

which contained $\text{Ru}(\text{NH}_3)_6\text{Cl}_3$. This experiment (10 mM DNA, 100 mM Na cacodylate buffer at pH 7.0, 5 mM $\text{Ru}(\text{NH}_3)_6\text{Cl}_3$, 0.5 mM spermine in the hanging-drop equilibrated against 45% MPD) first yielded regular hexagonal plates of size $0.1 \times 0.15 \times 0.20$ mm within 5 days. These plates did not change for atleast six months. When the experiment was viewed again after two years, the drop now contained only ring-shaped crystals. Further support for a template-directed growth mechanism comes from the observation in many of the present cases of a thin spike running from one side of the hexagonal ring to another as sketched in Figure 1b. This could be a remnant of the template.

To summarize, each ring-shaped crystal could be the residue of a flat hexagonal plate, the centre of which has somehow evenly dissolved back into the solution. This however, does not explain several features such as the notches seen at the six vertices of the hexagon. The role of the packing mode of DNA hexamers is also unclear. Further experiments are required to establish the molecular structure and packing of the DNA hexamers in these ring-shaped crystals and to arrive at satisfactory explanation for their formation.

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Variation in podophylloresin and podophyllotoxin contents in different populations of *Podophyllum hexandrum*

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Podophylloresin and podophyllotoxin contents in rhizomes of *Podophyllum hexandrum* were analysed with a HPLC system. The concentration of both resin as well as toxin contents varied in different populations, highest being in the populations collected from alpine regions. The quantities of the resin and the toxin contents were high during May–June compared to that in September–October. Four morphological variants with 1, 2, 3 and 4 leaves at fruit-bearing stage were identified in nature. The resin and the toxin contents were highest in plants with one leaf and lowest in those with four leaves. The results indicate morphological variability as well as variability in the active constituents, which could be explored to obtain superior types.

THE search for genetic diversity and eco-physiological studies of different variants of Indian *Podophyllum* (*Podophyllum hexandrum* Royle), a perennial herb, belonging to family Berberidaceae, has increased because this species is known to contain the highest content of podophyllotoxin^{1–3}. From a chemical point of view, podophyllotoxin belongs to the lignans, which are defined as dimerization products of two phenylpropane units linked by β -carbon atoms of their side chains⁴. These compounds originate from phenylpropanoid bio-

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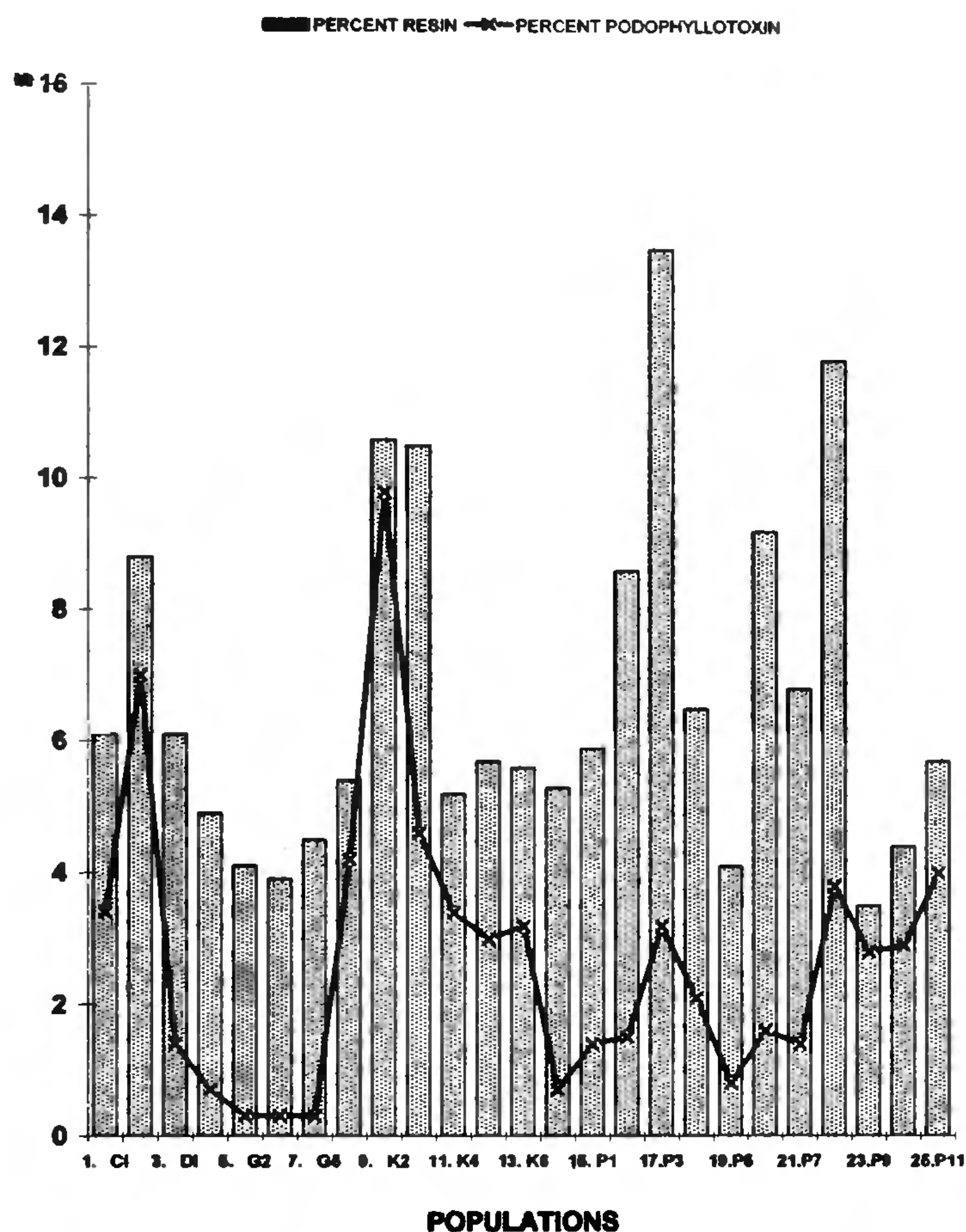


