# ENHANCEMENT OF OLEO-GUM RESIN PRODUCTION IN *COMMIPHORA WIGHTII* BY IMPROVED TAPPING TECHNIQUE

J. R. BHATT, M. N. B. NAIR and H. Y. MOHAN RAM
*Department of Botany, University of Delhi, Delhi 110 007, India.*

## ABSTRACT

The traditional tapping methods used for obtaining guggal, the oleo-gum resin from *Commiphora wightii*, are unproductive and destructive. An improved tapping technique, using 'Mitchie Golledge' knife, coupled with ethephon application has been devised. This method can enhance guggal production by about 22 times over that obtained in controls. The technique is inexpensive, safe, requires no specialized skills and can easily be taught to the tribals. The structure and orientation of secretory ducts in the main stem and the favourable season for tapping are described. Secretory ducts occur in association with secondary phloem in the stem. They are discontinuous, oriented parallel to the longitudinal axis of the stem and anastomose tangentially. April and May are peak months for guggal tapping as established by localization of resin in the sectioned material using bright field and epifluorescence microscopy.

## INTRODUCTION

*COMMIPHORA WIGHTII* (family: Burseraceae) is a drought- and salinity-tolerant small tree (figure 1) found in the semi-arid to arid areas of northwestern India. The plant exudes a medicinal oleo-gum resin called 'guggal' of commerce (figure 2) from cut ends of branches and wounded bark.

Used widely as an incense in religious ceremonies and as a fixative in perfumery, guggal is also considered astringent, demulcent, expectorant, alterative, stomachic, carminative, aphrodisiac and antiseptic[1].

Guggal has been used in Ayurveda since time immemorial for treating rheumatoid arthritis, heart ailments, neurological disorders, skin infections and obesity in humans[2]. The resinous portion of guggal carries significant anti-inflammatory, anti-rheumatic and hypocholesterolemic/hypolipaemic activity. It is also a rich source of steroids which may find use as an alternative raw material for the synthesis of important corticosteroid drugs such as dexamethasone and betamethasone[3]. A preparation by name 'Guglip' developed from guggal by the Central Drug Research Institute, Lucknow is reported to possess hypolipiemic activity equivalent to that of clofibrate (ethyl *p*-chlorophenoxyisobutyrate)—the present drug of choice[2]. Clofibrate is being discontinued and phased out in the USA on account of its toxic manifestations. Therefore, there is ample scope for introducing guglip on a commercial scale.

Guggal is collected from the wild and untended plants by tribal people, who use crude and haphazard methods of incising the main stem by an axe, often stripping off patches of bark. Heavy tapping injures the cambium and curtails the life-span of the tree on account of poor wound-healing. The yellowish-white, viscous exudate hardens in the warm air to a reddish brown or pale greenish masses on the surface of the tree. These are hand-picked and stored in goatskins or gunny bags. After the first collection has been made, the edge of the initial incision is re-cut to stimulate fresh oleo-gum resin flow. Subsequent collections are made from plants every 10–12 days during the rest of the tapping season.

To enhance the rate and the yield of guggal production after two or three collections, the tribal people are encouraged by local contractors to apply a paste around the incision. This paste is reported to contain a plethora of substances—horse or wild-ass urine, butter milk, hydrochloric acid, ammonium chloride, sodium chloride, sulphuric acid, alum, copper sulphate or even a sample of guggal itself. These substances are either used singly or in combinations[4]. Application of the paste doubles the exudate yield. However, the tree succumbs to the injurious tapping method and ceases to yield further. Recognizing the rapid and extensive depletion of the natural population of *C. wightii*, this species has been listed as a threatened plant of India[5].

The trade potential of guggal is large and promising[6]. Methods of tapping must be improved

and standardized to achieve optimum annual production of guggal on a sustained basis and to ensure the survival of the trees in the wild.

Ethephon (2-chloroethylphosphonic acid, an ethylene releasing synthetic chemical) is routinely used for stimulating latex flow in pará-rubber (*Hevea brasiliensis*). In the present study, we have extended its use for enhancing oleo-gum resin exudation in *C. wightii*. We have also established the favourable season for tapping, have used 'Mitchie Golledge' knife for incising the bark and have studied the pattern of arrangement of the secretory ducts in the main stem. A brief account of these aspects is given below.

