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Phytosanitary potential of ethanedinitrile: A fungicidal solution for wood pathogens in logs and timber traded internationally

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Abstract

Background: Phytosanitary treatments are essential in ensuring the safe trade of logs, lumber, or wood-based products by preventing the spread of harmful insects and pathogens across international borders. Methyl bromide and sulfuryl fluoride are effective fumigants which kill both invertebrate pests and plant pathogens which can damage exported logs. Both have adverse effects on the environment; therefore, less damaging alternatives should be investigated. Ethanedinitrile (EDN) has demonstrated efficacy against invertebrate pests in logs and does not damage the ozone layer or act as a greenhouse gas. However, its efficacy against plant pathogens has not been well tested.

Methods: This study tested the efficacy of EDN as a fumigant against 20 fungi and oomycetes associated with wood products, such as *Pinus radiata* D. Don logs. EDN was used as a fumigant in experimental chambers in which Petri plates were inoculated with a 5-mm agar disc from the growing edge of fungal and oomycete cultures. After treatment, mycelial growth was measured every 2–3 days for 14 days, after which EDN-treated discs were transferred to fresh media and growth was observed for an additional 14 days.

Results: Mycelial growth of all isolates was almost completely inhibited following EDN treatment at 50 g m^{-3} for 24 h, with no resumption of growth observed after transferring treated agar discs onto fresh media. The fungicidal effect of EDN was evident in both mycelium and putative survival structures, including oospores and chlamydospores.

Conclusions: These findings align with those of previous studies highlighting the potential of EDN as an alternative fumigant, offering broad-spectrum efficacy without the environmental drawbacks associated with methyl bromide and sulfuryl fluoride. The results suggest that EDN is an effective phytosanitary treatment for fungi and oomycetes that grow in logs, and that it could be used for managing pathogens associated with *P. radiata* logs or timber traded internationally. This research contributes to ongoing efforts to improve biosecurity measures in international wood trade.

Keywords: Disinfection; fungal pathogens; log fumigation; methyl bromide alternatives; oomycete; wood treatments

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Introduction

Phytosanitary treatments are critical for international trade, ensuring that commodities meet importing countries' biosecurity regulations to safeguard their natural resources from harmful insects and pathogens. These treatments are tailored to meet specific phytosanitary standards for each commodity, thereby preventing the introduction of invasive species. Recent research on postharvest disinfestation has underscored the importance of customised treatment approaches that target specific pests and pathways.

Ethanedinitrile (EDN) is a relatively new and promising fumigant, offering an environmentally sustainable and effective alternative to fumigants like methyl bromide (MB) and sulfuryl fluoride (SF) which have significant adverse environmental impacts. Unlike MB, which contributes to ozone depletion, or SF, which contributes to global warming, EDN provides an eco-friendly phytosanitary treatment solution (Shine & Kang 2023). EDN is registered to treat wood products in Australia, Malaysia, New Zealand, South Africa, South Korea, Russia, Turkey and Uruguay. It features advantageous characteristics, including efficacy against 22 insect species in nine families (Dowsett et al. 2004; Hooper et al. 2003; Lee et al. 2017a, b; Najar-Rodriguez et al. 2015, 2020; Park et al. 2014, 2021; Pranamornkith et al. 2014; Ramadan et al. 2020; Ren et al. 2006; Stejskal et al. 2017); eight species of nematodes in six families (Arbuzova et al. 2020; Douda et al. 2020; Lee et al. 2017b; Park et al. 2014; Seabright et al. 2020; Stevens et al. 2022; Uzunovic et al. 2021); three species of fungi in two families and one oomycete species (Uzunovic et al. 2021; Yang et al. 2023). EDN has been shown to penetrate deeply into treated pine (*Pinus radiata* D. Don) blocks (Ren et al. 2011; Hall & Adlam 2023); and its use leads to improved Workplace Health and Safety (WHS) for fumigators and bystanders compared with MB and SF (EPA 2022; Wood 2022).

