South African Avocado Growers' Association Yearbook 1987. 10:101-103
Proceedings of the First World Avocado Congress

The dynamics and distribution of phosphite in avocado trees treated with phosetyl-Al

JJ BEZUIDENHOUT, JM DARVAS and JM KOTZE
Westfalia Estate, PO Box 14, Duivelskloof
0835, RSA

SYNOPSIS
In a Fuerte orchard, the phosphite content of avocado trees injected with phosetyl-Al reached a maximum four weeks after treatment. The phosphite content was higher in the branches than in the roots or leaves. The half-life of the phosphite in Hass seedlings grown under glasshouse conditions, was estimated at 5,1 months. In the Fuerte trial, however, the half-life of phosphate varied between 0,8 and 2,2 months, depending on the plant organ analysed. Bacteria capable of oxidising phosphite to phosphate were isolated from avocado roots and leaves.

INTRODUCTION

Phosetyl-Al and related compounds are extensively applied in the control of root rot of avocados caused by *Phyto*phthora *cinnamomi* Rands. In plant tissues, phosetyl-Al degrades to ethanol and phosphite. The latter is the toxophore by either activating defense mechanisms in the plant (Bompeix & Saindrenan, 1984) or by acting directly on the fungus (Fenn & Coffey, 1984).

Phosphite is translocated throughout the plant and knowledge of its distribution in the plant is useful to determine optimum time for treatment. A further aim of this study was to investigate possible causes for the breakdown of phosphite. Plants cannot utilise phosphite as a phosphorous source (MacIntyre *et al,* 1950). Bacteria however, is capable of converting phosphite to phosphate (Malacinski & Konetzka, 1966). An attempt was thus made to isolate bacteria capable of oxidising phosphite to phosphate *in vitro* from avocado tissues.

MATERIALS AND METHODS

Treatments

Ten year-old-Fuerte trees were injected with phosetyl-Al as described by Darvas, Toerien & Milne (1984) at a rate of 0,4 g ai m-2 canopy. In a second trial, Hass seedlings grown under glasshouse conditions were injected at the same rate as the Fuerte trees.

Assay for phosphite

Phosphite was determined after methylation with diazomethane by gas-liquid-chromatography (glc), as described previously (Bezuidenhout, Korsten& Kotze, 1985) with minor modifications. A sample was homogenised using liquid nitrogen in a mortar. One g of the fine sample was added to 10 ml distilled water and further homogenised with a blender. The sample was centrifuged. To 2 ml methoxi-ethanol, 0,2 ml of the sample and 10 µl formic acid was added and treated with diazomethane. Ten ml formic acid was added when the solution turned slightly yellow, after which the methylated sample was analysed by glc, under the conditions presented in Table 1.

Isolation of bacteria

Root and leaf segments were dipped in 0,5 per cent sodium hypochlorite for 30 seconds and then thoroughly rinsed in sterile water. One g of the sample in 50 ml sterile water was homogenised with a blender under aseptical conditions. A serial dilution was made and plated out on SN1-agar. The plates were incubated at 15°C. After a week the colonies which developed were streaked out on fresh SN1-agar plates and tested for phosphite utilisation.

Phosphite utilisation

The isolated bacteria were transferred to a mineral solution consisting of ammonium chloride (0,2 per cent), potassium chloride (0,1 per cent), magnesium chloride (0,05 per cent) and chelated iron (0,001 per cent Fe-Na-EDTA), supplemented with glucose (1,0 per cent) and sodium phosphite (0,1 per cent). After incubation at 25°C for two weeks, the concentration of phosphite and phosphate was determined by either glc or colorimetrically with the chlorostannous blue colour method, as outlined by Jackson (1962).

Bacteria identification

The bacteria were identified according to the Bergey's Manual of Determinative Bacteriology (Buchanan & Gibbons, 1974).

RESULTS

The phosphite concentration in the branches and root samples taken from the Fuerte trees under field conditions, reached its peak four weeks after the injection of phosetyl-Al (Figure 1). In the mature leaves, however, the phosphite peak was much broader and ranged between four and eight weeks. Only two sample dates were available for fruit. In both cases the phosphite concentration was lower than in any of the other plant organs. A small amount (1-2 ppm) of phosphite could be detected in the samples at the onset of the injection cycle, which was probably due to a carry-over of phosphite from the previous season. In trees never treated with phosphite compounds, no phosphite was detected.

