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Walton, Andrew; Smith, Andy

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How effective are methods to induce or facilitate the natural resistance of temperate trees to exotic *Phytophthora* species? A systematic review

Andrew Walton and Andrew R. Smith 

School of Natural Sciences, Bangor University, Bangor, UK

ABSTRACT


Some of the most prominent exotic pathogens in temperate forest regions belong to the genus *Phytophthora*. The pathogen, which is nourished by the enzymatic destruction of living plant cells, can cause mortality in >150 plant species including many temperate forest trees. However, studies have demonstrated the natural disease resistance of trees can be directly induced or facilitated using methods to deploy biochemical compounds that include foliar sprays, trunk injection or bark application, and soil amendments. This systematic review identified and analysed the efficacy of novel treatments to induce a natural resistance against different *Phytophthora* species in temperate trees. Results showed that treatments reduced *Phytophthora* infection symptoms compared to controls in all but one of the experiments reviewed. Trunk injections demonstrated the highest cumulative efficacy with a pooled effect size of 1.85 ± 0.56 (Hedge's $g \pm 95\%$ confidence interval). Foliar sprays had the second highest efficacy, with a pooled effect size of 1.11 ± 0.28 . Finally, soil amendments had the lowest cumulative efficacy, with a pooled effect size of 0.61 ± 0.36 . This review supports the use of treatments on trees in nurseries, urban forests, orchards, and arboreta; however, success is dependent upon the application of optimal doses.

KEYWORDS

Systemic induced resistance;
foliar spray; trunk injection;
soil amendment

Introduction

Globalisation and anthropogenic activities over the past century, coupled with changes in environmental conditions, have facilitated the movement of tree pathogens into novel environments and exposed trees to diseases that they have not genetically evolved to resist (Wargo, 1995). Two-thirds of pathogens present in ecosystems are non-native globally (Waller, 2013), leading to pandemic-scale impacts as exemplified by the spread of pathogens like *Phytophthora* species. Derived from the Greek word for “plant killer”, *Phytophthora* species comprise water moulds called oomycetes that are related to algae whilst exhibiting properties similar to fungi such as filamentous hyphae and sporal reproduction (Hansen, 2015). The oomycetes colonise host plants via motile zoospores and are nourished via “the enzymatic destruction of living plant cells” (Hansen, 2015, p. 16). The process of pathogenesis manifestation is dependent upon the *Phytophthora* species, and includes defoliation,

CONTACT Andrew R. Smith  a.r.smith@bangor.ac.uk

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fine root damage, or lesions on inner bark tissues. The pathogen can cause mortality in more than 150 plant species, many of which are important temperate forest genera (Forest Research, 2021). With a capability to destroy nursery stock, field crops, mature trees, and entire forest stands (Zwart & Kim, 2012), some *Phytophthora* species have inevitably been designated as notifiable diseases in the UK (Department for Environment, Food and Rural Affairs (Defra), 2021).

Fortunately, recent studies have demonstrated methods by which the natural disease resistance of trees can be directly induced or facilitated by deploying naturally derived chemical elicitors. These included foliar sprays, trunk injections or bark applications, and soil amendments to promote, or prime, tree natural defences. Trees can increase their defensive response to disease via three distinct pathways: hypersensitive responses of trees to the pathogens themselves, known as systemic acquired resistance (SAR); via signalling molecules from organic compounds, known as systemic induced resistance (SIR); or as a result of rhizosphere interactions with micro-organisms, known as induced systemic resistance (ISR) (Eyles, Bonello, Ganley, & Mohammed, 2010). Additionally, although not necessarily inducing resistance as described above, direct applications of organic compounds to foliage, bark, or root systems have also been used to facilitate localised tree defence by directly suppressing pathogens. For instance, pure mulches (i.e. mulch derived solely from one tree species) have been shown to contain pathogen destroying cellulases and soil microbes (Percival, 2013). This field of study represents a potential paradigm shift in tree care from predominantly structural interventions, like pruning and felling, to solutions that help to improve the general physiology of trees. Although studies with contrasting results are evident throughout the scientific literature, a systematic review of such treatments, undertaken specifically in relation to *Phytophthora* species, is needed to synthesise the results and identify the optimum treatment and efficacy.

This systematic review has followed the Reporting standards for Systematic Evidence Syntheses (ROSES; Haddaway, Macura, Whaley, & Pullin, 2018) in environmental research methodology to identify studies regarding the efficacy of treatments against various *Phytophthora* species in temperate forest trees. Specifically, this review aims to answer the following questions:

- How effective are foliar sprays, stem injections, and soil amendments in inducing natural resistance to exotic *Phytophthora* species in temperate forest trees?
- What is the mechanism by which treatments work?
- Which species of *Phytophthora* are the treatments effective against?
- Which tree species have the treatments been used to protect?
- How many interventions are remedial and how many are preventative?

Materials and methods

Literature search

Studies of interest were captured from full text peer reviewed journals and grey literature in English using a predefined search string. Grey literature included documents and information that was not owned or published by commercial publishing

organisations. The following bibliographic databases and web search facilities were used: Web of Science Core Collection (<https://clarivate.com/>); Open Grey (<http://www.opengrey.eu/>); Open Access Theses and Dissertations (<https://oatd.org/>); and Google Scholar (<https://scholar.google.com/>). Databases were searched for relevant articles that have been published at any time.

The ROSES process and flow diagram outline the search, screening, and critical appraisal stages for article selection that were used to identify articles for the analysis (Figure 1). The search strategy was optimised during a scoping phase, which tried to find an appropriate balance between depth (number of papers found) and specificity (how well the papers matched the search criteria). Comprehensiveness was achieved once four articles present in a test list appeared in the cumulative results, and when the maximum number of additional relevant articles was identified within the lowest possible number of results overall (Garbelotto, Schmidt, & Harnik, 2007; Graham, 2011; Percival, 2013; Zwart & Kim, 2012). Search terms were concatenated using the Boolean operators “AND” and “OR”. Due to nuances in the way each database/search engine operated and subsequently filtered results, specific search strings were devised for each database as shown in Table 1.

Data extraction

Inclusion criteria: Title and abstract screening stage

Search results were exported into Excel (Microsoft Corporation, Albuquerque, NM, USA) and duplicates removed. Studies were included in the full-text screening stage if they included any combination of the population, exposure, or intervention criteria detailed in Table 2. If the study was conducted in a temperate forest biome as identified by reference to the temperate biome denoted in the NASA Earth Observatory forest map (Figure 2), or the species was known to thrive outdoors in the UK (all year round), then it would meet the population inclusion criteria. Evidence suggests that trees generally have natural immunity to pathogens with which they have co-evolved (Ghelardini et al., 2017; Hansen, 2015; Wargo, 1995): subsequently only invasive *Phytophthora* species, exotic to the tree species they were infecting, were included in this review.

Inclusion criteria: Full-text screening stage

Studies were included for data extraction if all the criteria in Table 3 were met and all relevant bibliographic information was available.

Data synthesis

Data synthesis consisted of a narrative summary with a meta-analysis component to analyse quantitative data as detailed below.