## MATERIALS AND METHODS

Thirty vigorous female trees of *C. wightii* (Arnott) Bhandari (Burseraceae) free of any visible defects and growing in ravines at Vasad and its adjoining areas of Gujarat State, India were selected for experimentation for three successive years. Twentyseven plants were treated with ethephon and three plants were maintained as distilled water-treated controls. Ethephon containing 400 g/l of 2-chloroethylphosphonic acid in various dilutions used in this study is subsequently referred to as the active substance. A preliminary trial was made in which different concentrations of the active substance were used and the safe dose was determined. As concentrations above 400 mg of active substance induced 'shoot desiccation' and 'die back', they were not used[7]. An aqueous solution of ethephon was used in dilutions containing 100, 200 and 400 mg of the active substance. For each concentration of ethephon, a set of three replicates was maintained—one set each for root feeding, stem injection and surface application of ethephon.

The following three methods of ethephon application were used. The plants were tapped after 48 h of ethephon treatment.

(a) Ethephon was fed to the plant through a root. By digging the earth in the vicinity of a tree, a small lateral root was traced and its tip portion was cleaned with water and then inserted through a split cork into a bottle containing ethephon solution. The bottles were removed prior to tapping.

(b) Ethephon was dispensed into holes (2 cm deep and 0.5 cm diameter) made by an increment borer in the trunk of trees near the base. Holes of required dimensions were also made with a chisel and hammer. The latter method is beneficial where borers are not available. One hole was made in each tree twice annually for three successive years. The opening was made such that it slanted downwards to prevent the backflow of the solution introduced. Fresh holes were not incited upon the healed up portions of the region of previous year's tapping but were made about 2–3 cm lateral to the healed region. The holes were covered with sealing wax after treatment. Control plants received distilled water in the holes.

(c) Ethephon was applied by soaking a piece of cotton in the solution of a specific concentration and placing it around the stem (after removing the outer bark) as a 3.5 to 4 cm wide band near the base of the tree. Cotton soaked in distilled water served as the control treatment.

The results of the three methods of tapping are presented in table 1.

Tapping can be described as 'controlled wounding' and is done by removing thin shavings of the bark. Tapping can be done at any given height and along the entire circumference so long as it does not girdle the tree. The following three types of tapping cuts were found to be useful in the present study (figures 3–5). (a) straight cut; (b) oblique cut; (c) multiple–'v' cut (it consists of three or five oblique cuts connected by a straight cut).

A multiple–'v' cut (figure 5) was possible only in plants with a straight bole. An oblique or straight cut (figures 3, 4) was useful in plants with uneven or crooked boles. The tapped portions gradually healed up (figure 6).

The oblique cuts were useful in preventing overflow of the exudation from the sides of the tapping cut. The oleo-gum resin that flows out on tapping is channelled into a coconut shell (cut into

**Figures 1–6.** **1.** Habit of the plant; **2.** A sample of guggal of commerce; **3.** A straight cut (arrow) and oblique-cuts (arrow-heads) on the main bole; **4.** An oblique cut (arrow) on the main stem from where the exudate is channelled into a coconut shell (C); **5.** A multiple-v cut (arrows) conveying the exudate to a coconut shell (C) fixed to the stem, and **6.** Healed up bark (arrow) after tapping.

**Table 1** *Mean amount of exuded guggal from treated plants at different concentrations of ethephon in December–January and April–May. Data pooled from three harvests in each season during three successive years*

| Active substance (mg) in administered solutions of ethephon | Stem injection: Amount of guggal in g | | Root feeding: Amount of guggal in g | | Surface application. Amount of guggal in g | |
|---|---|---|---|---|---|---|
| | April–May | December–January | April–May | December–January | April–May | December–January |
| 100 | 160 | 45 | 155 | 40 | 165 | 50 |
| 200 | 335 | 90 | 340 | 85 | 345 | 105 |
| 400 | 865 | 165 | 880 | 160 | 870 | 170 |

Average yield in control samples: April–May = 40 g; December–January = 12 g.

half) through a metal spout attached below the tapping cut (figures 3–5).

