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The Influence of Chitin- and Chitosan-Based Soil Amendments on Pathogen Severity of Apple and Pear Scab

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Abstract. Apple and pear scab are foliar pathogens of apple and pear trees. Unmanaged, yield and aesthetic losses can be severe. The risk of resistance associated with over-reliance on fungicides means novel pathogen management methods and products are increasingly required. Chitin and chitosan are widely recognised as induced resistance (IR) agents that trigger plant defence responses that in turn enhance plant resilience to pathogen ingress. A container and field trial was conducted using apple (*Malus sylvestris*) and pear (*Pyrus communis* 'Conference') respectively to assess the efficacy of a range of liquid and granular chitin/chitosan-based IR agents and fertilisers against apple and pear scab. A synthetic fungicide (penconazole) spray program used within the UK for apple and pear scab control was included for comparison. Application of chitin/chitosan IR agents at concentrations above 1% caused phytotoxicity. Limited efficacy as scab protectants was also demonstrated when chitin/chitosan IR agents were applied at concentrations of 0.25%. However, chitin IR agents when applied at 0.5% and 1% and chitosan IR agents at 1% demonstrated efficacy as scab protectants and resulted in an increased leaf chlorophyll content, increased fruit yield, and reduced leaf scab severity when compared against the watered control. Only one of three chitin/chitosan fertilisers evaluated demonstrated efficacy as scab protectants (liquid chitosan). A synthetic fungicide penconazole spray program provided the greatest protection against apple and pear scab in the container trials. However, under field conditions the degree of scab control following application of chitin and chitosan at 1% and the chitosan containing fertiliser liquid chitosan was statistically comparable to fungicide treated trees. Results suggest application of an appropriate chitin/chitosan IR agent(s) and fertiliser offers a useful addition to existing methods of apple and pear scab management under field and container conditions.

Keywords. Fungicides; Integrated Disease Management; Orchard Management; Pathogen Control; Plant Health Care; Urban Landscapes; *Venturia inaequalis*; *V. pirina*.

INTRODUCTION

Ornamental apple (*Malus* spp.) and pear (*Pyrus* spp.) varieties and cultivars are a popular planting choice for public and private urban landscapes throughout Europe and the USA. Both genera possess aesthetic (flower colour, attractive bark) and functional characteristics (hardy, adaptable to varied soil conditions, available in a wide range of sizes and shapes) desirable for urban landscape plantings (Percival 2018). During the growing season, however, *Malus* and *Pyrus* species are susceptible to attack by the foliar pathogens *Venturia inaequalis* (Cke.) Wint. and *V. pirina* Aderhold, which are the pathogens of apple and pear scab respectively (Cuthbertson and Murchie 2003; Villalta et al. 2004; Hailey and Percival 2014). Highly susceptible varieties can be severely defoliated by mid- to late-summer while heavily scabbed fruit becomes deformed and may fall before maturity (Percival and Boyle 2005; Aćimović et al. 2016). Within the UK, thousands of ornamental apple and pear trees exhibit symptoms of these 2 pathogens, which if untreated will result in annual defoliation leading to decline and, in the case of highly sensitive varieties, death (Percival 2018). Strategies for scab management within the UK urban landscape can be difficult to implement (Lainsbury 2020): in environmentally sensitive and high pedestrian traffic locations, the risk associated with spray drift from foliar applications of fungicides can be prohibitive or severely limiting. This risk could be mitigated by applying systemic fungicides to the soil or through stem infusion. However, at the time of publication, there are no stem-infused or soil-applied systemic fungicides that are registered for apple and pear scab

control within the UK urban landscape. Therefore, a soil-applied product with the capacity to reduce apple and pear scab disease severity would be valuable (Cuthbertson and Murchie 2003; Villalta et al. 2004).

