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Research Article

The Effect of Different Phosphite Spray Formulas on Phytotoxicity and Phosphite Uptake of Avocado and Control Efficacy against *Phytophthora* Root Rot

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Abstract

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Phytophthora root rot (PRR) caused by *Phytophthora cinnamomi* is one of the most important avocado diseases in Taiwan. Though foliar sprays of phosphonate fungicides have been used to manage this disease in other countries, the most effective application dosage and techniques have not been determined for avocado cultivars planted in Taiwan. To provide information for optimizing phosphite application strategies, this study investigated the effects of different phosphite spray formulas on avocado seedlings of various cultivars, focusing on phytotoxicity and phosphite uptake. The results showed that 0.1% and 0.2% phosphite caused mild (phytotoxicity score < 2) or no phytotoxic damage on seedlings of 6 avocado cultivars tested, including 'Choquette', 'Changan', 'Hung Shin Yuan', 'Zongpu Green Skin', 'Hall', and 'CAES3', while 0.5% phosphite could cause severe phytotoxic damage (phytotoxicity score ≥ 2) on 'Choquette', 'Changan', and 'Hall' under high temperature. In addition, pH had no obvious effect on phytotoxicity when the solutions were buffered to a pH ranging from 6.5 to 7.5. A detached root bioassay showed that, compared with the control, the colonization rates of *P. cinnamomi* were significantly lower in 'Changan' seedlings treated with phosphite and an adjuvant (0.2% phosphite plus Jia-Shou-Huo-Jhan (加收活展 , JSHJ), 0.5% phosphite plus JSHJ, or 0.5% phosphite plus S-408), and the root phosphite concentrations were above 180 μg g^{-1} for all these three treatments. Moreover, adding an adjuvant (JSHJ or S-408) to the phosphite solution reduced phytotoxic damage and increased the uptake of phosphite into the roots. The results generated from in this study will be helpful for the optimization of phosphite application strategies for the avocado industry.

Key words: Avocado, Adjuvant, Phosphite, *Phytophthora* root rot, Phytotoxicity.

INTRODUCTION

Phytophthora root rot (PRR) caused by *Phytophthora cinnamomi* is one of the most important avocado diseases not only in Taiwan but worldwide, resulting in tree decline, yield reduction, and even tree death. Methods currently used for management of PRR include the following: selection of good drainage sites; usage of resistant rootstocks, pathogen-free materials, and mulch; and implementation of proper irrigation practices, tree nutrition, and chemical controls (Dann *et al*. 2013).

Phosphonate fungicides (salts and esters of phosphite) have been shown to be effective

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in the management of avocado PRR (Pegg *et al*. 1985; Whiley *et al*. 2001; Ann *et al*. 2006; Dann *et al*. 2013). In plants, phosphonate fungicides ultimately dissociate into the relevant cations and phosphite anions (hydrogen phosphite [HPO_3^{-2}] and/or dihydrogen phosphite [$H_2PO_3^-$]), the latter of which are the active components involved in the suppression of *P.* spp. (Guest & Grant 1991; McDonald *et al*. 2001; Dann & McLeod 2021). The specific mode of action whereby phosphite suppresses avocado PRR is currently largely unknown, but it has been suggested that a sufficient level of phosphite in roots is required to protect the trees from serious PRR (Giblin *et al*. 2007; Dann *et al*. 2017). For example, in Australia, concentrations of 25–40 μg g^{-1} phosphite in avocado roots are considered necessary for root protection based on field observations (Giblin *et al*. 2007), while another study showed that at least 80 μg g^{-1} is required (Dann *et al*. 2017). The "sufficient level" of phosphite to suppress PRR might vary under different situations, as it could be related to the sensitivity of *P. cinnamomi* isolates to phosphite and the innate resistance level of rootstocks to PRR (Dann *et al*. 2017; Belisle *et al*. 2019). Therefore, the amount of phosphite required for effective control of PRR needs to be investigated for different cultivars and regions. Nevertheless, it has not been determined for avocado cultivars planted in Taiwan.

In avocado, phosphonate fungicides can be applied in various ways, including soil drench, trunk injection, foliar spray, and bark spray. There are a few peer-reviewed and non-peer-reviewed studies comparing the efficacy of different application methods. Soil drenches of phosphite had proved effective for PRR management in avocado seedlings (Ann *et al*. 2006). However, a study conducted with 10-year-old avocado trees in the field suggested that soil drenches only provided short-term (11 wk) protection (Pegg *et al*. 1985). Giblin *et al*. (2007) compared the effect of phosphonate trunk injections and bark sprays and found that root phosphite levels were generally higher in injected trees, although bark sprays also provided sufficient levels for controlling PRR. Trunk injections during the seasons when feeder roots are relatively strong sinks have been shown to have preventative and curative effects on avocado PRR (Pegg *et al*. 1985; Dann *et al*. 2013). Because of labor costs and possible trunk damage caused by injections, foliar spray application is considered an effective alternative (Dann *et al*. 2013; McLeod *et al*. 2018). In fact, for long-term and cost-effective control of PRR in Australia and South Africa, phosphonate fungicides are most often applied as foliar sprays or trunk injections (Dann *et al*. 2013; Masikane *et al*. 2020).

In Taiwan, to our best knowledge, only few growers apply phosphite to manage avocado PRR disease. Unlike that in other countries, phosphite is not applied annually during specific seasons, but applied as a soil drench when avocado trees are found to be in decline or as a foliar spray to apparent healthy trees at a rate of 0.1% phosphite before the rainy seasons to prevent PRR (unpublished data). Moreover, instead of using commercially available phosphonate products, most growers make phosphite solutions by dissolving equal weights of industrial grade phosphorous acid and potassium hydroxide into water right before application (Ann 2001). However, in a preliminary field trial, we found that spraying phosphite at a rate of 0.1% had limited effects on elevating the root phosphite concentration, and increasing application rate could lead to unacceptable phytotoxic damage, but the root phosphite concentration still did not reach a level sufficient for suppressing PRR (unpublished data).

