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Calcium and Silicon Fertiliser Influence on Fungicide Efficacy Against *Guignardia* Leaf Blotch and Apple Scab Management

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Abstract. Management of fungal diseases within urban landscapes relies heavily on repeat fungicide sprays. Environmental concerns have led to a need to eliminate or reduce fungicide use. Foliar sprays of calcium- (Ca) and silicon- (Si) based fertilisers have been shown to reduce symptom severity of several fungal pathogens. The aim of our research was to evaluate the influence of Ca chloride, Ca metasolate, tetra ethyl silicate, and potassium silicate fertilisers, applied independently and in combination with a synthetic fungicide (penconazole) at full and half strength, on apple scab (*Venturia inaequalis*) and *Guignardia* leaf blotch (*Guignardia aesculi*) symptom severity, as well as leaf chlorophyll content, fruit yield, and crown volume. Trials used container-grown *Aesculus hippocastanum* and field-grown *Malus domestica* cv. 'Golden Delicious'. Applications of Ca, Si, and penconazole sprays alone significantly reduced scab and leaf blotch severity compared to water-sprayed controls; however, a significant difference between the type of Ca and Si fertilisers was recorded. A combined mix of a Ca or Si fertiliser with a full or half dose of penconazole was more effective at reducing symptom severity of both foliar pathogens than a full or half dose of penconazole alone. Data analysed with Limpel's formula indicated positive synergistic effects between Ca and Si and penconazole in some, but not all, cases. The integration of Ca and Si foliar sprays as an alternative to, or additive with, penconazole for scab and leaf blotch management appears feasible based on results of our studies and may have applicability against other fungal pathogens.

Keywords. Disease Management; Fruit; Holistic Approach; Integrated Pathogen Management; Pathogen Control; Plant Health Care; Urban Landscapes.

INTRODUCTION

Foliar pathogens such as apple scab (*Venturia inaequalis*) and *Guignardia* leaf blotch (*Guignardia aesculi*, horse chestnut leaf blotch) represent a worldwide problem for ornamental trees planted into UK, US, and European landscapes (Hersh et al. 2012; Hantsch et al. 2013, 2014; Percival 2018). Heavy disease incidence can result in premature leaf drop, increased rate of crown dieback, and a reduction in carbon allocation to fine roots, trunk, and twigs (Villalta et al. 2004; Hantsch et al. 2013; Oliva et al. 2014). Repeat annual defoliations and subsequent loss of photosynthetic productivity can result in tree decline and sometimes death, resulting in expensive tree replacement costs (Tello et al. 2005; Oliva et al. 2014; Stravinskienė et al. 2015; Percival 2018). In addition, premature defoliation makes a tree aesthetically undesirable, and many of the functional benefits urban trees provide (absorbing pollutants and dust, UV protection through shading, evaporative cooling) are significantly reduced (Morgenroth et al. 2016; Vogt et al. 2017). To prevent foliage loss and reduction of functional qualities, repeat fungicide sprays are usually undertaken (Carisse and Dewdney 2002; Berrie and Xu 2003; Hailey and Percival 2014). However, new approaches to scab management are needed, as strains of *V. inaequalis* have developed resistance to synthetic fungicides. Similarly, public concern has increased over fungicide residues, and arborists manage trees in densely populated areas where potential fungicide contact with pedestrians could lead to litigation claims (Christiansen et al. 1999; Carisse and Dewdney 2002; Patocchi et al. 2004).

Calcium (Ca) is a major macronutrient in trees, important to the structural integrity of cell walls and plasma membranes (Khalifa et al. 2009; Fromm

2010). There is also scientific evidence showing that enhancing Ca concentrations in leaf, stem, and root tissue of plants with calcium fertilisers can reduce the severity of symptoms caused by several pathogenic fungi and bacteria (Springer et al. 2007; Percival and Haynes 2009). Pertinent examples include *Erwinia carotovora* subsp. *amylovora*, *Phoma exigua* (gangrene), *Fusarium solani*, *Botrytis cinerea* and *Neonectria galligena* of apple, *Monilinia fructicola* (brown rot of peach), *Venturia pirina* (pear scab), and *Guignardia* leaf blotch (Bain et al. 1996; Ippolito et al. 2005; Elmer et al. 2006; Khalifa et al. 2009).

Likewise, silicon (Si) fertiliser soil amendments and foliar sprays have proved effective in controlling pathogens of several plant species, including rice blast (*Magnaporthe grisea*, anamorph = *Pyricularia grisea*), grey leaf spot, pear scab (*V. pirini*), powdery mildew, *Rhizoctonia solani*, *Pythium aphanidermatum*, and *Sclerotinia homoeocarpa* (Hamel and Heckman 2000; Saigusa et al. 2000; Rondeau 2001; Uriarte et al. 2004; Wang et al. 2017; Percival 2018).

While the use of Ca and Si fertilisers alone to reduce disease severity has been studied, the Ca and Si fertilisers used in this study have received limited investigation. Similarly, the potential of Ca and Si fertilisers in combination with a synthetic fungicide to manage scab diseases of trees has received little, if any, investigation. Consequently, the purpose of these studies was, firstly, to investigate the association of Ca and Si fertilisers, singly and in combination with a synthetic fungicide (penconazole), as scab and leaf blotch protective compounds using container-grown and field-planted species of horse chestnut (*Aesculus hippocastanum*) and apple (*Malus domestica* cv. 'Golden Delicious'), known to be sensitive to *Guignardia* leaf blotch and apple scab infection, respectively; and secondly, to determine if the concentration of synthetic fungicide and/or the number of spray applications can be reduced without an impact on efficacy if a synthetic fungicide is combined with a Ca or Si fertiliser.