After cellulose, chitin is the second most abundant polysaccharide on the planet. Developments in fertiliser formulation have led to the manufacture of chitin and its partially deacylated derivative known as chitosan (Sharp 2013; Sharif et al. 2018). Both chitin and chitosan are found in, and can be sourced from, a variety of living organisms including the exoskeletons of arthropods and crustaceans and the cell walls of fungi (El Hadrami et al. 2010). Use of these chitin, chitosan, and/or fertilisers containing these 2 compounds as active ingredients has repeatedly been shown to be beneficial in the management of fungal diseases such as Fusarium wilt (biotrophic pathogen) in tomato and *Botrytis cinerea* in strawberry (Benhamou et al. 1994; Rabea et al. 2003). Similarly, control of the oomycete pathogens *Phytophthora capsici* on peppers (Xu et al. 2007) and *P. infestans* in potato (O'Herlihy et al. 2003) has been achieved following soil amendment with chitosan. Chitin and chitosan applied to plants in low concentrations as a soil amendment or foliar spray activates biochemical, genetic, and physical defence mechanisms which in turn have allowed plants to resist or tolerate a broad range of pathogenic fungi, bacteria, and viruses (Zhang et al. 2003; Radwan et al. 2012; Sharp 2013). This concept of using compounds to activate defence mechanisms is widely referred to as induced resistance. In addition, chitin and chitosan have been found to stimulate the activity of plant symbiotic microbes and promote the colonisation of root tissue by arbuscular mycorrhizal (AM) fungi and *Rhizobium* bacteria (Li et al. 2020; Volpe et al. 2020). This is a 2-fold benefit for the plant due to an increase in water and nutrients (notably nitrogen with *Rhizobium* symbiosis and phosphorus with AM symbiosis) and an alteration in the rhizosphere microbial equilibrium which disadvantages soil-borne pathogens (Bell et al. 1998; Murphy et al. 2000; Sharif et al. 2018).

Due to chitin and chitosan's low toxicity and environmental compatibility, chitin- and chitosan-based soil amendments offer opportunities for the control of fungal pathogens in urban landscape ecosystems through their induced resistance mode of action (Zhang et al. 2003). Likewise, given the relative low cost, these products offer an economically feasible option to protect newly planted and established mature trees through their incorporation into the bulk soil surrounding existing root systems and the rhizosphere, using, for example, air excavation technology (Percival and Graham 2021). However, care should be taken when selecting chitin- or chitosan-based fertilisers for plant protection purposes as efficacy can differ markedly between formulations.

Little information exists on the efficacy of soil-applied chitin and chitosan against pathogens of urban tree species. Consequently, this paper aims to generate original and novel data by answering the following questions:

1) Do chitin, chitosan, and chitin/chitosan-based commercially available fertilisers offer viable management options for scab protection?
2) What is the optimal chitin/chitosan/fertiliser concentration that can be applied to plants for maximal efficacy?
3) Can chitin/chitosan/fertiliser be phytotoxic to trees?

MATERIALS AND METHODS

Container Trials (2018)

Experiments used cell-grown bare-rooted stock of *Malus sylvestris* (crab apple, susceptible to apple scab [*Venturia inaequalis*]). Trees were approximately 90 cm high, ± 6 cm. Trees were purchased from a commercial supplier during December and January and planted upon delivery in February. Trees were potted into 10-L plastic pots filled with soil: loam texture, 23% clay, 44% silt, 30% sand, 3% organic matter, pH 6.6, and supplemented with the controlled-release nitrogen-based NPK fertiliser Basacote® Plus 9M 16-8-12(+2+TE)(Compo Expert UK Ltd, Stourport-on-Severn, England, UK) at a rate of 5 g L^{-1} of soil. At this stage, soil was also amended with chitin or chitosan at zero (control), 0.25%, 0.5%, 1.0%, 1.5%, and 2% by volume. *Malus sylvestris* trees from the same cell-grown bare-rooted stock were planted into nonamended soil and sprayed with a conventional fungicide (penconazole at 0.75 mL L^{-1}) at 4 growth stages identified as key application times for scab control under field conditions (Bevan and Knight 2001): namely, bud break (March 13), green cluster (April 12), 90% petal fall (May 19), and early fruitlet (June 12). Therefore, the container trials had 12 different treatments: chitin and chitosan at 5 different concentrations, a penconazole fungicide industrial

comparison, and a control, with 10 trees per treatment. Following potting, trees remained outdoors on a free-draining weed-suppressant fabric at the Bartlett Tree Research Site, Reading, Berkshire, England, UK (51°24'44"N, 01°56'18"W). Trees were subject to natural climatic conditions and watered as required. The experimental design adopted was a completely randomised block design. All trees were subjected to an insecticide program using the residual pyrethroid insecticide deltamethrin (product name Bandu) (Headland Agrochemicals Ltd, Saffron Walden, Essex, England, UK) where a 0.9 mL L^{-1} solution of deltamethrin was applied every 12 weeks commencing in May 2018 to September 2018. Insecticide sprays were applied using a 2-L handheld pump sprayer, and trees were sprayed until runoff, which generally required 30 to 40 mL of insecticide solution per tree.

Field Trials (2019)

The field site was established in 2010 and consisted of a 100-m^2 block planted with bare-rooted, standard *Pyrus communis* 'Conference' pear stock with a distance of 2 m between trees. Trees were staked and given supplementary water until establishment (2 years). Trees had an average height of 1.6 m ± 0.25 m at the time of the experiment, with mean trunk diameters of 5 cm ± 1.4 cm just above the graft. The trial site was located at the Bartlett Tree Research Site, Reading, Berkshire, England, UK (51°24'44"N, 01°56'18"W).

