Australasian Plant Pathology (2022) 51:245–249
https://doi.org/10.1007/s13313-022-00852-1

RESEARCH NOTE

Phosphonate applied as a pre-plant dip controls *Ceratocystis paradoxa* base rot of pineapple planting material

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Received: 3 August 2021 / Accepted: 18 January 2022 / Published online: 21 February 2022

Abstract

Base rot, caused by *Ceratocystis paradoxa*, can be a severe disease of pineapple planting material and can result in poor establishment of plants. Pre-plant dipping of pineapple crowns in potassium phosphonate is used to control *Phytophthora cinnamomi* root and heart rot and was tested for efficacy against base rot in this study. Dipping crowns in 0.5% potassium phosphonate for 2 min significantly reduced the severity of base rot on inoculated crowns in two trials with 'MD2' and '73–50'. Fresh crowns had a more marked response to potassium phosphonate than well-cured, dried crowns. In studies with amended media, the growth of *C. paradoxa* was not inhibited by phosphonate concentrations of up to 100 ppm. Growth was reduced at higher concentrations but phosphonate did not completely inhibit growth of *C. paradoxa* even at the highest rate of 6000 ppm. Potassium phosphonate failed to control incidental *Penicillium funiculosum* infection of basal crown tissue.

Keywords *Ananas comosus* · *Chalara paradoxa* · Butt rot · Potassium phosphonate

Base or 'butt' rot of pineapple (*Ananas comosus*) caused by *Ceratocystis paradoxa* (Dade) C. Moreau can be a serious disease of pineapple planting material (Rohrbach and Johnson 2003). Pineapples may be propagated from crowns (apical vegetative shoots), slips (lateral vegetative shoot from the peduncle below the fruit) and suckers (axillary vegetative shoot) (Rohrbach and Schmitt 2003). *C. paradoxa* can rot the entire piece of planting material and affected pieces are usually discarded by growers. However, where there is partial decay of the base (butt), the material may not be discarded and when planted growth will be severely curtailed due to reduced carbohydrate reserves in infected stems and the destruction of some root initials (Rohrbach and Johnson 2003). Infection occurs through wounds produced when planting materials are removed from the mother plant. Allowing wounds to heal and suberise (cure) by storing on mother plants in the field during dry weather provides good control of the disease (Rohrbach and Johnson 2003). However, mechanisation in the pineapple industry has increased the pace of farm operations meaning freshly removed planting material is planted with no time for curing. *C. paradoxa* can survive in the soil as chlamydospores in decaying pineapple trash and if uncured or untreated material is planted in infested soils losses can be extremely high Rohrbach and Johnson (2003). Freshly removed planting material for immediate planting must be treated with a suitable fungicide within 12 h of removal from the mother plant to avoid base rot (Rohrbach and Johnson 2003). Overseas, these fungicides have included benomyl or triadimefone (Rohrbach and Johnson 2003), while in Australia propiconazole is registered for use as a pre-plant dip (Australian Pesticides and Veterinary Medicines Authority 2021).

Anderson et al. (2012) found that a pre-plant dip of pineapple planting material in 0.5% potassium phosphonate reduced *Phytophthora cinnamomi* root and heart rot by 80–100% in a series of experiments with the pineapple cultivars Smooth Cayenne, MD2 and 73–50. Originally phosphonate was considered to only have activity against diseases caused by Oomycetes, but it has an effect against a much wider spectrum of diseases (Heaton and Dullahide 1990; Guest and Grant 1991; Guest et al. 1995; Norman et al. 2006). Rohrbach and Schenck (1985) when evaluating the effectiveness of phosphonate fungicides in Hawaii for the control of Phytophthora root and heart rot of pineapple, found that fosetyl-Al (also a phosphonate product) as a pre-plant dip at 1200 and 2400 ppm

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significantly reduced base rot of pineapple crowns caused by *C. paradoxa.*

In this study the activity of potassium phosphonate in vitro against *C. paradoxa* was investigated, as was the effectiveness of potassium phosphonate as a dip treatment of crowns for the control of base rot caused by *C. paradoxa*.