Meta-analysis

Data was extracted from manuscript tables and when presented only in the form of figures, data values were extracted using the online tool WebPlotDigitizer (Version 4.5; Rohatgi, 2021) and visual estimation. Only the largest mean effect for each experiment was recorded, as this demonstrates treatment efficacy when applied at optimal dosage

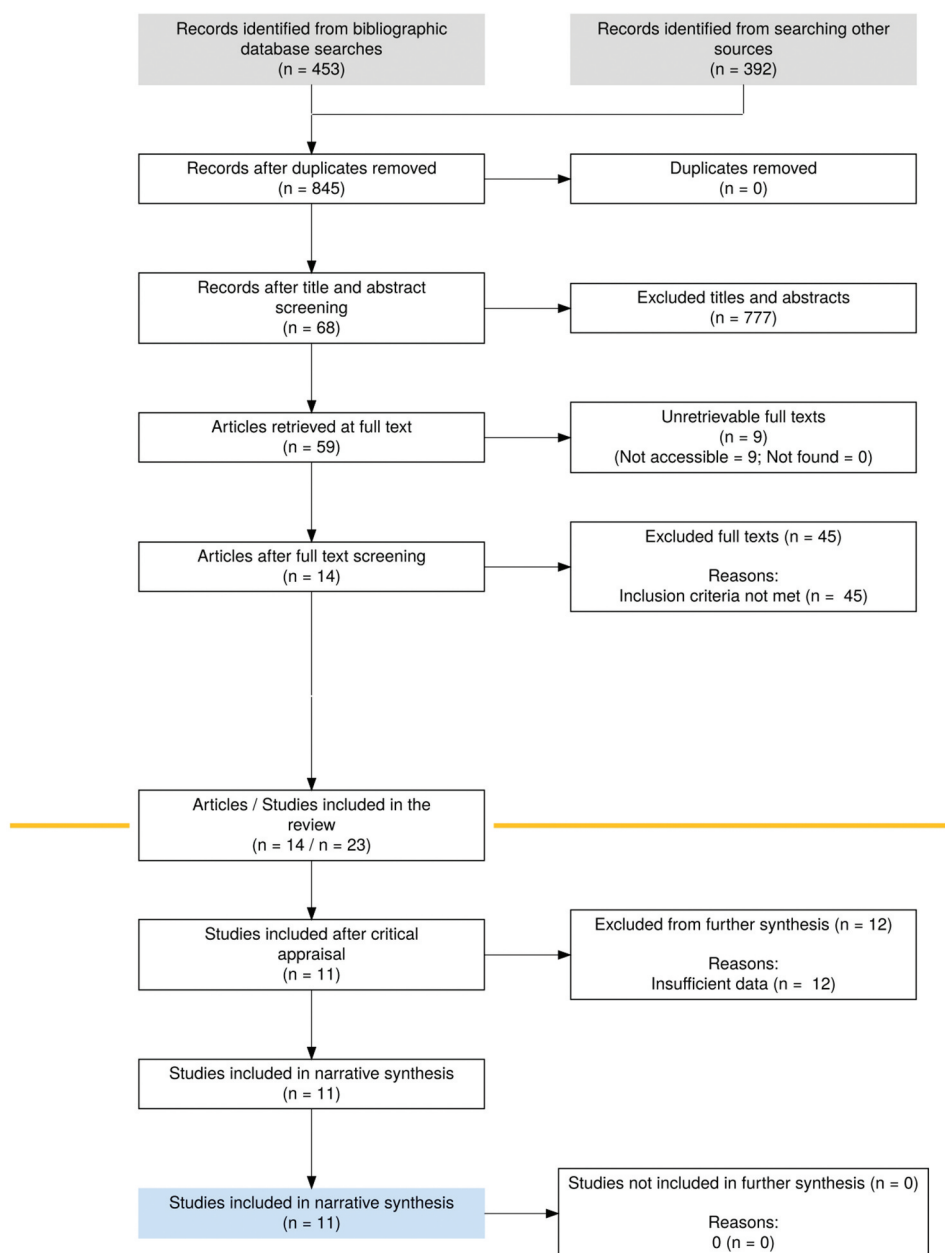


Figure 1. Flow diagram adopted from the RepOrting standards for Systematic Evidence Syntheses in environmental research showing literature sources and the search, screening and critical appraisal stages (Haddaway et al., 2018).

levels. Once all required data was extracted, the standard deviation for each experiment was calculated (Equation 1) where SD is the standard deviation, SE is the standard error, and N is the sample size.

$$SD = SE * \sqrt{N} \quad (1)$$

Table 1. Definition of the search terms used, date of search, and the database, or search engine, used to identify literature for inclusion in the study.

Date	Database	Search String
11 February 2021	Web of Science	TS = (("biochar amendment" OR "induced resistance" OR "acquired resistance" OR "systemic resistance" OR "foliar spray" OR "trunk injection" OR "bark application" OR "soil amendment" OR "pure mulch" AND (phytophthora OR "sudden oak death" OR "phytophthora root rot pathogens") NOT (tomato OR potato OR avocado OR pepper)).
	Open Access Theses and Dissertations	((("biochar amendment" OR "induced resistance" OR "acquired resistance" OR "systemic resistance" OR "foliar spray" OR "trunk injection" OR "bark application" OR "soil amendment" OR "pure mulch") AND (phytophthora OR "sudden oak death" OR "phytophthora root rot pathogens") NOT (tomato OR potato OR avocado OR pepper)).
15 February 2021	Open Grey	"biochar amendment" OR "induced resistance" OR "acquired resistance" OR "systemic resistance" OR "foliar spray" OR "trunk injection" OR "bark application" OR "soil amendment" OR "pure mulch*" AND phytophthora OR "sudden oak death".
17 February 2021	Google Scholar	"biochar amendment" OR "induced resistance" OR "foliar spray" OR "trunk injection" OR "bark application" OR "soil amendment" OR "pure mulches" AND "suppressing phytophthora" OR "sudden oak death" OR "phytophthora root rot pathogens".

Table 2. Eligibility criteria for title and abstract screening.

Question elements	Eligibility criteria
Population	<i>Included:</i> <ul style="list-style-type: none"> • Temperate tree species. • Any species grown outdoors (all year round) in the United Kingdom.
Exposure	<i>Included:</i> <ul style="list-style-type: none"> • Exotic <i>Phytophthora</i> species
Intervention	<i>Included:</i> <ul style="list-style-type: none"> • Foliar sprays • Trunk injections or bark applications • Soil amendments

Meta-analysis was conducted using R 4.0.3 (R Foundation for Statistical Computing, and R Core Team, 2019) via the online interactive shiny application MAVIS (Version 1.1.3; Hamilton, Aydin, & Mizumoto, 2021) for meta-analysis that supports both random and fixed effect modules via the meta (version 4.12.0; Schwarzer, 2021) packages of R. MAVIS was used to calculate an effect size, within the parameters of a fixed effects model, for each experiment, cumulatively for each treatment type, and cumulatively for all experiments overall using Hedges' *g* (Equation 2; Durlak, 2009) where *M1* and *M2* are the sample mean of samples 1 and 2, respectively, and *SDpooled* is the pooled standard deviation.