Figure 1. Resin and podophyllotoxin contents (per cent of rhizome dry weight) in 25 populations of *Podophyllum hexandrum* Royle.

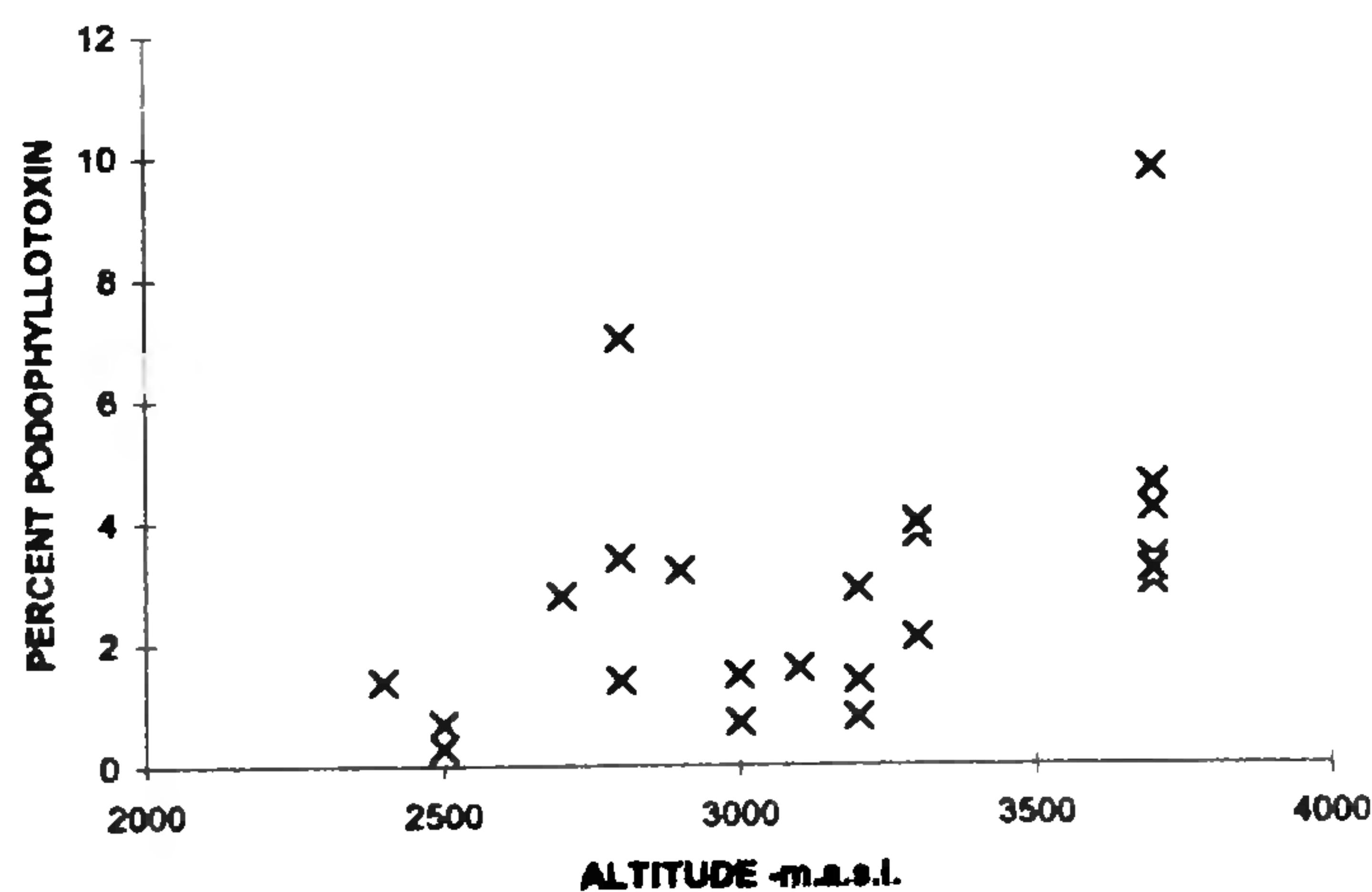


Figure 2. Podophyllotoxin contents in relation to altitude from where the populations were collected.