A phosphonate sensitivity test for *C. paradoxa* (isolate BRIP 53417) was conducted on corn meal agar (CMA) amended with potassium phosphonate concentrations of: 0, 1, 10, 50, 100, 500, 1000, 2000, 4000, 5000 and 6000 ppm active ingredient (a.i.). Plates were made using the method as described in Anderson et al. (2012). A 3 mm plug from a 4-day old culture on potato dextrose agar (PDA) was used to initiate cultures. Three single plate replicates were used for each concentration. Colony diameter measurements were made (subtracting 3 mm for the plug) and the daily growth rate for the three plates were averaged and compared by analysis of variance (Genstat 19th Edition).

Three experiments were conducted to examine the effect of phosphonate dipping on the colonisation of *C. paradoxa* on the base of inoculated crowns. Prior to treatment crowns were sliced across the base to expose a fresh surface before either: 1) dipping in 0.5% v/v a.i. potassium phosphonate (0.5% w/v, pH 6.5, Agri-Fos 600®, Agrichem, Australia) for 2 min before drying for 24 h; 2) inoculation with conidia of *C. paradoxa*, or; 3) both dipping in potassium phosphonate and inoculation with conidia (Table 1). The *C. paradoxa* inoculum was made by flooding approximately 2-week-old PDA cultures with sterile water, gently dislodging conidia, filtering through 4 layers sterile gauze and adjusting concentration with sterile water as required to either 1×10^4 or 1×10^6 conidia/mL (Table 1). Conidia were applied to the exposed bases of the crowns using a hand-held atomiser ensuring even application. Crowns were stored up-side-down with the cut bases exposed in high humidity (approx. 80%) (Fig. 1) for 5 days prior to removal for visual assessment. Assessments were made by cutting vertically through crowns and rating base rot severity on a 5-point scale. Data were analysed using ANOVA (Genstat 19th Edition) except for experiment 2 where the normality assumption underlying ANOVA was not met and therefore a Kruskal–Wallis (Genstat 19th Edition) test was applied.

In the in vitro experiment there was some inhibition of mycelial growth of *C. paradoxa* at potassium phosphonate concentrations greater than 100 ppm (Table 2). Increased concentrations gave greater inhibition but growth was not totally inhibited at concentrations tested. The 5000 ppm rate is equivalent to the pre-plant dip concentration.

Dipping the crowns in potassium phosphonate decreased the base rot incidence and severity after inoculation with *C. paradoxa* (Table 3, Fig. 1). In the first experiment with fresh crowns the pre-inoculation phosphonate dip reduced disease incidence from 55% down to 0% in crowns inoculated with 1×10^4 conidia/mL, and from 100% down to 5% in crowns inoculated with 1×10^6 conidia/mL (Table 3). Disease was more severe at the higher inoculum concentration in the non-dipped crowns (Table 3).

The second experiment (MD2, December 2011) was undertaken with aged and well cured crowns which had been dried on the mother plants in the field with good air

Table 1 Summary of experiments undertaken to examine effect of phosphonate dip on base rot caused by *C. paradoxa*

Experiment information	Experiment 1	Experiment 2	Experiment 3
Source of planting material			
Cultivar	'73–50'	'MD2'	'MD2'
Location	Beerwah, South East Queensland	Beerwah, South East Queensland	Mareeba, North Queensland
Date	September, 2010	December, 2011	February, 2012
Plant part	Crown	Crown	Crown
Replicates	20 fresh crowns per treatment	6 well cured crowns per treatment	20 crowns per treat, harvested by twisting leaving remnants of fruit tissue
Treatments			
Non-dipped, non-inoculated control	✓	✓	✓
Dipped, non-inoculated	✓	Not assessed	Not assessed
Non-dipped, inoculated 1×10^4conidia/mL	✓	Not assessed	Not assessed
Non-dipped, inoculated 1×10^6 conidia/mL	✓	✓	✓
Dipped, inoculated 1×10^4 conidia/mL	✓	Not assessed	Not assessed
Dipped, inoculated 1×10^6 conidia/mL	✓	✓	✓
Inoculated 1×10^6 conidia/mL, 24 h later dip in 0.5% phosphonate	Not assessed	✓	Not assessed
Inoculation	Inoculum was applied 24 h after treatment unless stated otherwise		
Incubation	After inoculation crown incubated at 28 °C, approximately 80% relative humidity for 5 days prior to assessment		