Weeds were controlled physically by hand and by using a hoe at 8 weekly intervals throughout the experiment. No supplemental watering or fertilisation was applied during the trial. Historically, trees suffered from pear scab infection on an annual basis. The trees were inspected in September 2018, and only those with greater than 30% of leaves affected with scab infection were included in the trial that commenced in spring 2019. Treatments were assigned to trees using a completely randomised block design with 5 replicates and consisted of an industry comparative fungicide spray of penconazole; a watered control; a chitosan or chitin top dressing soil amendment at 1.0% by volume; a soil-applied top dressing of crab meal; a soil-drench liquid chitosan; and a soil-drench chitin fertiliser (Agrinos 5-0-0). See Table 1 for full details. Fungicide application occurred at 4 growth stages (Bevan and Knight 2001), namely: bud break (March 9), green cluster (April 8), 90% petal fall (May 16), and early fruitlet (June 9).

All trees were subjected to an insecticide program using the residual pyrethroid insecticide deltamethrin, where a 0.9-mL L^{-1} solution of deltamethrin was applied every 12 weeks commencing in May 2019 until September 2019. Insecticide sprays were applied using a 10-L knapsack sprayer, and trees were sprayed until runoff, which generally required 200 to 250 mL of insecticide solution per tree.

Plant Vitality Assessments

Measurements were made towards the cessation of the growing season (late September/early October) in both container (2018) and field (2019) trials. To keep the physiological age of the leaves comparable throughout the experiment, plant vitality measurements were made only on fully expanded, mature, green-leaf tissue.

Leaf Chlorophyll SPAD Measurements

A Minolta chlorophyll meter SPAD-502 (Konica Minolta, Inc., Tokyo, Japan) was used at the midpoint of the leaf next to the main leaf vein. In all cases, SPAD measurements were taken from 6 leaves (2 from the top of the crown, 2 in the centre, and 2 at the base) per plant.

Leaf Chlorophyll Fluorescence

Measurements were made on 10 leaves per tree. Measurements were performed using a pocket Plant Efficiency Analyser (PEA) device (Hansatech Instruments Ltd, Norfolk, England, UK). Following dark adaptation (30 minutes), the fluorescence response was induced by a one-second flash of light (650 nm, 1500 µmol m^2 s^{-1}) provided by an array of 3 light-emitting diodes over a 4-mm diameter of leaf surface. The ratio of variable ($F_V = F_M - F_0$) to maximal fluorescence (F_M) was calculated. The multi-parametric parameter known as performance index (PI_{ABS}) was also calculated in accordance with the calculations review by Banks (2017) with extrapolated F_0 used.

Scab Severity

Scab severity of leaves and fruit was assessed visually on September 28 and 29 in both container (2018) and field (2019) trials. Leaf scab severity of each tree was rated using a visual indexing technique and ratings on the scale:

0 = No scab observed

1 = < 5% of leaves affected and no aesthetic impact

Table 1. Products, active ingredient, concentration, directions for use, and supplier of treatments[a] used.

Commercial name	Active ingredient	Concentration applied	Directions for use	Supplier
Topas	Penconazole	1.5 mL/L of water	See "Materials and Methods"	Syngenta UK Ltd, Fulbourn, Cambridgeshire, UK
Chitin	Chitin	120 g/m^2 under the canopy = 360 g per tree applied	Once as a top dressing in March or as buds swell	Tidal Vision, Bellingham, WA, USA
Chitosan	Chitosan	120 g/m^2 under the canopy = 360 g per tree applied	Once as a top dressing in March or as buds swell	Tidal Vision, Bellingham, WA, USA
Crab meal	Crab meal	0.75 kg under the canopy per tree applied	Once as a top dressing in March or as buds swell	Ocean Crest Seafoods Inc., Gloucester, MA, USA
Liquid chitosan	Chitosan	1.0 mL/5 L of water per m^2 under the canopy = 3 mL per tree applied	One weekly drench for the first three weeks, and then one drench per month throughout the growing season; start first drench in March or as buds swell	Viresco UK Ltd, Thirsk, North Yorkshire, UK
Agrinos 5-0-0	Chitin	0.8 g/L of water per m^2 under the canopy = 2.4 g per tree applied	Every 2 weeks throughout the growing season; start first drench in March or as buds swell	Hortifeeds, Park Farm, Kettlethorpe, Lincoln, UK
Control	Water	–	–	–

[a]All treatments based on manufacturers' recommended efficacy rates.

