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Induction of Precocious Flowering in Hevea brasiliensis *by the Combined Effects of Girdling and Paclobutrazol*

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While girdling or paclobutrazol treatment could individually induce precocious flowering in Hevea brasiliensis, *a combination of both treatments gave rise to an enhanced effect. The supplementation of high nitrogen fertiliser to these treatments did not increase precocious flowering. Compared with the Wickham clones, clones from the 1981 Germplasm collection showed a recalcitrance to flower-induction probably because of their state of juvenility. Induced male flowers could be successfully used for hand-pollination, whereas induced female flowers tended to give poor fruit-set. Paclobutrazol treatment had a side-effect in causing 'bark burst' (splitting and flaking of the bark) in some of the Germplasm plants although this was rarely observed among the Wickham clones. Repeated girdling was observed to have a protective effect against bark burst.*

The juvenile phase in *Hevea brasiliensis* lasts four to five years during which time the young plant does not flower. This poses a constraint to the plant breeder's efforts to shorten the breeding cycle of the tree. The induction of flowering in young *Hevea* plants provides an avenue for the reduction in duration between generations. In addition, various benefits relating to hand-pollination may also be derived from early flowering in young rubber plants. Among these are the convenience of hand-pollination from the ground (*i.e.* without having to climb full-grown trees), more effective pathogen control leading to better fruit-set and an extended duration of flowering which may help to overcome non-synchrony in flowering among clones. As induced flowering is not strictly confined to the two annual flowering seasons, it might be possible to carry out year-round hand-pollination, thus enabling greater flexibility in the deployment of field workers.

Trunk girdling[1-4] as a means of flower-induction in *Hevea* has been experimented on since the 1950s and has received renewed attention at the RRIM in the 1970s. More recently, treatment with paclobutrazol has been found to be an effective alternative method of flower-induction[5]. In this paper, the induction of precocious flowering in *Hevea* by girdling and paclobutrazol is examined. The study is based on three field trials, which were designed to evaluate the individual and combined effects of girdling and paclobutrazol on the induction of precocious flowering. In addition to this principal objective, *Trial 1* compared flower-induction in the Wickham clones with the more recently acquired Brazilian Germplasm clones. *Trial 2* sought to examine the effect of paclobutrazol dosage on flower-induction while *Trial 3* evaluated the effect of supplementing high levels of nitrogen fertiliser.

MATERIALS AND METHODS

Planting Materials and Flower-induction

Trial 1. Buddings of 377 plants from 19 clones from the 1981 Brazilian Germplasm collection and 528 plants from 25 Wickham clones were planted at 2 × 2 m spacing. When

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sixteen months' old, the plants of each clone were assigned to three treatments: paclobutrazol, repeated girdling and a combination of both paclobutrazol and repeated girdling. Each clone represented an experimental 'block' containing one 'plot' of each of the three treatments. A total of 138 Germplasm plants were girdled, 156 treated with paclobutrazol while 83 plants received both treatments. For the Wickham clones, the figures were respectively 197, 227 and 104.

Girdling was carried out by excising a fine (2 mm) ring of bark from the trunk to the depth of the cambium. The process was repeated at one to two month intervals as the wound healed over. Treatment with paclobutrazol [(2RS 3RS)-1-(4-chlorophenyl)-4.4-dimethyl-2-(1H-1.2.4-triazol-1-yl)penton-3-ol)] was by soil drench with 6 g a.i. applied per plant in two split doses of 3 g a.i. spaced three months apart. For each application, the compound was diluted with water such that 1 litre applied per plant contained the desired amount of active ingredient.

Trial 2. Plants of RRIM 921 and RRIM 922 were planted out at 3 × 3 m spacing and were treated to induce flowering when they were twenty-one months' old. A 'split plot' design with two replicates per clone was adopted. Each main plot had 'girdled' and 'ungirdled' sub-plots in which each plant received soil drenches of 0, 2.5 or 5.0 g a.i. paclobutrazol applied in two split doses 1.5 months apart. Girdling was carried out as in *Trial 1*. There were fourteen RRIM 921 and sixteen RRIM 922 plants per treatment. Preliminary statistical analysis indicated that the two clones (which are full sibs) responded similarly to the treatments with no significant clone-treatment interactions. Hence, the plants from the two clones were combined to give thirty plants per treatment for the final analyses.

