Jatropha* cake (3.0% N, 1.0% P, and 1.2% K). The shrubs were irrigated annually with 7 cm of water.

Plant and Soil Measurements

Seed yield was recorded for both years, whereas other plant measurements recorded pertained to the year following paclobutrazol application. The incremental height of each shrub was measured 16 months (in January 2007) after paclobutrazol application. *Jatropha curcas*, being deciduous, completely sheds its leaves annually and enters into a dormant phase at the end of the fruiting season every year (January at this geographic location). The increment in total branch length for the period between two successive leaf falls after treatment was computed by summing the length of new growth of all the individual branches per shrub. The mean internode length per shrub was determined from the total length of new shoots developed and total number of nodes formed in the corresponding period. The dry leaf litter fall of all the shrubs corresponding to the growing season of Year 2 was collected, weighed, and expressed in t ha^{-1}. During Year 2, chlorophyll from fully expanded (10th to 13th leaves from apex of branch) leaf samples was extracted with 80% acetone and absorbance values were measured at 663 and 646 nm with a spectrophotometer (Lichtenthaler and Wellburn 1983). Shoots with terminal inflorescences were tagged at the prebloom stage during the second week of September (Year 2) and the numbers of male and female flowers were counted at the time of anthesis to determine the M/F ratio in each shrub separately. All the branches bearing fruits in the entire reproductive season of Year 2 were tagged and counted to determine the percent of fruiting branches in the shrubs.

The harvesting of *Jatropha* capsules is spread over a span of time that varies with climate; in the present study it was effectively from the middle of August to the middle of January for both years of the experiment. The number of fruits set per shrub before and after 15 October was tracked over the whole fruiting season of Year 2. Harvested capsules from each shrub were collected, counted, sun-dried, and weighed, after which seeds were manually separated from the capsule shells and counted. Total numbers of infructescences per shrub were counted to compute the mean number of capsules per infructescence in Year 2. The seeds were sun-dried and weighed thereafter. Seed yields were expressed in g $shrub^{-1}$ or in t ha^{-1}. The mean weight per seed was calculated using seed weight per shrub and number of seeds per shrub. Percent kernels in seeds (by weight) was determined by manually separating kernels and the seed coat from sun-dried seeds. Oil was extracted from seed samples by the Soxhlet method using hexane as a solvent (distillation temperature range = 63–70°C) and expressed as a percentage of seed weight. Samples of seed lots harvested in Year 2 from the shrubs before and after 31 October 2007 were collected and analyzed for oil content separately.

To calculate nitrogen uptake by different plant parts, composite samples of seeds, capsule shells, and fallen leaves were collected with respect to treatment from all the replications. The samples were dried, powdered, weighed, and analyzed for total nitrogen by the semimicro-Kjeldahl method (AOAC (Association of Official Analytical Chemists) 1970) after the plant tissues were oxidized and decomposed by sulfuric acid with a digestion mixture [potassium sulfate (K_2SO_4)–copper sulfate ($CuSO_4$) 5:1]. Analytical values from the composite samples were then multiplied by the average sun-dried weight of the respective plant parts to estimate average nutrient offtake per shrub. A portion of the seeds were dried at 70°C for 24 h in an oven and wet digested in HNO_3–$HClO_4$, and the P concentration was determined by employing the vanadomolybdate phosphoric acid yellow color method (Jackson 1973), whereas the K concentration was estimated by flame photometry. The initial representative soil samples (0–0.60 m) were collected from the experimental plots during June 2004. Collected soil samples were air-dried, ground by wooden mortar and pestle, and passed through a 2-mm sieve. Soil pH and EC were determined using a 1:2.5 (w:v) soil–water ratio. Organic C was determined by the wet oxidation method (Walkley and Black 1934). Cation exchange capacity of soil was determined as described by Richards (1954). The contents of sand, silt, and clay particles were determined by the sedimentation method (Day 1965). Available N was determined by the alkaline-permanganate method (Subbiah and Asija 1956), whereas available P was extracted with sodium bicarbonate (Olsen and others 1954) and estimated using a spectrophotometer. Available K was extracted with neutral normal ammonium acetate (Hanway and Heidel 1952) and determined using a flame photometer. Available Cu, Fe, Mn, and Zn were extracted with DTPA (diethylenetriamine pentaacetic acid) (Lindsay and Norvell 1978) and estimated by ICP-OES (PerkinElmer Optima 2000 DV).

Data Analysis

The treatment effects were assessed by analysis of variance (ANOVA) using Minitab statistical software (Minitab, Inc., State College, PA). The 0.05 level of probability was used as the decision level for the acceptance or rejection of statistical significance in all analyses. Means and standard errors of five replicates were calculated and presented in tables or figures for each level of treatment. Where a significant difference was found with the ANOVA test, the significance of differences between means was tested using Duncan's Multiple Range Test (DMRT) using MSTAT-C statistical software (MSTAT-C 1991, Michigan State University, East Lansing, MI). A test of normality, the Anderson-Darling test, was applied to the residuals; if it deviated significantly from normality, then a suitable transformation was applied to the original data. Either Bartlett's test or Levene's test was used to test homogeneity of variance between treatments.