About 4 cm long stem segments (from control and treated plants) were transversely sectioned at a thickness of 25 $\mu$m on a sliding microtome. The stains[8–13] listed in table 2 were used to localize resin in the sections either with bright field or epifluorescence microscopy. Sections were stained in cavity blocks, washed thoroughly with distilled water and mounted on slides in glycerine jelly for bright field observation and in 50% glycerine for epifluorescence. For fluorescence-inducing illumination, a Zeiss standard research microscope was equipped with an IV FL epifluorescence condenser and an Osram HBO–50W high pressure mercury vapour lamp. Different filter sets (exciter filters, chromatic beam splitters and barrier filters) with excitation wavelengths of 515–560 nm, 450–490 nm and 365 nm respectively were employed to obtain maximum autofluorescence. The last excitation wavelength was most effective in producing autofluorescence. Seasonal variation in the resin yield was investigated by studying the fluorescence of resin in sectioned material during summer (April–May) and winter (December–January) for three successive years. For semi-thin sections, the duct-containing portions of the secondary phloem (1–2 cm long) were dissected from the bark and trimmed into 2 mm$^2$ pieces, while still immersed in the fixative and discarding the cut ends. The samples were fixed according to the fixation procedure described by Bhatt[14]. Sections (1 $\mu$m thick) were cut on a Polaron ultratome using glass knives and stained with 1% toluidine blue 'O' prepared in 1% borax[15].

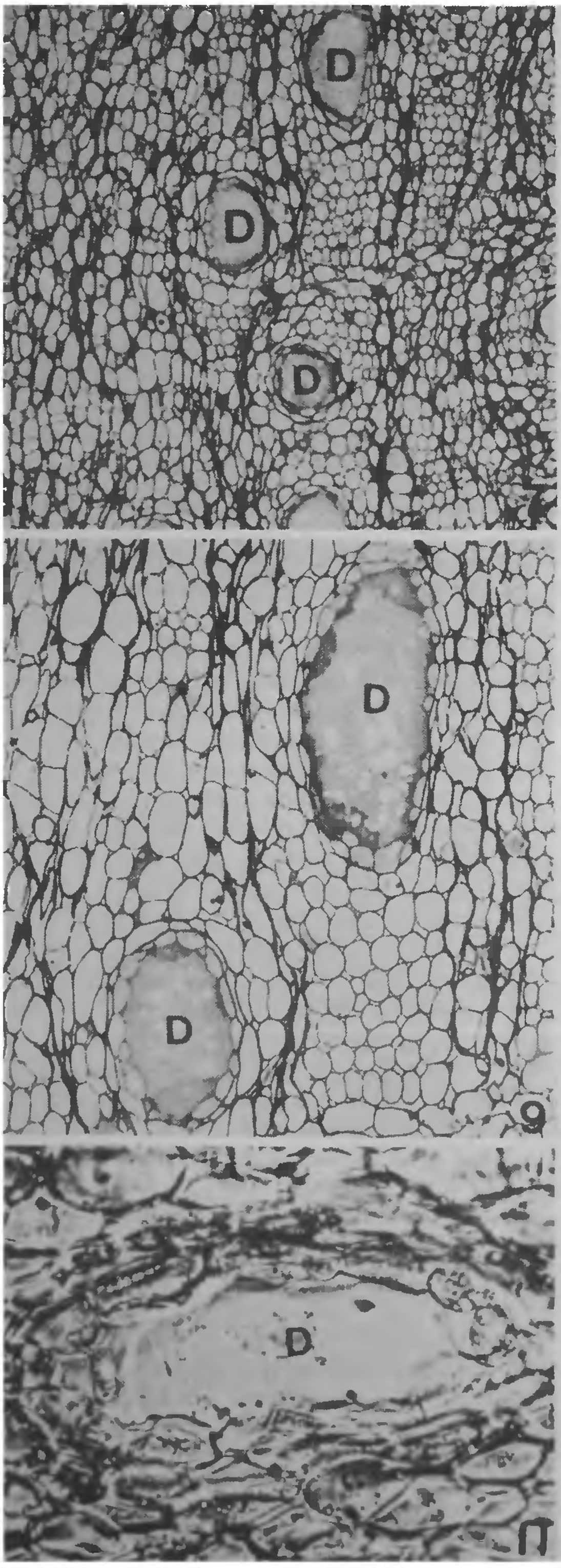
**Table 2** *Cytochemical tests employed on fresh stem sections of Commiphora wightii*

| Stain | Staining reaction with resin | Reference |
|---|---|---|
| Bright field | | |
| Nile blue | Red with patches of blue | Jensen[8] |
| Oil red O | Red | Gurr[9] |
| Osmium tetroxide | Black | Jensen[8] |
| Sudan black B | Blue-black | O'Brien and Mc Cully[10] |
| Sudan IV | Orange red | Johansen[11] |
| Epifluorescence | | |
| Auromine-O | Yellow | Heslop–Harrison[12] |
| Neutral red | Greenish white | Kirk[13] |

## OBSERVATIONS

A cross section of the bark shows secretory ducts in secondary phloem (figures 7–9). The ducts are discontinuous and run parallel to the long axis of the stem (figure 8). A single duct may branch or two adjacent ducts may coalesce (figure 10). The resinous secretion of the epithelial cells accumulates in the duct lumen and stains positively with sudan black B, sudan IV, osmium tetroxide, oil red O and nile blue (table 2).