Trial 3. Treatments to induce flowering in RRIM 901 plants, planted at 3 × 3 m spacing, commenced when the plants were fifteen months' old. There were three replicate blocks comprising 'normal nitrogen' and 'high nitrogen' sub-blocks. These sub-blocks were separated by two guard rows of plants to minimise fertiliser poaching effects. Manuring treatments commenced three months from planting and consisted of normal nitrogen application (285 g N per plant) and high nitrogen application (820 g N per plant) applied over eleven rounds in twenty-seven months. Basal dressings of P_2O_5 (230 g per plant), K_2O (260 g per plant) and MgO (80 g per plant) were also applied uniformly over the same frequency and duration.

Each sub-block consisted of 'girdled' and 'ungirdled' plots and each plot was divided into two sub-plots that received no paclobutrazol or soil drenching of 2 g paclobutrazol a.i. per plant in two split doses spaced three weeks apart. Girdling was carried out as in *Trial 1*.

Observations on Flowering

Flowering was most intense during the two annual flowering seasons (the main season in March/April and the secondary season in September/October). Although monthly observations on flowering were from the time when the treatments were applied, the major assessment of the responses to the flower-induction treatments was only made during the flowering season when the best treatment first induced flowering in more than 50% of the plants. The time taken to reach this stage varied among the three trials. In *Trial 1*, the main analysis was made during the secondary flowering season, thirteen months from the commencement of the treatments. In *Trials 2* and *3*, observations were made four to five months from the commencement of treatments (corresponding with the secondary flowering season) and again five months later (corresponding with the main flowering season).

RESULTS AND DISCUSSION

Trial 1. Effect of Girdling and Paclobutrazol on Germplasm and Wickham Clones

Induction of precocious flowering. It was observed that the Wickham clones were much more responsive to flower-induction than the Germplasm clones. Thirteen months after the commencement of flower-induction treatments, flowering had been observed in twenty-

four out of twenty-five Wickham clones. On the other hand, only six out of a total of nineteen Germplasm clones had flowered by this time. The delayed flowering of the Germplasm clones could be due to their still being in the juvenile state as the original Germplasm plants (the source of budwood) had been propagated from seeds relatively recently. Although the experimental plants were buddings the buds from which they were derived were still juvenile in nature. The Wickham clones on the other hand, were not constrained by the problem of juvenility.

The number of plants of the Germplasm clones and the number that flowered are summarised in *Table 1*. Similar data for Wickham clones are given in *Table 2*. Because a proportion of Germplasm clones did not respond to flower-induction, the performance of the clones that were successfully induced to flower could be more meaningfully assessed by excluding the non-flowering clones. A scoring of individual flowering plants among the six Germplasm clones that responded to flowering-induction showed flowering in 9% of the plants that had been treated with paclobutrazol. Of the plants that were repeatedly girdled, 19% flowered while flowering was observed in 47% of the plants receiving both treatments (*Table 1*). Among the 24 Wickham clones that responded to flower-induction, flowering was observed in 31% of the plants treated with paclobutrazol, 30% of the plants that were repeatedly girdled and 57% of the plants that received both treatments (*Table 2*).

The results in both the Germplasm and Wickham plants showed therefore that plants that were subjected both to repeated girdling and paclobutrazol flowered far more readily

TABLE 1. SUMMARY OF FLOWERING IN GERMPLASM PLANTS ACCORDING TO THE METHOD OF FLOWER-INDUCTION IN *TRIAL 1*

	Paclobutrazol	Repeated girdling	Paclobutrazol + Repeated girdling
Number of plants	156 (66)	138 (59)	83 (32)
Number of plants that have flowered	6	11	15
Percentage of plants that have flowered	4 (9)	8 (19)	18 (47)

Figures not within brackets refer to plants of all clones, whereas figures within brackets refer only to plants of clones that have been successfully induced to flower.