Fig. 1 Photograph of pineapple crowns inoculated with conidia and stored in high humidity to enable expression of base rot. Crowns on the left had been dipped in 0.5% potassium phosphonate prior to inoculation with 1×10^6 conidia/mL, crowns on the right received no treatment prior to inoculation

circulation, in dry conditions. In contrast to the first experiment, inoculation with conidia of *C. paradoxa* caused base rot in 100% of crowns which had been dipped in potassium phosphonate (Table 3). However, the pathogen did not infect the basal leaves of the potassium phosphonate dipped crowns (Table 3). Application of phosphonate 24 h after inoculation did not prevent destruction of stem or basal leaves by the pathogen (Table 3).

At the time of assessment of the third trial, there was a blue mould, visually distinct from the appearance of base rot, present on the bases of many of the crowns. The mould was identified as *Penicillium funiculosum* Thom by morphological methods. The base rot and blue mould were both assessed for incidence and severity. The *P. funiculosum* colonised the bases of the non-dipped, non-inoculated crowns (Table 3). The crowns which were inoculated with *C. paradoxa* had a 100% incidence of base rot and the symptoms were severe (Table 3). The crowns which had been dipped in potassium phosphonate and then inoculated had a mixture of base rot and blue mould (Table 3).

Table 2 Average daily growth rate of *C. paradoxa* growing on CMA amended with different concentrations of potassium phosphonate. Average daily growth rates followed by different letters indicate significant difference at P = 0.05

Phosphorous acid concentration (ppm)	Average daily growth rate (mm)
0	21.0 g
1	21.5 g
5	20.7 g
10	21.0 g
50	21.0 g
100	21.0 g
500	17.3 f
1000	15.3 e
2000	9.0 d
4000	4.2 a
5000	7.2 c
6000	6.0 b

This experiment indicates that *P. funiculosum* can colonise and damage the cut stem of the hybrid 'MD2'. In the non-dipped crowns inoculated with *C. paradoxa* the rapid damage caused by *C. paradoxa* masked the presence of *P. funiculosum*. The phosphonate dip treatment failed to reduce the incidence and severity of stem decay caused by *P. funiculosum*. The crowns used in this study had been harvested by twisting the crowns to remove them from the fruit (usual practice is to cut across the stem), leaving a portion of fruit tissue attached to the crown which was invaded by the fungus prior to trimming before treatment and inoculation.

In this experiment, when the crowns were examined with a hand lens it was found that they were infested with red mite which had caused damage when feeding on the white basal tissue of the lower leaves. When isolations were made from these feeding sites, *P. funiculosum* was recovered from all lesions. This indicates that these feeding sites had provided an entry wound for *P. funiculosum* which sporulated prolifically to provide inoculum for the cut stem of the crowns. Where *P. funiculosum* is present growers may need to invest in treatment with compatible fungicides or use cutting, rather than twisting, to remove crowns to prevent this occurring and affecting plant establishment.