Results

The *Jatropha* shrubs treated with paclobutrazol exhibited instant adverse affects, the most striking of which was compaction of inflorescences, whose flowers failed to develop into fruits during the fruiting season of Year 1 (Fig. 1a, b). The incremental height of the shrubs, measured 16 months after treatment application, was reduced significantly ($F_{4,\ 16} = 17.38$, $p < 0.001$) by paclobutrazol (Fig. 2a). There was a significant reduction ($F_{4,16} = 35.55$, $p < 0.001$) in total length of new branches per shrub that were formed during the period between the first and second leaf fall after paclobutrazol application (Fig. 2b) as a result of significant shortening ($F_{4,16} = 215.81$, $p < 0.001$) of internodes, with minimum lengths (0.83 cm) at the 1.25-g a.i. level in contrast to the maximum (2.75 cm) at the control level. Furthermore, leaf biomass formation during Year 2 was greatest ($F_{4,16} = 11.86$, $p < 0.001$) in untreated shrubs (Fig. 2c). These data indicate the suppressive effect of paclobutrazol on overall vegetative growth of *Jatropha*. However, statistically significant increases of 25–42% ($F_{4,16} = 18.45$, $p < 0.001$) and 22–47% ($F_{4,16} = 6.27$, $p = 0.003$) in chlorophyll *a* and *b* content, respectively, were detected in leaves of treated plants (Table 2).

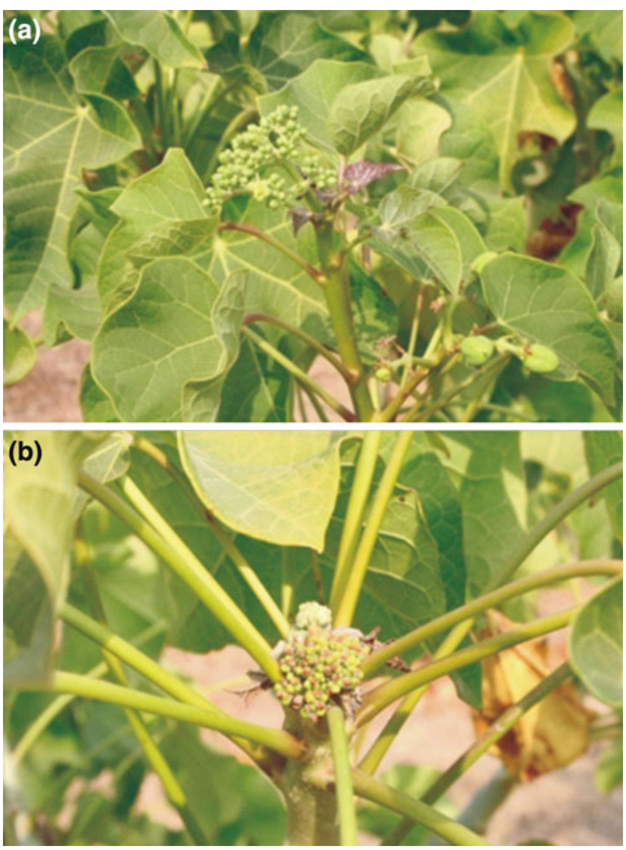

Fig. 1 Inflorescence structure of a representative normal *Jatropha* shrub (**a**) and paclobutrazol-treated shrub showing compaction of inflorescence due to shortening of peduncle (**b**) at 98 days after treatment

Although there were setbacks in flowering and fruiting activities in the application year, the depressive responses were no longer detected in Year 2. On the contrary, discernible improvement in reproductive efforts was noticed. Associated with a decline in aboveground vegetative productivity, there was a significant increase ($F_{4,16} = 8.78$, $p < 0.001$) in the number of fruited branches per shrub; an increase in the proportion of fruited branches within the shrubs; an increase ($F_{4,16} = 4.94$, $p = 0.009$) in the number of fruits (capsules) set per infructescence (Fig. 3); an increase ($F_{4,16} = 15.90$, $p < 0.001$) in the number of capsules per shrub; and an enhancement ($F_{4,16} = 19.54$, $p < 0.001$) in the capsule yield per shrub (Table 3). The average number of female flowers per inflorescence was increased (9.0 in untreated plants versus 13.4 at 1.25-g a.i. level) due to the application of paclobutrazol, albeit with marginal significance ($F_{4,16} = 2.32$, $p < 0.1016$). However, the M/F flower ratio in the inflorescences was significantly ($F_{4,16} = 6.46$, $p = 0.0027$) influenced by the treatments; it ranged from 8.8 to 12.8 in treated shrubs compared to the higher ratio of 15.4 recorded in the control (Table 3).

