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Induction and Control of Flowering in Hevea

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Unlike juvenile seedlings, young buddings can be induced to form flowers by stem girdling. The problem of die-back, frequently encountered in girdled trees, is overcome when fine girdles of 1.5 mm width are used to induce stress. Stress is maintained by repeated girdling at monthly intervals. 'Pinching' out apical buds is useful in increasing floral shoot formation in girdled trees. Growth substances generally are ineffective in hastening flowering, even when applied to girdled trees. Bending and root pruning were also ineffective. Clonal differences in amenability to floral induction are evident. Branches on source bushes are easily induced to flower by girdling. A preliminary study of a method of establishing cuttings from mature trees is described and the precocious flowering observed in these grafted plants is noted.

The growth and development of trees of *Hevea brasiliensis*, and of other species in the same genus, are characterised by an initial phase of purely vegetative growth, lasting usually from four to five years, before the onset of flowering. The transition from the vegetative to a reproductive phase is a well recognised feature of perennial tree species. This phenomenon of phase change has been the subject of numerous physiological investigations, partly at least in recognition of the very enormous increase in scope of plant breeding that can be achieved by induction and control of flowering in both juvenile and mature trees.

Trees of *Hevea brasiliensis* in Malaysia flower twice a year, usually between March to April and between August to September. The long juvenile period of non-flowering and the short periodic duration of flowering can be an impediment in plant breeding. The long juvenile duration also delays the incorporation of clones obtained from foreign rubber producing countries into locally existing germplasm. The lack of synchronisation between parent clones can sometimes be an impediment, particularly since satisfactory methods of pollen storage are lacking. Finally, the control of fungal pathogens and insects is an integral part of *Hevea* hand pollination programmes. Besides the convenience of hand pollination from the ground and reduction of the non-flowering juvenile period, it is conceivable that difficulties encountered in disease control in mature trees conventionally used in hand pollinations would be more easily overcome with small plants.

Several attempts have been made at induction of flowering in juvenile *Hevea* plants, no doubt in recognition of possible uses outlined above. Campaignolle and Bouthillon[1] reported that young *Hevea* could be induced to flower by gravimorphic and ring-barking treatments. These results were confirmed by de Silva and Chandrasekera[2], who in addition reported that photoperiodic induction methods were tried with inconclusive results. Camacho and Jimenez[3] reported that flowering was hastened when stress treatments such as bending and ring-barking were carried out in conjunction with foliar application of tri-iodobenzoic acid and coumarin. The more recent investigations of Ong[4] have confirmed the necessity of ring-barking for induction of flowering in juvenile *Hevea* plants. Tri-iodobenzoic acid, however, was found to be ineffective. On the other hand, coumarin and RH 531 were reported to enhance flowering in ring-barked trees.

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Ring-barking, as carried out in the investigation, appears to be important for induction of flowering in juvenile *Hevea*, but this ultimately results in serious die-back as shown in this paper. We have therefore 'screened' a variety of methods with the aim of evaluating the feasibility of these methods in practical plant breeding. In particular, attempts have been made to apply stress without causing die-back.

MATERIALS AND METHODS

The experiments were carried out in the Rubber Research Institute of Malaysia Experiment Station in Sungei Buloh. Experiments on girdling were carried out on budding and seedlings planted 0.9 × 2.4 m apart in a nursery. Root pruning experiments were carried out, on buddings planted 3.7 × 7.6 m apart.

A variety of stress treatments were used in attempts to induce flowering. They include

- Girdling – this refers to the removal of a ring of bark around the stem 15 – 20 cm above soil level. The width of bark removed varied from 1.5 mm to 5 centimetres. Due to wound healing it was found necessary to repeat the 1.5 mm girdles at monthly intervals, the girdles being spaced about 5 cm apart *(Figure 1)*. It was possible to avoid die-back while at the same time subjecting the trees to almost continual stress using 1.5 mm girdles. A girdling device[5] was used for fine girdling.