The results of samples taken from Hass seedlings 17 months after treatment with phosetyl-Al, are illustrated in Figure 2. The phosphite concentration of the old branches (1-3 cm in diameter) averaged 12,9 ppm, whereas young branches contained 5,7 ppm. Young leaves and leaf stems had slightly less phosphite than young branches. No difference ($p=0,95$) in the concentration could be detected between old leaves and roots. Bark and wood differed ($p=0,95$) in the phosphite concentration. The bark had a phosphite concentration three times lower than the woody portion of old branches.

Taking into account the mass of each portion of the Hass seedling, the total amount of phosphite present in the different portions was calculated (Figure 3).

Old branches stored most of the phosphite (50 per cent), followed by roots (30 per cent) and the remaining 20 per cent was located in young branches, leaves and leaf stems.

No ethylphosphonate was detected in either the Hass seedlings 17 months after injections with phosetyl-Al, or in the Fuerte trial from week four after treatment with phosetyl-Al. It was thus possible to calculate the half-life of the phosphite. An amount equivalent to 57,5 mg phosphite in the form of phosetyl-Al was injected into each Hass seedling. After 17 months, the total amount of phosphite was 5,36 mg. Assuming that the change in phosphite concentration with time follows an exponential function, the half-life of phosphite was calculated as 5,1 months for the

seedlings (Table 2). The half-life of phosphite in the different plant portions for Fuerte under field conditions, was calculated from the changes in the phosphite content between week six and 10 (Table 2).

When cell-free avocado leaf extracts were supplemented with 100 ppm phosphite, no change in the phosphite or phosphate content was detected 48 h after incubation. However, three genera of bacteria, ie *Alcaligenes, Pseudomonas* and *Seratia,* capable of converting phosphite to phosphate, were isolated from avocado root and leaf samples. According to the plate count technique, their numbers ranged from 100 to 400 per g tissue.

DISCUSSION

The results clearly indicate that phosphite is unevenly distributed among the plant organs. Branches store most of the phosphite when trees are injected with phosetyl-Al. A higher concentration of phosphite than detected was expected in the mature leaves. However, young flush had a higher concentration of phosphite than mature leaves a week after injections of phosphorous acid (Bezuidenhout, unpublished results).

Phosphite is not utilised by plants as a phosphorous source (Macintyre *et al,* 1950), which explains the relatively slow breakdown rates calculated for phosphite after injections of phosetyl-Al. However, bacteria are able to convert phosphite to phosphate (Malacinski & Konetzka, 1966), which may then act as a nutrient source for the plant. Three genera of bacteria capable of producing phosphate from phosphite *in vitro,* were isolated from avocado roots and leaves. In this context, the increase in the phosphate content of avocado leaves, which was observed after injection with phosphorous acid (Toerien & Slabbert, 1984), is significant. Further investigations are necessary to validate whether these bacteria can influence the phosphite content in avocado trees.

Table 1 Conditions for gas-liquid-chromatography for the determination of phosphite.

Column	type length temperature	3% SE-30 on 80/100 Chromosorb W 2m 140°C
Detector	type temperature	NPSD (Carlo Erba) 190°C
Injector	temperature	150°C
Carrier gas	type flow rate	N_2 40 mlh-t
Air		60 mlh^{-1}
Hydrogen		30 mlh^{-1}
Sample size		1µl

TABLE 2 The half-life of phosphite in Fuerte and Hass after injections with phosetyl-P

Experiment	Organ	Half-life (months)
Fuerte (Field)	Branches	1,1
	Roots	0,8
	Leaves	2,2
Hass (Glasshouse)	Whole plant	5,1

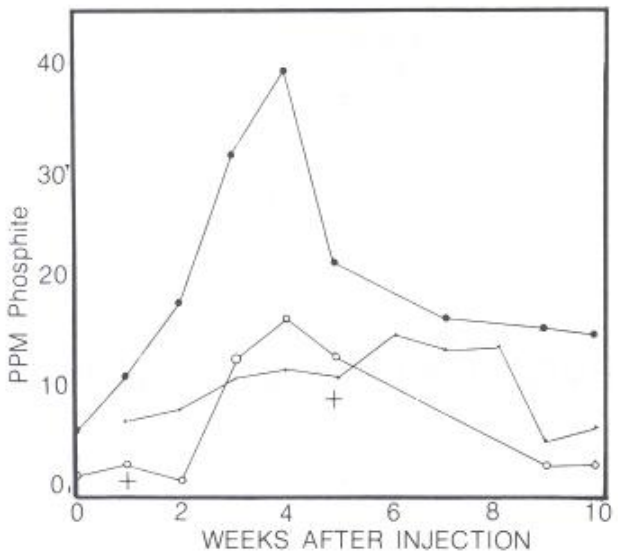

Fig 1 Phosphite concentrations in root (o), mature leaf (.), branch (•) and fruit (+) of Fuerte samples under field conditions following phosetyl-Al injections.