In Year 2, the number of seeds per capsule increased ($F_{4,16} = 4.33$, $p = 0.015$) with the application of paclobutrazol; the values recorded ranged between 2.6 and 2.8 in treated shrubs compared to 2.4 recorded in untreated controls. The mean weight per seed increased significantly ($F_{4,16} = 21.49$, $p < 0.001$) as a residual effect of paclobutrazol (Table 4). Given that there was no significant effect of treatments on seed volume detected (mean = 0.87 cc seed^{-1}, $F_{4,16} = 0.90$, $p = 0.488$), the reason for the increase in mean weight per seed was expected to be due to better filling of seeds. In accordance with this, we detected a marked enhancement ($F_{4,16} = 18.51$, $p < 0.001$) in the proportion of kernel (by weight) in the seeds (a proxy for seed filling) as a residual treatment effect in the second year (Table 4). Also, higher proportions of capsules (79–95%) were set before 15 October in the treated shrubs compared to capsules (48%) set in untreated shrubs up to that time (Table 4). Significant treatment differences were recorded for seed oil content, irrespective of harvest earlier ($F_{4,16} = 24.5$, $p < 0.001$) or later ($F_{4,16} = 11.8$, $p < 0.001$) in the season (Fig. 4).

Significant reduction ($F_{4,16} = 5.19$, $p = 0.007$) in seed yield per shrub was noticed in Year 1 (Fig. 5a). Furthermore, yield depression by paclobutrazol application prompted cessation of the treatments in the subsequent year because the minimum dose employed was also apparently detrimental. However, in the subsequent year there was significant improvement ($F_{4,16} = 18.3$, $p < 0.001$) in seed

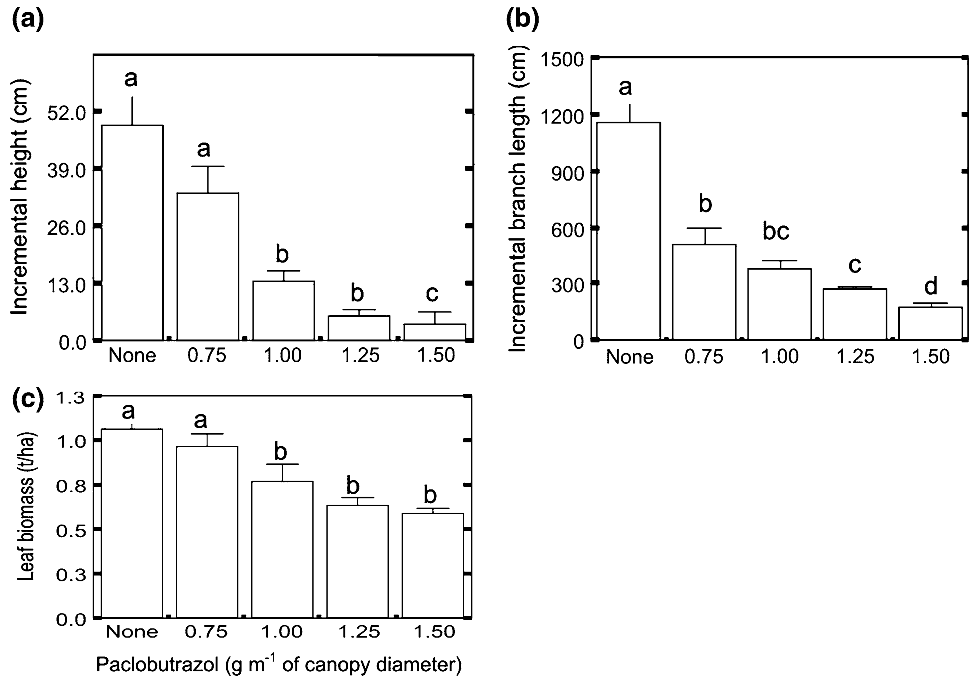

Fig. 2 Mean (± SEM) values for growth attributes of *Jatropha* as affected by paclobutrazol treatments. **a** Incremental height after 16 months of treatment. **b** Total incremental length of new branches per shrub formed during the period between two successive leaf falls after treatment. **c** Leaf biomass formation during Year 2. Values with different letters are significantly different at the $p < 0.05$ level

Table 2 Treatment effects of paclobutrazol on internodal length and leaf pigment content of *Jatropha* during year 2 (2007–2008)

Paclobutrazol (g m^{-1} of canopy diameter)	Internodal length (cm)	Leaf chlorophyll *a* (mg g^{-1})	Leaf chlorophyll *b* (mg g^{-1})
None	$2.75^{a} \pm 0.04$	$1.09^{c} \pm 0.04$	$0.38^{c} \pm 0.03$
0.75	$2.38^{b} \pm 0.10$	$1.44^{ab} \pm 0.04$	$0.51^{ab} \pm 0.01$
1.00	$1.41^{c} \pm 0.11$	$1.48^{ab} \pm 0.07$	$0.53^{ab} \pm 0.05$
1.25	$0.83^{d} \pm 0.01$	$1.55^{a} \pm 0.03$	$0.56^{a} \pm 0.02$
1.50	$0.88^{d} \pm 0.03$	$1.36^{b} \pm 0.05$	$0.47^{bc} \pm 0.02$