Though the phytotoxic effects of phosphite and its accumulation in avocado have been investigated in Australia, South Africa, and the United States of America (USA) (Ouimette & Coffey 1989; Whiley *et al*. 2001; Dann *et al*. 2017; Masikane *et al*. 2020), the scion and rootstock cultivars used in Taiwan are different from those used in the above-mentioned countries. Instead of 'Hass', cultivars widely planted in Taiwan include 'Hall' (「厚皃」), 'Choquette' (「秋殼」), 'Changan' (「章安」), 'CAES3' (「嘉選 3 號」), and 'Hung Shin Yuan' (「紅心圓」) (Liang *et al*. 2021), which are also often used as rootstocks. Different avocado cultivars might respond

differently to phosphite, but the phytotoxic effects of phosphite and its accumulation in avocado roots have never been assessed on the cultivars planted in Taiwan.

The efficacy of phosphite uptake via foliar spray can be enhanced by applying appropriate adjuvants. Hardy *et al*. (2001) demonstrated that, upon control of *P. cinnamomi* by foliar application of phosphite in natural ecosystems, adding an adjuvant to phosphite increased spray coverage, promoted spray retention, as well as reduced spray drift, evaporation, and wash-off. Rolando *et al*. (2014) analyzed the effects of four commercial adjuvants and showed that the organosilicone-based adjuvant enhanced the uptake of phosphite into *Pinus radiata*. Though foliar application of phosphite has been used on avocado in many countries, there have been limited studies investigating the effects of different adjuvants on the uptake of phosphite in avocado.

The overall objective of this study was to provide useful information for optimizing phosphite application strategies to manage avocado PRR. The first aim was to evaluate the foliar phytotoxicity of phosphite on various avocado cultivars when applied at different dosages and with different pH values. The second aim was to assess the effects of adjuvants on phytotoxicity and the uptake efficacy of phosphite into avocado. The third aim was to evaluate the relationship between phosphite concentrations in avocado roots and the control efficacy against PRR by using a detached root bioassay. The information obtained in this study will help to optimize the phosphite application strategies for avocado.

MATERIALS AND METHODS

Preparation of phosphite solutions, cultivation of avocado seedlings, and evaluation of phytotoxicity

Phosphite solutions were prepared by dissolving crystal phosphorous acid (H_3PO_3, Grace Fertilizer Co., Ltd., Taichung, Taiwan) in reverse osmosis water. The pH value was adjusted to 7.2 by using potassium hydroxide (KOH, Grace Fertilizer Co., Ltd.) unless otherwise stated.

Avocado cultivars analyzed included 'Choquette', 'Changan', 'Hung Shin Yuan', 'Zongpu Green Skin' (「中埔青皮」), 'Hall', and 'CAES3'. The seeds from each cultivars were grown in 4-inch pots in the greenhouse to generate the seedlings. To evaluate the phytotoxicity effects, avocado seedlings were sprayed until run off with 0%, 0.1%, 0.2%, or 0.5% phosphite solution, with each treatment involving five seedlings. After 2 wk, all leaves were cut from the plants and photographed together (Fig. 1). The total necrotic and unaffected areas of all leaves were then measured together by using Image J software (National Institute of Health, Bethesda, ML, USA) (Pride *et al*. 2020). Because the maximum total necrotic leaf area was about 25% of the total leaf area in this study, and the symptom looked severe when the necrotic leaf area was above 10%, the phytotoxicity score was rated on a scale of 0 to 4, where 0 = no necrosis, 1 = less than 2% necrotic leaf area, 2 = 25% necrotic leaf area, 3 = 5–10% necrotic leaf area, and 4 = more than 10% necrotic leaf area.

The experiment was conducted twice. In the first trial, the seedlings of 'Changan', 'Hung Shin Yuan', and 'Zongpu Green Skin' were 4–6 months old, while those of 'Choquette' were 13 months old. In the second trial, the seedlings of 'Changan', 'Hung Shin Yuan', 'Zongpu Green Skin', and 'CAES3' were 9–11 months old, while those of 'Choquette' and 'Hall' were 18 months old. The ambient temperatures in the greenhouse were recorded as 11–33℃ and 26–42℃ in the first and second trials, respectively. The ambient relative humidity was recorded as 39–94% in both trials.

Effect of pH on the phytotoxicity of phosphite sprays

To evaluate the effect of pH on the phytotoxicity of phosphite sprays, the seedlings of 'Hall', 'Zongpu Green Skin', and 'CAES3' were sprayed with 0.5% phosphite solution of different pH (6.5, 6.8, 7.2, or 7.5) and the phytotoxic ef-

Fig. 1. A representative photograph showing the pattern of phytotoxicity. The leaves were all from the same seedling, arranged from the oldest to the youngest in this photograph from top left to bottom right. This replicate seedling was 'CAES3' sprayed with 0.5% phosphite, in which the total necrotic area was calculated as 21%.

fects were evaluated as described in the previous section, with each treatment involving five replicate seedlings as well. The ambient temperature and relative humidity in the greenhouse were recorded as 18–40℃ and was 30–98%, respectively. The seedlings of 'Hall', 'Zongpu Green Skin', and 'CAES3' were about 16, 8, and 6 months old, respectively.

Inoculation of detached roots with *P. cinnamomi* zoospores and assessment of the colonization rate

The method for producing *P. cinnamomi* zoospores was adapted from Lonsdale *et al.* (1988). *P. cinnamomi* P69 was cultured on potato dextrose agar (PDA; Merck, Darmstadt, Germany) at 25℃ in darkness for 5 d. A Petri dish (9 cm in diameter) containing 2% V8 agar overlaid with moist miracloth (Merck, Darmstadt, Germany) was inoculated evenly with 10 pieces of agar blocks (2 × 2 mm) excised from the PDA culture. The plate was incubated in darkness for 7 d at 25℃, after which the miracloth was transferred to a 250 mL flask containing 100 mL of 2% V8 broth. The flask was shaken overnight in darkness at 25℃ at 160 rpm, and then

the miracloth was washed four times with 75 mL of mineral salt solution [0.01 M Ca $(NO_3)_2$, 0.005 M KNO_3, 0.004 M $MgSO_4$, and 1.3 mM Fe-EDTA] for 30 min at 160 rpm. After the final wash, 40 mL of the mineral salt solution was added to the flask, which was then shaken at 160 rpm overnight in darkness at 25℃. The miracloth was washed with 100 mL chilled (19℃) water, and then 10 mL of chilled water was added into the flask. The flask was incubated at 19℃ under light for 2–5 h to induce sporangium production and zoospore release. Zoospore concentration was quantified using a hemocytometer, and the concentration was adjusted to 1×10^4 zoospores mL^{-1} before use.