TABLE 2. SUMMARY OF FLOWERING IN WICKHAM PLANTS ACCORDING TO THE METHOD OF FLOWER-INDUCTION IN *TRIAL 1*

	Paclobutrazol	Repeated girdling	Paclobutrazol + Repeated girdling
Number of plants	227 (216)	197 (197)	103 (103)
Number of plants that have flowered	67	60	59
Percentage of plants that have flowered	30 (31)	30 (30)	57 (57)

Figures not within brackets refer to plants of all clones, whereas figures within brackets refer only to plants of clones that have been successfully induced to flower.

than those that had received either one of the treatments separately. In five clones (two Germplasm clones and three Wickham clones), flowering was induced only when the plants were both girdled and supplied with paclobutrazol: flower-induction was unsuccessful when only one of the treatments was applied. The combined effect between the two treatments was especially prominent among the Germplasm clones where the application of both treatments resulted in more than twice as many flowering plants as when either treatment was applied singly (*Table 1*). Even in the case of the Wickham plants, an almost two-fold discrepancy was attained (*Table 2*).

A more detailed clone-by-clone statistical comparison of the three flower-induction treatments was made using data from fifteen clones (4 Germplasm and 11 Wickham) that had at least five plants per clone to score for flowering success. The results, presented in *Table 3*, again showed that clonal success in flower-induction by combination of paclobutrazol and girdling was more than twice as high as that by either treatment applied singly. The difference was significant at $P<0.001$. No significant difference was observed between girdling and paclobutrazol applied separately. The combined effect between girdling and paclobutrazol was confirmed in *Trials 2* and *3*.

Having observed that paclobutrazol and girdling had a combined effect in inducing flowers, plants that had received either one of the two treatments (paclobutrazol or repeated

TABLE 3. PERCENTAGE OF FLOWERING PLANTS IN CLONES THAT HAVE BEEN SUCCESSFULLLY INDUCED TO FLOWER ACCORDING TO THE METHOD OF FLOWER-INDUCTION: SEPTEMPER 1987

	Clone	Paclobutrazol	Repeated girdling	Paclobutrazol + Repeated girdling
1.	RRIM 905	39	61	100
2.	GP 22/392	50	90	71
3.	RRIM 728	57	46	83
4	GT 1	50	56	80
5	PB 217	50	17	83
6.	RRIM 916	44	0	100
7.	RRIM 712	27	18	40
8.	GP 22/250	8	0	67
9	RRIM 901	9	0	60
10.	GP 10/169	0	14	50
11.	RRIM 913	0	13	33
12.	RRIM 902	0	0	43
13.	GP 22/243	0	0	25
14.	RRIM 802	0	0	17
15.	PR 261	0	0	14
	Mean	22.0	20.5	55.2

Clones with fewer than 5 plants in any of the three treatments have been excluded.

girdling) were additionally given the other treatment.

The trends in cumulative proportion of plants that had flowered up till the next flowering season in Germplasm and Wickham clones, meaned for the different flower-induction treatments, are given in *Figure 1*. It was evident that flowering in plants that had received paclobutrazol alone showed a further marked increase in flowering from the time they were girdled, thus displaying the combined effect of paclobutrazol and girdling. This trend was observed both in Germplasm clones and Wickham clones (*Figure 1*). On the other hand, plants that had received the girdling treatment alone did not show a similar upsurge in flowering when the paclobutrazol treatment was subsequently supplemented (*Figure 1*). It is therefore essential that where plants are to be treated both with paclobutrazol and by girdling, paclobutrazol application must precede girdling.

Availability of induced flowers for hand-pollination. The flowers obtained for the purpose of hand-pollination are the *raison d'être* of flower-induction. The greater propensity to flowering in the Wickham clones as compared with the Germplasm clones has been mentioned. Within each group, there is further variation in the intensity of flowering between clones. As noted previously[5], the highest flowering intensities corresponded with the two flowering seasons of the year, although flowers were also available at other times of the year. This trend was shown by both the Germplasm and Wickham clones irrespective of the method of flower-induction.

At the peak of the main flowering season in February, twenty-one months from the commencement of flower-induction, subjected to the most effective treatment (girdling + paclobutrazol) an average of 88% of the Wickham plants were in flower and the figure remained above 80% from February to June.

