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Note

Rapid method for the determination of abscisic acid applied to apple leaves

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Previous methods for the determination of abscisic acid (ABA) in plant material[1] were found unsuitable for the determination of (±)-ABA in leaves from apple trees sprayed with $2 \cdot 10^{-4}$ *M cis*-(±)-ABA[2]. This was either because of the presence of interfering peaks in the gas chromatogram or the time required for separation by thin-layer chromatography. A simple and rapid partition method was devised which removed materials giving interfering peaks and prepared the abscisic acid for gas chromatography (GC) without significant loss.

EXPERIMENTAL

Equipment

An MSE macerator fitted with a 100-ml flask was used to extract the leaves. The gas chromatograph was a Pye 104 instrument fitted with a ^{63}Ni detector operating with a pulse space of 15 μsec; the column was glass, 915 × 4 mm I.D. packed with 3% OV-17 on Chromosorb G AW DMCS (80–100 mesh) and operated at 195°C. A silanized glass wool plug was inserted in the injection port, which was at *ca.* 300°C; the detector temperature was 250°C.

Chemicals

These were Analytical Reagent grade, except for "Diazald" which was purchased from Aldrich (Gillingham, Great Britain). Pure (±)-ABA was donated by Roche Products (Welwyn Garden City, Great Britain), and a further quantity prepared and purified in the laboratory.

Procedure

Fully expanded leaves with a total fresh weight of *ca.* 8 g were macerated with 4 × 30 ml of dichloromethane and the extracts filtered through a Whatman No. 1 paper and combined. Half of this solution was transferred to a separating funnel and extracted with 4 × 10 ml, 5% aqueous ammonia solution. The aqueous extracts were combined, 10 mg butylated *p*-hydroxytoluene added (as an antioxidant) and the solution evaporated to dryness on a rotary evaporator at *ca.* 50°C. The residue was transferred to a 10-ml glass-stoppered tube with 3–5 ml diethyl ether and methylated with diazomethane solution, prepared from Diazald[3]. Methylation was assumed complete when the solution remained yellow for 10 min. The ether was removed at *ca.* 30°C with a current of dry air and the methyl ester dissolved in acetone (usually 2 ml) before a portion (usually 5 μl) was taken for gas chromatography.

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The remainder of the dichloromethane extract was used for a duplicate determination.

Extracts were protected from direct sunlight and stored, when necessary, in a refrigerator.

Full details of the field application and biological effects are published elsewhere[2], but briefly, the application was of 2×10^{-4} *M* (±)-ABA in a solution containing 1% acetone (used to dissolve the ABA initially) and 0.05% "Triton" wetting agent. One litre of solution was applied to each tree and these were 5-year-old spindle bush trees of Golden Delicious on M.9a rootstock.

RESULTS AND DISCUSSION

Typical chromatograms are shown in Fig. 1.

The recovery of (±)-ABA at the 1 ppm level from 8 g (fresh weight) of leaf, as determined by adding the acid immediately before maceration, was 88.5%. If no plant material was present, *i.e.*, if a dichloromethane solution of (±)-ABA was extracted, a much lower recovery was obtained indicating that plant material assisted in protecting the ABA from breakdown. The minimum detectable level of (±)-ABA was *ca.* 0.01 ppm, based on a signal-to-background noise level of 5:1. This was approximately the level of the endogenous ABA in the leaves during these experiments.

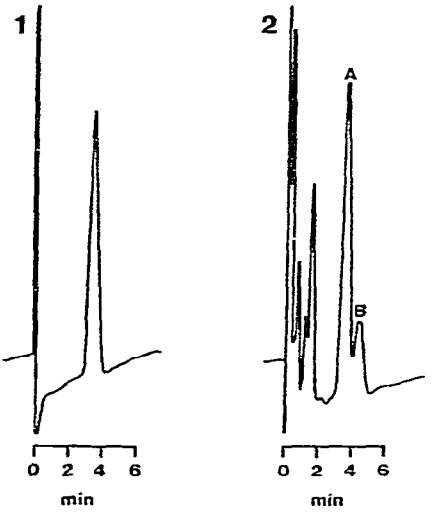

Fig. 1. Typical gas chromatograms of methylated abscisic acid: 1, 1.5 ng pure *cis*-(±)-ABA; 2, ABA extracted from leaves samples immediately after spraying. Peaks: A = *cis*-(±)-ABA; B = *trans*-(±)-ABA.

The mean concentration of ABA on the leaves immediately after sprays of $2 \cdot 10^{-4}$ *M* (±)-ABA solution was 0.58 ppm *cis*- and 0.13 ppm *trans*-ABA; a level which declined rapidly during the following 2 days.

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