Different letters indicate that the values are significantly different at the $p < 0.05$ level

yield per shrub, and depending upon the dose, the amount over that of controls ranged from 519% to 1127% (Fig. 5b). Analysis of the yield response to paclobutrazol in Year 2 revealed that shrubs treated with paclobutrazol at 1.25 g a.i. m^{-1} of canopy diameter in the previous year recorded a maximum seed yield (607.4 g $shrub^{-1}$ or 1.01 t ha^{-1}) compared to control shrubs, which recorded a dismal yield (49.5 g $shrub^{-1}$ or 0.08 t ha^{-1}). The maximum yielding treatment was, however, statistically on par with other treatments that had doses lower to it. The treatment at the highest concentration (1.5 g a.i. m^{-1} of canopy) produced significantly lower seed yield compared to all other lower concentrations, indicating a retardant effect of the residual concentration, but nevertheless a yield significantly higher than controls.

Increase in seed productivity might have been associated with an increase in nutrient assimilation by the shrubs and a favorable nutrient redistribution among the plant parts. In accordance with this, paclobutrazol-treated shrubs had statistically significant increases of 6–36% ($F_{4,16} = 5.0$, $p = 0.008$), 23–57% ($F_{4,16} = 8.8$, $p = 0.001$), and 6–46% ($F_{4,16} = 30.26$, $p < 0.001$) in seed N%, P%, and K%, respectively, during the second year (Table 4). Significant enhancement ($F_{4,16} = 21.3$, $p < 0.001$) in total N uptake by the separate parts of the shrub (leaves, seeds, and capsule shells) was recorded during Year 2 in paclobutrazol-treated plants (Fig. 6).

Discussion

Growth suppression by paclobutrazol (PBZ) had been reported in earlier studies on other plant species (Berova and Zlatev 2000; Blaikie and others 2004; Asin and others 2007). Reduced cell proliferation due to PBZ treatment may be responsible for restricted shoot growth (Haughan and others 1989). The effect of growth regulators may persist in woody plants for several years after application.

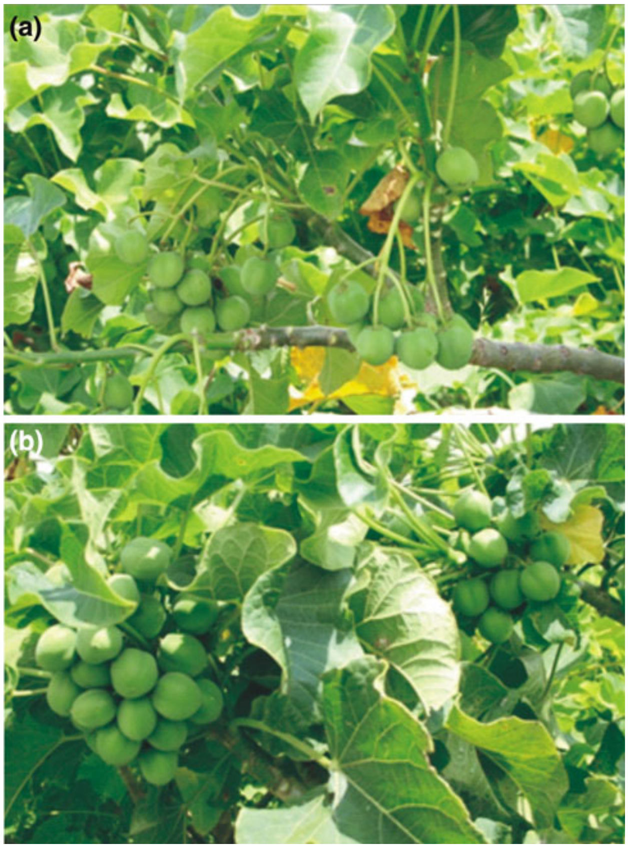

Fig. 3 Infructescence structure of a representative *Jatropha* at 375 days after treatment showing **a** fewer fruits in control shrubs and **b** compact bunch of more fruits in shrubs treated at 1.25-g a.i. paclobutrazol

It reportedly persisted up to 2–5 years in apple (Ma and others 1990), 1–3 years in peach (Erez 1986), and 1–2 years in apricot (Jacyna and others 1989) and citrus (Aron and others 1985). The restrictive effect of PBZ on shoot growth persisted up to 2 years in *Eucalyptus globulus* (Hasan and Reid 1995) and *Rhododendron catawbiense* and 3 years in *Kalmia latifolia* (Gent 1997). A one-time soil application of paclobutrazol and flurprimidol suppressed shoot elongation by 50–90% for up to 3 years after treatment in pecan *Carya illinoensis* (Wood 1988). In trials with apples on sandy loam soil, the effect of soil surface treatment at a rate of 2.0 g paclobutrazol m^{-2} was completely dissipated after 3 years. On heavier soils containing clay and organic matter, the effect persisted through the fourth and fifth seasons (Williams and others 1986).