**Figures 7–12.** **7.** A cross section of bark showing the presence of numerous secretory ducts (D) in association with secondary phloem (× 215); **8.** Parallel orientation of ducts (D) in the axis of the stem as seen in longitudinal section (× 185); **9.** An enlarged view of two ducts in cross section (× 410); **10.** Tangential anastomosis of two nearby ducts (D). Note the copious exudate in the duct lumen (× 385); **11.** Transverse section of duct (D) during December–January showing little or no contents in its lumen (× 380), and **12.** Duct (D) in cross section during April–May (stained with sudan IV) showing copious exudate in the lumen (× 385).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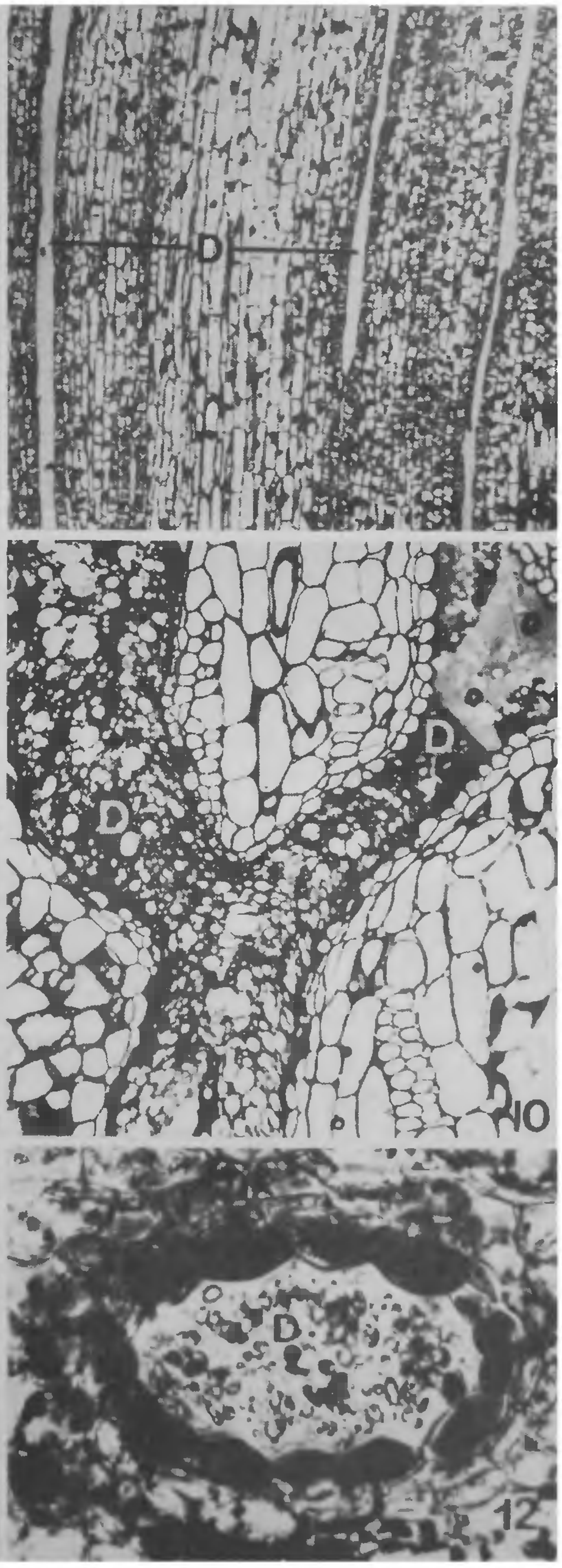
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During December–January, the ducts had little or no contents (figure 11) while during April–May the ducts had copious amount of secretion (figure 12) as observed under the bright field microscopy. The resinous material in the duct lumen showed strong white autofluorescence under UV light. Quenching or fading of autofluorescence occurred with long exposures. The resin gave yellow and greenish white fluorescence with auromine O and neutral red respectively. With the use of these two stains fluorescence becomes more stable and facilitates observation.