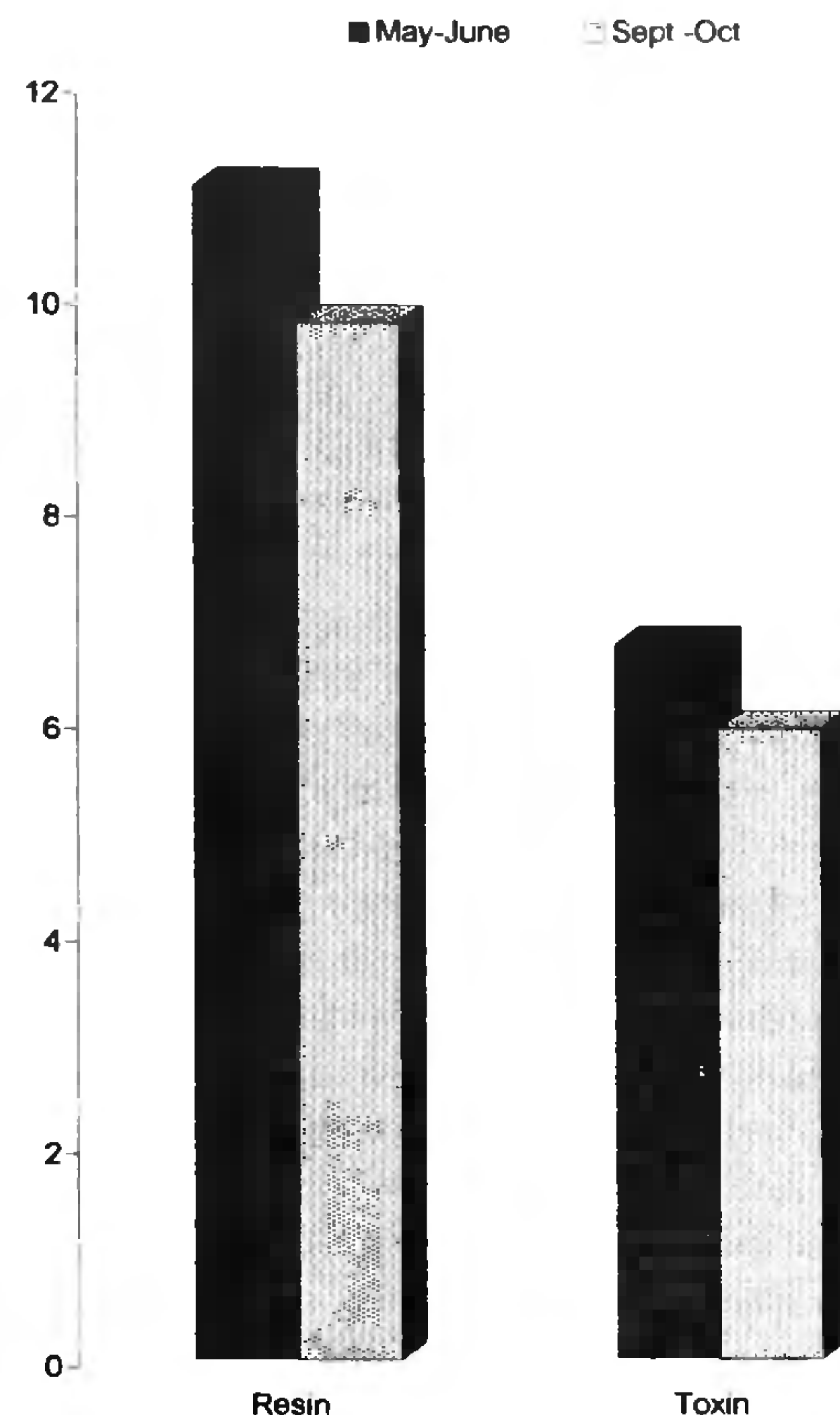


Figure 3. Resin and toxin contents in rhizomes collected during May-June and September-October.

