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A Simplified Technique for Detection of Paclobutrazol in Plant Sap Extracts, Using HPLC

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Abstract. The tree growth regulator paclobutrazol (PAC) was detected using a simplified high pressure liquid chromatography technique. PAC was detected in both xylem and phloem sap 6 months after trees were injected with the compound. PAC is believed to be exclusively xylem mobile in plants, thus its detection in the phloem during this experiment was unexpected. The technique presented is simple and effective and avoids the use of radioactive material and complicated purification processes before analysis.

Key Words. Paclobutrazol—*Pistachia chinensis*

Paclobutrazol (PAC) is a triazole compound and plant growth regulator that is applied as a foliar spray (El-Khoreiby et al. 1990, Lewis and Ju 1993), soil drench, (Kawabata and DeFrank 1993, Keever and Cox 1989) or, trunk injections (Cox 1990, Deans 1989).

The monitoring and detection of movement of PAC in vivo are difficult. [^{14}C]PAC tracer methods are complicated and potentially hazardous if trees are studied within the public domain. Similarly, gas chromatography is extremely complex and requires highly specialized equipment. Both of these methods are also labor intensive. A simpler approach is to analyze plant material utilizing high pressure liquid chromatography (HPLC).

This paper presents a method for detection and quantification of nonradioactive PAC in plant sap using HPLC.

Abbreviations: PAC, paclobutrazol; HPLC, high pressure liquid chromatography.

Materials and Methods

Pistachia chinensis Bunge (family Anacardiaceae) is a small tree (5–12 m) originating from China and the Philippines which has been planted extensively as a street tree. Trees were injected with Clipper® (20 g/liter PAC in 77.6% ethanol) in a range of dosages, 0, 1.25×10^4, 2.5×10^4, 5×10^4, or 7.5×10^4 mg of PAC. After 6 months, stem segments were harvested from the control trees and the trees injected with the highest concentration of PAC.

Stems were trimmed to 20 cm, and a girdle of tissue 20 mm long was removed to enable collection of xylem and phloem sap from separated sections of the segment.

The stem segment was placed in a pressure bomb within 15 min of harvesting. At a pressure of 3×10^3 m/kg/s (30 bar) the cut surface of the xylem exuded a watery sap, and the phloem exuded a clear resin.

The resinous exudate from the phloem was dried in an oven at 80°C for 1 h and the xylem sap collected in 50-μL microcapillary tubes. Samples were transferred separately to Eppendorf tubes containing 3 mL of HPLC-grade methanol. The methanol dissolved any crystallized PAC contained in the sap. The tubes were then shaken, centrifuged at 15,000 m/s (*g*) for 5 min, and the top layer was injected into an HPLC column.

The analysis system consisted of an LC1200 spectrophotometric HPLC detector (ICI Instruments) with a variable wavelength detector operating at 227 nm (Early and Martin 1988) with a 0.001% AUFS sensitivity factor. An LC1500 HPLC pump (ICI Instruments) operated at a flow rate of 1.0 cm^3/min. Material was injected through a Rheodyne model 7125 syringe-loading sample injector attached to an Alltech C_{18} (10U) reverse phase column (silica, 250 mm × 4.1 mm) with a C_{18} guard unit. The liquid phase of the system was methanol-deionized water (70/30 v/v).

Standard solutions from 1 to 10^{-8} mg/cm^3 PAC in absolute methanol were created from solid PAC. The purity of PAC was checked using ^{1}H nuclear magnetic resonance (65 Hz) and was assayed as 99.0% pure. These standards were used to determine relative concentrations of PAC in the xylem and phloem samples from *Pistachia*.

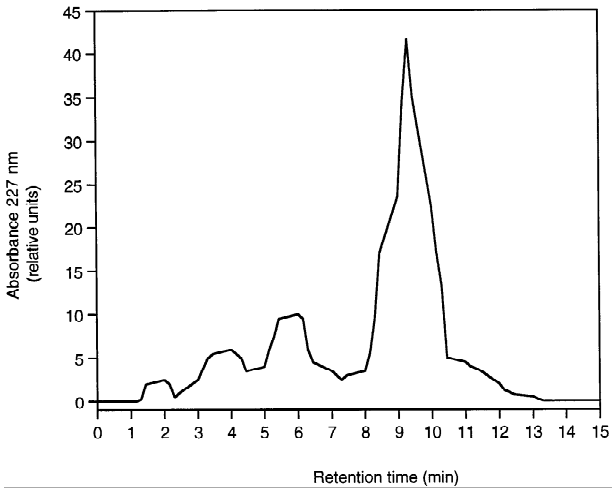

Fig. 1. Sample elution profile of sap from a PAC-injected *P. chinensis* tree. For equipment details, see the Materials and Methods section. The PAC peak occurs at 9 min 30 s.

Results

Using a plotter linked to the detector, PAC was identified by a distinct peak occurring about 9 min 30 s after column injection (Fig. 1).

Quantitative estimates of peaks against standard solutions indicated that the concentration of PAC in the xylem is between 10^{-6} and 10^{-4} mg of PAC/cm^3, whereas in the phloem it is near 10^{-8} mg of PAC/cm^3.

Peaks occurred at 2 and 4 min were indicative of a pressure gradient initiated at the time of injection and bore no relationship to the compounds originally within the sap extract. A larger peak encountered at 6 min probably represented a complex of compounds within the sap which could be neither separated nor identified. The peak returned near to the baseline before PAC was detected. A small artifical shoulder at the side of the PAC peak is a result of the low level of plotter resolution.

Discussion

The advantages of the technique presented here are that it is relatively inexpensive, safer than existing methods of detection utilizing radioactive tracers, less labor intensive, and relatively rapid.

Exudate from the phloem of *Pistachia* is resinous, and it is remarkable that such a simple technique has been so successful. In species that do not possess resin, obtaining results should be quite simple.

This experiment has shown that PAC is present in the xylem and phloem sap of *Pistachia* injected with the highest dosage (7.5×10^4 mg of PAC/cm^3 in 77.6% ethanol) 6 months after injection.

It is commonly believed that the synthetic triazole growth regulator PAC is exclusively xylem mobile, and the detection of PAC in phloem exudate is an unexpected discovery.

Detection of PAC in the phloem sap suggests a secondary pathway for the movement of the compound in *Pistachia.* It will be reported in a subsequent paper that PAC has been found to occur in both the xylem and phloem sap of *R. communis* L.

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