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 (field trial only) was calculated using 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).

Fruit Yield: Field Trial Only

Fruit was not thinned during July and August 2019; all were allowed to reach maturity. Fruit yield per tree was determined by weighing all of the fruit (symptomatic and asymptomatic) on each tree at harvest and dividing by the number of fruits per tree.

Statistical Analysis

All data were analysed using Analysis of Variance (ANOVA), and where appropriate, differences between means were determined using Tukey's Honest Significant Difference test (P = 0.05) using Genstat 19th edition (VSNi International, Hemel Hempstead, England, UK).

RESULTS

Container Trials

Damaging outbreaks of apple scab were recorded on control trees as indicated by an average leaf scab symptom severity rating of 3.8 on *M. sylvestris* at the cessation of the 2018 growing season (Table 2). None of the treated or control trees died as a result of scab attack during the course of the study. However, chitin

and chitosan applied at concentrations greater than 1.0% proved phytotoxic to the containerised apples. The degree of phytotoxic damage recorded meant accurate scab severity values could not be recorded. Similarly, due to phytotoxicity issues, leaf chlorophyll content (SPAD) and chlorophyll fluorescence F_V/F_M and PI_{ABS} values as measures of leaf photochemical and photosynthetic efficiency respectively were significantly lower ($P < 0.05$) than water-treated controls.

Limited efficacy as scab protectant compounds was demonstrated when chitin and chitosan were applied at 0.25%, where leaf scab severity, leaf chlorophyll content (SPAD), and chlorophyll fluorescence F_V/F_M and PI_{ABS} values were in virtually all cases not statistically different from water-treated controls. Chitin and chitosan applied at 1.0% were an effective scab protectant against scab on leaves, where leaf scab severity, leaf chlorophyll content (SPAD), and chlorophyll fluorescence F_V/F_M and PI_{ABS} values were most similar to the penconazole treatment, albeit significantly different for all metrics apart from F_V/F_M. In all cases, leaf scab severity was significantly lower than the controls, and leaf chlorophyll content (SPAD) and chlorophyll fluorescence F_V/F_M and PI_{ABS} values were significantly greater than the controls. Application of chitin at 0.5% significantly reduced leaf scab severity and significantly increased leaf chlorophyll content (SPAD) and chlorophyll fluorescence F_V/F_M and PI_{ABS} values compared to water-treated controls. However, application of chitosan at 0.5% had no significant effect on leaf scab severity, leaf chlorophyll content (SPAD), and chlorophyll fluorescence F_V/F_M and PI_{ABS} values compared to water-treated controls. Greatest reductions in leaf scab severity and increases in leaf chlorophyll content (SPAD) and chlorophyll fluorescence F_V/F_M and PI_{ABS} values were recorded in penconazole-treated trees. A highly significant effect of treatment was observed with a P-value < 0.001.

Field Trials

Damaging outbreaks of pear scab were recorded on control trees as indicated by leaf and fruit scab symptom severity ratings of 3.5 and 1.8 respectively at the cessation of the growing season (Table 3). None of the treated or control trees died as a result of scab attack during the course of the study. Likewise, no symptoms of phytotoxicity were recorded following application of chitin and chitosan at 1.0%, penconazole, crab meal, liquid chitosan, or Agrinos 5-0-0. Limited efficacy as scab protectant compounds was observed when the chitin and chitosan containing fertilisers

Table 2. The influence of penconazole and chitin/chitosan-based soil amendments on pathogen severity of apple scab (*Venturia inaequalis*) on *Malus sylvestris* (crab apple) containerised seedlings[a].

Treatment	Leaf scab severity[b]	SPAD	F_V/F_M	PI_{ABS}
Control (water)	3.8d	19.9c	0.700de	3.61d
Penconazole	1.0a	38.4g	0.813g	7.65h
Chitin (2.0%)	–	14.3ab	0.564b	1.47a
Chitin (1.5%)	–	15.9b	0.639c	2.75bc
Chitin (1.0%)	1.6b	34.9f	0.800fg	6.06f
Chitin (0.5%)	2.2c	31.3e	0.770f	4.97e
Chitin (0.25%)	3.6d	22.8d	0.728e	4.43e
Chitosan (2.0%)	–	13.5a	0.497a	1.34a
Chitosan (1.5%)	–	16.0b	0.550b	2.45b
Chitosan (1.0%)	1.5b	34.2f	0.803fg	6.82g
Chitosan (0.5%)	3.8d	19.4c	0.711de	3.17cd
Chitosan (0.25%)	3.7d	20.0c	0.690d	3.47d
Significance of Treatment (T)	< 0.001	< 0.001	< 0.001	< 0.001

[a]Numbers within a column followed by a common letter are not significantly different according to Tukey's Honest Significant Difference test ($P = 0.05$).
[b]Mean of 100 leaves from 10 trees (10 leaves per tree) placed in a completely randomised block design.