The compact nature of *Jatropha* shrubs due to suppression of aboveground growth caused by paclobutrazol application may be extremely desirable for a variety of reasons, including ease of tending the shrubs, better penetration of sunlight into the canopy, and averting lodging or breaking of top-heavy shrubs in areas prone to strong winds. *Jatropha*, a fast-growing plant, requires pruning to keep a low harvestable height that will minimize harvest costs. In the present investigation, paclobutrazol application was effective in reducing the branch growth and the height of the plants, which may possibly reduce the necessity to prune the shrubs in the long run. Results of one of our other experiments (unpublished data) in which pruning was carried out on different *Jatropha* accessions revealed that moderate pruning (30 cm from top) arrested the increase in height of the shrubs. However, there was a consequential loss of seed yield (up to a 96.5% reduction relative to nonpruned shrubs) in the immediate fruiting season, even though *Jatropha curcas* bears fruits terminally and there was a noticeable increase in terminal branching due to pruning. Whether paclobutrazol application after timely pruning of shrubs could result in higher fruiting numbers in the immediate season would be an important aspect to investigate in prospective studies. Growth reduction following soil injection of paclobutrazol was reported to persist in red and silver maple growing under electric wires in Pennsylvania so that pruning was not required for 10 years. Estimated percent reduction in biomass over the 10 years from 1984 to 1994 was 85% for red maple and 88% for silver maple (Burch and others 1996). Previous investigations on different crops showed that the foliage of PBZ-treated plants typically exhibits an intense dark green color due to enhanced chlorophyll synthesis (Belakbir 1998; Sebastian and others 2002)

Table 3 Effects of paclobutrazol applied in year 1 on the flowering and fruiting behavior of *Jatropha* in year 2

Paclobutrazol (g m^{-1} of canopy diameter)	Male:female flower ratio	No. of fruited branches per shrub	No. of fruits set per infructescence	No. of capsules per shrub	Dry capsule yield (g $shrub^{-1}$)
None	$15.4^{a} \pm 0.9$	$5.2^{c} \pm 0.7$ (16.7)	$5.2^{c} \pm 0.5$	$42.4^{c} \pm 8.4$	$90.9^{c} \pm 20.6$
0.75	$12.8^{ab} \pm 1.6$	$19.2^{a} \pm 2.4$ (51.9)	$7.2^{bc} \pm 0.1$	$303.4^{a} \pm 22.5$	$737.8^{a} \pm 38.7$
1.00	$11.8^{b} \pm 1.1$	$13.0^{b} \pm 1.1$ (47.1)	$8.6^{ab} \pm 1.1$	$326.8^{a} \pm 51.7$	$768.0^{a} \pm 116.0$
1.25	$8.8^{c} \pm 1.7$	$13.6^{b} \pm 2.5$ (51.1)	$10.2^{a} \pm 1.7$	$380.6^{a} \pm 22.9$	$900.5^{a} \pm 63.3$
1.50	$11.3^{bc} \pm 0.9$	$9.4^{bc} \pm 2.7$ (40.2)	$9.2^{ab} \pm 0.9$	$196.6^{b} \pm 33.8$	$449.6^{b} \pm 79.4$

Different letters indicate that the values are significantly different at the $p < 0.05$ level. Values in parentheses are percent of fruit-bearing branches in shrubs

Table 4 Treatment effects of paclobutrazol on the fruiting behavior and physicochemical characteristics of seeds during year 2 (2007–2008)

Paclobutrazol (g m^{-1} of canopy diameter)	No. of capsules set before 15 October	No. of seeds $capsule^{-1}$	Weight $seed^{-1}$ (g)	% Kernel in seeds	% N in seeds	% P in seeds	% K in seeds
None	$20.4^{c} \pm 3.4$ (48.1)	$2.43^{b} \pm 0.07$	$0.468^{b} \pm 0.006$	$45.1^{b} \pm 1.6$	$1.71^{b} \pm 0.03$	$0.36^{c} \pm 0.01$	$1.94^{d} \pm 0.08$
0.75	$285.4^{a} \pm 16.5$ (94.1)	$2.78^{a} \pm 0.04$	$0.604^{a} \pm 0.019$	$56.9^{a} \pm 2.0$	$1.91^{b} \pm 0.04$	$0.44^{b} \pm 0.02$	$2.84^{a} \pm 0.05$
1.00	$308.4^{a} \pm 49.3$ (94.4)	$2.77^{a} \pm 0.03$	$0.576^{a} \pm 0.005$	$56.6^{a} \pm 1.6$	$1.89^{b} \pm 0.08$	$0.45^{b} \pm 0.05$	$2.33^{b} \pm 0.04$
1.25	$361.2^{a} \pm 17.8$ (94.9)	$2.79^{a} \pm 0.03$	$0.574^{a} \pm 0.021$	$59.3^{a} \pm 0.9$	$2.33^{a} \pm 0.18$	$0.49^{b} \pm 0.01$	$2.18^{bc} \pm 0.08$
1.50	$154.6^{b} \pm 26.6$ (78.6)	$2.59^{ab} \pm 0.13$	$0.592^{a} \pm 0.019$	$55.8^{a} \pm 2.3$	$1.81^{b} \pm 0.10$	$0.57^{a} \pm 0.00$	$2.06b^{cd} \pm 0.02$