MATERIALS AND METHODS

Container Trials

Experiments used 4-year-old, cell-grown stock of *Aesculus hippocastanum*. Trees were 45 cm high, ± 4.5 cm. All trees were obtained from a commercial supplier (Blackmoor Estate, Blackmoor, Nr Liss, Hampshire, UK) during December to January as bare root stock and stored at 5 °C in a ventilated cold store prior to potting up in February. Trees were potted into 10.0-L plastic pots filled with soil (loamy texture, 23% clay, 44% silt, 30% sand, 3% organic carbon, pH of 6.6) and supplemented with the controlled release, nitrogen-based (N:P:K 20:8:8) fertiliser 'Enmag' (Salisbury House, Weyside Park, Goldmar, Surrey, UK) at a rate of 5.0 g kg^{-1} soil. Following potting, trees remained outdoors on a free-draining weed suppressant fabric at the University of Reading Shinfield Experimental Site, Reading, Berkshire, UK (51°43′N, −1°08′W). Trees were subject to natural climatic conditions and watered as required. The experiments were undertaken in 2016 and 2017. Throughout the experiments, 6 containerised trees per treatment were used. The experimental design adopted was a completely randomised block design.

Field Experiment

The apple trial site consisted of a 1.5-ha block of apple (*Malus domestica* cv. 'Golden Delicious') interspersed with individual trees of *Malus domestica* 'Red Delicious' and 'Gala' as pollinators. Planting distances were 3 m × 3 m spacing. The trees were planted in 2003 and trained under the central-leader system to an average height of 2.5 m ± 0.25 m, with mean trunk diameters of 12 cm ± 1.4 cm at 45 cm above the soil level. The trial site was located at the University of Reading Shinfield Experimental Site, University of Reading, Berkshire, UK (51°43′N, −1°08′W). The soil was a sandy loam containing 4% to 6% organic matter with a pH of 6.1, and available P, K, Mg, Na, and Ca were 55.3, 702.4, 188.2, 52.9, and 1,888 mg L^{-1}, respectively. Site management consisted of a 2-m-wide weed-free strip beneath the trees maintained with glyphosate (Roundup; Green-Tech, Sweethills Park, Nun Monkton, York, UK). No supplementary fertilisation was applied during the trials. A randomised complete block was utilised in the experimental design. Six trees per treatment were used. A minimal insecticide (deltamethrin, product name Bandu, Headland Agrochemicals Ltd., Saffron Walden, Essex, UK) program was applied every 2 months during the growing season, commencing in May 2016 and 2017 (Nicholas et al. 2003), a standard practice followed at the University of Reading experimental site for orchard pest control.

Treatments

Penconazole, calcium, and silicon sprays were applied using a 5-L pump action sprayer, and trees were sprayed until runoff. Treatments were applied 3 times per year at bud break (2016 March 19, 2017 March 27), 90% petal fall (2016 May 15, 2017 May 25), and early fruitlet (2016 June 3, 2017 June 6). Treatments for containerised stock and field trials are shown in Table 1.

In addition, and as an industrial comparative, 6 sprays of penconazole were applied on the same dates as above, and a further 3 sprays were applied 2 weeks after early fruitlet (2016 June 21, 2017 June 25), fruit swell (2016 July 5, 2017 July 9), and (2016 July 19, 2017 July 23). Ca and Si fertilisers were applied at the manufacturers' recommended rates. Penconazole was applied at 1 mL L^{-1} of water, manufacturer's recommended rate (full strength), and at 0.5 mL L^{-1} of water, half manufacturer's recommended rate (half strength).

Leaf and Fruit Scab/Blotch Symptom Severity

During each field trial, scab symptom severity of leaves and fruit was assessed commencing September 14 each year. For containerised stock trials, leaf blotch symptom severity was assessed commencing September 24 each year. Leaf scab and blotch severity for each tree was rated using a visual indexing technique and ratings on the scale: 0 = no leaf scab or blotch symptoms observed; 1 = less than 5% of leaves affected and no aesthetic impact; 2 = 5% to 20% of leaves affected with some yellowing, but little or no defoliation; 3 = 21% to 50% of leaves affected, significant defoliation and/or leaf yellowing; 4 = 51% to 80% of leaves affected, severe foliar discolouration; 5 = 81% to 100% of leaves affected with 90% to 100% defoliation. Scab severity on fruit (apple only) was calculated on the scale: 0 = no visible lesions; 1 = < 10% fruit surface infected; 2 = 10% to 25% fruit surface infected; 3 = > 26% to 50% fruit surface infected; 4 = > 50% fruit surface infected. The individual ratings for each tree in each treatment were used as a measure of scab severity for statistical analysis. Leaf scab severity ratings used in this study were based on UK and Ireland market standards for fungicide evaluation of scab control (Butt et al. 1990; Swait and Butt 1990). Fruit scab severity was based on a scale used by Ilhan et al. (2006). Ten leaves and five fruit (apple only) per tree randomly selected throughout the crown were used for scab and leaf blotch measurements, with the mean calculated per tree for statistical purposes.

Chlorophyll Measurements

A Minolta chlorophyll meter SPAD-502 was used. Chlorophyll was measured at the mid point of the leaf next to the main leaf vein. Calibration was obtained by measurement of absorbance at 663 and 645 nm in

Table 1. Fungicide, calcium fertilisers, and silicon fertilisers used, concentration applied, and frequency of application.

Active ingredient	Concentration applied	No. of applications
Penconazole*	1 mL L^{-1} of water (FS)	6
Penconazole	1 mL L^{-1} of water (FS)	3
Penconazole	0.5 mL L^{-1} of water (HS)	3
Tetra ethyl silicate	10 mL L^{-1} of water (FS)	3
Penconazole + tetra ethyl silicate	1 mL L^{-1} of water (FS) + 10 mL L^{-1} of water (FS)	3
Penconazole + tetra ethyl silicate	0.5 mL L^{-1} of water (HS) + 10 mL L^{-1} of water (FS)	3
Potassium silicate	10 mL L^{-1} of water (FS)	3
Penconazole + potassium silicate	1 mL L^{-1} of water (FS) + 10 mL L^{-1} of water (FS)	3
Penconazole + potassium silicate	0.5 mL L^{-1} of water (HS) + 10 mL L^{-1} of water (FS)	3
Ca chloride	2.5 g L^{-1} of water (FS)	3
Penconazole + Ca chloride	1 mL L^{-1} of water (FS) + 2.5 g L^{-1} of water (FS)	3
Penconazole + Ca chloride	0.5 mL L^{-1} of water (HS) + 2.5 g L^{-1} of water (FS)	3
Ca metasolate	2.5 g L^{-1} of water (FS)	3
Penconazole + Ca metasolate	1 mL L^{-1} of water (FS) + 2.5 g L^{-1} of water (FS)	3
Penconazole + Ca metasolate	0.5 mL L^{-1} of water (HS) + 2.5 g L^{-1} of water (FS)	3
Water (control)		3

All treatments based on manufacturers' recommended efficacy rates.