Agrinos 5-0-0 and crab meal were applied. In Agrinos 5-0-0 and crab meal treated trees at the cessation of the growing season, leaf and fruit scab severity symptoms, leaf chlorophyll content (SPAD), and chlorophyll fluorescence F_V/F_M and PI_{ABS} values were, in almost all cases, statistically comparable to water-treated controls. Application of chitin and chitosan at 1.0% and liquid chitosan, however, proved effective as scab protectant compounds. At the cessation of the growing season, there was a reduction in leaf and fruit scab severity and an increase in leaf chlorophyll content (SPAD) and chlorophyll fluorescence PI_{ABS} and F_V/F_M values. With the exception of liquid chitosan and crab meal fruit yield, the metrics were statistically comparable to the penconazole treatment and statistically lower (leaf and fruit scab severity symptoms) and higher (leaf chlorophyll content [SPAD] and chlorophyll fluorescence PI_{ABS} and F_V/F_M values) than water-treated controls. A highly significant effect of treatment was observed with a P-value < 0.001.

DISCUSSION

The effectiveness of triazole-based fungicide spray programmes against apple and pear scab under field conditions has been confirmed several times, although concerns have been raised regarding build-up of triazole resistance amongst scab populations (Jørgensen and Thygesen 2006; Deising et al. 2008; Percival et al. 2009; Aćimović et al. 2016). Despite this, penconazole is fully approved for apple and pear scab management under current UK pesticide legislation (Lainsbury 2020). Indeed, penconazole proved very effective for apple and pear scab control in both container and field studies (Percival et al. 2009). Results of this study support these conclusions with penconazole proving to be a highly effective scab protectant in terms of reduced leaf and fruit apple and pear scab severity and increases in leaf chlorophyll content and fruit yield compared to untreated controls.

The use of chitin and chitosan at a concentration of greater than or equal to 1.5% proved phytotoxic to container grown *M. sylvestris*. Previous research has also shown that chitin, chitosan, and chitin- and chitosan-based fertilisers can, at an inappropriate concentration, prove phytotoxic to plants. Consequently, it is now recommended that for plant protection purposes under field conditions, chitin and/or chitosan should be applied at less than or equal to 1.0% (D'Addabbo 1995; Radwan et al. 2012). In support of this recommendation, laboratory-based in vitro bioassays have shown that chitin and chitosan have optimal fungicidal, bactericidal, and antiviral properties when used at between 0.5% and 1.0% (Muzzarelli et al. 1990; Vasyukova et al. 2001; Rabea et al. 2005; El Hadrami et al. 2010).

The use of chitin and chitosan to control plant pathogens has been extensively explored with success heavily depending on the pathosystem, the used chitin/chitosan derivatives, concentration, and the applied formulation (i.e., soil amendment and foliar application, alone or in association with other treatments)(Ozbay and Newman 2004; Walker et al. 2004). For example, root rot caused by *Fusarium oxysporum*

Table 3. The influence of penconazole and chitin/chitosan-based soil amendments on pathogen severity of pear scab (*Venturia pirini*) on *Pyrus communis* 'Conference' (pear 'Conference') under field conditions[a].

Treatment	Leaf scab severity[b]	Fruit scab severity[c]	SPAD	F_V/F_M	PI_{ABS}	Fruit yield (kg)
Control (water)	3.5c	1.8b	36.4a	0.828a	7.47ab	8.44a
Penconazole	2.0a	1.0a	42.4b	0.834a	9.92c	10.80c
Chitin (1.0%)	2.1a	1.1a	44.1b	0.832a	9.96c	10.10abc
Chitosan (1.0%)	2.2a	1.0a	42.0b	0.832a	10.1c	10.56bc
Crab meal	3.0b	1.7b	41.9b	0.830a	7.65ab	8.92ab
Liquid chitosan	2.4a	1.3a	42.8b	0.834a	9.07bc	8.94ab
Agrinos 5-0-0	3.5c	1.9b	37.6a	0.832a	6.84a	9.24abc
Significance of Treatment (T)	< 0.001	< 0.001	< 0.001	< 0.445	< 0.001	< 0.001

[a]Numbers within a column followed by a common letter are not significantly different according to Tukey's Honest Significant Difference test ($P = 0.05$).
[b]Mean of 50 leaves from 5 trees (10 leaves per tree).
[c]Mean of 50 fruits from 5 trees (10 fruits per tree).