EDN has demonstrated broad efficacy across various commodities, with applications ranging from soil sterilisation and pre-planting pest management to the fumigation of logs as a quarantine treatment (Armstrong et al. 2014; Douda et al. 2021; Patar et al. 2023; Najar-Rodriguez et al. 2020). For instance, 12 concentrations of EDN ranging from 9 to 1,751 mg kg^{-1} soil were examined (Thalavaiasundaram et al. 2023) by injection into 1-L incubation jars containing 0.5-kg sandy loam or sandy soil sampled after 24 h. It was effective against several weed species, nematodes, pathogenic fungi, and oomycetes, including *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen, *Macrophomina phaseolina* (Tassi) Goid., *Pythium ultimum* Trow (syn. *Globisporangium ultimum* (Trow) Uzuhashi, Tojo & Kakish), and *Verticillium dahliae* Kleb. EDN exhibited a dose-dependent response, with higher concentrations generally resulting in increased pest control. Further, concentrations between 100 and 200 mg kg^{-1} were found to be highly effective in controlling *F. oxysporum* and *P. ultimum*, while lower concentrations were sufficient to control nematodes. However, the optimal EDN concentration varied depending on the specific pest and soil conditions (Thalavaiasundaram et al. 2023). Similarly, EDN dissolved in water applied in strawberry production systems through drip tapes placed 2.5 cm below the surface of raised beds achieved statistically significant control of *M. phaseolina* (the causal agent of charcoal rot of strawberries) and a weed species (Yu et al. 2020). EDN concentrations ranging from 224 to 560 kg ha^{-1} were shown to be effective and achieved complete control of the pathogen, outperforming conventional fumigants such as 1,3-D (1,3-dichloropropene) and chloropicrin (Yu et al. 2020). The effectiveness of EDN was demonstrated for controlling clubroot (*Plasmodiophora brassicae* Woronin) by fumigating oilseed rape growing in mesh-enclosed pots, with concentrations of 42 g m^{-3} for 12 and 24 h, resulting in 100% disease control at both application durations, whereas 35 g m^{-3} for 48 h achieved 93.6% control (Patar et al. 2023).

EDN has shown promise in the context of controlling wood pathogens. For example, oak (*Quercus rubra* L. or *Q. ellipsoidalis* E.J. Hill) logs of varying sizes, ranging from 15.2 to 98.0 cm in length and 9.1 to 46.1 cm in diameter, were fumigated to control the oak wilt fungus, *Bretziella fagacearum* (Bretz.) Z.W.de Beer, Marincowitz, T.A.Duong & M.J.Wingfield (syn. *Ceratocystis fagacearum* J.Hunt) (Yang et al. 2023). These logs were treated with 120 g m^{-3} EDN for 24, 48, or 72 h, which significantly reduced pathogen recovery, with complete eradication in smaller logs and up to 94.0% reduction in larger logs. The effectiveness of EDN was tested against four tree pathogens (*Heterobasidion annosum* sensu G.Cunn., *G. morbida*, *Phytophthora ramorum* Werre, De Cock & Man in't Veld and *B. fagacearum*) grown on barley grains and then fumigated at 10 and 20 °C. In this study, EDN killed all pathogens except *G. morbida* at a 50 g m^{-3} dose for 1 or 3 h (Uzunovic et al. 2021).

Given the substantial economic contribution of log exports to countries such as New Zealand, where this sector plays a critical role in the national economy, it is imperative to ensure the efficacy of phytosanitary treatments meets the requirements of the importing country by mitigating the risk of invasions of harmful pathogens. This is particularly relevant for industrial round wood (logs and poles) which, by volume, is the leading wood export product globally (UNECE/FAO 2023). As EDN gains approval in more countries as a registered fumigant for treating export wood products, demonstrating its efficacy against emerging phytosanitary threats is crucial. Notably, fungi and oomycetes associated with imported *Pinus radiata* D. Don logs pose significant risks to some countries, such as India and Australia. Australia's unwanted organism list includes *Ophiostoma novo-ulmi*, *Fusarium circinatum*, *Phytophthora kernoviae*, and *Phytophthora ramorum* (DAFF 2024), all of which are known to be associated with other tree species and therefore could be present on logs if those are exported from countries where these pathogens are present. The fungal tree pathogens on the unwanted list for India include the fungi *Heterobasidion annosum*, *Leptographium procerum* and *Ophiostoma ulmi*, as well as the oomycetes *Phytophthora cactorum*, *P.*

cryptogea and *P. ramorum* (MPI 2025). The present study aimed to evaluate the effectiveness of EDN in killing a range of fungal and oomycete tree pathogens, including most of these species, or closely related proxies for pathogens on these lists (DAFF 2024; MPI 2025) that are not present in New Zealand (Table 1).

A series of experiments was conducted to evaluate the fungicidal efficacy of EDN as a phytosanitary treatment for export logs, lumber or wood-based products which are currently fumigated with either phosphine or methyl bromide (NZFOA 2025). Phosphine needs to be applied under tarpaulins for 10 days to be effective (Armstrong et al. 2014). The selection of target organisms, including fungi and oomycetes, was guided by their known association with pine logs, using cultures sourced from authoritative collections in New Zealand (Scion National Forest Culture Collection (NZFS) and the Culture Collection of Plant & Food Research). The impact of EDN on the mycelial growth of these pathogens was examined following published protocols (Everett & Timudo 2007; Shin et al. 2017), using the dose described in Uzunovic et al. (2021) for their *in vitro* studies. The results of this present study contribute valuable data for the ongoing enhancement of biosecurity measures in international trade.