$$Hedges'g = \frac{M1 - M2}{SDpooled} \quad (2)$$

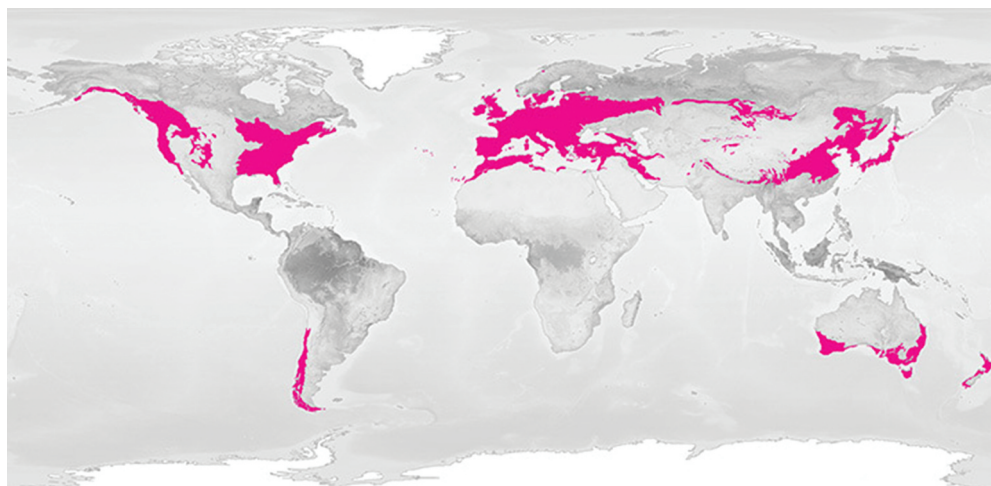


Figure 2. Map of the world with temperate forest biomes highlighted in pink (NASA Earth Observatory, 2021).

Table 3. Eligibility criteria for full-text screening stage.

Question elements	Eligibility criteria
Population	<p><i>Included:</i></p> <ul style="list-style-type: none"> • Temperate tree species • Any species grown outdoors (all year round) in the United Kingdom.
Exposure	<p><i>Included:</i></p> <ul style="list-style-type: none"> • Exotic <i>Phytophthora</i> species
Intervention	<p><i>Included:</i></p> <ul style="list-style-type: none"> • Foliar sprays • Trunk injection or bark application • Soil amendments
Data	<p><i>Included:</i></p> <ul style="list-style-type: none"> • Name of active compound • Timing of intervention (i.e. preventative or remedial)
Outcomes	<p><i>Included:</i></p> <ul style="list-style-type: none"> • Study group size • Mean efficacy of intervention vs control • Standard error of the mean (SE) • Statistical significance reported as $P < 0.05$
Language	<p><i>Included:</i></p> <ul style="list-style-type: none"> • English

For the purposes of interpretation, effect sizes that are ≤ 0.2 , ≥ 0.2 and ≤ 0.5 , and ≥ 0.8 can be considered as small, medium, and large effect sizes, respectively.

Narrative summary

In addition to collating quantitative efficacy data for the meta-analysis, relevant additional quantitative and qualitative information was collated to prepare a critical narrative

appraisal of the literature. Listed below are elements of the reviewed manuscripts that were identified for inclusion in the narrative review.

- Identify key characteristics of each *Phytophthora* species.
- Summarise how many tree species have been studied.
- Summarise how many treatments are remedial and how many preventative.
- Identify the type of resistance induced, i.e., SIR, ISR, SAR, or facilitative.
- Highlight any adverse effects on the trees being treated.
- Identify knowledge gaps for further study in the future.

Additional inputs to experiments such as irrigation, fertilisers, and/or pesticides were also recorded and highlighted as potential effect modifiers, with any final conclusions drawn in the narrative synthesis acknowledging their potential impact.

Study validity and publication bias

Following the article screening process, only studies from peer reviewed scientific journals were selected for inclusion in this study, which we believe decreases the chances of including erroneous results.

Funnel plots were used to visually estimate publication bias. Funnel plots show the study effect size estimates against sample size, which can be useful to assess the validity of a meta-analysis. In the absence of publication bias, the plot should appear symmetrical with results from small studies scattering widely at the bottom of the plot, and the effect size spread narrowing towards the top of the plot with an increase in study size, if bias exists a funnel plot will usually be skewed and asymmetrical (Egger, Davey Smith, Schneider, & Minder, 1997).

Results

Study search and screening

Searches in bibliographic databases Web of Science, Open Grey, and Open Access Theses and Dissertations generated 453 hits, with search engine Google Scholar generating an additional 392 hits. Altogether 845 records were generated and screened at the title and abstract stage using the CADIMA online evidence synthesis software for facilitating the conduct of systematic reviews (CADIMA., 2021). In total, 777 of the publications were excluded due to not meeting inclusion criteria, leaving 68 records for full-text screening. Nine of the full texts were unavailable due to a pay-wall restriction on access. Therefore, 59 full texts were retrieved and screened. Forty-five articles were excluded following full-text screening due to not meeting inclusion criteria. The remaining 14 full texts contained 23 separate studies that were then selected for inclusion for the data extraction phase. Following a critical appraisal and detailed data analysis, a further 12 studies were excluded due to the reported results not being statistically significant or because the standard error or standard deviation was not reported. Eleven studies remained after full-text screening and exclusions, containing results from a cumulative total of 27 experiments, which were included in the final meta-analysis and narrative synthesis.

Publication year

The majority of results (24) were published between 2012 and 2021, indicating a recent increase in scientific research relating to *Phytophthora* treatments (Figure 3). However, the earliest experiment included in this review was conducted in 1995, indicating a period of at least 26 years of research on this topic.

Meta-analysis

How effective are treatments?

Treatments reduced or limited *Phytophthora* infection symptoms compared to a control in all but one of the experiments (Table 4). In this case, a monoammonium phosphate soil amendment (Utkhede & Smith, 1995) exacerbated symptoms, producing a negative effect size of -0.18 but all other treatments yielded positive effect sizes of at least 0.2, with a large effect size recorded in 25 experiments. Nineteen of the experiments reviewed tested the efficacy of foliar sprays, which scored a large summary effect size of 1.11 (Figure 4) with a standard error (SE) of 12.995. Five experiments tested trunk injections, which scored a large summary effect size of 1.85 (Figure 5) with a SE of 2.381. Three experiments tested soil amendments, which scored a medium summary effect size of 0.61 (Figure 6) with a SE of 0.399. The summary effect size for all three treatment types was 1.04 (Figure 7) with a SE of 12.995.

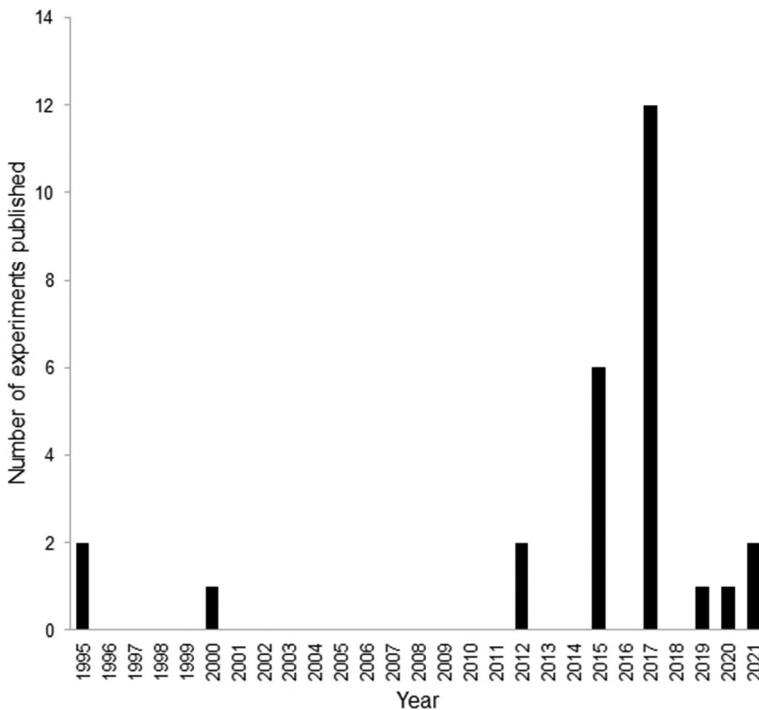


Figure 3. Histogram depicting the total number of experiments identified attempting *Phytophthora* control by inducing natural resistance published between 1995 and 2021.