FS = full strength, HS = half strength.

Tetra ethyl silicate, potassium silicate, Ca chloride, Ca metasolate (Orion Future Technology Ltd., Henwood House, Henwood, Ashford, Kent, UK).

* a protectant triazole fungicide with antisporulant activity commercially used for apple scab control (Syngenta Crop Protection UK Ltd., Whittlesford, Cambridge, UK).

a spectrophotometer (PU8800 Pye Unicam) after extraction with 80% v/v aqueous acetone (regression equation = 5.82 + 0.063x; r^2 adjusted = 0.94, $P < 0.001$) (Lichtenthaler and Wellburn 1983). Ten leaves per tree randomly selected throughout the crown were used for measurements and the mean calculated per tree for statistical purposes.

Fruit Yield and Crown Volume

Yield per tree was determined by weighing all fruit on each tree at harvest and dividing by the number of trees per treatment.

Crown volume (C_v) was estimated from the crown width (D) and crown depth (L) using the paraboloid form of the crown (Kupka 2007):

$$C_v = \pi\left(\frac{D^2L}{8}\right)$$

Data Analysis

Data were analysed using Genstat 13. Levene's (1960) test was used to determine the homogeneity of variances, and data were transformed (log [$y + 0.5$]) when necessary. Visual severity index data were transformed using arcsine ($\sqrt{[y/100]}$). Treatment significance from controls was separated using Fisher's least significant difference (LSD) at the $P < 0.05$ level. Limpel's formula as described by Richer (1987) was used to describe synergistic interactions between the reduced dose of penconazole and both calcium and silicon fertiliser combinations. The presence of synergism between Ca and Si fertiliser and fungicide was determined by using Limpel's formula:

$$E_e = X + Y - \frac{XY}{100}$$

where E_e is the expected effect from additive responses of two inhibitory compounds, and X and Y are the percentages of inhibition of each compound used alone. If the combination of the two agents produces any value of inhibition greater than E_e, then synergism exists (Lorito et al. 1993).

$$X + Y > E_e$$

RESULTS

Container-Grown Stock: *Guignardia* Leaf Blotch

Irrespective of Ca, Si, and penconazole combination, and whether applied at full or half strength, no leaf phytotoxic effects were recorded (data not shown). Naturally occurring outbreaks of *Guignardia* leaf blotch were recorded on foliage of control trees as indicated by a leaf severity rating of 3.5 and 3.2 at the end of the 2016 and 2017 growing seasons, respectively (Table 2). The greatest reductions in *Guignardia* leaf blotch severity on leaves was recorded following 6 repeat applications of the synthetic fungicide penconazole at full strength, where no leaf blotch was recorded in the 2016 and 2017 trials (Table 2). Likewise, 6 repeat applications of the synthetic fungicide penconazole at full strength had the most positive effect on leaf chlorophyll content and crown volume compared to all other treatments (Tables 3 and 4). Limited efficacy against *Guignardia* leaf blotch was recorded when penconazole at half strength (0.5 mL L^{-1}), Ca metasolate, and potassium silicate were applied 3 times during a growing season. In these instances, leaf blotch severity symptoms (Table 2), leaf chlorophyll content (Table 3), and crown volume (Table 4) were statistically comparable to water-treated controls. Combinations of penconazole at half strength (0.5 mL L^{-1}) with either Ca metasolate or potassium silicate, however, when applied 3 times during a growing season, in most instances, significantly reduced leaf blotch severity symptoms and significantly increased leaf chlorophyll content and crown volume compared to water-treated controls (Tables 2, 3, and 4). These effects were additive, not synergistic according to Limpel's formula. Three spray applications of penconazole at full strength (1.0 mL L^{-1}), tetra ethyl silicate, and Ca chloride, singly and in combination, significantly reduced leaf blotch severity symptoms and significantly increased leaf chlorophyll content and crown volume compared to water-treated controls (Tables 2, 3, and 4). Improved efficacy (reduced leaf blotch severity, increased leaf chlorophyll content and crown volume) was recorded using combinations of penconazole at full strength + tetra ethyl silicate or penconazole at full strength + Ca chloride compared to each product applied singly (Tables 2, 3, and 4). Similarly, 3 spray applications of penconazole at half strength (0.5 mL L^{-1}) + tetra ethyl silicate or penconazole at half strength + Ca chloride had greater efficacy compared to spray applications of penconazole at half strength alone. These effects were primarily additive, with one exception: penconazole at half strength + Ca chloride on leaf chlorophyll content, where a synergistic effect based on Limpel's formula was recorded (Table 3).