Young *Hevea* plants tend to branch sparsely. Flowering can therefore be increased in the plant by inducing multiple branching through pollarding. If this is carried out, new branching must be allowed to establish before treatment with paclobutrazol. Once the chemical is applied, branching is drastically inhibited. Another important consideration in implementing pollarding is the juvenility factor in the new outgrowth of shoots. This is especially important in the juvenile plants where the lateral buds close to the base of the stem are more juvenile in character than those more acropetally situated. In such instances, heavy pollarding tends to sustain juvenility by enhancing the development of the juvenile meristem: flowering can hence be delayed.

Fruit-set. Small scale hand-pollinations were carried out during the secondary flowering season (when the plants were about twenty-nine months' old) and the main flowering season five months later. Both induced male and female flowers were employed.

During the secondary flowering season, 1117 hand-pollinations involving nineteen crosses were completed using *induced male flowers* crossed with female flowers located on regular mature trees. In the main flowering season that followed, 1822 similar hand-pollinations involving nine crosses were carried out. Fruit-set in the female flowers on the mature trees for the two seasons were respectively 46.5 and 19.8 fruits per thousand pollinations. This success rate was comparable to that expected of ordianary hand-pollination on (uninduced) mature trees for the secondary and main flowering seasons. As a rough guide for comparison, an average fruit-set of 15.1 fruits per thousand pollinations was obtained for regular hand-pollinations (involving only flowers from mature trees) carried out by the RRIM in its breeding programme during the same main flowering season.

A total of 869 hand-pollinations involving thirteen crosses were carried out in the two flowering seasons using *induced female flowers*. None of the induced female flowers that were hand-pollinated set fruit. Nevertheless, numerous fruits that had arisen from natural (free) pollination were observed.

The observations suggest that while fruit-set in the induced plants was possible, the rate