Table 4. Summary of findings from the data extraction process with treatment effect size calculations. Only the largest mean effect within each experiment was recorded to demonstrate treatment efficacy when applied at optimal dosage levels. This was used to calculate an effect size using the Hedges' g formula (Equation 2).

Application method	Study	Active compound	Type of resistance	Intervention timing	Phytophthora species	Tree species	Tree age	Effect size
Foliar spray	Ali et al. (2000)	Potassium phosphonate	Facilitator	Remedial	<i>P. cinnamomi</i>	<i>Pinus radiata</i>	Sapling	10.32
	Berger et al. (2015)	<i>Bacillus amyloliquefaciens</i>	Facilitator	Preventative	<i>P. plurivora</i>	<i>Quercus robur</i>	Sapling	66.76
	Berger et al. (2015)	Phosphite	Facilitator	Preventative	<i>P. plurivora</i>	<i>Quercus robur</i>	Sapling	82.14
	Berger et al. (2015)	<i>Trichoderma atroviride</i>	Facilitator	Preventative	<i>P. plurivora</i>	<i>Quercus robur</i>	Sapling	51.73
	Rolando et al. (2017)	Phosphite	Facilitator	Preventative	<i>P. pluvialis</i>	<i>Pinus radiata</i>	Sapling	2.12
	Rolando et al. (2017)	Copper oxychloride	Facilitator	Preventative	<i>P. pluvialis</i>	<i>Pinus radiata</i>	Sapling	2.76
	Rolando et al. (2017)	Metalexyl-M	Facilitator	Preventative	<i>P. pluvialis</i>	<i>Pinus radiata</i>	Sapling	3.18
	Rolando et al. (2017)	Phosphite	Facilitator	Preventative	<i>P. pluvialis</i>	<i>Pinus radiata</i>	Sapling	1.77
	Rolando et al. (2017)	Copper oxychloride	Facilitator	Preventative	<i>P. pluvialis</i>	<i>Pinus radiata</i>	Sapling	0.94
	Rolando et al. (2017)	Metalexyl-M	Facilitator	Preventative	<i>P. pluvialis</i>	<i>Pinus radiata</i>	Sapling	2.12
	Rolando et al. (2017)	Phosphite	Facilitator	Preventative	<i>P. kernoviae</i>	<i>Pinus radiata</i>	Sapling	0.70
	Rolando et al. (2017)	Copper oxychloride	Facilitator	Preventative	<i>P. kernoviae</i>	<i>Pinus radiata</i>	Sapling	1.96
	Rolando et al. (2017)	Metalexyl-M	Facilitator	Preventative	<i>P. kernoviae</i>	<i>Pinus radiata</i>	Sapling	1.46
	Rolando et al. (2017)	Phosphite	Facilitator	Preventative	<i>P. kernoviae</i>	<i>Pinus radiata</i>	Sapling	1.09
	Rolando et al. (2017)	Copper oxychloride	Facilitator	Preventative	<i>P. kernoviae</i>	<i>Pinus radiata</i>	Sapling	2.75
	Rolando et al. (2017)	Metalexyl-M	Facilitator	Preventative	<i>P. kernoviae</i>	<i>Pinus radiata</i>	Sapling	1.39
	Solla et al. (2021)	Phosphite	SIR	Remedial	Phytophthora spp.	<i>Quercus robur</i>	Mature	0.91
	Solla et al. (2021)	Phosphite	SIR	Remedial	Phytophthora spp.	<i>Quercus ilex</i>	Mature	1.12
	Utkhede and Smith (1995)	Urea	Facilitator	Preventative	<i>P. cactorum</i>	<i>Malus domestica</i>	Sapling	0.20
	Stem injection	Berger et al. (2015)	<i>Bacillus amyloliquefaciens</i>	Facilitator	Preventative	<i>P. pluvialis</i>	<i>Fagus sylvatica</i>	Mature
Berger et al. (2015)		Phosphite	Facilitator	Preventative	<i>P. pluvialis</i>	<i>Fagus sylvatica</i>	Mature	3.37
Berger et al. (2015)		<i>Trichoderma atroviride</i>	Facilitator	Preventative	<i>P. pluvialis</i>	<i>Fagus sylvatica</i>	Mature	14.79
Gonzalez et al. (2020)		Fosetyl-Aluminium	SIR	Remedial	<i>P. cinnamomi</i>	<i>Quercus suber</i>	Young	0.88
Romero et al. (2019)		Fosetyl-Aluminium	SIR	Remedial	<i>P. cinnamomi</i>	<i>Quercus ilex</i>	Mature	0.85

(Continued)

Table 4. (Continued).

Application method	Study	Active compound	Type of resistance	Intervention timing	Phytophthora species	Tree species	Tree age	Effect size
Soil amendment	Utkhede and Smith (1995)	Monoammonium phosphate	Facilitator	Preventative	<i>P. cactorum</i>	<i>Malus domestica</i>	Sapling	-0.18
	Zwart and Kim (2012)	Biochar and salts of phosphorous acid	SIR	Remedial	<i>P. cactorum</i>	<i>Acer rubrum</i>	Sapling	1.8
	Zwart and Kim (2012)	Biochar and salts of phosphorous acid	SIR	Remedial	<i>P. cinnamomi</i>	<i>Quercus rubra</i>	Sapling	1.25

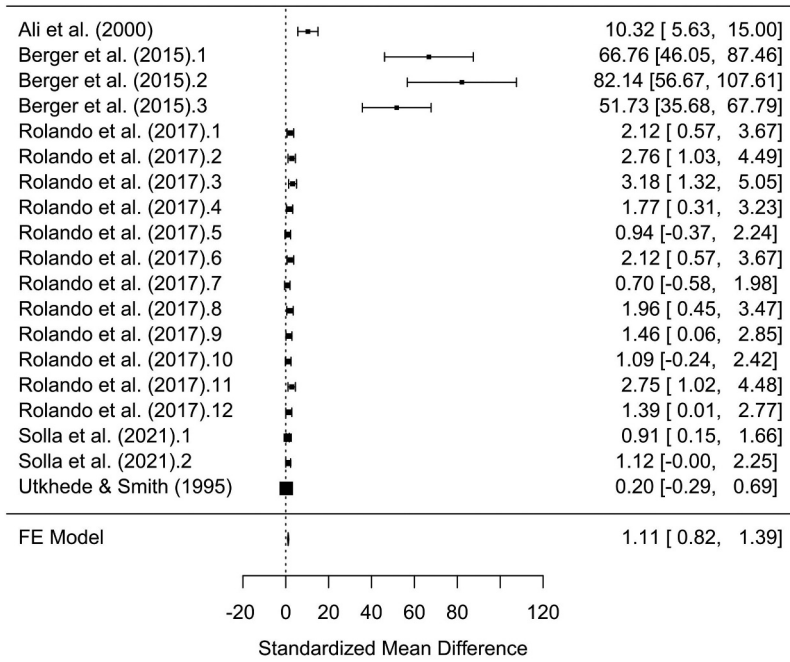


Figure 4. Forest plot of effect sizes for foliar spray treatments. The grand mean denoted by a diamond at the bottom is the summary effect of all the individual effect sizes.