In December–January, the resin content in the ducts was negligible as also detected by fluorescence microscopy (figure 13). April and May are peak months for guggal production as established by studying the fluorescence of resin in the sectioned material (figures 14–18).

Data regarding the quantity of guggal produced in response to ethephon treatment in December–January and April–May are given in table 1. A spectacular increase in yield occurred when the plants were treated with 400 mg of active substance over the control in both the seasons in three successive years. However, guggal yield was about five times higher in April–May than in December–January.

## DISCUSSION

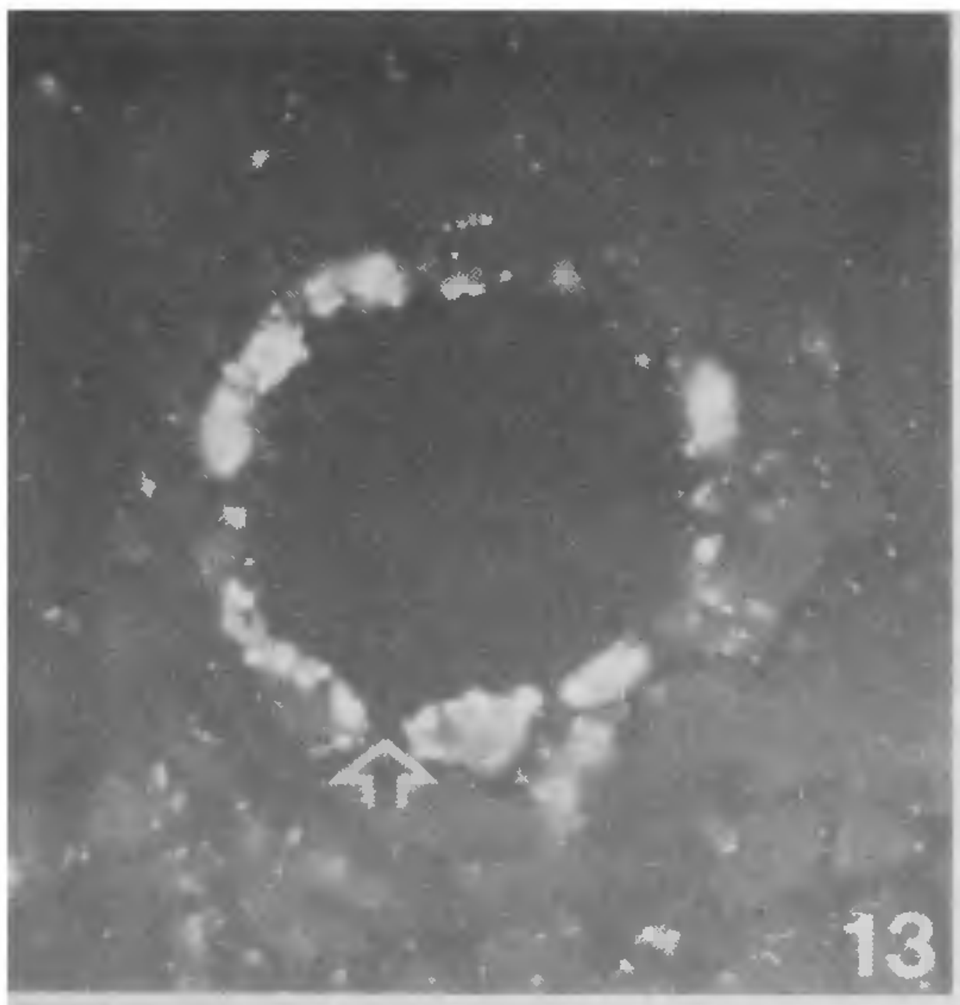
Much in use from ancient times, the exudate guggal of *C. wightii* has recently become important in national and global gum/gum resin trade because of its immense medicinal and other uses. But owing to lack of adequate scientific techniques of tapping and collection procedures, it has become difficult to optimize the utilization of this indigenous natural resource.