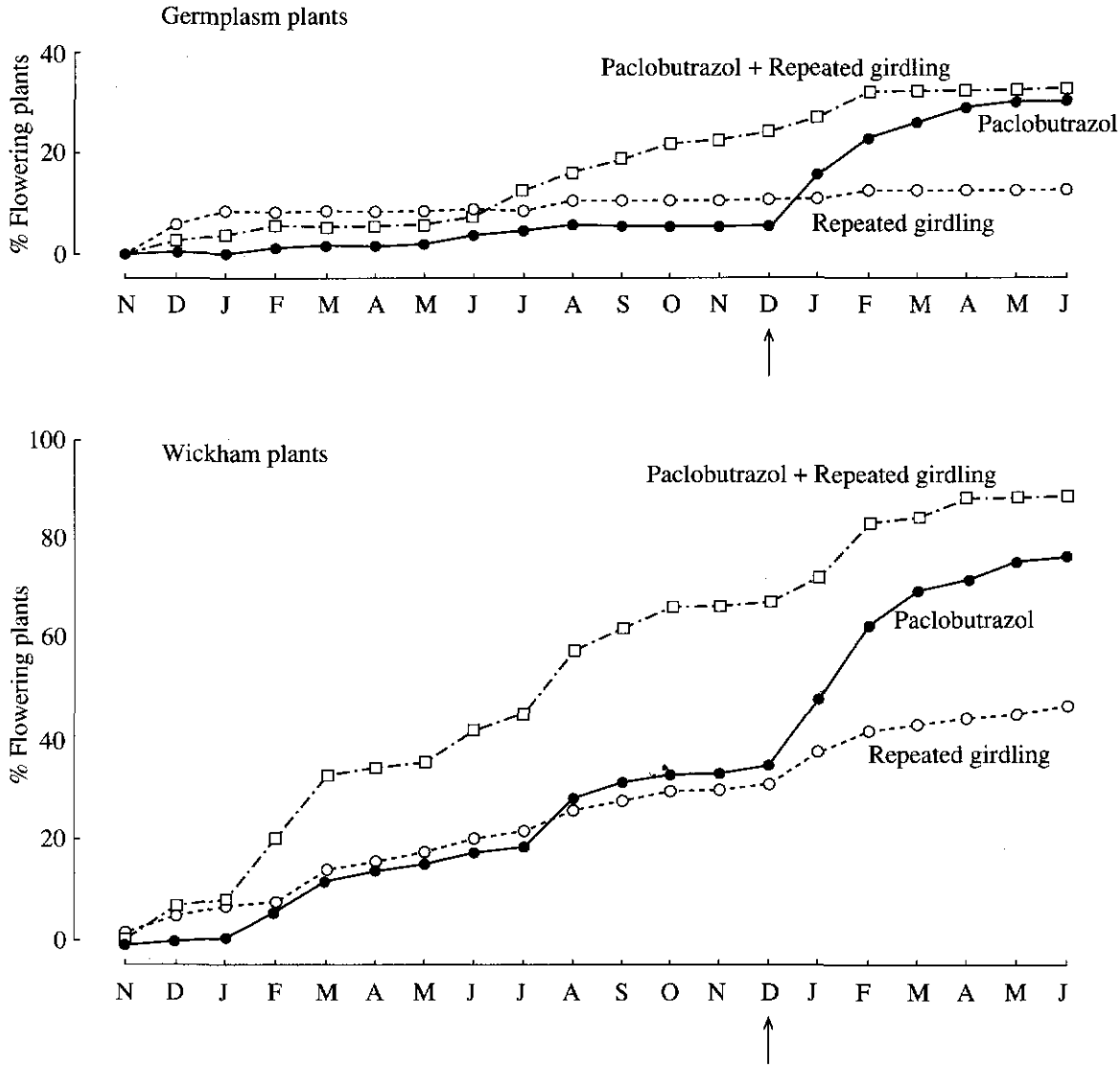

Figure 1. Induction of flowering in Germplasm plants and Wickham plants. Data represent cumulative proportion of flowering plants according to flower-induction treatments that were applied 3 months prior to the first reading (November) presented. Percentages are calculated for each clone and then meaned to give equal weightage to the different clones. Plants that initially received only girdling treatment were additionally treated with paclobutrazol at the time indicated by the arrow. Similarly, plants that initially received only paclobutrazol treatment were additionally girdled at the time indicated by the arrow.

of success is likely to be very poor. Hence, induced female flowers are not recommended for hand-pollination whereas male flowers can be gainfully exploited for this purpose.

Plant casualties. A number of the plants in the trial were afflicted with die-back and bark burst. Die-back occurred only in the girdled plants while bark burst (splitting and flaking of the bark) was observed only in plants that were treated with paclobutrazol (*Figure 2*). The two disorders were probably physiological in origin, arising from girdling stress and reaction to paclobutrazol respectively.

While incidences of die-back were confined to girdled plants, the afflictions were more severe when the plants were additionally treated with paclobutrazol. However, paclobutrazol treatment alone did not induce die-back. The affliction was severe among the Germplasm clones. A survey in early 1988 showed an incidence of 19% in plants that were girdled only, with the figure rising significantly ($P<0.05$) to 35% when paclobutrazol treatment was supplemented. Fortunately, among the Wickham clones, the incidence of die-back was much lower, being 4% and 6% respectively in girdled plants and girdled plants treated with paclobutrazol.

Bark burst *(Figure 2)* is a reaction to paclobutrazol that has not previously been reported in *Hevea*. Again, it was the Germplasm clones that were highly susceptible to the disorder. Bark burst was observed in 63% of Germplasm clones compared with 12% in the Wickham clones, the difference being significant at $P<0.01$. A similar discrepancy ($P<0.1$) was seen when plants from only the affected clones were scored. Ungirdled Germplasm plants had a 25% incidence of bark burst while only 8% of the ungirdled Wickham plants were similarly afflicted.

Girdled plants appeared to be resistant to bark burst. Combining scores of paclobutrazol-treated plants from the affected Germplasm and Wickham clones, only one out of 74 girdled plants suffered bark burst. On the other hand, thirty ungirdled plants out of 147 (20%) were so afflicted. Hence, girdling as a method of flower-induction had an ancillary function when used in conjunction with paclobutrazol in that it appeared to protect plants from bark burst induced by paclobutrazol. In the long run, nevertheless, it might be prudent to decrease the dosage of paclobutrazol and perhaps also the severity of girdling for flower-induction to minimise the incidence of die-bark or bark

Figure 2. Bark burst in a plant from the Germplasm collection treated with paclobutrazol.

burst. Otherwise, these afflictions are likely to become increasingly serious with time.