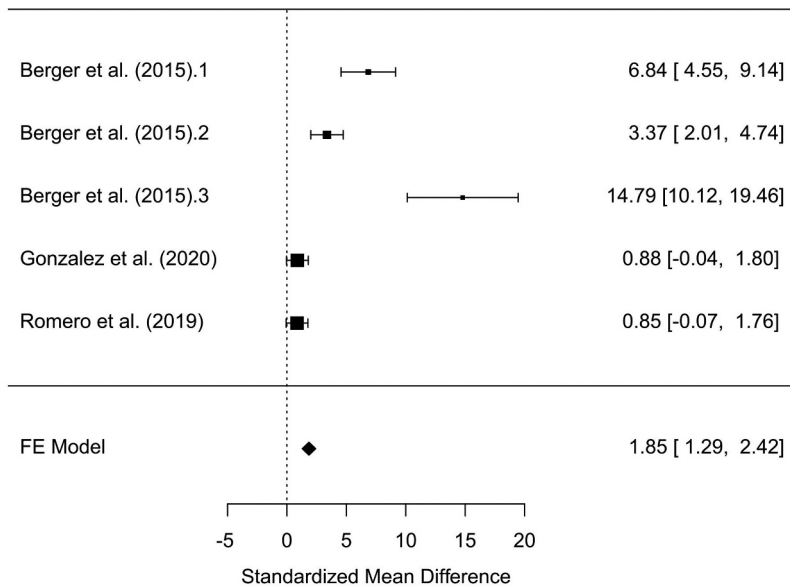


Figure 5. Forest plot of effect sizes for trunk injection and bark application of treatments. The grand mean denoted by a diamond at the bottom is the summary effect of all the individual effect sizes.

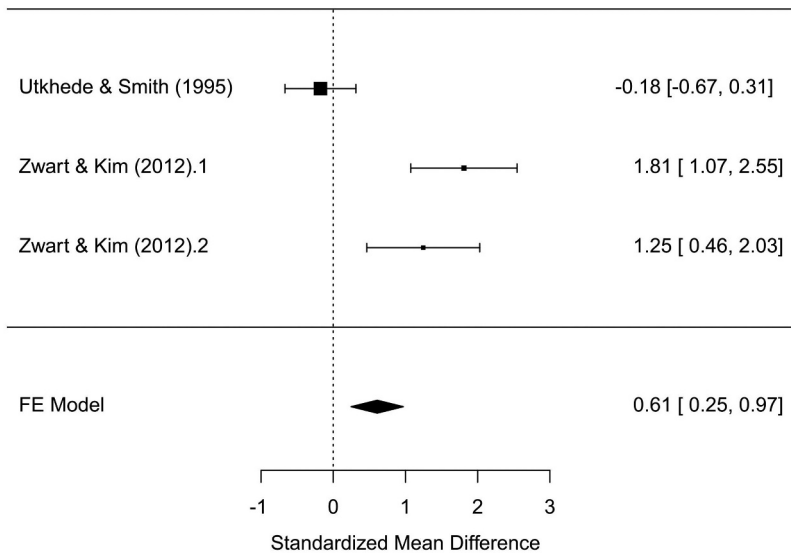


Figure 6. Forest plot of effect sizes for soil amendments. The grand mean denoted by a diamond at the bottom is the summary effect of all the individual effect sizes.

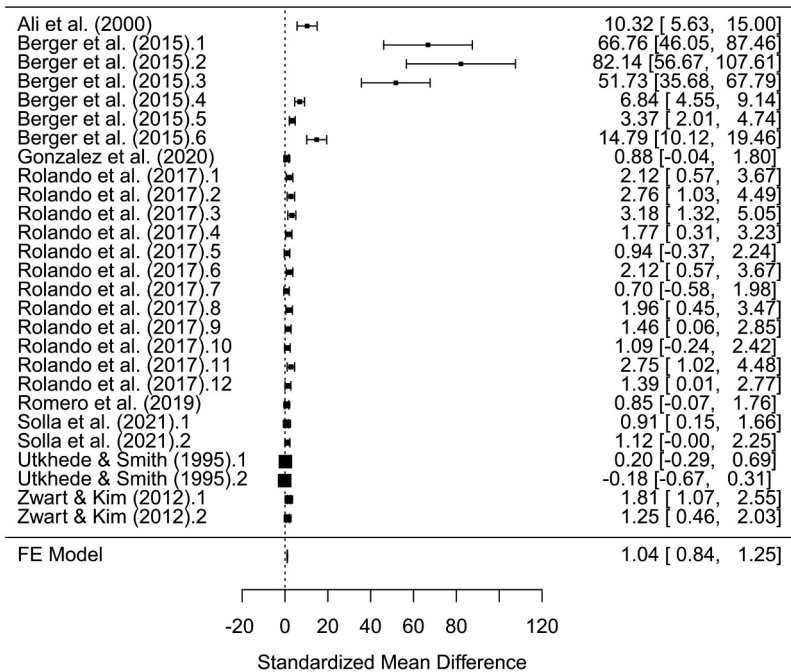


Figure 7. Forest plot of effect sizes for all treatments. The grand mean denoted by a diamond at the bottom is the summary effect of all the individual effect sizes.

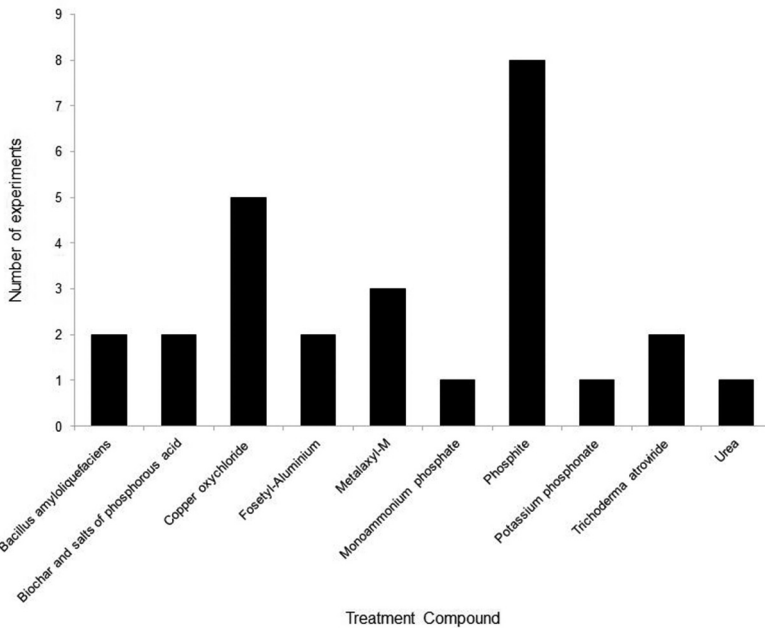


Figure 8. Histogram depicting the number of experiments categorised by active compound used.

How do the treatments work?

Treatment efficacy observed in six experiments (González, Romero, Serrano, & Sánchez, 2020; Romero, González, Serrano, & Sánchez, 2019; Solla et al., 2021; Zwart & Kim, 2012) was attributed to the process of SIR by the study authors. This process induces a tree's defensive response to *Phytophthora* species infections via signalling molecules contained within active compounds. These compounds were as follows: phosphite; fosetyl-aluminium; and biochar charged with salts of phosphorous acid. The mechanism behind a further 20 experiments in which the treatments reduced *Phytophthora* species symptoms was not specified. They were therefore categorised as having facilitated resistance to infections, either by directly suppressing pathogens locally or by another unspecified mechanism.