- Bending – this was carried out with the aid of 3 mm thick twine tied to wooden pegs in soil, the upper half of the stem being kept horizontal by this means.

- Root pruning – this refers to pruning of lateral or tap roots. The root system was first exposed up to a depth of 50 cm and about the same distance laterally. Roots were pruned with a saw and a dressing of 'Fomac 2' (containing quintozene PCNB) was brushed liberally onto the cut surfaces to prevent fungal infections.

A variety of growth substances were also used in attempts to hasten flowering. For foliar application, all chemicals were dissolved in a small volume of alcohol and diluted as required with distilled water. The final concentration of ethanol in the spray was 2% (volume/volume). The chemicals were sprayed onto the leaves of plants at weekly intervals on four occasions, using a hand sprayer. For application onto roots, growth substances were dissolved in a small volume of ethanol and formulated in 'Fomac 2' upto the required concentrations. The roots were lightly scraped and the formulations were applied by brush.

The method of 'seedling-grafting; previously described in brief[6], was a modification of methods developed initially by Pakianathan[7]. Essentially this was done by first cutting off the epicotyl of two- to three-week-old seedlings 2 cm above the cotyledons and grafting the cut end of the remaining seedling onto an exposed piece of cambium on the shoot of a mature tree. The graft was secured in a mixture of soil and sawdust (1:1) with polythene *(Figure 2)* and kept moist by periodic watering. The grafted shoots were excised 15 cm from the graft union and transferred to polybags, which were maintained under shade and mist. Ooi[8] has used similar methods of grafting on source bushes for propagation of *Hevea.*

Treated plants were observed at weekly intervals. In all experiments, the numbers of plants flowering were recorded. Developing fruits (arising from open pollination) were 'bagged' in nylon pockets and the

Figure 1. An eighteen-month-old budding girdled repeatedly at monthly intervals. The 1.5 mm girdles are spaced 5 cm apart, the first girdle being the lowermost.

seeds were collected for germination tests after the fruits had dehisced. Seed germination was tested in nursery beds under shade.

Ethephon was obtained from Amchem Products Inc., while the other growth substances were obtained from Sigma Chemical Company.