Bark burst was not observed in *Trials 2* and *3* where lower dosages of paclobutrazol were applied to Wickham clones.

Trial 2: Effect of Paclobutrazol Dosage on Precocious Flowering

In view of incidences of bark burst and (in conjunction with girdling) die-bark sometimes observed with paclobutrazol usage in *Trial 1*, a study was carried out to determine if flower-induction was effective with reduced paclobutrazol application. Paclobutrazol was applied at the rate of 0, 2.5 or 5 g per plant with or without girdling.

The results of the trial are given in *Figure 3*. During the secondary flowering season, barely four months after flower-induction treatments commenced, girdling was observed to be

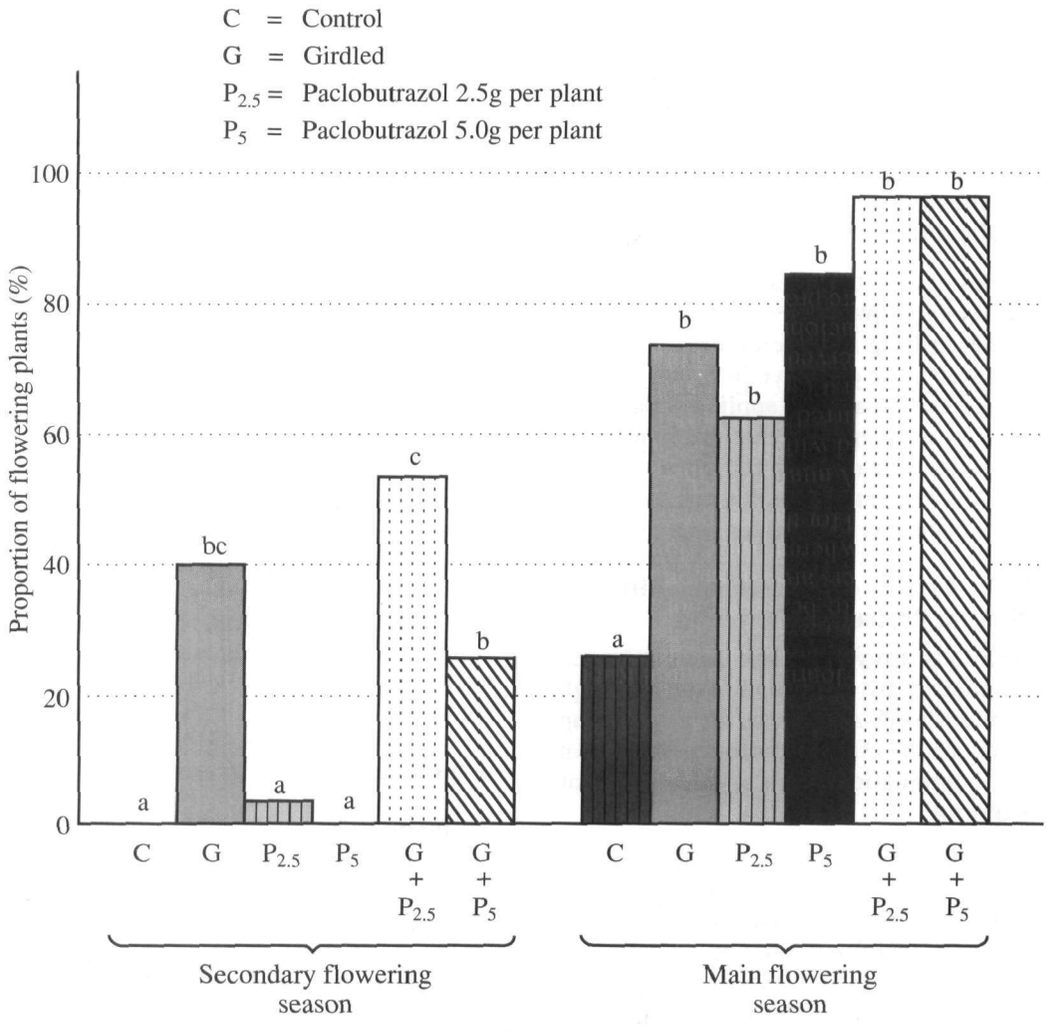

Figure 3. Effect of girdling and two dosages of paclobutrazol on the induction of precocious flowering in two flowering seasons. Values in each season bearing the same letter are not significantly different at $P<0.05$.

effective in inducing flowering. With this treatment, 40% of the plants flowered. The best response, with 54% flowering plants, was when girdling was complemented by paclobutrazol applied at the rate of 2.5 g per plant. The higher rate of paclobutrazol application (5 g per plant) gave poorer response.