For the assessment of the colonization rate, three root tips (4 cm in length) were collected per seedling. After washing with tap water and rinsing with sterile water, the root tips were inoculated with *P. cinnamomi* P69 zoospores. The inoculation method was adapted from Van der Merwe & Kotzé (1994). Zoospore suspension (50 μL) was added to a 200 μL PCR tube. A detached avocado root tip was placed into the tube, with the root tip touching the bottom of the tube, and incubated in a moisture chamber at 25℃ for 2 h. Subsequently, the roots were transferred to Petri dishes with moist filter paper and incubated at 25℃ for 2 d. Colonization rates were determined by aseptically cutting the root tip into 10 segments (each ≈ 4 mm in length) after surface disinfection for 10 s in 70% ethanol. The root segments were then plated sequentially on a selective medium, PARPNB-PDA (PDA containing 10 mg L^{-1} pimaricin, 200 mg L^{-1} ampicillin, 8 mg L^{-1} rifampicin, 10 mg L^{-1} pentachloronitrobenzene, 50 mg L^{-1} nystatin, and 25 mg L^{-1} a.i. benomyl), adapted from Jung *et al.* (2000). After incubation at room temperature for 2 d, the number of segments from which *P. cinnamomi* grew was counted. The colonization rate was calculated using the following formula: colonization (%) = (the number of segments colonized/10) × 100%. The average percentage of three root tips from one seedling was shown as the value for one replicate.

Effects of adjuvants on phytotoxicity, root phosphite accumulation, and *P. cinnamomi* colonization

'Hall', 'Choquette', and 'Changan' seedlings were sprayed until runoff with 0%, 0.2%, or 0.5% phosphite solution with 0.05% (v/v) adjuvant: Jia-Shou-Huo-Jhan (加收活展, JSHJ) (Sinon Co., Taichung, Taiwan) or S-408 (Microgreen, Ltd., Taichung, Taiwan). The labeled composition of JSHJ was "blend of polyethylene alkyl aryl ether and sodium salt of dialkyl sulfosuccinate" and that for S-408 was "10% silicone-isohexadecyl". Phosphite solutions without the adjuvants were also applied. There were five seedlings in each treatment. Two weeks after the treatment, the phytotoxic effects were assessed as aforementioned. Because growers are advised not to spray phosphite during leaf flush, phytotoxicity scores were also rated only on mature leaves.

In the trails using 'Choquette' and 'Changan', three root tips were collected per seedling for the assessment of *P. cinnamomi* colonization rate as aforementioned. The rest of the roots were sent to SGS Taiwan Ltd. for phosphite extraction and quantification. Because of technical limitations, at least 5 g of dry roots were required for processing each sample. Therefore, the roots from five replicates in each treatment were combined as one sample to be analyzed.

The ambient temperatures and relative humidity were recorded as 16–39℃/43–94%, 10–37℃/29–94%, and 15–40℃/27–92% in the trials of 'Hall', 'Choquette', and 'Changan', respectively. The seedlings of 'Hall', 'Choquette', and 'Changan' were about 15, 14, and 6 months old, respectively, in this experiment.

Quantification of phosphite in avocado roots

Roots obtained as described in the previous section were washed with tap water, blotted dried, and weighed to measure the fresh weights. Subsequently, they were placed in a brown paper bag, and dried at 50℃ in an oven for 5 d, followed by

measurement of the dry weights. Then, the dried roots were sent to SGS Taiwan Ltd. for phosphite extraction and quantification. The analytical method was adapted from McLeod *et al*. (2018) with the following modifications: (1) In the last step of phosphite extraction, the filtrate was centrifuged at 14,000 g for 40 min instead of 20 min. (2) For the liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis, the mobile phase consisted of solvent A (0.1% formic acid and 5 mM ammonium acetate in water) and solvent B (0.1% formic acid and 5 mM ammonium acetate in methanol). The LC gradient was as follows: 0 min, 99% A and 1% B; 2.5 min, 50% A and 50% B; 8.5 min, 30% A and 70% B; 12.5 min, 0% A and 100% B; 15.5 min, 0% A and 100% B; 15.6 min, 99% A and 1% B; and 18 min, 99% A and 1% B, with a flow rate of 0.3 mL min^{-1}. The column temperature was held at 50℃. The injection volume was 3 μL. The electrospray ionization source conditions in the negative ionization mode were as follows: a capillary voltage of 2.5 kV, source temperature of 150℃, and desolvation temperature of 400℃. The cone and desolvation gas flow rates were 150 and 800 L h^{-1}, respectively. Phosphite was detected using multiple reaction monitoring mode with the 80.9 > 63 transition at a collision energy of 20 eV. A recovery rate of 90% was used to adjust the LC/MS-MS value. Because most previous studies presented phosphite concentrations based on fresh weight (McLeod *et al*. 2018), the dry weight phosphite concentration was converted to fresh weight concentration according to the moisture content of each sample (85–90%). The total root phosphite quantity for each sample was then obtained by multiplying the root phosphite concentration by fresh weight.

Effect of adjuvants and pH on the phytotoxicity of phosphite sprays

'Zongpu Green Skin' and 'CAES3' were used for evaluating the effect of pH on the phytotoxicity when phosphite was applied with an adjuvant. Seedlings were sprayed with 0.5% phosphite plus 0.05% JSHJ solution at different pH values (6.5, 6.8, 7.2, and 7.5) until runoff. There were five replicate seedlings in each treatment. The ambient temperature and relative humidity in the greenhouse were recorded as 17–42℃ and 41–93%, respectively. The seedlings of 'Zongpu Green Skin' and 'CAES3' were about 8 and 6 months old, respectively, in this experiment.