Ten different active compounds were identified during the meta-analysis (Figure 8). Phosphite was the most common active compound, appearing in eight experiments on its own, and a further two experiments in combination with biochar (phosphorous acid and phosphite are interchangeable designations). Twenty treatments were applied to trees before they became infected with *Phytophthora* species (Table 2), acting as a preventative measure that helped to limit the development of symptoms. Seven treatments were applied remedially, after symptoms appeared, to reduce lesion size or defoliation levels.

Which *Phytophthora* species did the treatments work against?

Treatments were tested against five different species of *Phytophthora* including *P. cinnamomi*, *P. kernoviae*, *P. plurivora*, *P. pluvialis*, and *P. cactorum* (Figure 9). Two experiments did not specify the species of *Phytophthora* being treated.

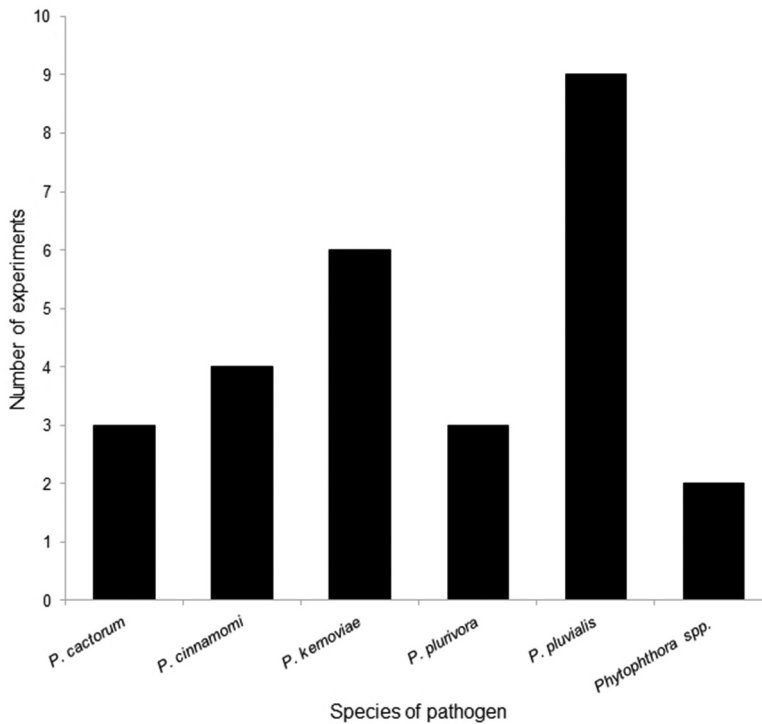


Figure 9. Histogram depicting the number of experiments conducted categorised by *Phytophthora* species.

Phytophthora cinnamomi

Four experiments, from four separate studies (Ali, Smith, & Guest, 2000; González et al., 2020; Romero et al., 2019; Zwart & Kim, 2012), measured treatment efficacy of foliar sprays, trunk injections, and soil amendments against *P. cinnamomi*. Studies reported symptoms of the disease to include wilting needles and branches, root decay, stem cankers, and tree death when left untreated. This review identified four different tree species as being susceptible to the disease: *Pinus radiata*, *Quercus suber*, *Quercus ilex*, and *Quercus rubra*. A remedial foliar spray (Ali et al., 2000), deploying a combination of potassium phosphonate and Bion, was observed to have the highest efficacy against the disease with a large effect size of 10.32. However, Ali et al. (2000) observed that it did not completely prevent root infection, nor did it eradicate the pathogen from the soil.

Phytophthora kernoviae

Six experiments, from one study (Rolando, Dick, Gardner, Bader, & Williams, 2017), measured treatment efficacy of foliar sprays against *P. kernoviae*. The study reported disease symptoms in *Pinus radiata* to include severe defoliation. A preventative foliar spray, deploying copper oxychloride, was observed to have the highest efficacy against the disease with a large effect size of 2.75.

Phytophthora plurivora

Three experiments, from one study (Berger, Czarnocka, Cochard, Oszako, & Lefort, 2015), measured treatment efficacy of foliar sprays against *P. plurivora*. The study reported disease symptoms in *Quercus robur* to include low crown density, fine and lateral root rots, collar and trunk canker, tarry spots, wilting, and branch dieback. A preventative foliar spray, deploying phosphite, was observed to have the highest efficacy against the disease with a very large effect size of 82.14.

Phytophthora pluvialis

Nine experiments, from two studies (Berger et al., 2015; Rolando et al., 2017), measured treatment efficacy of foliar sprays and trunk injections against *P. pluvialis*. Studies reported disease symptoms in *Pinus radiata* and *Fagus sylvatica* to include severe defoliation, fine and lateral root rots, collar and trunk canker, tarry spots, wilting, and branch dieback. A preventative trunk injection, deploying fungus *Trichoderma atroviride* (Berger et al., 2015), was observed to have the highest efficacy against the disease with a large effect size of 14.79.

Phytophthora cactorum

Three experiments, from two studies (Utkhede & Smith, 1995; Zwart & Kim, 2012), measured treatment efficacy of foliar sprays and soil amendments against *P. cactorum*. Studies reported disease symptoms in *Acer rubrum* and *Malus domestica* to include root rot, stem cankers, phloem damage leading to girdling, and tree death. A remedial soil amendment, deploying biochar and phosphorous acid (Zwart & Kim, 2012), was observed to have the highest efficacy against the disease with a large effect size of 1.8.

Which tree species were the treatments used on and how old were they?

This review found eight different tree species that were susceptible to *Phytophthora* species (Figure 10), all of which were also receptive to various interventions. The majority of the experiments (13) featured *Pinus radiata* and various *Quercus* species (8), but *Fagus sylvatica* (3), *Malus domestica* (2), and *Acer rubrum* (1) were also subject to successful interventions. These species represent four different taxonomic families: *Fagaceae*, *Pinaceae*, *Rosaceae*, and *Sapindaceae*.

Twenty of the experiments involved sapling trees, categorised as 0–2 years old, one experiment involved a young tree (i.e. 3–10 years old), and six experiments involved mature trees (i.e. 20 years or older) (Figure 11).

Were any adverse effects of treatment observed?

Monoammonium phosphate was observed to have a detrimental impact on *Malus domestica* (Utkhede & Smith, 1995) with mean disease incidence increasing as a result of its application via soil amendment. Zwart and Kim (2012) also reported increased disease incidence in *Acer rubrum* and *Quercus rubra* resulting from biochar and phosphorous acid soil amendments if applied at dosages of 10% or 20% soil volume. The optimum dosage was 5% soil volume.

