A simple, rapid and inexpensive Chemical Method for the detection Phosphite in plant tissue

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Aim to develop a simple technique to detect phosphite in plant tissues to complement the hi-tech, costly HPLC method.

Method

1.Glass fiber filter paper discs (0.5 cm, Whatman GF/B) were saturated with an acidified silver nitrate reagent (1 M $AgNO_3$ and 1 M HNO_3, 25:1) and dried in the dark for 2 hours at 60^0C.
2.Approximately 100 mg of fresh plant material was macerated in 2 volumes of deionised water.
3.Approximately 100 µL of aqueous supernatant from plant tissues was extracted for 30 min at 25^0C with ½ volume of PVPP.
4.20 µl (one drop) of PVPP treated aqueous plant extract was adsorbed onto the middle of a dried silver nitrate saturated disc, and incubated in a Petri dish in the dark at 25^0C for 30 mins.
5.Phosphite in the plant samples was quantified by visual comparison with standards that had been prepared in the same way.

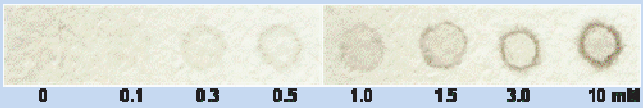

Figure 1. Series of phosphite standards (0 - 10 mM) in deionised water. Aqueous phosphite solution (20µL) was adsorbed on to dried silver nitrate reagent saturated Whatman GF/B disks and incubated in the dark for 1 hour at room temperature.

Plant species and material	Plant extract standards A Control	B 1 mM Phi	C 3 mM Phi	D 0.3% phi spray	E Estimated Phi concentration (mM) in 0.3% sprayed sample	F GLC phosphite analysis (mM) Control	0.3% sprayed
Jacksonia sternbergiana Leaf					1-3	0.01	1.94
Lupinus angustifolius Leaf					1-3	0.004	1.137
Lupinus angustifolius Root					< 1	na	na
Pultenaea reticulata [1] Leaf		na			> 3	0.008	35
Adenanthos cygnorum Leaf		na			1-3	0.003	2.1
Banksia grandis [2] Leaf					na	na	na
Lambertia inermis Leaf					> 3	0.003	3.15
Beaufortia elegans Leaf					< 1	0.004	0.318
Beaufortia squarrosa Leaf		na			< 1	0.004	0.38
Eucalyptus gomphocephala Leaf					< 1	0.004	0.621
Eucalyptus gomphocephala Root					1-3	0.023	0.683
Hypocalymma robustum [1] Leaf		na			> 3	0.023	54
Persea americana [2] Leaf					na	na	na
Persea americana [2] Root					na	na	na
Arabidopsis thaliana Leaf					>3	na	na
Arabidopsis thaliana Root					< 1	na	na

Table 1: **Detection of phosphite in plant tissues.** **A**. extracts from untreated control plants; **B.** extracts as in A but spiked with 1mM phosphite; **C.** extracts as in A but spiked with 3 mM phosphite; **D.** extracts from plants sprayed with 0.3% phosphite (equivalent to 36.6 mM phosphite) 1 week before analysis; 20 uL of plant extract was adsorbed on to a silver nitrate saturated disc and incubated for 1 hour in the dark at room temperature. **E**. Estimated phosphite concentration using the silver nitrate test, on material from phosphite sprayed (0.3%) plants; **F.** GLC phosphite analysis of plant extracts from plants sprayed with 0.3% phosphite and unsprayed controls. All phosphite concentrations shown are mM. [1] = severe leaf burn observed on foliage of phosphite sprayed plants, indicating phosphite accumulation. [2] = Field grown plants. na = not analysed.

Results

1. Phosphite detection is based on the concentration dependent reduction of a silver nitrate reagent by phosphite to a visible grey-black precipitate of elemental silver.
2. Colour development compared with standards showed that levels above 1 mM could be detected in leaves and roots.☺
3. Phosphite estimates were comparable to the levels detected using HPLC. ☺
4. A few species had compounds that interfered with the detection of the colour change.☹

Features of the detection method

Cheap, easy to use in lab or field.

Requires only a small amount of plant material (50 mg).

Results are available within an hour.

Levels of phosphite above 1 mM can be detected (equivalent to XX µg phosphite).

References: