



Evaluation of Inducing Agents and Synthetic Fungicide Combinations for Management of Foliar Pathogens of Urban Trees

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Abstract. Unmanaged, foliar pathogens of urban trees can be detrimental to tree health and aesthetics. Overreliance on synthetic fungicides increasingly means alternative means of pathogen management are now required. The purpose of these studies was to investigate the efficacy of 3 commercially available agents, harpin protein, salicylic acid derivative, and liquid chitosan, which can initiate induced resistance (IR) in plants. IR agents were applied independently and in combination with a synthetic fungicide (boscalid + pyraclostrobin) against 2 foliar pathogens (*Venturia pirina* and *Guignardia aesculi*) under field conditions with *Pyrus communis* ‘Williams’ Bon Chrétien’ and horse chestnut (*Aesculus hippocastanum*) acting as tree hosts. These agents were tested over 3 consecutive years. In 4 of 5 field studies, the use of an IR agent alone reduced pathogen symptom severity, increased fruit/seed yield, and enhanced leaf chlorophyll content. In virtually all studies, application of boscalid + pyraclostrobin at 2/3 strength plus an IR agent provided the same degree of pathogen control as boscalid + pyraclostrobin at full strength. Application of boscalid + pyraclostrobin at 1/3 strength plus an IR agent provided a reasonable degree of foliar pathogen control. Results showed that a combined mix of an IR agent with a 1/3 reduced dose of boscalid + pyraclostrobin was as effective at reducing symptom severity of 2 foliar pathogens as boscalid + pyraclostrobin applied at full strength, provided at least 4 sprays were applied during a growing season.

Keywords. Fungicides; *Guignardia*; Orchard Management; Pathogen Control; Plant Health Care; Synergism; Urban Landscapes; *Venturia*.

INTRODUCTION

Throughout the world, foliar diseases such as anthracnose, mildews, rusts, and sooty moulds occur on most species of trees and shrubs planted in urban landscapes. Symptoms of these diseases include scabs, spots, blotches, blights, needle casts, blisters, and curls (Hersh et al. 2012; Hantsch et al. 2013, 2014). Heavy pathogen symptomology can result in a reduction in leaf photosynthetic productivity, loss of carbohydrates, premature leaf drop, decreased tree growth and seed yield, increased rate of crown dieback, and a reduction in carbon allocation to fine roots, trunk, and twigs (Villalta et al. 2004; Tubby and Webber 2010; Hersh et al. 2012; Hantsch et al. 2013; Oliva et al. 2014). Repeat annual defoliations and subsequent loss of photosynthetic productivity can ultimately result in tree decline and sometimes even death (Percival 2018). To prevent foliage loss and ornamental disfigurement, repeat fungicide sprays are usually undertaken

(Carisse and Dewdney 2002; Hailey and Percival 2014). However, public and government demands to reduce fungicide use, stimulated by greater awareness of environmental consequences (e.g., spray drift, soil contamination), human health issues (contamination of the food chain), and collateral damage to nontarget organisms, has placed a greater emphasis on the development of reduced fungicide management strategies (Gozzo 2003; Fobert and Després 2005; Ilhan et al. 2006; Hailey and Percival 2014). Increased legislative restrictions on the registration, use, and application of fungicides has also resulted in more fungicides in Europe being withdrawn on an annual basis than are released onto the commercial market (BCPC 2019). This in turn means an overreliance on fewer fungicides, which increases the selection pressure for fungicide resistance in surviving pathogen populations (Ishii 2006).

Application of specific biological and/or chemical agents to plants can lead to the induction of local and systemic resistance to subsequent pathogen attack (Walters et al. 2013). An alternative approach to fungicides is to make use of the plants' own resistance mechanisms. Induced resistance (IR) is characterized by a restriction of pathogen growth and suppression of symptoms compared to non-induced plants infected with the same pathogen. IR onset is associated with an accumulation of salicylic acid (SA) at sites of infection and with the coordinated activation of a specific set of genes encoding pathogenesis-related proteins. Developments in plant protection technology have led to the formulation and commercialization of a range of IR agents. These include SA, marketed in the UK under the trade name Rigel-G®, or an SA functional analog (acibenzolar-*S*-methyl [ASM]), marketed in Europe under the trade name Bion® and in North America as Actigard®. Other examples of IR agents are the harpin protein, marketed in the US as Messenger®, and probanazole, marketed in Japan as Oryzemate®. Treatment of plants with these products induces resistance and activates the same set of pathogenesis-related genes as SA (Spoel and Dong 2012; Pieterse et al. 2014). However, these IR agents are generally less effective than standard synthetic fungicides for total pathogen control (Agostini et al. 2003; Krokene et al. 2008; Percival and Haynes 2008; Percival et al. 2009). This has led to the suggestion that a more appropriate role for IR agents would be in combination with a reduced dose of synthetic fungicide to achieve control comparable or significantly higher than stand-alone applications of fungicides at full dose (Bécot et al. 2000; Van Loon et al. 2002; Ilhan et al. 2006). This in turn is likely to reduce potential environmental impacts and extend the working life of existing fungicide products.

In this paper, we report the results of a number of field experiments conducted over 3 consecutive years to determine the potential of 3 commercially available IR agents, namely, harpin protein, salicylic acid derivative, and liquid chitosan. All were applied singly and in combination with a synthetic fungicide to control 2 foliar pathogens widely encountered in urban landscapes, namely, pear scab (*Venturia pirina*) against *Pyrus communis* 'Williams' Bon Chrétien' and *Guignardia* leaf blotch (*Guignardia aesculi*) against horse chestnut (*Aesculus hippocastanum*).

MATERIALS AND METHODS

Field Trials (2016 to 2018)

The pear trial site consisted of a 0.90 ha block of *Pyrus communis* 'Williams' Bon Chrétien,' identified as sensitive to *Venturia pirina* infection, interspersed with individual trees of *Pyrus communis* 'Beth' and 'Concorde.' Planting distances were based on 2 m × 2 m spacing. Trees were planted in 2003 and trained under a 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 horse chestnut trial site consisted of a 2.0 ha block of horse chestnut (*A. hippocastanum*) located adjacent to the pear trial site. Planting distances were based on a 2 m × 2 m spacing. The trees were planted as large standards in 1999 and trained under a central-leader system to an average height of 4.5 m ± 0.35 m with mean butt diameters of 50 cm ± 10 cm. Both pear and horse chestnut trial sites were located at the University of Reading Shinfield Experimental Site, University of Reading, Berkshire (51°43', -01°08'). At both trial sites, the soil was a sandy loam containing 4% to 6% organic matter, pH 6.6, with available P, K, Mg, Na, and Ca nutrients at 60.0, 693.7, 190.1, 48.8, and 2542 mg/L, respectively. Weeds were controlled chemically using glyphosate (Roundup; Green-Tech, Sweethills Park, Nun Monkton, York, UK) throughout each growing season. No watering or fertilisation was applied to plants during any of the trials. Historically, both pear and horse chestnut trees suffered heavily from pear scab and *Guignardia* leaf blotch infection, respectively, on an annual basis. Prior to trials commencing, trees were inspected late in the growing season (September) of the previous year, and only those trees visually rated with a high degree of fungal infection, i.e., > 50% of leaves infected, representing high foliar discolouration and scab/leaf blotch infection, were used the following year. Five independent experiments were performed once over three years, with each one or two experiments occurring in a different year. The treatments (1 water control, 1 IR agent [salicylic acid, harpin protein, liquid chitosan], 1 fungicide [boscalid + pyraclostrobin], 3 fungicide + IR combinations) were applied in 8 randomized complete blocks with a single tree as the experimental unit, giving a total of 48 observations per response variable.

Materials Tested for Efficacy

Signum (a.i. 27% boscalid + 7% pyraclostrobin; BASF plc, Cheadle Hume, Cheadle, Cheshire, UK).

Messenger (a.i. Harpin protein; EDEN Bioscience Corporation, N. Bothell, Washington, USA).

Rigel-G (a.i. Salicylic acid [SA]; Orion Future Technology Ltd, Henwood House, Henwood, Ashford, Kent, UK).

Liquid Chitosan (a.i. Chitosan; Viresco [UK] Ltd, Thirsk, North Yorks, UK).

Water (control).

Spray Treatments

Salicylic acid (SA) was used at a concentration of 3 mL/L of water, harpin protein (HP) at 0.005 g/L, and liquid chitosan (LC) at 1 mL/L, in line with manufacturers' labelled rates. The synthetic fungicide boscalid + pyraclostrobin was applied at 0.9 g/L (labelled rate) and at 2/3 and 1/3 labelled rate. Foliar sprays of each product were applied until runoff using a hand-sprayer (Cooper Pegler, Watling Street, Clifton upon Dunsmore, UK). Treatments, hosts, and year the experiment occurred are shown in Table 1. For consistency between years, all fungicide and IR agents were applied 4 times during a growing season at key treatment times for leaf pathogen management (Bevan and Knight 2001),

namely, bud break (mid-March), flower opening/green cluster (late-April), 90% petal fall (mid-late May), and early fruitlet (mid-late June). Prior to spray treatments, plants were inspected to ensure no visible symptoms of leaf infection were apparent. During spray treatments, polythene screens 2.5-m high were erected around each tree to prevent dispersal of sprays and possible cross contact. The base of the tree was covered with a 0.5 m × 0.5 m polythene mulch to prevent any soil percolation.

Insect Pest Management

A minimal insecticide program to prevent pest infestation was performed based on the pyrethroid insecticide deltamethrin (product name Bandu; Headland Agrochemicals Ltd, Saffron Walden, Essex, UK) which was applied every 6 weeks during each growing season. Trees were sprayed until runoff.

Treatment Efficacy

The degree of protection conferred by each treatment was assessed by recording 3 parameters towards the end of the growing season (25–30 September).

Leaf/Fruit Pathogen Symptom Severity

Severity of symptoms caused by *V. pirini* and *Guignardia aesculi* on leaves was assessed with a visual index

Table 1. Experimental treatments, timing, and host plants.

Treatment and concentration of compounds per L of water	Year and host		
	2016 <i>Pyrus</i> and <i>Aesculus</i>	2017 <i>Pyrus</i> and <i>Aesculus</i>	2018 <i>Pyrus</i>
Salicylic acid (3 mL)		X	
Harpin protein (5 mg)		X	
Boscalid + pyraclostrobin (0.9 g)	X	X	X
Boscalid + pyraclostrobin (0.9 g) + Harpin protein (5 mg)		X	
Boscalid + pyraclostrobin (0.6 g) + Harpin protein (5 mg)		X	
Boscalid + pyraclostrobin (0.3 g) + Harpin protein (5 mg)		X	
Boscalid + pyraclostrobin (0.9 g) + Salicylic acid (3 mL)		X	
Boscalid + pyraclostrobin (0.6 g) + Salicylic acid (3 mL)		X	
Boscalid + pyraclostrobin (0.3 g) + Salicylic acid (3 mL)		X	
Liquid chitosan (1 mL)		X	
Boscalid + pyraclostrobin (0.9 g) + Liquid chitosan (1 mL)		X	
Boscalid + pyraclostrobin (0.6 g) + Liquid chitosan (1 mL)		X	
Boscalid + pyraclostrobin (0.3 g) + Liquid chitosan (1 mL)		X	
Water control	X	X	X

and rating on the scale: 0 = No leaf pathogen symptoms observed; 1 = < 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 symptom severity on fruit 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. Leaf pathogen symptom severity ratings used in this study were based on UK and Ireland market standards for fungicide efficacy evaluations (Butt et al. 1990; Swait and Butt 1990). Fruit scab symptom severity was based on a scale used by Ilhan et al. (2006).

Leaf Chlorophyll Content

Measurement of leaf chlorophyll content or “leaf greenness” was used as a proxy to quantify disease pathogen severity. A commercially available chlorophyll content meter was used to measure greenness based on optical responses when a leaf is exposed to light. This in turn is used to estimate foliar chlorophyll concentrations (Kariya et al. 1982). Instantaneous and nondestructive readings are based on the quantification of light intensity (peak wavelength approximately 650 nm: red light-emitting diode [LED]) absorbed by the tissue sample. A second peak (peak wavelength approximately 940 nm: infrared LED) is emitted simultaneously with red LED to compensate for thickness of the leaf (Hoel 1998). Chlorophyll content measurements (SPAD) were made only on fully expanded mature leaves. In all cases, SPAD measurements were taken from 10 leaves randomized throughout the canopy per tree. A Minolta chlorophyll meter SPAD-502 (Minolta Camera Co., Osaka, Japan) was used. Chlorophyll was measured at the mid-point of the leaf next to the main leaf vein (Hoel 1998). Calibration was obtained by measurement of absorbance at 663 and 645 nm in a spectrophotometer (PU8800 Pye Unicam, Cambridge, UK) after extraction with 80% v/v aqueous acetone (regression equation = $5.58 + 0.053x$; $r^2 \text{ adj} = 0.94$, $P = < 0.001$) (Lichtenthaler and Wellburn 1983).

Fruit Yield

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

Statistical Analysis

As mean leaf and fruit pathogen severity was assessed visually, symptom severity values for all fungicide and IR agent treatments singly and in combination were transformed using the Arcsin (\sin^{-1}) transformation. All data to include fruit yield, seed dry weight, and leaf chlorophyll content was then analyzed by using the nonparametric Kruskal-Wallis One-Way Analysis of Variance. Ranks and means were separated by the Kruskal-Wallis Multiple Comparison Z-test ($P \leq 0.05$).

RESULTS

Pyrus communis ‘Williams’ Bon Chrétien’ (2016)

The IR agent SA alone significantly reduced ($P < 0.05$) leaf and fruit scab severity symptoms but had no significant effect on fruit yield and leaf chlorophyll content compared to the water-treated control trees (Table 2). The most effective treatment was boscalid + pyraclostrobin at 0.9 g/L + SA. In this instance, leaf and fruit scab symptom severity were reduced from 3.5 and 2.2 to 0.5 and 0.0, respectively ($P < 0.05$), while pear fruit yield and leaf chlorophyll content were increased from 10.1 and 26.8 to 14.0 and 44.5, respectively ($P < 0.05$). Reductions in leaf and fruit scab symptoms and increased fruit yield and leaf chlorophyll content were statistically comparable ($P < 0.05$) between boscalid + pyraclostrobin at 0.9 g/L (labelled rate) and boscalid + pyraclostrobin at 0.6 g/L (2/3 labelled rate) + SA. Application of boscalid + pyraclostrobin at 0.3 g/L (1/3 labelled rate) + SA significantly reduced ($P < 0.05$) leaf scab severity symptoms and significantly increased leaf chlorophyll content but had no significant effect on fruit yield (Table 2).

Aesculus hippocastanum (2016)

Boscalid + pyraclostrobin and boscalid + pyraclostrobin + SA treatments were effective in significantly reducing leaf blotch symptom severity ($P < 0.05$) and increasing seed dry weight ($P < 0.05$; Table 3) compared to water-treated controls. Only the treatment

Table 2. The influence of fungicide and salicylic acid combinations applied as foliar sprays on symptom severity of pear scab (*Venturia pirini*) on *Pyrus communis* 'Williams' Bon Chrétien.'

Treatment	Chlorophyll content	Leaf scab symptom severity ^z	Fruit scab symptom severity ^z	Fruit yield
Water (control)	26.8a	3.5d	2.2e	10.1a
Salicylic acid (SA)	33.2ab	1.9bc	1.3cd	11.8abc
Boscalid + pyraclostrobin 0.9 g/L	42.4cd	1.0ab	0.2ab	13.6bc
Boscalid + pyraclostrobin 0.9 g/L + SA	44.5d	0.5a	0.0a	14.0c
Boscalid + pyraclostrobin 0.6 g/L + SA	40.8bcd	1.2abc	0.8bc	12.9bc
Boscalid + pyraclostrobin 0.3 g/L + SA	35.0bc	2.0c	1.8de	11.5ab

^zAll values mean of 80 leaves (10 leaves per tree) and 40 fruits (5 fruits per tree) from 8 randomized complete blocks with a single tree replication per block. Numbers within a column followed by a common letter are not significantly different according to the Kruskal-Wallis Multiple Comparison Z-test ($P \leq 0.05$).

Table 3. The influence of fungicide and salicylic acid combinations applied as foliar sprays on symptom severity of *Guignardia* leaf blotch (*Guignardia aesculi*) on horse chestnut (*Aesculus hippocastanum*).

Treatment	Chlorophyll content	Leaf blotch symptom severity ^z	Mean seed dry weight
Water (control)	33.8a	3.2c	4.8a
Salicylic acid (SA)	37.9ab	2.4bc	6.8b
Boscalid + pyraclostrobin 0.9 g/L	41.5ab	0.5a	7.9b
Boscalid + pyraclostrobin 0.9 g/L + SA	46.6b	0.0a	8.3c
Boscalid + pyraclostrobin 0.6 g/L + SA	40.1ab	0.8a	7.7bc
Boscalid + pyraclostrobin 0.3 g/L + SA	38.0ab	2.3b	6.8b

^zAll values mean of 80 leaves (10 leaves per tree) from 8 randomized complete blocks with a single tree replication per block. Numbers within a column followed by a common letter are not significantly different according to the Kruskal-Wallis Multiple Comparison Z-test ($P \leq 0.05$).

boscalid + pyraclostrobin at 0.9 g/L + SA significantly ($P < 0.05$) increased leaf chlorophyll content. All remaining boscalid + pyraclostrobin and boscalid + pyraclostrobin + SA treatments had no significant effect on leaf chlorophyll content (Table 3). Application of SA alone had no significant effect on leaf blotch symptom severity and leaf chlorophyll content but significantly increased seed dry weight (Table 3). Significant differences between treatments were recorded. Greatest efficacy ($P < 0.05$) against leaf blotch symptom severity resulted from the treatments boscalid + pyraclostrobin and boscalid + pyraclostrobin labelled rate and 2/3 labelled rate + SA. In this instance, leaf blotch severity was reduced from 3.2 to 0.5, 0.0, and 0.8, respectively. Boscalid + pyraclostrobin (1/3 labelled rate) + SA significantly reduced leaf blotch symptom severity from 3.2 to 2.3 ($P < 0.05$) compared to water-treated control trees.

***Pyrus communis* 'Williams' Bon Chrétien' (2017)**

The IR agent HP alone significantly ($P < 0.05$) reduced leaf and fruit scab symptomology but had no significant effect on leaf chlorophyll content and pear yield compared to the water-treated trees (Table 4). No significant effect on fruit scab symptomology, leaf chlorophyll content, and fruit yield was recorded when boscalid + pyraclostrobin was applied at 0.3 g/L (1/3 labelled rate) + HP. However, a significant reduction in leaf scab symptomology was recorded. All remaining treatments (boscalid + pyraclostrobin [labelled rate] with and without HP, boscalid + pyraclostrobin [2/3 labelled rate] + HP) significantly reduced ($P < 0.05$) leaf and fruit scab symptomology and significantly increased leaf chlorophyll content and pear fruit yield compared to the water-treated pear trees. No significant differences between these treatments were recorded (Table 4).