Data analysis

The phytotoxicity scores were analyzed by using RStudio version 2022.02.3 (PBC, Boston, MA, USA). A Kruskal-Wallis test with a significance level of $P = 0.05$ was used to compare distributions of scores across treatments. If the Kruskal-Wallis test was significant, post-hoc analysis was performed using the Holm-corrected Dunn test to analyze differences between treatments.

The root colonization percentage data were transformed by arcsine transformation and subjected to one-way analysis of variance (ANOVA) followed by Fisher's least significant difference (LSD) test using SAS Enterprise Guide version 7.15 (SAS Institute Inc., Cary, NC, USA).

RESULTS

Phytotoxicity of phosphite sprays on different cultivars of avocado

Prior to phosphite application, all the avocado seedlings were healthy without any symptom of foliar necrosis. One day after the seedlings were sprayed with 0.2% or 0.5% phosphite, leaf discoloration, the initial symptom of phytotoxic effect, appeared on the margins of young leaves (Fig. 2A). Two days after application, the discolored area expanded and turned darker (Fig. 2B). Five days after application, the discolored area no longer expanded but dried up. Moreover, some young leaves became distorted due to the phytotoxicity of phosphite (Fig. 2C). Mild phytotoxic damage was observed as leaf tip burning and small necrotic spots on leaves (Fig. 2D), while large, dried areas developed in the case of more severe phytotoxic damage (Fig. 2E). Abscis-

sion of the dried spots was also observed in some cases (Fig. 2F). In addition, the degree of phytotoxicity might vary from leaf to leaf on the same seedling. Generally, younger leaves tended to be more susceptible to phosphite phytotoxicity and had higher percentages of necrotic area (Fig. 1).

In the first trial to analyze the phytotoxic effects of phosphite sprays, application of 0.1%, 0.2%, and 0.5% phosphite caused mild or no leaf burn on all cultivars (Table 1). However, the phytotoxic damage was more severe in the second trial. Phosphite applied at 0.5% caused mild leaf burn on 'Hung Shin Yuan', 'Zongpu Green Skin' and 'CAES3' (phytotoxicity score < 2), while it caused over 2% (score 2) necrotic leaf area on 'Choquette' and 'Hall', and over 5% (score 3) necrotic leaf area on 'Changan' (Table 1).

Effect of pH on the phytotoxicity of phosphite sprays

Assays to determine the phytotoxic effects of phosphite solutions with various pH values on avocado were conducted with 0.5% phosphite on 'Hall', 'Zongpu Green Skin', and 'CAES3'. The phytotoxicity scores of 'Hall' and 'Zongpu Green Skin' were close to values obtained from assays of 0.5% phosphite without adjuvant in Table 1; the score for 'Hall' was about 2.4, while that for 'Zongpu Green Skin' was about 1–2. However, the average phytotoxicity score of 'CAES3' (3.2) was higher in this experiment compared to that shown in Table 1 (1.0 from Trial 2). There was no significant difference in the average phytotoxicity scores among different pH values for each cultivar as determined by the Kruskal-Wallis test (Table 2).

Effects of adjuvants on phytotoxicity, root phosphite accumulation, and *P. cinnamomi* colonization

The average phytotoxicity scores were significantly lower for 'Hall' seedlings sprayed

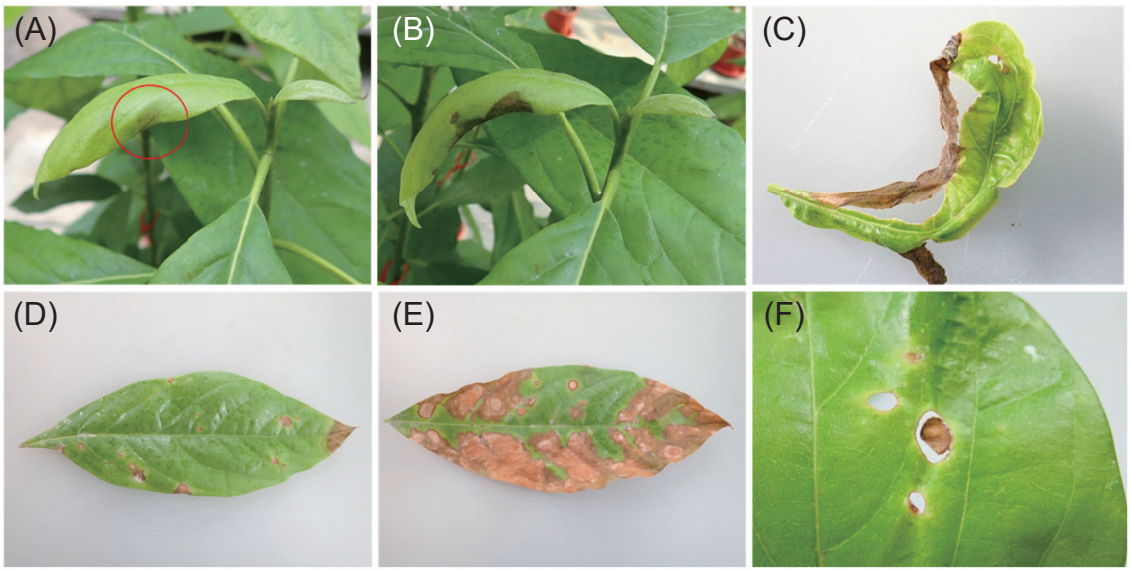

Fig. 2. Symptoms of phytotoxic damage caused by phosphite sprays on avocado leaves. (A) One day after application of a phosphite foliar spray, initial discoloration could be observed on the margins of new leaves of 'Choquette' sprayed with 0.2% phosphite. (B) Two days after application, the discolored area turned darker and the area enlarged. (C) New leaves of 'Zongpu Green Skin' sprayed with 0.5% phosphite distorted due to phytotoxicity. (D) Leaf tip burning and small necrotic spots observed on 'Zongpu Green Skin' leaves showing mild phytotoxicity damage from 0.5% phosphite. (E) Leaves of 'CAES3' with large necrotic areas, indicating severe phytotoxicity damage from 0.5% phosphite. (F) Abscission of the necrotic spots from 'Choquette' sprayed with 0.5% phosphite.