Field Trials: *V. inaequalis*

Irrespective of Ca, Si, and penconazole combination, and whether applied at full or half strength, no leaf phytotoxic effects were recorded (data not shown). Damaging outbreaks of leaf and fruit scab symptoms were recorded on control trees, where visual index values of 4.7 and 4.2 (leaves) and 2.6 and 2.8 (fruit) were recorded at the end of the 2016 and 2017 growing seasons, respectively (Tables 2 and 5). The greatest reductions in leaf and fruit scab symptom severity were recorded following 6 applications of the synthetic fungicide penconazole at full strength, where no leaf scab was recorded in 2016 and no fruit scab in 2016 and 2017 (Tables 2 and 5). This treatment also had the greatest effect on fruit yield (Table 5), leaf chlorophyll content (Table 3; 2016 growing season only), and crown volume (Table 4) compared to all other treatments. Limited efficacy was recorded when penconazole at half strength (0.5 mL L^{-1}), Ca metasolate, or potassium silicate alone was applied 3 times during the growing season. Leaf and fruit scab severity symptoms, fruit yield, and leaf chlorophyll content were, in most cases, not significantly different from water-treated controls (Tables 2, 3, and 5). However, penconazole at half strength (0.5 mL L^{-1}), Ca metasolate, or potassium silicate alone did significantly increase crown volume compared to water-treated controls (Table 4). Penconazole at half strength (0.5 mL L^{-1}) in combination with either Ca metasolate or potassium silicate, when applied 3 times during a growing season, in most instances, significantly reduced leaf and fruit scab severity symptoms (Tables 2 and 5) and significantly increased fruit yield (Table 5), leaf chlorophyll content (Table 3), and crown volume (Table 4) compared to water-treated controls. These effects were additive based on Limpel's formula. Three spray applications of penconazole at full strength (1.0 mL L^{-1}), tetra ethyl silicate, or Ca

Table 2. The influence of calcium and silicon fertilisers and synthetic fungicide combinations applied as foliar sprays on the severity of *Guignardia* leaf blotch (*Guignardia aesculi*) on *Aesculus hippocastanum* (container-grown stock) and apple scab (*Venturia inaequalis*) on *Malus domestica* cv. 'Golden Delicious' (field trials).

	A. hippocastanum **Leaf blotch symptom severity index**		***M. domestica*** **cv. 'Golden Delicious' Leaf scab symptom severity index**	
Treatment	**2016**	**2017**	**2016**	**2017**
Water (control)	3.5d	3.2g	4.7j	4.2h
Penconazole (1 mL × 6 sprays)	0.0a	0.0a	0.0a	0.5a
Penconazole (1 mL)	2.0c	1.8cd	2.2de	2.1def
Penconazole (0.5 mL)	3.1d	3.0fg	4.2ij	3.7gh
Tetra ethyl silicate (10 mL)	1.8c	2.0de	2.8efgh	2.9fg
Penconazole (1 mL) + tetra ethyl silicate (10 mL)	0.9b (S^{yn})	1.3bc (A^{dd})	1.0bc (S^{yn})	1.2abc (S^{yn})
Penconazole (0.5 mL) + tetra ethyl silicate (10 mL)	1.6bc (A^{dd})	1.7cd (A^{dd})	2.6efg (A^{dd})	2.6ef (A^{dd})
Potassium silicate (10 mL)	3.0d	2.7fg	3.6hi	3.8h
Penconazole (1 mL) + potassium silicate (10 mL)	1.3bc (A^{dd})	1.5bcd (A^{dd})	1.5bcd (A^{dd})	1.8bcde (A^{dd})
Penconazole (0.5 mL) + potassium silicate (10 mL)	2.8d (A^{dd})	2.5ef (A^{dd})	3.4ghi (A^{dd})	3.6gh (A^{dd})
Ca chloride (2.5 g)	1.7c	1.8cd	2.4def	2.4ef
Penconazole (1 mL) + Ca chloride (2.5 g)	0.8b (S^{yn})	1.0b (S^{yn})	0.8ab (S^{yn})	1.0ab (S^{yn})
Penconazole (0.5 mL) + Ca chloride (2.5 g)	1.5bc (A^{dd})	1.9cde (A^{dd})	1.6bcd (S^{yn})	2.0cde (A^{dd})
Ca metasolate (2.5 g)	3.1d	2.9fg	3.3fghi	3.7gh
Penconazole (1 mL) + Ca metasolate (2.5 g)	1.6c (A^{dd})	1.6bcd (A^{dd})	1.9cde (A^{dd})	1.4bcd (A^{dd})
Penconazole (0.5 mL) + Ca metasolate (2.5 g)	2.8d (A^{dd})	2.5ef (A^{dd})	3.5ghi (A^{dd})	3.7gh (A^{dd})
Treatment	< 0.001	< 0.001	< 0.001	< 0.001

Lower case letters indicate significant differences between means for each evaluation date by LSD ($P = 0.05$).

S^{yn} = synergistic effect according to Limpel's formula. A^{dd} = additive effect if values did not exceed Limpel's formula but were greater than the effect of each product alone.

All values mean of 6 trees, 10 leaves per tree.

Leaf scab and blotch severity scale: 0 = no leaf scab or blotch symptoms observed; 1 = less than 5% of leaves affected and no aesthetic impact; 2 = 5% to 20% of leaves affected with some yellowing, but little or no defoliation; 3 = 21% to 50% of leaves affected, significant defoliation and/or leaf yellowing; 4 = 51% to 80% of leaves affected, severe foliar discolouration; 5 = 81% to 100% of leaves affected with 90% to 100% defoliation.

chloride, singly and in combination, significantly reduced leaf and fruit scab severity symptoms (Tables 2 and 5) and significantly increased fruit yield, leaf chlorophyll content, and crown volume compared to water-treated controls (Tables 3, 4, and 5). Greater efficacy (reduced leaf and fruit scab severity, increased leaf chlorophyll content, fruit yield, and crown volume) was recorded using combinations of penconazole at full strength + tetra ethyl silicate or penconazole at full strength + Ca chloride compared to each product applied singly (Tables 2, 3, 4, and 5). Similarly, 3 spray applications of penconazole at half strength (0.5 mL L^{-1}) + tetra ethyl silicate or penconazole at half strength + Ca chloride had greater efficacy as scab protectant compounds compared to spray applications of penconazole at half strength alone (Tables 2, 3, 4, and 5). These effects were primarily additive, with two exceptions: penconazole at half strength + Ca chloride on leaf scab severity symptoms (Table 2) and crown volume (Table 4), where a synergistic effect based on Limpel's formula was recorded.