TABLE 1: List of fungi and oomycetes treated with ethanedinitrile

Culture no.	Family	Genera and species	Common name	Reference record for New Zealand
NZFS[1]4711	Echinodontiaceae	*Amylostereum areolatum* (Chaillet ex Fr.) Boidin	-	Burnip et al. (2010)
CC[2]863	Ceratocystidaceae	*Ceratocystis fimbriata* Ellis & Halst.	Black rot of sweet potato	Slade (1960)
NZFS5495	Nectriaceae	*Corinectria fuckeliana* (C. Booth) C.González & P.Chaverri	Pine flute canker	Dick & Crane (2009)
NZFS3787	Botryosphaeriaceae	*Diplodia sapinea* (Fr.) Fuckel	Sphaeropsis blight	Curtis (1926)
NZFS5245	Mycosphaerellaceae	*Dothistroma septosporum* (Dorog.) M.Morelet	Red band needle blight	Gilmour (1967)
NZFS5479	Ophiostomataceae	*Grosmannia huntii* (Rob.-Jeffr.) Zipfel, Z.W.Beer & M.J.Wingf.	-	Reay et al. (2005)
NZFS24	Bondarzewiaceae	*Heterobasidion araucariae*	-	Anonymous (2024)
NZFS626	Ophiostomataceae	*Leptographium procerum* (W.B.Kendr.) M.J.Wingf.	White pine root decline	Reay et al. (2005)
NZFS3676	Ophiostomataceae	*Leptographium radiaticola* (J.J.Kim, Seifert & G.H.Kim) M.Procter & Z.W.de Beer	-	Hausner et al. (2000)
NZFS169	Ophiostomataceae	*Leptographium truncatum* (M.J.Wingf. & Marasas) M.J.Wingf.	Black stain root disease	Reay et al. (2005)
NZFS820	Rhytismataceae	*Lophodermium conigenum* (Brunaud) Hilitzer	Lophodermium needle cast	Anonymous (2024)
NZFS5029	Ophiostomataceae	*Ophiostoma floccosum* Math.-Käärik	-	Reay et al. (2005)
NZFS3380	Ophiostomataceae	*Ophiostoma piceae* (Münch) Syd. & P.Syd.	Vascular mycosis of oak	Nkuekam et al. (2012)
NZFS5362	Peronosporaceae	*Phytophthora aleatoria* P.M.Scott, R.L.McDougal & P.M.Taylor	-	Scott et al. (2019)
NZFS4462	Peronosporaceae	*Phytophthora citricola* Sawada	Black hop root rot	Waterhouse (1957)
NZFS2564	Peronosporaceae	*Phytophthora cryptogea* Pethybr. & Laff.	Tomato foot rot	Anonymous (2024)
NZFS5513	Peronosporaceae	*Phytophthora kernoviae* Brasier, Beales & S.A.Kirk	-	Studholme et al. (2019)
NZFS5337	Peronosporaceae	*Phytophthora pluvialis* Reeser, W.Sutton & E.M.Hansen	Red needle cast	Brar et al. (2018)
NZFS108D	Teratosphaeriaceae	*Teratosphaeria cryptica* (Cooke) Crous & U.Braun	Eucalyptus leaf blotch	Anonymous (2024)
NZFS5392	Nectriaceae	*Thyronectria pinicola* (Kirschst.) Jaklitsch & Voglmay	-	Anonymous (2024)

[1] NZFS = Scion National Forest Culture Collection courtesy of Dr Darryl Herron, Curator.
[2] CC = Culture Collection of Plant & Food Research.

Methods

Preparation of fungi and oomycete cultures

Difco® potato dextrose agar (PDA; Fort Richard Laboratories Ltd, Auckland, New Zealand) was used as a growth medium in the Petri plates for all fungi, except oomycetes, which were grown on V8 agar (Riberio 1978).

After 10 days growth, a 5-mm diameter agar disc of mycelium was removed from the edge of growing colonies of fungi and oomycete cultures (Table 1) and placed in the centre of six Petri plates containing agar the day before treatment for treatment replicates 2, 3 and 4 (on 29, 30, 31 August 2023, respectively), and 2 days before treatment for replicate 1 (24 August 2023). The Petri plate lids were removed in a laminar flow cabinet (Gelman Sciences, HLF 120, Ann Arbor, Michigan, USA). Plates were placed inside thoroughly cleaned and sterilised (with 70% ethanol) 28-L fumigation chambers (Labconco® # 5530000, Kansas City, Missouri, USA). The chamber was a modified fibreglass vacuum desiccator cabinet (Labconco®). The chamber is equipped with a 3-way vacuum release valve, a hinged door, and a stainless steel (SS) pan. Modifications to the chamber door included extra gaskets and clamps to ensure gas tightness. The ports allowed introduction and sampling of the fumigant and eventual evacuation of the EDN from the chamber. The air inlet port was modified to allow the use of a syringe to inject the fumigant dosage. The 20 Petri plates were placed in six specially designed racks (20 cultures per rack), which were placed in each of six fumigation chambers (six replicates) in a laminar flow cabinet. Lids were removed and left inside the chambers, and the chambers were sealed. Sealed fumigation chambers were placed in a temperature-controlled room set to 15 ± 0.4 °C for 12 h before the treatment to acclimate the organisms.