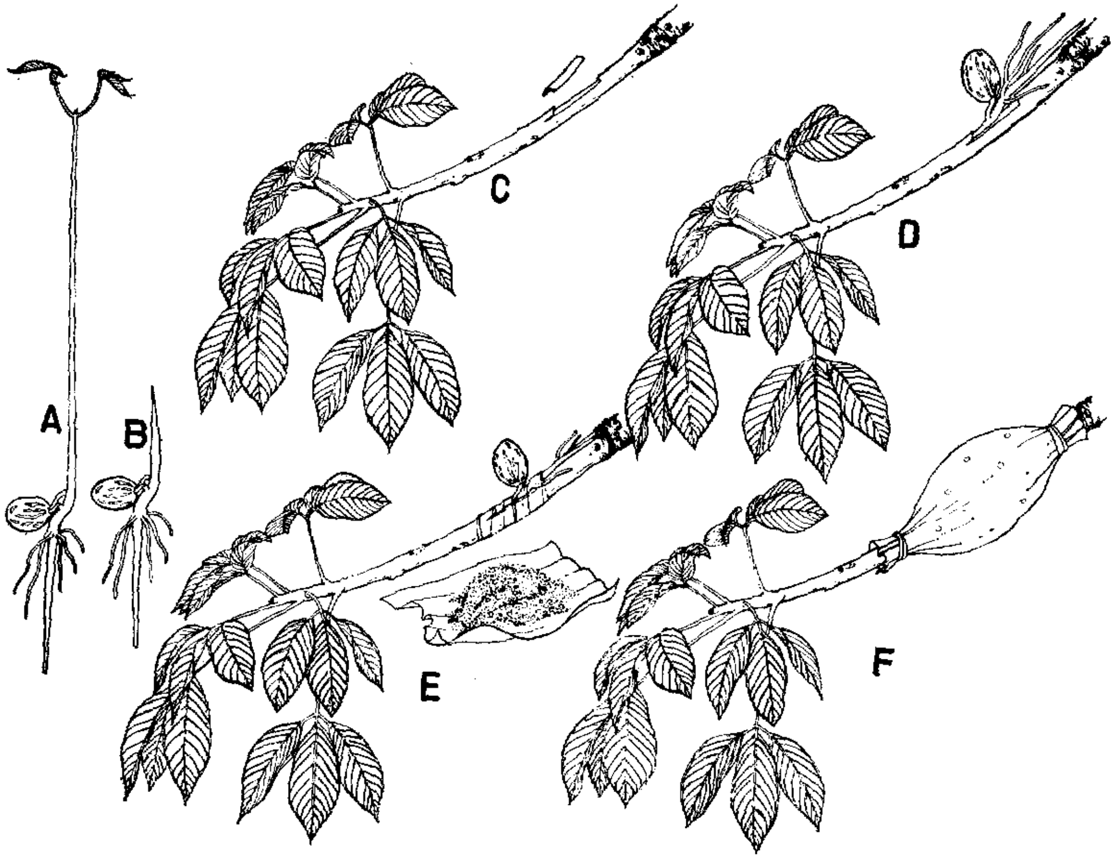

Figure 2. Stages in seedling-grafting. Shoots were selected from ten-year-old mature trees. A : Two-week-old seedling ; B : Seedling after excision of epicotyl ; C : Shoot prepared to receive seedling-graft ; D : Seedling grafted onto shoot ; E : Seedling-graft secured with polythene tape ; F : Graft wrapped up in sawdust with polythene.

RESULTS

Flower Induction in Young Buddings and Seedlings

Eighteen-month-old buddings and seedlings of RRIM 600 and PB 5/51 were girdled approximately 15–20 cm above soil level by removal of a 5 cm ring of bark. Two trees from each clone and two seedlings were used for treatments indicated in *Table 1*. A variety of nucleic acid base analogues, in addition to the naturally occurring inhibitor abscisic acid, were tested for their efficiency in induction of flowering against tri-iodobenzoic acid as control, which at the time the experiments were initiated was known to be effective in enhancing flower formation in girdled trees[4].

TABLE 1. FLOWERING IN GIRDLED EIGHTEEN-MONTH-OLD SEEDLINGS AND RRIM 600 AND PB 5/51 BUDDINGS AS OBSERVED FOURTEEN MONTHS AFTER GIRDLING

Treatment	Number of trees flowered[a]		
	RRIM 600	PB 5/51	Seedling (unselected)
Control I: Ungirdled	–	–	–
Control II: Girdled (5 cm) + tri-iodobenzoic acid (1000 p.p.m.)	2	2	–
Girdled (5 cm) + Abscisic acid (50 p.p.m.)	2	1	–
Girdled (5 cm) + 2-Thiouracil (100 p.p.m.)	–	–	–
Girdled (5 cm) + 4,5-diamino 2-thiopyrimidine (100 p.p.m.)	–	–	–
Girdled (5 cm) – 6-mercaptopurine (100 p.p.m.)	–	1	–
Girdled (5 cm) + 8-azaguanine (100 p.p.m.)	1	1	–
Girdled (5 cm) + 6-azauracil (100 p.p.m.)	1	1	–
Girdled (5 cm) + 5-flourouracil (100 p.p.m.)	1	1	–

[a]Number of replicates per treatment = 2.

Flowering was first observed in buddings in October, fourteen months after girdling and chemical treatment as indicated in *Table 1*. These observations indicated that none of the nucleic acid base analogues were as effective as tri-iodobenzoic acid treated trees. None of the trees treated with thiouracil produced flowers. Trees treated with the nucleic acid base analogues were visibly retarded in growth and it did not appear from this that retardation of vegetative growth could lead directly to flowering. None of the seedlings, treated in the experiment flowered.