Table 4. The influence of fungicide and harpin protein combinations applied as foliar sprays on symptom severity of pear scab (*Venturia pirini*) on *Pyrus communis* 'Williams' Bon Chrétien.'

Treatment	Chlorophyll content	Leaf scab symptom severity ^z	Fruit scab symptom severity ^z	Fruit yield
Water (control)	31.5a	3.9c	1.8c	11.4a
Harpin protein (HP)	37.2ab	2.5b	1.3b	13.0abc
Boscalid + pyraclostrobin 0.9 g/L	44.1bc	0.8a	0.0a	14.8bc
Boscalid + pyraclostrobin 0.9 g/L + HP	48.8c	0.2a	0.0a	15.0c
Boscalid + pyraclostrobin 0.6 g/L + HP	40.2bc	1.0a	0.5a	13.4abc
Boscalid + pyraclostrobin 0.3 g/L + HP	36.1ab	2.2b	1.6bc	12.0ab

^zAll values mean of 80 leaves (10 leaves per tree) and 40 fruits (5 fruits per tree) from 8 randomized complete blocks with a single tree replication per block. Numbers within a column followed by a common letter are not significantly different according to the Kruskal-Wallis Multiple Comparison Z-test ($P \leq 0.05$).

Table 5. The influence of fungicide and harpin protein combinations applied as foliar sprays on pathogen severity of *Guignardia* leaf blotch (*Guignardia aesculi*) on horse chestnut (*Aesculus hippocastanum*).

Treatment	Chlorophyll content	Leaf blotch symptom severity ^z	Mean seed dry weight
Water (control)	30.4a	3.0c	5.0a
Harpin protein (HP)	36.6ab	2.0b	7.2b
Boscalid + pyraclostrobin 0.9 g/L	45.2cd	1.2ab	8.3b
Boscalid + pyraclostrobin 0.9 g/L + HP	51.4d	0.8a	8.4b
Boscalid + pyraclostrobin 0.6 g/L + HP	43.9bcd	1.5ab	8.0b
Boscalid + pyraclostrobin 0.3 g/L + HP	37.2abc	1.8b	7.5b

^zAll values mean of 80 leaves (10 leaves per tree) from 8 randomized complete blocks with a single tree replication per block.

Numbers within a column followed by a common letter are not significantly different according to the Kruskal-Wallis Multiple Comparison Z-test ($P \leq 0.05$).

***Aesculus hippocastanum* (2017)**

Application of the synthetic fungicide boscalid + pyraclostrobin and IR agent HP alone or in combination, irrespective of labelled rate, was effective in significantly ($P < 0.05$) increasing mean seed dry weight compared to the water-treated control trees (Table 5). No significant differences between these treatments were recorded. Application of the IR agent HP alone significantly reduced ($P < 0.05$) leaf blotch symptom severity but had no significant effect on leaf chlorophyll content compared to the water-treated control trees. The treatments (boscalid + pyraclostrobin [labelled rate] with and without HP, boscalid + pyraclostrobin [2/3 labelled rate] + HP) significantly reduced ($P < 0.05$) leaf blotch symptomology and significantly increased leaf chlorophyll content. No significant differences between these treatments were recorded (Table 5). No significant effect on leaf chlorophyll content was recorded when boscalid + pyraclostrobin was applied at 0.3 g/L (1/3 labelled rate) + HP.

However, a significant reduction in leaf blotch symptom severity was recorded.

***Pyrus communis* 'Williams' Bon Chrétien' (2018)**

Application of the IR agent liquid chitosan (LC) alone significantly ($P < 0.05$) reduced leaf scab symptomology but had no significant effect on fruit scab symptomology, leaf chlorophyll content, and fruit yield compared to the water-treated control trees (Table 6). All remaining treatments (boscalid + pyraclostrobin [labelled rate] with and without LC, boscalid + pyraclostrobin [1/3 labelled rate] + LC, boscalid + pyraclostrobin [2/3 labelled rate] + LC) significantly reduced ($P < 0.05$) leaf and fruit scab symptomology and significantly increased leaf chlorophyll content and pear fruit yield compared to the water-treated pear trees. No significant differences between boscalid + pyraclostrobin (labelled rate) with and without LC were recorded with respect to these 3 parameters.

Table 6. The influence of fungicide and liquid chitosan combinations applied as foliar sprays on symptom severity of pear scab (*Venturia pirini*) on *Pyrus communis* 'Williams' Bon Chrétien.'