Gas chromatograph parameters

EDN was measured using an Agilent 7890 (Agilent Technologies, USA) gas chromatograph equipped with a flame ionisation detector (GC-FID). The conditions used were the same as those used by Brierley et al. (2019), who validated a GC-FID method to quantify EDN in air.

Fumigation trials

Ethanedinitrile (EDN™ 97%) was supplied by Dravslovka Services NZ (Auckland, New Zealand). EDN was applied using a 2 L syringe through an injection port into three sealed fumigation chambers, which were placed in a controlled-temperature room maintained at 15 ± 0.4 °C. Each chamber received a dose of 50 g m^{-3} of EDN (equivalent to 4.2 g or 636 mL EDN per chamber). The chambers were then sealed for 24 h. Three additional chambers served as untreated controls, into which 636 mL of air was injected.

Gas samples (1 mL) were withdrawn from the fumigation chambers after 30 min. from gas injection to allow gas equilibration and then at hourly intervals throughout the first day of treatment, yielding between five and nine samples depending on the start time. Additional samples were collected 1–3 times on the second day, and the last sample was collected just before 24 h had been completed. The samples were immediately analysed using GC-FID, with the instrument calibrated before each experiment. The experiment was repeated four times.

Following the 24-h exposure, the chambers were ventilated by passing air through at a flow rate of approximately 28 L m^{-1} for 2 h. A HEPA filter was attached to the input pipe of each chamber during ventilation to minimise airborne microbial contamination.

For each replicate, 20 different fungal and oomycete cultures were fumigated (Table 1). Once the ventilation was completed (determined by GC analysis showing no EDN remaining inside the chamber), the fumigation chambers were opened inside a fume hood to replace the Petri plate lids and for measurements.

CT values

The sum of areas of trapezoids method was used to estimate the CT (concentration × time) product (TPFQ 2010). This method calculates the sum of the areas of trapezoids formed by consecutive concentration readings and a rectangle representing the initial concentration from 0 to the first reading. The triangle in the first trapezoid (between fumigant injection and the first reading) when the gas reaches equilibrium is not included in the calculation. This method was chosen because it accurately represents the total fumigant exposure by considering the area under the curve from the initial injection to the first reading and excludes the part of the graph that has not reached equilibrium.

Efficacy assessments

The mycelial diameters of fungi and oomycetes on the plates were measured (two measurements at right angles) inside the fume hood every 1-4 days for 14 days after treatment, as per standard protocols (Everett & Timudo 2007; Shin et al. 2017).

Statistical analysis

Growth rates (mm day^{-1}) were calculated for each isolate in each replicate by determining the slope of the linear portion of the mycelial diameter growth curves using the SLOPE function in Microsoft® Excel®. Data were graphically displayed using the graphics package Origin® 2025 (OriginLab® Corporation, Massachusetts, USA).

The mixed effects model analysis of variance (ANOVA) function of MINITAB® version 21.3.1 (Minitab Pty Ltd, Sydney, Australia) was used to compare growth rates across treatments (EDN-treated versus untreated controls) and between isolates, with replicate measurements as a random factor. The normality of each analysis was confirmed by visual assessment of residual plots. Test results were considered significant at $P < 0.05$. All data are presented as means with standard errors.

Results

Fungal and oomycete growth

Mycelial growth of all fungi and oomycetes treated with EDN was almost completely inhibited, whereas mycelial growth of untreated controls continued (Figure 1). A few replicate plates of some cultures in the first treatment replicate showed a brief resumption of growth (for a single assessment date), but then growth stopped. These were *Amylosterum areolatum* (one plate), *Grosmannia huntii* (two plates), *Leptographium radiaticola* (one plate), *Lophodermium conigenum* (one plate), *Ophiostoma piceae* (one plate) and *Phytophthora cryptogea* (two plates). For the second treatment replicate, only one oomycete, *P. pluvialis* (one plate), resumed growth. In the third treatment replicate, one fungus, *Corinectria fuckeliana* (one plate), showed a small amount of growth a few days after treatment, but this stopped almost immediately. No fungi or oomycetes resumed growth in the fourth treatment replicate.

After 14 days, the inoculation discs were removed from the treated plates and placed on fresh PDA or V8 agar. There was no resumption of growth for any of the species or replicates after another 14 days.

Aerial contamination occurred in 97/240 of the untreated control Petri plates, but the colonies, apart from one instance, did not compete with the inoculated fungi or oomycetes. The slow-growing culture of *Dothistroma septosporum* was outcompeted by aerial contaminants on one Petri plate for the third treatment repeat; hence this replicate was removed from the analysis. EDN eliminated all aerial contaminants from the 240 treated Petri plates. Possible sources of this contamination were air injected into the chambers using a non-sterile syringe, and the replacement of Petri plate lids after treatment in a fume hood containing unfiltered air. It was not possible to sterilise the EDN application syringe due to concern that residual sterilant could affect the precision of application. Despite spraying the air in the container in which the fumigation chambers were located with 70% ethanol, aerial contaminants were still present. These fungal contaminants were identified from culture morphology as *Penicillium* spp.