Table 1. Phytotoxic effects of phosphite sprays on various avocado cultivars.

	Phytotoxicity score (M ± SD)[z]					
Phosphite app. rate (%)	'Choquette'	'Changan'	'Hung Shin Yuan'	'Zongpu Green Skin'	'Hall'	'CAES3'
Trial 1						
0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	not tested	not tested
0.1	0.2 ± 0.4	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0	not tested	not tested
0.2	0.2 ± 0.4	0.4 ± 0.5	0.2 ± 0.4	0.0 ± 0.0	not tested	not tested
0.5	0.8 ± 0.4	1.0 ± 0.0	1.2 ± 0.4	1.0 ± 0.7	not tested	not tested
Trial 2						
0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
0.1	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0	0.2 ± 0.4	0.4 ± 0.5
0.2	1.0 ± 0.0	1.0 ± 0.7	1.0 ± 0.7	1.0 ± 0.0	0.8 ± 0.4	0.8 ± 0.4
0.5	2.0 ± 1.2	3.0 ± 1.4	1.2 ± 1.4	1.8 ± 0.8	2.4 ± 1.3	1.0 ± 0.0

[z] Mean (M) and standard deviation (SD) were derived from five replicates.

Table 2. Phytotoxic effects of 0.5% phosphite sprays with various pH values on avocado.

	Phytotoxicity score (M ± SD)[zy]				
	Without adjuvant			With JSHJ[x]	
pH value	'Hall'	'Zongpu Green Skin'	'CAES3'	'Zongpu Green Skin'	'CAES3'
6.5	2.8 ± 1.3	1.2 ± 0.4	3.0 ± 1.4	1.0 ± 0.0	0.8 ± 0.4
6.8	2.8 ± 0.4	1.0 ± 0.7	3.2 ± 0.4	1.0 ± 0.0	1.0 ± 0.0
7.2	2.4 ± 0.9	1.0 ± 0.7	3.2 ± 0.8	0.8 ± 0.4	1.0 ± 0.0
7.5	3.8 ± 0.4	1.2 ± 0.4	2.4 ± 0.5	1.0 ± 0.0	1.0 ± 0.0

[z] Mean (M) and standard deviation (SD) were derived from five replicates.
[y] No significant difference among the average phytotoxicity scores of different pH values for each cultivar as determined by the Kruskal-Wallis test.
[x] JSHJ: Jia-Shou-Huo-Jhan (加收活展).

with 0.5% phosphite plus JSHJ or S-408 as compared with those sprayed with 0.5% phosphite without adjuvant (P = 0.036 and 0.014, respectively) (Table 3). The average phytotoxicity score for applying 0.2% phosphite plus S-408 was also significantly lower than that with 0.2% phosphite without adjuvant (P = 0.029) (Table 3). The adjuvants had less effect on phytotoxicity on 'Choquette' and 'Changan', which were less susceptible to phosphite phytotoxicity than 'Hall'. The addition of either JSHJ or S-408 adjuvant reduced phytotoxicity scores for both the 0.2% and 0.5% phosphite sprays, but only the 0.5% phosphite spray with JSHJ or S-408 on mature leaves of 'Choquette' significantly dropped the scores (Table 4). In contrast, analysis performed with 'Changan' indicated that only the 0.2% phosphite spray plus S-408 significantly lowered the scores (Table 5). In some plants, phytotoxic damage was observed only on immature leaves. In 'Choquette', no phytotoxic damage was observed on mature leaves of seedlings treated with phosphite plus adjuvant sprays. Application of JSHJ or S-408 alone caused no phytotoxic damage.

In control plants without phosphite spray application, the root phosphite concentrations were very low, with 1.6 and 1.7 μg g^{-1} for 'Choquette' (Table 4) and 'Changan' (Table 5), respectively. In contrast, the root phosphite concentrations and quantities were all elevated by over

Table 3. Effect of adjuvants on the phytotoxicity on 'Hall' seedlings.

Phosphite app. rate (%)	Adjuvant	Phytotoxicity score (M ± SD)[z]
0.0	-	0.0 ± 0.0
0.2	-	0.8 ± 0.4 a
0.2	JSHJ[y]	0.2 ± 0.4 ab
0.2	S-408	0.0 ± 0.0 b
0.5	-	2.2 ± 0.8 a
0.5	JSHJ	1.0 ± 0.0 b
0.5	S-408	0.8 ± 0.4 b

[z] Mean (M) and standard deviation (SD) were derived from five replicates. Values with the same phosphite application rate followed by different letters are significantly different according to Holm-corrected Dunn test ($P < 0.05$).
[y] JSHJ: Jia-Shou-Huo-Jhan (加收活展).

Table 4. Effects of adjuvants on phytotoxicity, root phosphite accumulation, and *Phytophthora cinnamomi* colonization on 'Choquette' seedlings.

Phosphite app. rate (%)	Adjuvant	Phytotoxicity score (M ± SD)[z]	Phytotoxicity score on mature leaves (M ± SD)[z]	Root phosphite conc. (μg g^{-1})	Total root phosphite quantity (mg)[y]	Root colonization (M ± SD, %)[x]
0.0	-	0.0 ± 0.0	0.0 ± 0.0	1.6	0.1	60.0 ± 15.5 a
0.0	JSHJ[w]	0.0 ± 0.0	0.0 ± 0.0	not tested	not tested	not tested
0.0	S-408	0.0 ± 0.0	0.0 ± 0.0	not tested	not tested	not tested
0.2	-	0.4 ± 0.5 a	0.4 ± 0.5 a	18.7	1.4	46.7 ± 20.5 ab
0.2	JSHJ	0.2 ± 0.4 a	0.0 ± 0.0 a	78.7	7.3	44.0 ± 23.5 ab
0.2	S-408	0.0 ± 0.0 a	0.0 ± 0.0 a	18.8	2.2	41.3 ± 8.0 ab
0.5	-	1.0 ± 0.7 a	1.0 ± 0.7 a	17.2	1.8	36.0 ± 14.4 b
0.5	JSHJ	0.4 ± 0.5 a	0.0 ± 0.0 b	111.8	10.9	35.3 ± 9.6 b
0.5	S-408	0.4 ± 0.5 a	0.0 ± 0.0 b	78.7	4.8	28.0 ± 11.7 b

[z] Mean (M) and standard deviation (SD) were derived from five replicates. Values with the same phosphite application rate followed by different letters are significantly different according to Holm-corrected Dunn test ($P < 0.05$).
[y] The total quantity of phosphite from five replicate seedlings in each treatment.
[x] M and SD were derived from five replicates. Values followed by different letters are significantly different according to Fisher's least significant difference test ($P < 0.05$).
[w] JSHJ: Jia-Shou-Huo-Jhan (加收活展).