Five months later during the main flowering season, all treatments showed good flowering response, with every flower-induction treatment attaining over 60% flowering plants. Even a fair proportion of the untreated control plants flowered. The best treatments were the ones which combined girdling with paclobutrazol. There was no difference between the two dosages applied, 2.5 g or 5 g per plant. Girdling and paclobutrazol applied separately though showing lower responses, were not statistically different from the best treatments.

The results of this trial were consistent with the observation in *Trial 1* that the treatment with girdling and paclobutrazol applied together was more effective in flower-induction than either treatment applied separately. This study also showed that paclobutrazol applied at the rate of 2.5 g per plant was not less effective than at double this rate. A dosage of 2 g per plant was adopted for *Trial 3*.

Trial 3. Effect of Increased Nitrogen Fertiliser on Precocious Flowering

Precocious flowering has been reported to be induced by the application of high nitrogen fertiliser[6]. A trial was therefore set up to investigate the effect of nitrogen on its own and in combination with girdling and/or paclobutrazol.

After five months of treatment, the best flowering was seen in plants that received paclobutrazol *(Figure 4)*. The best treatments were where paclobutrazol application was combined with girdling. In the secondary flowering season that followed, the efficacy of paclobutrazol with girdling was maintained, but high nitrogen and girdling (in the absence of paclobutrazol) were also showing positive responses.

The results of this trial were again consistent with the earlier observations that paclobutrazol and girdling had a combined effect in the induction of precocious flowering. High nitrogen level on its own had a limited effect on flower-induction but did not add much further to the effects already attained with girdling plus paclobutrazol. Nevertheless, it must be appreciated that the enhancing influence of extra nitrogen on flowering is a relatively slow and gradual process, usually requiring some thirty months before response becomes manifested, as observed in other studies[6]. In the present investigation, the extra nitrogen manuring treatment had been carried out for just seventeen months at the time of flowering observation, and possibly its effect on flowering had yet to be expressed.

CONCLUSIONS

The main findings from observations made in the three trials are as follows.

Methods of Precocious Flower-induction

While repeated girdling or paclobutrazol treatment individually induced precocious flowering in *Hevea*, a combination of both treatments gave rise to a further enhanced effect. A suitable paclobutrazol dosage for *Hevea* plants of about fifteen months' old is 2 g a.i. per plant applied as a soil drench. Paclobutrazol treatment should precede the commencement of repeated girdling. High nitrogen fertiliser application on its own had some effect in flower-induction, but did not add to the effect achieved by the combination of girdling and paclobutrazol.

Response to Precocious Flower-induction

A wide variety of *Hevea* clones is amenable to precocious flower-induction. Clonal differences can be expected in the degree and earliness of the response. Clones from the Germplasm collection showed a recalcitrance to flower-induction. The most probable explanation is that the plants had not yet outgrown their juvenile phase. In the same manner, induction of precocious flowering is likely to be problematic in young seedlings or young

