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Paclobutrazol Is Phloem Mobile in Castor Oil Plant (*Ricinus communis* L.)

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Abstract. It is commonly believed that the synthetic triazole growth regulator paclobutrazol (PAC) is exclusively xylem mobile within plants. By contrast, the triazole amitrole and many natural growth regulators are phloem mobile. This raises some doubt as to whether PAC must necessarily be exclusively xylem mobile. PAC was introduced into castor oil plants (*Ricinus communis* L.) through their hollow petioles. PAC was detected in xylem and phloem sap collected above the point of introduction but not in xylem sap below this point. This finding shows that PAC is not exclusively xylem mobile as believed previously. These results also raise the possibility of introducing PAC into plants in a different way so that it is carried by both the xylem and phloem and thus optimizing its effectiveness.

Key Words. Paclobutrazol—*Ricinus communis*

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 injection (Cox 1990, Deans 1989). PAC inhibits three enzymatic steps from *ent*-kaurene to *ent*-kaurenoic acid in the gibberellin biosynthesis pathway (Luster and Miller 1993).

[^{14}C]PAC has been shown to move through the plant via the transpiration stream (Quinlan and Richardson 1986) to the leaves where it is not exported but rather degraded (Early and Martin 1988). These authors even suggested the possibility that the stems, trunks, and roots could serve as potential reservoirs for PAC carryover in trees, although they did not examine this directly, nor did they consider that PAC could appear in the roots of a plant only if transported there by the phloem.

Many naturally occurring growth regulators are transported through the phloem system. PAC is also a synthetic growth regulator so there is no reason to assume it could not follow a similar pathway merely because our methodology of introducing the compound presumes its xylem mobility.

In 1995 (Witchard unpublished data) and I analyzed senesced leaves from deciduous trees treated with PAC. We found that far from being reservoirs of PAC, the leaves actually contained none, whereas the shoots contained small amounts of PAC, presumably exported from the leaves before their senescence.

This paper shows that in castor oil plant (*Ricinus communis* L.), PAC is transported through both phloem and xylem pathways.

Materials and Methods

R. communis L. (castor oil plant, family Euphorbiaceae) is a tall herbaceous shrub with palmatifid leaves born on long petioles. *Ricinus* petioles are hollow at maturity, and material injected into these structures creates its own reservoir. The plant also freely exudes phloem sap when cut.

With a 10-mL hypodermic syringe and needle, 5 mL of Clipper® (commercial formulation of 20 g/liter PAC in 77.6% ethanol) was introduced into a petiole of 9-month-old plants. Sap was collected at two points on the stem: 20 cm above the introduction point and 20 cm below the introduction point.

Phloem sap was collected by bleeding the phloem, which involves slicing the bark so as to cut only the phloem tissue (see Zimmermann and Milburn 1983). Cuts were made 10 min and 16 h after introduction of PAC, and the exuding sap was collected in 50-μL microcapillary tubes. The quantity of exudate collected ranged from 5 to 12.5 μL.

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

Table 1. Estimated amount of PAC (mg/cm^3) detected in stem exudates from *R. communis* taken 20 cm above/below the point of introduction, after 10 min and 16 h

	Xylem		Phloem	
	10 min	16 h	10 min	16 h
+20 cm	2.4	3.6	1.6	2.4
−20 cm	N.D.[a]	N.D.	0.8	1.6

[a] N.D., not detected.

Xylem sap was collected by severing the test plant and taking the shoot to the laboratory in a partially sealed, clear plastic bag. Shoots were placed in a pressure bomb at 10×10^3 mg/kg/s (10 bar) for 5 min followed by 15×10^3 m/kg/s (15 bar) for 5 min. Sap was collected in 50-μL microcapillary tubes in quantities similar to those exuded by the phloem.

Sap was transferred from microcaps to Eppendorf tubes containing 1 mL of high pressure liquid chromatography (HPLC) grade methanol. This process dissolved any crystallized PAC contained in the sap. Tubes were shaken, centrifuged at 10,000 m/s^2 (*g*) for 5 min, then the top layer was injected into an HPLC column. The analysis system was a spectrophotometric HPLC detector with a variable wavelength detector operating at 227 nm (Early and Martin 1988) with a 0.001% AUFS sensitivity factor. An HPLC pump operated at a flow rate of 1.0 cm^3/min. Material was injected through a syringe-loading sample injector attached to a reverse phase column (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). Using a plotter linked to the detector, PAC was identified by a distinct peak occurring about 9 min 30 s after column injection.

Results

PAC was detected in both xylem and phloem sap extracted above the point of introduction of the compound. PAC was detected in phloem sap extracted below the point of introduction but was not detected in xylem sap extracted below the point of introduction. The concentration of detected PAC increased between the two sample periods but was always lower in the phloem (Table 1).

Discussion

This experiment has shown that PAC can be transported via the phloem pathway in *R. communis* L.

The interior surface of the hollow petiole in *Ricinus* is xylem tissue, so simple diffusion could explain the movement of PAC into the xylem. There is no obvious mechanism for movement into the phloem, but it might be achieved via another diffusive mechanism between the xylem and phloem, similar to that proposed for inorganic ions by Pate (1975) and Pate et al. (1974).

The mobility of the triazole PAC in the phloem has been determined empirically by Brudenell et al. (1995) using the Kleier model of phloem mobility of xenobiotics. PAC was estimated to be relatively immobile within the phloem (Baker personal communication). This determination appears to contradict the findings of this experiment, but empirical derivations of data as used by Brudenell et al. (1995) may not always reflect accurately the biologic activity of all of the compounds they tested. For example, it is somewhat anomalous that data for another triazole, amitrole, indicates that it is phloem mobile in *Ricinus* (Bromilow et al. 1987).

Now that we know that PAC is phloem mobile we should reassess the methodology surrounding its introduction into plants. Recent techniques have refined the use of pressurized trunk injection equipment to introduce compounds into the xylem (Deans 1989, Navarro et al. 1992, Stone 1994), but in light of this new evidence, we might question whether that method optimizes movement of PAC within the plant.

Similarly, making use of phloem transport might enhance the effectiveness of PAC. Whereas the xylem pathway would have the most immediate effect on the inhibition of gibberllin biosynthesis, the phloem pathway may be slower and so extend the time during which PAC acts on the tree. The effective concentration or volume injected into the three may be reduced if both the xylem and phloem pathways can be used.

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