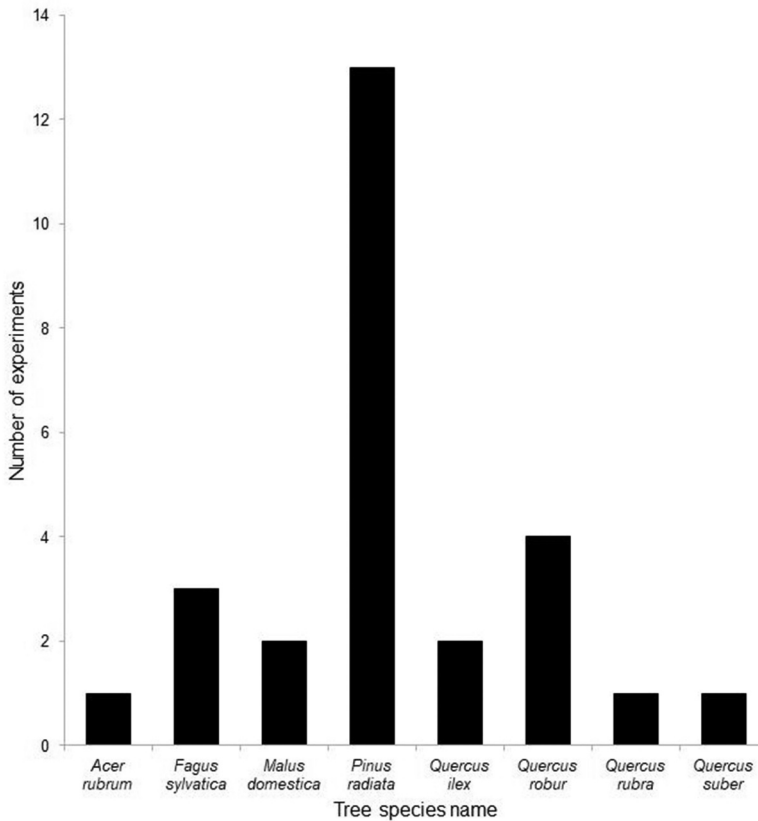


Figure 10. Histogram depicting the number of experiments conducted categorised by tree species.

Were there any potential effect modifiers in experiments?

The Utkhede and Smith (1995) study infers that both pesticides and herbicides were applied to trees being studied but does not state this explicitly. These factors may have influenced results, but it is not possible to definitively say so. No other potential chemical effect modifiers were identified during the review. However, the data shows significant differences in edaphic and environmental conditions between studies, for instance, five studies were conducted under field conditions and six under laboratory conditions (i.e. pot grown in a controlled environment). The external validity of studies conducted under laboratory conditions is open to scrutiny since it could be argued that results are not necessarily transferable to field conditions. This is considered further in the discussion section.

Publication bias

Funnel plots can be used to discern the potential for publication bias. For instance, Sterne et al. (2011) state that a meta-analysis may be considered free of publication bias if results are predominantly located within the funnel and they are scattered symmetrically. A funnel plot of effect sizes (depicted by black dots) from all experiments

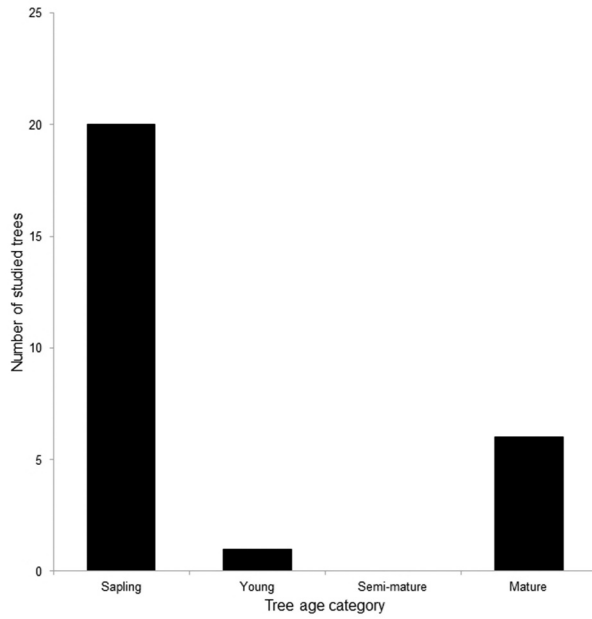


Figure 11. Histogram depicting the number of studied trees within four age categories: sapling (0–2 years old), young (3–10 years old), semi-mature (10–20 years old), and mature trees (>20 years old).

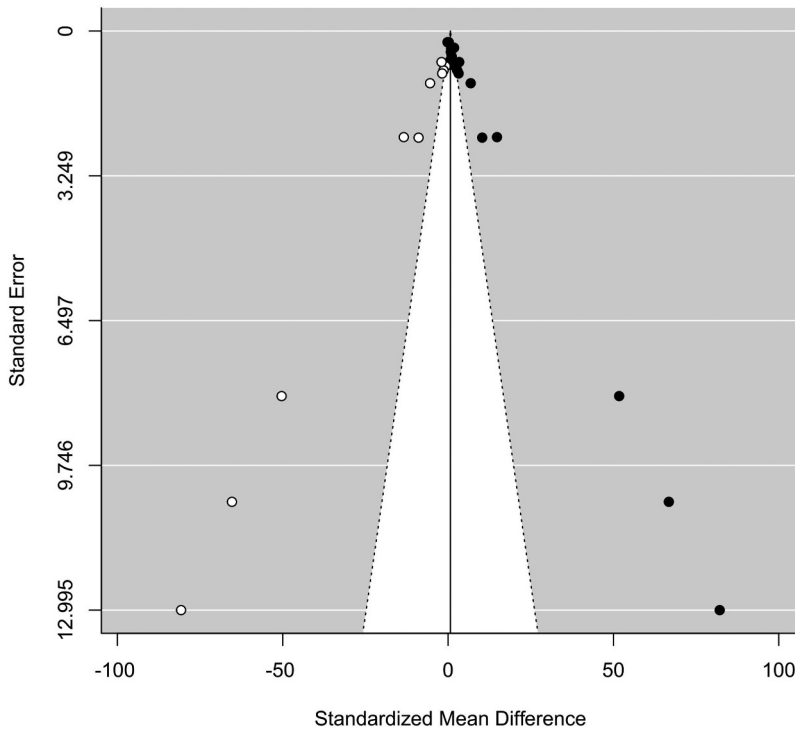


Figure 12. Funnel plot of effect sizes from individual studies. The plot was produced using a weighted regression with multiplicative dispersion model using standard error as the predictor.

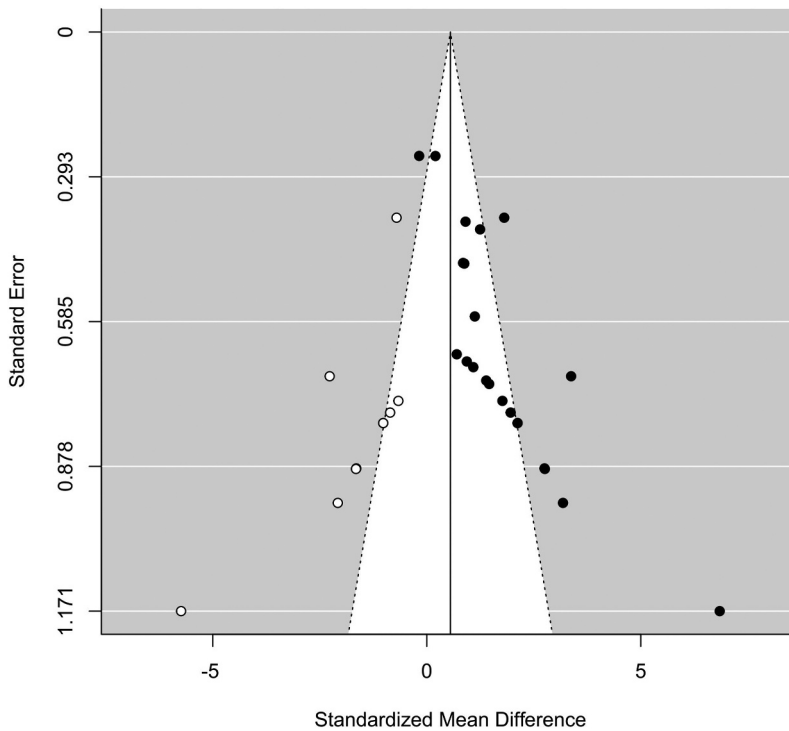


Figure 13. Funnel plot of effect estimates from individual studies with the five largest outliers removed. The plot was produced using a weighted regression with multiplicative dispersion model using standard error as the predictor.

included in the meta-analysis, using a fixed effects model and standard error as the predictor is shown in [Figure 12](#). It appears to indicate a high probability of publication bias since the results are scattered asymmetrically. There are also outliers with effect sizes considerably larger than the majority, suggesting that the standard error of the mean is high (12.995). In the funnel plot shown in [Figure 13](#) the five largest outliers are no longer included. In this version, approximately two-thirds of results are clearly within the funnel whilst the standard error of the mean is significantly lower (1.171).