Rohrbach and Schenck (1985) using 2% V-8 juice as a substrate in an in vitro study, found that fosetyl –Al and phosphorous acid at rates of 1200 ppm and 2400 ppm inhibited growth

Table 3 Effect of potassium phosphonate on the incidence and mean severity of base rot caused by *C. paradoxa* in crowns of pineapple hybrids

	73–50 Sept 2010		MD2 Dec 2011		MD2 Feb 2012			
	Base rot		Base rot		Base rot		Blue mould	
Treatment	Incidence	Severity[A]	Incidence	Severity[B]	Incidence	Severity	Incidence	Severity[C]
Non-dipped, non-inoculated control	0/20	0.00 a	0/6	0 a	0/20	0 a	19/20	38.5
Dipped, non-inoculated	0/20	0.00 a	n.a	n.a	n.a	n.a	n.a	n.a
Non-dipped, inoculated 1×10^4conidia/mL	11/20	0.59 b	n.a	n.a	n.a	n.a	n.a	n.a
Non-dipped, inoculated 1×10^6 conidia/mL	20/20	3.36 c	6/6	5 b	20/20	4.65 c	- [†]	- [†]
Dipped, inoculated 1×10^4 conidia/mL	0/20	0.00 a	n.a	n.a	n.a	n.a	n.a	n.a
Dipped, inoculated 1×10^6 conidia/mL	1/20	0.003 a	6/6	2 ab	6/20	0.35 b	20/20	29.0
Inoculated 1×10^6 conidia/mL, 24 h later dip in 0.5% phosphonate	n.a		6/6	5 b	n.a	n.a	n.a	n.a
P (l.s.d)		< 0.001		< 0.001		< 0.001		0.089

n.a. = not assessed, Severity was assessed as 0 = no infection; 1 = slight colonisation of cut stem; 2 = complete colonisation of cut stem; 3 = complete colonisation of cut stem + one basal leaf; 4 = complete colonisation of cut stem + one to three basal leaves; 5 = complete colonisation of cut stem + all basal leaves

[A] Mean severity for each treatment (not including controls) were compared using ANOVA Genstat 19th Edition, data were square root transformed, back transformed means are presented

[B] As underlying assumption of normality for an ANOVA was not met a Kruskal–Wallis test was applied and a Bonferroni adjustment undertaken to enable multiple comparisons in R

[C] blue mould severity was assessed as percentage stem invasion

[†] base rot extensive and any blue mould symptoms were obscured

of *C. paradoxa* (no growth at 2400 ppm) and attributed base rot control solely to the fungitoxic activity of the chemicals. In the current study where CMA was used as the substrate there was considerable growth at 5000 ppm potassium phosphonate. It is possible that the isolate of *C. pardoxa* used in these studies is less sensitive to phosphonate, there is no literature comparing a large collection of *C. paradoxa* isolates for sensitivity to phosphonate.

The dip treatment failed to control base rot of stem tissue in well cured crowns but it prevented infection of their basal leaves. Potentially the inoculum was taken up more effectively in the dried crowns, but potassium phosphonate has been shown to induce defence responses in a range of plant/pathogen systems (Dann and McLeod 2021). Further investigations to determine if a defence response is stimulated by potassium phosphonate application to crowns would be useful.

This study shows that a pre-plant dip treatment of fresh pineapple planting material with potassium phosphonate is effective for the control of base rot caused by *C. paradoxa.* As this treatment will also protect young plants against Phytophthora root and heart rot it will help prevent irregular establishment of a new planting.

Acknowledgements This project was funded by Horticulture Australia Limited, using the pineapple research and development levy and contributions from the Australian Government. We thank Carole Wright for assistance with statistical analysis. An anonymous reviewer is acknowledged for improvements to the manuscript. We acknowledge our wonderful colleague Mr Col Scott (deceased) who helped us develop and undertake this work with a focus on practical outcomes for pineapple growers.

Author contributions K.P. and J.A. contributed to the study conception, design and analysis. Material preparation and data collection were performed by K.P., J.D. and J.A. Data analysis was performed by J.A. The first draft of the manuscript was written by K.P. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript. Mr Col Scott contributed significantly to the work but passed away prior to paper submission.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions.

Data availability The datasets generated and analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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