Growth rate analysis

The mycelial growth rate of EDN-treated fungal and oomycete isolates was compared with untreated controls using ANOVA. ANOVA results indicated that treatment ($F = 532.36$, df = 1 and 135, $P<0.0001$) and isolate ($F = 6.07$, df = 19 and 135, $P<0.0001$) were highly significant factors affecting growth rates (Table 2). It was expected that individual fungal and oomycete isolates would show different growth rates. However, no significant differences were found between replicates ($F = 0.21$, df = 3 and 135, $P=0.892$), confirming consistency among replicate measurements (Table 2).

EDN concentrations during fumigation were analysed for consistency across replicates (Figure 2). A target concentration of 50 g m^{-3} was achieved in all chambers, with mean concentrations showing minimal variation between replicates (Figure 3). CT values over the 24-h treatment period were calculated for each replicate and are presented alongside the minimum final concentrations of EDN after 24 h (Table 3), as well as initial EDN concentration. Overall average sorption loss during the fumigations was 69.5% of the administered EDN dose.

Discussion

During our experiments, EDN killed fungi and oomycetes growing on agar. The almost complete inhibition of growth in treated plates, followed by no resumption of growth when transferred to fresh media, denotes fungicidal rather than fungistatic action of EDN. The brief spurts of growth that occurred, especially in the first treatment replicate, occurred for one day only for both species of *Leptographium* and for *Ophiostoma piceae*, *Phytophthora cryptogea*, *P. pluvialis,* and *Corinectria fuckeliana.* These growth spurts may be due to the development of survival structures before treatment. The delay between inoculation and treatment in the first replicate probably allowed these survival structures to form, as evidenced by the more pronounced growth in the first replicate than in the subsequent replicates. However, the lack of regrowth when discs from these cultures were transferred to fresh media suggested that EDN effectively eradicated any survival structures that may have formed, confirming its systemic fungicidal properties.

This is particularly significant given the biosecurity implications for international trade, especially for log exports. Our study has shown that EDN efficacy is not limited to the three fungal and one oomycete species on logs previously shown (Uzunovic et al. 2021; Yang et al. 2023), but is also effective against a further 15 fungal species from an additional seven fungal families and five more species of the oomycete *Phytophthora*. The ability of EDN to control both the mycelia and potential survival structures (e.g. oospores and chlamydospores) in oomycetes highlights its potential as a phytosanitary treatment; however, further studies are required to confirm this.

Our results align with the findings of Thalavaiasundaram et al. (2023), who demonstrated the efficacy of EDN across a variety of soil-borne pathogens, including *Fusarium oxysporum* and *Pythium ultimum*. Similar to our results, EDN had a fungicidal effect across different pathogen species, with no pathogen re-growth post-treatment. Although Thalavaiasundaram et al. (2023) focused on soil pathogens, our study expands the application to pathogens associated with traded logs, adding to the versatility of this fumigant in various biosecurity contexts. Moreover, the effectiveness of EDN in controlling both fungi and oomycetes in this study adds to the findings of Patar et al. (2023), who demonstrated complete control of *Phytophthora brassicae* in oilseed rape, further supporting the role of EDN as a potential fumigant for plant pathogen management.

The significant reduction in fungal growth observed here supports the work of Uzonovic et al. (2021), in which application of EDN completely inhibited fungal growth of