In trees that flowered the leaves had a characteristic yellowish-green colour. This, however, did not resemble the colouration seen in leaves in mature trees prior to wintering. Neither was leaf-fall evident in the young buddings before flowering. Between the two clones studied, flowering was generally more profuse in PB 5/51. Fruit development was periodically seen in almost all the trees that flowered *(Figure 3)*. Severe die-back, often leading to tree death, was observed in the girdled trees eighteen months after commencement of flowering. Tree death was partly inevitable as new shoots developing below the girdle were routinely pruned off.

In efforts to prevent die-back, the use of a much narrower girdle, 1.5 mm wide, was investigated. It was found that healing was almost complete at the end of one month and the trees were therefore girdled again, 5 cm above the first girdle. Girdling was continued at monthly intervals.

Repeated 1.5 mm girdling was compared with 5 cm girdling, using eighteen-month-old buddings of RRIM 600, PB 5/51, RRIM 623 and PB 260. Observations made four to nine months after initial stress indicated that repeated fine-girdling induced flowering and subsequently fruit formation *(Table 2)*. Flowering in the repeatedly girdled trees was not accompanied by severe branch die-back as seen in trees that had been stressed with 5 cm girdling. On the other hand, flowering was more profuse and continuous in the severely girdled trees. Removal of the apical

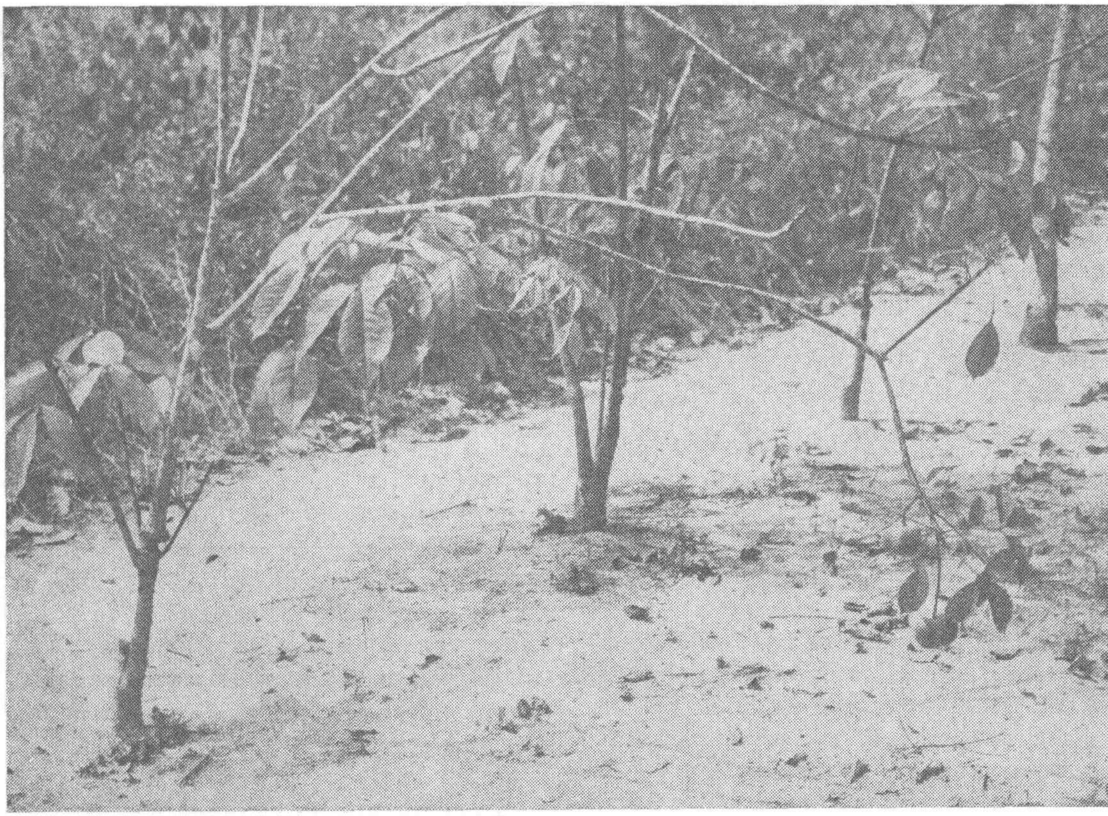

Figure 3. Simultaneous flowering and fruiting in an eighteen-month-old budding of PB 5/51. This tree with a 5 cm wide girdle suffered severe dieback eventually.

