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The effect of phosphite on meiosis and sexual reproduction

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Abstract. The fungicide phosphite was found to reduce pollen fertility in Australian and exotic species. In the perennial *Dryandra sessilis* the reduction was evident for a year after treatment with 2.5-10 gL^{-1} phosphite sprayed to run off. *Pterochaeta paniculata*, an annual, showed reduced pollen fertility in flowers that opened 16-30 days after spraying. The horticultural species *Petunia hybrida* and *Tradescantia virginiana* also displayed reduced pollen fertility after phosphite treatment. Pollen mother cells of *Tradescantia* had a significant percentage of abnormal first and second divisions and micronuclei in the microspores for up to one month after spraying. There was evidence that phosphite induced premature tapetum breakdown in *Petunia* but not *Tradescantia*. The percentage of abnormal meiotic cells and the frequency of premature tapetum breakdown appeared insufficient to account for the high levels of pollen infertility observed after phosphite treatment.

Introduction

Phosphite is the most cost effective, widely used fungicide for control of *Phytophthora* in natural ecosystems and horticultural crops (Coffey and Bower 1984; Wicks and Hall 1988; Ouimette and Coffey 1989; Guest and Grant 1991; Shearer 1994). Apart from providing relatively long-term control of the pathogen (Shearer *et al.* 1991) it is noted for its generally low phytotoxicity on vegetative parts of plants (Barrett and Grant 1997). However, little is known about its effect on sexual reproduction, though several other fungicides, including the well known Benlate (active ingredient Methyl (butylcarbamoyl)-2-benzimidazole carbamate), have been shown to cause aberrant pollen development, reduced pollen germination and altered patterns of synthesis of tapetal proteins (Church and Williams 1977; Ries 1978; Gentile *et al.* 1978; He and Wetzstein 1994; He *et al.* 1996). It is necessary to understand the effects of phosphite on sexual reproduction as the compound is an important tool in the fight to conserve rare plant species threatened with *Phytophthora*. Consequently, we have examined the effect of phosphite on a number of annual and perennial species in jarrah (*Eucalyptus marginata*) forest and from northern sandplains Kwongan vegetation (Fairbanks *et al.* 2001, 2002a). Detail will only be given of the effect of phosphite on pollen of a typical annual species *Pterochaeta paniculata* and a perennial *Dryandra sessilis* and in the model species *Petunia hybrida* and *Tradescantia virginiana*.

Methods

Experimental design

Plants species utilised in the field were in the jarrah forest at Jarrahdale 48 km SE of Perth, Western Australia, from the northern sandplain at Eneabba 250 km north of Perth, or, in the case of the horticultural species, plants were grown in the glasshouse at Murdoch University. Phosphite treatments involved spraying foliage to run off with the stated concentrations of phosphite (Foli-R-Fos 400. Unitec, Australia, mono-di-potassium phosphite) mixed with 0.25% synertol oil (Organic Crop Protectants, NSW, Australia) to enhance adhesion of droplets.

Pollen fertility was assessed by exposure of pollen grains to fluorescein diacetate (Widhlom 1972) and pollen germination by sowing pollen on nutrient medium (Alexander and Ganeshan 1989). As both sets of data gave similar results, only pollen fertility will be presented here.

Pollen assessment of native species

Dryandra sessilis is a member of the Proteaceae, a tall sclerophyllous shrub with sessile flowers in terminal clusters. Plants were sprayed with 0, 2.5, 5 and $10gL^{-1}$ phosphite in winter when they were in flower. There were 5

plants per concentration. Pollen fertility was assessed 6 weeks later then one and two years later. The phosphite content in shoot tips, of 2 plants per concentration, 1 and 6 weeks and one year after treatment was assessed using HPIC analysis (Roos *et al.* 1999).

Pterocheata paniculata was chosen as an example of an annual understorey species. It is a small composite reaching 18 cm high and flowers between July and November. Ten plants per concentration were sprayed with 0, 2.5, 5 and $10gL^{-1}$ phosphite when vegetative, and a second set of replicates when the inflorescences had initiated but before anthesis. Pollen fertility was assessed 4 to 30 weeks after treatment.

Pollen assessment of model species

The effect of 0-20 gL^{-1} phosphite on pollen fertility of the horticultural model species *Tradescantia virginiana* (6 plants per concentration) and *Petunia hybrida* (8 plants per concentration) was examined by spraying potted plants in a glasshouse and examining pollen fertility 1 -28 days later.