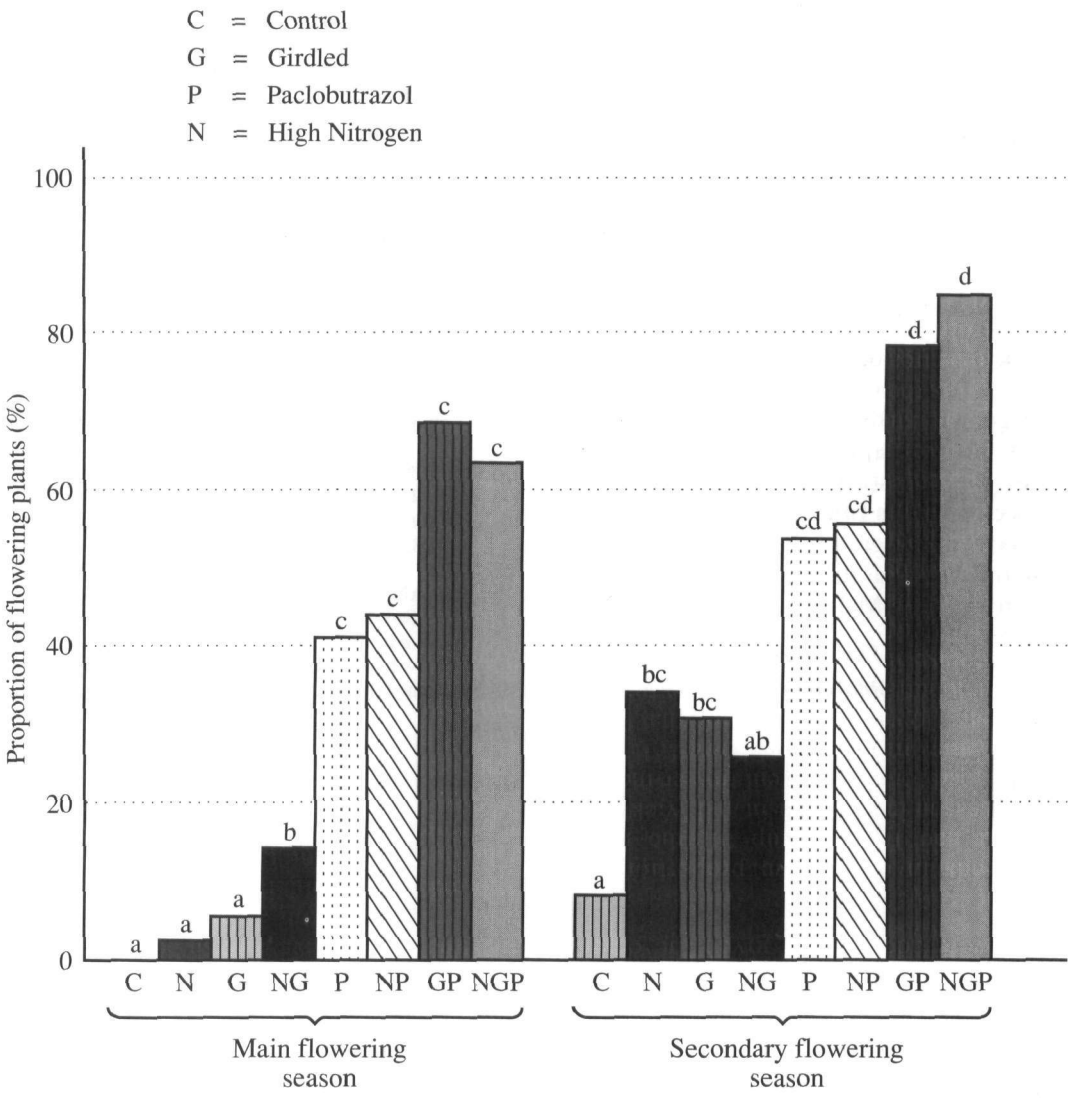

Figure 4. Effect of girdling, paclobutrazol and high nitrogen fertiliser on the induction of precocious flowering in two flowering seasons. Values in each season bearing the same letter are not significantly different at $P<0.05$.

buddings derived from seedlings that are still in their juvenile phase of development.

Use of Induced Flowers for Hand-pollination

Induced *male* flowers used in the hand-pollination of female flowers on mature trees gave fruit-set comparable to male flowers harvested from mature trees. On the other hand, induced *female* flowers set fruit poorly when hand-pollinated. Therefore, induced male flowers are useful for hand-pollination whereas female flowers are not.

Adverse Effects of Flower-induction

Paclobutrazol caused bark burst in some of the Germplasm plants but this malady was rare in the Wickham plants. Repeated girdling protected paclobutrazol-treated plants against bark burst. However, girdling itself tended to cause some dieback, this again being confined largely to Germplasm clones.

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