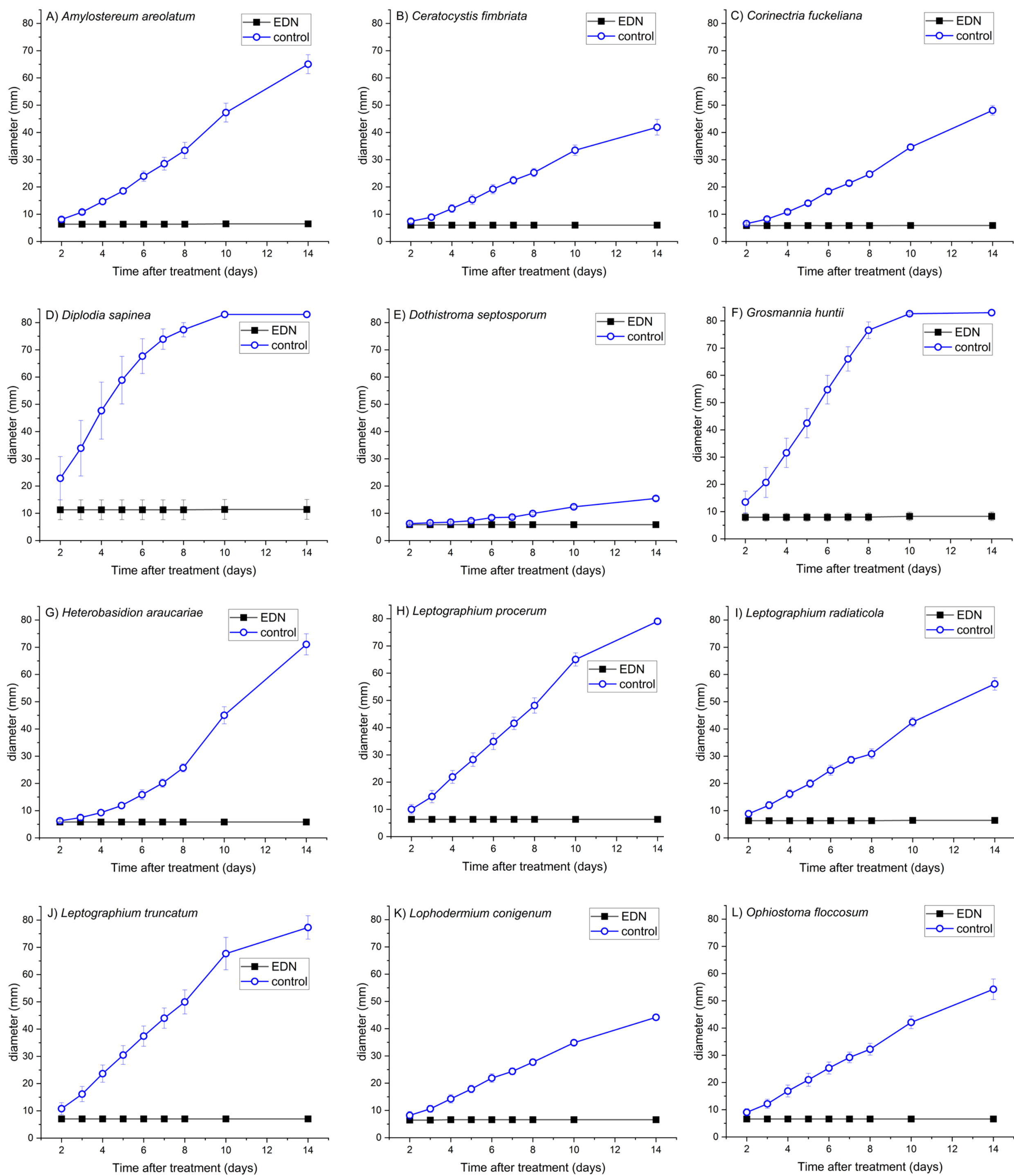

FIGURE 1: Mycelial growth (colony diameter in mm every 1–4 days) of fungi on Difco® potato dextrose agar and oomycetes on V8 agar in 83 mm diameter Petri plates. Plates were inoculated with 5-mm diameter agar discs containing mycelia. A measurement of 5 mm indicates no growth. There was a delay between discs placed on agar and treatment for the first of the four repeated treatments, with some growth occurring, accounting for initial measurements greater than 5 mm. When mycelium approached the edge of the Petri plate, growth slowed. Blue circles are untreated controls, and black squares are treated with EDN (50 g m^{-3} for 24 h). Values are the mean ± standard error of three Petri plate replicates treated on four separate occasions.

