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Evaluation of Electrolysed Oxidizing Water for European Pear Rust Management on Pear (*Pyrus* cv. Conference)

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Abstract. Pear rust is a foliar pathogen of ornamental and fruiting pear trees. Unmanaged, yield and aesthetic losses can be severe. Over-reliance on synthetic fungicides means novel means of pathogen management are required. Field trials were conducted using pear (*Pyrus* cv. Conference) to assess the efficacy of electrolysed oxidizing water (EO water) as a rust protective compound. A synthetic fungicide (boscalid + pyraclostrobin) spray program used for pear rust management was included for comparison. Each treatment was applied 4 times prior to the visible appearance of rust (April through June, i.e., preventatively). Studies were conducted in 2017 and repeated in 2018. Efficacy of EO water as a rust protectant compound was confirmed (increased leaf chlorophyll content, reduced leaf rust incidence and severity). The degree of leaf rust severity protection conferred was not statistically different from a boscalid + pyraclostrobin spray program. Results suggest 4 spray applications of EO water provides a useful addition to existing methods of pear rust management under field conditions that may have applicability against other foliar pathogens.

Keywords. Fungicides; *Gymnosporangium*; Integrated Disease Management; Orchard Management; Pathogen Control; Plant Health Care; Urban Landscapes.

INTRODUCTION

Ornamental pear (*Pyrus* spp.) varieties and cultivars are a popular choice for public and private urban landscapes throughout Europe and the USA (Santamour and McArdle 1983; Rahman et al. 2011). Pear trees are hardy, adaptable to varied soil conditions, and are available in a wide range of sizes and shapes with predominantly white flowering characteristics (Yun et al. 2009). Species exist to suit most purposes, from small urban gardens, entry courtyards, and parking strips, to public parks and highway plantings (Rahman et al. 2014). Likewise, the planting of community orchards in urban landscape schemes now means fruiting pears are increasingly planted by community groups within towns and cities throughout Europe and parts of the US (King and Clifford 2008; Kalb et al. 2011; Ames 2013). However, an exception is Callery pear (*Pyrus calleryana*), which has emerged as a significant invasive species in North America and has been added to noxious plant lists in some states (e.g., Ohio), although this is not the case in the UK and Europe (Culley and Hardiman 2007).

Pear leaf infection caused by European pear rust (*Gymnosporangium sabinae* [Dicks] G. Winter) is an obligatory pathogen of fruiting and ornamental pear species as well as their varieties and cultivars. The first symptoms of infection appear as yellow spots on the upper side of young leaves that generally start to protrude seven days after infection (Dong et al. 2006). Gradually, these circular shaped spots become thickened and turn bright orange, sizing up to 2 cm in diameter (Ormrod et al. 1984). One individual leaf may have several lesions depending on the infection pressure, leaf age, and susceptibility of the variety. During the growing season, lesion spread decreases leaf photosynthetic area, and leaves may curl and drop prematurely (Naqvi 2004). If infection pressure is high and occurs over several years, trees can become predisposed to attacks by secondary pathogens. In addition, poor fruit set or premature fruit drop can occur, reducing fruit quality and yield. Genetic resistance between pear species is limited, and no biocontrol options are currently available (Fitzner and Fischer 2005; Ivarsson 2011). Consequently, strategies for pear rust management are reliant upon repeated systemic fungicide sprays. Such an option is not available for pears growing within European urban landscapes, as currently there are no synthetic fungicides registered or permitted for use (Percival 2018; BCPC 2020). Therefore, the development of an effective treatment to slow pear decline due to European pear rust and to protect valuable trees from infection is required. A potential alternative to fungicides is the use of electrolysed oxidizing (EO) water (Buck et al. 2002; Al-Haq et al. 2003; Forghani 2019). EO water is generated by electrolysis of a dilute solution of sodium chloride in an electrolysis chamber where anode and cathode electrodes are separated by a nonselective membrane made from nonwoven polyester fabric (Al-Haq et al. 2005). Water collected from the anode (EO water) has unique properties, such as high oxidation-reduction potential, low pH, presence of hypochlorous acid, and, importantly, has been shown to have strong bactericidal and fungicidal activity. EO water has been successfully used as a contact fungicide and for general sanitation in commercial glasshouses and is regarded as an environmentally benign alternative to fungicides (Al-Haq et al. 2002; Abbasi and Lazarovits 2006). Importantly, EO water presents negligible toxicity to humans, an important factor when applying plant protection agents in densely populated urban areas. Due to its mode of action, i.e., physical rather than chemical, it is less likely to result in the development of pathogen resistance (Mueller et al. 2003; Al-Haq et al. 2005; Forghani 2019).