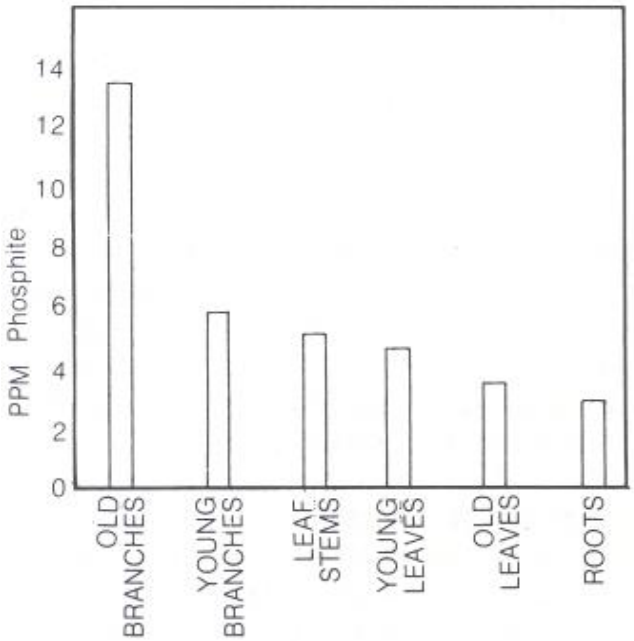

Fig 2 The phosphite content in the different plant portions of Hass seedlings 17 months after injection with phosetyl-Al.

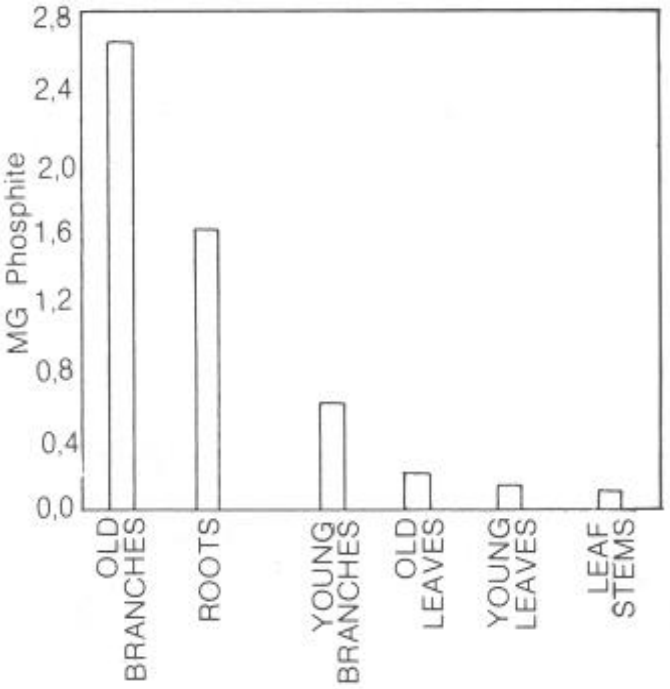

Fig 3 Total amount of phosphite in different portions of the Hass seedlings after injecting phosetyl-Al.

REFERENCES

1 Bezuidenhout, JJ, Korsten, L & Kotzé, JM, 1985. Monitoring phosphorous compounds in avocado tissues. *S Afr Avocado Growers' Assoc Yrb,* **8**, 100-102.

2 Buchanan, RE & Gibbons, NE, 1974. *Bergey's Manual of Determinative Bacteriology.* 8th Edition. The Williams and Wilkins Company, Baltimore.

3 Fenn, ME & Coffey, MD, 1984. Studies on the *in vitro* and *in vivo* antifungal activity of phosetyl-Al and phosphorous acid. *Phytopathology,* **74,** 606-611.

4 Guest, DJ & Bompeix, G, 1984. Phosetyl-Al as a tool in understanding the resistance response in plants. *Phytophthora Newsletter,* **12**, 62-69.

5 Jackson, ML, 1962. *Soil Chemical Analysis.* Prentice-Hall. Englewood Cliffs, NJ.

6 Maclntyre, WH *et al,* 1950. Fertilizer evaluation of certain phosphorus, phosphorous and phosphoric materials by means of pot culture, *Journal American Society* Agrono **42**, 543-549.

7 Malacinski, G & Konetzka, WA, 1966. Bacterial oxidation of orthophosphite. *Journal of Bacteriology,* **91**, 578-549.

8 Toerien, JC & Slabbert MJ, 1984. Phosphorous nutrition of avocados through trunk injection, a preliminary report. *S Afr Avocado Growers' Assoc Yrb,* **7**, 96.