Effect of phosphite on pollen mother cell meiosis

Pollen mother cell meiosis was examined in flower buds of the phosphite treated *Tradescantia* (described above). Buds were fixed in alcohol:acetic acid (3:1) for 24 hours and then transferred to 70% alcohol and stored at 4°C. Pollen mother cells and microspores were stained in 2% aceto-orcein.

Effect of phosphite on tapetum development in model species

The stages of tapetal maturation were examined in buds of *Petunia hybrida* and *T. virginiana* after spray to run-off of 0, 10 or 20 gL^{-1} phosphite. Buds were removed 7 days after treatment and classified into different size classes (Fairbanks 2001), fixed in 3% gluteraldhyde in 0.025 M phosphate buffer (pH 7), dehydrated through 30-100% acetone and embedded in Spurr's epoxy resin (Spurr 1969). Resin blocks were sectioned at 1.5 μ and stained in 1% methylene blue and 1% azur II in 1% borax for 5 mins (Richardson *et al.* 1960).

Results

Effect of phosphite on pollen fertility in native plants

Fertility of pollen in flowers of *D. sessilis* that opened 6 weeks after spraying was reduced and the effect was still marked in flowers one year later (Fig. 1). Two years after spraying pollen fertility was normal. One week after spraying there was 218.7 (± 2.2) $\mu g\ g^{-1}$ phosphite in the shoot tips. This dropped to 68.4 ± 68.4 $\mu g\ g^{-1}$ after 6 weeks, and no phosphite was detected after one year. No phosphite was detected in the control plants.

Pterochaeta paniculata sprayed when vegetative showed a reduction in pollen fertility when they flowered 16-30 weeks later. Plants sprayed when inflorescences were in bud were also affected (Fig. 2). Phosphite was more phytotoxic to plants in the vegetative condition, and at $10gL^{-1}$ killed 15% of the plants.

Effect of phosphite on pollen fertility of model species

Phosphite was shown to have a similar effect on pollen fertility of the two horticultural species. There was a depression in *Tradescantia* for up to 2 weeks while in *Petunia* pollen fertility was zero in most tests for up to 3 weeks (Figs. 3,4).

Effect of phosphite on pollen mother cell meiosis

Pollen mother cell meiosis in *Tradescantia* revealed significantly ($P < 0.05$) more abnormal meiotic cells in plants treated with 10 or 20 gL^{-1} phosphite than in control anthers. The types of abnormalities observed included bridges and stickiness, lagging chromosomes in the 1st and 2nd divisions of meiosis and micronuclei in the uninucleate microspores (Fairbanks *et al.* 2002b). There were more abnormal cells in 1st and 2nd division of meiosis for up to a week after spraying and more abnormal microspores for up to 1 month after spraying (Table 1).

Effect of phosphite on tapetum development in model species

In *Tradescantia* the tapetal dis-integration is of the amoeboid type. There was no difference between the timing of the stages of disintegration in control and phosphite treated plants. *Petunia* tapetal cells disintegration is of the glandular type (Fairbanks 2001). Amongst buds 12mm long, those from the control plants had 53% with intact tapetum and 47% with partially disintegrated tapetum. The proportion was similar in the plants treated with $10gL^{-1}$ phosphite while in buds from plants treated with $20gL^{-1}$ phosphite 55% had partially degenerated tapetum and in 45% of buds the tapetum had degenerated entirely.

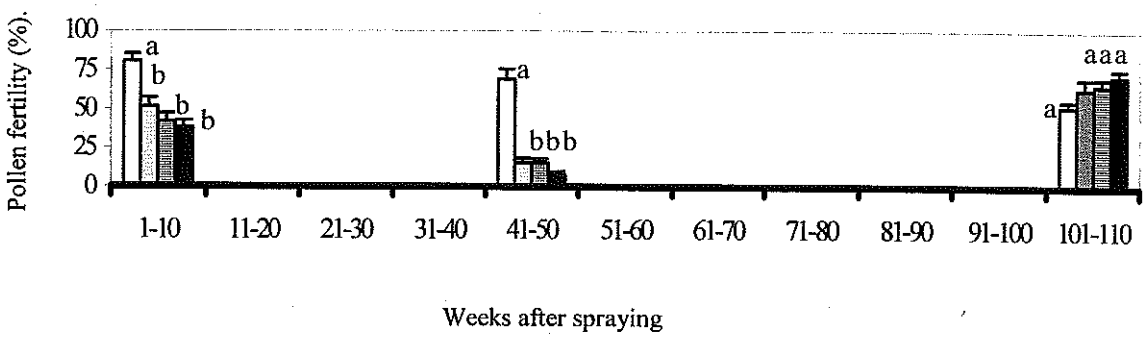

Fig. 1. Pollen fertility of the perennial *Dryandra sessilis* after application of □ 0, ▨ 2.5, ▩ 5, ■ 10 gL^{-1} phosphite in winter 1997. Bars indicate standard errors of means. Bars within the same assessment, with the same letters are not significantly different.

