RESEARCH COMMUNICATIONS

Table 4. Trace elements content in the coins and an average of ore sample of Agarundula

<table>
<thead>
<tr>
<th>Element</th>
<th>Min. and max. in the coins (ppm)</th>
<th>Ore sample range (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Co</td>
<td>&lt;10</td>
<td>&lt;30</td>
</tr>
<tr>
<td>Ni</td>
<td>&lt;10</td>
<td>&lt;30</td>
</tr>
<tr>
<td>Zn</td>
<td>10-30</td>
<td>&lt;100</td>
</tr>
<tr>
<td>Cu</td>
<td>35-120</td>
<td>40-150</td>
</tr>
<tr>
<td>Ag</td>
<td>40-600</td>
<td>10-25</td>
</tr>
</tbody>
</table>


ACKNOWLEDGEMENTS: The cooperation received from the Institute of Archaeological and Cultural Research Institute is gratefully acknowledged. I thank Shri B. N. Tandon, Archaeological Survey of India, New Delhi, for guidance; Shri R. K. Sharma, Archaeological Survey of India, Dehra Dun and Shri K. G. Ragade, Hyderabad for valuable suggestions.

Received 26 August 1991; revised accepted 25 January 1992

Consequences of nectar robbing in the pollination ecology of *Vitex negundo* (Verbenaceae)


Department of Environmental Sciences, Andhra University, Waltair 530 003, India

More than 70% flowers of *Vitex negundo* were punctured by the wasps; *Rynchium* and *Ropalidia.* As a result very low percentage of pollination (9) and fruit set (7) was recorded in perforated flowers as against 96% pollination and 76% fruit set in the nonperforated flowers.

FLORAL nectar is offered as a reward for the biotic pollinating agents. However, certain insects do not enter the flowers via the opening of the corolla (perianth), but bite or prick a hole through it and get the nectar from the outside. Such insects in effect rob the nectar as they do not help in pollination. Once an opening has been made, other insects, which would otherwise follow the regular path also use this opening and thus throw the pollination system out of balance. Such blossoms might be a total loss for the plant as they are not likely to be pollinated. But, there are conflicting views on the effects of nectar robbing on plant's pollination. Darwin, Kerner, Barrows, showed examples in which nectar robbing was deleterious to plants while, Hawkins, Macior and Free found no effect of nectar robbing on plant pollination. In this plant we enquire into the consequences of such nectar robbing on the pollination of *Vitex negundo* L., a member of the family Verbenaceae.

*yegundo* plants flower almost throughout the year; the flowers anthes between 0830 and 1300h, and produce nectar at dawn and complete by dusk. About 22 species of diurnal insects visit the flowers during this period. Among them, *Rynchium metallicum* and *Ropalidia* spp. start foraging at 0530 at dawn. These were seen puncturing the corolla tubes just above or at the juncture of the calyx and corolla tubes. These holes are single, ovoid in shape and are easily accessible to all types of foragers. By the time the other foragers appear on the plant the *Rynchium* and *Ropalidia* spp puncture more than 70% of the flowers in the bud condition itself. Even the other foragers having free access to nectar through the regular entrance search for 'back doors' at the flower bases. If they happened to find the holes at the base, take the nectar without rendering any pollination. This type of marauding is seen with bees and ants. The ants were seen licking the calyx cups of the one-day-old flowers in addition to the perforated fresh flowers.

The plants available at Visakhapatnam formed the study material. After the cessation of the foragers' activity on five plants, a total of 651 stigmas were collected at random from perforated and nonperforated flowers, and mounted in lactophenol aniline-blue. The number of stigmas with and without pollen loads was counted and the percentage of stigmas pollinated was calculated. Perforated and nonperforated flowers (n = 500 each) were labelled at random on five plants and were scored for fruit set after a week.