Few, if any, studies exist evaluating EO water under field conditions and against a pathogen of urban landscape trees. Consequently, aims of this study were to investigate the efficacy of EO water for its potential plant protective properties against European pear rust under field conditions.

MATERIALS AND METHODS

Field Site and Experimental Trees

The pear trial site consisted of a 0.75 ha block of *Pyrus* cv. Conference. Planting distances were based on 2 m × 2 m spacing. The trees were planted in 2010 and trained under a central-leader system to an average height of 2.0 m ± 0.20 m, with mean trunk diameters of 4.0 cm ± 0.8 cm at 45 cm above the soil level. The trial site was located at Aston Manor Orchard, Tiverton, Devon, UK (50°53′37″N, 3°29′53″W).

Fifteen soil cores from the trial site were taken to a depth of 20 cm and radius of 5 cm based on an 8.0 m "W" pattern as stipulated under UK soil sampling procedures (Tytherleigh 2008). The soil was a sandy loam containing 5.2% organic matter, with a pH of 6.6 and available P, K, Mg, Na, and Ca of 51.3 mg/L, 680.9 mg/L, 199.6 mg/L, 48.3 mg/L, and 2,329 mg/L, respectively. Weeds were controlled chemically using glyphosate (Roundup; Green-Tech, Sweethills Park, Nun Monkton, York, UK) throughout experiments. No watering or fertilisation was applied during the trial. Due to a monoculture planting site, the pear trees suffered from pear rust infection on an annual basis. A minimal insecticide program based on the residual pyrethroid insecticide deltamethrin (product name Bandu; Headland Agrochemicals Ltd., Saffron Walden, Essex, UK) was applied every 2 months commencing in May 2017 to September 2017. All sprays were applied using a 10-L knapsack sprayer at 70 mL deltamethrin (Bandu; Barrettine Group, St. Ivel Way, Warmley, Bristol, UK) per 100 L of water. Trees were sprayed until runoff, generally 0.40 L of insecticide per tree.

EO Water and Fungicide Treatments

EO water and fungicide (boscalid + pyraclostrobin, 0.9 g/L of water [trade name Signum; Barrettine Group, St. Ivel Way, Warmley, Bristol, UK]) treatments were applied 4 times over 2 growing seasons, namely, April 28, May 09, May 28, and June 10 in 2017, and April 26, May 11, May 28, and June 12 in 2018 (Ivarsson 2011). Two guard trees were located between each treated tree to prevent dispersal of sprays and possible cross contact with other trees. Ten trees per treatment were used in a completely randomised block design. Foliar sprays of EO water and boscalid + pyraclostrobin were applied until runoff using a 10-L knapsack sprayer (Cooper Pegler; Agratech NW Ltd., Waterfoot, Rossendale, UK), generally 0.40 L of product per tree.

Plant Vitality Assessments

Measurements were made towards the cessation of the growing season (24–26 September 2017; 25–27

September 2018). 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 (Minolta Camera Co., Osaka, 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. 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 = $5.68 + 0.066x$; r^2 adj = 0.95, $P \leq 0.001$) (Lichtenthaler and Wellburn 1983).

Rust Incidence and Severity

The degree of protection conferred by each treatment was assessed by recording rust incidence and severity at the cessation of the growing season.

At each assessment, 100 leaves and 30 fruits were chosen arbitrarily from different sides of a tree. A leaf or a fruit was considered to be infected if at least one visible rust lesion was present.