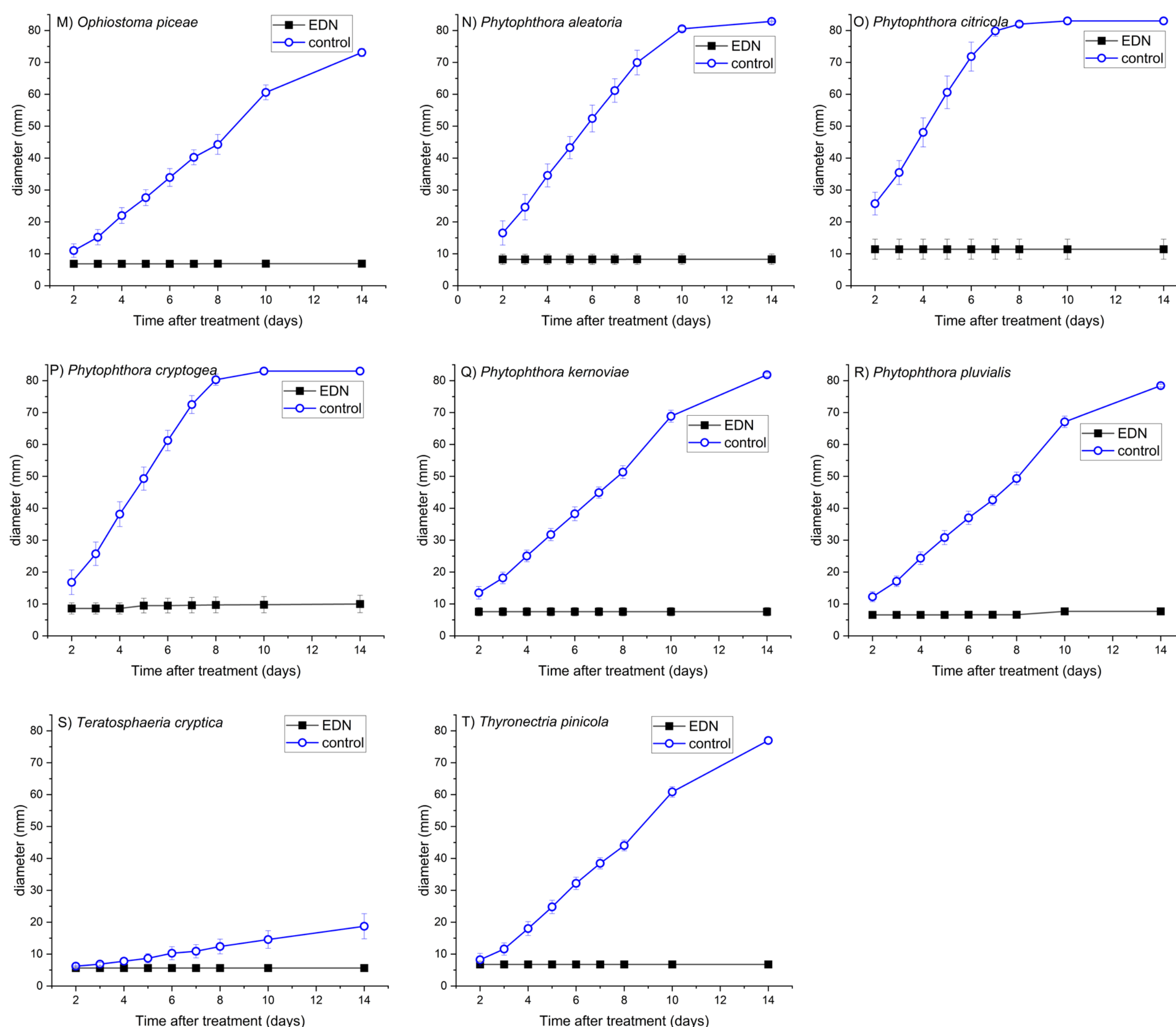

FIGURE 1 *continued*: Mycelial growth (colony diameter in mm every 1–4 days) of fungi on Difco® potato dextrose agar and oomycetes on V8 agar in 83 mm diameter Petri plates. Plates were inoculated with 5-mm diameter agar discs containing mycelia. A measurement of 5 mm indicates no growth. There was a delay between discs placed on agar and treatment for the first of the four repeated treatments, with some growth occurring, accounting for initial measurements greater than 5 mm. When mycelium approached the edge of the Petri plate, growth slowed. Blue circles are untreated controls, and black squares are treated with EDN (50 g m^{-3} for 24 h). Values are the mean ± standard error of three Petri plate replicates treated on four separate occasions.

TABLE 2: Analysis of variance for growth rate (mm day^{-1}) for 20 isolates of fungi and oomycetes treated with ethanedinitrile compared with untreated controls.

Source	DF	Adj. SS	Adj. MS	F-Value	P-Value[1]
Treatment	1	1313.16	1313.16	532.36	0.000
Isolate	19	284.48	14.97	6.07	0.000
Replicate	3	1.53	0.51	0.21	0.892

[1] Results were considered statistically significant at P<0.05.

FIGURE 2: Concentration (g m^{-3}) of ethanedinitrile (EDN) in each fumigation chamber immediately after injection and at subsequent intervals. The treatment replicates were for: 24 August (A); 29 August (B); 30 August (C); and 31 August 2023 (D).

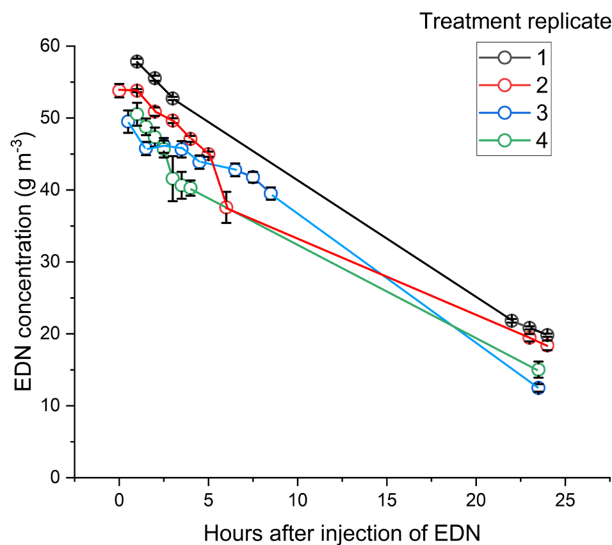

FIGURE 3: Ethanedinitrile (EDN) concentration (g m^{-3}) for all treatment replicates of fungi and oomycetes immediately after injection and subsequently. Values are presented as the mean ± standard error.