10 folds after treatment with phosphite spray. In addition, the quantities of phosphite were all higher in 'Choquette' (Table 4) and 'Changan' (Table 5) seedlings treated with phosphite plus adjuvants compared to treatment with phosphite at the same rate without adjuvants.

Generally, the addition of JSHJ yielded higher root phosphite quantities than S-408 under the same phosphite application rate, and the treatments with 0.5% phosphite yielded higher root phosphite quantities than those with 0.2% phosphite with the same adjuvant. The only one exception was 'Changan' seedlings sprayed with 0.5% phosphite plus JSHJ, in which the root phosphite quantity (6.9 mg) was lower than that of seedlings sprayed with 0.2% phosphite plus JSHJ (9.2 mg) or 0.5% phosphite plus S-408 (8.5 mg). In 'Choquette', treatment with 0.5% phosphite plus JSHJ yielded the highest root phosphite concentration (111.8 μg g^{-1}) and quantity (10.9 mg), while in 'Changan', treatment with 0.2% phosphite plus JSHJ yielded the highest root phosphite quantity (9.2 mg), and treatment with

Table 5. Effects of adjuvants on phytotoxicity, root phosphite accumulation, and *Phytophthora cinnamomi* colonization on 'Changan' seedlings.

Phosphite app. rate (%)	Adjuvant	Phytotoxicity score (M ± SD)[z]	Phytotoxicity score on mature leaves (M ± SD)[z]	Root phosphite conc. (μg g^{-1})	Total root phosphite quantity (mg)[y]	Root colonization (M ± SD, %)[x]
0.0	-	0.0 ± 0.0	0.0 ± 0.0	1.7	0.1	62.7 ± 27.5 a
0.0	JSHJ	0.0 ± 0.0	0.0 ± 0.0	not tested	not tested	not tested
0.0	S-408	0.0 ± 0.0	0.0 ± 0.0	not tested	not tested	not tested
0.2	-	0.8 ± 0.4 a	0.6 ± 0.5 a	35.2	1.6	39.3 ± 23.3 abc
0.2	JSHJ	0.4 ± 0.5 ab	0.2 ± 0.4 a	183.1	9.2	18.7 ± 11.9 cd
0.2	S-408	0.0 ± 0.0 b	0.0 ± 0.0 a	104.0	5.8	40.0 ± 13.9 abc
0.5	-	1.2 ± 0.4 a	1.2 ± 0.4 a	108.5	5.7	42.0 ± 20.6 ab
0.5	JSHJ	1.0 ± 0.0 a	1.0 ± 0.0 a	199.1	6.9	13.3 ± 8.5 d
0.5	S-408	1.0 ± 0.0 a	1.0 ± 0.0 a	190.8	8.5	29.3 ± 15.9 bcd

[z] Mean (M) and standard deviation (SD) were derived from five replicates. Values with the same phosphite application rate followed by different letters are significantly different according to Holm-corrected Dunn test ($P < 0.05$).
[y] The total quantity of phosphite from five seedlings in each treatment.
[x] M and SD were derived from five replicates. Values followed by different letters are significantly different according to Fisher's least significant difference test ($P < 0.05$).

0.5% phosphite plus JSHJ yielded the highest root phosphite concentration (199.1 μg g^{-1}) (Tables 4 and 5).

The root colonization rates were significantly lower in the 'Choquette' seedlings sprayed with 0.5% phosphite alone, 0.5% phosphite plus JSHJ, or 0.5% phosphite plus S-408 as compared with the control, and the root phosphite concentrations ranged from 17.2 to 111.8 μg g^{-1} for these treatments. The three treatments with 0.2% phosphite resulted in a similar range of root phosphite concentrations (18.7–78.7 μg g^{-1}), but the root colonization rates were not significantly lower than that of the control (Table 4). In the 'Changan' seedlings sprayed with 0.2% phosphite plus JSHJ, 0.5% phosphite plus JSHJ, or 0.5% phosphite plus S-408, the average root colonization rates were all lower than 30%, which was significantly different from that in the control (62.7%), and the root phosphite concentrations were all above 180 μg g^{-1} (Table 5).

Effects of adjuvants and pH on the phytotoxicity of phosphite sprays

When 0.5% phosphite was applied along with the adjuvant JSHJ, the average phytotoxicity scores were 1.0 or 0.8 for all pH values for both 'Zongpu Green Skin' and 'CAES3' seedlings, and there were no significant differences in the average phytotoxicity scores among the treatments with pH values ranging from 6.5 to 7.5, as determined by the Kruskal-Wallis test (Table 2).

DISCUSSION

Instead of being uniformly planted with the 'Hass' cultivar, most avocado orchards in Taiwan are planted with various cultivars. Therefore, at the beginning of this study, various cultivars were sprayed with phosphite solutions at different rates to evaluate the phytotoxic effects of phosphite on them. The results suggested that susceptibility to phosphite might vary among different cultivars. As there are diverse cultivars planted in Taiwan, more than what were included in this study, the susceptibility of more avocado cultivars to phosphite needs to be investigated in the future. Besides, genetically identical grafted plants should be used for the assessment of susceptibility to provide more reliable results. Before then, growers should test the application rate on a small scale before

applying it to the whole orchard. In addition, the phytotoxic damage was more severe in the second trial, in which the ambient temperature in the greenhouse was higher than that in the first trial. The results suggested that the occurrence of phytotoxicity would increase when phosphite was applied under high temperature (above 35℃). Therefore, growers should be more careful when applying foliar phosphite sprays under high temperature.