Rust severity of leaves and fruit was assessed visually. Leaf rust severity of each tree was rated using a visual indexing technique and ratings on the scale: 0 = no rust 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. Leaf rust severity ratings used in this study were based on UK and Ireland market standards for fungicide evaluation of foliar pathogen control (Butt et al. 1990; Swait and Butt 1990).

Fruit Yield

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

Experimental Design and Statistical Analysis

Mean rust severity values for all treatments were transformed using the Arcsin (sine $^{-1}$) transformation. All data were analysed using ANOVA, and the differences between means were determined using LSD ($P = 0.05$) using the Genstat program (VSN International Ltd., Hemel Hempstead, UK). Rust severity values are presented here to ease interpretation of these data. The binary data obtained for rust incidence (0 for no rust and 1 for rust present) was subjected to survival analysis statistics using the Wilcoxon-Gehan method. This experimental design was adopted in line with Official Recognition of Efficacy Testing Organisations in the United Kingdom guidelines for product efficacy testing and analysed as a randomised complete block design.

Table 1. Comparison of boscalid + pyraclostrobin and electrolysed oxidizing water on managing *Gymnosporangium sabinae* on *Pyrus* cv. Conference (2017).

Treatment	Leaf chlorophyll SPAD	Fruit yield (kg) tree	Rust incidence	Leaf rust severity
Water (control)	29.9a	14.5a	72.0c	2.7b
Electrolysed oxidizing water	35.5b	15.9a	31.0b	1.0a
Boscalid + pyraclostrobin (0.9 g/L)	37.0b	15.7a	12.0a	0.5a
Treatment	0.032	0.224	< 0.001	< 0.001

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

Rust incidence = total number of fruit and leaves with rust symptoms/number of leaves (100) and fruit (30) examined.

SPAD values mean of 10 trees, 25 leaves per tree.

Fruit yield mean of 10 trees.

Leaf rust severity scale: 0 = no rust 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.

RESULTS

Damaging outbreaks of pear rust were recorded on control trees at the cessation of the 2017 and 2018 growing seasons as indicated by leaf rust severity ratings of 2.7 and 2.5, respectively (Tables 1 and 2). None of the treated or control trees died as a result of rust attack during the course of the study. No symptoms of phytotoxicity or the presence of pear rust infection on pear fruit was recorded following EO water and fungicide treatments in both the 2017 and 2018 field trials. Therefore, for reasons of clarity, this data is not shown. No significant effect of EO water or boscalid + pyraclostrobin on pear yield (kg) was recorded in either field trial compared to water-treated controls. The effectiveness of EO water and boscalid + pyraclostrobin on rust incidence and severity of leaves, as well as leaf chlorophyll content, was confirmed in both the 2017 and 2018 trials. In all cases, leaf rust incidence and severity was significantly ($P < 0.05$) lower than water-treated controls, and leaf chlorophyll content was significantly higher ($P < 0.05$) than water-treated control trees. There was no statistical difference ($P < 0.05$) in the degree of efficacy between EO water and boscalid + pyraclostrobin with respect to leaf chlorophyll content and leaf rust severity values in either the 2017 or 2018 field trials. However, boscalid + pyraclostrobin resulted in a significantly lower rust incidence value compared to EO water in both the 2017 and 2018 field trials. In all cases except fruit yield, a significant effect of treatment was recorded (Tables 1 and 2).