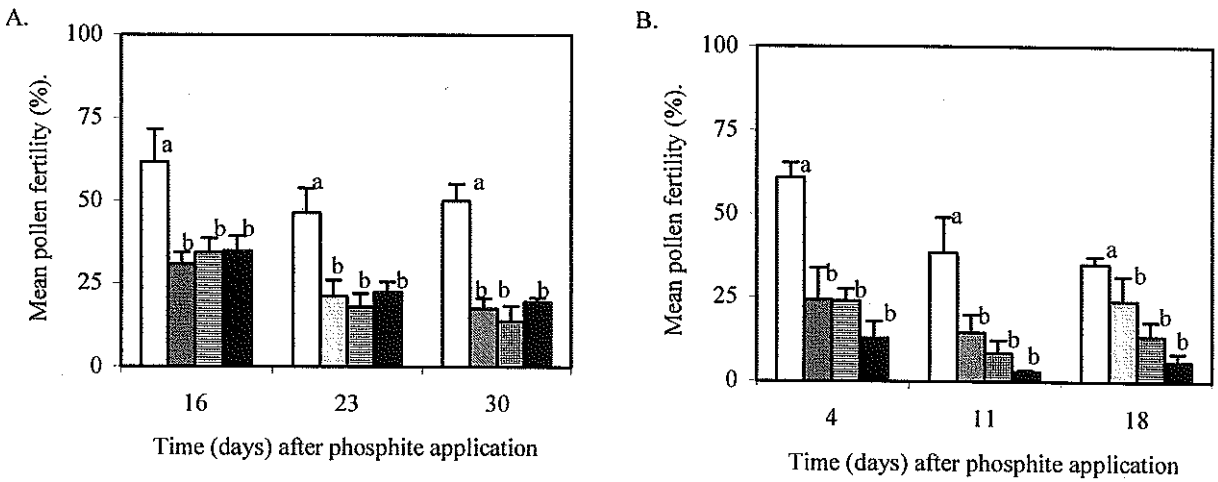

Fig. 2. Pollen fertility of the annual *Pterocheata paniculata* sprayed with □ 0, ▨ 2.5, ▩ 5, ■ 10 gL^{-1} phosphite when (A) vegetative and (B) between flower initiation and anthesis. Bars indicate standard errors of means. Bars within the same assessment, with the same letters are not significantly different. Figure from Fairbanks *et al*, 2001a with permission.

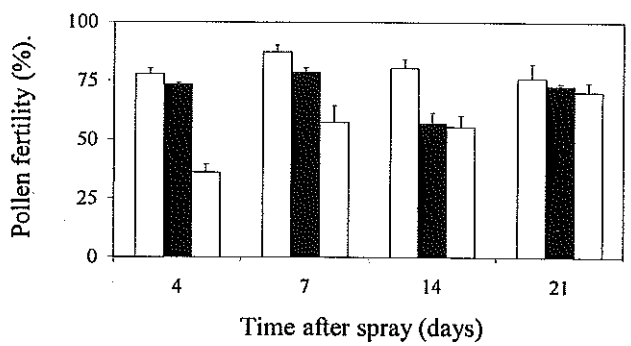

Fig. 3. The effect of phosphite sprayed at □ 0, ■ 10, □20 gL^{-1} on pollen fertility of *Tradescantia virginana*. Bars indicate standard errors of means.

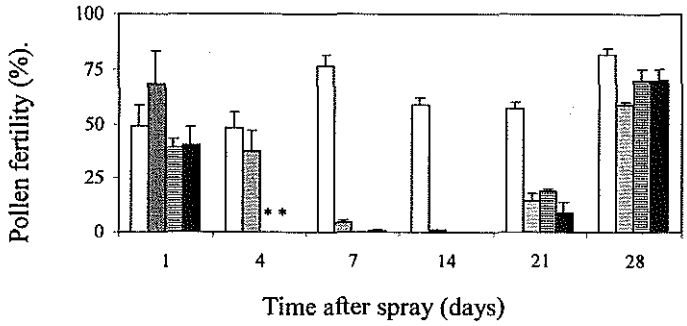

Fig. 4. The effect of phosphite sprayed at □ 0, ▨ 2.5, ▩ 5, ■ 10 gL^{-1} on pollen fertility of *Petunia hybrida*. * no pollen available. Plants treated with 2.5 gL^{-1} phosphite had 1% pollen fertility at day 14. Plants treated with 5 and 10 gL^{-1} phosphite had 0% pollen fertility at days 7 and 14. Bars indicate standard errors of means.