An inconsistent result in this study was that the average phytotoxicity score of 'CAES3' sprayed with 0.5% phosphite was only 1.0 as shown in Table 1, but it was 3.2 in Table 2. One possible explanation for this discrepancy is that the 'CAES3' seedlings were older in the former experiment (10 months old) than in the latter (6 months old). The effects of plant age on phytotoxicity should be investigated in future studies to test this hypothesis.

It is suggested that the most effective times to apply phosphite are the stages when the spring and summer flush leaves have matured because feeder roots are acting relatively as stronger sinks at these two stages (Dann *et al*. 2013). In this study, we also found that phosphite could cause deformation of immature leaves. The findings further support the advice that growers should avoid applying phosphite when there are immature leaves on the trees.

In Taiwan, most growers make phosphite solutions by dissolving industry grade H_3PO_3 and KOH at equal weights into water right before application. However, making the solution with different sources of water might result in different pH values. For example, the pH values of phosphite solutions made by dissolving equal weights of H_3PO_3 and KOH into reverse osmosis water (pH 6.8), underground water (pH 7.4), and a different source of underground water (pH 7.9) were 6.45, 6.5, and 7.5, respectively (unpublished data). To provide guidelines for growers for preparation of phosphite solutions, whether it is necessary to adjust the pH value to a certain range was investigated in this study. Whiley *et al*. (2001) compared the phytotoxicity of foliar-applied phosphite solutions with different pH values ranging from 6.8 to 7.6 and showed that the least phytotoxic damage on 'Hass' leaves was observed at pH 7.2. Therefore, it was suggested that foliar-applied phosphite solutions be buffered to pH 7.2 before application (Dann *et al*. 2013). However, we found no significant difference among phosphite treatments with different pH values ranging from 6.5 to 7.5. One possible explanation for this discrepancy is that the sensitivity of the cultivars used in this study to phosphite solutions with various pH values was different from that of 'Hass'. Another possible explanation is that the sensitivity of seedlings in the greenhouse to phosphite was different from that of mature trees in the field. Further studies should be conducted in the field with 'Hass' and grafted mature trees to test these hypotheses.

During the experiments, we observed that phosphite sprays formed droplets on the leaves due to their slight hydrophobicity, and the droplets left sticky patches when they dried up. Therefore, we speculate that the uneven distribution of phosphite on leaves might increase the risk of phytotoxicity and that prompted us to investigate the possibility of adding adjuvants to reduce the phytotoxic damage.

Though the effect of adding adjuvants into foliar phosphite sprays has been tested in a few studies (Hardy *et al*. 2001; Rolando *et al*. 2014), very few studies focused on the effects of adjuvants on avocado. Ouimette & Coffey (1989) added 0.1% Triton B-1956 as an adjuvant to a foliar spray of potassium phosphonate, but there was no comparison of the phosphite uptake between the treatments with or without the adjuvant. A non-peer-reviewed study showed that applying phosphite with the adjuvant Agral® or Nufilm® significantly increased the risk of phytotoxicity (Whiley *et al*. 2001). To our best knowledge, the present study is the first one investigating the effect of an adjuvant on phytotoxicity and uptake of phosphite on avocado simultaneously. Our

study showed that adding adjuvants not only decreased the risk of phytotoxicity, but also increased the uptake of phosphite into roots. In the experiments with 'Choquette' and 'Changan', spraying 0.2% phosphite containing adjuvants even resulted in a higher total root phosphite quantity than spraying 0.5% phosphite without adjuvant, which means less chemical would be needed if spraying with adjuvants and the cost of application could be reduced. However, it is possible that different adjuvants might have different effects on phytotoxicity when applied with phosphite, but only two adjuvants were tested in this study. The effect of more other adjuvants warrants further investigation. As there is a wide range of active ingredients in adjuvants, and some of them, such as Agral® or Nufilm®, might increase the risk of phytotoxicity (Whiley *et al*. 2001), growers should test combinations on a small scale before applying them to the whole orchard.

The root phosphite concentrations were all relatively lower in 'Choquette' than those in 'Changan' seedlings treated with phosphite. It is possible that different cultivars might have different abilities to accumulate phosphite in roots, which was suggested by Dann *et al*. (2017). This variation could also possibly result from the differences in root mass between the two cultivars. The root fresh weight per treatment (five seedlings) for 'Choquette' was 60–120 g but was 30–60 g for 'Changan' (data not shown). As 'Choquette' seedlings had greater root mass than 'Changan', the absorbed phosphite might have been diluted to a lower concentration. This could also explain why spraying 'Choquette' with 0.2% phosphite alone yielded the same phosphite concentration as that with 0.2% phosphite plus S-408: the seedlings in the latter treatment happened to have a greater root mass. This observation might imply that the ratio of roots to leaves could influence the final concentration of root phosphite.

Generally, the treatments with JSHJ yielded a higher total root phosphite quantity than with S-408 under the same phosphite application rate, which means JSHJ might be a better adjuvant than S-408 when only their ability to enhance uptake efficiency is considered. The finding that spraying 'Changan' with 0.5% phosphite plus JSHJ did not result in a higher root phosphite quantity than spraying with 0.2% phosphite plus JSHJ or 0.5% phosphite plus S-408 is confusing. One possible explanation is that seedlings in this treatment happened to have less leaf area for absorbing phosphite. Despite the inconsistency, phosphite uptake was consistently enhanced by adding adjuvants in this study.

In the detached root bioassay, the root phosphite concentrations of 'Changan' seedlings were all above 180 $\mu g\ g^{-1}$ for the 0.2% phosphite plus JSHJ, and 0.5% phosphite plus JSHJ or S-408 treatments, in which the root colonization rates were all significantly lower compared with those of the control. This result might suggest that a high concentration of phosphite in roots is necessary for controlling PRR. However, the "sufficient" level of root phosphite concentration still could not be determined, because the colonization rates between the 0.2% phosphite and 0.5% phosphite plus S-408 treatments were not significantly different, even though there was a difference of 155.6 $\mu g\ g^{-1}$ in the root phosphite concentration between them.