In the present study, guggal exudation has been enhanced by 22-times over the control as a result of treatment with 400 mg of active substance of ethephon (root feeding) during April–May. The response to ethephon application varied with the season. The yields were low in December–January and copious in April–May. The tree refoliates with the onset of monsoon and subsequently bears flowers and fruits. As the energy demanding processes[16] like bud growth/shoot development and flowering/fruiting occur during June–July and September–January respectively, the only period during which the reserve metabolites are understandably high is April–May. This seems to explain why greater exudate yields are obtained in April–May than during any other time in the year. As guggal yields are about 5-times higher in April–May than in December–January, in response to treatment with 400 mg of active substance, we suggest that *C. wightii* should be tapped for commercial purposes in April–May and be given rest in the remaining part of the year. It is worthwhile noting here that none of the treated plants in the present work showed any visible injury symptoms even after being tapped twice annually for three consecutive years. Not only is the production of oleo-gum resin less during refoliation, flowering and fruiting stages of the plant but additionally the young leafy/flowering shoots are avidly browsed by the wildlife and sometimes also lopped for fodder. Hence, the overall returns will not be economical if the plants are tapped at any other time in a year excepting April–May.

Ethephon is inexpensive, indigenously manufactured, easily available, safe and is used in agriculture and horticulture as a plant growth-regulator the world over[17–20]. The projected gains of employing ethephon for enhanced guggal production are shorter tapping cuts, reduced tapping frequencies, survival of the tapped plants and optimum production on a sustained basis.

Ethephon application has been reported to result in the formation of characteristic heartwood polyphenols in *Rhus*[21]; resin enrichment in pines[22]; copious gum exudation in *Prunus*[7] sp., *Azadirachta indica*[23] and *Anogeissus latifolia*[24]; increased Kino formation in *Eucalyptus*[21] sp. and stimulated gum-resinosis in *Mangifera indica*[25]. There is a decline in responses observed with repeated ethephon stimul-