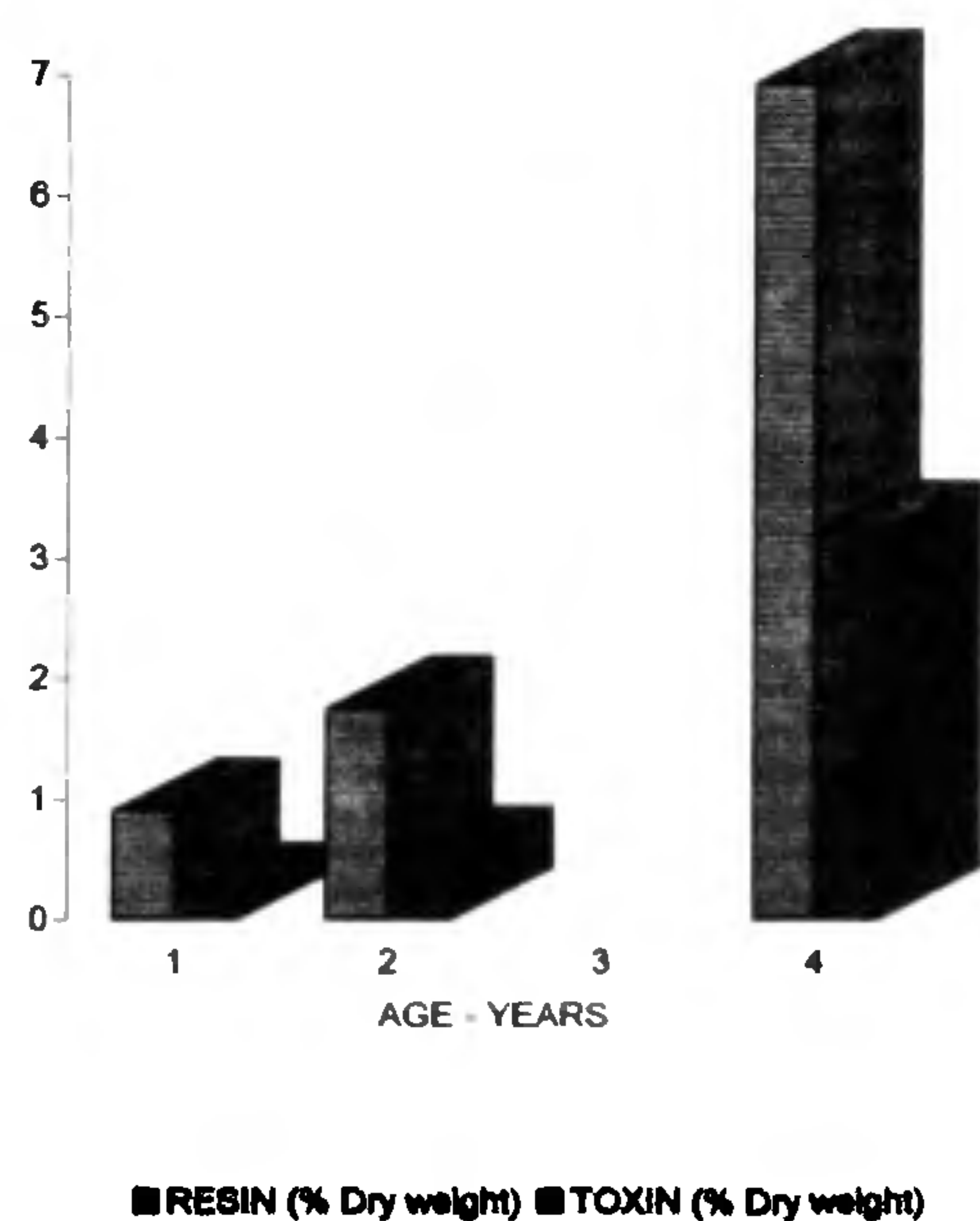


Figure 4. Resin and toxin contents in rhizomes collected from 1, 2 and 4-year-old plants grown in nursery at 2400 m.a.s.l.

synthesis route via shikimic acid pathway. The lignans occurring in *Podophyllum* possess anti-tumour properties, podophyllotoxin being the most active cytotoxic

compound. Recently, it has been reported that different populations of this species show considerable variations in seed characters, isoenzyme and polypeptide photosyn-

Table 1. Resin and toxin contents in rhizomes of four morphological variants of *Podophyllum hexandrum* Royle

Plant type	Resin content (% of dry weight)	Podophyllotoxin content (% of dry weight)
Leaf-type	11.52	8.26
Leaf-type	8.38	7.86
Leaf-type	4.40	6.20
Leaf-type	2.39	5.18

thetic rates and leaf morphology⁵⁻⁷. However, except for one report by Chatterjee¹, there is hardly any work on resin and podophyllotoxin variability with respect to location, season and age of the *Podophyllum* plants. This report was aimed to identify the locations where *Podophyllum* is found in Central Himalaya (populations) having the highest toxin contents so that these could be used for further multiplication and cultivation.

Mature individual plants, showing variability in leaf size and number, were collected during May–June and September–October from 25 populations in Central Himalaya at altitudes ranging from 1800 to 3800 m.asl. Rhizomes of individual populations were washed with a fine jet of water till the soil was completely removed. All the samples were dried at 40°C to constant weight. After grinding to fine powder, the dry powder of the rhizomes was extracted with ethanol in Soxhlet apparatus. The ethanol extract was distilled in vacuum to remove the solvent and weighed. The residue obtained was dissolved in the minimum volume of absolute ethanol and precipitated by acidulated water. The resin obtained was filtered and weighed for the total resin content. It was then dissolved in HPLC grade methanol (0.01 mg in 10 ml, Merck Indian Ltd.). A 20 µl aliquot of each sample was injected into a Beckman System Gold HPLC. A column packed with Silica C-18 (4.5 × 250 mm) was used. Methanol at 1.0 ml/min was used as mobile phase. Podophyllotoxin was detected at λ_{max} of 290 nm. Podophyllotoxin 1, 2, 4, 8, 10 and 20 ppm from Sigma Chemicals, USA, was used as standard.