buds in the repeatedly girdled trees appeared to offset this difference, floral shoots being seen one month after 'pinching' *(Figure 4)*.

TABLE 2. EFFECTS OF GIRDLING AND BENDING ON FLOWER INDUCTION IN EIGHTEEN-MONTH-OLD BUDDINGS

Treatment	No. trees treated	No. trees flowered
Bending	20	None
Repeated girdling (1.5 mm)	20	8
Bending + repeated girdling (1.5 mm)	20	8
5 cm girdling	20	7

Five replicate buddings of PB 5/51, RRIM 600. RRIM 623 and PB 260 were used per treatment.

Bending of trees was conspicuously ineffective in inducing formation of flowers.

The following growth substances were also applied onto repeatedly girdled trees to study their effects on flowering: tri-iodobenzoic acid (1000 p.p.m.), abscisic acid (50 p.p.m.), ethephon (10 p.p.m.) and chlorocholine chloride (100 p.p.m.). There was no clear indication that these chemicals were effective in enhancing flowering in repeatedly girdled trees *(Table 3)*.

The induction of flowering by repeated girdling varied with clones: PB 5/51 and PB 260, generally showing better responses than RRIM 600 and RRIM 623 *(Table 4)*.

Figure 4. Profuse development of floral inflorescences after removal of the apical end in a repeatedly girdled tree.

The viability of seeds obtained from the treated juvenile trees also appeared to be clonally dependent. All seeds obtained from RRIM 600 trees failed to germinate, while only 29% of seeds obtained from PB 5/51 germinated successfully *(Table 5)*. Seeds obtained from RRIM 623 and PB 260 were considerably superior, showing 78% and 62% germination success respectively. Examination of seeds obtained from RRIM 600 trees revealed poor development of the embryo and perisperm; it is plausible that this was partly at least a consequence of poor nutrient status of the stressed juvenile trees used in this experiment.

Flower Induction Using Source Bush Branches

Efforts were made to induce flowering in source bushes with the aim of utilising these for hand pollination from the ground. Vertical branches (about eighteen-month-old) in eight-year-old clonal source bushes were selected and girdled, the width of bark removed ranging from 2 mm to 5 centimetres. The branches which were 4 – 5 m tall, were girdled at a height of about 1 metre. Flowering was observed six months after girdling. Observations continued for another six months and results are summarised in *Table 6*. Not all clones flowered. Of those that flowered, flowering was more profuse

TABLE 3. EFFECTS OF GIRDLING AND FOLIAR APPLICATION OF GROWTH SUBSTANCES ON FLOWERING IN EIGHTEEN-MONTH-OLD BUDDINGS

Treatment	No. trees treated	No. trees flowered
Control	8	0
RG	20	8
RG + CCC	8	2
RG + CCC + TIBA	8	2
RG + CCC + TIBA + ABA	8	3
RG + CCC + ABA	8	0
RG + CCC + TIBA + ABA + ethephon	8	4
RG + TIBA + ABA	8	4
RG + TIBA	8	4
RG + ABA	8	2
RG + ABA + ethephon	8	4
RG + CCC + ethephon	8	3
RG + TIBA + ethephon	8	3
RG + ethephon	8	2

RG = Repeated girdling (1.5 mm)
CCC = Chlorocholine chloride (1000 p.p.m.)
TIBA = Tri-iodobenzoic acid (1000 p.p.m.)
ABA = Abscisic acid (50 p.p.m.)
Ethephon (500 p.p.m.)
Each treatment comprised of an equal number of each of the following clones: PB 5/51, RRIM 600, RRIM 623 and PB 260.