Although the funnel plots presented here are asymmetric, Sterne et al. (2011) suggested multiple potential sources of asymmetry beyond publication bias. The most likely source within this meta-analysis is selective outcome reporting since only the largest mean effect within each experiment was recorded to demonstrate treatment efficacy when applied at optimal dosage levels. This may explain why only two results are scattered to the left-hand side of the solid vertical line (which signifies the threshold for little or no effect) since only one negative effect size and one small effect size appear in the data. Furthermore, Sterne et al. (2011) advise that tests for funnel plot asymmetry may not provide an accurate indication of publication bias if study sizes are similar. The majority (21) of experiments within this meta-analysis contained sample sizes of either five or 10 trees per experiment.

Discussion

Evidence reviewed here suggests a strong basis for the use of treatments against various *Phytophthora* species on infected trees in nurseries, urban forests, orchards, and arboreta, in both a preventative and remedial capacity. However, success appears to be dependent upon the application of treatments at optimal doses.

Trunk injections demonstrated the highest cumulative efficacy, whilst foliar sprays demonstrated the second highest efficacy, and soil amendments the lowest. However, there were only five and three experiments conducted for trunk and soil amendments, respectively, compared to 19 studies using foliar sprays. The SE of the mean was also lower for summary effect sizes on trunk injections and soil amendments than it was for foliar sprays, largely due to three outliers identified in the Berger et al. (2015) study. This suggests that the results are indicative but not definitive when it comes to comparing the efficacy of treatment types. If more of the studies identified during the search phase of this review reported numerical variance around the mean efficacy data, it would have been possible to include a greater number of studies in the meta-analysis, increasing the sample size for all treatments and therefore improving the reliability of findings. Despite this shortfall in data acquisition, all three treatment types were shown to have a medium to high level of efficacy overall.

Ali et al. (2000) observed that the most effective treatment within their study did not completely prevent root infection, nor did it eradicate *Phytophthora* from the soil. As all studies included in this review were conducted over finite periods, it is possible that trees could become reinfected over time and that, therefore, treatments may need to be re-applied intermittently. Further research is needed to establish the efficacy and longevity of all three treatment types. In the case of treatments eliciting SIR, it would be pertinent to discover whether immune responses are triggered indefinitely or for finite periods of time.

Implications for policy and management

Given that most experiments (20) involved saplings, the findings of this review may be most relevant to practitioners involved in woodland creation projects. For instance, tree nurseries supplying cell grown and bare root saplings could utilise foliar sprays and soil amendments to prevent or remediate *Phytophthora* species infection prior to sending trees into the field. Foresters planting the trees could also prepare planting areas with a soil amendment of biochar, for example, to increase the resilience of woodlands before they are created. The results of this review are also meaningful for practitioners working with established trees since young and mature trees were responsive to treatments. For instance, practitioners responsible for urban forests, orchards, or arboreta might consider trunk injections or foliar sprays for individual specimen trees in the early stages of *Phytophthora* infection.

The cost-effectiveness of such interventions requires further research, but this review demonstrates that alternatives to the pruning and felling of diseased trees now exist. This review therefore recommends updating best practice arboricultural guidance, such as the British Standard 3998 (BSI, 2010), with information on the benefits and practical application of treatments including foliar sprays, trunk injections, and soil amendments in treating *Phytophthora* based tree diseases.

Implications for research

Study validity

Six of the studies were conducted under laboratory conditions (Ali et al., 2000; Berger et al., 2015; Rolando et al., 2017; Zwart & Kim, 2012), potentially undermining the external validity of the results since they are not necessarily transferable to field conditions. This is because edaphic and environmental conditions are variable in the field, putting additional stresses on trees which could reduce the efficacy of treatments. It could be counter-argued, however, that since these experiments were conducted under controlled conditions, there were less effect modifiers potentially skewing results. Either way, the results provide a platform for further research wherein experiments could be duplicated under field conditions.

Phosphite as an important component in tree resistance to *Phytophthora* species

This review appears to demonstrate a correlation between phosphite and improved tree health, particularly as it is shown to elicit SIR or facilitate resistance to *Phytophthora* species in various tree species. Phosphite is an active ingredient in 10 of the experiments included in this review, and observed to be a by-product of fosetyl-aluminium in a further two experiments (González et al., 2020; Romero et al., 2019). Therefore, future research could explore the relationship between phosphite, trees, and a variety of pathogens, with a view to clarifying the mechanisms behind improved tree resistance to *Phytophthora*, and how phosphite could be used to manage or prevent landscape-scale outbreaks of disease.

Scope for further research in future

The majority of research included in this review was conducted in the last decade, indicating that the use of treatments against *Phytophthora* species is an emerging field of study. This may partially explain why only eight tree species are covered by the review, despite the fact there are known to be some 150 temperate plant species affected by the pathogen. That said, studies were excluded from the meta-analysis due to incomplete data sets; these included studies that investigated the effects of treatments on *Aesculus hippocastanum* (Percival, 2013), *Castanea sativa* (Del Maso, Cocking, & Montecchio, 2017), *Juglans regia* (Gentile, Valentino, & Tamietti, 2009), *Prunus armeniaca*, and *Prunus avium* (Turkalmaz and Dervis, 2017). Therefore, the potential for *Phytophthora* treatments across a wide taxonomic range is considerable. These excluded studies also tested treatments against a wider range of *Phytophthora* species including *P. cambivora*, *P. palmivora*, and *P. criticola*. For the purposes of systematic reviews in the future, this review recommends journal editors and study authors strive for greater uniformity in the presentation of findings for instance, by including important elements of datasets such as variance (e.g. standard error and standard deviation). This will enable larger, wider ranging meta-analyses to be undertaken.

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Data availability statement

The full dataset that support the findings of this study are available from the authors upon request.

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Notes on contributors

Andrew Walton is a Lantra qualified professional tree inspector and a Forestry Commission Woodland Officer. His areas of interest include ecosystem function, silviculture, urban forestry, forest policy, invasive species, and forest resilience to pests and diseases. He has an MSc in Forestry obtained at the School of Natural Sciences, Bangor University.

Andrew R. Smith is a Reader in Forestry at the School of Natural Sciences, Bangor University. His research interests encompass forest ecology, soil biogeochemistry, plant-soil interactions, forest pests and diseases, root biology and mycorrhizal symbiosis. He has a BSc in Forest Ecology and PhD in Forest Biogeochemistry both from the University of Wales.

ORCID

Andrew R. Smith  <http://orcid.org/0000-0001-8580-278X>

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