f.sp. radicis lycopersici in tomato was suppressed using chitosan amendments (Lafontaine and Benhamou 1996). Similarly, chitosan utilised as a soil amendment was shown to control Fusarium wilts in many plant species (Rabea et al. 2003). Applied at an optimal concentration, chitosan delayed disease development, leading to a reduced plant wilting in tomato (Benhamou et al. 1994). Similar results were reported in forest nurseries suffering from *F. acuminatum* and *Cylindrocladium floridanum* infections which were reduced following the use of chitosan as a soil amendment (Laflamme et al. 2000). In 3 separate field trials, foliar sprays of chitosan were shown to provide a higher degree of control of *Pseudomonas syringae* pv. *actinidiae* on avocado compared to commercially available copper hydrochloride and copper oxychloride (Scortichini 2014). Foliar applications of chitosan were also shown to induce resistance against pitch canker (*F. circinatum*) and Diplodia tip blight (*Sphaeropsis sapinea*) in *Pinus radiata* with the authors concluding that chitosan offers potential for induced resistance in forest nursery disease management (Reglinski et al. 2004). The control of oomycete pathogens has also been achieved with chitosan treatment, with *Phytophthora capsici* controlled on peppers (Xu et al. 2007) and *P. infestans* in potato (O'Herlihy et al. 2003). It has been shown that chitin and chitosan induce host defence responses in both monocotyledons and dicotyledons. These responses include enhanced lignification of leaves, phytoalexin biosynthesis, generation of reactive oxygen species, expression of early responsive and defence-related genes, callose formation, and synthesis of proteinase inhibitors (Kuchitsu et al. 1993; Vasyukova et al. 2001; Wojdyla 2004; Sharp 2013). Although the mechanistic bases of each chitin/chitosan/fertiliser evaluated in these studies was not investigated, results published here are the first to show that applications of chitin and chitosan possess useful scab protectant properties when applied as soil amendments or foliar sprays.

In line with other research findings, chitin applied at 0.5% and 1.0%, and chitosan applied at 1.0%, provided a useful degree of efficacy as a scab protectant compound under containerised and field conditions, i.e., reduced leaf and fruit (field trials only) scab development: the main proxy of scab success or aggressiveness. In terms of practical disease control, the frequency of application is a crucial consideration in terms of labour, chemical usage, and equipment. Although greater degrees of scab control were achieved by the fungicide treatment in the container pot trials, in the case of the field trial the degree of scab control recorded via a single chitin or chitosan soil amendment was statistically equivalent to that of the fungicide treatment. These findings, combined with the relative low cost of chitin or chitosan compared to synthetic fungicides, mean the chitin- and chitosan-based products hold promise commercially to protect trees in large-scale urban forestry planting projects. Future research projects can be directed to further evaluate these products on larger trees and other species.

A difference in efficacy between the commercially available chitin/chitosan fertilisers used in this study was recorded. Application of liquid chitosan for example reduced leaf and fruit scab severity by 31% and 28% respectively, while the chitin/chitosan fertilisers crab meal and Hortifeeds (Lincoln, England, UK) had limited effect on scab severity. Differences in the degree of pathogen protection between commercially available chitin/chitosan fertilisers are consistent with other researchers (Ozbay and Newman 2004; El Hadrami et al. 2010). The degree of resistance induced has been shown to be heavily influenced by the type of chitosan (pure or artificially modified), its degree of polymerisation, the host, the chemical and/or nutrient composition of the substrates, and environmental conditions (Walker et al. 2004; Sharp 2013). For example, in some studies, oligomeric chitosans (pentamers and heptamers) have been reported to exhibit a better antifungal activity than larger units (Rabea et al. 2003). In others, the antimicrobial activity increased with the increase in chitosan molecular weight (Kulikov et al. 2006). Results of this study show that care should be taken when selecting a chitin- or chitosan-based fertiliser for plant protection purposes as efficacy can differ markedly between formulations.