Table 1. Effect of phosphite on *Tradescantia virginiana* microspore meiosis up to 28 days after treatment with phosphite applied as 0, 10 and 20 gL^{-1} spray to run-off.

		% Abnormal Cells[A]		
Days after treatment	Phosphite con. (gL^{-1})	Division 1	Division 2	Uninucleate microspores (micronuclei)
4	0	1.5[a]	1.6[a]	2.0[b]
	10	6.6[b]	7.0[b]	5.6[ab]
	20	8.7[b]	6.9[b]	7.0[b]
7	0	1.8[a]	1.3[a]	4.6[a]
	10	11.1[b]	7.8[b]	9.3[ab]
	20	12.2[b]	13.0[b]	20.1[b]
14	0	3.3[a]	3.1[a]	3.1[a]
	10	3.8[a]	3.1[a]	6.8[ab]
	20	4.9[a]	4.6[a]	8.6[b]
21	0	5.5[a]	2.7[a]	3.4[a]
	10	8.0[a]	3.0[a]	3.6[a]
	20	13.0[a]	2.7[a]	8.5[b]
28	0	3.1[a]	2.6[a]	3.0[a]
	10	4.0[a]	2.5[a]	6.1[b]
	20	3.7[a]	3.2[a]	4.7[ab]

[A]Three to 14 meiotic anthers per treatment were analysed. Means followed by different letters are significantly different at the 95% confidence level dependent on harvest time, SE in parenthesis.

Table 2. Species showing a reduction in pollen fertility after treatment with at least one concentration of phosphite (2.5 to 20 gL^{-1}). Data from Fairbanks 2001.

Location	Habit	Family	Species
Jarrah forest	Perennial	Proteaceae	*Dryandra sessilis*
		Proteaceae	*Adenanthos barbiger*
		Rhamnaceae	*Trymalium ledifolium*
		Sterculiaceae	*Lasiopetalum floribundum*
		Rutaceae	*Boronia cymosa*
		Euphorbiaceae	*Phyllanthus calycinus*
	Annual	Asteraceae	*Pterocheata paniculata*
		Asteraceae	*Hyalosperma cotula*
		Asteraceae	*Podotheca gnaphalioides*
Northern sandplain	Perennial	Myrtaceae	*Eremaea astrocarpa*
		Dilleniaceae	*Hibbertia hypericoides*
		Polygalaceae	*Comesperma calymega*

Discussion

Treatment of plants with phosphite caused a reduction of pollen fertility in several annual and perennial species from both the Australian native flora and horticultural species. These data supported information from other species showing that the effect was widespread (Table 2). A reduction in pollen fertility of variable extent and duration has been recorded for a number of perennial and annual species belonging to Asteraceae, Dilleniaceae, Euphorbiaceae, Goodeniaceae, Myrtaceae, Papilionaceae, Polygalaceae, Proteaceae, Rhamnaceae, Rutaceae and Sterculiaceae families, from the jarrah forest and the northern sand plain (Fairbanks *et al.* 2001, 2002a).

Meiotic abnormalities observed in first and second division as a result of phosphite would have caused abnormal microspores and contributed to the reduction in pollen fertility. Tapetum development and disintegration is precisely timed during normal pollen maturation and early tapetum disintegration is a well-known cause of pollen sterility in several species with male sterile flowers (Echlin 1971; Izhar and Frankel 1971). In the species with a glandular-type disintegration of the tapetum, premature disintegration of the tapetum may also contribute to pollen infertility. No effects on tapetum were observed in the species with an amoeboid –type disintegration. The extent of the damage to pollen cannot be fully accounted for by the percentages of abnormal meiotic cells or the frequency of early tapetum breakdown. The mechanisms active in *D. sessilis* must be effective after one year, even in the absence of detectable levels of phosphite in the shoot tips.

Our results suggest that caution should be exercised in applying phosphite to rare and endangered species and that spraying just before or during the flowering season should be avoided.

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