DISCUSSION

There is a significant body of literature that describes Si and Ca nutrition reducing the intensity and severity of fungal and bacterial pathogen symptomology. Examples include rice blast (*Magnaporthe grisea*, anamorph = *Pyricularia grisea*), grey leaf spot, powdery mildew, *Rhizoctonia solani*, *Pythium aphanidermatum*, *Sclerotinia homoeocarpa*, *Phoma exigua* var. foveata, brown rot of fruit trees (*Monilinia fructicola*), apple scab (*V. inaequalis*), pear scab (*V. pirina*), and fireblight (*Erwinia carotovora* subsp. *atroseptica*)(Uriarte et al. 2004; Ippolito et al. 2005; Elmer et al. 2006; Springer et al. 2007; Khalifa et al. 2009; Wang et al. 2017; Percival 2018). In support of this, results of this study show that applications of Si- and Ca-based fertilisers alone reduced leaf blotch and apple scab severity of containerised and field-grown trees, respectively, when applied 3 times during a growing season. Supplementing plants with Si and/or Ca has been shown to act primarily in two ways. The first is by physical alterations to leaf structure caused

Table 3. The influence of calcium and silicon fertilisers and synthetic fungicide combinations applied as foliar sprays on leaf chlorophyll content (SPAD) of *Aesculus hippocastanum* (container-grown stock) with *Guignardia* leaf blotch (*Guignardia aesculi*) and *Malus domestica* cv. 'Golden Delicious' (field trials) with apple scab (*Venturia inaequalis*).

	A. hippocastanum SPAD		*M. domestica* cv. 'Golden Delicious' SPAD	
Treatment	**2016**	**2017**	**2016**	**2017**
Water (control)	36.5a	34.6a	28.8a	27.1a
Penconazole (1 mL × 6 sprays)	47.0g	46.9h	38.6g	36.9de
Penconazole (1 mL)	40.5abcdef	39.8cde	32.1bcd	32.8bc
Penconazole (0.5 mL)	37.5ab	35.9ab	30.5ab	31.5b
Tetra ethyl silicate (10 mL)	39.0abcd	39.4bcde	31.6abc	32.0bc
Penconazole (1 mL) + tetra ethyl silicate (10 mL)	43.0defg (A^{dd})	44.1fgh (A^{dd})	33.4bcd (A^{dd})	34.6bcd (A^{dd})
Penconazole (0.5 mL) + tetra ethyl silicate (10 mL)	40.0abcdef (A^{dd})	41.5ef (A^{dd})	34.1cdef (A^{dd})	32.9bc (A^{dd})
Potassium silicate (10 mL)	38.8abc	36.8abc	31.3ab	32.5bc
Penconazole (1 mL) + potassium silicate (10 mL)	43.3efg (A^{dd})	44.0fgh (A^{dd})	33.8cde (A^{dd})	35.0bcde (A^{dd})
Penconazole (0.5 mL) + potassium silicate (10 mL)	39.2abcde (A^{dd})	41.1defg (A^{dd})	32.4bcd (A^{dd})	32.1bc (A^{dd})
Ca chloride (2.5 g)	41.4bcdef	39.8cde	35.1def	33.9bcd
Penconazole (1 mL) + Ca chloride (2.5 g)	43.5fg (A^{dd})	44.7gh (A^{dd})	36.9efg (A^{dd})	35.1cde (A^{dd})
Penconazole (0.5 mL) + Ca chloride (2.5 g)	42.8cdef(A^{dd})	47.0h (S^{yn})	37.1fg(A^{dd})	38.2e (A^{dd})
Ca metasolate (2.5 g)	38.9abc	37.4abcd	30.9abc	32.8bc
Penconazole (1 mL) + Ca metasolate (2.5 g)	42.2cdef (A^{dd})	41.5efg (A^{dd})	33.1bcd (A^{dd})	34.6bcd (A^{dd})
Penconazole (0.5 mL) + Ca metasolate (2.5 g)	39.1abcde (A^{dd})	40.1cdef (A^{dd})	32.7bcd(A^{dd})	33.5bcd (A^{dd})
Treatment	< 0.001	< 0.001	< 0.001	< 0.001

Lower case letters indicate significant differences between means for each evaluation date by LSD ($P = 0.05$).

S^{yn} = synergistic effect according to Limpel's formula. A^{dd} = additive effect if values did not exceed Limpel's formula but were greater than the effect of each product alone.

All values mean of 6 trees, 10 leaves per tree.

by Si or Ca deposition in cell walls (Olsson 1988; Chardonnet et al. 2000; Kim et al. 2002; Zhang et al. 2006; Cai et al. 2008; Hayasaka et al. 2008; Wang et al. 2017). Si application, for example, leads to a pronounced cell silicification and larger papillae formation in leaves, enhanced Si layer formation in plant epidermal cell walls, and increased cuticular layer thickness. With respect to Ca, Ca-mediated alterations in leaf structure include improved structural integrity of the cell wall and middle lamella, increased cell wall strength, and enhanced resistance to enzymatic digestion from extra-cellular enzymes produced by fungal pathogens. Ca is also known to directly affect some pathogens by interfering with spore germination, germ tube elongation, and fungal cell wall thickness. Other studies show that Si and Ca play a biochemical role in mediating plant resistance to pathogens (Rodrigues et al. 2003; Liang et al. 2005; Rodrigues et al. 2005; Cai et al. 2008; Sun et al. 2010; Dallagnol et al. 2011). These include Si- and Ca-induced accumulation of soluble phenolics, lignin, peroxidase, chitinase, phytoalexins, and pathogenesis-related proteins. Although the basis of Ca- and Si-mediated resistance was not part of this study, Si- and Ca-enhanced leaf blotch and apple scab resistance recorded in our investigations may partially be caused by the role of these compounds inducing physiological and morphological alterations to leaf structure that in turn act as a physical barrier against fungal penetration, coupled with the promotion of inherent plant defence systems.