Treatment	Chlorophyll content	Leaf scab symptom severity ^a	Fruit scab symptom severity ^a	Fruit yield
Water (control)	28.4a	4.0c	2.2b	9.5a
Liquid chitosan (LC)	32.4ab	3.6b	1.8b	10.9abc
Boscalid + pyraclostrobin 0.9 g/L	42.8c	0.5a	0.5a	13.6c
Boscalid + pyraclostrobin 0.9 g/L + LC	46.1c	0.5a	1.0a	13.0c
Boscalid + pyraclostrobin 0.6 g/L + LC	39.2bc	2.5b	1.0a	12.5bc
Boscalid + pyraclostrobin 0.3 g/L + LC	30.7a	3.5b	1.8b	10.0ab

^aAll values mean of 80 leaves (10 leaves per tree) and 40 fruits (5 fruits per tree) from 8 randomized complete blocks with a single tree replication per block. Numbers within a column followed by a common letter are not significantly different according to the Kruskal-Wallis Multiple Comparison Z-test ($P \leq 0.05$).

However, a significant difference between boscalid + pyraclostrobin (labelled rate) with and without LC and boscalid + pyraclostrobin (2/3 labelled rate) + LC was recorded with respect to leaf scab symptomology (Table 6).

DISCUSSION

Aesthetic damage such as symptoms of leaf blotches and scabs caused by fungal pathogens is viewed as a high priority by professionals managing urban landscapes, as aesthetics (leaf colour, silhouette, shape) is a major characteristic by which trees are selected and planted (Hersh et al. 2012; Hantsch et al. 2013, 2014). However, loss of leaf photosynthetic area caused by these pathogens can also have a detrimental influence on the short- and long-term survival of trees (Hersh et al. 2012). Excess photosynthetic carbohydrates are predominantly stored in the sapwood and the bark (Mangel et al. 2000), and it is important for trees to have a large carbohydrate reserve for future growth and tolerance to abiotic stressors such as drought, salinity, and waterlogging (Martínez-Trinidad et al. 2009). In addition, the amount of reserve carbohydrates in young trees and their root systems is crucial for successful establishment following out-planting (Ritchie and Dunlop 1980; Struve 1990). The process of recovery following root severance is dependent on the ability of a tree to manufacture abundant photosynthetic carbohydrates, such as sucrose (Lonsdale 2001; Lindqvist and Asp 2002). While it could be argued that mature trees will be in a position to withstand repeated loss of leaf photosynthetic area due to their larger storage reserves within the tree trunk and

root system (Pallardy 2008; Martínez-Trinidad et al. 2009), it is debatable whether smaller trees (≤ 2.5 m high) are capable of such long-term survival.

Our results demonstrated that currently labelled fungicides are effective against pear scab (*Venturia pirina*) and *Guignardia* leaf blotch (*Guignardia aesculi*) under field conditions, provided adequate spray coverage and frequent sprays are applied. Under these conditions, symptom control ranged from 75% to 100%. Boscalid plus pyraclostrobin is a systemic fungicide with protective and curative properties that has been shown to possess efficacy against a broad range of foliar pathogens under *in vitro*, glasshouse, and field conditions (Percival et al. 2009; Burnett et al. 2010; LaMondia 2015). Consequently, boscalid plus pyraclostrobin has approval for the management of foliar leaf pathogens under glasshouse, orchard, and urban landscapes (BCPC 2019). In contrast, although it is now well documented that pre-treatment of plants with various chemical and biological agents (e.g., virulent or avirulent pathogens, non-pathogens, cell wall fragments, natural plant extracts, and synthetic chemicals) can induce resistance to subsequent pathogen attack, efficacy is more uncertain. Induced resistance rarely leads to complete pathogen control, more often resulting in, for example, a reduction in lesion numbers and size (Walters et al. 2005). Despite this, the prospect of broad-spectrum disease control using the plant's own resistance mechanisms has led to increasing commercial interest in the development of available IR agents which mimic natural inducers of resistance (Walters et al. 2005; Eyles et al. 2010). Our study, like others, found that the IR agents harpin

protein and salicylic acid reduced disease symptoms by 25% to 58%, whereas liquid chitosan reduced disease symptoms by 10% to 18%. These results indicate that IR agent efficacy may differ between products.