However, the amendment of soil with chitin/chitosan-based fertilisers presents a number of desirable options for individuals involved in disease management within urban forestry landscapes. Both products are degraded enzymatically and are non-toxic to the beneficial rhizosphere biota at low concentrations, and both induce the symbiotic exchange between plant and microbes (Escudero et al. 2017). In addition, chitosan is a polysaccharide-based biopolymer, which stimulates the activity of plant symbiotic microbes,

resulting in the alteration of rhizosphere microbial equilibrium, thus disadvantaging any soil-borne plant pathogens (Bell et al. 1998; Murphy et al. 2000; Sharif et al. 2018). Furthermore, chitosan bio-fertilisers are available commercially from different manufacturers worldwide. Soil amendments are simple and relatively inexpensive to perform and allow for the direct delivery of a proportionate quantity of chitin and chitosan to a tree. Amendments can be applied at the time of planting or through the use of vertical mulching, air-spading, or radial trenching around established trees. Providing the correct amount of chitin and chitosan are applied, results of this study indicate that chitin/chitosan fertilisers induce positive plant growth effects, i.e., enhanced leaf chlorophyll content and fruit yield. Additional advantages of soil applications are that they are discrete and there is no spray drift—important considerations when applying plant protection agents to trees located in densely populated urban landscapes.

In conclusion, the results of this study provide evidence that chitin and chitosan applied at 1.0% possess efficacious properties against apple and pear scab when applied as soil amendments and demonstrate potential as a method of disease management or as a component of integrative pest management under field conditions. Care should be taken when selecting a chitin- or chitosan-based fertiliser, as efficacy can differ markedly between products.

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Résumé. La tavelure du pommier et du poirier sont des agents pathogènes foliaires des pommiers et des poiriers. En l'absence de mesures phytosanitaires, les pertes de rendement et d'esthétique peuvent être sévères. Le risque d'une résistance aux fongicides associé à une dépendance excessive à l'égard de ces derniers signifie que de nouvelles méthodes et produits pour la gestion des pathogènes sont de plus en plus nécessaires. La chitine et le chitosane sont largement reconnus comme des agents de résistance induite (RI) qui déclenchent des réactions de défense des plantes qui, à leur tour, améliorent la résistance des plantes à l'avènement des pathogènes. Des essais en contenants et sur le terrain ont été menés respectivement sur des pommiers (*Malus sylvestris*) et des poiriers (*Pyrus communis* 'Conference'), afin d'évaluer l'efficacité d'une gamme d'agents RI et de fertilisants liquides et granulaires à base de chitine/chitosane contre la tavelure du pommier et du poirier. Un programme de pulvérisation de fongicide synthétique (penconazole), utilisé au Royaume-Uni pour lutter contre la tavelure des pommes et des poires, a été inclus à des fins de comparaison. L'application d'agents RI à base de chitine/chitosane à des concentrations supérieures à 1 % a provoqué de la phytotoxicité. Une efficacité limitée pour la prévention de la tavelure a également été constatée lorsque des agents RI à base de chitine/chitosane ont été appliqués à des concentrations de 0,25 %. Toutefois, les agents RI à base de chitine appliqués à 0,5 % et 1 % ainsi que les agents RI à base de chitosane appliqués à 1 % ont démontré leur efficacité pour la prévention de la tavelure et ont entraîné une augmentation de la teneur en chlorophylle des feuilles, un rendement accru de la fructification et une réduction de la gravité de la tavelure sur les feuilles par rapport aux plants-témoins simplement irrigués. Seul un des trois engrais à base de chitine/chitosane évalués a démontré son efficacité pour la prévention de la tavelure (chitosane liquide). Un programme de pulvérisation du fongicide synthétique penconazole a fourni la meilleure protection contre la tavelure du pommier et du poirier dans les essais en contenants. Cependant, dans des conditions de terrain, le degré de contrôle de la tavelure après l'application de chitine et de chitosane à 1 % ainsi que du fertilisant contenant du chitosane liquide, était statistiquement comparable à celui des arbres traités avec le fongicide en ce qui a trait à la gravité de la tavelure des fruits et des feuilles. Les résultats suggèrent que l'application d'agents RI appropriés à base de chitine/chitosane et d'un engrais constitue une alternative utile aux méthodes existantes de gestion de la tavelure du pommier et du poirier dans des conditions de terrain et de contenants.