A difference in efficacy between the Si and Ca fertilisers used in this study was recorded. Application of Ca chloride, for example, reduced leaf blotch and scab severity by up to 50%, while Ca metasolate reduced leaf blotch and scab severity by 20% to 30%. Similarly, tetra ethyl silicate reduced leaf blotch and scab severity by up to 50%, while potassium silicate reduced leaf blotch and scab severity by less than 20%. Differences in the degree of pathogen protection between commercially available Si and Ca fertilisers are consistent with other research (Wojcik 2001; Wang et al. 2017; Percival 2018). A role of the anion attachment (chloride, metasolate, etc.) may play an influential part in conferring protection. For example, chloride is widely used as a means of sterilising stored food products against bacterial and fungal infection. Ca chloride at higher concentrations also acts as a caustic salt that can corrode sprayer parts

Table 4. The influence of calcium and silicon fertilisers and synthetic fungicide combinations applied as foliar sprays on crown volume of *Aesculus hippocastanum* (container-grown stock) and *Malus domestica* cv. 'Golden Delicious' (field trials).

	Crown volume (m^3)	
Treatment	***A. hippocastanum***	***M. domestica* cv. 'Golden Delicious'**
Water (control)	0.15a	0.98a
Penconazole (1 mL × 6 sprays)	0.29f	1.73f
Penconazole (1 mL)	0.18abc	1.19abc
Penconazole (0.5 mL)	0.16ab	1.13ab
Tetra ethyl silicate (10 mL)	0.21cde	1.34bde
Penconazole (1 mL) + tetra ethyl silicate (10 mL)	0.23e (A^{dd})	1.46de (A^{dd})
Penconazole (0.5 mL) + tetra ethyl silicate (10 mL)	0.21cde(A^{dd})	1.49def (A^{dd})
Potassium silicate (10 mL)	0.16ab	1.11ab
Penconazole (1 mL) + potassium silicate (10 mL)	0.21cde (A^{dd})	1.41cde (A^{dd})
Penconazole (0.5 mL) + potassium silicate (10 mL)	0.17ab (A^{dd})	1.18abc (A^{dd})
Ca chloride (2.5 g)	0.19bcd	1.27bcd
Penconazole (1 mL) + Ca chloride (2.5 g)	0.22de (A^{dd})	1.52def (A^{dd})
Penconazole (0.5 mL) + Ca chloride (2.5 g)	0.23e (A^{dd})	1.55ef (S^{yn})
Ca metasolate (2.5 g)	0.18abc	1.20abc
Penconazole (1 mL) + Ca metasolate (2.5 g)	0.21cde (A^{dd})	1.43cde (A^{dd})
Penconazole (0.5 mL) + Ca metasolate (2.5 g)	0.19bcd (A^{dd})	1.19abc (A^{dd})
Treatment	< 0.001	< 0.001

Lower case letters indicate significant differences between means for each evaluation date by LSD ($P = 0.05$).

S^{yn} = synergistic effect according to Limpel's formula. A^{dd} = additive effect if values did not exceed Limpel's formula but were greater than the effect of each product alone.

All values mean of 6 trees.

(Fixen 1993). The influence of protein-based amino acids (metasolate) and their derivatives as plant protection compounds has received growing attention over the past 10 years. The β-amino acid derivative blasticidin S, isolated from the soil actinomycete *Streptomyces griseochromogenes*, has been used commercially on a large scale as a fungicide against *Magnaporthe grisea* (rice blast). The serine derivative mildiomycin from *Streptoverticillium rimofaciens* was shown to have strong activity against powdery mildew diseases of several different crops, e.g., *Blumeria graminis* (wheat powdery mildew) and *Uncinula necator* (grape powdery mildew). Dipeptide nitropeptin, produced by *Streptomyces xanthochromogenus*, displayed strong activity against *Magnaporthe grisea* (rice blast), while Rhizocticin A, isolated from *Bacillus subtilis* ATCC 6633, controlled *Botryotinia fuckeliana* (grey mould) on grape in field trials (Lamberth 2016). Finally, the polyoxin isolated from *Streptomyces cacaoi* var. *asoensis* are an important class of peptidyl nucleosides that interfere with fungal cell wall synthesis by blocking chitin synthetase. Polyoxin B is effective against a number of fungal pathogens of ornamentals, vegetables, and fruits, e.g., *Alternaria kikuchiana* (pear black spot), while polyoxin D is marketed as Zn salt for the control of *Rhizoctonia solani* (rice sheath blight). Similarly, polyoxin L is highly active against *Alternaria mali* (apple cork spot)(Lamberth 2016). The anion attachment to a cation has also been shown to be important in influencing plant uptake and translocation of Si and Ca, which in turn conferred greater protection against brown rot of cherry via Si- and Ca-mediated alterations to leaf structure and topography (Wojcik 2001; Khalifa et al. 2009; Wang et al. 2017). Results of this study show that care should be taken when selecting Ca- or Si-based fertiliser(s) for plant protection purposes, as efficacy can differ markedly between formulations.

Within Europe, suppliers and growers of apples as well as tree nursery producers adopt a very low/zero tolerance policy towards any form of fungal infection. As a result, the economics of tree and fruit production require frequent application of synthetic fungicides throughout the growing season (Didelot et

Table 5. The influence of calcium and silicon fertilisers and synthetic fungicide combinations applied as foliar sprays on fruit-scab severity and yield of *Malus domestica* cv. 'Golden Delicious' (field trials).