The relationship between the root phosphite concentration and colonization rate in the experiment with 'Choquette' seedlings was also inconsistent, as treatments resulting in the same root phosphite levels had significantly lower colonization rates than the control in some cases but not in others. A possible explanation is that none of the root phosphite levels in 'Choquette' seedlings treated with phosphite sprays reached a level sufficient to make a difference in control efficacy. In addition, because of technical and financial limitations (approximately 195 USD per sample), the roots from each treatment were mixed together for phosphite quantification, instead of each replicate seedling being analyzed separately.

The variation of root phosphite concentration among replicate seedlings could be high because the root mass of each seedling was different, and this might result in the inconsistency between the root phosphite concentration and colonization rate. It is also possible that other mechanisms such as induced resistance might be involved. More research needs to be conducted to clarify the relationship between root phosphite concentration and colonization rate and to investigate the specific mechanisms relating to how phosphite inhibits colonization and that will be useful for managing avocado PRR.

Phosphite is frequently used as an environmentally friendly protectant for controlling various plant diseases in Taiwan, but no grower quantifies phosphite concentration in plant tissues before or after application, mostly because there is no facility providing phosphite quantification service in Taiwan, and phosphite is thought to control diseases by inducing resistance at low concentrations (Ann 2001). We propose that quantification of phosphite in avocado is important for the following reasons. Firstly, there is evidence that a sufficient level of phosphite in roots is required to suppress avocado PRR (Giblin *et al*. 2007; Dann *et al*. 2017). In fact, the current strategy of phosphonate application in Australia involves quantifying phosphite concentration in feeder roots before and after application for growers to determine whether they should apply or re-apply phosphonate fungicides, which might be a good model for phosphite application in Taiwan. Secondly, although potassium phosphonates are considered harmless to human health, some countries still set a maximum residue limit (MRL) for potassium phosphonate products in avocado. For example, the European Food Safety Authority (EFSA) considers an acceptable daily intake (ADI) of phosphonic acid to be 1 mg kg^{-1} applicable, and the MRL of phosphonic acid in avocado was set at 50 mg kg^{-1} (European Food Safety Authority *et al*. 2020). Though in Taiwan there is no MRL set for avocado in the domestic market and no avocado is currently exported, accurate phosphite quantification techniques might become necessary in the future, especially if the industry intends to pursue foreign markets.

Several analytical methods have been published for quantifying phosphite in plant tissues, including high performance ion chromatography, LC-MS/MS, and gas chromatography–mass spectrometry (Roos *et al*. 1999; Barrett *et al*. 2003; Berkowitz *et al*. 2011; McLeod *et al*. 2018). The advantages and disadvantages of these methods have been reviewed in McLeod *et al*. (2018). LC-MS/MS was chosen as the analytical method in this study because it is used by commercial laboratories for quantifying phosphite in avocado roots in Australia and New Zealand. This study adapted and introduced the analytical method for phosphite to a commercial laboratory (SGS Taiwan Ltd.) in Taiwan. We hope that sending roots for phosphite quantification will become a common practice for avocado growers to optimize the application of phosphite in the future.

In conclusion, this study suggested that: (1) the susceptibility to phosphite phytotoxicity might vary among different avocado cultivars; (2) the pH value of a phosphite spray might have limited effect on phytotoxicity when the solution is buffered to a pH between 6.5 and 7.5; and (3) adding adjuvants might reduce the risk of phytotoxicity and increase the uptake of phosphite. Since the experiments were all conducted with non-grafted seedlings in the greenhouse, they might not represent exactly the effects on grafted mature trees in the field. Therefore, future studies with mature trees are warranted. The analytical method used for phosphite quantification and the results derived from this study could provide important information for optimization of phosphite application strategies and future studies on managing avocado PRR.

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不同亞磷酸配方對酪梨之藥害程度、亞磷酸吸收及根腐病防治效果之影響

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摘要

梁鈺平、倪蕙芳。2023。不同亞磷酸配方對酪梨之藥害程度、亞磷酸吸收及根腐病防治效果之影響。台灣農業研究 72(2):81–96。

由 *Phytophthora cinnamomi* 造成之根腐病 (*Phytophthora* root rot) 為台灣酪梨最重要之病害。雖然在國外，以葉面噴施亞磷酸防治酪梨根腐病由來已久，但針對國內酪梨品種，至今仍缺乏有效施用亞磷酸劑量及技術之研究。為優化亞磷酸防治酪梨根腐病施用策略所需資訊，本研究以國內常見品種之酪梨苗測試不同亞磷酸配方對酪梨葉片藥害及亞磷酸吸收之影響。實驗結果顯示，於測試的 6 個品種 (「秋穀」、「章安」、「紅心圓」、「中埔青皮」、「厚皃」及「嘉選三號」) 上，葉面噴施 0.1% 及 0.2% 之亞磷酸於酪梨苗造成之藥害均極輕微 (藥害級數 < 2)，而 0.5% 亞磷酸則可能於高溫下對「秋穀」、「章安」及「厚皃」造成嚴重藥害 (藥害級數 ≥ 2)。若亞磷酸已中和至 pH 值介於 6.5–7.5 之間，則 pH 值對藥害程度影響無顯著差異。於「章安」之離根接種實驗結果顯示，噴施 0.2% 亞磷酸 + 加收活展、0.5% 亞磷酸 + 加收活展，或 0.5% 亞磷酸 + S-408 處理之根系 *P. cinnamomi* 感染率均顯著低於對照組，且此些處理酪梨根系之亞磷酸濃度均大於 180 μg g^{-1}。此外，於亞磷酸溶液中添加展著劑 (加收活展或 S-408) 可減少藥害並增加根部對亞磷酸之吸收。本研究結果有助於優化亞磷酸在酪梨產業的應用策略。

關鍵詞：酪梨、展著劑、亞磷酸、根腐病、藥害。

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