Different letters indicate that the values are significantly different at the $p < 0.05$ level. Values in parentheses are percent of capsules set before 15 October 2008

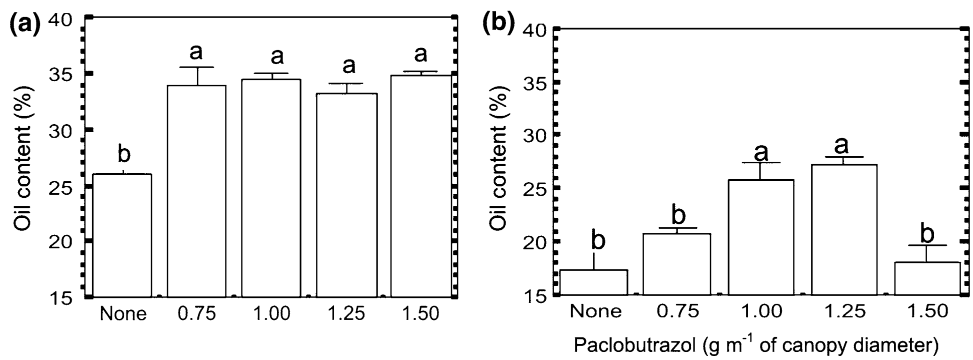

Fig. 4 Effect of paclobutrazol application on mean oil content (±SEM) of seeds harvested during Year 2. **a** Before 31 October. **b** After 31 October

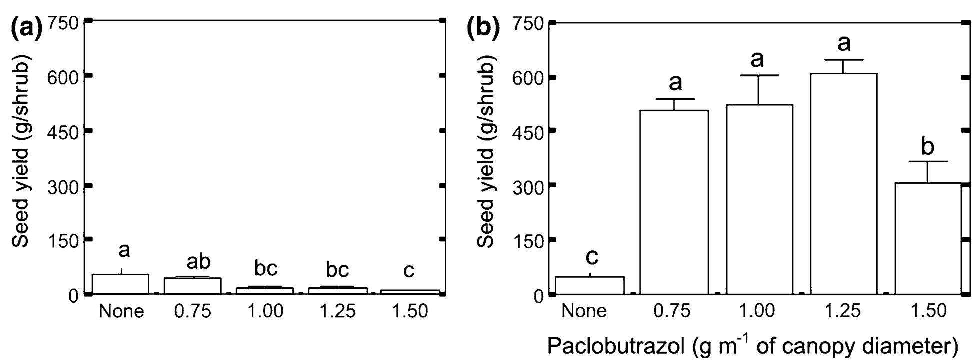

Fig. 5 Mean (± SEM) values of seed yield per shrub as affected by paclobutrazol application in **a** application year (2006–2007) and **b** subsequent year (2007–2008). Values with different letters are significantly different at the $p < 0.05$ level

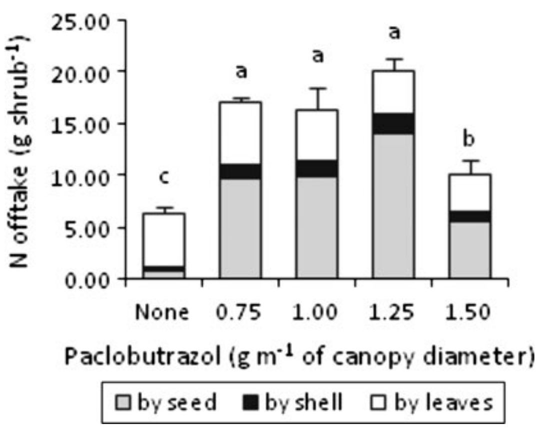

Fig. 6 Nitrogen uptake per shrub by different plant parts during year 2 as influenced by paclobutrazol treatments. *Error bar* indicates SEM for total N removal by annually separate plant parts (capsules and leaves)

and/or more densely packed chloroplasts per unit leaf area (Khalil 1995). A similar explanation also agrees with the increased chlorophyll *a* and *b* contents in the current investigation.

It was shown that the seed and oil productivity of *Jatropha* shrubs treated with paclobutrazol increased significantly. Paclobutrazol applied at 250 mg increased fruit yield and fruit number in alternate years for up to 4 years in apples (Khurshid and others 1997). In another study, 1-year-old plum, sour and sweet cherry, apricot, and pear were treated with a single application of paclobutrazol to the root collar tissue. Over 3 years this treatment reduced shoot growth of all five species, the fruiting of plum trees increased fourfold, and advanced flowering was clearly evident in pear trees (Grochowska and Hodun 1997). Evidently, the response of *Jatropha* to PBZ treatment was in Year 2 and, therefore, it is obvious that the proper dose should have been less than the lowest dose used, the optimization of which may be addressed in prospective studies along with scheduling.