Of the 651 flowers collected at full anthesis on the five plants, 520 were perforated, 131 nonperforated. Screening of the stigmas under a light microscope revealed that 9% of the stigmas of perforated flowers and 96% of the stigmas of nonperforated flowers contained pollen loads (Table 1). Under the circumstances

Table 1. Corolla perforation versus pollination and fruit set in *V. negundo*

<table>
<thead>
<tr>
<th>Corolla condition</th>
<th>No. of flowers observed</th>
<th>No. of stigmas showed pollen</th>
<th>Flowers pollinated (%)</th>
<th>No. of flowers observed</th>
<th>No. of fruits set</th>
<th>Fruit set (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perforated</td>
<td>520</td>
<td>46</td>
<td>9</td>
<td>500</td>
<td>35</td>
<td>7</td>
</tr>
<tr>
<td>Non-perforated</td>
<td>131</td>
<td>126</td>
<td>96</td>
<td>500</td>
<td>380</td>
<td>76</td>
</tr>
</tbody>
</table>

690

CURRENT SCIENCE, VOL. 62, NO. 10, 25 MAY 1992
Micronucleus induction in a mammalian cell line subjected to X-radiation and hyperthermia as studied by cytokinesis block method

P. Uma Devi and C. Streffer

Department of Radiobiology, Kasturba Medical College, Manipal 576 119, India

The micronuclei induction in a human tumour cell line 4197 in vitro was studied after treatment with 0.4 Gy of X-rays, 43°C for 1 h, or X-rays followed by heat, using the cytokinesis block (CB) method with cytochalasin B (CyB). Irradiation produced significantly higher number of binucleate cells (BNC) with micronuclei than hyperthermia. Hyperthermia after radiation reduced the number of cells entering division and further cell progression and also markedly increased the number of BNC bearing more than two micronuclei. This test appears to be a good method to study the relative sensitivity of the mitotically active cells to different treatments.

The micronucleus technique is a viable alternative to chromosome aberration analysis for evaluating the cytogenetic damage in cells induced by chemical and physical agents. Micronuclei are formed fromacentric chromosome fragments1 or whole chromosomes2, lagging behind during the anaphase separation and hence excluded from the daughter nuclei. Therefore, at least one cell division is needed before the micronuclei can be produced. However, the conventional micronucleus technique does not give accurate information, since the cells that have divided only once cannot be distinguished from the cells that have undergone more than one division or no division at all. Therefore, counting the micronuclei per nucleus or per 100 nuclei, as is generally done, will not reveal the actual chromatin injury due to treatments. To overcome this problem in human lymphocytes, Fenech and Morley3 developed a method to block cytokinesis using cytochalasin which does not hinder karyokinesis, so that the once-divided cells can be identified as binucleate cells and the micronuclei score in these cells can be done without ambiguity. In vitro studies demonstrated that the cytochalasin block method is more sensitive than the conventional micronuclei assay4,5. Ramalho et al.6 have confirmed that the frequency of micronuclei detected by this method is a suitable indicator of the frequency of chromosomal aberrations induced by ionizing radiations. Fenech et al.7 also concluded that the cytokinesis block (CB) micronucleus assay may have the potential to complement metaphase analysis of chromosomes for estimating chromosome damage in human lymphocytes following in vitro irradiation. We have used the CB method to study the in vitro sensitivity of a human tumour cell line to radiation and hyperthermia.

The tumour cell line 4197 originally derived from a human oral carcinoma was established in vitro at the Institute for Medical Radiobiology, University Clinics Essen, Germany. The cells were grown in minimum essential medium (MEM, Gibco) with 20% fetal calf serum. Twenty-four-hour-old cultures were used for the experiment. In a preliminary test, six samples containing 2 × 10⁶ cells each were incubated in 5 ml MEM in 35-mm culture dishes. Cytochalasin B (Sigma, Germany) in DMSO was added at 1 μg/ml to each 24-h-old culture and incubated again. Two samples were removed at 24 h, 48 h and 72 h and processed for micronuclei assay by the method described by Shibamoto et al.8. Briefly, the cells were fixed in 1% glutaraldehyde in phosphate buffer after aspirating the medium, treated...