DISCUSSION

European pear rust is a widely distributed and destructive pathogen of ornamental and fruiting pears (Ivarsson 2011). Fungicide-resistant strains of the pathogens and public health are two major concerns for urban landscape managers and justify the need for alternative methods for pathogen control (Carisse and Dewdney 2002; Patocchi et al. 2004; Kikuhara et al. 2019). Our evaluation of EO water as a potential alternative to fungicides clearly demonstrated that EO water could control pear rust on *Pyrus* cv. Conference. Although it could be argued that specific cultural management (soil decompaction, mulching, irrigation) may delay or lower pear rust outbreaks by enhancing tree vitality, control is still primarily achieved by the use of synthetic fungicides (Ivarsson 2011; Kikuhara et al. 2019). Development of fungicide-resistant strains of pear rust have recently been reported, which provides further credence to the search for fungicide alternatives (Kikuhara et al. 2019). Results of this study demonstrate that EO water provided a high degree of efficacy as a pear rust protectant compound under field conditions when applied 4 times during April through June. In support of this result, EO water has been shown to possess strong fungicidal activity against a range of foliar pathogens, such as *Botrytis cinerea* on geranium, powdery mildew on gerbera daisy, brown rot (*Monilinia laxa*) on peach, as well as the soil-borne pathogen *Fusarium oxysporum* f. sp. *lycopersici* on tomato (Al-Haq et al. 2001; Mueller et al. 2003; Al-Haq et al. 2005; Abbasi and Lazarovits 2006). In addition, EO water has been shown to

Table 2. Comparison of boscalid + pyraclostrobin and electrolysed oxidizing water on managing *Gymnosporangium sabinae* on *Pyrus* cv. Conference (2018).

Treatment	Leaf chlorophyll SPAD	Fruit yield (kg) tree	Rust incidence	Leaf rust severity
Water (control)	30.4a	12.6a	76.0c	2.5b
Electrolysed oxidizing water	36.1b	13.4a	27.0b	0.8a
Boscalid + pyraclostrobin (0.9 g/L)	36.8b	14.5a	10.0a	0.4a
Treatment	0.029	0.128	< 0.001	< 0.001

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

Rust incidence = total number of fruit and leaves with rust symptoms/number of leaves (100) and fruit (30) examined.

SPAD values mean of 10 trees, 25 leaves per tree.

Fruit yield mean of 10 trees.

Leaf rust severity scale: 0 = no rust 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.

possess strong bactericidal properties reducing pathogen severity symptoms of *Xanthomonas campestris* pv. *vesicatoria* (bacterial spot of tomato) and *Streptomyces scabies* (potato scab)(Abbasi and Lazarovits 2006). Indeed EO water is frequently used as a sterilising agent within the food, agricultural, and medical industries (Al-Haq et al. 2005; Forghani 2019). However, prior to our study, no full field trials of EO water for pear rust control had been conducted. EO water also possesses a number of other beneficial properties to include a rapid kill of fungal spores and hyphae, in addition to several bacteria. No residues exist that could result in soil contamination, or in the case of fruit pears for human consumption, contamination of the food chain (Forghani 2019). EO water is also fully compatible with a range of fungicides and insecticides, permitting use within an integrated pest management system to complement, enhance the efficacy of, or replace certain fungicides currently used for pathogen management (Mueller et al. 2003; Abbasi and Lazarovits 2006). Due to the sterilising nature of EO water, concern about phytotoxicity has been raised (Buck et al. 2003). However, research has shown that if EO water is applied weekly, then no symptoms of phytotoxicity develop. If multiple applications of EO water occur during a week, however, marginal phytotoxicity (peripheral leaf burn, leaf cupping) has been observed (Mueller et al. 2003; Guentzel et al. 2011). Another limitation of EO water is that it possesses no systemic properties (Mueller et al. 2003; Al-Haq et al. 2005), so complete coverage of foliage is required for total pathogen management, which is difficult when dealing with mature trees with a large canopy. Research also indicates that EO water is preferably used as a preventative spray treatment rather than a curative one (Al-Haq et al. 2001; Mueller et al. 2003; Abbasi and Lazarovits 2006). Despite these limitations, results of this study show that EO water has applicability against pear rust under field conditions and may offer potential in the management of other pathogens of concern within the arboricultural industry.