Paclobutrazol [(2RS, 3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1H-1,2,4-triazol-1-yl) pentan-3-ol] retards growth by interfering with gibberellin biosyntheses by inhibiting the oxidation of ent-kaurene to ent-kaurenoic acid through inactivating cytochrome P450-dependent oxygenases (Hedden and Graebe 1985; Graebe 1987). It also simultaneously possesses florigenic properties (Asin and others 2007), which probably increased the flowering and fruiting in *Jatropha*. Enhanced productivity due to paclobutrazol has been reported earlier in several plant species (Senoo and Isoda 2003; Blaikie and others 2004; Asin and others 2007), but the magnitude of increase found in *Jatropha curcas* is comparatively much higher. Increased early fruiting due to paclobutrazol has been reported for few other plant species (SalazarGarcia and VazquezValdivia 1997; Berova and Zlatev 2000). Fruits formed later than the rainy season may experience a diminished supply of soil moisture and nutrients which may lead to poor uptake and seed filling, especially in unfertile soils with low water-holding capacity. In accordance with our presumption, we detected lower oil content in seeds harvested after October 2008. The variation in oil content, evident due to differences in the timing of harvesting, is of particular interest and it would be advantageous to advance the fruiting and harvesting season so as to obtain seeds with higher oil content.

The comparison between vegetative and reproductive behavior of control and treated shrubs indicates that paclobutrazol influenced the way biomass was partitioned among plant organs, probably by redistributing assimilates and directing the majority of assimilates toward reproductive growth. A much higher percentage of total nitrogen was distributed to the capsules in the treated shrubs. It is evident from Fig. 6 that the higher N content observed in treated shrubs (Table 4) was predominantly due to significantly enhanced total N uptake by the *Jatropha* plant parts. Paclobutrazol application resulted in greater export of N compounds to reproductive parts of the plant, as shown by the increase in its concentration in the seeds and uptake thereof. The increase in stored N compounds may explain the beneficial effects of paclobutrazol on fruit set and fruit growth (Blanco 1990). Due to the paucity of arable land, *Jatropha* cultivation is being emphasized only on wastelands which have innately poor soil fertility. Our results suggest that large quantities of plant nutrients are removed annually by *Jatropha*, even when the plantations are immature, and biomass detached in the form of shells, seeds, and leaves is comparatively less in juvenile stages. A considerable fraction of available soil nutrients (up to 23:5:24 N:P:K kg ha^{-1}) is removed by seeds only, and the nutrient exodus will be enormous once the plantations are mature and higher productivity is achieved, leading to further degradation of the lands, contrary to the stated objective of reclaiming wastelands. Nutrient recycling by plowing back the de-oiled cake containing most of the lost nutrients is being suggested to maintain soil fertility. Oil extraction or biodiesel production, if done in a decentralized manner at the farm level, would best achieve this objective as then cake can be utilized directly and cost-effectively as manure in the *Jatropha* plantation itself (Ghosh and others 2007).

The initial unambiguous findings from this study should enable researchers of various disciplines to look into the problem of low productivity of this species with an entirely new perspective by relating seed yield to endogenous hormones, the levels of which can be regulated. This finding provides a technique to induce desired physiological effects in *Jatropha* shrubs that are advantageous to realizing the maximum oil potential of this hitherto unexplored plant species. Future work could emphasize understanding the phenomenon behind differential flowering and fruiting behavior among various accessions and their response to different internal hormonal configurations affected by similar or different compounds.

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References

AOAC (Association of Official Analytical Chemists) (1970) Official methods of analysis. AOAC, Washington, DC

Aron Y, Monselise SP, Goren R, Costo J (1985) Chemical control of vegetative growth in citrus trees by paclobutrazol. HortScience 20:96–98

Asin L, Alegre S, Montserrat R (2007) Effect of paclobutrazol, prohexadione-Ca, deficit irrigation, summer pruning and root pruning on shoot growth, yield, and return bloom, in a 'Blanquilla' pear orchard. Sci Hortic 113:142–148

Belakbir A (1998) Yield and fruit quality of pepper (*Capsicum annum* L.) in response to bioregulators. HortScience 33:85–87

Berova M, Zlatev Z (2000) Physiological response and yield of paclobutrazol treated tomato plants (*Lycopersicon esculentum* Mill.). Plant Growth Regul 30:117–123

Blaikie SJ, Kulkarni VJ, Muller WJ (2004) Effects of morphactin and paclobutrazol flowering treatments on shoot and root phenology in mango cv. Kensington Pride. Sci Hortic 101:51–68

Blanco A (1990) Effects of paclobutrazol and of ethephon on cropping and vegetative growth of "Crimson Gold" nectarine trees. Sci Hortic 42:65–73

Burch PL, Wells RH, Kline WL III (1996) Red maple and silver maple growth evaluated 10 years after application of paclobutrazol tree growth regulator. J Arboric 22:61–66