	Fruit scab symptom severity index		Fruit yield per tree (kg)	
Treatment	2016	2017	2016	2017
Water (control)	2.6c	2.8c	6.2a	6.0a
Penconazole (1 mL × 6 sprays)	0a	0a	8.1f	8.7e
Penconazole (1 mL)	1.4cde	1.6bcd	7.1bcde	7.0bc
Penconazole (0.5 mL)	2.2ef	2.3cd	6.7abcd	6.9abc
Tetra ethyl silicate (10 mL)	1.8cdef	1.8cd	7.3bcdef	6.9abc
Penconazole (1 mL) + tetra ethyl silicate (10 mL)	0.9b (A^{dd})	0.8ab (S^{yn})	7.7ef (A^{dd})	7.7cd (A^{dd})
Penconazole (0.5 mL) + tetra ethyl silicate (10 mL)	1.6cdef (A^{dd})	1.4bc (A^{dd})	6.5abc (A^{dd})	6.8abc (A^{dd})
Potassium silicate (10 mL)	2.3f	2.4d	6.6abc	6.4ab
Penconazole (1 mL) + potassium silicate (10 mL)	1.3cd (A^{dd})	1.4bc (A^{dd})	7.3bcdef (A^{dd})	7.6c (A^{dd})
Penconazole (0.5 mL) + potassium silicate (10 mL)	2.0cdef (A^{dd})	2.1cd (A^{dd})	6.8abcd (A^{dd})	6.8abc (A^{dd})
Ca chloride (2.5 g)	1.3cd	1.5bcd	7.4cdef	7.1bcd
Penconazole (1 mL) + Ca chloride (2.5 g)	0.5ab (S^{yn})	0.7ab (S^{yn})	7.9ef (A^{dd})	8.0d (A^{dd})
Penconazole (0.5 mL) + Ca chloride (2.5 g)	1.2bc (A^{dd})	1.6bcd (A^{dd})	7.3bcdef (A^{dd})	7.3bcd (A^{dd})
Ca metasolate (2.5 g)	2.4f	2.2cd	6.4ab	6.6ab
Penconazole (1 mL) + Ca metasolate (2.5 g)	1.2bc (A^{dd})	1.3b (A^{dd})	7.5def (A^{dd})	7.6cd (A^{dd})
Penconazole (0.5 mL) + Ca metasolate (2.5 g)	2.1def (A^{dd})	1.9cd (A^{dd})	7.0bcde (A^{dd})	6.5ab (A^{dd})
Treatment	< 0.001	< 0.001	< 0.001	< 0.001

Lower case letters indicate significant differences between means for each evaluation date by LSD ($P = 0.05$).

S^{yn} = synergistic effect according to Limpel's formula. A^{dd} = additive effect if values did not exceed Limpel's formula but were greater than the effect of each product alone.

All values mean of 6 trees, 5 fruits per tree.

Fruit scab severity scale: 0 = no visible lesions; 1 = < 10% fruit surface infected; 2 = 10% to 25% fruit surface infected; 3 = 26% to 50% fruit surface infected; 4 = > 50% fruit surface infected.

al. 2007; Beresford et al. 2012; Hrabětová et al. 2017). Results of this study show that the synthetic triazole fungicide penconazole, when applied 6 times during the growing season, was highly effective in reducing apple scab and leaf blotch severity, where in most instances, 100% control was achieved. The effectiveness of penconazole against fungal pathogens under laboratory and field conditions has been confirmed elsewhere (Percival et al. 2009; Derbalah 2011; Beresford et al. 2012). However, over-reliance on synthetic fungicides has led to decreased pathogen sensitivity, coupled with public demands to reduce fungicide use based on a greater awareness of environmental and health issues. This has placed a greater emphasis on the development of non- or reduced fungicide pathogen control strategies (Gozzo 2003; Villalta et al. 2004; Hailey and Percival 2014). Three methods of achieving this objective are by (i) reducing the number of sprays per growing season, (ii) reducing fungicide dosage, and (iii) adding non-fungicide additives such as fertilisers to enhance efficacy while not compromising plant quality and aesthetics. Results of this study show that a half-strength application of penconazole applied 3 times over a growing season had little influence on reducing leaf blotch and apple scab severity compared to water-treated controls. Consequently, this treatment cannot be recommended. Application of penconazole 3 times over a growing season at full strength, however, reduced leaf blotch and apple scab severity by 40% to 50%. Such a reduction may be acceptable for the production, supply, and/or consumption of apple fruit sold under an organic or naturally produced label, as scab severity levels tend to be less stringent (Bevan and Knight 2001). Similarly, with ornamental *Aesculus* spp. planted for aesthetic reasons within the town and city landscape industry, lower leaf blotch levels may be acceptable (Percival et al. 2009). A key finding of this study, however, was that addition of Ca- and Si-fertilisers, especially Ca chloride and tetra ethyl silicate, to penconazole at full or half strength enhanced efficacy compared to each product applied alone. Not only was leaf blotch and scab severity reduced, but leaf chlorophyll content, fruit yield, and crown volume increased. In most instances, these increases were additive, but in other cases a synergistic response occurred, especially when penconazole was combined with Ca chloride. Synergism related to plant protection agents such as fungicides has been defined as “the simultaneous action of two or more compounds in which the total response of an organism to the pesticide combination is greater than the sum of the individual components” (Burpee and Latin 2008). While many investigations addressing synergy in fungicide mixtures exist, most have been limited to laboratory studies using cultured fungal pathogens (Huang and Chen 2008). Few studies demonstrate synergistic interactions between fungicides at the field level (Burpee and Latin 2008). Our field trial results show that while the degree of control recorded was not as great as the conventional approach to leaf blotch and scab management, i.e., 6 repeat fungicide sprays where 100% control was achieved, in most cases, addition of Ca chloride or tetra ethyl silicate to penconazole at full strength reduced leaf blotch and scab severity by 75% to 85%. Addition of Ca chloride or tetra ethyl silicate to penconazole at half strength reduced leaf blotch and scab severity by 45% to 65%. A search of the literature indicates that there is clearly a lack of reports of fungicide synergism in trees. Our findings confirm that foliar Ca and Si applications alone or in combination with a full or reduced dose of synthetic fungicide offer a means of reducing the number of fungicide sprays currently used for pathogen management that can be implemented with existing spray technologies and may have applicability for the control of other foliar pathogens of ornamental and urban trees not explored within this study. Results of this study, however, do suggest that there is a high probability for individuals involved with the management of fungal pathogens in urban landscapes, as well as workers in other tree-related industries (horticulture, forestry, nursery tree production), to take advantage of fungicide synergism to control apple scab and *Guignardia* leaf blotch.