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Résumé. La rouille du poirier est un agent pathogène foliaire des poiriers ornementaux et fruitiers. Sans son contrôle, les pertes de rendement et d'esthétique peuvent être importantes. La dépendance excessive à l'égard des fongicides synthétiques signifie que de nouveaux moyens de gestion des agents pathogènes sont nécessaires. Des essais sur le terrain ont été menés en utilisant le poirier (*Pyrus* cv. Conference) pour évaluer l'efficacité de l'eau comburante électrolysée (ECE) comme composé contre la rouille. Un programme de pulvérisation du fongicide synthétique (boscalid + pyraclostrobine) utilisé pour la gestion de la rouille du poirier a été inclus sur une base comparative. Chaque traitement a été appliqué quatre fois avant l'apparition observée de la rouille (d'avril à juin, c'est-à-dire à titre préventif). Des études furent menées en 2017 puis répétées en 2018. L'efficacité de l'eau comburante électrolysée comme composé antirouille a été confirmée (augmentation de la teneur en chlorophylle des feuilles, réduction de l'incidence et de la sévérité de l'atteinte par la rouille des feuilles). Le degré de protection acquis contre la rouille des feuilles n'était pas statistiquement différent du programme de pulvérisation de boscalid + pyraclostrobine. Les résultats suggèrent que quatre applications par pulvérisation d'eau comburante électrolysée constituent un complément utile aux méthodes existantes de lutte contre la rouille du poirier dans des conditions de terrain et qui peuvent également s'appliquer à d'autres agents pathogènes foliaires.

Zusammenfassung. Birnenrost ist ein Blattkrankheitserreger von Zier- und Fruchtbirnenbäumen. Unkontrolliert können Ertrags- und Ästhetikverluste schwerwiegend sein. Da man sich zu sehr auf synthetische Fungizide verlässt, sind neue Mittel zur Bekämpfung des Erregers erforderlich. Es wurden Feldversuche mit Birne (*Pyrus* cv. Conference) durchgeführt, um die Wirksamkeit von elektrolysiertem oxidierendes Wasser (EO-Wasser) als Rostschutzmittel zu bewerten. Ein synthetisches Fungizid-Spritzprogramm (boscalid + pyraclostrobin), das zur Bekämpfung von Birnenrost eingesetzt wird, wurde zum Vergleich herangezogen. Jede Behandlung wurde viermal vor dem sichtbaren Auftreten von Rost (April bis Juni, d. h. präventiv) angewendet. Die Studien wurden im Jahr 2017 durchgeführt und 2018 wiederholt. Die Wirksamkeit von EO-Wasser als Rostschutzmittel wurde bestätigt (erhöhter Blattchlorophyllgehalt, reduziertes Auftreten und Schweregrad von Blattrost). Der Grad des gewährten Schutzes vor Blattrostschwere war statistisch nicht unterschiedlich zu einem Spritzprogramm mit boscalid + pyraclostrobin. Die Ergebnisse deuten darauf hin, dass vier Sprühanwendungen von EO-Wasser eine nützliche Ergänzung zu den bestehenden Methoden der Birnenrostbekämpfung unter Feldbedingungen darstellen, die möglicherweise auch gegen andere Blattpathogene anwendbar sind.

Resumen. La roña es un patógeno foliar de peras ornamentales y frutales. Las pérdidas estéticas y de rendimiento pueden ser graves. La excesiva dependencia de fungicidas sintéticos significa que se requieren nuevos medios de gestión de patógenos. Se realizaron ensayos de campo utilizando pera (*Pyrus* cv. Conference) para evaluar la eficacia del agua oxidante electrolizada (agua EO) como un compuesto protector contra la oxidación. Se incluyó un programa de pulverización de fungicida sintético (boscalid + pyraclostrobin) utilizado para el manejo de la roña de la pera para la comparación. Cada tratamiento se aplicó cuatro 4 veces antes de la aparición visible del daño (abril a junio, es decir, preventivamente). Los estudios se realizaron en 2017 y se repitieron en 2018. Se confirmó la eficacia del agua de EO como compuesto protector contra la enfermedad (aumento del contenido de clorofila de la hoja, reducción de la incidencia y gravedad del daño de la hoja). El grado de protección de la gravedad del daño de la hoja conferido no fue estadísticamente diferente de un programa de pulverización boscalid + pyraclostrobin. Los resultados sugieren que cuatro aplicaciones con pulverización de agua EO proporcionan una adición útil a los métodos existentes de gestión de la oxidación de pera en condiciones de campo que pueden tener aplicabilidad contra otros patógenos foliares.