Day PR (1965) Particle fraction and particle size analysis. In: Black CA et al (eds) Methods of soil analysis part 1. ASA, Madison, pp 545–567

Erez A (1986) Growth control with paclobutrazol of peaches grown in meadow orchard system. Acta Hortic 160:217–224

Fairless D (2007) Biofuel: the little shrub that could—maybe. Nature 449:652–655

Gent MPN (1997) Persistence of triazole growth retardants on stem elongation of *Rhododendron* and *Kalmia*. J Plant Growth Regul 16:197–203

Ghosh A, Chaudhary DR, Reddy MP, Rao SN, Chikara J, Pandya JB, Patolia JS, Gandhi MR, Adimurthy S, Vaghela N, Mishra S, Rathod MR, Prakash AR, Shethia BD, Upadhyay SC, Balakrishna V, Prakash CR, Ghosh PK (2007) Prospects for jatropha methyl ester (biodiesel) in India. Int J Environ Stud 64:659–674

Graebe JE (1987) Gibberellin biosynthesis and control. Annu Rev Plant Physiol 38:419–465

Grochowska MJ, Hodun M (1997) The dwarfing effect of a single application of growth inhibitor to the root-stem connection—“the collar tissue”—of five species of fruit trees. J Hortic Sci 72:83–91

Hanway JJ, Heidel H (1952) Soil analysis methods as used in Iowa State College soil testing laboratory. Iowa Agric 57:1–31

Hasan O, Reid JB (1995) Reduction of generation time in Eucalyptus globules. Plant Growth Regul 17:53–60

Haughan PA, Burden RS, Lenton JR, Goad LJ (1989) Inhibition of celery cell growth and sterol biosynthesis by the enantiomers of paclobutrazol. Phytochemistry 28:781–787

Hedden P, Graebe JE (1985) Inhibition of gibberellin biosynthesis by paclobutrazol in cell-free homogenates of *Cucurbita maxima* endosperm and *Malus pumila* embryos. J Plant Growth Regul 4:111–122

Jackson ML (1973) Soil chemical analysis. Prentice Hall, New Delhi

Jacyna T, Sparrow SM, Dodds KG (1989) Paclobutrazol in managing mature cropping apricot trees. Acta Hortic 240:139–142

Jonschaap REE, Corre WJ, Bindraban PS, Brandenburg WA (2007) Claims and Facts on *Jatropha curcas* L., Report 158. Plant Research International B.V., Wageningen

Khalil IA (1995) Chlorophyll and carotenoid contents in cereals as affected by growth retardants of triazole series. Cereal Res Commun 23:183–189

Khurshid T, McNeil DL, Trought MCT, Hill GD (1997) The response of young ‘Braeburn’ and ‘Oregon Spur Delicious’ apple trees growing under an ultra-high density planting system to soil-applied paclobutrazol: I. Effect on reproductive and vegetative growth. Sci Hortic 72:11–24

Kochhar S, Kochhar VK, Singh SP, Katiyar RS, Pushpangadan P (2005) Differential rooting and sprouting behaviour of two Jatropha species and associated physiological and biochemical changes. Curr Sci 89:936–939

Lichtenthaler HK, Wellburn AR (1983) Determinations of total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Biochem Soc Trans 11:591–592

Lindsay WL, Norvell WA (1978) Development of DTPA soil test for Zn, Fe, Mn, and Cu. Soil Sci Soc Am J 42:421–428

Ma FW, Wang JC, Rong W (1990) Effect of plant growth regulators on in vitro propagation of apple cultivar Fuji. J Fruit Sci 7:201–206

Olsen SR, Cole CV, Watanabe FS, Dean LA (1954) Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular 939. U.S. GPO, Washington, DC

Richards LA (1954) Diagnosis and improvement of saline alkali soils, handbook no. 60. USDA, Washington, DC

SalazarGarcia S, VazquezValdivia V (1997) Physiological persistence of paclobutrazol on the ‘Tommy Atkins’ mango (*Mangifera indica* L.) under rainfed conditions. J Hortic Sci 72:339–345

Sebastian B, Alberto G, Emilio AC, Jose AF, Juan AF (2002) Growth, development and color response of potted *Dianthus caryophyllus* cv. Mondriaan to paclobutrazol treatment. Sci Hortic 94:371–377

Senoo S, Isoda A (2003) Effects of paclobutrazol on dry matter distribution and yield in peanut. Plant Prod Sci 6:90–94

Subbiah BV, Asija GL (1956) A rapid procedure for the determination of available nitrogen in soils. Curr Sci 25:259–260

Walkley AJ, Black IA (1934) Estimation of soil organic carbon by chromic acid titration method. Soil Sci 37:29–38

Williams MW, Curry EA, Greene GM (1986) Chemical control of vegetative growth of pome and stone fruit trees with GA biosynthesis inhibitors. Acta Hortic 179:453–458

Wood BW (1988) Paclobutrazol, uniconazole, and flurprimidol influence shoot growth and nut yield of young pecan trees. HortScience 23:1026–1028