**Figures 13–18.** **13.** Cross section of duct showing fluorescence of the meagre resin (arrow) in the duct epithelial cells during December–January (×310); **14.** Autofluorescence of exudate in the duct (arrow) in cross section during April–May (×315); **15–18.** Fluorescence of resin in ducts from ethephon-treated samples. Clear, stable and intense fluorescence of resin (arrow) with auromine-O (**15**), and neutral red (**16**) employed as flurochromes. (Figure **15** × 325; figure **16** × 320; D, Duct.); **17.** Fluorescence of resin (stained with auromine-O) in two nearby ducts (D) in cross section (×310). Note that only the exudate and the epithelium are fluorescent, and **18.** Stable, intense fluorescence of resin (arrow) stained with auromine-O (flurochrome). The resin droplets are not sharply delineated (×340).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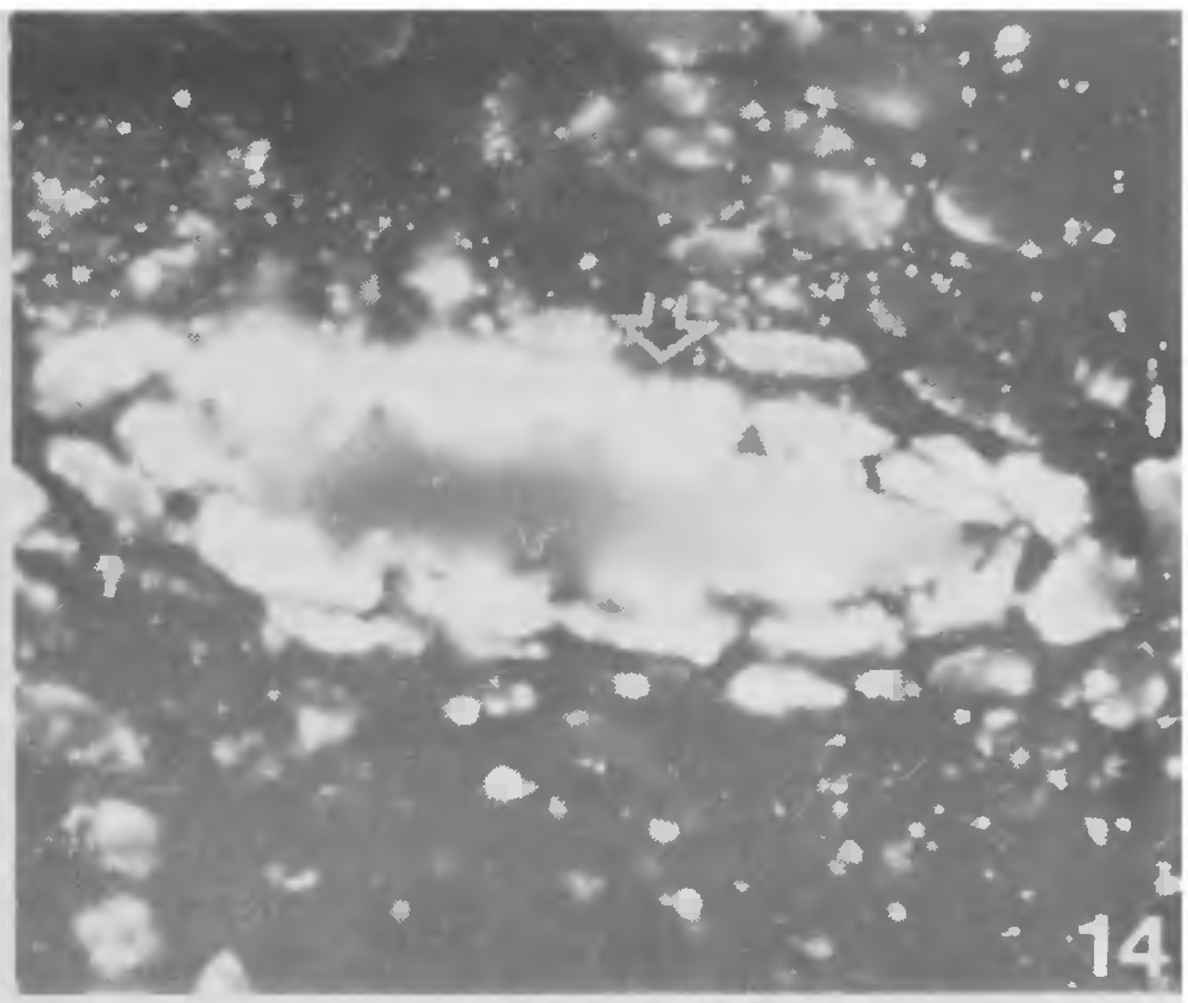
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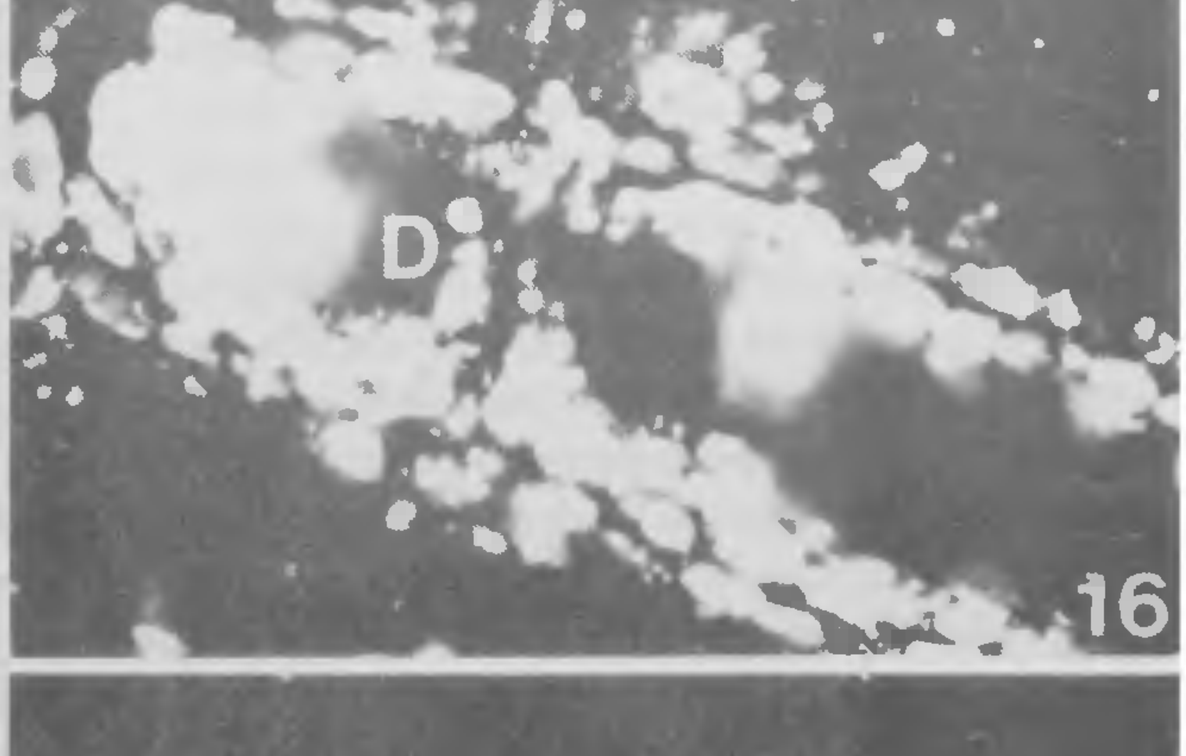
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ation in the case of pará rubber trees[26,27]. This is yet to be proved for *C. wightii* by conducting long-term experiments with repeated use of ethephon, involving the same experimental plants. Moreover, *C. wightii* exists in three forms, i.e. male, female and andromonoecious[28]. It will be worthwhile to examine the comparative responses of these three forms to repeated ethephon treatment. As explained earlier, only the female plants growing at Vasad (Gujarat State) were used in the present study. Responses of provenances growing in different geoclimatic regions of India also need to be evaluated if the present work has to be extended at the field level.