Zusammenfassung. Apfel- und Birnenschorf sind Blattkrankheitserreger an Apfel- und Birnbäumen. Werden sie nicht bekämpft, kann es zu erheblichen Ertrags- und ästhetischen Einbußen kommen. Das Risiko einer Fungizidresistenz, das mit dem übermäßigen Einsatz von Fungiziden verbunden ist, bedeutet, dass zunehmend neue Methoden und Produkte zur Bekämpfung des Erregers erforderlich sind. Chitin und Chitosan sind weithin als Mittel der induzierten Resistenz (IR) anerkannt. Sie lösen pflanzliche Abwehrreaktionen aus, die wiederum die Widerstandsfähigkeit der Pflanzen gegen das Eindringen von Krankheitserregern erhöhen. In einem Container- und einem Feldversuch mit Äpfeln (*Malus sylvestris*) bzw. Birnen (*Pyrus communis* ‚Conference') wurde die Wirksamkeit einer Reihe von flüssigen und granulierten IR-Mitteln auf Chitin/Chitosan-Basis und Düngemitteln gegen Apfel- und Birnenschorf untersucht. Zum Vergleich wurde ein synthetisches Fungizid-Sprühprogramm (Penconazol) herangezogen, das im Vereinigten Königreich zur Bekämpfung von Apfel- und Birnenschorf eingesetzt wird. Die Anwendung von Chitin/Chitosan-IR-Mitteln in Konzentrationen über 1 % verursachte Phytotoxizität. Eine begrenzte Wirksamkeit als Schorfschutzmittel wurde auch nachgewiesen, wenn Chitin/Chitosan-IR-Mittel in Konzentrationen von 0,25 % angewendet wurden. Chitin-IR-Wirkstoffe in Konzentrationen von 0,5 % und 1 % sowie Chitosan-IR-Wirkstoffe in einer Konzentration von 1 % erwiesen sich jedoch als wirksame Schorfschutzmittel und führten im Vergleich zur bewässerten Kontrolle zu einem erhöhten Blattchlorophyllgehalt, einem höheren Fruchtertrag und einer geringeren Schorfbildung. Nur eines der drei untersuchten Chitin/Chitosan-Düngemittel (flüssiges Chitosan) erwies sich als wirksames Schorfschutzmittel. Ein Spritzprogramm mit dem synthetischen Fungizid Penconazol bot in den Containerversuchen den besten Schutz gegen Apfel- und Birnenschorf. Unter Freilandbedingungen war der Grad der Schorfbekämpfung nach der Anwendung von Chitin und Chitosan in einer Dosierung von 1 % und dem chitosanhaltigen Dünger Flüssigchitosan jedoch statistisch gesehen mit den fungizidbehandelten Bäumen in Bezug auf den Schweregrad des Frucht- und Blattschorfs vergleichbar. Die Ergebnisse deuten darauf hin, dass die Anwendung eines geeigneten Chitin/Chitosan-IR-Mittels und eines Düngemittels eine nützliche Ergänzung zu den bestehenden Methoden der Apfel- und Birnenschorfbekämpfung unter Feld- und Containerbedingungen darstellt.

Resumen. La roña de manzana y pera es un patógeno foliar de manzanos y perales. Las pérdidas no gestionadas, estéticas y de rendimiento, pueden ser severas. La resistencia a los fungicidas asociada con la dependencia excesiva significa que se requieren cada vez más nuevos métodos y productos de gestión de patógenos. La quitina y el quitosano son ampliamente reconocidos como agentes de resistencia inducida (IR) que desencadenan respuestas de defensa de las plantas que a su vez mejoran la resistencia a la entrada de patógenos. Se realizó un ensayo de contenedor y de campo utilizando manzana (*Malus sylvestris*) y pera (*Pyrus communis* 'Conference') respectivamente para evaluar la eficacia de una gama de agentes IR líquidos y granulares a base de quitina/quitosano y fertilizantes contra la roña de manzana y pera. Se incluyó para la comparación un programa de pulverización de fungicida sintético (penconazol) utilizado en el Reino Unido para el control de la costra de manzana y pera. La

aplicación de agentes IR quitina/quitosano a concentraciones superiores al 1% causó fitotoxicidad. También se demostró una eficacia limitada como protectores de la costra cuando se aplicaron agentes IR quitina/quitosano a concentraciones del 0,25%. Sin embargo, los agentes IR de quitina cuando se aplicaron al 0,5% y 1% y los agentes IR de quitosano al 1% demostraron eficacia como protectores de la costra y dieron lugar a un aumento del contenido de clorofila foliar, un mayor rendimiento del fruto y una reducción de la severidad de la costra foliar en comparación con el control regado. Solo uno de los tres fertilizantes de quitina/quitosano evaluado demostró eficacia como protectores de la roña (quitosano líquido). Un programa de aerosol de fungicida sintético penconazol proporcionó la mayor protección contra la roña de manzana y pera en los ensayos de contenedores. Sin embargo, en condiciones de campo, el grado de control de la costra después de la aplicación de quitina y quitosano al 1% y el quitosano líquido que contiene quitosano fertilizante fue estadísticamente comparable a los árboles tratados con fungicidas en la severidad de la roña de frutas y hojas. Los resultados sugieren que la aplicación de un agente IR y fertilizante de quitina/quitosano ofrece una adición útil a los métodos existentes de manejo de roñas de manzana y pera en condiciones de campo y contenedor.