Resin and podophyllotoxin contents in the rhizomes of different populations are shown in Figure 1. While the maximum resin contents were found in population 17.P3

and lowest in population 23.P9, toxin contents were highest in population 9.K2 and lowest in population 5.G2 to 7.G5. Therefore, there seems to be no direct correlation between resin and toxin contents. Per cent toxin contents seem to be higher at high altitudes (Figure 2). However, some populations from lower elevations also show high toxin contents, indicating that the differences in resin and toxin contents are not totally due to altitudinal of growth. Cross-breeding experiments could show if these variations are genetic.

As the number of plants available were highest in population 10.K3, the seasonal variations both in resin as well as toxin contents were studied only in this population. Contrary to the earlier report¹, we found higher contents in rhizomes collected early in the season (May–June, Figure 3). To study the variations in resin and toxin contents with the age of plants, rhizomes of plants raised from seeds in nursery were analysed in September. The data are shown in Figure 4. Both resin and toxin contents increased with the age of the plant.

As reported earlier, we found four morphological variants of this species with 1, 2, 3 and 4 leaves at maturity in nature. Rhizomes of these plants were analysed in September. The results are shown in Table 1. Both resin and toxin contents were highest in plants with one leaf and lowest in those with four leaves. Chromatographic profiles clearly indicate that the four variants differ even in the number of components. These results are indicative of genetic variability in the four types of plants.

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