The results of the present study were achieved under severe inoculum pressure, as a monoculture planting existed during field trials. Under these conditions, the application of a synthetic fungicide at full strength in combination with an IR agent in general provided a greater reduction in disease symptomology (2% to 20%) than application of a synthetic fungicide at full strength. However, this reduction was rarely significant at $P < 0.05$. Such a small increase in efficacy raises a dilemma. On one hand, it is unlikely to prove cost-effective to combine products. On the other, both of the fungicides used in our studies have specific modes of action and as such may lead to the rise of fungicide-resistant strains of pathogens (Gozzo 2003; Vallad and Goodman 2004; Fobert and Després 2005; Witzell and Martin 2008). As a consequence, their use requires a resistance management strategy, such as tank mixing or alternating with other fungicides. Combining a fungicide with an IR agent that possesses different modes of action may therefore help reduce the build-up of pathogen resistance and prolong the commercial life of an existing fungicide before it is withdrawn. Within Europe, loss of registered commercial fungicides is of concern, as a stage has now been reached where fungicide withdrawal from commercial use exceeds the registration of new fungicides, placing even greater selection pressure on those fungicides still available for use (BCPC 2019).

One of the key results of this investigation is that in virtually all studies, application of a fungicide at 2/3 strength plus IR agent provided the same degree of pathogen control as a fungicide at full strength. This suggests that IR agents offer the potential to control foliar pathogens using a reduced input of synthetic fungicide. Previous research using a combination of fungicides and IR agents against pathogens of crop plants supports this finding. For example, a mixture of a strobilurin fungicide and ASM was shown to be effective in controlling *Albugo occidentalis* and increasing leaf quality in spinach (Leskovar and Kolenda 2002), while a mixture of ASM and mancozeb was shown to have potential to provide protection against *Claviceps africana* on sorghum, especially where fungal isolates resistant to the usual fungicide treatment, triadimenol, were present (Ryley et al.

2003). Gent and Schwartz (2005) found that integration of ASM and biological control agents with copper hydroxide could be used to replace less desirable fungicides without compromising effective control of *Xanthomonas* leaf blight on onion. Work by LaMondia (2009) demonstrated that the addition of the IR agent ASM was effective in a spray program that reduced lesions per plant by up to 99% compared to the non-treated control against blue mould of tobacco. Similar results have been reported by other researchers (Cole 1999; Perez et al. 2003). In addition, LaMondia (2009) also found that substituting 2 or 3 applications of ASM for the fungicides dimethomorph + mancozeb treatments in a spray program decreased blue mould lesions compared with fungicide-only treatments by 28% to 94%. In addition, systemic activity was considered very desirable, as spray coverage was difficult to achieve in shade tobacco (LaMondia and Horvath 2001). In addition, as the mode of action of IR agents is indirect, selection pressure on pathogens is not exerted, and thus insensitivity does not arise, and no fungicide residues would exist within plants. This is important when using fungicides against foliar diseases of fruit bearing crops, i.e., the use of IR agents has little, if any, direct antimicrobial activity, presents no known human toxicity issues, and is environmentally benign in comparison to pesticide alternatives (Vallad and Goodman 2004; Walters 2009; Eyles et al. 2010).

CONCLUSION

In conclusion, 5 independent field experiments performed over 3 years with 1 or 2 experiments per year show that applications of IR agents alone are useful in reducing the symptomology of 2 foliar pathogens frequently encountered in urban landscapes. Combinations of an IR agent with as little as a 1/3 reduced dose of fungicide still significantly reduced the severity of symptoms on both leaves and fruit. This suggests that an IR agent plus a reduced dose of fungicide offers a way to reduce fungicide use and may also reduce the risk of fungicide resistant pathogen strains developing by decreasing fungicide selection pressure. These findings would be useful to professionals involved in urban landscape management, as they indicate synthetic fungicide usage can be reduced, but adequate control retained, with the additional advantage that IR agents in general cost 40% to 80% less than conventional fungicides.

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Résumé. Les agents pathogènes foliaires non contrôlés peuvent être préjudiciables à la santé et à l'esthétique des arbres. La dépendance excessive à l'égard des fongicides synthétiques signifie que des moyens alternatifs de gestion des pathogènes sont maintenant nécessaires. Le but de ces projets de recherche était d'étudier l'efficacité de trois agents disponibles dans le commerce, soit la protéine harpine, un dérivé de l'acide salicylique et le chitosane liquide, qui peuvent initier une résistance induite (RI) chez les végétaux. Des agents RI furent appliqués individuellement et en combinaison avec un fongicide synthétique (boscalide + pyraclostrobine) contre 2 pathogènes foliaires (*Venturia pirina* et *Guignardia aesculi*) dans des conditions réelles avec *Pyrus communis* 'Williams' Bon Chrétien' et le marronnier d'Inde (*Aesculus hippocastanum*) agissant comme arbres hôtes. Ces agents furent évalués durant trois années consécutives. Pour 4 de ces 5 études de terrain, l'utilisation d'un agent RI en solo a permis de

réduire la gravité des symptômes de l'agent pathogène, d'augmenter la production des fruits et des semences et d'améliorer la teneur foliaire en chlorophylle. Dans pratiquement tous les projets, l'application de boscalide + pyraclostrobine aux deux tiers de la concentration avec l'addition d'un agent RI a permis d'obtenir le même degré de contrôle des pathogènes que le boscalide + pyraclostrobine à pleine puissance. L'application de boscalide + pyraclostrobine au tiers de la concentration plus un agent RI a permis un degré appréciable de contrôle des pathogènes foliaires. Les résultats ont montré que la combinaison d'un agent RI avec le tiers de la dose de boscalide + pyraclostrobine était aussi efficace pour réduire la gravité des symptômes des 3 pathogènes foliaires que le boscalide + pyraclostrobine appliqué à pleine puissance, à condition qu'un minimum de 4 pulvérisations soient effectuées pendant la saison de croissance.