In the main bole, the secretory ducts are found only in association with secondary phloem and hence the tapping cut should not be deeper than the thickness of the bark. In traditional tapping methods, the main stem is haphazardly wounded. The exuded guggal drops to the ground owing to the mishapen and crooked branches of the plant. The dried guggal collected from the ground is of low commercial grade as it is invariably mixed with soil. The sloping cuts given by the Mitchie Golledge knife and the use of coconut shell ensure better collection of the exuded guggal and keep it free from sand and bark.

The most favourable months for guggal tapping are the dry months of April–May as established by localization of resin in the sectioned material. Although the histochemical reactions (listed in table 2) are not absolutely specific, they can be used for *in situ* localization of resin. The positive reactions from these tests can be attributed to long chain lipids, aliphatic hydrocarbons with ester or cetonic functions, triglycerides, steroids and free fatty acids[29]. The positive reaction to sudan IV indicates that free fatty acids and eventually resiniferous acids may occur in the secretion. Nile blue further distinguishes between neutral lipids (stained red) and acidic lipids (stained blue).

Osmium stains the resin black. To intensify the stain, sections were exposed to a 60 W incandescent light for 15–20 min. Hayat[30] reported blackening of the complex formed by the reaction of unsaturated lipids with $OsO_4$ when the samples were dehydrated through alcohols. After staining with $OsO_4$, we may expect some variation in osmiophilia of resin droplets due to slight differences in tissue processing or in species differences in the composition of resin. Caution is required in interpreting osmiophilic material as resin because even the intracellular tannins take up the stain. The contrast in $OsO_4$ treated sections is higher than with other methods listed here. However, $OsO_4$ is corrosive and carcinogenic.

Auromine O and neutral red give better fluorescence of resin than its autofluorescence because the fluorescence is stable and this facilitates observation. The fluorescent techniques described here can also be used to quantify resin in sections microfluorometrically, for identifying individual plants with high and low yields.

Sudan black B and sudan IV are prepared in alcohol in which the small resin droplets are readily soluble. Further, these dyes have the tendency to crystallize out of solution during differentiation. The advantage of using nile blue, auromine-O and neutral red over the other stains (table 2) lies in the use of aqueous solutions so that even the smaller resin droplets are not dissolved out during staining. With regard to clarity and retention of staining for resin, we have found nile blue for bright field and auromine-O for epifluorescence microscopy to be the best. Kirk[13] used neutral red as a lipid fluorochrome and Heslop–Harrison[12] employed auromine-O for detecting cuticle by fluorescence microscopy. We have extended their use for localizing resin under our experimental conditions. Werker and Fahn[31] have also described the use of sudan IV, sudan black B, nile blue and crystal violet in detecting resin in fresh pine tissues.

*C. wightii* occupies inhospitable areas of deserts where conventional agriculture is not feasible and where poverty is rife and economic conditions are poor. With better management, guggal plant could bring additional employment and improved welfare to tribals and other underprivileged people.

In arid regions, wood of *C. wightii* is used as fuel. Evidently, *C. wightii* would become a suitable and remunerative crop for semi-arid and arid regions of India if elite provenances are selected, propagated and tapped scientifically under the scheme for young scientists.

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## ANNOUNCEMENT

### SYMPOSIUM ON DEVELOPMENT WITHOUT DESTRUCTION

'Nature conservators' in collaboration with Department of Botany, University of Kashmir, Srinagar is organizing a Symposium on 'Development without Destruction' at Kashmir from October 17 to 20, 1989. Further details can be had from: Prof. V. Kaul, Director and Convenor of the Symposium, Department of Botany, University of Kashmir, Srinagar 190 006; or Dr S. R. Verma, General Secretary, Nature Conservators, 502 Kailash Bhawan, Civil Lines, Meerut Road, Muzaffarnagar 251 001.