TABLE 4. CLONAL DIFFERENCES IN AMENABILITY TO FLOWERING IN EIGHTEEN-MONTH-OLD BUDDINGS BY REPEATED GIRDLING

Clone	No. plants treated	No. plants flowered	No. of plants flowered (%)
RRIM 600	34	6	18
RRIM 623	34	7	21
PB 5/51	34	20	59
PB 260	34	18	53

TABLE 5. GERMINATION SUCCESS IN SEED SAMPLES COLLECTED FROM REPEATEDLY GIRDLED TREES

Clone	No. of seeds collected	No. of seeds germinated	Success (%)
RRIM 600	9	0	0
RRIM 623	9	7	78
PB 5/51	21	6	29
PB 260	66	41	62

in the severely girdled trees, as in NAB 17. Flowering in the latter clone was continuous throughout the period of observation, such that all stages of floral shoot development could be seen in one induced branch. As in previous experiments severe girdling led to eventual die-back of shoots.

Establishment of Flowering Plants from Mature Materials

Efforts were also made to develop plants from cuttings taken from mature flowering trees. Rooting under mist and shade gave poor results. In view of this, another approach was adopted in which two- to three-week-old seedlings were grafted onto the shoots of mature trees as described earlier. Observation at the end of one month after grafting revealed that twenty-five of fifty such grafts were successful. These were transferred to polybags and maintained under shade and mist. Six plants were found to produce floral inflorescences there weeks after transplanting. One of these is shown in *Figure 5*. Leaf development was noticed in addition to development of floral inflorescences. Fruit set, however, was not observed. The subsequent viability of these flowering plants, nevertheless, was poor. It appeared that this was due primarily to root rot, after two to three months of transplanting, the cause of which was not identified.

TABLE 6. EFFECT OF GIRDLING ON FLOWER FORMATION IN EIGHTEEN-MONTH-OLD BRANCHES ON EIGHT-YEAR-OLD SOURCE BUSHES

Clones	Treatment	No. of branches	No. of flowering branches
NAB 17	Control	10	0
	2 cm girdling	10	10
	3 cm girdling	10	9
	4 cm girdling	10	10
	5 cm girdling	10	10
	6 cm girdling	10	5
RRIM 719	Repeated 1.5 mm girdling	20	10
RRIM 723	Repeated 1.5 mm girdling	25	3
RRIM 717	Repeated 1.5 mm girdling	25	0
FX 349	Repeated 1.5 mm girdling	25	1
AVROS 2037	Repeated 1.5 mm girdling	40	0
RRIM 719	5 cm girdling	10	1
RRIM 717	5 cm girdling	10	0
FX 349	5 cm girdling	10	0
AVROS 2037	5 cm girdling	10	0
RRIC 110	Repeated 2 mm girdling	5	5
	Repeated 8 mm girdling	5	2
	Repeated 16 mm girdling	5	4
RRIC 102	Repeated 2 mm girdling	5	0
	Repeated 8 mm girdling	5	0
	Repeated 16 mm girdling	5	1
RRIC 101	Repeated 2 mm girdling	5	0
	Repeated 8 mm girdling	5	0
	Repeated 16 mm girdling	5	0
PR 309	Repeated 2 mm girdling	5	0
	Repeated 8 mm girdling	5	0
	Repeated 16 mm girdling	5	0
PR 306	Repeated 2 mm girdling	5	3
	Repeated 8 mm girdling	5	3
	Repeated 16 mm girdling	5	3
PR 302	Repeated 2 mm girdling	5	0
	Repeated 8 mm girdling	5	0
	Repeated 16 mm girdling	5	1
BPM 26	Repeated 2 mm girdling	5	1
	Repeated 8 mm girdling	5	5
	Repeated 16 mm girdling	5	2
BPM 24	Repeated 2 mm girdling	5	3
	Repeated 8 mm girdling	5	2
	Repeated 16 mm girdling	5	3
BPM 3	Repeated 2 mm girdling	5	1
	Repeated 8 mm girdling	5	1
	Repeated 16 mm girdling	5	4
BPM 1	Repeated 2 mm girdling	5	0
	Repeated 8 mm girdling	5	0
	Repeated 16 mm girdling	5	0