TABLE 3: CT values for treatment replicates of fungi and oomycetes in g h m^{-3} over 24 h, initial concentration, and minimum final concentration (g m^{-3}) after 24 h.

Treatment replicate	Initial concentration (g m^{-3})	CT (g h m^{-3}) over 24 h	Minimum final concentration (g m^{-3}) after 24 h
1	57.8	889.7	20.0
2	53.8	763.5	18.5
3	49.5	758.2	11.5
4	50.5	698.7	14.5
Mean ± SE	52.9 ± 1.88	777.5 ± 40.18	16.2 ± 1.93

tree pathogens from inoculated barley grains. Moreover, Yang et al. (2023) reported up to 100% reduction in pathogen recovery of EDN-treated logs (c. 12 cm diam.) naturally infected with *B. fagacearum*, demonstrating penetration of a fungicidal dose of EDN into these logs. Although pathogens were not completely eradicated from larger logs (c. 35 cm diam.), there was a 94% reduction in *B. fagacearum* infecting both the inner and outer sapwood. Adjustment of the EDN concentration or exposure duration may improve these results for larger logs, as well as how long after felling the logs are treated. EDN penetrates less effectively into recently cut logs with a high moisture content (Armstrong et al. 2014, CSIRO et al. 1996, Hall et al. 2018, Hall & Adlam 2023) suggesting greater efficacy when treating drier logs.

EDN has been shown to penetrate under the bark of *Pinus radiata* blocks with approximately 30% of the initial dose absorbed by the logs (Hall & Adlam 2023). Hall et al. (2018) showed that when logs were treated 4 weeks after harvest, c. 90% of the EDN was absorbed when treated at 10°C. Further studies are required to determine the most effective EDN dose for killing pathogenic fungi and oomycetes in logs, but it is likely to differ from the dose used in the *in vitro* studies reported here, due to absorption into logs.

While our study focused on *in vitro* cultures, the complete inhibition of growth across all isolates further supports the view that EDN has the potential to offer broad-spectrum protection against fungal pathogens following penetration into logs, as supported by the results of others (Hall & Adlam 2023; Uzonovic et al. 2021; Yang et al. 2023). Further studies to examine the effects of EDN on inoculated wood pieces or naturally infected logs are warranted to confirm these inferences before commercial use against fungal pathogens.

Furthermore, the absence of significant variation in growth rates between treatment replicates suggests that EDN treatment was highly consistent across all experimental runs. The controlled EDN concentrations achieved in the chambers, with minimal variation between replicates, reinforce this consistency. The CT values, ranging from 698.7 to 889.7 g h m^{-3}, were sufficient to ensure complete pathogen inhibition in all replicates, as indicated by the lack of re-growth in treated cultures. These CT values for EDN are in a similar range to those reported by Najar-Rodriguez et al. (2020), that is, 451-818 g h m^{-3}, for commercial-scale tarpaulin fumigations applied as 120 g m^{-3} for 24 h, which successfully controlled ≥ 190,000 insects from all life stages of the black pine bark beetle (*Hylastes ater* Paykull).

The ability to control a wide range of fungal and oomycete pathogens with a single treatment protocol using EDN demonstrates its potential as a phytosanitary tool, offering a viable alternative to currently used fumigants. Unlike methyl bromide, EDN does not deplete the ozone layer (Shine & Kang 2023), and compared to phosphine its relatively short treatment time makes it a more efficient option for industry stakeholders.

Conclusions and Recommendations

This study provides evidence that EDN is an effective fumigant for controlling fungi and oomycetes associated with *P. radiata* logs. There is some evidence that survival structures, as well as mycelia, may be killed by EDN. Its fungicidal action and its greenhouse gas friendly profile make EDN an effective tool for enhancing biosecurity measures in international trade. These findings contribute to the growing body of literature supporting the use of EDN across different commodities and highlight its potential to replace more harmful fumigants currently used in the forestry sector. However, before practical use can be recommended, the efficacy of EDN in treating inoculated wood and whole logs must be examined.

Competing interests

Dr Matthew Hall and Dr Kambiz Esfandi are currently employed by Draslovka Services Pty Ltd (Australia), a group of companies owned by Draslovka s.a. Kolin (Czech Republic) that manufactures ethanedinitrile (EDN™). As indicated by the author’s affiliation, Dr Esfandi was employed by Plant & Food Research (New Zealand) (now the New Zealand Institute for Bioeconomy Science Limited) when this research was completed. It is the opinion of the authors that this perceived conflict of interest has had no impact on the study or its findings.

Authors’ contributions

KRE was the primary author. KRE, CdV, KE, LJ designed and established the novel protocols and adapted insect chambers and protocols to pathology use. CdV and KRE maintained fungal cultures, assessed and analysed results. CB and KE operated the gas analyser and applied the EDN. LJ provided advice from her entomological

studies to apply to this pathology study. KE and CdV provided text, editorial comments and improvements to the manuscript. MS and LJ made and designed the Petri plate racks.

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