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Résumé. La gestion des maladies fongiques en milieu urbain repose en grande partie sur des pulvérisations répétées de fongicides. Les préoccupations environnementales ont conduit à la nécessité d'éliminer ou de réduire l'utilisation des fongicides. Il a été démontré que les pulvérisations foliaires d'engrais à base de calcium (Ca) et de silicium (Si) réduisent la gravité des symptômes de plusieurs champignons pathogènes. L'objectif de notre recherche était d'évaluer l'influence des engrais de chlorure de calcium, de métasolate de calcium, de silicate de tétraéthyle et de silicate de potassium, appliqués séparément ou en combinaison avec un fongicide synthétique (penconazole) à pleine ou à demi-dose, sur la gravité des symptômes de la tavelure du pommier (*Venturia inaequalis*) et de la brûlure des feuilles *Guignardia* (*Guignardia aesculi*), ainsi que sur la teneur en chlorophylle des feuilles, production des fruits et le volume du houppier. Des essais ont été effectués avec des *Aesculus hippocastanum* cultivés en pot et des *Malus domestica* cv. 'Golden Delicious' cultivés en plein champ. Des applications de Ca, Si et penconazole pulvérisés seuls ont réduit de manière significative la gravité de la tavelure et de la brûlure des feuilles par rapport aux témoins pulvérisés avec de l'eau; toutefois, une différence significative entre le type d'engrais Ca et Si a été notée. Une combinaison d'engrais Ca ou Si avec une dose complète ou une demi-dose de penconazole s'est avérée plus efficace pour réduire la gravité des symptômes des deux pathogènes foliaires qu'une dose complète ou une demi-dose de penconazole utilisé seul. Les données analysées avec l'algorithme de Limpel ont indiqué des effets synergiques positifs entre Ca, Si et le penconazole dans certains cas, mais pas tous. L'intégration de pulvérisations foliaires de Ca et Si comme alternative ou en addition avec du penconazole pour la gestion de la tavelure et de la brûlure foliaire semble possible sur la base des résultats de nos recherches et pourrait être applicable contre d'autres pathogènes fongiques.

Zusammenfassung. Das Management von Pilzkrankheiten in städtischen Landschaften hängt stark von regelmäßigen Fungizidspritzungen ab. Aufgrund von ökologischen Bedenken ist es notwendig, den Einsatz von Fungiziden zu eliminieren oder zu reduzieren. Blattspritzungen mit Düngemitteln auf Kalzium- (Ca) und Silizium- (Si) Basis haben gezeigt, dass sie die Symptomschwere verschiedener Pilzkrankheiten reduzieren. Ziel unserer Forschung war es, den Einfluss von Ca-Chlorid-, Ca-Metasolat-, Tetraethylsilikat- und Kaliumsilikat-Düngern, die unabhängig voneinander und in Kombination mit einem synthetischen Fungizid (Penconazol) in voller und halber Stärke ausgebracht wurden, auf die Symptomschwere von Apfelschorf (*Venturia inaequalis*) und *Guignardia*-Blattfleckenkrankheit (*Guignardia aesculi*) sowie auf den Blattchlorophyllgehalt, den Fruchtertrag und das Kronenvolumen zu untersuchen. In den Versuchen wurden im Behälter gezüchtete *Aesculus hippocastanum* und im Freiland gezüchtete *Malus domestica* cv. 'Golden Delicious' verwendet. Anwendungen von Ca-, Si- und Penconazol-Spritzungen allein reduzierten signifikant die Schorf- und Blattfleckenschwere im Vergleich zu den mit Wasser besprühten Kontrollen; es wurde jedoch ein signifikanter Unterschied zwischen der Art der Ca- und Si-Dünger festgestellt. Eine kombinierte Mischung aus einem Ca- oder Si-Dünger mit einer vollen oder halben Dosis Penconazol war wirksamer bei der Reduzierung der Symptomschwere beider Blattkrankheitserreger als eine volle oder halbe Dosis Penconazol allein. Die mit der Limpel-Formel analysierten Daten zeigten in einigen, aber nicht in allen Fällen positive synergistische Effekte zwischen Ca und Si und Penconazol. Die Integration von Ca- und Si-Blattspritzungen als Alternative zu Penconazol oder als Zusatz zu Penconazol zur Behandlung von Schorf und Blattflecken scheint auf der Grundlage der Ergebnisse unserer Studien machbar zu sein und könnte auch gegen andere fungale Krankheitserreger anwendbar sein.

Resumen. El manejo de enfermedades fúngicas dentro de los paisajes urbanos depende en gran medida de la repetición en la aplicación de aerosoles fungicidas. Las preocupaciones ambientales han llevado a la necesidad de eliminar o reducir el uso de fungicidas. Se ha demostrado que los aerosoles foliares de fertilizantes a base de calcio (Ca) y silicio (Si) reducen la gravedad de los síntomas de varios patógenos fúngicos. El objetivo de nuestra investigación fue evaluar la influencia del cloruro de Ca, Ca metasoato, silicato de tetra etílico y fertilizantes de silicato de potasio, aplicados de forma independiente y en combinación con un fungicida sintético (penconazol) a fuerza plena y media de gravedad del síntoma, en roña de manzana (*Venturia inaequalis*) y mancha foliar (*Guignardia aesculi*), así como el contenido de clorofila de la hoja, rendimiento de la fruta y volumen de la copa. Los ensayos utilizaron *Aesculus hippocastanum* cultivado en contenedores y *Malus domestica* cv 'Golden Delicious'. Las aplicaciones de los aerosoles ca, y penconazol por sí solas redujeron significativamente la gravedad de la roña y la mancha de las hojas en comparación con los controles rociados con agua; sin embargo, se registró una diferencia significativa entre el tipo de fertilizantes Ca y Si. Una mezcla combinada de un fertilizante Ca o Si con una dosis completa o media de penconazol fue más eficaz para reducir la gravedad de los síntomas de ambos patógenos foliares que una dosis completa o media de penconazol solo. Los datos analizados con la fórmula de Limpel indicaron efectos sinérgicos positivos entre Ca y Si y penconazol en algunos, pero no en todos los casos. La integración de los aerosoles foliares Ca y Si como alternativa o aditivo con penconazol para el manejo de roñas y manchas de hojas parece factible en base a los resultados de nuestros estudios y puede tener aplicabilidad contra otros patógenos fúngicos.