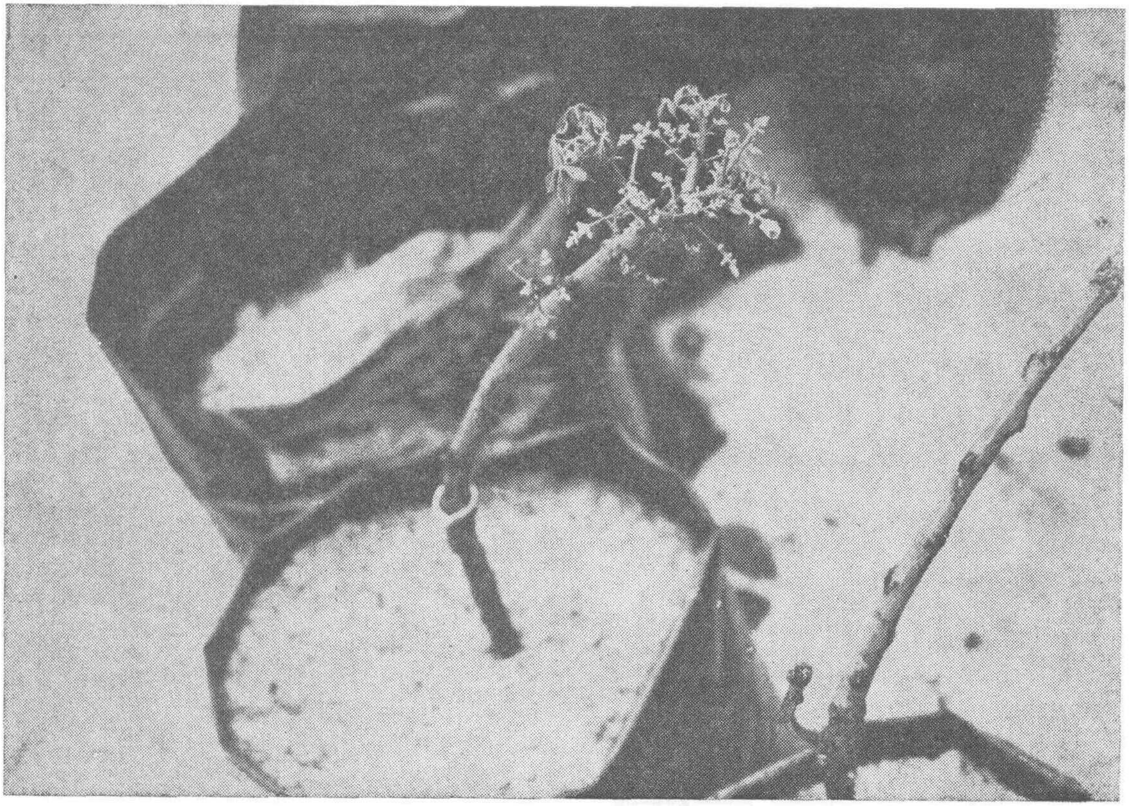

Figure 5. A seedling-grafted shoot from a mature tree with flowers and expanding leaves.