Zusammenfassung. Unkontrollierte, blattbildende Krankheitserreger an Stadtbäumen können die Gesundheit und Ästhetik der Bäume beeinträchtigen. Das übermäßige Vertrauen in synthetische Fungizide bedeutet, dass zunehmend alternative Mittel zur Bekämpfung von Krankheitserregern erforderlich sind. Ziel dieser Studien war es, die Wirksamkeit von drei kommerziell erhältlichen Wirkstoffen zu untersuchen: Harpin-Protein, Salicylsäure-Derivat und flüssiges Chitosan, die eine induzierte Resistenz (IR) in Pflanzen auslösen können. Die IR-Mittel wurden unabhängig voneinander und in Kombination mit einem synthetischen Fungizid (Boscalid + Pyraclostrobin) gegen 2 Blattpathogene (*Venturia pirina* und *Guignardia aesculi*) unter Feldbedingungen mit *Pyrus communis* 'Williams' Bon Chrétien' und Rosskastanie (*Aesculus hippocastanum*) als Baumwirte angewendet. Diese Mittel wurden über drei aufeinanderfolgende Jahre getestet. In vier von fünf Feldstudien reduzierte die Anwendung eines IR-Mittels allein die Schwere der Pathogensymptome, erhöhte den Frucht-/Samenertrag und verbesserte den Chlorophyllgehalt der Blätter. In praktisch allen Studien lieferte die Anwendung von Boscalid + Pyraclostrobin in 2/3 Stärke zusammen mit einem IR-Mittel den gleichen Grad an Pathogenkontrolle wie Boscalid + Pyraclostrobin in voller Stärke. Die Anwendung von Boscalid + Pyraclostrobin

in 1/3-Stärke zusammen mit einem IR-Mittel lieferte ein angemessenes Maß an Kontrolle von Blattpathogenen. Die Ergebnisse zeigten, dass eine kombinierte Mischung eines IR-Mittels mit einer um 1/3 reduzierten Dosis Boscalid + Pyraclostrobin bei der Reduzierung der Symptomschwere von 3 Blattpathogenen genauso wirksam war wie Boscalid + Pyraclostrobin in voller Stärke, vorausgesetzt, es wurden mindestens 4 Spritzungen während einer Wachstumsperiode durchgeführt.

Resumen. Los patógenos foliares administrados sin manejo a los árboles urbanos pueden ser perjudiciales para la salud y la estética de los árboles. El uso excesivo de fungicidas sintéticos hace que ahora se requieran medios alternativos de gestión de patógenos. El propósito de estos estudios fue investigar la eficacia de 3 agentes disponibles comercialmente: proteína dearpin, ácido salicílico y quitosano líquido, que puede iniciar la resistencia inducida (IR) en las plantas. Los agentes IR se aplicaron de forma independiente y en combinación con un fungicida sintético (boscalid + piraclostrobina) en contra de 2 patógenos foliares (*Venturia pirina* y *Guignardia aesculi*) en condiciones de campo con *Pyrus communis* 'Williams' Bon Chrétien' y castaño de Indias (*Aesculus hippocastanum*) actuando como anfitriones. Estos agentes fueron probados durante 3 años consecutivos. En 4 de 5 estudios de campo, el uso de un agente IR por sí solo redujo la gravedad de los síntomas patógenos, el aumento del rendimiento de la fruta/semilla y el contenido mejorado de clorofila de la hoja. En prácticamente todos los estudios, la aplicación de boscalid + piraclostrobina a 2/3 de resistencia más un agente IR proporcionó el mismo grado de control patógeno que boscalid + piraclostrobina a plena resistencia. La aplicación de boscalid + piraclostrobina a 1/3 de resistencia más un agente IR proporcionó un grado razonable de control del patógeno foliar. Los resultados mostraron que una mezcla combinada de un agente IR con una dosis reducida de boscalid + piraclostrobina fue tan eficaz para reducir la gravedad de los síntomas de 3 patógenos foliares como boscalid + piraclostrobina aplicada a plena resistencia, siempre que se aplicaran al menos 4 aerosoles durante una temporada de crecimiento.

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