Effects of Stress and Chemical Application on Roots

Application of stress to the trees either by pruning off the taproot or lateral roots, or by application of growth substances to the root system was not found to be an effective means of inducing formation of flowers *(Table 7)*. Among the growth

TABLE 7. EFFECTS OF ROOT PRUNING AND REPEATED GIRDLING ON TWENTY-SIX-MONTH-OLD BUDDINGS OF RRIM 600

Treatments	No. of trees flowered[a]		
	Control (no girdling)	2 mm repeated girdling	16 mm repeated girdling
Control	0	7	10
Dressing with TIBA	1	10	10
Dressing with ABA	1	8	10
Dressing with coumarine	2	8	10
Dressing with ethephon	1	10	10
Dressing with CCC	0	10	10
Dressing with fungicide (Santa A)	0	10	10
Pruning off lateral roots	1	9	8
Pruning off main roots	0	8	9

[a]A total of ten trees were used per treatment.

TIBA = Tri-iodobenzoic acid (1000 p.p.m.)
CCC = Chlorocholine chloride (1000 p.p.m.)
ABA = Abscisic acid (50 p.p.m.)
Santa A = 3% mercury oxide

substances tested, coumarine was most effective. None of these treatments, however, were as effective as girdling. Root pruning, either of the main root or of the lateral roots led to unstable anchorage of the trees and staking was sometimes necessary. It was observed in the same experiment that flowering was more profuse in trees stressed with 16 mm wide girdles, reflecting probably the greater stress of the wider girdles *(Table 8)*.

TABLE 8. EFFECTS OF WIDTH OF GIRDLING ON PRODUCTION OF SHOOTS BEARING FLORAL INFLORESCENCES IN TWENTY-SIX-MONTH-OLD RRIM 600 BUDDINGS

Treatment	Total no. of floral shoots/tree[a]		
	June	July	August
Ungirdled	0	0	0.6
2 mm repeated girdling	0.1	0.5	13.3
16 mm repeated girdling	1.9	3.4	22.8

[a]Each figure is a mean of ninety trees.

DISCUSSION

Buddings can clearly be induced to produce flowers by girdling while seedlings of the same age are recalcitrant in this respect. This difference is possibly related to the well recognised fact that bud-grafting itself reduces juvenility and favours precocious flower formation. It is quite conceivable that the graft union itself is an area of stress of a type that favours reduction of juvenility.

The results reported here clearly indicate that girdling is the most effective means of inducing flowers. It is also evident that flower formation in juvenile buddings is not necessarily dependent on stresses that will also cause eventual tree death. This was seen in repeatedly girdled trees which flowered and also survived the stress of the fine but effective girdles. Though flowering was less profuse in the trees that were repeatedly subjected to 1.5 mm girdles, it appeared that this shortcoming could be overcome by 'pinching'. The latter procedure also offers a possible means of controlling flowering in girdled trees.

The application of growth substances, either to the leaves or to roots of non-girdled trees proved disappointing in induction of flowering in our investigations. Stress treatments such as root pruning were also ineffective. Root-diseased trees, on the other hand, are known to flower. It is likely that in these trees pathogenic destruction of feeding roots is also involved, as the diseased trees eventually die. Juvenile plants in water-logged conditions have also been reported to flower[9]. Thus it is possible that the degree of stress induced by pruning off lateral or taproots in this investigation was insufficient to induce flowering. Restriction of root growth using pots might be a useful method of inducing flowers in young *Hevea*. Preliminary experiments have been promising[10] and further trials are in progress.

The induction of flowering in source-bushes raises a practical possibility of utilising these in plant breeding. Source bushes have numerous shoots and hand pollination from the ground, with the use of ladders if necessary, can be conveniently carried out. In addition, disease control from the ground can also be more effectively achieved.

Cuttings obtained from mature flowering trees sometimes do not root easily. Grafting of seedling root systems onto mature shoots was carried out successfully. The flowering in these plants, approximately 60 cm tall, was quite profuse and further investigation with these materials is obviously desirable.

The recalcitrance of *Hevea* seedlings to induction of flowering is an obvious problem that merits further attention. Synchronisation of flowering might be achieved by grafting various clonal buds onto mature trees. Preliminary investigations suggest that this might be a practical approach. These and other investigations are currently in progress.

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